



*Workshop on the results of the Project:
Monitoring the pollution of water through non-target
screening on the Rhine*



Workshop
18.09.2023
ICPR Koblenz

A new harmonized approach for non-target screening – exploring its potential to track contaminants in environmental monitoring

AUE Basel-City, BfG, ICPR, LANUV, LUBW, RWS



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Project Partners

International Commission for the Protection of the Rhine (ICPR)

Amt für Umwelt und Energie Base-Stadt (AUE-Basel)

Landesanstalt für Umwelt Baden-Württemberg (LUBW)

Bundesanstalt für Gewässerkunde (BfG)

Landesamt für Natur, Umwelt und Verbraucherschutz des Landes Nordrhein-Westfalen (LANUV)

Rijkswaterstaat (RWS) – Ministerie van Infrastructuur en Waterstaat (RWS)

IT-Developer

enviBee GmbH

<https://www.envibee.ch/>



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Organizations that have participated in the workshop

Bayer AG (Germany)

<http://www.bayer.com>

Bayerisches Landesamt für Umwelt (Germany)

<https://www.lfu.bayern.de/index.htm>

Berliner Wasserbetriebe (Germany)

<https://www.bwb.de/de/index.php>

Bezirksregierung Düsseldorf (Germany)

<https://www.brd.nrw.de/>

BUND Hessen Arbeitskreis Wasser (Germany)

<https://www.bund-hessen.de/ueber-uns/organisation/arbeitskreise/wasser/>

Bundesamt für Umwelt (Switzerland)

<https://www.bafu.admin.ch/bafu/de/home.html>

Fernwasserversorgung Elbaue-Ostharz (Germany)

<https://www.feo.de/>

F. Hoffmann-La Roche AG (Switzerland)

<https://www.roche.com/>

Hessisches Landesamt für Naturschutz, Umwelt und Geologie (Germany)

<https://www.hlnug.de/>

Het Waterlaboratorium N.V. (Netherlands)

<https://www.hetwaterlaboratorium.nl/en>

Internationale Arbeitsgemeinschaft der Wasserwerke im Rheineinzugsgebiet

<https://en.iawr.org/>

Internationale Kommission zum Schutz der Elbe/ Mezinárodní Komise pro Ochranu Labe (Czech Republic/Germany), <https://www.ikse-mkol.org/en/>

KWR Water Research Institute (Netherlands)

<https://www.kwrwater.nl/en>

Landesuntersuchungsanstalt Sachsen (Germany)

<https://www.lua.sachsen.de/>

Niedersächsischer Landesbetrieb für Wasserwirtschaft, Küsten- und Naturschutz (Germany)

<https://www.nlwkn.niedersachsen.de>

Povodí Vltavy s. p. (Czech Republic)

<https://www.pvl.cz/>

Regiowasser e.V. (Germany)

<https://www.akwasser.de/>



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Swiss Federal Institute of Aquatic Science and Technology (Eawag) (Switzerland)

<https://www.eawag.ch/en/>

Technologiezentrum Wasser (TZW) (Germany)

<https://tzw.de/>

Umweltbundesamt (Austria)

<https://www.umweltbundesamt.at>

University of Chemistry and Technology, Prague (Czech Republic)

<https://www.vscht.cz/?jazyk=en>

Vereniging van Rivierwaterbedrijven (RIWA-Rijn) (Netherlands)

<https://www.riwa-rijn.org/en/>

Zweckverband Landeswasserversorgung (Germany)

<https://www.lw-online.de>

Abbreviations

BITBW	IT Baden-Württemberg
DDA	Data Independent Acquisition
DIA	Data Independent Acquisition
EPs	Emerging Pollutants
EU	European Union
GB	Gigabyte
HRMS	High Resolution Mass Spectrometry
InChI	International Chemical Identifier
IWAP	International Warning and Alarm Plan Rhine
LAN	Local Area Network
MS¹	Mass Spectra from the first spectrometer
MS²	Mass Spectra from the second spectrometer (fragment masses)
NTS	Non-Target Screening
QC	Quality Control
RAM	Random Access Memory
SMILES	Simplified Molecular Input Line Entry System
SSD	Solid State Drive
FOF MS	Time of Flight Mass Spectrometry
TPs	Transformation Products
vCPUs	Virtual Central Processing Units
WFD	Water Framework Directive

Background of the Project

Since 1800, the number of known synthesized anthropogenic compounds increases exponentially and doubles every 16 years (Llanos et al., 2019). At the end of the last century, there were more than 100 000 newly synthesized compounds per year (Llanos et al., 2019). Newly synthesized substances are gradually researched, registered, industrially produced, and commercially applied on the market. In line with this development, new compounds of anthropogenic origin are continuously discovered in the environment, e. g. (Sauvé and Desrosiers, 2014). This poses a problem since these compounds are further transformed depending on their persistency in the environment via biotic and abiotic transformation processes (e. g. via microbial transformation, photolysis, oxidation etc.) into an unknown number of transformation products (TPs) e.g., (Seiwert et al., 2016). These TP draw attention to numerous scientific studies since they can have greater adverse effects (persistency, bioaccumulation, toxicity) on the environment (plants, animals, humans) than the original compounds (Noguera-Oviedo and Diana, 2016; Schulze et al., 2020). Emerging pollutants (EPs) and their TP represent a challenge for environmental monitoring since target screening is confronted with an increasing number of known and unknown compounds e.g., (Vasilachi et al., 2021). In addition to the effort of environmental institutions in developing target screening techniques, focusing on river-specific contaminants and regular updates of the environmental legislation (e. g. Water Framework Directive (WFD)), non-target screening is becoming increasingly important as a complement to approach by means of target or suspected analysis (Hollender, 2019).

The states in the Rhine river basin have therefore successfully applied for a EU-funded project in order to further improve new analytical approaches, known as non-target screening (NTS) allowing to simultaneously access thousands of compounds and to develop a new approach for an international,



centralized, innovative and fast data processing system allowing for evaluation and storage of non-target screening data that can be applied in the daily routine of the monitoring stations – allowing them to compare data across laboratories and to share their knowledge about suspected compounds.

Purpose of the NTS Workshop

The NTS Workshop was organized in order to present and discuss the achievements of the project on Non-Target Screening (NTS) at the River Rhine funded via the EU LIFE programme. Together with our international project partners, we have developed a harmonized evaluation method and a visualization tool allowing for detection and efficient tracking of emerging contaminants over large areas. By providing a comprehensive information base, the NTS Tool is intended to equip decision-makers, particularly environmental protection authorities, with essential information for effective environmental protection measures. Another aim of the workshop was to define requirements for a follow-up project that the project partners and other interested parties have agreed on.

Description of the NTS Tool developed within the project

The NTS Tool consists of the following parts: monitoring stations, the harmonized LC-HRMS analytical method, the IT infrastructure (a cloud, a synchronization tool, and a terminal server), the pollutant databases (a *Joint Screening Database* and an *Interest List*), the software (the commercial software EnviMass and a new tool for a search and visualization of data from all stations).

Monitoring stations

The monitoring stations are located in Basel-Weil am Rhein (CH, D), Karlsruhe (DE), Koblenz (D), Bad Honnef (D), Lobith (NL) and Kleve-Bimmen (D). All stations have a high sampling frequency between 5 and 30 minutes and provide 24 hours composite samples for further analysis. It is planned that in 2024 three further stations from Luxemburg and France will join the NTS monitoring network.

Harmonized analytical method

The harmonized analytical method is based on an exact chromatographical regime and recommended mass spectrometry (MS) parameters. The method requires the presence of 26 internal standards in each sample allowing for data normalization and quality control. Resulting data have a high comparability across monitoring stations. As tested on a chosen set of 145 relevant micropollutants (pesticides, pharmaceuticals, their metabolites and environmental TPs), the method and applied instruments allow to detect their presence starting at concentrations between 10 and 100 ng/L.

Screening databases

The *Joint Screening Database* is listing EPs known by the individual institutions. The database is used for suspect screening and includes parameters crucial for detection (MS^1 spectra, fragment masses, retention times etc.) and classification of these compounds (pesticides, pharmaceuticals, metabolites etc.). Moreover, the database contains unambiguous EPs identifiers (e. g., project specific ID, the Simplified Molecular Input Line Entry System (SMILES), the International Chemical Identifier (InChI)).

The *Interest List* allows to select, label and keep monitoring of compound profiles of interest (time-intensity development of a specific unknown compound). Adding further information to the *Interest List* such as MS^2 fragments, elemental composition (figure 2), allows for gradual identification of these compounds. At present, each monitoring station has its own interest list. A merge of these lists is planned for daily operations in spring 2024.



Data transfer and processing

Measurement files generated by each laboratory are manually uploaded to the BITBW Cloud. These files are automatically transferred for further processing to the terminal server located at the LUBW. The server is equipped with Windows 10 and has 64 GB of a RAM, 12 vCPUs, 10 Gbit LAN network connection. The system disc is based on SSD (700 GB) and a HDD with a size of 7 TB is used as data storage. The measurement files need to be manually loaded into a measurement station specific project. The measurement mode, sample type, and its role in processing are automatically distinguished based on the name of each file. The setup of the data processing is harmonized across monitoring stations and is manually initiated. The software enviMass allows to process data from different instruments (TOF and Orbitrap instruments) as well as MS² data resulting from data dependent (DDA) and data independent acquisition (DIA) acquisition modes.

Data visualization

After processing, the crew of each monitoring station can examine the results in their own project. Here one can inspect the QC parameters, the detection of individual MPs, confidence of this detection, estimated concentration (optional), time-intensity development (known as profile) of both, known and unknown compounds; and associated profiles (showing emissions with similar time-intensity patterns). The results in individual projects are synchronized with a central elastic search database. A visualization tool accesses this database and allows a search for NTS features and their time-intensity profiles across all participating monitoring stations. All users can access the results either via the station specific enviMass projects or via the visualization tool.

Exemplified emission profiles

To show the functionality and current state of development of the NTS Tool, the following emission profiles were presented:

Emission of an UV filter belonging to the group of “Uvinul” compounds

The compound, 2-ethoxyethyl(2Z)-2-cyano-2-[3-(3-methoxypropylamino)cyclohex-2-en-1-ylidene]acetate (CAS: 1419401-88-9), is a micropollutant that was detected and identified by AUE-Basel in September 2022. This compound is a by-product of a modified industrial manufacturing process and was unintentionally released into the River Rhine. It is also listed as DTXSID901019869 and belongs to the group of UV Filters known as “Uvinul”, which is used in personal care products and plastics applications.

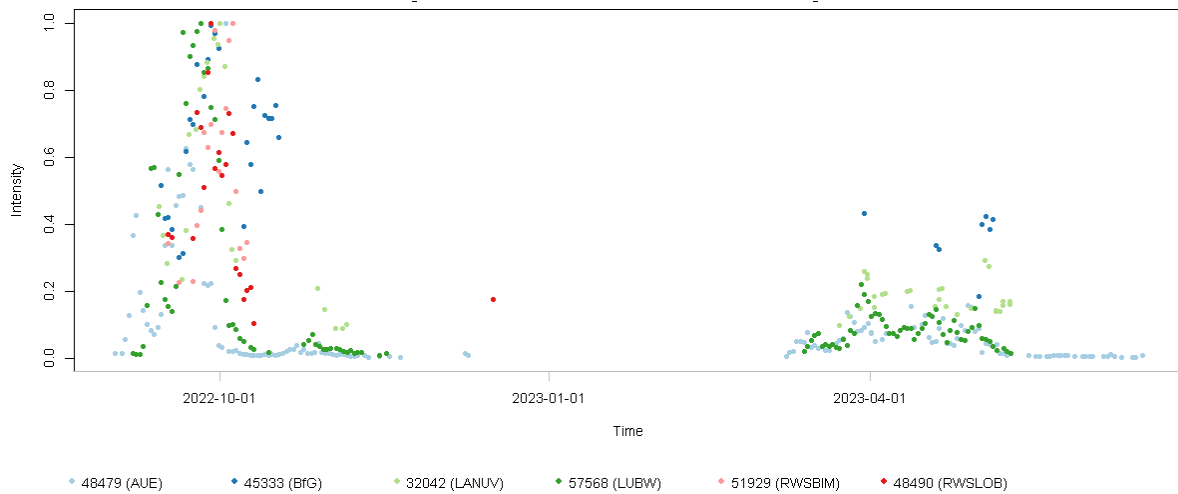


Figure 1 Time-intensity diagram of the precursor adduct ion $[M-H]^-$ of the new micropollutant DTXSID901019869 as exported from the visualization tool.

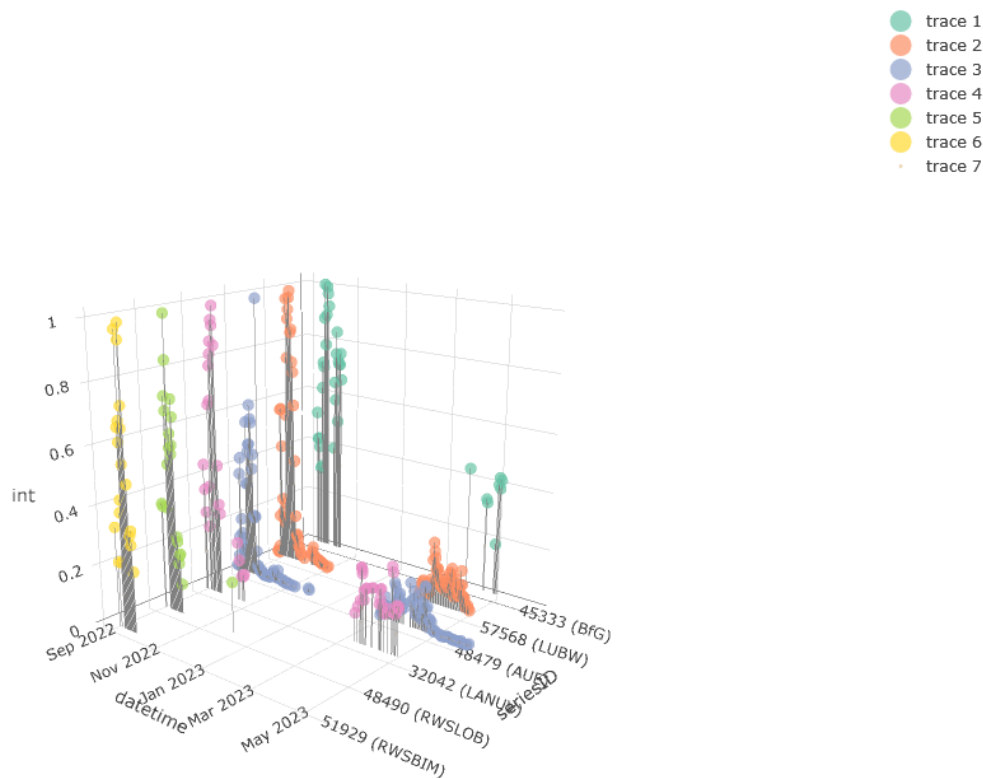


Figure 2 Time-intensity diagram of the precursor adduct ion $[M-H]^-$ of the new micropollutant DTXSID901019869 (3D View) as exported from the visualization tool.

Emission of the 1-Propene-1,1,3,3-tetracarbonitrile

1-Propene-1,1,3,3-tetracarbonitrile (CAS: 36589-04-5) as a micropollutant in the River Rhine was identified by AUE-Basel in 2014. The compound is a byproduct of chemical production. Since 2014, the

production process has been optimized, and the emission of the compound has been significantly reduced. The emission of the compound, detected by means of the NTS Tool, is most likely a result of a wastewater treatment issue. Compounds included in the screening database, as well as masses of still unknown compounds, are tracked by the NTS Tool, and the results are available to the involved authorities for further investigation.

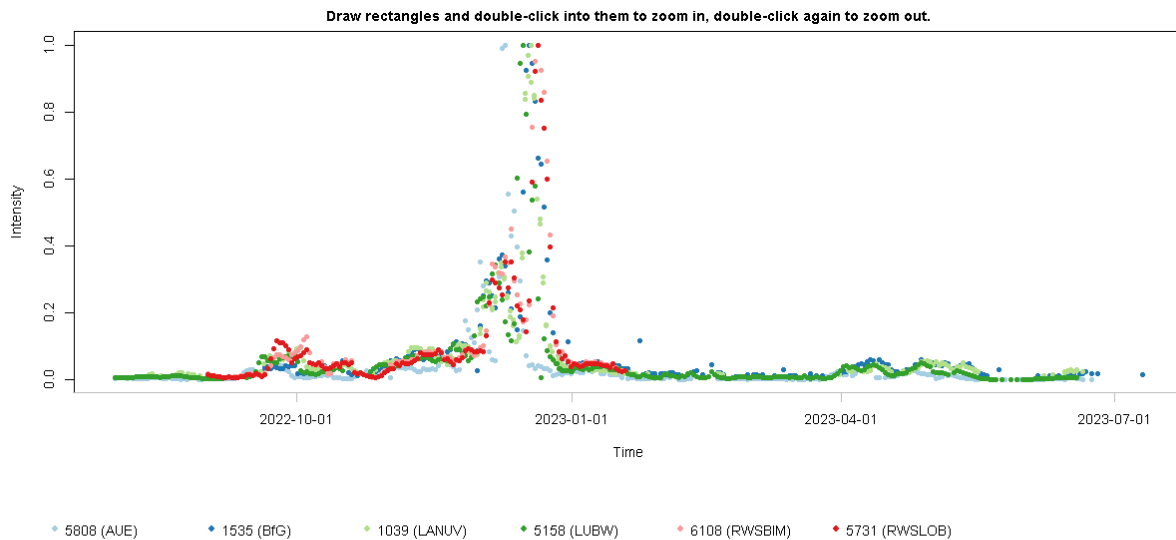


Figure 3 Time-intensity diagram of the precursor adduct ion $[M-H]^-$ of the 1-Propene-1,1,3,3-tetracarboxitrile as exported from the visualization tool.

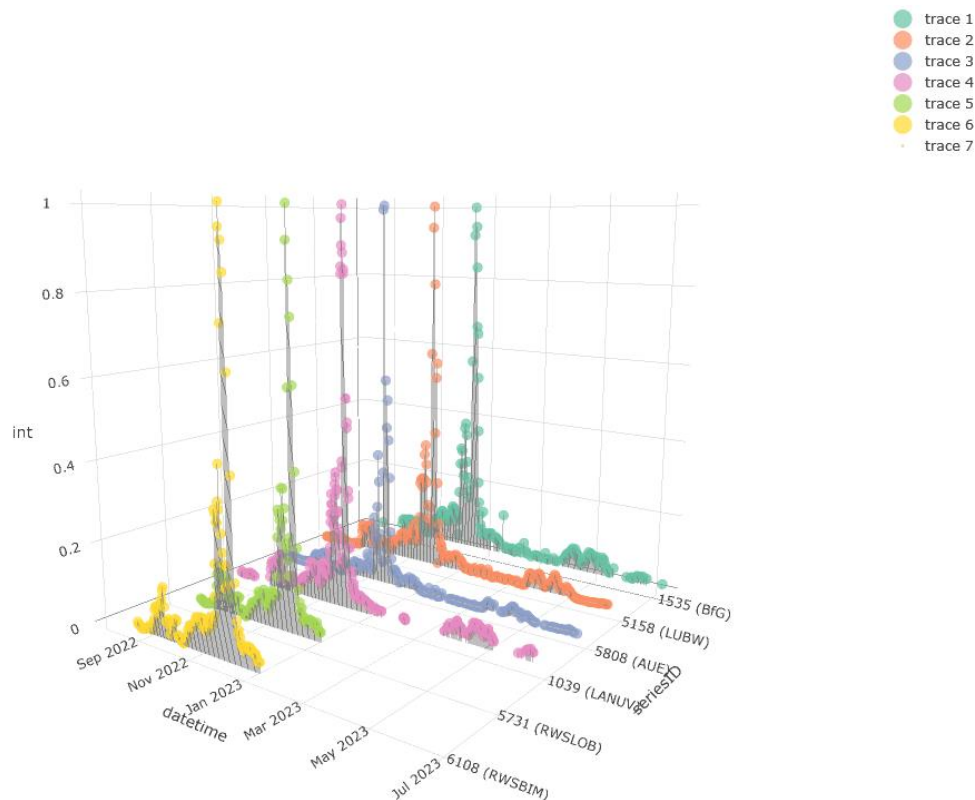


Figure 4 Time-intensity diagram of the precursor adduct ion $[M-H]^-$ of the 1-Propene-1,1,3,3-tetracarboxitrile (3D view) as exported from the visualization tool.

Points of discussion

Technical questions regarding the NTS Tool

How is the MS² information used?

- The software enviMass supports both acquisition methods (DDA, DIA). If the information about fragments is a part of the *Interest List* or the *Joint Screening Database* list in enviMass, then these fragments can be searched in the measurement data at the enviMass level as well as on the level of the visualization tool across all stations.

Is the database the same for everybody?

- The visualization tool uses a database that is synchronized with all enviMass projects located on the server. Each enviMass project contains the measurement data as well as the *Joint Screening Database* and *Interest List* (database of still unidentified compounds/features). In principle, these databases should be the same for each project, but they are not automatically updated which results in slight differences.

How do you choose the features?

- One can search for any feature or its time-intensity profile using the visualization tool. The users usually search for profiles with recently increasing trends detected in their monitoring station specific enviMass projects. When found, they use the visualization tool to see the occurrence of this feature at other stations.

Will the alarm monitoring using the NTS Tool be performed daily?

- There is currently still a lack of real-time monitoring. Currently, the evaluation of NTS data is conducted retrospectively and the data from individual monitoring stations varies in age.
- It is intended to further develop the NTS tool towards a real-time monitoring suitable for alarm monitoring in scope of the follow-up project (2024-2029).
- In case of alarm monitoring, there will always be a certain delay (at least 24 hours) since the laboratories need to measure the samples, upload the measurement files at the server and the software needs to process them and show the time-intensity trends.

Are intensities normalized?

- Yes, intensities are normalized already in individual enviMass projects using 26 internal standards. The visualization tool provides time-intensity profiles of individual stations in 2D and 3D diagrams. Each profile is normalized on the 0 to 1 scale.

What are the most important features and profiles?

- On the level of the individual station, trends can be searched for in recorded time-intensity profiles in projects as well as via the Visualization Tool. Most users search for recently increasing trends and profiles with highest average intensities.

Questions regarding the opportunities of NTS in environmental protection

When do you expect the authorities to take an action? Based on which data? Is identification and quantification required?

- Competent authorities should pay attention when a laboratory records an increasing signal of a feature over time. The full identification is not necessary in the beginning. Trends of unknown features often correlate with already known substances. This can be used to identify the pollution sources. Prioritization of features according to the trend should be the first step.
- Authorities should take action when the new features occur in drinking water. Then, the source of this features must be identified. If they should take an action when the features occur in ground water is still a matter of discussion.
- There are legal obligations. The Water Framework Directive (WFD) requires that water quality should improve and must not deteriorate. The River Rhine is a source of drinking water. In addition, this is the responsibility of the companies (industry) for not causing any water quality deterioration not of the authority.
- For discussion with the industry, an identification of the substance (elucidation of the chemical formula) is needed. Companies should provide a list of chemical formulas they produce or use for production together with their amounts per year.

Which data should be shared with industry and how? Which channels can be used for that?

- The competent authorities should contact the companies; they are responsible for their emissions.
- For instance, the monitoring station in Basel contacts individual companies (when a feature with suspicious trend is detected).
- Moreover, once a year, there is a local round table with representatives of individual companies.
- This is a good practice example that could be applied elsewhere along the River Rhine!

Which information would be of interest for water suppliers?

- The occurrence of unknown features is of great interest for water suppliers.
- The polluters cannot omit all emissions. One should focus mainly on the toxic compounds released into the environment. For this, the structure of the substances needs to be known.
- Authorities should compare the occurrence of compounds between different matrixes (drinking water, ground water, and surface water). If found in these matrixes, one should track it back to the source.
- The law for drinking water or ground water is stricter than the law for surface water.

Is there a way to link the non-target signals with toxicity endpoints?

- This is not fully possible. One approach would be to estimate the toxicity of the compound based on its fragments (moieties) with known toxic effects.

Where do you see NTS in 5 or 10 years? What improvements do you expect?

- Toxicity tests are needed, even if the structure of the compound is known. To conduct only screening is not enough.
- Lists of substances are endless, the list of sources is shorter and the number of measures is even shorter.
- NTS should be better linked to the measures. The goal should always be to choose the best measure. NTS can help to identify the most effective measures.
- NTS could develop towards a live monitoring directly on site.

Questions regarding the follow-up project and cooperation with other projects

What are the requirements to join the (follow-up) project in 2024?

- Environmental protection agencies and other institutions can participate either directly or indirectly in the (follow-up) project.

Institutions participating directly

- Direct partners are participating with financial contributions to the project (staff costs for a scientific assistant/project coordinator, server costs and costs for further software development).
- Each institution conducts LC-HRMS measurements. (The frequency and location of the sampling are currently being discussed.)
- All institutions involved in the project will use a harmonized LC-HRMS method. This method is required in order to ensure the comparability of the data and their final alignment. This allows to track contaminants across all participating monitoring stations. This requires the appropriate measuring equipment, chemicals must be ordered and a license of the software is needed.

Institutions participating indirectly (associated partners)

These institutions are not involved in the project financially, but they can participate in the following activities:

- Topic-related participation on analytical meetings
- Possibility to exchange suspect screening lists
- Cooperation in the identification of newly discovered substances
- Participation in round robin tests and quality control measurements

The indirect participation will NOT include:

- Access to the data visualization tool
- Access to the software and data of project partners
- Access to the analytical results

Direct cooperation with drinking water operators and industry is not yet planned. However, possibilities for cooperation can be examined by 2029.

Will a search in data from discharges from industry also be performed?

- No, the project is focused on a monitoring of the River Rhine and its tributaries. If conspicuous features will be discovered, the individual authorities will act according to their competences.
- At the moment, there is still a lack of real-time monitoring, and the data are evaluated retrospectively.

Conclusions

- The high degree of harmonization / standardization and exchange between the laboratories is the special feature of the EU-funded project.
- Harmonized methodology and standardized data processing allow easier and more efficient information exchange between state authorities, laboratories, and monitoring stations.
- New monitoring stations can join the project.
- In environmental monitoring, prioritization is crucial. Prioritization of detected substances based on their toxicity must be the focus. Coupling the identification of compounds with toxicity tests is a necessary step. The NTS should be more closely linked to the measures to keep the Rhine water clean.
- The NTS Tool is intended to, in the medium-term, reduce the workload of the Rhine monitoring stations conducting NTS.
- The data analysis and visualization within the NTS Tool is currently conducted retrospectively. NTS along the Rhine should be further developed towards a real-time monitoring. This would open up valuable possibilities for use in alarm monitoring, including within the framework of the International Warning and Alarm Plan (IWAP) Rhine.
- A comparison with features found in drinking water and groundwater with those in wastewater can help to identify compounds with high mobility and persistence.
- Cooperation with third parties (drinking water suppliers, industry) should be expanded. Effective communication with the industry is highly beneficial in this regard.
- The annual round table in Basel (Switzerland) is a good practice example. NTS can make a contribution, here.
- Cooperation with other NTS projects and the exchange of data can further advance the NTS on the Rhine.
- It still needs to be clarified under which conditions the NTS Tool can be made available to authorities in other river basins. A dissemination of the method would advance environmental monitoring as a whole.



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