NORMAN Non-target screening (NTS) prioritisation scheme for ranking thousands of contaminants of emerging concern in effluent wastewater collected from Europe

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Abstract
Prioritisation of contaminants of emerging concern (CECs) remains a challenging task of primary importance for environmental managers and the scientific community as regards the definition of priority actions for pollution prevention & control and for the allocation of resources to address current knowledge gaps. The NORMAN prioritisation scheme combines the traditional risk-based ranking process with the preliminary application of a decision tree, which allows the allocation of substances into six action categories, based on the knowledge gaps and actions needed to fill them, e.g. development of more powerful analytical methods, launch of monitoring campaigns and performing additional ecotoxicity tests. The ranking within each category is then evaluated by occurrence, hazard and risk criteria. The tremendous improvements in high-resolution mass spectrometry and the development of advanced chemometric tools resulted in the update of the NORMAN prioritisation scheme, so that it incorporates the automatic retrieval of the occurrence of CECs through retrospective suspect screening. The objective of the study was to present a) the updated NORMAN prioritization scheme and the modifications introduced and b) the application of the scheme for the prioritization of more than 40,000 CECs in 46 effluent wastewater samples collected from Europe.
Keywords: NORMAN prioritization scheme, retrospective suspect screening, effluent wastewater, Digital Sample Freezing Platform (DSFP)

1. Introduction

The term “contaminants of emerging concern” (or simply "emerging contaminants") refers to chemicals that have been detected in the environment and can potentially have detrimental effects in the aquatic life. Emerging contaminants are not included in routine monitoring programmes at European level but may be candidates for future regulation depending on their (eco)toxicity, potential health effects, public perception, and frequency of occurrence in environmental compartments. A task of primary importance for decision-makers and environmental managers is to prioritize the emerging contaminants, both as regards the definition of priority actions for pollution prevention and control, and for the allocation of resources to address the yet existing knowledge gaps in a cost-effective way.

Experts on prioritization of emerging contaminants (working group 1) of NORMAN network deal with the topic from 2009 and proposed a prioritization decision system, which is based on the allocation of the substances in six action groups: (i) integration in routine monitoring and derivation of legally binding EQS (Priority pollutants), (ii) screening studies needed for information about current exposure, (iii) rigorous hazard assessment needed, (iv) improvement of analytical methods is required, (v) both screening studies and rigorous hazard assessment are needed and (vi) monitoring efforts should be reduced. The substances within these categories are then ranked based on exposure, hazard and risk indicators **(Dulio V. and von der Ohe P.C. 2013).**

The objective of the study is to present the upgrade of the decision algorithm, which was mandated by the developments in the field of non-target screening (NTS), in which NORMAN has started a number of highly strategic activities, including the Digital Sample Freezing Platform (DSFP) **(Alygizakis et al., 2019a),** which allows better exploitation of NTS high-resolution-mass spectrometry data, using retrospective suspect screening.

2. Material and Methods

2.1. Wastewater treatment plants

The pilot study was conducted on the samples obtained from 46 composite effluent wastewater samples collected from Danube river basin (August 2017) **(Alygizakis et al., 2019b)** and from a national effluent

wastewater sampling campaign that took place in Germany (May 2018) **(Freeling et al., 2019)**.

2.2. Sample preparation and Instrumental analysis

Samples were cleaned up and pre-concentrated 4,000-fold on an Atlantic HLB-M Disk using HORIZON SPE-DEX 4790 (USA) with 47 mm disk holder. The sample preparation protocol involved rinsing of Atlantic HLB-M disks with isopropanol, Milli-Q water, methanol and ethyl-acetate, loading of the samples and extraction with methanol and ethyl acetate sequentially and repeated for three times. Extracts were evaporated using gentle stream of nitrogen and reconstituted with 500 µl 50:50 MeOH:water for UHPLC-ESI-QTOF analysis. Before instrumental analysis extracts were filtered through RC syringe filters of 4 mm diameter and 0.2 µm pore size (Phenomenex, USA). UHPLC-ESI-QTOF analyses were performed using a UHPLC apparatus (Dionex UltiMate 3000 RSLC, Thermo Fisher Scientific, Dreieich, Germany), coupled to the QTOF-MS mass analyser (Maxis Impact, Bruker Daltonics, Bremen, Germany). Chromatographic separations were performed on an Acclaim RSLC C18 column (2.1 x 100 mm, 2.2 µm) from Thermo Fisher Scientific (Dreieich, Germany) preceded by a guard column of the same packaging material, kept at 30˚C. Gradient program, ESI parameters and mobile phases are summarized

3. Results

The focus before the upgrade of the prioritization system was put on few hundred substances for which concentration levels were available **(NORMAN EMPODAT)**. The transition from few hundreds to few hundred thousand substances **(NORMAN SusDat list)** had the obvious consequence that for the great majority of the compounds, the data required to support any possible decision-making process were completely lacking and it was therefore difficult to discriminate, within such a large list of CECs, those that deserve the highest priority attention. This was the reason of the connection of the prioritization decision algorithm with NORMAN DSFP **(Alygizakis et al., 2019a)**, which can feed the prioritization system with occurrence data through retrospective suspect screening. To implement the connection of the two tools, a series of modifications were introduced in both. Among the most important modifications in DSFP was (i) the introduction of a weighted equation to judge whether there is sufficient identification evidence to claim unambiguously identified compounds in the digitally archived data and (ii) the introduction of a semi-quantification system for the detected suspects based on structure similarity and daylight fingerprints. The semi-quantification approach was validated based on results from target-screening. In the prioritization decision tree, a new query was inserted ("Sufficient identification proof?"), which differentiates between compounds that were unambiguously identified (Category 2&5) and compounds for which identification is not proven with sufficient confidence (allocated to Category 4 – action improvement of analytical method).

The compounds of categories 2 & 5 were allocated to sub-categories (++, +, - and ---) based on an estimate of their spatial occurrence and potential risk of exceedance of the provisional no effect concentration (PNEC). Category 2 was used for cases, in which experimental PNEC was available, whereas category 5 was used in cases, in which only the predicted PNEC (P-PNEC) was available.

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