Beyond target chemicals: updating the NORMAN prioritisation scheme to support the EU chemicals strategy with semi-quantitative suspect/non-target screening data

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Abstract

Background Prioritisation of chemical pollutants is a major challenge for environmental managers and decision-makers alike, which is essential to help focus the limited resources available for monitoring and mitigation actions on the most relevant chemicals. This study extends the original NORMAN prioritisation scheme beyond target chemicals, presenting the integration of semi-quantitative data from retrospective suspect screening and expansion of existing exposure and risk indicators. The scheme utilises data retrieved automatically from the NORMAN Database System (NDS), including candidate substances for prioritisation, target and suspect screening data, ecotoxicological effect data, physico-chemical data and other properties. Two complementary workflows using target and suspect screening monitoring data are applied to first group the substances into six action categories and then rank the substances using exposure, hazard and risk indicators. The results from the 'target' and 'suspect screening' workflows can then be combined as multiple lines of evidence to support decision-making on regulatory and research actions.

Results As a proof-of-concept, the new scheme was applied to a combined dataset of target and suspect screening data. To this end, > 65,000 substances on the NDS, of which 2579 substances supported by target wastewater monitoring data, were retrospectively screened in 84 effluent wastewater samples, totalling > 11 million data points. The final prioritisation results identified 677 substances as high priority for further actions, 7455 as medium priority and 326 with potentially lower priority for actions. Among the remaining substances, ca. 37,000 substances should be considered of medium priority with uncertainty, while it was not possible to conclude for 19,000 substances due to insufficient information from target monitoring and uncertainty in the identification from suspect screening. A high

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degree of agreement was observed between the categories assigned via target analysis and suspect screening-based prioritisation. Suspect screening was a valuable complementary approach to target analysis, helping to prioritise thousands of substances that are insufficiently investigated in current monitoring programmes.

Conclusions This updated prioritisation workflow responds to the increasing use of suspect screening techniques. It can be adapted to different environmental compartments and can support regulatory obligations, including the identification of specific pollutants in river basins and the marine environments, as well as the confirmation of environmental occurrence levels predicted by modelling tools.

Keywords Contaminants of emerging concern, Retrospective suspect screening, Chemical prioritisation, Environmental risk assessment, NORMAN Database System

Graphical Abstract
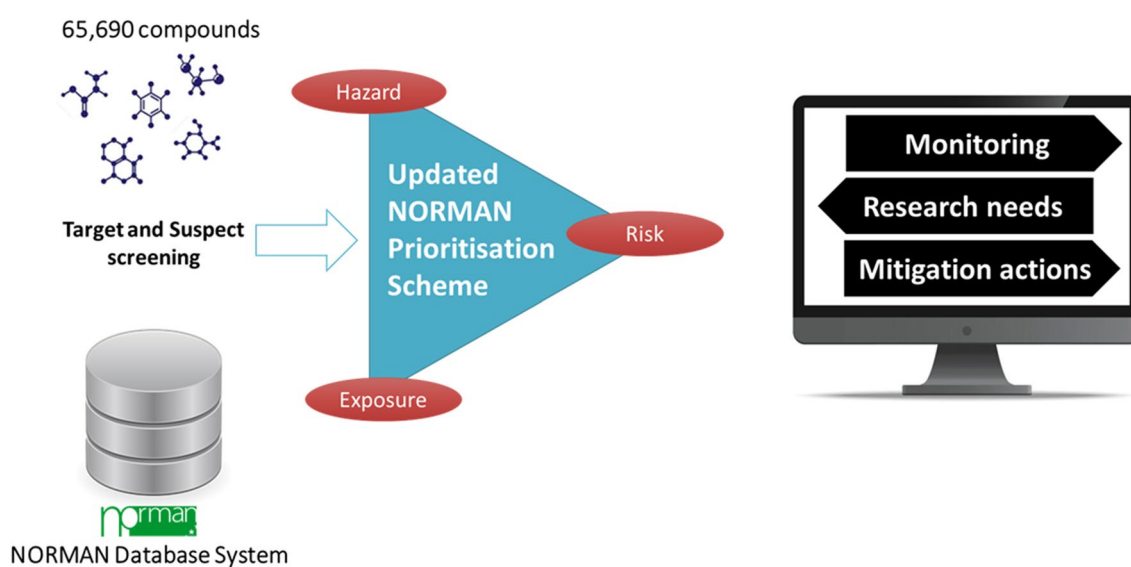
Background

Prioritisation of chemicals is of primary importance for environmental managers and decision-makers, both for defining priority actions for pollution prevention and control, and for allocating resources to address existing knowledge gaps in a cost-effective way. In the context of European and national legislation related to chemicals, monitoring data could be used more systematically and effectively for this purpose. However, given the large number of chemicals suspected to be present in the environment, data availability and data quality are limiting factors in the decision-making process [1]. The lack of knowledge on chemical exposure to humans and the environment is also recognised by the European Commission and Member States of the European Union (EU) in several policy documents and action plans [2].

The *EU Chemicals Strategy for Sustainability towards a toxic-free environment* was adopted by the European Commission in October 2020 [3] as part of the implementation of the Green Deal. It proposes a clear roadmap and timeline to tackle the current knowledge gaps and make chemicals legislation more effective for the safe and sustainable use of chemicals. One area of particular importance is the innovative use of chemical monitoring and hazard data to provide decision-makers with multiple lines of evidence for the identification of substances associated to specific chemical groups, endpoints (e.g. endocrine disruption, neurotoxicity, persistency), uses and sources that must be tackled as a priority.

The NORMAN network has pursued a similar vision since 2005, with the development of a number of interconnected databases which form the *NORMAN Database System* (NDS; https://www.norman-network.com/nds/) for the systematic collection of data on environmental monitoring, physico-chemical and hazard properties and quality targets to support the identification of

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relevant contaminants of emerging concern (CEC) in different environmental media. The prioritisation of chemicals has been a key activity in the NORMAN network, allowing the categorisation and ranking of chemicals according to different occurrence, hazard and risk indicators [4–6]. The efforts of NORMAN to improve knowledge about the environmental occurrence and potential risks of CECs are constantly evolving to accommodate new scientific developments. An important development is the increasing use of suspect and non-target screening techniques (both performed on data acquired using non-target analysis, as opposed to target analysis) in the chemical analysis of CECs [7]. Some crucial developments such as retention time indexing, substance curation, chemical domain calculation, semi-quantification and harmonised identification scoring system have been achieved over the last few years [7].

The aim of this article is to present and discuss an updated version of the NORMAN prioritisation scheme, which integrates recent developments such as retrospective suspect screening and predictive modelling tools into the prioritisation approach for the assessment of CECs. The original prioritisation workflow (Sect. "The NORMAN Database System and original prioritisation scheme"), designed to work exclusively with target monitoring data, has now been extended to integrate target and suspect/non-target data in a systematic and transparent way as two separate lines of evidence. This updated version of the NORMAN prioritisation framework was then applied in a suspect screening case study involving 84 wastewater (WW) sites across Europe, considered as hotspots of riverine contamination (Sects. "Materials and methods" & "Results and discussion"). Collectively, this demonstrates how the use of various databases that are part of the NDS can support a holistic, large-scale prioritisation of the several thousands of substances that are regularly present in the aquatic environment [1].

The NORMAN database system and original prioritisation scheme

The original version of the NORMAN prioritisation scheme [4–6], based on target monitoring data, is embedded in the *NDS.* It is directly supported by the Substance Database (*SusDat*), the Chemicals Occurrence Data (*EMPODAT),* the Ecotoxicology Database (*ECOTOX),* and the *Substance Factsheets*, explained in the following paragraphs.

The Substance Database (*SusDat*, https://www.norman-network.com/nds/susdat/) constitutes the NORMAN inventory of chemical substances for prioritisation. It is the result of the merging of all lists of environmentally relevant substances regularly contributed by NORMAN partners and NORMAN-connected activities as part of the NORMAN Suspect List Exchange initiative (NORMAN-SLE, https://www.norman-network.com/nds/SLE/) [8]. *NORMAN-SLE* contains 111 lists (as of 29 Nov. 2023) from different fields (e.g. per-/polyfluoroalkyl substances (PFAS), pharmaceuticals, pesticides, and transformation products (TPs)). The different lists are systematically combined and curated (i.e. removal of duplicates, removal of salts, neutralising, etc.) [8] before final integration into *SusDat*.

The *EMPODAT* Database (https://www.norman-network.com/nds/empodat/) was created in 2005 to host target monitoring data from different data sources, gathered in a standard format to facilitate data comparability and exploitation across Europe and beyond. *EMPODAT* provides the prioritisation tool with geo-referenced occurrence data from target monitoring studies of chemical contaminants conducted as part of research projects, national monitoring programmes, etc. in a variety of environmental matrices. More than 95 million records for over 4500 substances are available in *EMPODAT* (July 2023) thanks to the voluntary contribution of NORMAN members. Data are mainly from the aquatic environment (fresh water, wastewater effluents, marine water, groundwater, sediment and biota), while efforts are underway to improve the coverage of other compartments (e.g. soil, ambient air, indoor air and dust).

The NORMAN Ecotoxicology Database (*ECOTOX*, https://www.norman-network.com/nds/ecotox/) is a module designed for the systematic collection and evaluation of experimental ecotoxicity studies [e.g. ecotoxicity endpoints such as Lethal Effect Concentrations 50% (LC50) or No-Observed Effect Concentrations (NOEC)], as well as the compilation of existing quality targets, also referred to as the "Lowest" Predicted No-Effect Concentrations (PNEC). An in silico toxicity prediction model [9] based on the three basic trophic levels (i.e. algae, fish and crustacean), provides provisional PNECs for 93,613 of the *SusDat* substances (July 2023) with little or no experimental toxicity data. All *SusDat* substances are provided with predicted PNECs and/or experimentally based quality targets to calculate risks in support of the prioritisation of these substances.

Finally, physicochemical properties and other substance characteristics, such as the partitioning coefficients between octanol and water (K_{ow}), organic carbon and water (K_{oc}), the bioconcentration factor (BCF) and the degradation half-time (DT50), available on the NORMAN *Substance Factsheets* of the *NDS* (https://www.norman-network.com/nds/factsheets/) are used as key parameters for the classification of the substances based on the persistence, mobility and bioaccumulation (P, M, B) criteria. The physicochemical properties of substances
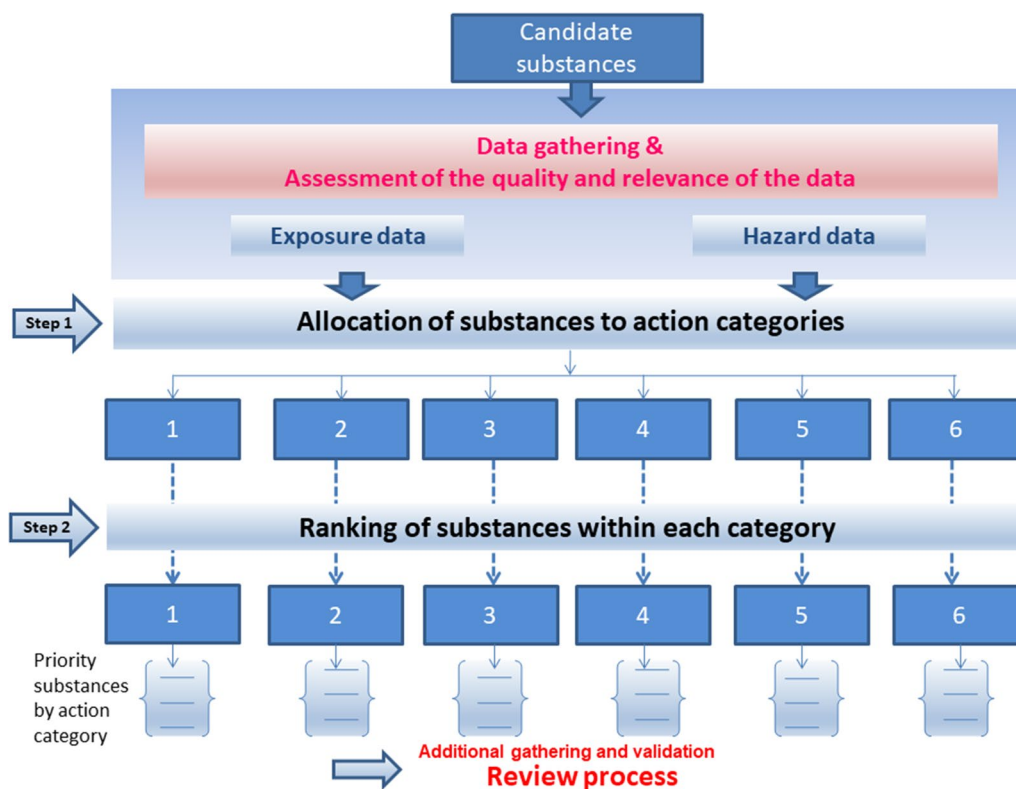
Fig. 1 Overview concept of the original two-step NORMAN prioritisation scheme for categorisation and ranking of emerging substances

are also crucial to support the selection of suitable instrumentations for analytical measurements.

The *NORMAN prioritisation scheme* involves a two-step approach (Fig. 1) where the substances are first allocated to action categories corresponding to specific knowledge gaps. The substances in each action category are subsequently ranked based on occurrence, hazard and risk indicators. Six main action categories have been identified, based on the most commonly identified knowledge gaps (Table 1). Note that there is no hierarchical ranking among the different categories; each corresponds to a particular knowledge gap.

Substances in Category 1 are of highest priority for control and mitigation measures (indicated with red shading in Table 1). For applications in the context of the Water Framework Directive, Category 1 substances should be proposed as candidates Priority Substances (PS), whereas top-ranked Category 2 substances would be priority Watch List candidates for improved knowledge about exposure levels and spatial distribution in the environment. Category 3 substances are also of high interest to decision-makers because there is sufficient evidence that they are frequently present in the environment. However, rigorous hazard assessment is necessary before final conclusions can be made about the associated risks. For

Category 4 substances, the quality of the monitoring should be improved before further actions are taken. For Category 5 substances both screening and rigorous hazard assessment are needed. Finally, for Category 6 (indicated with green shading in Table 1), monitoring efforts could be reduced, but mixture risks should be checked for substances frequently detected.

In this approach each substance is categorised based on its own 'knowledge-gap' profile, which is defined in relation to existing information and gaps in knowledge (e.g. insufficient spatial information on exposure levels, insufficient experimental data to assess adverse effects, or inadequate performance of analytical methods for environmental measurements). In this way, it is possible to prioritise less-investigated substances with a focus on the actions needed to reduce current knowledge gaps, whereas conventional prioritisation processes would tend to discard less-investigated substances or leave them on "stand-by" because of a lack of data.

It is important to stress that there is no hierarchical order of priority among the different categories. With the exception of Categories 1 and 6, which reflect the situation where all the information needed is available, the other categories (indicated by grey shading in Table 1) correspond to critical knowledge gaps which need to

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Table 1 Six action categories (original prioritisation scheme) identified based on common knowledge gaps

Cat ID	Current situation / knowledge gaps	Current situation (+) Yes; (-) No; (?) Maybe	Action categories
1*	Sufficient evidence of exposure and exceedance of no-effect thresholds (experimental quality target)	(+) Exceedance of quality targets (+) Sufficient analytical capabilities (+) Sufficient exposure data (+) Experimental effect data	Control measures required / Integration in routine monitoring and enforcement of legally-binding quality standards
2	Hazard assessment is based on experimental data BUT few monitoring data	(?) Exceedance of quality targets (+) Sufficient analytical capabilities (−) Sufficient exposure data (+) Experimental effect data	Watch List / screening studies needed to acquire info about levels of exposure and spatial distribution
3	Evidence of exposure BUT hazard assessment is based on predicted (eco)toxicity data (P-PNEC)	(?) Exceedance of quality targets (?) Sufficient analytical capabilities (+) Sufficient exposure data	Rigorous hazard assessment required before a conclusion on risk can be drawn
		(−) Experimental effect data	
4	Analytical capabilities not yet satisfactory	(?) Exceedance of quality targets (−) Sufficient analytical capabilities (−) Sufficient exposure data (?) Experimental effect data	Improvement of analytical methods required
5	No or few monitoring data AND hazard assessment is based on predicted (eco)toxicity data (P-PNEC)	(?) Exceedance of quality targets (+) Sufficient analytical capabilities (−) Sufficient exposure data (−) Experimental effect data	Screening studies AND rigorous hazard assessment needed
6*	Sufficient evidence of non-exceedance of no-effect thresholds (experimental quality targets)	(−) Exceedance of quality targets (+) Sufficient analytical capabilities (+) Sufficient exposure data (+) Experimental effect data	Monitoring efforts could be reduced, but mixture risks should be checked for substances frequently detected

Red shading: high priority for further action. Grey: additional information needed. Green: low priority, monitoring could be reduced. *) Cat.1 and 6 have two sub-categories, A and B, see Fig. 2

be addressed by decision-makers. It is also important to point out that substance categorisation and prioritisation is an iterative process that involves a periodic revision of the priority substances in each category whenever new information and/or more reliable data are generated or feedback from applied reduction measures is available.

The original NORMAN prioritisation scheme, operational since 2011, was applied to prioritise 500 substances in four river basins (Danube, Elbe, Scheldt and Llobregat), using the datasets collected in the EU MODELKEY project [6]. The scheme was also used by NORMAN to provide recommendations to the European Commission for the prioritisation of the substances on the first European surface water Watch List [10] and for developing a groundwater Watch List [11]. It has been adopted by regulatory agencies in France [12, 13] and Slovakia [14] for prioritisation studies at the national level [15]. More recently, the risk indicators of the NORMAN Prioritisation Framework have been used to identify risk drivers related to surfactants and their transformation products in wastewater [16] and in passive samplers in the Danube River [17]. However, this is all based on target chemical analysis results. As discussed above, there is a clear need to integrate suspect and non-target screening data due to the increasing application of these methods in monitoring [7]. These integration efforts to formulate the updated *NORMAN Prioritisation Scheme* are described in the next sections.

Materials and methods
Input data for the prioritisation scheme
In addition to the *EMPODAT*, *ECOTOX* and *Substance Factsheets* modules of the NDS, already presented in Sect. "The NORMAN Database System and original prioritisation scheme", the *NDS* offers several features which are essential for the exploitation of non-target high-resolution mass spectrometry data using retrospective suspect screening approaches [18]. The following additional modules are the key data sources to enable the extension of the NORMAN Prioritisation scheme to data generated by non-target screening techniques.

First of all, *SusDat*, which provides the inventory of candidate substances for suspect screening, with 120,513 unique chemical structures as of 13 Jan. 2024, is also the source of the mass spectrometric and retention information for the identification of substances with non-target screening (NTS) techniques (e.g. predicted retention time index, predicted electrospray ionisation fragmentation). This information is also retrievable from other *NDS* modules, including the *Digital Sample Freezing Platform* (*DSFP)*, which uses this information to search for the occurrence of substances in digitally frozen samples as explained in more detail below.

The *DSFP* (https://dsfp.norman-data.eu/) was developed in 2017 to share non-target screening high-resolution mass spectrometry (NTS-HRMS) data and is also the reference database for suspect screening monitoring data [19]. Within the *DSFP* it is possible to retrieve qualitative and semi-quantitative occurrence data for the chemical substances in the *SusDat* from "digitally frozen" environmental samples. Retrospective suspect screening of CECs is performed by searching for the exact mass of the most probable adduct ion and fragments' ions of a given substance (the contents of *SusDat* are available as a drop-down menu) in combination with the retention time index (RTI) [20] in the full scan spectra of the digitally archived samples. In this way, the DSFP allows for screening of presence of virtually any substance detected by HRMS in any environmental matrix, provided that the analytical method is fit for purpose.

The fragmentation pattern of substances present in *MassBank EU* (https://massbank.eu/MassBank/) is used to support identification where available, whereas for substances with unknown fragmentation behaviour, in silico predicted fragments are generated with CFM-ID 4.0 [21] and used.

Indicators for substance categorisation and prioritisation
Three types of indicators are applied to first categorise CECs into action categories, then rank them within each action category: exposure, hazard and risk indicators. The indicators reflect the physicochemical properties, ecotoxicity and occurrence data of the candidate substances, while the absence of certain information reflects existing data gaps.

Exposure indicators
The exposure indicators used in the categorisation phase assess whether the quality and quantity of the available monitoring data are sufficient to allow an adequate exposure assessment for the highly ranked CECs. The first two indicators are the number of countries and number of sites analysed, which reflect the level of investigation of a substance (well monitored substances vs. insufficiently monitored substances) against a defined reference value. In the light of current knowledge and experience, for an application for water monitoring at EU level, the workflow requires the availability of recent monitoring data (collected within the last 6 years) from at least 4 countries and 100 monitoring sites. These reference values can be adapted according to the objective of the study and the target compartments (see SI, Appendix B, Section S1).

Another indicator is the number of sites at which the substance was detected above the Limit of Quantification (LOQ). It indicates whether the exposure is

widespread or only a local problem based, as above, on a defined reference value. In the application of the WFD, at least 50 sites with results > LOQ are required. Again, the reference value can be defined according to the objective of the study.

Finally, the last indicator allows to check the compatibility of the applied analytical methods with the defined lowest PNEC (most reliable quality target agreed by experts) or environmental quality targets for a given compartment and target species. If the concentration of the substance is reported as "< LOQ" and the LOQ is above the "Lowest PNEC", the available monitoring data will not be sufficient to exclude a potential risk. For these chemicals, further monitoring is needed, and analytical methods should be improved to assess the real risk of the substance (i.e. Cat.4).

In the case of 'sufficiently monitored' substances with low quantification levels (less than 50 sites > LOQ), it is essential to investigate whether the infrequent quantification is due to very low (or zero) exposure levels or inadequate data quality (i.e. LOQ > lowest PNEC for the given contaminant), like e.g. in the case of the class of highly toxic pyrethroids [22]. A substance is defined as 'sufficiently' supported by 'good quality data' if there are at least 100 sites where measurements have been performed with an LOQ below the PNEC. This threshold aligns with the minimum number of sites required to classify a substance as "sufficiently monitored".

After the categorisation step, the substances are prioritised using specific indicators, which are meant to reflect the level of occurrence of the substances and their spatial distribution in the environment (e.g. frequency of observations above LOQ (FoQ), i.e. the number of sites with concentration > LOQ divided by the total number of investigated sites). An exposure index (see SI, Appendix B, Sect. 2.1) can also be used to rank the substances in Cat.2, 4 and 5 for which monitoring data are lacking. The same indicators can be used in both the categorisation and prioritisation steps.

In the case of retrospective suspect screening of substances from digitally archived NTS data in the *DSFP*, the frequency of observations above LOQ (FoQ) is replaced by the frequency of appearance (FoA), which is the frequency with which a given suspect substance is detected in the investigated sites (i.e. signals above noise and sufficient identification points, see IP score, below).

Hazard indicators

The hazard indicators used in the categorisation phase assess whether the quality and quantity of the available effect data are sufficient to allow for a rigorous hazard assessment of the candidate CECs. The workflow distinguishes between experiment-based and model-based quality targets. In the absence of quality standards derived by regulatory authorities, the workflow checks whether the available (eco)toxicity data are sufficient to establish a reliable and experiment-based quality target (e.g. PNEC) in the given environmental compartment. Note that for regulatory actions, acute effect data are not always considered sufficient to derive legally binding quality standards due to high assessment factors.

Following the categorisation step, a set of additional hazard indicators are used to prioritise the substances within the different action categories. The final hazard score is based on indicators considering:

- the carcinogenic, mutagenic and reprotoxic (CMR) classification,
- the persistent, bioaccumulative and toxic (PBT)/ very persistent and very bioaccumulative (vPvB) and persistent, mobile and toxic (PMT)/very persistent and very mobile (vPvM) classification (which can be combined in a persistent, mobile, bioaccumulative, toxic (PMBT) indicator),
- the potential of the substance to cause endocrine disrupting effects (ED).

These indicators are then weighted and combined according to the objectives of the prioritisation study (e.g. human health, wildlife protection objectives) to derive the final score. The details and sources used to assign the corresponding scores are reported in the SI, Appendix B, Section S2.2. The hazard indicators are independent of the analytical technique, i.e. target or suspect / non-target screening and therefore not changed in the update of the prioritisation scheme.

Risk indicators

The risk indicators used in the NORMAN prioritisation scheme are consistent with the Risk Quotient concept shown in Eq. 1:

$$RQ_i = \left(\frac{MEC95(i)}{Lowest\ PNEC(i)} \right), \tag{1}$$

where i refers to chemical i, MEC_{95} is the 95th percentile of the maximum measured environmental concentrations (MEC_{site}) of all sites monitored, and the *lowest PNEC* (most reliable quality target agreed by experts) refers to the safety threshold that should not be exceeded for chemical i in a given matrix.

Based on the risk quotient concept, three main indicators are applied to rank the substances in terms of potential risk (according to the data available); see detailed explanation in the SI, Appendix B, Section S2.3:

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- the Extent of Exceedance (EoE) of the *lowest PNEC* (to address the *intensity* of impacts associated to single substances exposure above the lowest PNEC).
- the spatial Frequency of Exceedance (FoE) of the *lowest PNEC* (to address the *spatial* impact associated to single substances exposure above the lowest PNEC).
- the frequency of Mixture Risks Contribution (MRC) (to address the frequency of occurrence of substances at concentration levels just below the *lowest PNEC*).

The three risk indicators are applied for the categorisation and prioritisation of substances (both target monitoring data and suspect screening data from *DSFP*). However, in the case of suspect screening data, their application relies on semi-quantified data, derived using the approach described in [23].

Exploitation of suspect/non-target screening data to improve prioritisation of insufficiently monitored substances

In addition to the indicators described in the previous section, two new approaches have been introduced to enable the application of this prioritisation scheme to NTS data: the identification point (IP) scoring system and the semi-quantification method.

The *identification point system (IP score)* [24] makes it possible to determine and communicate the *confidence of identification* of a substance according to the common approach in the environmental field [25] in an automated, concise and unambiguous manner. A machine learning approach based on random forest classifiers was used to efficiently filter substances with insufficient identification evidence.

The technical details behind the construction of this indicator are explained elsewhere [24]. For the *semi-quantitative analysis,* the method applied is based on a system-independent workflow which can provide estimation of concentrations at levels as low as 0.5 µg/L [26]. Briefly, detected suspected substances are semi-quantified based on the standard addition curve of the structurally most similar target substance. To find the structurally most similar target substance, 2D-linear fragment descriptors based on the original definitions of atom pairs and atom sequences were calculated [27], using the Tanimoto coefficient as the similarity distance function.

Application to a case study on wastewater effluent samples

The updated prioritisation scheme (see Sect. "The updated/expanded NORMAN prioritisation scheme") was applied to effluent wastewater samples as a proof-of-concept using the spectral data from the samples available in the *NDS*, i.e. target data in *EMPODAT* and suspect screening data in *DSFP*. The suspect screening data were obtained from retrospective screening of the 65,690 substances present in *SusDat* at the time (September 2021) on 84 wastewater effluent samples collected at 56 sites in 12 countries (Germany, Austria, Czech Republic, Slovakia, Hungary, Croatia, Serbia, Romania, Bulgaria, Ukraine, Greece and Cyprus), between 2017 and 2021 [28, 29].

The samples were collected in various multi-national campaigns, including the ITN-ANSWER project, SOLUTIONS project [30], Transformation Products of Emerging Pollutants in the Aquatic Environment (TREMEPOL), German national monitoring campaign 2018 [16] and Joint Danube Survey 4 (JDS4) [16, 31–33]. The target monitoring dataset included the data labelled as "Wastewater effluent" in *EMPODAT* from 2009 to 2021, i.e. 165,612 data points for 2579 substances. The full dataset (target plus suspect screening) included > 11 million data points. A dilution factor of 5 was applied to all concentration data (to convert the "wastewater concentration" into a "diluted waterbody concentration") before input into Eq. 1 for calculation of the risk quotient [34].

Results and discussion
The updated/expanded NORMAN prioritisation scheme
Figure 2 gives an overview of the extended categorisation workflow using target (Fig. 2A) and suspect screening data (Fig. 2B).

The prioritisation workflow starts from the assessment of the data available in the *NDS* (i.e. target monitoring data in *EMPODAT* and ecotoxicity data in the *ECOTOX* database), using the criteria defined in the target prioritisation scheme (Fig. 2A). If there is *sufficient evidence* available from target monitoring data to conclude about the level of concern of the substance, categorisation and prioritisation can already be made. Decision-makers can still opt to include information from the suspect screening workflow (Fig. 2B) if they wish to use this data as an additional line of evidence (e.g. additional sites, countries or years to extend the scale of the spatial or temporal assessment).

In the case of *insufficient* or complete lack of target data in *EMPODAT,* the suspect screening workflow (Fig. 2B) is applied for a preliminary assessment of the level of concern and as a trigger for follow-up actions. The first step is to check whether there is sufficient confidence in the identification of the substance from digitally archived data, using a new query: "*IP score above threshold?*", which was introduced into the new version of the decision tree. The classifier provides a response based on the collected evidence in the NTS-HRMS data. An
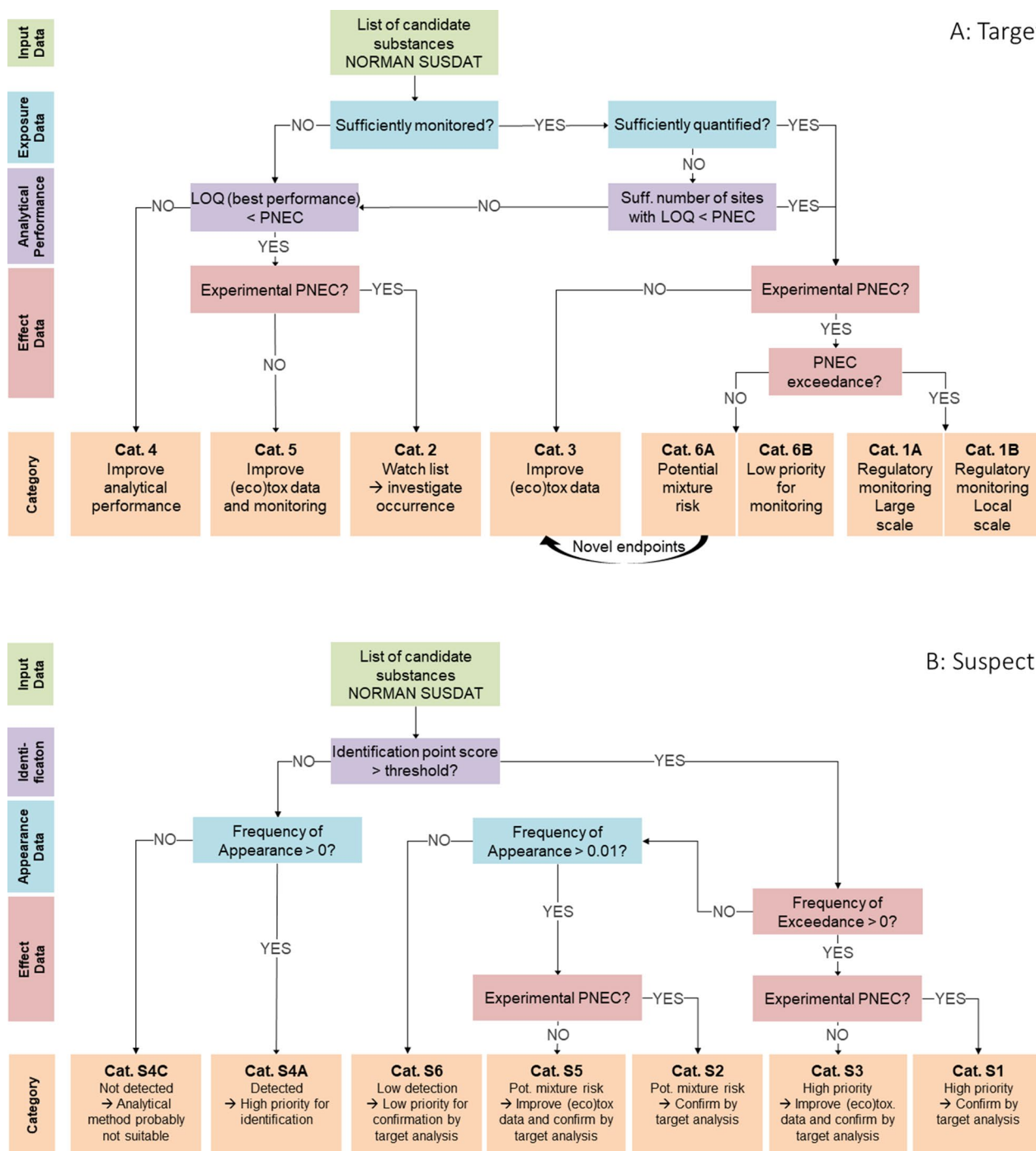
Fig. 2 NORMAN scheme for categorisation and prioritisation of CECs using: A target and B suspect screening data. The scheme in the figure illustrates only the categorisation part of the workflow, assuming that the substances are then ranked within each action category. (NOTE: Cat S4B is not currently in use, see Table 2)

IP score cut-off value (IP > 0.50), corresponding to Level 3 [25] is applied as a minimum requirement for identification of the substance. Substances that pass this query are submitted to additional queries in the decision tree to group the substances based on an estimate of their relative occurrence (i.e. FoA) and potential risk (i.e. FoE) and the existence of an experimentally based or predicted lowest PNEC. Each category can be associated to a well

Table 2 Six action categories identified based on exploitation of suspect screening monitoring data

Cat ID	Current situation / knowledge gaps	Current situation (+) Yes; (-) No ; (?) Maybe	Action categories
S1	Evidence of exposure and exceedance of no-effect thresholds. Hazard assessment is based on expermental data.	(+) Exceedance of quality targets (+) Sufficient analytical capabilities (+) Frequent positive detections (+) Experimental effect data	High priority for confirmation of identity with target analysis and monitoring action
S2	Low evidence of exceedance, BUT potential contribution to mixture risk. Hazard assessment is based on experimental data.	(−) Exceedance of quality targets (+) Sufficient analytical capabilities (+) Frequent positive detections (+) Experimental effect data	Medium priority for confirmation of identity with target analysis and monitoring action
S3	Evidence of exposure and exceedance of no-effect thresholds BUT hazard assessment is based on predicted data (P-PNEC)	(+) Exceedance of quality targets (+) Sufficient analytical capabilities (+) Frequent positive detections (−) Experimental effect data	High priority for confirmation of identity with target analysis and monitoring action recommended after revision of no-effect threshold
S4A	Evidence of exposure BUT insufficient confidence in the identification of the substance	(?) Exceedance of quality targets (−) Sufficient analytical capabilities (+) Frequent positive detections (?) Experimental effect data	High priority for improvement of analytical method for confirmation of identity with target analysis
S4C*	No evidence of exposure and insufficient confidence	(?) Exceedance of quality targets	Analytical method potentially not suitable / improvement of
Cat ID	Current situation / knowledge gaps	Current situation (+) Yes; (-) No ; (?) Maybe	Action categories
	in the identification of the substance and/or the applied analytical method	(−) Sufficient analytical capabilities (−) Frequent positive detections (?) Experimental effect data	the analytical method required
S5	Low evidence of risk, BUT potential contribution to mixture risk. Hazard assessment is based on predicted data (P-PNEC)	(?) Exceedance of quality targets (?) Sufficient analytical capabilities (+) Frequent positive detections (−) Experimental effect data	Medium priority for confirmation of identity with target analysis and monitoring action *after revision of no-effect threshold*
S6	Low evidence of exposure and non-exceedance of no-effect thresholds	(−) Exceedance of quality targets (+) Sufficient analytical capabilities (−) Frequent positive detections (?) Experimental effect data	Currently low priority for confirmation of identity and monitoring action

Table 2 (continued)

Red: high priority for analytical confirmation and monitoring action. Orange: medium priority for analytical confirmation and monitoring action. Grey: analytical improvements necessary before conclusions can be reached. Green: low priority for further action. *S4B is not displayed because it was foreseen for lower confidence identification case that may be included in a future version

identified action as indicated in Table 2. It is important to highlight that the choice of an IP-score cut-off value equivalent to "level 3" identifications has been intentionally made in alignment with the objective of this prioritisation framework (to prioritise substances for future action). The primary aim is to establish a "safety net" to identify potential new hazards that warrant further identification efforts.

The substances in Cat.S1 (immediately recommended for action) and Cat.S3 (action recommended after revision of the *lowest PNEC*) are important, because they represent a potential risk as individual substances (potential risk drivers). It is a priority to confirm their identity and environmental concentrations with target analysis, and they should be regarded as top candidates for monitoring action. The substances in Cat.S2 and Cat.S5 can be considered as potential contributors to mixture risks (i.e. mixture risk drivers). Individually they do not exceed quality standards, but they may represent a potential risk due to their frequent presence and co-occurrence in the environment at levels just below the lowest PNEC (i.e. $0.1 \leq RQ_i < 1$). Confirmation with target analysis is therefore recommended (relevant for Watch List). Since substances in Cat.S3 and Cat.S5 are prioritised based on PNECs derived from in silico predictions, improvement of the ecotoxicity dataset (extended literature research or generation of additional experimental toxicity values on relevant species) is recommended to increase the robustness of the prioritisation, before monitoring actions are taken. In fact, an experiment-based PNEC in the same order of magnitude would immediately result in a re-classification of the substance into Cat.S1. For the substances in Cat.S4A that do not pass the query "*IP score above threshold?*", further analytical actions (e.g. ion mobility, other types of spectrometric and chromatographic techniques, etc.) are needed to improve the identification process and confirm the identity of the substances before further monitoring actions can be started.

Substances in Cat.S4C, for which no signal is detected (FoA = 0, i.e. IPs = 0), reflect the case where either the substance does not occur in the environment or the analytical method is not suitable for the substance (there is a need for improving the analytical method (equivalent to Cat.4 in the target monitoring-based workflow). In contrast, substances classified as Cat.S6 (identified with sufficient confidence, i.e. IP score > threshold) are considered to fall within the applicability domain of the analytical method and FoE = 0 indicates no risk. NORMAN

places high importance to the definition of the chemical space covered by the analytical methods including sample preparation, suitability of chromatography and ionisability of substances. This is reflected in the predictions available for substances in the SusDat database. In the current workflow, evaluating the applicability domain of the analytical method for substances identified with insufficient confidence (IP score < threshold) should be conducted as a subsequent step. However, there are plans to explicitly define the applicability domain of the analytical method upfront for all SusDat substances in the near future.

Where non-target data are available in both positive and negative ionisation modes, the same workflow is run on the suspect screening datasets from each mode individually and the results from the two parallel categorisation process are then compared. The final "suspect" action category is assigned based on a worst-case outcome (i.e. more stringent category).

Combining results from target and suspect screening prioritisation workflows

The added value of the two complementary workflows is that the results from the 'target monitoring' and 'suspect screening' prioritisation workflows using the same or different monitoring data sets can be combined. Thus it is possible to analyse the different outcomes and then assign an 'overall action category' to each substance and a final score, based on multiple lines of evidence, as illustrated in Table 3 (please note that the numbers in the table refer to the results of the case study described in Sect. "Application to a case study on wastewater effluent samples").

The red area in the table refers to substances which are identified as Cat.1 or Cat.3, Cat.S1 or Cat.S3 in the respective workflows. Substances with insufficient or no evidence of risk from target monitoring data (i.e. all categories except Cat.1 and Cat.3) and which appear as frequently occurring in suspect screening (Cat.S2, Cat.S4A, Cat.S5) are of medium priority for confirmation with target monitoring (orange "overall action category"). The shaded orange region indicates "medium priority with uncertainty" for substances for which target monitoring data are absent and suspect screening data indicate insufficient evidence in the identification of the substance (i.e. 4A). The green area corresponds to substances with low evidence of the PNEC exceedance and/or very low frequency of detection. This is the case for substances

Table 3 Combined prioritisation of results from target and suspect screening workflows for 65,682 substances in *SusDat* in 84 wastewater samples of the case study

Target category	Exceeds PNEC	Sufficient. analytics	Sufficient exposure data	Experimental. effect data	Mixture Risk	Cat. S1	Cat. S3	Cat. S2	Cat. S5	Cat. S4A	Cat. S4C	Cat. S6	Total substances
					Exceeds PNEC	+	+	-	-	?	?	-	
					Suff. analytics	+	+	+	+	-	-	+	
					Frequently detected	+	+	+	+	+	-	-	
					Exp. effect data	+	-	+	-	?	?	?	
Cat. 1	+	+	+	+	+	3	n.a.	16	n.a.	12			31
Cat. 3	?	?	+	-	?	n.a.	1	n.a.	44	81	17	2	145
Cat. 2	?	+	-	+	?	8	n.a.	49	n.a.	197	91	2	347
Cat. 5	?	+	-	-	?	n.a.	12	n.a.	277	979	486	17	1771
Cat. 4	?	-	-	?	?	4			1	72	68		145
Cat. 6A	-	+	+	+	+	1	n.a.	10	n.a.	9	1		21
Cat. 6B	-	+	+	+	-	1	n.a.	28	n.a.	63	17		109
No data	-	-	-	-	-	3	472	26	5744	37907	18672	289	63113
Total substances						20	485	129	6066	39320	19352	310	**65682**

Priority Legend:	677	high	7455	medium	326	low
			37907	medium w/ uncertainty	19317	uncertainty

assigned to Category 6 in both workflows or substances with insufficient data in target analysis (Cat.2, Cat.5) and low frequency of detection in suspect screening. Finally, the grey region indicates uncertainty for substances for which target monitoring data are insufficient (or not available) and suspect screening data indicate that the analytical method is not suitable for the substance (i.e. Cat.4C).

In general, suspect screening data acquired with high-resolution mass spectrometry is only available for more recent samples, while target data collected from different sources may cover longer timeframes and reflect more scattered spatial patterns. Increasing frequency of appearance in suspect screening might indicate increasing trends or the onset of environmental exposure that may have been overlooked in target monitoring programmes. In contrast, lower concentration levels or fewer observations as compared to older target data might indicate a downward trend or phasing out of certain chemicals.

The categorisation/prioritisation algorithm for the 'target monitoring' prioritisation workflow is already built in the *NDS*, thus enabling automated prioritisation of all emerging substances contained in the *SusDat*, within the various categories, but can also be applied manually to other substances and datasets not included in the *NDS*. The integration of the NTS prioritisation workflow in the NDS is currently under way (planned to be finalised by the end of 2024). The prioritisation tool in the *NDS* allows easy updating of the categorisation and subsequent ranking of the substances in the event of inclusion of new substances or new data in the database. The final results can be exported as an Excel or csv file and can be checked by expert judgement.

Prioritisation of substances within each category
Once the substances have been allocated to one of the various action categories, the ranking of the substances within each action category is obtained using the set of indicators shown in Table 4.

The list of indicators in Table 4 are related to the exposure, hazard and risk assessment. Details on the derivation of the indicators and associated scores are included in the SI, Appendix B, Section S2). Since the objectives (i.e. actions) differ from one category to another (e.g. Category 4 for improvement of analytical performance; Category 3 and 5 for improvement of hazard assessment),

Table 4 Prioritisation indicators for the ranking process based on target monitoring and suspect screening data

Indicators			Application to categories	Value	Sub-score	Final score
Exposure	$Expo_{target}$	FoQ	All categories	0.00—1.00	$Expo_{target} = FoQ + EI$ (optional)	Expo score $= Expo_{target} + Expo_{suspect}$
		EI (exposure index)	Optional for Cat.2,4,5 (target monitoring)	0.00—1.00		
	$Expo_{suspect}$	FoA	All categories	0.00—1.00	$Expo_{suspect} = FoA$	
Hazard	$Haz_{Human\ Health}$	CMR	All categories	0.00—1.00	Haz score is counted only once in the final score	Haz score[1] $= CMR + ED + PBT/vPvB + PMT/vPvM$
		ED		0.00—1.00		
	$Haz_{Other\ properties\ of\ concern}$	PBT /vPvB		0.00—1.00		
		PMT/vPvM		0.00—1.00		
Risk	$Risk_{target}$	FoE_{target}	All categories	0.00—1.00	$Risk_{target}$[2] $= FoE_{target} + MRC_{target} + EoE_{target}$	Risk score $= Risk_{target} + Risk_{suspect}$
		MRC_{target}				
		EoE_{target}	Only Cat.1, 3 and 6	0.00—1.00		
	$Risk_{suspect}$	$FoE_{suspect}$	All categories	0.00—1.00	$Risk_{suspect}$[2] $= FoE_{suspect} + MRC_{suspect} + EoE_{suspect}$	
		$MRC_{suspect}$				
		$EoE_{suspect}$		0.00—1.00		
Final score (target + suspect screening)						**$= Expo + Haz + Risk$**

NOTE: (1) For the calculation of the hazard score the indicators and the associated weights can be adjusted according to specific prioritisation objectives; (2) in the current algorithm, each indicator has the same weight in the calculation of the risk score. However, a lower weight (1/2) could be given to the MRC indicator as compared to the FoE, as proposed by Sauer et al. [17], in order to reflect that the relevance of a substance contributing based on mixture risks (i.e. RQ between 0.1 and 1) is lower compared to a substance exceeding the PNEC as individual substance (RQ > 1)

the prioritisation indicators may differ from one category to another as well.

For the prioritisation workflow based on target monitoring data, the score of a substance is calculated using the following equations (see "Sub-score" column), depending on the type of action category:

– Final score$_{target}$ (Cat. 1, 3, 6) $=$ Expo_score (FoQ) + Risk_score (FoE + MRC + EoE)
– Final score$_{target}$ (Cat. 2, 4, 5) $=$ Expo_score (FoQ + EI) + Risk_score (FoE + MRC)

For the suspect screening prioritisation workflow, the same scoring system is applied for all categories:

– Final score$_{suspect}$ (all categories) $=$ Expo_score (FoA) + Risk_score (FoE + MRC + EoE)

The hazard score can be added as a supplementary part of the final score, by either adding up all individual scores (see below), or by multiplying it with the exposure score to derive a combined exposure and hazard score, similar to the risk score. The indicators and their weights can be adjusted, if needed, to address specific prioritisation objectives.

The final score, reflecting the results from both lines of evidence, will then be calculated as a sum of the individual scores, defined by the respective action categories in the 'target monitoring' and 'suspect screening' workflows.

– Final score $=$ Expo_score (Expo$_{target}$ + Expo$_{suspect}$) + Haz_score + Risk_score (Risk$_{target}$ + Risk$_{suspect}$)

In the case of substances for which target monitoring data are not available, the prioritisation score will be based on one line of evidence only, and the score will be a lower value.

Application to a case study on wastewater effluent samples
A total of 65,690 substances were screened in the case study involving 84 WW effluent samples. As expected, a high proportion (96% of the substances, 63,113 substances) were not covered by target monitoring data in *EMPODAT* (Table 3). Even considering the substances for which target monitoring data were available (2569), only 306 substances (less than 15%) can be considered as sufficiently investigated, i.e. assigned to Cat.1, Cat.3, Cat.6A and Cat.6B (monitored in ≥ 4 countries and at least 100 sites as defined in the NORMAN scheme [5]). Using the extended prioritisation workflow, it was possible to obtain evidence about the environmental occurrence for further 6,534 substances (sum of the substances assigned to Cat.S1, Cat.S2, Cat.S3, Cat.S5 and Cat.S6 in suspect screening, with no target monitoring data in *EMPODAT*) out of the 63,113 substances not covered by target data. Even if there is still a large percentage of substances (about 90% of the list of candidates from the suspect screening) with uncertainty in the identification (58,679 substances corresponding

to the sum of Cat.S4A and Cat.S4C), it was possible to detect a signal for more than 39,000 substances (Cat. S4A), and in this way identify the features with highest exposure and risk scores (i.e. FoA and FoE) to be selected for further elucidation of the structure.

Most of the substances in the candidate list (64,825 chemicals) had only predicted PNECs at the time of the study, which means that most of the frequently found substances were allocated to Category S3 (frequently found and evidence of risk) or Category S5 (frequently found with low or no evidence of risk), and a relatively small percentage was assigned to Category S1, Category S2 or Category S6.

Based on categorisation results (prior to the final prioritisation), 677 substances would fall in the red zone (high priority for further actions), 7455 in the orange zone (medium priority) and 326 in the green zone with potentially lower priority for actions. Among the remaining substances, more than 37,000 substances should be considered of medium priority with uncertainty while approximately 19,000 substances are classified as "uncertain" (i.e. no data or insufficient data from target monitoring and uncertainty in the identification from suspect screening or analytical method not appropriate). Overall, since the evidence collected with suspect screening was not sufficient to conclude on the identity or even appearance of a large number of candidate substances, efforts are still needed to improve the analytical capabilities to yield additional information for better enrichment, analysis, detection and finally structural identification in suspect screening.

Examples of representative substances in various combinations of categories from both approaches are shown in Table 5: and discussed further below for interpretation. Details of the screened substances are included in the SI, Appendix A.

Category S1: frequent PNEC exceedances
Category S1 substances are contaminants for which priority actions would be required at the level of regulatory monitoring and control of emissions, once confirmed by target analysis. Among the 20 substances in Category S1, two well-known pharmaceuticals *(diclofenac* CAS RN: 15307−86-5; *carbamazepine* CAS RN: 298-46-4) and one biocide (*bendiocarb* CAS RN: 22781-23-3) were already prioritised as Category 1 substances in the target prioritisation workflow, meaning that there is sufficient evidence from target monitoring data to demonstrate their potential risk to the environment. The frequencies of appearance of these substances obtained by target and suspect screening were consistent. Here the suspect screening approach could provide complementary additional evidence for prioritisation of these chemicals. For *diclofenac*

and *carbamazepine*, while both substances have the same final score, their individual risks profiles are actually quite different: diclofenac is more often found to exceed the PNEC (FoE: 0.43; MRC: 0.21), while carbamazepine is more often present at concentration levels close to the PNEC (FoE: 0.14; MRC: 0.45), i.e. a potential mixture risk contributor.

Amongst the highest ranked substances, there are several substances for which the information from available target monitoring data were insufficient (8 substances) or not available (3 substances). In particular, the conclusions based on the suspect screening prioritisation could provide evidence on industrial chemicals such as *8-hydroxyquinoline (CAS RN: 148-24-3)* (used as antiseptic drug and antifungal agrochemical), *nonanoic acid* (CAS RN: 112-05-0) (high tonnage biocide used in cosmetics and personal care products) and *oxacycloheptadec-10-en-2-one* (CAS RN: 28645-51-4) (flavouring and fragrance agent) for which no data were available in *EMPODAT*.

In the investigated sites of our study *17beta-estradiol* (CAS RN: 50-28-2) and other oestrogens, e.g. *17beta-trenbolone* (CAS RN: 10161-33-8, steroid), were detected in wastewater at relatively high concentration levels above the PNEC, which has led to their assignment to Category S1. The same substances were assigned to Category 4 in the target prioritisation scheme. Although local exceedances were observed also in the target dataset, an insufficient analytical performance (LOQ > PNEC) resulted in a limited number of sites where the substances were quantified. Therefore, they did not meet the more stringent criteria for allocation to Category 1 in the target prioritisation workflow (i.e. > 50 sites > LOQ). This example shows the added value of combining suspect and target monitoring data to identify substances of higher priority for improvement of analytical methods. However, it is important to be aware of the limitations of screening methods. Since they are not as sensitive as dedicated target methods, the detection of the two steroids in suspect screening might be a false positive result and should be verified before further action.

Finally, *climbazole* (CAS RN: 38083-17-9 antifungal agent) and *propyphenazone* (CAS RN: 479-92-5, pharmaceutical, analgesic) were both classified as Category 6A in target monitoring, meaning that they were frequently quantified at concentration levels not exceeding the PNEC, but still close to the threshold value. This is in line with the conclusion from the suspect screening data where these substances appear as frequently quantified but with lower frequency of exceedance of the PNEC (FoE: 0.01 for both substances). In this case the combined use of target and suspect screening data provides more robust information before further actions are taken.

Table 5 Examples of representative substances of concern in the various prioritisation categories for the present wastewater case study

Substances	CAS RN/ NORMAN SusDat ID	Final score[1] (suspect screening)	Final category (suspect screening)	Final category (target monitoring)[2]	Remarks
Diclofenac	15307-86-5 NS00000212	1.65	S1	1A	Pharmaceutical, non-steroidal anti-inflammatory drug (NSAID)
Carbamazepine	298-46-4 NS00000207	1.65	S1	1A	Pharmaceutical, antiepileptic agent, anticonvulsant
Bendiocarb	22781-23-3 NS00001535	0.35	S1	1A	Biocide, insecticide, carbamate
8-Hydroxyquinoline	148-24-3 NS00010316	1.42	S1	N/A	Industrial chemical, disinfectant
Bisphenol A	80-05-7 NS00008865	1.01	S1	2	Industrial chemical, phenol, plasticiser, ED substance
Nonanoic acid	112-05-0 NS00009752	0.96	S1	N/A	REACH; ≥ 1 000 to < 10 000 tonnes/year; biocide; cosmetics and personal care products
Oxacycloheptadec-10-en-2-one	28645-51-4 NS00013096	0.90	S1	N/A	Industrial chemical
4-Methyl-1H-benzotriazole	29385-43-1 NS00010509	1.16	S2	2A	Industrial chemical, corrosion inhibitor
Clarithromycin	81103-11-9 NS00008649	1.13	S2	1A	Antibiotic
5-Methyl-1H-benzotriazole	136-85-6 NS00008943	0.99	S2	6A	Industrial chemical, corrosion inhibitor
Tris(2-butoxyethyl) phosphate	78-51-3 NS00010389	0.93	S2	2A	Industrial chemical, phosphate plasticiser, vPvM
N-Methyl-2-pyrrolidone	872-50-4 NS00009178	0.92	S2	2A	Industrial chemical, plasticiser
Diisopropanolamine	110-97-4 NS00001427	0.85	S2	N/A	Antibacterial drug, pesticide
Vinyl neodecanoate	51000-52-3 NS00007080	0.82	S2	N/A	REACH ≥ 10 000 to < 100 000 tonnes/year; adhesives and coatings, machine wash detergents, fragrances
1,2-Propylene oxide	75-56-9 NS00009434	0.79	S2	N/A	Antibacterial drug, fungicide
Methacrylamide	79-39-0 NS00003892	0.75	S2	N/A	Industrial chemical, polymer production
Melamine	108-78-1 NS00010262	0.74	S2	2A	Industrial chemical, vPvM and PMT
O-Desmethylvenlafaxine	93413-62-8 NS00000330	0.18	S2	2A	Transformation product, antipsychotic drug, antidepressant
Mesotrione	104206-82-8 NS00000240	0.05	S2	N/A	Herbicide, pesticide
Meclofenamic acid	644-62-2 NS00000697	1.49	S3	5A	Pharmaceutical, NSAID, anti-inflammatory drug
Allyl alpha-ionone	79-78-7 NS00011942	1.46	S3	N/A	REACH (pre-registered); fragrance; food additive; flavouring agent;
1H-Benzotriazole, 5,5'-methylenebis-	15805-10-4 NS00020749	0.56	S3	N/A	Transformation product of benzotriazole
2-(2H-Benzotriazol-2-yl)-4,6-bis(1,1-dimethylpropyl)phenol	25973-55-1 NS00010624	0.14	S3	N/A	REACH; (UV-328) is an ultraviolet (UV) stabiliser with a phenolic group connected to the benzotriazole structure, on the Stockholm Convention for Persistent Organic Pollutants
Octabenzone	1843-05-6 NS00005821	1.39	S4A	N/A	UV stabiliser

Table 5 (continued)

Substances	CAS RN/ NORMAN SusDat ID	Final score[1] (suspect screening)	Final category (suspect screening)	Final category (target monitoring)[2]	Remarks
Pethoxamid	106700-29-2 NS00000317	0.81	S4A	4	Herbicide, pesticide, chloroaceta-mide
17Alpha-ethinylestradiol	57-63-6 NS00008601	0.71	S4A	4	Steroid
Spinosad	168316-95-8 NS00003609	0.49	S4A	2A	Insecticide, pesticide
Triticonazole	31983-72-7 NS00010104	0.24	S4A	4	Fungicide, pesticide
1-Methylbenzotriazol	13351-73-0 NS00000460	1.05	S5	5A	Industrial chemical, corrosion inhibitor
2,5-Dimethyl-2-ethylhexanoic acid	24353-79-5 NS00001375	0.92	S5	N/A	Herbicide, plant growth regulator
4-Hydroxy-3-methoxybenzalde-hyde	121-33-5 NS00009754	0.76	S5	N/A	Vanillin, flavourings, food, perfumes and pharmaceuticals
2,4,6-Triaminotoluene	88-02-8 NS00039235	0.00	S6	N/A	Industrial chemical
Dimethyl sulfoxide	67-68-5 NS00001957	0.00	S6	N/A	Organic solvent

[1] Final score suspect screening (all categories) = Expo_score (FoA) + Risk_score (FoE + MRC + EoE)

Category S2: priority substances with potential contribution to mixture toxicity

A total of 130 substances were classified in Category S2 based on the suspect screening data. For 26 of them, no target occurrence data existed, and they were therefore detected as potentially relevant substances only via suspect screening. Amongst them are several high-volume industrial chemicals (such as *methacrylamide*, and *vinyl neodecanoate* with CAS RN 79-39-0 and 51000-52-3) and antimicrobial agents (such as *diisopropanolamine* and *1,2-propylene oxide* with CAS RN 110-97-4 and 75-56-9). The prioritisation based on suspect screening data provided additional evidence on the environmental occurrence of substances of high PMT/vPvM potential, such as *tris(2-butoxyethyl) phosphate* (CAS RN: 78-51-3), *1,3-diphenylguanidine* (CAS RN: 102-06-7), *melamine* (CAS RN: 108-78-1) which were not supported by sufficient monitoring data in EMPODAT, and some widely used triazoles, such as *5-methyl-1H-benzotriazole*, *4-methyl-1H-benzotriazole* and *1H-benzotriazole* (CAS RN: 136-85-6; 29385-43-1 and 95-14-7).

The semi-quantified concentration levels from suspect screening in Category 2 do not exceed the PNEC values, but monitoring of potential mixture effects should be considered using the MRC indicator. Substances with a higher contribution to mixture risks receive a higher score within the list of Category 2 substances. This is the case for *clarithromycin* (CAS RN: 81103-11-9) which had an MRC of 0.39, meaning that it was detected at 39% of the sites at concentration levels in the range of 10% of the

PNEC. Likewise, *sulfamethoxazole* (CAS RN: 723-46-6) was frequently found in both target and suspect screening (95 and 80% of the sites, respectively), with exceedances only at local level in target analysis. No exceedance was observed in suspect screening, but 7% of the sites had concentration levels in the range of 10% of the PNEC, meaning that sulfamethoxazole would be prioritised as a potential driver of mixture risk in a combined suspect screening and target analysis prioritisation scheme.

Category S3: priority substances for further hazard assessment

As many as 485 substances were classified as Category S3 based on the suspect screening data, meaning that these substances are frequently occurring in wastewater at semi-quantified concentration levels above the PNEC, although an uncertainty exists associated with the predicted PNEC values. Most of these substances (472) are not supported by target monitoring data in EMPODAT. Among these more frequently occurring substances, some require particular attention, such as *allyl alpha-ionone* (CAS RN: 79-78-7) which is a suspected carcinogen under the ECHA Annex III inventory. Often there are no agreed experimental PNECs for these substances to protect aquatic ecosystems. Among pharmaceuticals, *meclofenamic acid* (CAS RN 644-62-2), an anti-inflammatory, analgesic drug similar to diclofenac (NSAID), and the antihypertensive agent *bisoprolol* (CAS RN 66722-44-9) exceeded the predicted PNECs in 36% and 13% of the samples, respectively. In the case of

5-methyl-2-(1-methylbutyl)-5-propyl-1,3-dioxane (CAS RN 80480-24-6), a fragrance used in cleaning products and cosmetics, 3 valid short-term toxicity studies on invertebrates are reported by ECHA, with an EC50 of 1.8 mg/L, leading to an estimated PNEC of 1.8 µg/L for freshwater (in line with the P-PNEC of 0.84 µg/L used in this study). The concentration values (dilution factor 5) showed exceedances for 10 out of 86 wastewater samples, suggesting the need to look in more detail on the ecotoxicity data for this substance, which might be potentially relevant for further target monitoring. Finally, *galaxolidone (CAS RN: 256393-37-0)*, a metabolite of *galaxolide* (a polycyclic musk widely used in soaps and cosmetics) was found in 18% of the samples (5% above the predicted PNEC). No experimental ecotoxicity data exist for this substance to our knowledge. Its parent substance, *galaxolide* (CAS RN: 1222-05-5), is classified in Cat.6A in the target prioritisation workflow (and in Cat.4A in suspect screening), meaning that the concentration levels do not exceed the PNEC, but they are still close to the threshold (i.e. potential contributor to mixture risks). The (eco)toxicity of degradation products of high tonnage chemicals ($\geq 1\,000$ to $< 10{,}000$ tonnes per annum) should hence be verified. Overall, suspect screening data provided a useful line of evidence for the identification of potential risks caused by the occurrence of substances not supported by sufficient target monitoring data in *EMPODAT*. Improvement in hazard assessment is required to confirm the environmental risk of these substances, which would potentially require regulatory scrutiny.

Category S4: priority substances for further analytical improvement

In total, 58,680 substances were classified as Category S4 substances, which means that the applied analytical approach was not able to collect enough evidence for elucidation of their identity and potentially the analytical method was not appropriate or sensitive enough for the substance. Among them, 19,360 are Category 4C substances, which were never detected. The other 39,320 substances in Category 4A showed FoA above 0, although with relatively low IP scores (i.e. their identification confidence is low). Over 96% of these substances were not studied in target analysis due to lack of reference standards in our case study, one example being the photostabiliser *octabenzone* (CAS RN: 1843-05-6) used for a variety of plastic systems, including food packaging materials. However, the list of S4 substances also includes some well-known contaminants such as *azithromycin (CAS RN: 83905-01-5), fipronil (CAS RN: 120068-37-3), diuron* (CAS RN: 330-54-1), *PFOS* (CAS RN: 1763-23-1) and *triclosan* (CAS RN: 3380-34-5), which are prioritised as Category 1 substances based on target monitoring

data available in *EMPODAT*. Indeed, suspect screening could not collect sufficient evidence to confirm the identity of these substances. The reasons for this shortcoming are likely due to analytical issues: e.g. poor fragmentation (common for substances detected in negative ionisation mode such as the above-mentioned substances), matrix effects in complex matrix (here wastewater) and limitations in the performance of the instrument (mass resolution obtained with the QTOF techniques used in this study is known to be lower compared to Orbitrap mass analysers).

In spite of the inherent limitations of the analytical instrumentation, the intensity of the signals are well aligned with the results from target monitoring, i.e. *azithromycin* (FoA 0.68; FoE 0.46; EoE 0.25) and *fipronil* (FoA 0.66; FoE 0.48; EoE 0.25) are frequently quantified with higher degree of PNEC exceedance, while *PFOS* (FoA 0.84; FoE 0.17; EoE 0.1) is frequently quantified with lower degree of PNEC exceedance and finally, *triclosan* (FoA 0.35; FoE 0.00 and EoE 0.00) is less frequently found in wastewater and at lower levels. In conclusion, suspect screening revealed substances that need enhanced chemical analysis to confirm their detection and quantification in suspect screening. Investigation to confirm the environmental presence of Category S4A substances could be focused on the ones that have high FoA from suspect screening (for example there are 470 Cat.S4A substances with FoA > 0.75 and no standard from the target analysis in this case study) and confirmed low PNECs (about 50 substances with experimental PNEC values below 1 µg/L) or PBT or PMT properties. With the confirmation of selected Category S4A substances by target screening, some of them could be moved to target Category 1, which would trigger follow-up actions in the regulatory context.

The Category S4C might contain substances for which the applied LC–electrospray ionisation (ESI)-HRMS analytical method is not suitable at all, e.g. substances that need specific enrichment or separation methods or can only be detected with GC–electron ionisation (EI)-HRMS due to, e.g. missing heteroatoms. This could be estimated from the physicochemical properties, molecular formula and molecular structure of the substances. Substances with high exposure index (high usage), which do not fall in the applicability domain of the screening method, should be selected for application or development of dedicated methods. For example, LC–ESI-HRMS/MS, currently the most common technique, could be complemented with GC–atmospheric pressure chemical ionisation (APCI)-HRMS/MS analysis.

Category S5: priority substances for further monitoring and improved hazard assessment

Approximately 6000 substances were assigned to Category S5. Based on the predicted PNECs and semi-quantified concentrations, these substances showed no exceedance of safety thresholds, which is not surprising given the multitude of substances present in our environment. The vast majority of the Category S5 substances were either supported by insufficient target monitoring data in *EMPODAT* (277 substances) or not supported at all by target monitoring data (the remaining 5744 substances). Amongst the highest ranked substances (based on FoA and MRC) there are several members of the class of *benzotriazoles* and their transformation products, widely applied as corrosion inhibitors in washing and cleaning products, de-icers, surface coatings, cutting fluids, etc., and already highlighted in Category S2 as ubiquitous contaminants in wastewater. Among the top-ranked substances there are also several representatives of the amines and amides chemical classes, registered as high-volume industrial chemicals. One example is *N-ethyl-2-methylbenzenesulfonamide* (CAS RN: 1077-56-1) used as pigment and plasticiser in various consumer products such as paints but also cosmetics [35], which was found in 95% of the samples. Another example is *N-ethyl-4-menthane-3-carboxamide* (CAS RN: 39711-79-0), a food additive (fragrance), which was frequently found (95% of the samples) at concentration levels close to the predicted PNEC (MRC: 0.23). Category S5 includes, amongst others, 23 substances, e.g. *geranyl acetate* (CAS RN: 105-87-3), *1,4-benzenediamine* (CAS RN: 106-50-3), *linalyl acetate* (CAS RN: 115-95-7), which are identified as suspect endocrine disrupters by ANSES/DEDuCT (NORMAN-SLE S99 ANSESEDC List [36] and as ingredients in cosmetics (NORMAN-SLE S13 EUCOSMETICS List von der Ohe and Aalizadeh [35]) and which are also highly present in the market (KEMI exposure index > 0.7). Experimental effect data (PNEC) need to be integrated in the *NDS* to allow for a proper risk assessment of all these substances, starting from those with highest MRC scores.

Category S6: low-priority substances according to current knowledge status

Suspect screening could identify 310 substances of 'low priority' with sufficient confidence for which target monitoring data are lacking in *EMPODAT*. This number represents a low percentage of the list of > 65,000 candidate substances investigated in this study. Surprisingly none of the 130 Category 6 substances from the target prioritisation scheme are found in Cat.S6. Nevertheless, the conclusions from target and suspect screening remain well aligned. These 130 substances were assigned either to Category S4C, meaning that they were not detected, most likely because the method was not appropriate, or to Cat.S4A or S2 with very low FoA scores, meaning that their occurrence in wastewater is not significant. One substance was assigned to Cat.S1. It is worth recalling that Cat.6A in the target prioritisation scheme corresponds to substances with risk quotient (RQ = MEC95/lowest PNEC) below 1, but still above 0.1.

Current knowledge gaps for exposure and hazard assessment to prioritise CECs for which actions are needed

As shown above, the use of suspect screening data in the prioritisation workflow provided additional lines of evidence to prioritise under-investigated contaminants in the environment. The novelty in the upgraded scheme is primarily in the concurrent use of target and suspect/non-target screening data available in the *NDS* for an integrated assessment and prioritisation of chemicals. The scheme provides a simple workflow which facilitates decision-making regarding under-investigated substances. The system scrutinises both the frequency and the level of detection to assess chemical occurrence, and compares concentrations to an environmental quality threshold, the lowest PNEC, to appraise the severity of the chemical risks to the environment. While the current study was focused on the aquatic environment, the same scheme can be adapted to any other environmental compartment (e.g. terrestrial). The substances contained in *SusDat* are automatically allocated to action categories via this prioritisation scheme, thereby providing evidence for the identification of potential priority CECs in wastewater (or the given matrix in question) and a preliminary assessment of their environmental risks.

Knowledge gaps for the determination of chemical exposure and hazard still exist, and the following actions are recommended to fill such gaps and optimise current prioritisation efforts:

(1) Improvement of the suspect screening (higher IP score where sufficient information is available) could result in more certain chemical occurrence data. This could be achieved using machine learning-based models in retention time prediction [37] and substance structure identification [38]. In cases of insufficient fragmentation, smarter data acquisition methods can be (and in the interim have been) developed by instrument vendors, such as AcquireX in Orbitrap systems [39] and in Iterative MS2 in Agilent TOF systems.

(2) The application of the same scheme can be extended to GC–APCI-HRMS techniques, to look beyond LC–ESI/APCI-HRMS data acquisition [7]. A further refinement of the current prioritisation

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framework is already under way that will integrate a preliminary step to define the most appropriate instrumentation for each substance based on its physicochemical properties. This improvement will enable the coverage of a wider chemical space via the application of several complementary methods and will increase the confidence in the results.

(3) More comprehensive compilation of agreed quality targets by governmental authorities should be sought. In the absence of experimental toxicity data, ecotoxicity endpoints should be predicted with the use of validated quantitative structure–activity relationship (QSAR) models, covering a wider range of taxa to allow derivation of PNECs of higher relevance and reliability. Comprehensive experimental datasets covering a well-defined chemical domain are essential to develop reliable QSAR models for PNEC derivation [40]. Data from in vitro assays, e.g. the high-throughput screening in vitro toxicity database from the United States Environmental Protection Agency (US EPA) can be used to extend the hazard categorisation directly (if available in the database) or with machine learning-based predictions [41]. In case the chemical domain of a certain group of chemicals is not yet covered by the models (e.g. PFAS in vivo toxicity), additional experimental data should be generated to expand the chemical domain of the original model.

Improving the availability and sharing of existing information, including raw and spectral data, chemical occurrence and effect data would facilitate collaboration and improvement in prioritisation efforts. The NDS is an example of comprehensive data dissemination strategy which provides various interconnected modules to foster the knowledge exchange to improve the prioritisation of CECs, and the prioritisation approach presented here shows how this data sharing can support complementary efforts at community level beyond international borders.

Conclusions

Given the increasing importance of suspect and non-target screening in environmental studies, the NORMAN prioritisation scheme has been expanded to exploit the potential of these new data. These updates cover substances that are in the domain of contaminants of emerging concern, which are by definition affected by lack of data. The categorisation / clustering process has been designed to specifically address such aspects and data gaps. Since the prevailing consensus was that having some information is preferable to having none at all, it was collectively decided to integrate uncertain data

related to both hazard and exposure data in dedicated categories and treat this information as an additional line of evidence. This updated scheme was applied to a combined dataset of > 65,000 chemicals with target analysis and suspect screening data from European wastewater samples. The prioritisation workflow using suspect screening data revealed 20 substances in Category S1 (highest priority for confirmation/target monitoring), 129 in Category S2 (medium priority, potential mixture toxicity risk), 485 in Category S3 (high priority based on predicted effect data), 58,680 in Category S4 (improvement of analysis required), 6066 in Category S5 (medium priority, improvement of effect data required) and 310 in Category S6 (lowest priority for target monitoring). Combining the results from the suspect screening and target analysis workflows as two lines of evidence revealed that 677 substances were high priority for further actions (red zone), 7455 were medium priority (orange zone) and 326 were in the green zone with potentially lower priority for actions. Among the remaining substances, more than 37,000 should be considered of medium priority with uncertainty, while the conclusion remains uncertain for ~ 19,000 substances due to insufficient data from target monitoring and uncertainty in the identification from suspect screening.

Further actions were suggested for the chemicals according to their assigned category. The suspect screening data showed good agreement with target screening data in terms of categorisation for highly prioritised chemicals and provided additional lines of evidence for tens of thousands of chemicals currently not frequently investigated. The study demonstrated that the integration of suspect screening data to the target prioritisation approach provides a more comprehensive basis for environmental risk assessment. With the infrastructure of the NORMAN Network, such risk assessment can also be performed in a retrospective manner. The combined use of these innovative tools, integrated in the *NDS* platform, can also serve as an early warning system for identifying CECs that start to appear in the environment. If a periodic NTS monitoring is established, it will soon be possible to also identify time trends. The proposed categorisation and prioritisation method is based on a comprehensive list of classification criteria and indicators, taking into account key aspects agreed by the scientific community for exposure and risk assessment. Future perspectives include e.g. enhancing the robustness of the statistical framework for this scheme further and the refinement of the hazard score. Finally, it should be highlighted that the workflow presented here can be applied to various environmental compartments and matrices, including freshwater, marine waters, sediments, biota, biosolids or reused waters as reclaimed water for

irrigation, to rank the critical groups of chemicals that are of potential environmental concern. It will be an important contribution to a more efficient use of environmental monitoring data for the prioritisation of monitoring and regulatory actions, as envisaged in the EU chemicals strategy for sustainability.

Disclaimers
The conclusions expressed in this paper represent the expert judgement of the authors, but not necessarily the opinion of their affiliation.

Abbreviations

AF	Assessment factor
APCI	Atmospheric pressure chemical ionisation
B	Bioaccumulation criterion
BCF	Bioaccumulation factor
CAS	Chemical Abstract Service
CEC	Contaminant of Emerging Concern
ChemSec	The International Chemical Secretariat (https://chemsec.org/)
CLP	Classification, Labelling and Packaging Regulation
CMR	Carcinogenic, mutagenic and reprotoxic
DEDuCT	Database of Endocrine Disrupting Chemicals and their Toxicity Profiles
DSFP	Digital Sample Freezing Platform
DT50	Half-life (time it takes for an amount of a compound to be reduced by half through degradation)
ECHA	European Chemicals Agency
ED	Endocrine disruptor
EI	Electron ionisation
EMPODAT	The NORMAN Chemicals Occurrence Database (formerly "EMerging POllutants DATabase")
EoE	Extent of Exceedance of the lowest PNEC
ESI	Electrospray ionisation
EU	European Union
FoA	Frequency of appearance
FoE	Frequency of Exceedance of the lowest PNEC
FoQ	Frequency of Quantification
GC	Gas chromatography
HRMS	High-resolution mass spectrometry
IP score	Identification point score
KEMI	Swedish Chemicals Agency
KEMI EI	KEMI exposure index
Koc	Partitioning coefficient between organic carbon and water
Kow	Partitioning coefficient between octanol and water
LC	Liquid chromatography
LC50	Lethal Concentration 50 (concentration in water having 50% chance of causing death to aquatic life)
LOQ	Limit of quantification
ME95	95Th percentile of the maximum measured environmental concentrations (MECsite) of all sites monitored
MEC_site	Measured maximum environmental concentration at one site
MRC	Mixture risks contribution
NDS	NORMAN Database System
NOEC	No-observed effect concentrations
NORMAN	Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances
NORMAN-SLE	NORMAN Suspect List Exchange
NSAID	Non-steroidal anti-inflammatory drug
NTS	Non-target screening
OECD	Organisation for Economic Co-operation and Development
P	Persistence criterion
PBT	Persistent, bioaccumulative and toxic
PFAS	Per- and polyfluoroalkyl substances
PMT	Persistent, mobile and toxic
PNEC	Predicted no-effect concentration
P-PNEC	Provisional predicted no-effect concentration (based on modelled data)
PS	Priority substances (under the Water Framework Directive, 2000/60/EC)
QTOF	Quadrupole time-of-flight (QTOF) mass spectrometry (MS)
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals (EU regulation)
RQ	Risk quotient
RTI	Retention time index
SPIN database	Substances in preparations in the Nordic countries
SusDat	NORMAN Substance Database
SVHC	Substances of very high concern
T	Toxicity criterion
TP	Transformation product
UNECE	United Nations Economic Commission for Europe
UNEP	United Nations Environment Programme
US EPA	United States Environmental Protection Agency
UV	Ultraviolet
VEGA HUB	Virtual models for property evaluation of chemicals within a global architecture (https://www.vegahub.eu/)
vPvB	Very persistent and very bioaccumulative substances
vPvM	Very persistent and very mobile substances
WW	Wastewater

Supplementary Information
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> Supplementary Material 1.
>
> Supplementary Material 2.

Availability of data and materials
The datasets analysed during the current study are available in the NORMAN Database System in the various modules as described throughout the article (https://www.norman-network.com/nds/).

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Declarations

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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