

Target Screening of Chemicals of Emerging Concern (CECs) in Surface Waters of the Swedish West Coast

Pedro A. Inostroza ^{1,*}, Eric Carmona ², Åsa Arrhenius ¹, Martin Krauss ², Werner Brack ^{2,3} and Thomas Backhaus ¹

¹ Department of Biological and Environmental Sciences, University of Gothenburg, 40530 Gothenburg, Sweden

² Department of Effect Directed Analysis, Helmholtz Centre for Environmental Research—UFZ, Permoserstrasse 15, 04318 Leipzig, Germany

³ Department of Evolutionary Ecology and Environmental Toxicology, Faculty of Biological Sciences, Goethe University Frankfurt, Max-von-Laue-Strasse 13, 60438 Frankfurt am Main, Germany

* Correspondence: pedro.inostroza@gu.se

Abstract: The aquatic environment faces increasing threats from a variety of unregulated organic chemicals originating from human activities, collectively known as chemicals of emerging concern (CECs). These include pharmaceuticals, personal-care products, pesticides, surfactants, industrial chemicals, and their transformation products. CECs enter aquatic environments through various sources, including effluents from wastewater treatment plants, industrial facilities, runoff from agricultural and residential areas, as well as accidental spills. Data on the occurrence of CECs in the marine environment are scarce, and more information is needed to assess the chemical and ecological status of water bodies, and to prioritize toxic chemicals for further studies or risk assessment. In this study, we describe a monitoring campaign targeting CECs in surface waters at the Swedish west coast using, for the first time, an on-site large volume solid phase extraction (LVSPE) device. We detected up to 80 and 227 CECs in marine sites and the wastewater treatment plant (WWTP) effluent, respectively. The dataset will contribute to defining pollution fingerprints and assessing the chemical status of marine and freshwater systems affected by industrial hubs, agricultural areas, and the discharge of urban wastewater.



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1. Summary

Streams, rivers, and coastal areas are characterized by the occurrence of mixtures of hundreds of organic chemicals occurring at low concentrations [1]. These mixtures consist of a wide variety of unregulated organic chemicals, including pharmaceuticals and personal-care products (PPCPs), pesticides, surfactants, industrial chemicals, and transformation products, referred to as chemicals of emerging concern (CECs) and originating from human activity [2]. At environmental concentrations, CECs are known to have deleterious effects on aquatic life, ranging from microbes [3] to higher vertebrates [4] and from genes to the landscape genetics of non-target organisms [5].

CECs enter streams, rivers, and coastal areas due to discharges from wastewater treatment plants (WWTPs) [6], individual drains, industrial facilities [7], run-off from agricultural and residential areas, and accidental spills [8]. Once in the aquatic environment, CECs can be detected in different environmental compartments (i.e., water and sediment), including biota [9,10]. Although most CECs are not highly persistent, their continuous

release into the aquatic environment, in small but significant amounts, makes many of them “pseudo-persistent” [11,12].

Data on the occurrence of CECs are critical for conducting environmental-risk assessments to assess the ecological status of water bodies and prioritize chemicals for further studies. Information on marine CECs is scarce, particularly in the context of identifying emission sources. Several emission sources usually contribute to the toxicant load in marine areas, leading to mixing zones with an enormous complexity of exposure, while the individual CECs are often diluted. In combination, this adds to the analytical challenges.

In this study, we describe a sampling campaign that analyzed and quantified CECs of different chemical classes in surface waters along the Swedish west coast using an on-site large volume solid phase extraction (LVSPE) device. This dataset contributes to defining pollution fingerprints and assessing the chemical status of marine and freshwater systems affected by large industrial hubs, agricultural areas, and the discharge of urban wastewater.

Stenungsund is a municipality of 27,000 inhabitants located on the Swedish west coast, approximately 40 km north of Gothenburg. It is home to the largest chemical cluster in Sweden, centered around a steam cracker that produces ethylene and various fuel gases, which are then used by a range of companies for the production of polyethylene, polyvinyl chloride (PVC), amines, detergents, and various other chemicals. Additionally, there are several harbors situated along the coast. The municipality, itself, discharges its treated sewage effluents into the fjord (Hakefjord). Consequently, a range of petrochemicals, surfactants, microplastics, heavy metals, and antifouling paint residues have been found in the waters and sediments of the area, including phthalates, chlorobenzenes, chlorophenols, PCBs, cadmium, copper, mercury, tributyltin (TBT), and pharmaceuticals and other chemicals from household use [13,14].

We collected surface-water samples from six marine and two freshwater sites, plus one WWTP effluent site, using an on-site large volume solid phase extraction device [15] in October 2020 (fall season). We selected marine sites (M1–M6) based on their relative location to urban and industrial areas along the Hakefjord. Freshwater sites represented inland contributions from agricultural areas and small settlements (F1), and the industrial hub and Stenungsund city (F2). In addition, the effluent from the Strävliden WWTP was used as a point source of chemicals in our study (F3; Figure 1).

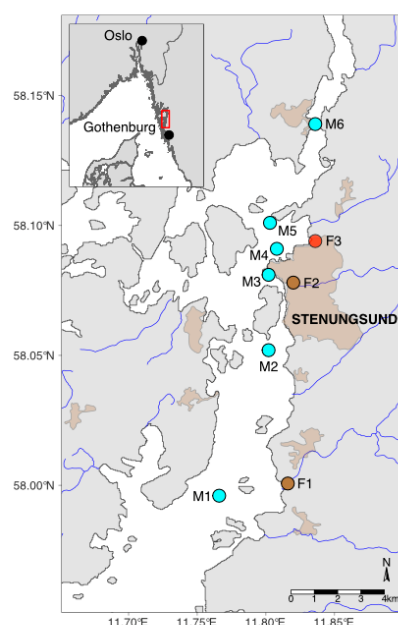


Figure 1. Location of sampling sites through the Hakefjord on the Swedish west coast. Marine sites (M1–M6) are marked in blue, freshwater sites (F1–F2) in brown, and WWTP effluent (F3) in red. Urban areas are depicted in light brown.

2. Data Description

The data reported in this study originates from surface-water samples collected down to five meters below the surface for marine samples and down to one meter below the surface for freshwater samples. The effluent sample was collected directly from the WWTP effluent pipe. The effluent later discharges into the marine environment at site M5. The data are reported as tabular data in both Rdata (RDS) and CSV (UTF-8 tab delimited) formats, and can be accessed at [16]. The CAS Registry Number (CAS RN), International Chemical Identifier (InChI) and its hashed InChIKey counterpart, and Simplified Molecular Input Line Entry System (SMILES) identifiers [17] are provided to easily access public databases containing detailed information on substances; this includes, for instance, the US National Institutes of Health (PubChem). The mechanism-of-action information was retrieved from literature [18] and gaps were filled through searching in the Elsevier Bibliographic Database (Scopus) and Google Scholar using terminology, which included (“chemical_name”) AND (“mode of action”) AND (“mechanism of action”).

The CSV file contains one sheet with the columns defined in Table 1 and a summary of the detected and quantified CECs in Table 2.

Table 1. Column description.

Columns	Description
chemical_name	Name of the emerging chemical.
cas_number	CAS Registry Number used as the chemical identifier.
InChIKey	Textual identifier for chemical substances.
SMILES	Line notation for the chemical structure.
molecular_weight	Molecular weight of the substance (g/mol).
DTXSID	Distributed structure-searchable toxicity-substance identifier.
type	Type of chemical (parent or transformation product).
main_source	Urban areas, agriculture-derived chemicals, or multiple sources.
compound_class_level_1	Use category (e.g., pharmaceutical, pesticide, biocide, etc.).
compound_class_level_2	Sub-use category (e.g., antibiotic, herbicide, plasticizer, etc.).
compound_class_level_3	Sub-use category (e.g., benzodiazepine, organophosphate, etc.).
alternative_class	Known alternative use (e.g., veterinary pharmaceutical, additive, etc.).
MoA	Mechanism of action of the chemical.
detection_limit	Level of quantification in ng/L.
ESI_mode	Electrospray mode (positive or negative).
M1	Concentration at M1 in ng/L.
M2	Concentration at M2 in ng/L.
M3	Concentration at M3 in ng/L.
M4	Concentration at M4 in ng/L.
M5	Concentration at M5 in ng/L.
M6	Concentration at M6 in ng/L.
F1	Concentration at F1 in ng/L.
F2	Concentration at F2 in ng/L.
F3	Concentration at F3 in ng/L.

Table 2. Summary of data provided.

	M1	M2	M3	M4	M5	M6	F1	F2	F3
Detected CECs	73	77	74	67	79	61	73	118	226
Quantified CECs	68	66	64	57	68	52	68	114	224

3. Methods

3.1. Sample Collection

Water samples (100 L) were collected at 9 sampling sites at the listed coordinates (Table 3). CECs were extracted by pumping the water through an on-site large volume solid phase extraction device (LVSPE, MAXX Mess-und Probenahmeteknik GmbH,

Rangendingen, Germany). The LVSPE consisted of a vacuum-sampling system, a filtration unit (Sartopure GF+ MidiCaps, 0.65 µm in separation-size, Sartorius) connected to a dose-glass chamber (500 mL), and a tailor-made, solid-phase extraction (SPE) cartridge filled with 10 g of neutral sorbent polystyrene divinylbenzene co-polymer (Chromabond HR-X, Macherey Nagel, Düren, Germany) [15]. We followed the recommendations of Nanusha et al. [19] and Machate et al. [20] for the cartridge conditioning, extraction, and Liquid Chromatography–High Resolution Mass Spectrometry (LC–HRMS) analysis.

Table 3. Information on different sampling sites. Geographic coordinates are in decimal degrees (WGS84).

Sampling Site	Type	Latitude (N)	Longitude (E)
M1	Marine	57.99630	11.766283
M2	Marine	58.05225	11.802267
M3	Marine	58.08136	11.802533
M4	Marine	58.09178	11.808867
M5	Marine	58.10185	11.803717
M6	Marine	58.13981	11.836833
F1	Agricultural stream	58.00076	11.816983
F2	Industrial stream	58.07856	11.820018
F3	WWTP effluent	58.09492	11.836374

3.2. Sample Preparation and Extraction Procedure

All samples were prepared and extracted following the recommendations of Nanusha et al. [19]. Briefly, Liquid Chromatography–Mass Spectrometry (LC–MS) grade methanol, formic acid, and ammonium formate were purchased from Merck (Rahway, NJ, USA), and LC–MS grade water from Thermo-Fisher (Waltham, MA, USA). LC–MS grade ethyl acetate and 7 N ammonia in methanol were obtained from Sigma-Aldrich (St. Louis, MO, USA). Reference standards were purchased from various suppliers with a purity of higher than 97%.

The tailor-made SPE cartridges were preconditioned with methanol/ethyl acetate (1:1, *v/v*), methanol, and LC–MS grade water before the water sampling. After the sampling, cartridges were kept at 4 °C and transported to the laboratory, where they were purged with nitrogen to remove water, freeze-dried, and stored at −20 °C for extraction. Blanks were also prepared using the LVSPE device, similar to the samples.

The analytes were eluted from each cartridge with methanol/ethyl acetate 1:1 (*v/v*, 500 mL each, neutral fraction), methanol containing 2% of 7 N ammonia in methanol (500 mL, acidic fraction), and methanol with 1% of formic acid (500 mL, basic fraction). The pH of both acidic and basic fractions was adjusted to 7 ± 0.5 by adding formic acid or 7 N ammonia in methanol. The eluates were filtered (GF/F Whatman) to remove precipitates and reduced to dryness using a rotary evaporator (40 °C water bath) and a gentle stream of nitrogen. Subsequently, the samples were transferred to methanol and adjusted to a final enrichment factor of 1000. For the LC analysis, 100 µL of the aliquots of these extracts were mixed with 10 µL of an internal standard mixture containing 40 isotope-labelled compounds (1 µg/mL), 30 µL of methanol, and 60 µL of water. The remaining extracts were stored at −20 °C for ecotoxicological investigations.

3.3. Target Chemical Screening

Information on CECs in the marine environment is extremely limited; therefore, the target list for the analysis consisted of chemicals typically found in European freshwater systems. Target screening was conducted for 861 chemicals using an UltiMate 3000 LC system (Thermo Scientific, Waltham, MA, USA) coupled to a quadrupole-Orbitrap MS (Q Exactive Plus, Thermo Scientific, Waltham, MA, USA) with a heated electrospray ionization (ESI) source. For 150 of the 861 target chemicals, the analysis was conducted by applying a

retrospective analysis, that is, the samples measured up to 1 year earlier were quantified with newly measured calibration standards as described by Muschket et al. [21].

3.4. Liquid Chromatography HRMS

Liquid-chromatographic separation was performed on a Kinetex C18 EVO column (50 × 2.1 mm, 2.6 µm in particle-size) equipped with a pre-column (C18 EVO 5 × 2.1 mm) and an inline filter as described by Nanusha et al. [19]. The mobile phase consisted of 0.1% of formic acid (eluent A) and methanol containing 0.1% of formic acid (eluent B), which was used at a flow rate of 300 µL/min. After 1 min of elution with 5% of eluent B, the fraction of eluent B was linearly increased to 100% within 12 min, after which the mobile phase was kept at 100% of eluent B for 11 min. Subsequently, the column was rinsed with a mixture of isopropanol + acetone 50:50/eluent B/eluent A (85%/10%/5%) to remove hydrophobic-matrix constituents from the column. Finally, the column was re-equilibrated to initial conditions for 5.7 min. An injection volume of 5 µL was used, and the column was operated at 40 °C. The heated ESI source and the transfer capillary were both operated at 300 °C with a spray voltage of 3.8 kV, a sheath gas-flow rate of 45 a.u., and an auxiliary gas-flow rate of 1 a.u. The full-scan MS1 was recorded in the m/z range of 100–1500 with a nominal resolving power of 140,000 (referenced to m/z 200). For compound confirmation, data-dependent MS/MS acquisition was performed at a resolving power of 70,000 in additional runs. The MS was calibrated externally every 2 days using the calibration mixtures of the vendor. The mass accuracy was always within 5 ppm for all analyses. All MS and MS/MS analyses were performed in ESI-positive (ESIpos) and ESI-negative (ESIneg) mode.

3.5. Data Analysis

ProteoWizard (version 3.0.19324-f948194c2) was used to convert the LC–HRMS raw data into mzML format (centroid mode) [22]. Subsequently, peak detection, sample alignment, and target-compound annotation were performed using MZmine (V 2.38) [23], as detailed in [24]. We used an in-house R package (MZquant, version 0.7.8) to perform blank correction, calibration, and quantification. Blank-peak elimination and blank-intensity thresholds were calculated according to the procedures conducted in Machate et al. [20]. Lastly, a series of method-matched calibration standards ranging from 0.5 to 5000 ng/L were used. The calibration standards were treated the same way as the water samples. The target compounds were quantified using the internal standards with the nearest retention time. The method detection limits (MDLs) were determined based on the US-EPA procedure [25].

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