Baltic Marine Environment Protection Commission

Working Group on the State of the Environment and Nature Conservation

Online, 5-9 October 2020

STATE & CONSERVATION 13-2020

Document title	Proposal for regular screening of hazardous substances in the Baltic Sea region
Code	4J-14-Rev.1
Category	DEC
Agenda Item	4J – Progress of relevant HELCOM expert groups and projects
Submission date	28.9.2020
Submitted by	Secretariat
Reference	STATE & CONSERVATION 12-2020 Outcome paragraph 4J.58 and 59, and document 4J.17.

This document has been revised to reflect discussion at EN-HZ 13-2020 (and the comments received to that meeting from other Expert groups, e.g. PLC and CG PHARMA). All changes are shown in red text in the document.

Background

The Ministerial Declaration of the 2018 Brussels HELCOM Ministerial Meeting (Paragraph 35) agreed "to identify the scale of problems of contaminants of emerging concern". This proposal develops an approach that would facilitate an understanding of this issue of contaminants in the Baltic Sea and proposes a longer-term plan for regular wide-scope screening to support the assessment of state in the Baltic Sea. The approach is directly compatible to ongoing projects in OSPAR (The OSPAR CONNECT Project) and in other European Seas (e.g. the LIFE APEX and EMBLAS Projects). Independent of this technical proposal, below, the HELCOM Expert Group on Hazardous Substances (EN-HZ) will also discuss how the output from such screening can be effectively integrated with broader HELCOM processes in their autumn meeting 2020 (21-25 September 2020). This proposal supports proposed work under the ongoing update of the Baltic Sea action plan, as outlined in Existing HELCOM actions to be transferred to the updated BSAP (Heads of Delegation, document 4-9).

The proposal has mainly been developed within the EN-HZ. An earlier version has been shared with two HELCOM Working Groups (STATE & CONSERVATION 12-2020 (Document 4J-17 and outcome paragraphs 4J.58-59) and PRESSURE 12-2020 (Document 10-5 and outcome paragraphs 10.16-22) and the comments resulting from those processes incorporated into this version. This version has also been shared with Experts in EN-HZ, the HELCOM Correspondence Group on Pharmaceuticals (CG PHARMA), and HELCOM Pollution Load Compilation (PLC)). The proposal aims to address the occurrence of emerging substances in the marine environment, and an evaluation of the risk posed, as well as providing a broader indication of the possible sources of these compounds, or routes of these substances entering the marine environment, should resources allow.

The assessment carried out using this screening approach should provide an overview of an extensive number of hazardous and potentially hazardous (e.g. substances of concern) substances. The selection of wide-scope target substances that can be determined and suspect substances that can be screened for their semi-quantitative presence/absence in samples with this approach is at present >2400 and >65000, respectively. These numbers are steadily increasing. The list of substances covers a large number of priority and concern substances identified through policy initiatives and research studies. The assessment would also therefore address an extensive number of substances that are not covered by the existing selection of 11 HELCOM indicators on the topic of hazardous substances. The proposal here outlines how this information could be developed into a HELCOM surveillance indicator (an early warning system) as well as providing a broader assessment of hazardous substance in, or with potential to have detrimental effects on, the Baltic Sea environment. This approach therefore has the ability to support overall status assessments (e.g. HOLAS III) and support work in relation to other policies (e.g. the Marine Strategy Framework Directive (MSFD)).

Currently no external funding opportunities suitable for this project proposal have been identified.

The Secretariat was informed of two laboratories used by the Contracting Parties for equivalent screening work and both were contacted by the Secretariat. One laboratory, the Norwegian Institute for Air Research (NILU), has prepared an equivalent costing and technical proposal. This has been recently shared with EN-HZ and is also provided in Annex 3 of this document for information.

~~The document has also been shared with EN-HZ (meeting on 23 September 2020), any revisions or comments will be transferred to State and Conservation directly after that meeting.~~

The EN-HZ 13-2020 meeting discussed the newly proposed method and costing from NILU and considered that the preferred option was initial proposal (i.e. lab in Athens), based on the following factors: 1) the cost is slightly lower per sample, 2) the approach applied would then be identical for HELCOM and OSPAR regions, 3) Contracting Parties taking samples in both regions wish to use a common laboratory including only requiring to send samples once, 4) uncertainty on the analytical an data solutions for the NILU proposal, 5) the assessment would be carried out via the same approach as other large scale regional and EU-wide assessments (also done at the Athens laboratory).

Please also note that this document will also be shared with the PRESUSRE Working Group.

Action requested

The Meeting is invited to:

- review the technical details of the proposal and propose improvements where relevant,
- indicate, using table in Annex II, if participation is anticipated and what samples are considered, e.g. number, matrix, spatial distribution if possible (EN-HZ suggest this should be completed later once the project is endorsed and ready to be initiated),
- explore the possibility of securing national resources to carry out the work.
- Endorse the submission of the document to HOD 59-2020 for consideration and approval.

Proposal for regular screening of hazardous substances in the Baltic Sea region

General introduction

The proposal presented here provide a general context for the organization of a wide-scope and suspect screening project across the Baltic Sea to take place during 2021 (sampling and data generation), and also lays out a proposal for regular regional campaigns to maintain a good overview of hazardous or potentially hazardous substances, especially those of emerging concern, that may enter the Baltic Sea environment. The proposal offers the possibility to develop such an assessment within a causal framework, should resources and capacity be available, and offers a focus for sampling (e.g. a primary sampling matrix) to ensure the strongest possible regional comparability. The project is also well aligned with ongoing work in OSPAR.

In brief the proposal considers the following aspects, further details provided below.

- Biota in the marine environment will be the priority targeted for sampling.
- Other sampling matrix types will be additive, where resources and capacity are available.
- A common approach and laboratory will be used across the region (if possible also matching to other regions).
- The project will support the development of a surveillance indicator (where possible combining national data with the data generated in this project).
- Future regular screening should take place a minimum once per assessment period (e.g. every six years).

The project proposal aims to establish common approaches and a regional survey through wide-scope and suspect screening. The selection of samples (spatial distribution and type) will be determined by national decisions (e.g. the resources available and thus the total number of samples that can be analyzed) and the following aspects aim to guide the selection so that a harmonized Baltic Sea wide assessment can be carried out. In the current format the individual Contracting Parties would carry out the sample collection and shipping to the chosen laboratory (costs not reflected in this project description) and the analysis would be then carried out. The analysis would be paid for by each Contracting Party independently in this current format, based on the fact that the project relies on separate resource allocation from within each Contracting Party wishing to participate. This approach has also been applied in the respective OSPAR project.

Selection of appropriate samples

Each Contracting Party will identify the total number of samples for which resources can be assigned. From this the spatial distribution aspect needs to be considered (i.e. what spatial coverage is wished for), with the focus on the primary sampling matrix type (biota, see table below). A balance between spatial distribution and including other non-biota sampling matrix types may also be relevant if resources permit (i.e. if good spatial coverage is met and resources permit would other matrix types also be included from the same location or pathway). In addition to the aspect of including other sampling matrix types to support an understanding of causal frameworks/pathways it may be relevant to consider different matrix types when targeting specific substances (e.g. aspect such as hydrophobicity or bioaccumulation).

It is proposed that the total number of samples should ideally be 4-10+ per Contracting Party to allow a reasonable regional/spatial coverage to be developed, focused on biota. Additional samples from other matrix types as well as historic samples (e.g. from biota specimen banks to establish temporal trends of pollution) are also considered valuable, if resources permit. Other factors that may be valid to consider may include transboundary rivers.

A proposed approach to select and prioritize samples across the region is given below in Table 1. Additional supporting information based on comments received to the draft version are included in Annex I of this document.

Table 1. Proposed approach for identifying samples and matrix types that should be prioritized in sampling to give a harmonized Baltic Sea wide overview. Priority is indicated to make sure that the most harmonized approach can be applied across the whole Baltic Sea region (also comparable with approach in OSPAR where biota samples are used).

EN-HZ initiated discussion on the best sampling matrix to apply in the HELCOM region (including comments received), so as to provide the best harmonized approach and comparable data. The meeting did not reach a final conclusion, though adjusted the table below, and agreed that a core team will reconvene and address the issue at the latest in early November to make a final proposal and guide the regional sampling effort.

Priority selection	Sampling matrix	Details
First choice*	Biota	Blue mussel – *Mytilus edulis* Macoma – *Macoma balthica* Where above not present: Zebra mussel - *Dreissena polymorpha* Herring ~~Flounder~~ ~~Cod, Perch, Eelpout~~
	Sediments in marine environment	
Second choice	WWTP effluents (focus on large WWTPs and ones that discharge close or direct to the sea)	Whole water or passive samplers
	Water in marine environment	Whole water or passive samplers
Third choice	River water	Whole water or passive samplers
	River sediment(ation)	Sediment traps
Fourth choice	WWTP sludges	
Other knowledge areas	Higher trophic species (e.g. mammals, birds)	NA
	Hotspots	NA
	Land based sources	NA
	Sea-based sources	NA
	Air	NA
Other aspects	Higher trophic species (e.g. mammals or birds)	There is a possibility that the LIFE APEX project could support some additional samples. Interested parties are invited to inform the Secretariat (owen.rowe@helcom.fi) by 30 October, so that a discussion can be planned with relevant LIFE APEX contacts on how to optimise the species of choice and the geographical distribution of the samples.

***First choice category should be selected by all Contracting Parties if taking just a single sample. Multiple first choice samples can also be taken to give good spatial coverage. Other selections (Second, Third or fourth) should only be taken in addition to a First**

choice selection (i.e. to support an understanding of a causal framework) for those Contracting Parties wishing to expand their assessment.

EN-HZ also noted that at the stage of establishing the locations of samples for collection it would also be valuable for the national Experts involved in the process to share information, via EN-HZ, so that the best spatial coverage and sample distribution can be achieved. The aspect of paired reference and polluted sites would also be considered at this stage.

Priority identification to ensure a harmonised Baltic Sea wide approach is carried out based on the following logic: biota represent an end point for major bioaccumulation and biomagnification processes, substances that enter the food web and have potential direct toxic effects to biota or may enter human food chains are of high relevance, a single sampling matrix will ensure comparable data across the region, resources can be focused towards a common comparable approach, and compatibility with work in OSPAR.

Sample collection and preparation

Sample collection and preparation is not represented in the costing provided as this will differ between each Contracting Party and depend on sample selection and placement (e.g. if integrated with existing monitoring work). A procedure for how to collect and prepare samples, as well as how to transport them to the laboratory, has been developed and tested by the LIFE APEX project and detailed information will be provided prior sampling.

Laboratory for sample analysis

All samples will be run by the same laboratory to ensure identical methodology and conditions. This aspect is key to an effective quantitative (wide-scope target screening) and semi-quantitative (suspect screening) analysis as proposed

The wide-scope target and suspect screening campaign will include the following stages:

- National Experts (e.g. from HELCOM EN-HZ, PLC, CG PHARMA, or other) will identify the most appropriate location and sampling matrix utilizing the priority guidance above. The national resource allocation will determine the number of samples per Contracting Party.
- Sampling will ideally be carried out across a single identified sampling season (i.e. during 2021). All samples will be dispatched (by each Contracting Party) to a common laboratory for analysis.
- Analysis will be carried out in the following four steps:
    - STEP 1: Wide-scope target screening analysis (>2400 substances with LC-HR-MS and GC-APCI-HR-MS*)
    - STEP 2: Suspect screening (>65000 substances*) and semi-quantification with LC-HR-MS with quality check
    - STEP 3: GC-APCI-HR-MS for retrospective screening for identification of unknowns
    - STEP 4: Report on overall findings (including comparison against available EQS and PNEC values in database) and data collation, per CP.
- Data would be provided to relevant databases or institutions.
- EN-HZ (PLC and CG Pharma) will compile an overview of the information in the form of a surveillance (early warning) indicator. Rules for estimating risk and possible follow up procedures will also be explored.

***NOTE: the steps in this proposal are based on the approach developed under NORMAN and the laboratory carrying out OSPAR analysis. The number of substances that the analytical approach is currently able to detect is steadily increasing also, thus the substance numbers listed here are likely on the low end of the actual analytical range by 2021 (possibly >90,000). The current list of substances that can be assessed with this approach is available at this link. For an overview of wide-scope target substances, please click the tick box for S21 UATHTARGETS and then click search at the bottom of the page.**

Data storage and handling

Data generated by this approach would be provided in a report (including a series of heat maps, visualizations and tables) per Contracting Party (based on the NORMAN approach). The data could be maintained under password protected conditions in the NORMAN Database System (https://www.norman-network.com/nds/) until the Surveillance indicator was published and then made public subsequent to this. Within the NORMAN Database System it is also possible to 'freeze' the collected high resolution mass spectrometry data (fingerprints of typically hundreds of currently known as well as unknown chemicals present in each analysed sample) in the Digital Sample Freezing Platform (DSFP; https://norman-data.net/Verification/) module and then re-analyze these historic data against future updates of the database, if needed (e.g. should the information allowing for identification of more 'known' substances in the database increase). No additional cost for maintaining the data in the NORMAN Database System is envisaged. There are also possibilities to incorporate the data, at least in the form used and presented in the HELCOM surveillance indicator via the HELCOM Map and Data Service (MADS) also. Digitally 'frozen' data can be reevaluated as the database grows in the future without any need for new sampling and analytical efforts.

Project costings

NORMAN network laboratory (Athens) costing

The costs provided in this section are based on the NORMAN proposal (as used in the OSPAR region). The equivalent cost is offered to HELCOM. The analysis would be carried out at the University of Athens laboratory that is part of the NORMAN Network.

Step 1: 600 Euros per sample

Step 2: 850 Euros per sample

Step 3: 200 Euros per sample

Step 4: 250 Euros per Contracting Party (i.e. a summary of all samples per Contacting Party)

Overall a single sample would cost 1650 Euros plus 250 Euros for the report – a sum of 1900 Euros. Additional samples would represent an increase of 1650 Euros each as the report covers all samples and data per Contracting Party irrespective of the number of samples analyzed. In case, the raw (not lyophilized) samples would be provided, additional 25 Euros per sample would be charged for their processing and lyophilization.

It is important to note that the costs above do not include costs for shipping samples to the selected laboratory or for the actual sample collection and preparation.

Full details of the above 'offer' would be provided as part of any contract agreement for the project.

Other laboratory options

Further information will be added once available.

Frequency for proposed regular assessment

This proposal mainly focusses on the application of a screening campaign in 2021, in particular with direct relevance to HOLAS III. However, carrying out such screening campaigns, especially regular and repeated campaigns, has high value. It is proposed that wide-scope screening should be carried out in an increasingly coordinated regional manner at 6-year intervals (e.g. once per 6-year assessment period). It is also considered important that the process and output are reviewed after the first assessment procedure to ensure lessons learned are considered prior to initiating any subsequent campaign.

The data generated from such an approach would provide a solid basis for a preemptive assessment (e.g. an early warning system) and while trends are not explicitly possible to determine with such analyses it would offer a broad overview of substances that are increasing or emerging in the Baltic Sea environment and may need to be appropriately addressed. Such knowledge could support identification of substances of concern, requirements to review and update priority lists, identify the potential need for new indicators, support alignment across HELCOM Expert Groups (e.g. EN-HZ, PLC) and other organizations of relevance (e.g. EMEP), as well as offer insights into the potential need for new measures.

Outcomes and potential for cooperation

There are a number of potential outcomes in this work, in addition to the specific overview of substances derived from the wide-scope target and suspect screening, for example:

- A HELCOM surveillance indicator – 'Screening-derived hazardous and potentially hazardous substances in the Baltic Sea'. This indicator would act as an early warning system to highlight substances at the regional and sub-regional scale that may be at high relative levels (and are not currently addressed by existing HELCOM indicators).

Support for HELCOM processes such as work of the Working Groups (e.g. State and Conservation and PRESSURE) and Expert Groups (e.g. EN-HZ, PLC and CG PHARMA) and ongoing processes related to:

- modernising the HELCOM strategy for hazardous substances. This could for example take place via the development of a process (e.g. evaluation rules) to address and review substances with higher relative concentrations and how to integrate the screening-derived knowledge into broader HELOCM processes.
- Support for other regional and global assessments, such as gaining a broad overview of hazardous substances in European Seas. In addition it may have relevance for work under other policies siuch as the Water Framework Directive (WFD) and processes to review and update threshold values such as the EQS values applied by HELCOM Contracting Parties that are also EU Member States. Similarly, it is likely valuable information in line with the aims of the EU Biodiversity Strategy 2030.
- A comparative study (and possible publication) of substances between the HELCOM and OSPAR regions.

Annex I: supporting information related to priority setting and samples within a 'causal framework'.

'Causal framework'	**Components**	**Sampling related comment received**	**Priority in this proposal (ranking)**
Inputs	Air	Rely on EMEP (was proposal at PLC, as hard to do effective full spatial coverage in this project)	Lowest (8)
	WWTPs (effluent)	Sampling viable, focus especially on those with direct marine inputs	Medium (4)
	WWTPs (sludge)	Sampling viable, focus especially on those with direct marine inputs	Lower (7)
	Land	Diffuse in general	Not directly (via rivers/run off)
	Hotspots	Possible to target if identified, sampling possibly hard	Not directly (via rivers/run off)
	Maritime	Diffuse in general	Not directly (into marine waters)
Pathways (that are not direct)	Rivers (water)	Sampling possible in water	Medium (5)
	Rivers (sediment)	Sampling possible in sediment(ation)	Lower (6)
'End points' or status	Water (sea)	Sampling viable	Medium (3)
	Sediments	Sampling viable	High (1)
	Biota	Sampling viable	High (1)
	Higher trophic species (e.g. mammals or birds)	Sampling viable	Not discussed directly, but may be possible to add selected via LIFE APEX project.

Annex II: supporting information related to priority setting and samples within a 'causal framework'.

EN-HZ 13-2020 proposed that this table should be completed on a single occasion (not in separate stages under different groups) once the project proposal has been approved and when the sample selection process is underway.

Contracting Party	Participation?		Total number of samples?	Sample selection (i.e. from priority table above)	Sample location (i.e. sub basin or sampling station)	Sampling year	Sample type	Additional information	Other national screening projects during 2016-2021
	Yes	No	n	Add new rows as needed	Add new rows as needed	2021 or earlier?	Reference or 'test'	Free text	Data of possible relevance for HOLAS III (free text)
Denmark									
Estonia									
Finland									
Germany									
Latvia									
Lithuania									
Poland									
Russia									
Sweden									

Annex III: Methodology and costing provided by Norwegian Institute for Air Research (NILU)

Selection of instrumental analytical methods

Figure 1 shows schematically the application range of the different available MS-techniques, and it is obvious that only the combined application of both GC- and LC-MS will provide sufficient coverage for the majority of possible environmental pollutants.

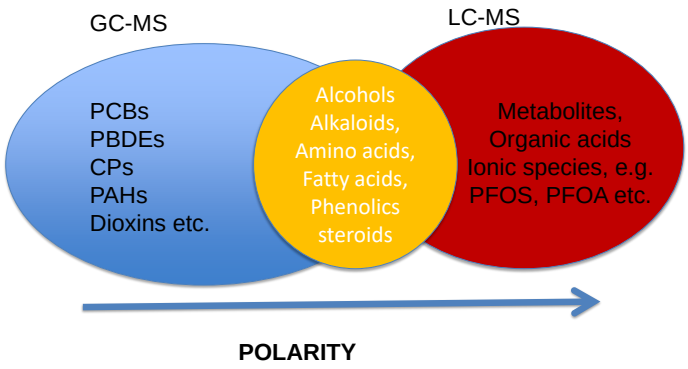

Figure 1: Application range for GC-MS and LC-MS techniques

Sample preparation

To enable high sensitivity and selectivity in a suspect and non-target screening analyses, optimized clean-up and extraction methods will be used. To cover a broadest possible range of compound groups two different extraction and clean-up methods will be applied, one, which is optimized for non-polar and very lipophilic compounds like PCBs, PAHs and other classical POPs, and another, which is optimized for polar compounds like different polar metabolites, pharmaceuticals, modern pesticides and biocides, PFAS, and bisphenols.

Prior extraction samples will be spiked with a number of isotopically labelled internal standards, that will enable quantitative (targeted) analyses of a number of compound groups semiquantitative analyses the possible new compounds identified in the task as a result of suspect and non-target screening.

Instrumental analyses and data processing

State of the art analytical techniques, high and ultrahigh resolution mass spectrometers (QTOF and Orbitrap) coupled to liquid and gas chromatography will be used for characterisation of organic substances.

Stage 1 - Suspect screening (>200 000 substances) and semi-quantitative analyses (>40000 substances)

To improve the understanding and facilitate the use of the data produced in the suspect screening workflow, Schymanski et al. proposed 5 levels of confidence of communicating the results.

This was adapted by the Norman network and demonstrated in different projects[1, 2] and will be used in this study (Figure 2).

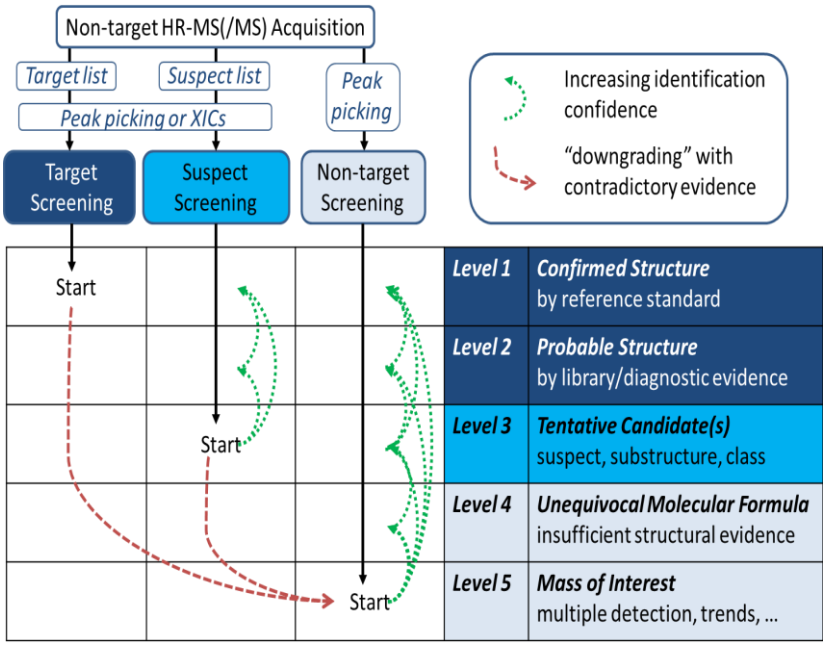

Figure 2. Level of confidence when reporting the data in target, suspect and non-target screening. Adapted from [3].

In this step, a high-quality suspect screening (a probable structure, level 2 of identification confidence[3]) will be conducted through a comparison of the raw data with one or more relevant libraries with mass spectra and application of retention time indices. A number of relevant libraries (both open source and commercially available) will be applied with the scope exceeding 250 000 substances.

List of compounds with level 2 will further be enhancement by suspect screening with the lists of relevant suspects compounds without available mass spectra, this resulting with the level 3 confidence level. Semi-quantitative analyses will be conducted with a coverage of approximately 40 000 of typical environmental substances.

Stage 2 - Wider scope target analyses

Compounds from the groups covered by spiked isotopically labelled compounds will be quantified. In addition, an intelligent prioritisation strategy (based on occurrence in all samples and relative abundance) will be applied for further quantification. 20 most common substances identified in all samples that were not covered by the earlier quantitative and semi-quantitative approaches will be selected and (depending on commercial availability of analytical standards) quantified in the samples.

Stage 3 – retrospective analyses of the stored raw data for the identification of unknowns

At this stage various approaches will be used for retrospective analyses of raw data. Priority will be given to identification of chlorinated and perfluorinated substances[4]. With samples from different level of a food chain (if available) bioaccumulation (even chemicals not possible to identify) will be highlighted.

Stage 4 – reporting

Reporting will include brief details of the procedures applied and the list of compounds identified with the level of identification according to Schymanski et al.[3], and (where available) the concentrations of compounds measured with a given level of analytical uncertainty.

Price:

0 – 9 samples – contact for details

10-49 samples - €1900/sample

>50 – contact for details

References:

1. Schymanski, E.L., et al., *Non-target screening with high-resolution mass spectrometry: critical review using a collaborative trial on water analysis.* Anal Bioanal Chem, 2015. **407**(21): p. 6237-55.
2. Rostkowski, P., et al., *The strength in numbers: comprehensive characterization of house dust using complementary mass spectrometric techniques.* Analytical and Bioanalytical Chemistry, 2019.
3. Schymanski, E.L., et al., *Identifying small molecules via high resolution mass spectrometry: communicating confidence.* Environ Sci Technol, 2014. **48**(4): p. 2097-8.
4. Koelmel, J.P., et al., *Toward Comprehensive Per- and Polyfluoroalkyl Substances Annotation Using FluoroMatch Software and Intelligent High-Resolution Tandem Mass Spectrometry Acquisition.* Analytical Chemistry, 2020. **92**(16): p. 11186-11194.