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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". Furthermore, it is also proposed that screening should be used to support a regional evaluation of substances of emerging concern in the actions currently proposed to be transferred to the updated Baltic Sea Action Plan (HOD 58-2020 document 4-9): "Address substances of emerging concern by commencing recurrent screening campaigns [starting from 2021] including broad analytical techniques such as suspect screening and non-target screening methods".

This project proposal develops an approach that would facilitate an understanding of this issue, screening for contaminants, in particular ones not regularly monitored or those of emerging concern, in the Baltic Sea via an initial wide screening campaign. The lessons learned from this initial campaign, and approaches under discussion within EN-HZ (e.g. EN-HZ 13 document 5-1), could also offer possibilities for longer-term integration of screening, and the output from such campaigns, within the HELCOM structure (and to address BSAP and other policy aims). The proposed 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).

The proposal outlined in this document has mainly been developed within EN-HZ, but has also been commented by HELCOM PLC, HELCOM CG PHARMA, HELCOM PRESSURE (12 and 13-2020) and HELCOM State and Conservation (12 and 13-2020). The document below incorporates the comments received from these sources.

In addition, an application was submitted to the NEFCO Baltic Sea Action Plan Fund to support this regional screening campaign, under the title: Pre-EMPT - Screening for hazardous substances to pre-empt deteriorated status. The NEFCO application aims to provide the analytical costs for 86 screening samples (based on the pricing offer from the University of Athens laboratory) that could be distributed between the Contracting Parties and across the region, as deemed most appropriate. It should be noted that the NEFCO application is not considered as an alternative to this project proposal but as a complimentary process, i.e. it would, if funded, provide additional support to Contracting Parties to carry out the broadest spatial (and environmental) screening possible (e.g. filing gaps etc). On this basis, this proposal for regional wide-scope screening is presented to secure approval for the process and to initiate necessary national discussions and planning, independent of the NEFCO application process.

The document contains project proposal to initiate regular screening of hazardous substances in the Baltic Sea region. It also contains a participation table (Annex I); description of methods proposed for application in the project (Annexes II and III) and extended sample priority selection and possibilities (Annex IV).

Action requested

The Meeting is invited to:

- approve the project proposal and explore what national resources can be assigned to participation;
- approve the selection of the laboratory of preference (i.e. Athens laboratory);
- agree on the timeline to initiate national processes starting from the completion of the participation table (Annex III) so that the project planning can be furthered in early 2021.

Project proposal to initiate regular screening of hazardous substances in the Baltic Sea region

Aim

The proposal aims, via the implementation of wide-scope and suspect screening, to provide an overview of hazardous substances (including those that are not currently monitored or of emerging concern) in the Baltic Sea marine environment, at the broadest spatial coverage possible. To do this a common sampling matrix is considered so that a core pool of directly comparable data can be developed across the region. The data, and lessons learned, from this project will allow follow up actions and integration of regular screening campaigns in the HELCOM framework for hazardous substances.

It is known that due to the large spatial distribution of samples, environmental gradients, species distributions, and the fact that resources are unlikely to be available to address all sampling matrix types across the marine region, that there will be need for both a clear focus sampling matrix but also flexibility (further details are provided below).

To support a clear focus for the core regional screening samples (i.e. establishing a core spatially distributed and comparable sample series across the region) the marine environment, specifically marine biota (and primarily bivalves), will be the primary focus for sampling effort. This does not preclude the addition of other samples of national interests in addition, but this is proposed to facilitate the comparative regional aspect required for the base of this project and alternative options are provided below to guide selection where needed.

General introduction

The proposal presented here provides 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). The project is also well aligned with ongoing work in OSPAR.

The project proposal aims to establish a common approach 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). The following aspects aim to guide the selection so that a harmonized Baltic Sea wide assessment can be carried out. In this project individual Contracting Parties would carry out the sample collection and shipment to the chosen laboratory (NOTE: these costs not reflected in this project description). The sample analysis would also be paid for by each Contracting Party. Thus, each Contracting Party would identify the samples they intent to collect, carry out the collection and shipment to the laboratory selected, and pay the analytical costs per sample sent.

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 screening approach would provide an overview of an extensive number of hazardous and potentially hazardous (e.g. substances of concern) substances. Currently wide-scope target substances (>2,400 substances) can be determined in addition to suspect substances (>65,000 substances) that can be screened for their semi-quantitative presence/absence in each sample. The number of substances is also 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.

Furthermore, the proposal 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)).

Laboratory selection

All samples should 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 original draft project proposal included a single project plan and costing (University of Athens laboratory). The relevant information from the University of Athens laboratory (plan and costing) has been extracted from the original draft proposal and provided in Annex II below. At State and Conservation 12-2020 (Outcomes paragraph 4J.59) it was requested in comments received after the meeting that the Secretariat explore additional laboratory and pricing options, in particular with a focus on finding a laboratory within the HELCOM region. Via EN-HZ the Secretariat was informed of two laboratories used by the Contracting Parties for equivalent screening work and both were contacted. One laboratory, the Norwegian Institute for Air Research (NILU), although not specifically within the HELCOM region itself, was able to provide an equivalent costing and technical proposal. This proposal is provided below in Annex III.

The EN-HZ 13-2020 meeting (Outcomes paragraphs 4.1-4.15) discussed the newly proposed method and costing from NILU (Annex III) and considered that the preferred option was initial proposal (i.e. University of Athens, Annex II), 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 (OSPAR CONNECT is to be analysed at the University of Athens laboratory), 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). Similar opinions were expressed by Contracting Parties at State and Conservation 13-2020 (Outcomes paragraph 4J.72) and PRESSURE 13-2020, though it was also noted a competitive tendering process may be needed for some Contracting Parties if not centrally/externally funded (Outcomes paragraph 4.45 and 4.41).

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 - bivalves). A table of priority selection and sampling matrix options is provided below (Table 1).

Where bivalves (i.e. Blue mussels, Zebra mussels or Limecola (Macoma)) are not possible to sample it is proposed that selected fish species are the secondary target (Flounder, Eelpout, or if required Perch). The proposed selection is based on discussion within the EN-HZ sub group and the logic that maintaining a biota aspect as the secondary category (especially selecting less dispersive/ migratory fish species) as this maintains the samples within a single ecosystem component and also increases likely policy application and relevance (i.e. biota are a common target of policy initiative for hazardous substances).

Where such samples are not available, or should Contracting Parties select additional samples on top of the primary core spatially distributed samples, then sediments are also considered the tertiary option.

Table 1. Sample matrix type and selection priority.

Priority selection	**Sampling matrix**	**Details**
First choice*	Biota	Blue mussel – *Mytilus edulis* Macoma/Limecola – *Limecola balthica* Zebra mussel - *Dreissena polymorpha*
Second choice	Biota	Flounder, Eelpout Perch
Third choice	Sediments in marine environment	Sediments in marine environment

It is proposed that the total number of samples per Contracting Party should ideally be >3. This is to allow a reasonable regional/spatial coverage to be developed, with a focused on the common sampling matrix.

It should also be noted that sampling for both contaminated and known reference sites within the samples collected would provide added value.

Possible additional samples

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 in addition to the primary core regional screening samples proposed above, if resources permit. This will be dependent on external funding and also on national decisions related to resource allocation and sample selection. In addition to the aspect of including other sampling matrix types (e.g. water, sediments, other biota) to support an understanding of causal frameworks/pathways, transfer and biomagnification in food webs, or entry points to the human food chain, it may be relevant to consider different matrix types when targeting specific substances (e.g. aspect such as hydrophobicity or bioaccumulation). Other potential sampling matrix types are provided below in Annex IV).

Sample collection and preparation

Sample collection and preparation is not represented in the costings provided (as described above) 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.

In addition, once the number and type of samples has been identified per Contracting Party (latest during February 2021), i.e. via completion of the participation table (in Annex I) the EN-HZ sub-group, and other relevant participants, will convene to finalise the specifics of the sample collection (e.g. aspects such as seasonality, placement, size, number of individuals, and other such details if required). This process will ensure a high level of consistency and comparability across the region and where possible the broadest spatial spread.

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 suggested that wide-scope screening could be carried out in an increasingly

coordinated regional manner at 6-year intervals (e.g. once per 6-year assessment period). It is however 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 framework for hazardous substances. This could for example take place via the development of a process (including a guideline) for recurrent screening campaign. Further, the results of the campaign will be utilized to address and review substances with higher relative concentrations (e.g. evaluation rules) with subsequent integration of the screening-derived knowledge into broader HELCOM 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 such 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: Participation table and additional relevant information.

The table below should be completed to inform on national plans to participate in the project. This will support planning for the strong regional perspective of the project in early 2021 and collate relevant information for a possible surveillance indicator. Contracting Parties are invited to complete the participation table and send it to the Secretariat (owen.rowe@helcom.fi) by [Friday 28 January] at the latest to enable further planning and coordination of the project.

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** Data of possible relevance for HOLAS III (free text)
	Yes	No	n	Add new rows as needed	Add new rows as needed	2021 or earlier?	Reference or 'test'	Free text	
Denmark									
Estonia									
Finland									
Germany									
Latvia									
Lithuania									
Poland									
Russia									
Sweden									

Annex II: Summary overview of approach and costing for University of Athens laboratory (as provided via NORMAN)

Generalized flow of analysis

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

- National Experts, i.e. the EN-HZ sub-group on screening, and other relevant participants, (e.g. additional participants from HELCOM EN-HZ, PLC, CG PHARMA, or other) will identify the most appropriate location and other key aspects to facilitate the broad spatial coverage.
- 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 (the below is based on the proposal from the University of Athens :
    - 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 will 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 (University of Athens). 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 re-evaluated as the database grows in the future without any need for new sampling and analytical efforts.

Project costings

NORMAN network laboratory (University of 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.

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.

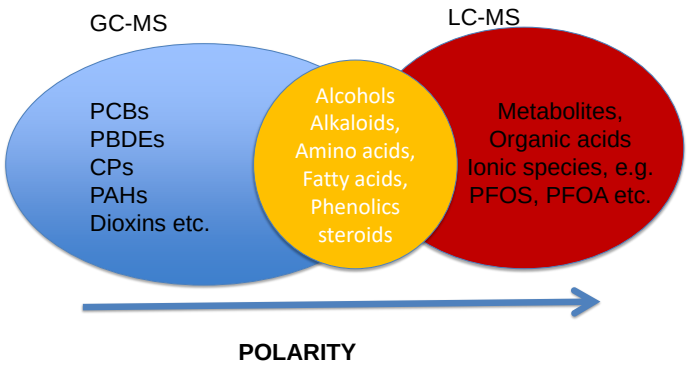

Figure 1: Application range for GC-MS and LC-MS techniques

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).

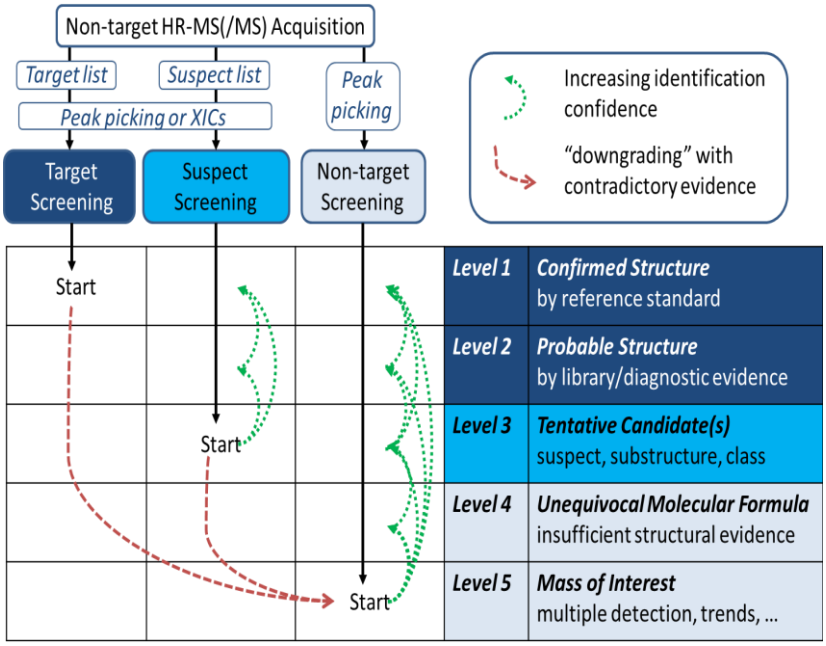

Figure 2. Level of confidence when reporting the data in target, suspect and non-target screening. Adapted from [3].

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.

Annex IV: Extended sample priority selection and possibilities

Priority selection	**Sampling matrix**	**Details**
First choice*	Biota	Blue mussel – *Mytilus edulis* Macoma/Limecola – *Limecola balthica* Zebra mussel - *Dreissena polymorpha*
Second choice	Biota	Flounder, Eelpout Perch
Third choice	Sediments in marine environment	Sediments in marine environment
Fourth 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
Fifth choice	River water	Whole water or passive samplers
	River sediment(ation)	Sediment traps
Sixth 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
	Transboundary rivers	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. Please also note the Screening study on hazardous substances in marine mammals proposal/offer by Germany.

***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.**