

## NORMAN Joint Programme of Activities (JPA 2025)

## List of scientific activities organised by the NORMAN network in 2025

We are proud to present the scientific programme which will be promoted by the NORMAN network in 2025.

The *NORMAN Joint Programme of Activities* (JPA) is defined every year by the Steering Committee, after consultation with the membership (General Assembly meeting and e-mail survey).

The final JPA and the associated budget are approved by the Steering Committee, using the following criteria:

- the level of interest of the members (results of the survey)
- the relevance of the research topic to European environmental policies
- the balance between different sectors / fields of interest
- the relative value of the proposed in-kind contribution vs amount of resources required.

For this JPA the Steering Committee has approved a budget of € 247,500, based on the expected income from membership fees of the Founding and Ordinary members. These resources will be allocated for scientific and coordination activities and regular updating and maintenance of the NORMAN Database System.

The JPA of the NORMAN network is financed by the contributions of its members (membership fees and members' in-kind contributions), always with a view to maximising synergies between research teams in the field of contaminants of emerging concern (CECs) and improving the science-to-policy interface at national, European and international level.

Exceptional income will be granted by the ICPDR to the NORMAN Association in 2025 as a contribution in support of NORMAN's participation in the experimental activities of the 5th Joint Danube Survey (JDS5) to be started in June 2025.

A summary description of each activity is presented in the following sections.



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## NORMAN NDS – Maintenance and upgrading

#### NORMAN Database System (Activity coordinated by EI, <a href="mailto:slobodnik@ei.sk">slobodnik@ei.sk</a>)

The NDS is a joint activity of all NORMAN members and at the core of the NORMAN activities, providing data and tools to fulfil its goals and visions. In many aspects it supports the vision of the EU Common Data Platform where reliable data of transparent origin are used at the risk assessment of chemical substances in the environment.

The NDS consists of 13 integrated databases modules:

- 1. Suspect List Exchange https://www.norman-network.com/nds/SLE/
- 2. Substance Database https://www.norman-network.com/nds/susdat/
- 3. Chemical Occurrence Data (EMPODAT) https://www.norman-network.com/nds/empodat/
- 4. Ecotoxicology Database https://www.norman-network.com/nds/ecotox/
- 5. Digital Sample Freezing Platform (DSFP) https://norman-data.net/Verification/
- 6. Substance Factsheets https://www.norman-network.com/nds/factsheets/
- 7. MassBank Europe https://massbank.eu//MassBank/
- 8. Passive Sampling https://www.norman-network.com/nds/passive/
- 9. Antibiotic Resistance Bacteria/Genes https://www.norman-network.com/nds/bacteria/
- 10. SARS-CoV-2 in sewage https://www.norman-network.com/nds/sars\_cov\_2/
- 11. Bioassays Monitoring Data https://www.norman-network.com/nds/bioassays/
- 12. Indoor Environment https://www.norman-network.com/nds/indoor/
- 13. Prioritisation https://www.norman-network.com/nds/prioritisation/

All NDS modules can be searched either individually or starting from the module 'Search All Databases' (https://www.norman-network.com/nds/common/), where any substance from SusDat can be searched and displayed with all available data for this substance in any of the database modules.

#### Progress of the activities in 2024

All NDS modules underwent continuous update in 2024. Some highlights are illustrated below. For the other modules, details are provided in the dedicated sections (see "Suspect List Exchange "; "Digital Sample Freezing Platform"; "MassBank Europe"; "Bioactivity Database").

The **EMPODAT Database** was enlarged for more than 600,000 new data entries and contained 96,625,121 data in the end of 2024, with monitoring data for 4,687 substances. A close cooperation with PARC, PROMISCES, SPRINT, CONNECT 2 and EU4EMBLAS projects have been established resulting in inflow of valuable datasets in harmonised formats. Several datasets are still not publicly accessible until finalisation of the projects and related reports/publications. A significant effort has been put into implementation of the automated protocol requested by the EC JRC for transfer of EMPODAT into IPCHEM on an annual basis.

In the **Ecotoxicology Database**, new EQS and PNEC values were uploaded and available PNECs underwent continuous revision with a special effort to remove duplicate entries. A DCT for PNECs from studies/literature was created and all NORMAN members were invited to contribute, especially with the experimental values. Based on the specific request of the WG-8 (marine mammals) and WG-7 (terrestrial environment), rat toxicity threshold values predictions were produced for SusDat substances using VEGA QSAR model. The values are ready to be integrated into both the database and automated prioritisation tool in 2025. Several models have been identified within the network as suitable candidates to provide reliable predictions for (eco)toxicity threshold values. There is a need to decide how to incorporate these values for numerous toxicity endpoints (e.g. ToxAl aiming at 105 different endpoints) into the Ecotoxicology database, which is currently based on aquatic ecotoxicity-derived threshold using the three 'regulatory' trophic levels (algae, daphnia, fish). At present, the marine and terrestrial endpoints are of particular interest to NORMAN members. These actions will be organised as part of the new CWG focused on Data Science for Monitoring of Emerging Environmental Substances.

In the **Antibiotic Resistant Bacteria/Genes Database** five new DCTs have been developed for ARG and ARB matrices (water, soil, sewage sludge, plant crop, air). New data were added as reported in Alygizakis et al., 2024, DOI: <u>https://doi.org/10.1016/j.watres.2024.121689</u> (see also WG-5 section).

The NORMAN Indoor Environment Database has been updated and upgraded DCT is available for providing new data (https://www.norman-network.com/nds/indoor/downloadDCT.php) in 2025. For an overview, see Haglund at al., 2024, DOI: <u>https://doi.org/10.1016/j.scitotenv.2024.177639</u> (see also WG-6 section).

The prototype of the new module, **EMPODAT-SUSPECT** (see WG-1), was tested in 2024 with a test-set of 58 million suspect screening data. It is expected that the full version will be ready in 2025 and gradually filled out with suspect screening data from HRMS chromatograms archived in DSFP (>5,000 samples as of December 2024; screening of

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>95,000 substances in each sample; assigning IP score to all detected substances (see JPA 2024 DSFP); total >475 million data entries).

## Planned activities for 2025 (and beyond)

In addition to maintaining and continuously populating all database modules, the tasks for 2025 include:

- **Development of the 'Hazards and Properties' database module** (in collaboration with WG-1) (see WG-1 section).
- Further development of the API portal (<u>https://www.norman-network.com/nds/api/</u>) for automated data sharing with external databases: API are currently available for NORMAN SusDat, EMPODAT, Ecotoxicology Database and Passive Sampling Database and will be further developed in order to cover all modules in 2025.
- Assigning DOIs to all contributed datasets in the NDS: a system for creating online accounts to assign DOIs to all contributed datasets has been developed for EMPODAT in 2024 and tested using https://doi.datacite.org/. In 2025, accounts will be created for each NDS module and for each contributed dataset.
- Further interlinking of NDS modules and quality check of all input data (collaboration with WGs).
- Update of Passive Sampling module: the NORMAN Passive Sampling Database contained 4,213 target analyses data entries in the end of 2024. New target and suspect screening data will be generated as a result of the NORMAN collaboration with the 5<sup>th</sup> Joint Danube Survey (JDS5) in 2025 (see "CWG-PS" and "Joint Danube Survey 5" sections). Additionally, the development of a Passive Sampling – SUSPECT module is also planned.
- Development of a database module for archiving data obtained through data mining tools from publications, patents and grey literature: several NORMAN members already use text mining tools to obtain data from peer-reviewed or grey literature for various purposes, e.g. for establishing early-warning systems for chemicals in the environment. However, the experience shows that these data are in various formats, difficult to compare, often aggregated and without sufficient data quality information. The aim is to establish a workflow for their processing into a harmonised data format as well as the creation of a common repository for such data within the NDS. Already collected data on metals and organic pollutants in apex predators from the TerrraChem project could be used as a starting point (as a possible joint collaboration with TerraChem project).
- Development of a new module for collection of training and validation datasets for models feeding various parts of the prioritisation framework of NORMAN (see "Blueprint for linking ecotoxicity to different levels of biodiversity damage" section). This activity will be discussed with the newly created CWG on Data Science for environmental monitoring of emerging substances (see "CWG-Data Science" section).
- Correction of spatial data in EMPODAT based on the curation by CleanGeoStreamR script and development of strategy for flagging correction of original data (collaboration with UFZ): Automated curation of spatial data in EMPODAT was presented at the AI workshop using CleanGeoStreamR script developed at UFZ Leipzig (open access at GitHub). It has been agreed to process the latest version of EMPODAT (>96 million data entries) with the tool to correct wrongly assigned coordinates, misspellings of station/river/sea region names etc. in the originally provided data. In the test run, more than 107 million corrections were made. The plan for 2025 to incorporate these corrections into EMPODAT and develop a strategy for flagging the corrected data. Ultimately, the script could be given to data providers for automated raw data treatment prior to sending it to the NDS.

#### **Development of the NORMAN Database System 2.0**

A particular focus will be given in 2025 to optimising and revising the NDS structure to better align with Big Data requirements. The objectives include enhancing query performance, creating APIs, and facilitating data management in Big Data environments.

The data will be in the virtual server provider with daily backups using operating system UBUNTU Linux 24.04 Long-Term-Support. The source code would be published on GitHub, and thus all members of the network could contribute to its further development.

The database will include the installation of Docker Service, PostgreSQL Database, connection to GitHub, PHP/LARAVEL framework, and SSL certificate for secure browsing (https) and periodic management and server health-checks.

Compared to current server solution it would include faster disks, high-performing database, GitHub support, and monthly management which offers tailored adaptability to new requests and using Docker Service, with which the entire database can be moved to different server within minutes (for more details see <a href="https://kubernetes.io/">https://kubernetes.io/</a>).

The task for update of the NDS and its continuous upgrade in 2025 include:



- Speedup the search process and improve user interface
- Integrate API for external users
- Consolidate data and data links between SusDat and other databases
- Running on new servers
- Changed from SSD drives to NVME drives 10x speedup in data extraction
- Running dedicated database solution
- Migration from current MariaDB to new PostgreSQL the ability for data partitioning to search group 100-1000x speedup
- Server resources not shared with database
- Changed back-end architecture
- Moving from pure PHP to Laravel framework standardised code writing
- Publication of the source code on GitHub
- Laravel back-end comes with professional API capabilities
- Queued exports: User will get an email, that export of data is ready to be downloaded
- User access and activity log auditing
- Each user will have his/her own dashboard and activity log
- Differentiation of individual admin/user roles for data maintenance, e.g., only users associated with specific project will have access to unpublished data.

## NORMAN Suspect List Exchange (NORMAN-SLE)

NORMAN Suspect List Exchange (Leader: LCSB, Luxembourg emma.schymanski@uni.lu)

The NORMAN Suspect List Exchange (NORMAN-SLE) is an initiative of the 2015 JPA and is an established central access point for NORMAN members (and others) to find suspect lists relevant for their environmental monitoring questions. The NORMAN-SLE and the compiled suspect list "NORMAN SusDat" have become the cornerstone / database for all NORMAN Database System (NDS) modules and prioritisation efforts. Both have been very highly prioritised by members in recent surveys. The split between NORMAN SusDat and NORMAN-SLE is primarily for logistical reasons (to clearly demarcate data gathering and delivery, the NORMAN-SLE, and the role of a substance database (NORMAN SusDat). The NORMAN-SLE activity involves the addition of new lists to the Suspect List Exchange, which then feed into SusDat, which is the chemical database behind all NDS modules. The 2022 NORMAN-SLE publication (DOI: 10.1186/s12302-022-00680-6) bringing together 97 co-authors, confirms the relevance and breadth of the community involvement. By the end of 2024, the NORMAN-SLE contained >120 lists. The latest statistics are here.

In 2025 we plan to continue and expand on the efforts from recent years, including:

- Website maintenance plus development of a new interface to "modernise" the SLE
- Addition of new lists from external contributors when they become available
- Addition/update of contents in existing lists as required
- Addition of new lists strategically selected to fill identified knowledge gaps
- Addition/update of lists to specifically save/register/link transformation product information
- Archiving of all datasets on Zenodo (https://zenodo.org/communities/norman-sle)
- Deposition of all substances in PubChem
- Integration of lists into PubChem
- Automation of various aspects of the SLE (statistics, curation, etc)
- Continuing development of new strategies to deal with UVCBs
- Further development of strategies to deal with tentative/unknown/related structures
- Work together with the NORMAN SusDat team to develop open software/packages/approaches for curation / merging between NORMAN-SLE and NORMAN SusDat
- Publication: NORMAN-SLE update publication (new lists, developments since 2022) in ~2025.

## MassBank Europe and RMassBank

MassBank Europe and RMassBank - Continuous development and upgrade of MassBank Europe and related activities (Emma Schymanski & Anjana Elapavalore (LCSB Luxembourg) in co-operation with Tobias Schulze (UBA Berlin), Steffen Neumann (IPB Halle) and Michael Stravs (Eawag)). Contact: <u>massbank@massbank.eu</u>

- Upload of mass spectra to MassBank (all NORMAN members welcome to contribute)
- Further maintenance / development of RMassBank
- Further maintenance / development of MassBank server platform (e.g. database and applications programming

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interface, curation of records, import and export of records, standardisation of curation rules – MassBank3 is under development at IPB-Halle and entering test phase)

- Improved cross-integration of MassBank and NORMAN-DSFP (see DSFP JPA)
- Fostering the collaboration with MassBank.JP members, reinitiated in the past years
- Fostering the integration of MassBank with other mass spectral and metadata platforms (e.g. MoNA, PubChem, GNPS, US EPA CompTox, NORMAN SusDat, RforMassSpectrometry, etc.)
- Fostering the discussion with vendors for better integration of vendors' software with MassBank
- Continuing the discussion on prioritised compounds missing in MassBank (matches to SLE, priority mixtures etc.).
- Making MassBank more FAIR (e.g. better integration of ontologies, linked to NFDI4chem)
- Integration of MassBank in NFDI4chem as central repository (2020-2025)
- MassBank EU publication scoping and planning under discussion with key Consortium members.

## NORMAN DSFP

## DSFP maintenance and upgrading (Leader: El alygizakis@ei.sk)

DSFP has been developed from a prototype to a production-ready system with enhanced informatic capabilities over the last years. DSFP enables archiving, processing, analysing, data mining and retrieving information for thousands of contaminants of emerging concern (CECs) contained in high-resolution mass spectrometry (HRMS) data. The DSFP is ready to support challenging and ambitious goals of NORMAN such as the application of non-target screening prioritisation and the automatic retrieval of chemical exposure in early-warning systems for chemicals. The database is a unique effort to collect HRMS data from environmental samples, facilitating the risk assessment of CECs in support of regulators and policy-makers. The DSFP, as part of the NORMAN Database System (NDS), is a valuable asset for the future activities of the NORMAN Association.

In 2024, several significant achievements were made. DSFP output elements were harmonised using controlled vocabularies for metadata, component lists, and screening outputs. A metadata schema was defined for all DSFP entities (<u>https://dsfp.norman-data.eu/data-schema</u>). The screening process for collections was debugged and optimised, and screening and indexing were applied to five collections. APIs were enhanced, and a new API for instant search was introduced. A web analytics collection system was installed, and submission forms were revised. A map of contributions was added to the main page, and users from various projects received support to ensure effective use of DSFP.

The continuous development of DSFP's functionalities is crucial for expanding its usage and further enriching the database. Enhancements that improve DSFP's efficiency and encourage researchers to upload their data, thereby increasing the collection of HRMS data, remain a key objective. To address this, the JPA 2025 proposal focuses on maintaining and advancing DSFP's functionalities:

- Preparation of a manuscript describing the new technologies utilised in upscaling DSFP
- Screening and indexing all collections in DSFP to enable instant search capabilities and populate EMPODAT-SUSPECT
- Developing a functionality for batch import of new samples
- Further integration with DataCite (assigning DOI to each contributed dataset)
- Creation of a machine learning plugin for analysing and further exploiting NTS data
- Exploration of collaboration opportunities with other major platforms (e.g., MassBank consortium, GNPS)
- Improving guidance documents and producing instructional videos for DSFP
- Maintaining and supporting current and future users, including fostering connections with PARC and other EUfunded projects involving NORMAN members.

## **NORMAN BioActivity Database**

**NORMAN BioActivity Database (BADB)** (Leader: KWR Water Research Institute <u>Tessa.Pronk@kwrwater.nl</u> <u>Astrid.Reus@kwrwater.nl</u>. <u>Miina.Yanagihara@kwrwater.nl</u> in collaboration with UBA <u>Peter.VonderOhe@uba.de</u> and EI <u>slobodnik@ei.sk</u>)

Bioactivity data are of interest to NORMAN for the interpretation of monitoring data (e.g. iceberg modelling) and the interpretation of chemical water quality with *in vitro* bioassays.



By the end of 2024, the BADB contained over 1,000 entries. All NORMAN members were invited to test the functionality and performance of the module, as well as the newly developed Data Collection Template, and to share their datasets. The first, official version of the database is scheduled for launch in 2025.

To accelerate feeding of contents and inspire the use of the BADB, a one-day workshop will be organised in 2025 for all interested NORMAN members and other potential users. The workshop will:

- showcase several lines of research that can be done with this type of data (iceberg modelling for mixture toxicity assessment, trigger value derivation for water quality monitoring, potency ranking, etc.)
- enable a discussion for new applications, relevant links of the BADB to other datatypes and databases
- lead to a research paper on Bioactivity data and its past, present, future.

As part of the JPA 2025, the outcomes of the workshops will enable an action plan to go beyond merely compiling information on bioassays responses and substances' potencies. Once a critical mass of data becomes available, we intend to introduce a tool for deriving EBTs, in analogy to the existing "PNEC derivation module" in the ECOTOX database. Furthermore, in analogy with the CRED module, we aim to implement a mechanism for verifying the reliability of bioassay data, based on the provided metadata. This will be comparable to the established reliability scoring system thanks to alignment with the metadata from the SCIRAP tool (<u>SciRAP - Start</u>).

## **WG-1** Prioritisation of CECs

**Working Group N°1:** Prioritisation of CECs (Activity coordinated by INERIS <u>valeria.dulio@ineris.fr</u> in collaboration with El <u>slobodnik@ei.sk</u>, <u>alygizakis@ei.sk</u> and UBA <u>peter.vonderohe@uba.de</u>).

The prioritisation activities of WG-1 constitute an ongoing effort to integrate various NORMAN activities in alignment with the objectives of the EU Chemicals Strategy and the Zero Pollution Action Plan. The tasks of WG-1 aim to:

- Ensure that all relevant information for assessment of chemical risks is maintained and regularly updated
  Integrate and exploit this information to identify lists of chemicals in need of priority actions (each priority list corresponds to an action)
- Continuously upgrade the prioritisation scheme (integration of innovative techniques) to enhance the value of the prioritisation work, particularly for the early identification of warning signals.

In 2025 the focus of WG-1 will address the following topics:

## Task 1: Support the prioritisation work of the Commission services at European level and provide comment on relevant consultation documents (PARC, EWS, where relevant) (ALL)

NORMAN participates as a stakeholder in the WG Chemicals of DG ENV and intends to further contribute to the activities related with the review of the list of WFD Priority Substances and the Watch List.

NORMAN WG-1 is committed to collaborate actively with PARC partnership on the following prioritisation-related topics:

- Collaboration in the development and implementation of the mechanism for priority setting in environmental and multi-source monitoring – *MonitoringFrame project* (PARC T4.2.1). The NORMAN scheme was recognised as an efficient and pragmatic way to address regulatory questions while addressing existing data gaps. The concept was further developed in the Prioritisation tool designed within the *MonitoringFrame* project. Further collaborative actions remain open for discussion.
- Early Warning System for Europe (EWS): NORMAN already contributes by providing signals obtained from retrospective suspect screening of HRMS data (DSFP) and target monitoring data (EMPODAT), using indicators customised for the identification of early warning signals. NORMAN can also contribute with experimental and predicted data of hazardous properties as well as identification potential risks and observed impacts from research papers and other public sources (see Task 10).

Task 2: Collection and prediction of compound-specific information in support of prioritisation (UBA / EI / NKUA / DERAC / INERIS) (ongoing activity to be pursued in 2025)

After finalisation of the harmonised ecotox DCTs, the extraction and **compilation of** <u>additional</u> experimental ecotoxicity data from <u>model training sets</u>, will be started in 2025 (see also task 3). Regulatory quality targets for various matrices, including re-use, soil and marine waters, will also be collected and compiled (see also task 3).

Moreover, data on physico-chemical and fate properties (e.g. Kow, Koc, BCF), as well as hazardous classifications (i.e. related to ED, CMR, PBT, PMT) will be retrieved e.g. from REACH registration dossiers and future CLP dossiers and integrated in the Substance Factsheets to support the hazard score in the prioritisation module (see

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task 4). For this purpose, a specific DCT has been developed in 2024. In case of lack of experimental data on these hazards, **prediction models using artificial intelligence will be developed and used to fill the gaps.** The P, B, T, CMR and ED scores were assigned to 76,269 compounds in the SusDat list by UBA and EI using the JANUS model. However, difficulties to run the model for the remaining ca. 30,500 chemicals persist. Hence, it is proposed to develop a **quantitative model for persistence**, based on a set of **8000 reported degradation half-lives for the OECD 302 tests**. The models will allow to **predict the DT50 in water for all compounds in SUSDAT** that are covered by the model domain. The experimental training dataset will also be uploaded.

In order to ensure that only reliable predictions are reported in the Substance Factsheets and used for the prioritisation of chemicals which lack experimental data, more general actions are planned as part of the new CWG focused on Data Science for Monitoring of Emerging Environmental Substances. Here **experts in modelling and machine learning should work to reach consensus on the use of the predictive models to be used and rules to define their application domain.** 

## Task 3: Collection of existing PNECs and deep learning-based toxicity predictions for use in NORMAN PNECs (UBA / EcotoxCentre / DERAC / El) (ongoing activity to be pursued in 2025)

After the successful extension of prediction models for 3 additional fish species, 2 crustaceans and one insect species for acute effects, we propose to extend the models also to chronic effects in fish, daphnia and algae for aquatic species with sufficiently large experimental datasets. The final aim is to enable a more robust statistical approach for the derivation of freshwater P-PNECs and their subsequent conversion for application to other matrices, like sediments and biota, e.g. by using Koc and BCF (see task 2). Thereby, the use of chronic-based endpoints will allow to lower the AF for deriving the P-PNEC, which will be appreciated by many regulatory bodies that are using the prioritisation results of NORMAN.

For 2025, it is therefore planned to continue with the predictions of toxicity values for both existing compounds and those that have recently been added to SusDat, in order to be up to date with the list of Lowest PNECs. The upload of existing PNEC and the derivation of new PNECs will also include **MAC-EQS values.** This will allow the evaluation of peak exposure concentrations, such as those resulting from the German small water body monitoring project.

It is also planned to derive more robust **PNEC marine values** to support prioritisation of compounds in the **marine environment** (delayed from JPA 2024).

WG-1 will continue to promote and coordinate the participation of Ecotoxicity Experts to derive and approve (*i.e.* vote for) new or revised Lowest PNEC values for SusDat substances, with a specific focus on substances that were highly prioritised in EU projects using the NORMAN Prioritisation Framework. The aim is to progressively replace predicted PNEC values for substances prioritised in Cat 3 and 5, by experimentally-based PNEC values.

# Task 4: Consolidation of the Physico-Chemical and Hazard properties module and its integration in the NORMAN Database System and Prioritisation Tool (UBA, INERIS, EI in collaboration with WG-1 partners)

Physico-chemical and hazard properties are essential for deriving the hazard scores (PBT/vPvB, PMT/vPvM, CMR, ED) used in the NORMAN prioritisation framework. Data are already collected in the NDS Factsheets for various relevant parameters (Kow, Koc, BCF, DT50, etc.), mainly from the US EPA CompTox DashBoard.

An increasing number of models (e.g. JANUS), including machine learning (ML)-based models (e.g. ToxAI), and tools are being developed to assess the Persistence (P), Bioaccumulation (B), Mobility (M), and Toxicity (T) criteria and classify the substances in line with the REACH and CLP requirements.

To provide a comprehensive overview of available resources and improve data assessment, a **Phys-Chem and Hazard properties** module was created. These sub-modules will store raw data and metadata in a harmonised format, as well as existing classifications. The module will also serve as a platform for transparent assessment of data sources, thereby facilitating consensus on robust classifications for prioritisation. Additionally, it will allow for the selection of different scenarios (e.g., use of conservative, robust, average data).

The design of the prototype started in 2024 (INERIS and UBA) and was discussed with the WG-1 participants. The work will be pursued in 2025 in consultation with the WG-1 partners and the programming will be performed by EI.

Task 5: Compilation of data / information regarding 'Use categories' and 'Chemical Functional Use' for all SusDat compounds (LCSB / INERIS / UBA / EI) (activity postponed and revised from 2024)



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We propose to integrate a new "Use" module into the NDS to ensure that substances in SusDat will have suitable information about uses and functional categories. This module will be linked with the Substance Factsheets, SusDat and the Prioritisation tool. This new functionality will enable searching in the NDS by e.g., all pesticides, all herbicides; all pharmaceuticals, all antibiotics; or by, e.g. all PFAS, pyrethroids, PAHs, ionic surfactants.

The task proposed in the previous JPAs remained on stand-by due to the complexity of reaching a consensus on creating a controlled vocabulary for use categories and functional classes. It is now proposed to adopt a data-driven approach, choosing a bottom-up rather than a top-down methodology, which will ensure that these tasks deliver preliminary data upon which to develop appropriate controlled vocabularies.

The task will involve:

- Pilot efforts to retrieve "Use" information from PubChem and other sources (e.g. Wikipedia, Wikidata, CompTox) and integrate it into a temporary "Use" module (working space)
- Organise the collected information to create a list of use categories and functional classes, along with their associated synonyms (ontology)
- Programming of a workflow resulting from the pilot efforts for the transfer of the information retrieved from PubChem and other sources into the actual prototype of the future "Use" module
- Integration and operational implementation of the prototype in the NDS (EI).

## Task 6: Prioritisation framework: follow-up activities to integrate and test the new workflow as part of the Prioritisation Tool in the NDS (EI, INERIS, UBA, ALL) (ongoing activity to be pursued in 2025)

In 2024, critical steps have been taken for the development of the necessary infrastructure (back-end) to implement the online prioritisation tool. The chemical occurrence database now has a new EMPODAT API (<u>https://www.norman-network.com/nds/empodat/api\_matrix\_index.php</u>) that allows fast retrieval of target screening data. The DSFP has made publicly available the data schemas used in the platform (<u>https://dsfp.norman-data.eu/data-schema</u>). Among the schemas, the DSFP Detection schema allows for hosting detected suspected compounds, their IP scores and sub-scores, and the semi-quantified concentration levels in a flexible JSON file for a given compound and collection. As of November 2024, four collections have been screened for all SusDat compounds, formatted using the DSFP Detection schema, and are ready for data retrieval through the DSFP API (<u>https://server.norman-data.eu/search/detections/all</u>). Therefore, the infrastructure is ready, and it is possible to operationalise the link between target and wide-scope suspect screening investigations.

The focus of 2025 will be on:

- Applying the workflow: Implementing the workflow published as a scientific article in 2024 https://doi.org/10.1186/s12302-024-00936-3
- API adaptation (if necessary): Making necessary modifications to the APIs to ensure they align with the workflow's requirements and purpose
- Enhancing visualisation (see Task 8): Applying the workflow within the Prioritisation Tool will produce more complex outputs, necessitating effective visualisation. The visualisation dashboard developed in 2024 will be further refined and fully integrated into the Prioritisation Tool
- Quality Assurance: Testing the workflow with the wastewater case study to validate the quality and reliability of the outputs
- **Expanding applications:** Extending the workflow's application to a different case study to demonstrate its versatility and robustness.

## Task 7: Improvement of the features of the Prioritisation Tool (expert consultation and programming activities) (ALL) (EI, INERIS, UBA, UFZ, NKUA, OVAM, DERAC, LCSB, KWR)

The Prioritisation Tool provides as an output a table file. The integrated NTS prioritisation scheme yields rich outputs and a table is not sufficient for comprehensive explanation of the output. More advanced data visualisation and exploration tools are required for in-depth analysis. In 2023 at the Database workshop in Athens, some visualisation options have been discussed and proposed for testing. In 2024, a visualisation dashboard was developed (available at <a href="https://norman-data.eu/Prioportal">https://norman-data.eu/Prioportal</a>) and presented during the WG-1 meeting (November 2024).

In 2025, the visualisation dashboard will be further enhanced with additional features and tools, enabling users to perform a wide range of statistical operations more effectively. Enhancements in related tasks, such as the inclusion of use categories and information on tonnage bands, will create additional opportunities for further refinement. The dashboard will be finalised and integrated to the Prioritisation Tool. It will allow the users to draw more conclusions about the prioritisation results (within the action categories and beyond). The dashboard will be further leveraged to improve the visualisation functionalities of other modules of the NDS.

## Task 8: Prioritisation of data from Joint Danube Survey 5 (collaboration with the ICPDR) (INERIS, UBA, EI in

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## collaboration with WG-1 partners) (end of 2025 - 2026)

Prioritisation of contaminants in the Danube River Basin with the following specific objectives:

- Identification of River Basin Specific Pollutants (update)
- Proposal for a candidate Watch List
- Changes / Progress observed from the previous Surveys

The work will be based on the extended NORMAN Prioritisation Framework using target and suspect screening data. Moreover, the improved PNECs (additional endpoints and species from ToxAI tool) will be applied.

## Task 9: Testing state of the environment indicators in the Danube River Basin (NORMAN collaboration with Joint Danube Survey 5) (EI, UBA, INERIS and ALL) (2025-2026)

The aim is to integrate the risks of individual substances at specific sites into an overall assessment (*i.e.* mixture toxicity) by mapping the concurrent presence of chemical contaminants across various compartments in Europe. For example, determining "how many compounds exceed the lowest PNEC at each site" or assessing "the total mixture risk at each site." These approaches could serve as new chemical indicators to measure progress in environmental quality, e.g., as a result of remediation measures and inform policy decisions (cf. pesticides indicator of the EEA) about the trends. This would allow NORMAN to, e.g., support the Zero pollution monitoring report of the EEA. Moreover, analysis of datasets regarding the minimum (most toxic compounds) compared to the total effect (mixture risk) might allow to derive an indication of a retrospective mixture allocation factor (MAF) in support of the COM EGD initiative.

**Task 10: NORMAN contribution to Early Warning System** (UBA, EI, INERIS, KWR, OVAM, EAWAG, SLU, NKUA) NORMAN has been working actively on EWS concept development for many years, with NormaNEWS, DSFP, prioritisation scheme by action category, etc. NORMAN can therefore significantly contribute together with PARC to the on-going collective efforts in building an EWS for Europe.

NORMAN already contributes by providing signals obtained from retrospective suspect screening of HRMS data (DSFP) and target monitoring data (EMPODAT), using indicators customised for the identification of early warning signals.

With this proposal WG-1 intends to develop a concept to support the EWS for Europe by creating automated signals based on non-target and target screening data for compounds in the NDS, as well as through the development of a DCT to compile additional evidence from research papers and other public sources regarding emerging hazards (e.g. new effects), sources (e.g. new products), increasing exposure (e.g. first or increasing detection in DSFP) or observed impacts.

These signals should be compiled and stored in a new EWS module. For the first time, an online form will allow experts to register their findings in a harmonised way. These "signals" can then be analysed by experts (for specific topics), or by the next generation of large language models (LLM), also known as General Artificial Intelligence (GAI), which are expected to emerge in 2025. Even if the next generation models are not yet able to link the multiple lines of evidence from the various signals submitted, NORMAN should start to compile the information needed to be ready once the GAI is available.

#### Task 11: Collaboration with other WGs to support prioritisation of contaminants in new compartments

- Prioritisation of contaminants in soil (WG-1 and WG-7): (see WG-7).
- Prioritisation of contaminants in the marine environment (WG-1 and WG-8): (see WG-8)
- Prioritisation of contaminants in reused matrices (WG-1 and WG-5) (ongoing task to be pursued in 2025).

## WG-2 Bioassays

Working Group N°2: The value of bioassays and biomarkers in water quality monitoring programmes (Activity coordinated by Goethe University Frankfurt, Henner Hollert <u>Hollert@bio.uni-frankfurt.de</u> and Sarah Johann <u>johann@bio.uni-frankfurt.de</u>)

Further to the successful workshop, "Innovative Approaches for Environmental Monitoring of Chemical Pollution and Biodiversity – Linking Biodiversity Loss and Chemical Pollution", in December 2023, WG-2 participants recognised the importance of continuing and intensifying their collective efforts to investigate the connection between chemical pollution and ecosystem degradation, as evidenced by biodiversity loss. An abstract submitted at the SETAC Europe 2025 in Vienna was accepted for a poster presentation and two joint manuscripts are in progress. During the WG-2 meeting in October 2024, a mentimeter poll was conducted to obtain input from the 40 participants as a joint basis for the later discussions on further directions and areas of work for the WG2. Participants suggested several areas for future work. These included: standardisation of bioassays (e.g. sample extraction/preparation), expanding databases on chemicals, ecosystem effects, and bioassay results as an initial effort; enhancing ecological knowledge on



ecosystem functioning and impact pathways to better understand and predict the effects of chemicals at the ecosystem or even catchment level; and developing bioassays that are relevant at the ecosystem level. Current models, which focus on organism and sub-organism ecotoxicology, are insufficient for predicting ecosystem-level impacts. It was concluded that predictive models should be further developed to predict the effects of chemicals at the higher level. A progressive approach should be adopted, starting with effects at the population and community levels, and eventually encompassing entire ecosystems and catchments.

Based on these considerations the focus of WG-2 in 2025 will cover the following tasks.

## Task 1: Two-day Workshop of WG-2 in summer 2025

A broad range of topics will be discussed during a two-day workshop of WG-2 in summer 2025 in Frankfurt, including contributions of WG-2 to the NORMAN Bioactivity database, the recent developments in the field of AI, links between WG-2 activities on bioassays and WG-3 on EDA, innovative behavioural assays, eDNA-and eRNA-based methods, including transcriptomics, adverse outcome pathways, as well as the further implementation of effect-based methods in European chemical regulations. Furthermore, the workshop will include a specific session to discuss the draft of the joint publication on "Remobilisation of pollutants during extreme flood events" (see Task 4).

## **Task 2 Joint Publications**

An poster presentation prepared by WG-2 will be presented at the SETAC Europe 2025, Vienna. Two joint manuscripts are in progress as a follow-up to the "Chemical Pollution and Biodiversity" workshop (Frankfurt 2023): 1) A joint manuscript "Overview of large projects and research networks linking chemical pollution and biodiversity loss in Europe", based on the keynotes at the workshop in 2023. 2) A second manuscript will be developed as a NORMAN position paper. The objective of this manuscript is to: (i) discuss the linkage between chemical pollution and biodiversity changes and to examine the implications of the evolving role of chemical pollution within the conceptual framework of planetary boundaries, thereby addressing the EU strategies on biodiversity and chemicals for sustainable chemical markets, as well as the EU One Health concept; (ii) bring together existing expertise and methods on chemical pollution and biodiversity, promote cooperation, combination of existing databases on both fields and joint studies. A draft will be discussed at the two-day workshop of WG-2 in Summer 2025 in Frankfurt.

Task 3 Joint investigation of suspended particulate matter from the environmental specimen bank using a battery of effect-based methods (follow-up JPA 2024) (Leader: University Frankfurt Hollert@bio.uni-frankfurt.de and Sarah Johann johann@bio.uni-frankfurt.de and Francisco Sylvester sylvester@bio.uni-frankfurt.de in collaboration with UBA jan.koschorreck@uba.de, Fraunhofer-IME bernd.goeckener@ime.fraunhofer.de, University of Örebro, SLU, EAWAG and others)

Since 2005, suspended particulate matter (SPM) samples are systematically collected from major German rivers, including Rhine, Elbe and Danube for the German Environmental Specimen Bank (https://umweltprobenbank.de/en/, Zizka et al 2022, ESEU, <u>https://doi.org/10.1186/s12302-022-00618-y</u> and Fliedner et al. 2022, STOTEN, <u>http://dx.doi.org/10.1016/j.scitotenv.2022.158430</u>. The samples have been analysed for a range of inorganic and organic pollutants with target, cumulative and Non-Target Screening methods. In addition, DNA metabarcoding data from the SPM samples are available for fish and macrozoobenthos communities. Additionally, fish and SPM from the Environmental Specimen Bank were also analysed using transcriptomics in early life stages of Danio rerio (JPA 2024).

The JPA 2024 and a PARC activity from ORU, UBA and SLU could identify severe temporal differences in the effectpatterns of the SPM. The aim of the applied JPA for 2025 is a **joint investigation of suspended particulate matter from the environmental specimen bank using a broad battery of effect-based methods and to establish a strong cooperation between NORMAN WG2 and PARC** in order to deepen the understanding of temporal trends of effects of SPM from the Environmental Specimen Bank. Selected effect-based methods of the NORMAN/Solutions biotest battery (Brack et al.2019, ESEU, <u>https://doi.org/10.1186/s12302-019-0192-2</u>, e.g., PFAS, ER, p53, antiAR-CALUX, <u>microEROD, FET, Ames</u>) will be carried out on the extracted suspended matter samples. In order to identify the drivers of toxicity, EBM data will be compared to the already existing chemical exposure and EDA data of the SPM and the transcriptome data of the investigated fish from the environmental specimen bank using mass balance calculations and also Al-based methods.

#### Task 4: Follow-up tasks started in the previous JPAs

- Remobilisation of pollutants during extreme flood events (ongoing) (Leader: Goethe University Frankfurt Hollert@bio.uni-frankfurt.de). Six sediment and suspended sediment samples, out of ca. 150 samples collected in the Eifel region further to the summer 2021 flood event, were selected for this study. The extracts were distributed to the 13 labs participating in this ring study for detailed and comprehensive toxicological and chemical profiling to evaluate the impact of such an extreme flood event in respect to the goals of the European Water Framework Directive. The evaluation and statistical analysis of the results by Goethe University have been completed. The evaluation, statistical analysis of the results and drafting a manuscript for a joint publication is

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under preparation by Goethe University. The WG-2 workshop in 2025 will provide participating labs the opportunity to meet and discuss the results and the draft for the joint publication as well as the elaboration of proposals for consideration of flood-driven pollutant remobilisation in the implementation of the WFD. Finally, as agreed in the JPA, the project leader will feed the chemical and bioanalytical monitoring data in the NORMAN databases (EMPODAT and bioassays modules).

- Ecosystem level effects of CECs on aquatic ecosystems (database / WG) (Leader: Wageningen Environmental Research <u>paul.vandenbrink@wur.nl</u> in collaboration with IMDEA <u>andreu.rico@imdea.org</u> / <u>andreu.rico@uv.es</u>). A paper presenting the outcomes of the NORMAN workshop: *Improving the use of (semi-field data for the risk assessment of chemicals* (November of 2022) is under preparation under the leadership of Paul van den Brink (WUR) and Andreu Rico (expected to be published in 2025).

Support the work of the Commission (EBM – CIS WFD Activity) (Leader: Goethe University Frankfurt Hollert@bio.uni-frankfurt.de)

NORMAN WG-2 will continue to support the activities of the Commission as regards the implementation of Effect-Based Methods in the *Recast Water Framework Directive* and other relevant directives.

## WG-3 Effect-directed analysis

Working Group N°3: Effect-directed analysis for hazardous pollutant identification (Activity coordinated by UFZ Iker Alvarez Mora <u>iker.alvarez-mora@ufz.de</u> and VU Frederic Béen, <u>f.m.been@vu.nl</u>)

Task 1: Integration of computational toxicity driver prioritisation tools to support non-target screening workflows in HT-EDA – Part II: Designing endpoint-specific fractionation methods based on predicted toxicity (activity already started in 2024)

Toxicity prediction tools like MLinvitroTox, as shown in previous WG-3 activities, significantly advance HT-EDA. Using these tools, HRMS analysis of a sample provides a preliminary insight into the distribution of potentially active compounds within a chromatographic run.

For certain endpoints with specific receptors (e.g., AR, ER, AhR...), it is common for most potentially active compounds to cluster in specific chromatographic regions (regions of interest). Identifying these regions through toxicity prediction would allow the chromatographic fractionation conditions to be tailored, thereby increasing the likelihood of successfully identifying the effect drivers.

In collaboration with PARC Task 4.3.E01, this approach will be evaluated on effluent samples from ongoing pan-European study. The objective of this study is to test MLinvitroTox to obtain preliminary information that can guide the design of tailored, endpoint-specific chromatographic conditions for fractionation in HT-EDA.

The work will be performed according to the following steps:

Activity 1: Identify the endpoints of interest (primarily those being tested within the ongoing PARC 4.3\_E01 project) and define the chromatographic conditions (particularly column types) to be tested in this approach.

Activity 2: Use the analyses conducted within PARC to apply toxicity prediction for the agreed endpoints and identify cases where toxicity distribution is concentrated in regions of interest. This will involve using the NTS workflow developed during the 2024 activities (<u>Alvarez-Mora et al., 2025</u>), which will also include MS/MS diagnostic information prediction (<u>Codrean et al., 2023</u>) to improve the applicability of MLinvitroTox.

Activity 3: Fractionate the samples and test them under both the standard HRMS conditions and new conditions adapted to the identified regions of interest. This will allow evaluation of the approach's effectiveness for the selected endpoints.

The results of this JPA will be published in a publication showing the benefits of applying toxicity prediction before designing the EDA experiment. Depending on the results obtained for the different endpoints on the WWTP samples, the identification of the effect drivers of these samples could be part of a second publication.

This activity builds on the initiatives started with the 2024 JPA, aiming to develop efficient workflows that enhance the identification of effect drivers in complex mixtures through HT-EDA, taking it to a new level.

Additionally, it provides an ideal scenario for the application and validation of several tools developed within NORMAN, particularly in the CWG-NTS. Furthermore, in line with the collaboration agreement between NORMAN and PARC, this activity will complement an ongoing project in PARC with new ideas emerging from NORMAN, fostering mutual benefit.



## WG-4 Nano- and micro-scale particulate contaminants

Working Group N°4: Nano- and micro-scale particulate contaminants (Activity coordinated by Eawag – Ralf.Kaegi@eawag.ch and NIVA Bert.vanBavel@niva.no and Vilde Kloster Snekkevik vilde.snekkevik@niva.no)

Over the past years, WG-4 has devoted significant effort to improve validation and harmonisation of common measurement methods and monitoring tools for microplastics in the environment in support of the EU's Green Deal and Plastic Strategy. The effort was made collectively with smart coordination of activities and finding synergies with other EU-funded projects and standardisation bodies. Various interlaboratory studies were organised in collaboration with QUASIMEME, EUROqCHARM and PlasticTrace EU project. In 2024 an ILS for the analysis of MP in environmental matrices was organised in collaboration with ISO TG 147/SC2 /JWG1, and the PlasticTrace project. Significant steps forward were made thanks to the development and testing of "sandwich filters" specifically designed to disentangle the measurement variations associated to sample preparation from the actual measurement and data processing.

These activities will be pursued in 2025 with a focus on the following initiatives.

## Task 1: Workshop and Feasibility Study: NORMAN Database for Microplastic Analysis

The particle-based quantification of microplastic particles in or extracted from complex matrices relies on an accurate assignment of experimental spectra to available, well-characterised (reference) spectra. Currently no central database exists, and information is fragmented and limited due to different data treatment software or instrument makers. Infrared (IR) absorption spectra databases (FT-IR or direct IR) often lack compatibility due to differences in spectral resolution, data formats, and material ranges.

Limited reference spectra can hinder the identification of uncommon or novel microplastics. The quality of spectra in databases can vary significantly. Factors such as particle size, shape, and surface contamination can affect the spectra, leading to misidentification. Additionally, environmental degradation of plastics can alter their spectral characteristics. Manual spectrum comparison is time-consuming and error-prone, while automated analysis depends on the quality of the underlying databases.

For that, WG-4 will organise a workshop to assess the current needs from instrument and software providers and from end users for establishing a database of reference spectra from different polymer types and differently aged variants. Such a workshop will address the following questions:

- Which databases are already available and for which instruments and software applications?
- What is needed, required (from the perspective of the instrument providers, software developer's and researchers (end users))?
- Is it envisaged that NORMAN should host a new module in the NDS dedicated to microplastics? How could such a platform be integrated into and maintained by the NORMAN network?

There is currently a need for a common repository dedicated to microplastics data as well as the definition of common protocols / standardisation for data and metadata collection. The issue is to decide whether NORMAN should / could host a new module in the NDS dedicated to microplastics. The feasibility of including a FTIR, RAMAN, MS and monitoring data module to the NORMAN databases will be investigated together with preparation of implementation.

### Task 2: Sourcing of plastic materials of interest for future ILS

So far, the focus of interlaboratory studies in the field of microplastic particles has primarily been on relatively 'simple' materials/ polymers. However, there is an urgent need to include more challenging materials and particle morphologies (fibres, paint particles, tire particles, Biodegradable Plastic Particles (BPP)).

The proposed activity will involve the acquisition and testing of polymeric materials, which are of interest / concern at EU level. After sourcing the different materials from commercial sources and research repositories, feasibility tests will be conducted using sandwich filter and / or soda tablet approaches. We will summarise the results of the feasibility experiments in a report and will recommend the most suitable materials for an ILS to be organised in 2026. The timeline for assessing the different materials is as follows: Fibres Q1 (2025), Tire particles Q2 (2025), Biodegradable Plastic particles Q3 (2025), Paint particles Q4 (2025), Feasibility report Q4 2025.

## Task 2: Follow-up activities previous JPAs

(Micro)plastics – leaching of additives and non-intentionally added substances (NIAS) (Leader: TNO andrea.brunner@tno.nl . NKUA, nalygizakis@chem.uoa.gr). This task is organised in collaboration with the NTS-CWG (This study was already approved under JPA 2024, see "(Micro)plastics – leaching of additives and non-intentionally added substances" – JPA 2024 ).



## WG-5 Water reuse and policy support

Working Group N°5: Water reuse and policy support (Activity coordinated by DERAC, France <u>genevieve.deviller@derac.eu</u> in collaboration with LTU, Sweden <u>lian.lundy@ltu.se</u> and EI, Slovakia <u>alygizakis@ei.sk</u>).

Interest in data on biological and chemical CECs linked to reuse practices (e.g. wastewater, stormwater, sludge for agriculture) has grown with the EU Circular Economy and Zero-Pollution Action Plans. New EU regulations promote reuse to address water scarcity. However, the lack of local impact assessment of CECs hinders widespread implementation. In line with WG-5's mandate, three database enrichment projects will continue in 2025, with a new focus on stormwater reuse.

## Task 1: The Antibiotic Resistance Bacteria and Genes Database

The NORMAN ARB&ARG (launched in 2021) currently contains 3741 data points from 11 countries (cf. Making Waves: The NORMAN ARB&ARG – an invitation for collaboration to tackle antibiotic resistance").

The goals for 2025 are to: 1) Intensify efforts to collect, harmonise, and upload ARB&ARG data from key scientific publications; 2) Improve functionalities of the database; 3) Attempt to launch monitoring campaigns (e.g., JDS5) for ARB&ARGs and A&TPs to enrich the database with new data; and 4) pursue the incorporation of NGS data into the database.

## Task 2: Scoping potential for stormwater reuse

A key impact of a warming climate is changes in rainfall patterns, with increases in the frequency of both summer droughts and winter rainfall events predicted for much of Northwestern Europe. This is a particular challenge for urban areas, where their largely impermeable surfaces reduce recharge of water bodies (contributing to water scarcity) and enhances surface runoff volumes (exacerbating flood risks and degrading water quality). Hence the same urban areas cam become a hot spot for both floods and droughts, raising the key question: can stormwater be used to meet non-potable water demands? Whilst regulations and policies are in place to facilitate wastewater reuse, the potential to collect, treat and store stormwater at an urban scale has yet to be robustly assessed. This new activity builds on the stormwater reuse component of the reuse article being developed under Task 3 by undertaking a systematic review of current knowledge on stormwater reuse from opportunities and challenges perspectives.

In 2025, we plan to: 1) organise an online meeting to discuss stormwater reuse interests and current projects within NORMAN; 2) scope interest and, if of interest; 3) plan an international stormwater reuse review article to report on current practices, treatment technologies and future opportunities; a pan-European stormwater monitoring campaign.

#### Task 3: Databases for CEC risk characterisation in reused environmental matrices

The risks linked to chemicals in reused matrices like water and sewage sludge are mostly unknown and occurrence data as well as quality targets are needed to characterise and prioritise those risks. The risks linked to chemicals in reused matrices like water and sewage sludge are mostly unknown and occurrence data as well as threshold values are needed to characterise and prioritise those risks. Significant effort has been made in 2024 to enrich EMPODAT database with new datasets on reclaimed water; to collect new quality targets for water reused for agricultural irrigation and for soil for various protection targets (which were also used to derive quality targets for sewage sludge); to prepare a peer review publication on the risk characterisation and prioritisation of CECs in reused waters, including stormwater and reclaimed water. The activities planned for 2025 are the following:

- Collection and publication of new occurrence data on stormwater, reclaimed water and sewage sludge in EMPODAT based on literature reviews.
- Collect and publish new quality targets for soil and convert these into quality targets for sewage sludge used in agricultural fertilisation for the Ecotoxicology database.
- Conduct risk characterisation and prioritisation of CECs in sewage sludge for agricultural fertilisation.
- Publish the risk characterisation and prioritisation of CECs in reused waters, including stormwater and reclaimed water, in a peer-reviewed journal.

## WG-6 CECs in the indoor environment

**Working Group N°6: CECs in the indoor environment** (Activity coordinated by RECETOX <u>lisa.melymuk@recetox.muni.cz</u> in collaboration with Vrije Universiteit Amsterdam <u>pim.leonards@vu.nl</u> and University of Antwerp <u>adrian.covaci@uantwerpen.be</u>).

The activities of WG-6 focus on investigating contaminants of emerging concern in both, the indoor environment (i.e. indoor air and dust) and outdoor air. Additionally, they aim to improve the harmonisation of measurement and sampling methods. In 2024, a significant achievement was marked by the publication of a paper by Haglund P. et al. This paper

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presented the results of the NORMAN collaborative trial on non-target screening (NTS) techniques for analyzing organic chemicals in dust. Additionally, it provided a comprehensive overview of the organic chemicals present in indoor dust across Europe.

In 2025, the activities of WG-6 will focus on the following tasks.

Task 1: Updating indoor data collection templates and harmonising metadata in support of indoor environment projects (Leader: RECETOX, Lisa Emily Melymuk, <u>lisa.melymuk@recetox.muni.cz</u>)

Indoor environments are a key area of interest for both the NORMAN network and other on-going initiatives/projects (e.g., PARC, IDEAL cluster of Horizon Europe projects on indoor air). Many of these projects look to the NORMAN database structure for guidance on structures, metadata and data collection templates for indoor and other databases. For example, the IPCHEM indoor module has incorporated NORMAN indoor database structures related to building categorisation and sampling parameters in its data structures (Kephalopoulos et al., 2022; https://doi.org/10.1016/j.ijheh.2020.113515). The increasing number of projects and growing interest in indoor environments necessitates updates to the data and metadata structures related to indoor air and dust data to ensure that the NORMAN database system remains a relevant repository of newly generated indoor data, from NORMAN projects as well as other indoor projects in which NORMAN partners are involved.

Objectives: This proposal aims to bring together key stakeholders in the generation and harmonisation of indoor data to critically evaluate the indoor data structures in the NORMAN indoor database and update metadata structures and data collection templates where needed.

The tasks in 2025 will include: 1. Practical evaluation and testing of existing NORMAN indoor data structures and DCTs in the context of newly generated indoor data.; 2. Workshops with experts outside NORMAN; 3. Publication preparation.

Task 2: Combining Wastewater-Based Epidemiology (WBE) and indoor dust analysis for comprehensive evaluation of chemical exposure risks in indoor environment (Leader: University of Bath, Barbara Kasprzyk-Hordern <u>bkh20@bath.ac.uk</u>, UFZ, <u>saskia.finckh@ufz.de</u>, Sara Castiglioni, Istituto Mario Negri, <u>sara.castiglioni@marionegri.it</u>, RECETOX, Lisa Emily Melymuk, <u>lisa.melymuk@recetox.muni.cz</u>) (study already approved under JPA 2024)

Water-based chemical mining, also known as Wastewater-Based Epidemiology (WBE), utilises metabolic biomarkers of exposure and effects produced collectively by studied populations. These biomarkers ultimately end up in urban water, providing a timely and complementary tool to traditional biomonitoring approaches. Similarly, chemicals emitted indoors are present in indoor air and dust, which is yet another, important matrix enabling chemical exposure studies.

This proposal (already part of the JPA 2024) aims to combine the investigation of indoor environments (DUST) and human community exposure via wastewater analysis (WBE) to provide a new, holistic framework for comprehensive understanding of human exposure risks to hazardous chemical mixtures in indoor environments.

The tasks and objectives planned for 2025 include:

- Based on an initial screening, it was concluded that there is little overlap between the WBE and DUST datasets. The aim is to identify commonalities in tested chemical targets and the geographies covered
- Organisation of a workshop to establish common sampling/sites protocols for WBE-DUST integration
- Based on the workshop results, plan the first WBE-DUST pan-European study. This study will focus on human exposure to chemical mixtures in indoor environments through a sample-sharing exercise between the WBE and DUST communities
- We will consider writing a perspective paper on opportunities in human exposure studies through combined monitoring of WBE and DUST within the "One Health" context. Our goal is to develop a solid WBE-DUST framework.

This activity will be carried out as a collaboration among two key ongoing activities in Europe:

- WBE pan-European monitoring program planned in 2024 in PARC (4.3\_E01 Mining chemical information in wastewater for human community and environmental exposure assessment)
- SCORE network activities focused on annual WBE monitoring campaigns.

#### Task 3: Follow-up tasks started in the previous JPAs, as listed below.

- CT on Passive air sampling and wide-scope suspect/non-target screening for organic substances in air (in collaboration with PS-CWG): The laboratories were invited to participate in the study (June 2024). Further to registration of the participants, the deployment of the passive PDMS foam sampler adsorbents was organised during summer. A preparatory workshop took place on 10 October 2024 before distribution of the exposed samplers to the participants in November 2024 together with the SOP and reporting templates for LC and GC. The results are expected to be submitted by the end of March 2025, with processing to be carried out throughout

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the remainder of the year. (Action leader: <u>adria.sunyer@aces.su.se</u> ; <u>jon.martin@aces.su.se</u> Stockholm University).

**CT on dust sampling methods:** The plan was to analyse selected chemical groups (chlorinated paraffins, POPs, plasticisers, and OPFRs) and complete the study with a publication. In 2021, two datasets were finalised – chlorinated paraffins and POPs. However, the samples assigned for analysis of plasticisers and PFRs by the University of Antwerp are still pending dispatch from Vrije Universiteit Amsterdam (2024). It is envisaged to complete this study and publish the results in peer-reviewed paper. (Action leader: <u>pim.leonards@vu.nl</u> Vrije Universiteit Amsterdam).

## WG-7 Soil and terrestrial environment

Working Group N°7: CECs in the terrestrial environment (Activity coordinated by UBA <u>Annegret.Biegel-Engler@uba.de</u>) and OVAM Laetitia Six <u>laetitia.six@ovam.be</u>

The proposal for a European Soil Monitoring Law (SML) was approved by the European Parliament in April 2024, followed by the approval by the European Council in June 2024. It is expected that upon completion of the trialogues, the SML will be approved in 2025, and the Directive will need to be implemented at Member States level shortly after. The long-term objective of this Directive is to achieve healthy soils by 2050.

The SML focusses on 3 pillars: (1) setting up a soil monitoring framework to assess the soil health throughout Europe; (2) sustainable soil management; and (3) risk assessment and management of contaminated sites.

The activities of WG-7 in 2025 will be targeting pillar 1: "soil monitoring". In the original proposal by the EU Commission, the "organic contaminants" to monitor were not defined. Hence, the European Council advised that, in order to ensure the protection of soil from pollution by emerging substances (CEC, contaminants of emerging concern) that can pose significant risk to human and animal health and can contaminate surrounding air, surface waters and groundwater, mechanisms to detect and assess such substances should be established.

A **watch list**, as in use in the Water Framework Directive (WFD) for surface and groundwater, should be developed for monitoring soil contamination. The substances or groups of substances to be placed on the 'soil watch list' should be substances for which the available information indicates that they could pose a significant risk at Union level to, or via, the soil environment, and for which the monitoring data are currently insufficient.

The number of substances or groups of substances to be monitored and analysed under the watch lists should not be limited.

Though the watch list is also up for discussion in the trialogues, the current proposal is that the EU Commission, in cooperation with the Member States, shall establish an **indicative watch list of soil contaminants**, having a high potential to affect soil health, human health or the environment. Member States may then adapt their soil contamination descriptor accordingly.

## Task 1: Recommendations for the definition of a watch list for monitoring of the soil contamination

To support the collective effort behind the future implementation of the SML, WG-7 aims to convene NORMAN members, experts in soil science and prioritisation mechanisms. The goal is to discuss and reach a consensus on a watch list for monitoring soil contamination. This effort will be based on the latest scientific knowledge regarding the potential risks of contaminants to soil health, human health, and the environment. In addition, WG-7 will closely consider monitoring experiences and available data from Member States to ensure the watch list reflects current understanding and addresses the most pressing concerns in soil contamination.

The plan is to organise this task in two steps:

- Short-term: The project will produce a list of soil contaminants that should be monitored with high priority. These contaminants will be selected based on scientific knowledge, with a strong focus on their analytical feasibility (e.g., availability of harmonised methods, LOQ achievable by routine analytical laboratories) and positive economic aspects ("low hanging fruit").
- Long-term: WG-7 experts will develop a prioritisation mechanism for soil contaminants based on predefined protection goals. This mechanism will incorporate input from innovative monitoring approaches, such as suspect screening and effect-based methods, to define the watch list. It is expected to result in a longer watch list of contaminants recommended for soil monitoring, including substances that still present analytical challenges.

Various workshops will be organised both in person and virtually to stimulate constructive and active discussions among members. We also plan to seek collaboration with stakeholders such as DG ENV, JRC, and CEN. Working groups will be formed based on the defined topics.



## WG-8 Marine environment

Working Group N°8: Marine environment (Leader: DCU <u>fiona.regan@dcu.ie</u> in collaboration with Marine Institute <u>Brendan.McHugh@Marine.ie</u>)

WG-8 on Marine Environment plays a pivotal role within the NORMAN network. Since 2020/2021, this group has gathered a multi-disciplinary community of experts from academia and environmental agencies, all active in the field of contaminants of emerging concern in the marine environment. Task areas now identified include: 1) Prioritisation of sea-specific contaminants for the four European Sea Regions; 2) Systematic sharing of published and proposed marine biota, water and sediment ecotoxicity threshold values for inclusion in the NORMAN Ecotoxicology Database; 3) Scoping of new activities; 4) Collaboration with OSPAR (see also OSPAR JPA) and HELCOM on monitoring, trends and effects of substances in the marine environment.

Building on the work plan initiated in the previous JPA, WG-8 activities in 2025 will focus on the following tasks, among others.

#### Task 1: WG Coordination – Meetings

It is planned to hold regular scheduled. All NORMAN members will be informed and will have the opportunity to participate. These meetings agree the WG-8 workplan, cross-cut with other relevant WGs and gather smaller groups to address each of the tasks. The WG 8 roadmap will continue to be a key focal point of WG-8. This 5-years plan now covers all relevant topics identified by the WG and links with other WGs, in particular, key aspects related to prioritisation/identification of sea-specific contaminants, mixtures risk assessment, status indicators to monitor the changes of the marine environment, optimisation of monitoring strategies (use of non-target screening, bioassays, passive sampling).

## Task 2: Prioritisation of sea-specific contaminants for European Sea Regions

Pursue efforts in respect of the prioritisation of substances identified in the North-East Atlantic and Baltic Sea under a series of tiers (using the NORMAN Prioritisation Framework as a starting basis). The work will be carried out through the following tiers: Tier 1: OSPAR CONNECT, OSPAR CONNECT2 and HELCOM PreEMPT projects; - Tier 2: LIFE APEX, UBA-HELCOM, OSPAR CONNECT, OSPAR CONNECT2 and HELCOM PreEMPT projects; - Tier 3: ICES monitoring data, LIFE APEX, UBA-HELCOM, OSPAR CONNECT, OSPAR CONNECT, OSPAR CONNECT2 and HELCOM PreEMPT projects; - Tier 3: ICES monitoring data, LIFE APEX, UBA-HELCOM, OSPAR CONNECT, OSPAR CONNECT, OSPAR CONNECT2 and HELCOM PreEMPT projects; - Tier 3: ICES monitoring data, LIFE APEX, UBA-HELCOM, OSPAR CONNECT, OSPAR CONNECT, OSPAR CONNECT2 and HELCOM PreEMPT projects.

In collaboration with WG-1 all marine samples (with a specific focus on biota and sediment matrices) will be retrospectively re-run with a list of >95,000 suspects in DSFP for presence of these substances in GC-HRMS and LC-HRMS chromatograms. The data will be uploaded in the EMPODAT SUSPECT database.

Collation of relevant projects and legacy monitoring data will continue through 2025 to enhance prioritisation processes (e.g. systematic inclusion of data on metals in this or future prioritisation schemes). Through 2025 the WG and Marine specific team will investigate adjustments of the prioritisation scheme taking into account the specificities of the marine environment. Funding was sought and is externally approved for these specific activities and will enable alignment with partner and policy reporting requirements.

Besides the classical Frequency of Appearance (FoA), Frequency of Exceedance of PNEC (FoE) and Extent of Exceedance of PNEC (EoE), alternative indicators taking into account persistence and bioaccumulation properties of the substances and new or modelled thresholds for marine mammals, early warning approaches, KEMI Hazard Score etc..) will be further explored for integration in the prioritisation framework specific to the marine environment.

#### Task 3: Data gathering

Through 2025 efforts will continue to be made to improve the population of the databases with the data relevant to the marine environment, with a specific focus on data on emerging substances in the Arctic and Mediterranean environment and on data from the ICES database into both LIFE APEX and NORMAN Database System.

#### Task 4: Ocean Decade event (timeframe in line with Joint Danube Survey 5)

WG-8 sub-committee will continue to plan an Ocean Decade event on chemicals. This should also include members of ERICs and other relevant groups. A suggested timeframe will be to kick-off in line with the Joint Danube Survey 5 organised in summer 2025 – with an event in November/December. Where feasible we will create linkages with passive sampling and ecotoxicology groups to start looking at effects and indicators of effects rather than single contaminant responses.

Task 5: Explore funding opportunities



In 2025 there will be a call for funding relevant to WG-8 in HORIZON Europe that we will work toward. A sub-group will explore this and other funding avenues in 2025. This group continues to draft a WG-8 scoping paper to be submitted in May 2025.

## **OSPAR-NORMAN** Collaboration

MIME 2024 supported the ongoing collaboration with the NORMAN WG Marine environment (WG-8) and noted the valued contribution of collaboration particularly via CONnECT 1 and 2 projects and associated assessment products. Through 2024 OSPAR (MIME) continued to foster collaborative linkages with both HELCOM and NORMAN and MIME 2024 further ratified continued exploration of these collaborative efforts. Continuation of this institutional collaboration between OSPAR and the NORMAN Association will continue to support the implementation of OSPAR's North-East Atlantic Environmental Strategy (NEAES) 2030, specifically strategic objectives (See OSPAR Commission JPA) on:

- developing a revised approach to managing the OSPAR Lists of Chemicals for Priority Action and Substances of Possible Concern (LCPA and LSPC)
- further developing and identifying marine-relevant assessment criteria for hazardous substances, including for emerging contaminants, working closely with relevant experts, particularly in the WG Chemicals under the WFD Common Implementation Strategy.

# WG-8 Monitoring, trends and effects of substances in the marine environment of the North-East Atlantic (OSPAR Commission)

Monitoring, trends and effects of substances in the marine environment of the North-East Atlantic (Leader: OSPAR Commission. contact person <u>Brendan.McHugh@Marine.ie</u>, Marine Institute)

Recent years has seen strong collaboration between OSPAR and the NORMAN Association, OSPAR's Hazardous Substances and Eutrophication Committee (HASEC) agreed to collaborate with the NORMAN Association intersessionally, to prevent pollution by hazardous substances, by eliminating their emissions, discharges and losses, to achieve levels that do not give rise to adverse effects on human health or the marine environment with the ultimate aim of achieving and maintaining concentrations in the marine environment at near background values for naturally occurring hazardous substances and close to zero for human made hazardous substances.

OSPAR's Working Group on Monitoring and on Trends and Effects of Substances in the Marine Environment (MIME) will ensure an effective collaboration with NORMAN Association linked with the following actions:

- Close out of the CONnECT 1 and 2 project initiatives on wide-scope target and suspect screening and prioritisation
- Peer review publication in 2025 of outputs from CONnECT
- Ecotoxicology and marine thresholds
- Passive sampling
- List of chemicals for priority action and its annex
- Chemicals in top predators and in polar regions
- Ongoing development of the proposed tiered approach (WG-8 Task 2)
- Participation in the marine specific prioritisation group.

## **CWG-NTS Non-target Screening Cross-Working Group Activity**

**CWG-NTS:** Cross-Working Group Activity on Non-target Screening (Activity coordinated by Eawag juliane.hollender@eawag.ch in collaboration with El <u>slobodnik@ei.sk</u>, University of Athens Nikolaos Thomaidis, <u>ntho@chem.uoa.gr</u>, LCSB - Luxembourg <u>emma.schymanski@uni.lu</u>).

The actions undertaken by the CWG-NTS have been pivotal in enhancing the performance of NTS methods, as well as the quality and reliability of NTS data. These efforts have contributed to establishing NTS techniques as an essential tool for elucidating the risks posed by chemicals to environmental ecosystems and human health. Among others, significant achievements were made in 2024 with the publication of various papers disseminating the final results of key activities initiated in previous JPAs, such as the ILS on semi-quantification methods (L. Malm et al., 2024) and the NTS studies for characterisation of European house dust contaminants (P. Haglund et al., 2024).

While preparing this JPA, our network became aware of the launch of the NEXUS activity focused on measuring the exposome. NORMAN members participated in the NEXUS survey on methods and tools for exposome characterisation and aim to actively contribute to the upcoming workshop, where the survey results will be discussed and used as a basis for designing a ring trial. The NTS-CWG will seek synergies between NORMAN and NEXUS in activities such as ring trials, methods and tools for improving exposome assessment.

The following actions will be carried out as part of the CWG-NTS Activity in 2025.



## Identification Scoring System for GC-HRMS (NILU / EI / NKUA)

The identification of emerging contaminants using high-resolution mass spectrometry (HRMS) through non-target screening (NTS) has seen significant advancements in terms of reproducibility, automation, and harmonisation, particularly for liquid chromatography (LC) systems. Meanwhile, gas chromatography (GC) -based workflows have not consistently achieved the same level of progress. Existing identification point (IP) scoring systems are tailored to LC workflows, limiting their applicability to gas chromatography methods. This gap in methodology undermines the reproducibility, automation, and harmonisation of NTS approaches across analytical platforms. Drawing insights from recent methodological advancements such as those presented in Koelmel et al. (2022), this task seeks to adapt and refine the scoring framework for GC-specific workflows, ensuring compatibility while maintaining the rigor of LC-derived standards.

The planned activities for 2025 will cover:

- Generation of a GC-EI-HRMS dataset by selected laboratories covering different types of GC-EI-HRMS instrumentation (in addition, raw data from ongoing activities, e.g. from GC-HRMS JPA 2024, will be used in 2025)
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- Adaptation of the IP scoring system currently available for LC-HRMS to integrate GC-EI-HRMS-specific parameters such as isotopic patterns, retention index, and fragment ion ratios
- Development of a framework that harmonises scoring criteria across LC- and GC-based NTS methods, emphasising automated and transparent confidence level assignments
- Validation of the updated framework through collaboration with participating laboratories and testing on real-world datasets
- Organisation of an online workshop
- Preparation of a manuscript for submission to a peer-reviewed journal detailing the harmonised scoring system and its application in multi-platform workflows. The manuscript will be published as gold open access. Manuscript preparation and publication may take place in 2026.

## On going activities related to the NORMAN Database System and MassBank Europe

- NORMAN Suspect Lists Exchange (LCSB) (see "NORMAN-SLE" above)
- NORMAN SusDat database: Database development and maintenance (EI, NKUA) (see "NDS")
- Digital Sample Freezing Platform upgrading (EI) (see "NORMAN DSFP")
- Finalisation and testing of EMPODAT SUSPECT database a new NDS module to host suspect screening results (EI) (see "NDS" and "WG-1 Task 6")
- MassBank Europe Continuous development and upgrade (LCSB, Eawag and IPB Halle) (see "MassBank").

#### Follow-up of activities started in previous JPAs:

- **CT** on Passive air sampling and wide-scope suspect/non-target screening for organic substances in air (University of Stockholm) (in collaboration with PS-CWG): A preparatory workshop and deployment of the passive PDMS foam sampler adsorbents took place in the first half of 2024. Exposed samplers were distributed to the participants in November 2024. The results of the labs are expected by March 2025. The activities will be pursued in 2025 as outlined in the JPA proposal.
- CT for the intercomparison of Ion mobility separation data (SLU): Progress of the tasks, as of December 2024: In May 2024, participant laboratories submitted their data, resulting in nearly 7,000 CCS values for 75 reference standards. During the latter half of 2024, the CT organising team held numerous meetings to discuss data processing and analysis. They curated and analyzed the received datasets and began producing the initial set of results. These activities will continue to progress as outlined in the JPA proposal. (see "Collaborative Trial for the intercomparison of Ion mobility separation data. Development of open-access CCS databases for IMS" JPA 2023)
- Intercomparison study on PS and suspect and NTS for PFASs (QAEHS / RECETOX / VU). 20 participating laboratories have submitted their data in the first half of 2024 (target and non-target analysis for PFAS chemicals in these passive sampler extracts). Analysis and interpretation of the results is ongoing. The second part of the ILS has also been initiated. Two types of PS devices have been deployed and will allow to obtain a second series of extracts planned to be distributed to participants in 2025 (this task is carried out in collaboration with WG-6 and CWG-PS)
- Machine learning-supported algorithms for assessment of the spectral quality and diagnostic information of MS2 data acquired with LC-ESI-HRMS (VU, UBA): Progress of the tasks, as of December 2024: the spectral quality prediction model has been improved. Data from MassBank, MoNA and various custom/in-house MS2 libraries as well as SIRIUS CSI:FIngerID for the prediction of molecular fingerprints, have been integrated into the model. Additionally, new predictors, specifically fragments and neutral losses derived directly from the MS2 data, have been incorporated. Based on the findings from the modeling activities, such as the selection of appropriate chemical space regions, a list of compounds has been selected. These compounds will be used in 2025 to spike samples that will be shipped to NORMAN partners for analysis. The activities will be pursued in 2025 as outlined in the JPA proposal. (see "Assessing the spectral quality and diagnostic information of MS2 data acquired with LC-ESI-HRMS")

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- (Micro)plastics leaching of additives and non-intentionally added substances (NIAS) (TNO, NKUA, EI) (see "(Micro)plastics leaching of additives and non-intentionally added substances" JPA 2024): (micro)plastic leachates for different types of polymers and for different leaching conditions were provided by TNO. Various meetings were organised to consolidate the list of substances (plastic additives from selected SLE-lists) to be used for HR-MS suspect screening. The leachate extracts were distributed to 15 participating labs, with six labs submitting their results. The data evaluation (EI) will continue in 2025, with the finalisation and publication of the study results expected by the end of 2025.
- Development of the NORMAN GC-HRMS workflows (NKUA, NILU and EI) (see "NORMAN GC-HRMS workflows" JPA 2021): Reference standards for ca. 100 GC-amendable compounds relevant for various regulatory purposes have been purchased in 2024 and distributed for analysis by different GC-HRMS techniques (NKUA, NILU, BOKU...). Their HRMS spectra will be uploaded into MassBank Europe in 2025. Development and testing of MS-DIAL-based workflow to produce GC-HR-MS spectra is ongoing. The GC functionality for DSFP was programmed and successfully tested.
- Integration of computational toxicity driver prioritisation tools to support NTS workflows in HT-EDA (EAWAG, EI) (see "Bringing together NTS and toxicity Cooperation between *DSFP* and *MLinvitroTox*, *MS2Tox* and other tools" JPA 2024): the activities started in 2024, and some preliminary results were presented at the Artificial Intelligence workshop (Leipzig, October 2024). This activity is ongoing.
- Expanding and validating the chemical space of non-target screening workflows (NKUA) (see "Expanding and validating the chemical space of non-target screening workflows" JPA 2023)). At the end of 2023 the team was working at drafting of a paper presenting the features of the models. No actions were taken for the organisation of the trial. This task is currently in stand-by.

## **CWG-PS Passive Sampling Cross-Working Group Activity**

**CWG-PS - Passive Sampling Cross-Working Group Activity** (Activity coordinated by NIVA <u>lan.Allan@niva.no</u> and INRAE <u>cecile.miege@inrae.fr</u>)

In recent years, the work of the cross-working group activity on passive sampling has been instrumental to (i) advancing the use of passive sampling in research and regulatory contexts, (ii) helping interaction between PS experts and those in charge of monitoring, (iii) evaluating the performances of laboratories in the analysis of emerging pollutants from PS extracts. In 2024, the group was able to complete, submit and publish the publication of the PS – biota paper (Passive sampling in support of biota monitoring of hydrophobic substances under the Water Framework Directive) (Allan I. J. et al., 2025) <a href="https://doi.org/10.1016/j.jhazmat.2024.136672">https://doi.org/10.1016/j.jhazmat.2024.136672</a>.) This paper was initiated as the outcome of the online workshop on how PS can contribute to biota monitoring held in December 2020. In this paper, we argue that PS can play a role as a first step of a tiered approach in compliance testing for WFD priority substances with EQS set for biota/fish. Many of the 16 co-authors are NORMAN members.

The following actions will be carried out as part of the CWG-PS Activity in 2025.

Task 1: Intercomparison study on passive sampling and NTS for PFASs (Phase II – continuation) (Leader: QAEHS <u>k.sarit@uq.edu.au</u> in collaboration with RECETOX <u>branislav.vrana@recetox.muni.cz</u>, University of South Bohemia <u>rgrabic@frov.jcu.cz</u>, NIVA <u>lan.Allan@niva.no</u> and University of Amsterdam <u>s.samanipour@uva.nl</u>)

This project was launched in 2023 with the aim to address and identify some of the limitations and opportunities with the passive sampling and broad scale non-target analysis of PFASs.

Phase I (organised as full in-kind contribution by QAEHS, UQ) involved the deployment of one type of passive sampler at a highly contaminated site in Australia along with grab sample collection. Homogenised passive sampler and grab sample extracts, along with reference standards and blanks were sent to participating laboratories for analysis in Sep/Oct 2023 and results from the labs were submitted in the first half of 2024. The analysis and interpretation of the results was under way (end of 2024).

In parallel the second phase of the project was initiated. Phase II, already approved under JPA 2024, involves the codeployment of two different passive samplers at a PFAS-contaminated surface water site in Czech Republic. The site was selected to represent a range of PFAS sources and profiles. The aim is to further examine: (i) the performance of different passive samplers for a range of PFAS; (ii) the extraction techniques used by each lab (since Phase I only examined differences in analysis and reporting); and (iii) analytical aspects, such as chromatography-mass spectrometry methods.

The JPA 2025 updated workplan includes:

- Nov 2024: Deployment of samplers
- March 2025 homogeneity test of sample extracts for approximately 10 PFAS using target analysis
- April 2025 distribution of passive samplers to participants

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- June 2025 receive data from participants.

- Results processing and evaluation expected by during Q3-Q4 2025.

Task 2: Analytical assessments of organic micropollutants in the Danube River using a combination of passive sampling, targeted analysis, non-target screening (NORMAN collaboration in Joint Danube Survey JDS5) (Leader: RECETOX, <u>branislav.vrana@recetox.muni.cz</u>)

NORMAN has already successfully demonstrated applicability of PS for chemical and effect-based monitoring of a broad range of organic chemicals and their prioritisation in the previous JDS3 and JDS4 surveys (Novák et al., 2018; Vrana et al., 2018, Šauer 2023 et. al. 2023, Beggs et al. 2023) and the PS methodology developed by NORMAN has now become an integral part of Danube surveys.

The proposed application of temporally integrative sampling approach will result in samples that provide a representative picture of pollution situation at 11 defined sites of the Danube River (selected out of the 49 JS5 sites) where other in depth investigations will be performed in parallel to PS activity. Passive samplers from water will be investigated by target chemical analyses (priority and river basin specific pollutants), suspect and non-target screening mutually agreed among project partners.

Three types of passive samplers will be applied: for hydrophobic, polar compounds, and a specific sampler for PFAS, respectively. The samplers for hydrophobics will be based on silicone materials, whereas passive samplers for polar compounds will be made from available SPE extraction adsorbents (Affinisep HLB disks). For PFAS, validated MPT samplers developed at the University of Queensland will be applied.

Samplers will be deployed for approximately 12 weeks during the period May-August 2025. Similarly to JDS4 we propose deployment of samplers at stationary sites (dams, bridge pillars, buoys). The extended deployment period will allow integrative accumulation of chemicals in passive samplers to better detectable levels. The principle of stationary PS methodology remains equal to the methodology applied in JDS4, and the results can be evaluated using usual passive sampler calibration parameters and compared to results obtained in JDS4. Samples (from polar, non-polar and PFAS passive samplers) will undergo:

- Analysis of selected target priority substances; river basin specific pollutants
- Wide-scope target analysis
- Suspect screening and NTS of extracts from passive samplers.

Objectives:

- Repeating sampling of trace organic pollutants after 6 years using the methodology successfully tested in JDS4
- Comparing data with the available 2019 baseline in the Danube, thereby continuing a temporal trend monitoring of relevant substances (incl. new priority substances – e.g. pyrethroids)
- Monitoring previously identified toxicity drivers in complex pollutant mixtures present in the Danube River
- Comparing bioaccumulative substances in passive sampler extracts from water and biota (chemometer approach)
- Monitoring contribution of large urban WWTP effluents to the water quality in the Danube.

#### Schedule:

- 1. Steering group meeting in March 2025 finalising the methodology (RECETOX).
- 2. Sampling with a battery of passive samplers installed in the JDS5 (May August 2025)
- 3. Analysis of the passive samplers in partner laboratories (2025-2026)

## Data Science for Monitoring of Emerging Environmental Substances

NORMAN has been substantially contributing to collecting data on emerging substances. This includes organising high-resolution mass spectrometry data for known contaminants into MassBank, curating and publishing suspects lists, combining bioassay data to NORMAN SusDat, collecting and processing data for substance prioritisation activities, etc. At the same time, new data science tools, often called AI, are changing the search=, application, and collection of new data.

In October 2024, in Leipzig, a NORMAN network AI workshop, "Artificial Intelligence for environmental monitoring, assessment and prioritisation of chemicals and their mixtures", took place. Among specific AI tools, future AI activities in NORMAN were discussed. One idea was to establish a working group focused on application and development of AI tools that can benefit NORMAN. Such tools are already developed by members of the NORMAN network, and the cross-working group would provide a platform for directly collecting, comparing, and combining these tools as well as developing the tools further collectively. As AI impacts data handling, modeling, and interpretations, it is relevant for all working groups in NORMAN and suggests the formation of a cross-working group.

The activities planned for 2025-2026 will focus on:



- Mapping the interests, experiences, and on-going AI based research activities of NORMAN members and identifying the areas where NORMAN has most to contribute to the research scene and the science-policy interface. The mapping will be carried out through a member survey.
- A webinar summarising the findings from the survey, mapping the most critical areas
- NORMAN summer school on using existing AI tools inside the network
- In conjunction with the summer school, we suggest carrying out the second NORMAN workshop on "Artificial Intelligence for environmental monitoring, assessment and prioritisation of chemicals and their mixtures", with a focus on the areas mapped based on the survey.

This CWG will directly link with the activities on SusDat, MassBank, DSFP as the AI tools can potentially allow even better use of the data deposited in these databases and may aid curation. The AI tools may also allow us to indicate gaps in chemical space coverage by the existing data and direct further activities acquiring new data regarding effects (bioassay endpoints, effect-directed analysis), analytical characterisation (HRMS, chromatography, IMS), as well as sampling (biota, marine environment, indoor environment).

# Blueprint for linking ecotoxicity to different levels of biodiversity damage, building on and integrating various NORMAN data streams

Blueprint for linking ecotoxicity to different levels of biodiversity damage, building on and integrating various NORMAN data streams (Activity coordinated by Environmental Institute Peter Fantke <u>peter@substitute.dk</u> in collaboration with RIVM <u>Leo.Posthuma@rivm.nl</u>, UBA <u>Gabriele.Treu@uba.de</u> and Goethe Frankfurt University Henner Hollert <u>Hollert@bio.uni-frankfurt.de</u>)

Various efforts within and outside the European Union are currently working on linking chemical ecotoxicity effects at organism level to damage at ecosystem level to provide a quantitative relationship between chemical exposure and biodiversity loss. Despite some available metrics and data, currently no operational workflow exists that can support prioritisation of chemicals based on damage on biodiversity. This is partly due to the variety of existing damage level indicators ranging from species loss to damage on ecosystem functioning, but also because currently useful data streams are rather disconnected, and modelling approaches focus on merely individual indicators and pathways. A small-scale workflow showcasing how data and models can be effectively integrated into a kind of a blueprint of how to connect chemical ecotoxicity to various levels of biodiversity damage is hence urgently needed. Such a blueprint could then serve as a sort of template for conducting larger-scale analyses for various types of regions, ecosystems and stressors, which would ultimately serve as complementary line of evidence in the prioritisation of chemicals for substitution and phase-out.

Activities and expected outcomes for 2025 / 2026:

The main goal of the proposed activity is to create an **operational «blueprint» approach for quantitatively linking chemical pollution to different levels of damage on biodiversity**. Different steps will be followed. We will start by developing a conceptual overview of how to link chemical pollution to damage on biodiversity (i.e. measurable impact expressed in terms of what we value about ecosystems) at different levels, including genetic diversity, species diversity, functional diversity and ecosystem services, and how relevant data and models can be integrated. Next, quantitative links will be created and metrics defined to translate ecotoxicity effects into biodiversity damage. Finally, an **illustrative case study will be developed** with an operational workflow defined to quantify biodiversity damage, with **recommendations how to apply this workflow more broadly**. The steps translate into specific tasks:

- Task 1: Develop a consistent impact pathway for chemicals and their impact on ecological functions and biodiversity at different levels of damage and clarify how different types of data and modelling steps can be integrated into this impact pathway framework.
- Task 2: Define a set of quantitative, mostly complementary biodiversity damage metrics that are suitable for possible implementation in policy frameworks (e.g. the Cooperate Sustainability Reporting Directive, CSRD) and discuss approaches to translate ecotoxicity impacts to different biodiversity damage levels, building on existing studies and data for species richness, genetic diversity, functional diversity and damage on ecosystem services from public databases (e.g. RIVM species monitoring data, Monarch Initiative data on generic variance, DISPERSE Functional Trait database).
- Task 3: Execute an illustrative source-to-damage quantification case study, based on good available data (e.g. building on data from SOLUTIONS project/Joint Danube Survey 4 (2019 and from the recent JPAs of WG-2 on linking EBMs, exposure and eDNA data for samples from the Environmental Specimen Bank, https://umweltprobenbank.de/en/), and discuss requirements for expanding to a wide range of chemicals, ecosystems and regions. Given the data (e.g. NORMAN Ecotoxicology database; to be extended for specific ecotoxicity threshold values for additional specific endpoints, cf. JPA 2024 WG-1 Prioritisation) and approaches already available for specific biodiversity damage modelling elements (e.g. msPAF-to-PDF translation), an initial focus will be on freshwater aquatic biodiversity. It is planned to adapt this workflow on terrestrial ecosystems, if possible, mainly via strong interaction with related EU-funded projects, such as TerraChem, in which EI, UBA and

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#### RIVM are involved.

Expected outcomes are to (a) build on and integrate various existing data streams (such as biomonitoring, target/suspect-screening and ecotoxicity effect test data available in the NORMAN Database System), (b) an aligned definition of biodiversity damage metrics ranging from damage on species richness, genetic diversity, functional diversity and ecosystem services, (c) recommendations for how to scale up the proposed workflow and how to integrate the proposed metrics for chemical prioritisation efforts, and (d) a quantitative link between ecotoxicity data and biodiversity damage as new line of evidence for chemicals management.

## Integrating persistence testing and assessment

How to integrate persistence testing and assessment within the NORMAN network (Activity coordinated by Technical University of Denmark Philipp Mayer philm@dtu.dk)

Persistence is becoming increasingly important within the environmental risk assessments and management of chemicals. Persistent chemicals (1) can remain in the environment for extended periods of times, (2) can be prone to long range transport, (3) have time to distribute and contaminate other media including groundwater if mobile and (4) are notoriously difficult to clean-up. Persistence is thus one of the most important criteria to prioritise organic pollutants as well as identify hazardous chemicals of emerging concern.

Biodegradation and persistence are traditionally assessed in standardised single substance tests. However, several NORMAN partners are conducting biodegradation and persistence research where multiple chemicals are tested simultaneously. Birch et al conducted for instance biodegradation tests of around 50 hydrocarbons covering 5 and 9 orders of magnitude in Kow and Kaw, and determined persistence based on peak area ratios between biotic and abiotic test systems (Birch et al, 2018). This research has since been extended to a much wider range of chemicals (Birch et al, 2023) and very recently also to complex discharge samples (Møller, 2024). Several NORMAN partners are testing multiple more polar chemicals and then using chemical specific analysis to determine biodegradation kinetics, persistence and even transformation products. We foresee significant new research opportunities in the cross-field between persistence research and non-targeted analysis.

The aim for 2025 is to start discussions so that we at the next general assembly can present concrete ideas and plans for the next years. Within 2025 we envisage:

- Formation of a small core-group by March 2025, and then exploring interest within NORMAN partners to join, participate & contribute (larger interest group) by June 2025.
- Discussion items within prioritising groups: How can this new type of persistence testing inform the activities of the NORMAN prioritisation group? How can the NORMAN prioritisation group help during the substance selection within upcoming biodegradation testing and research of chemicals?
- Discussion items within non-target screening group: Are some of the strong analytical groups within NORMAN interested to engage in coupling persistence testing of complex mixtures with suspect and non-targeted screening? Can the new type of persistence testing help prioritising the suspect screening and non-targeted screening within NORMAN, by focusing on the most persistent chemicals in the chromatograms.
- Discussions on how to integrate future persistence research activities within the current NORMAN structure, facilitate collaboration between NORMAN partners and support future collaborative proposals.

This NORMAN activity is envisaged to be complementary to the activities of the SETAC persistence group. The strongest representation in the SETAC persistence group is industry and contract research institutes serving industry. We envisage that this NORMAN group will have a much stronger representation from Universities, National Research Institutes and National Environmental Protection Agencies. The SETAC persistence group has a focus on the testing of chemicals, whereas the NORMAN Network has a focus on prioritising, monitoring and assessing pollutants of emerging concern.

## NORMAN – ICPDR Joint Danube Survey 5 (JDS5)

NORMAN - ICPDR Joint Danube Survey (JDS5) (Activity coordinated by Environmental Institute slobodnik@ei.sk)

The International Commission for the Protection of the Danube River (ICPDR; 14 European countries and EU) organises large-scale Joint Danube Surveys (JDS) each six years since 2001. Preparation, implementation and outcomes of the surveys are subject to approval by Water Directors of all involved countries and EC DG Environment. NORMAN Association has contributed with a range of scientific activities to JDS3 (2013), in close cooperation with the EU SOLUTIONS project, and JDS4 (2019). More than 140 organisations and laboratories, including those from the NORMAN network, participated in the JDS4. Majority of the data obtained with the state-of-the-art chemical and biological monitoring and analytical methodologies fed back to NORMAN and allowed, *i.a.*, for prioritisation of the

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Danube River Basin Specific Pollutants (RBSPs) using NORMAN prioritisation tools and establishment of the baseline of pollution by microplastics, antimicrobial resistance (AMR) bacteria/genes and Rare Earth Elements (REE) at the basin scale. A regulatory concept of using Effect-based Trigger (EBT) values for a battery of bioassays applied for analysis of effluent wastewaters has been successfully tested.

The key objectives of JDS5 are:

- Producing comparable information on selected water quality elements for the Danube River including the major tributaries on a short-term basis
- Providing an opportunity for a basin-wide harmonisation and training in WFD-related monitoring
- The outcomes of the JDS5 should cover the information gaps as necessary for the 2027 update of the Danube River Basin Management Plan
- Public awareness-raising for a healthier and cleaner Danube.

The activities of NORMAN members in JDS5 aim (not exhaustively) at:

- Verification of use of alternative methods for pollutant analysis with the view of potential reducing WFD monitoring costs in future. This can include e.g., screening methods, effect-based monitoring tools, the use of passive sampling or biomarkers
- Investigation of the potential for introducing the proposed new holistic approach for the regulation of chemicals in the aquatic environment under the WFD, including those regulated by the (updated) UWWTD
- Analysis of updated list of WFD priority substances, Watch list 4/5 substances and RBSPs proposed in JDS4 (lead NKUA)
- Update of the list of RBSPs for the Danube River Basin using updated NORMAN Prioritisation Framework (lead INERIS)
- Analysis of all parameters required by updated UWWTD including micropollutants, microplastics, ecotoxicological effects and AMR (lead UFZ).

The overall goal is to demonstrate the practicality of use of the new analytical techniques in the regulatory framework. JDS5 offers NORMAN experts an excellent opportunity for testing different analytical and monitoring tools on a large transboundary river basin. Also, time-trends of pollution can be followed regarding results from previous surveys and the data can feed various models requiring corresponding datasets on chemical pollution, nutrient pollution, hydrology, physico-chemical parameters, temperature profiles, BQEs, biodiversity (eDNA), adverse ecotoxicological effects etc.

### Description of the proposed activity:

Main bulk of JDS5 activities is planned to be carried out in June – August 2025, however some of the samples will be collected in monthly interval already since April 2025 (e.g. phytoplankton). Most of the water, biota (fish and molluscs) and sediment samples will be taken by the JDS5 National Teams at 49 sites and submitted for a full-scale biological (WFD Biological Quality Elements and zooplankton), chemical (WFD priority substances, Watch list 4 and 5 substances, RBSPs, etc.), radiological and hydromorphological assessment. Samples requiring special treatment in field (e.g. large volume solid-phase extraction; microbiology/AMR, specific eDNA/eRNA water samples etc.) will be collected by six Longitudinal Survey teams. Analyses of microplastics in suspended particulate matter (SPM) and river water (fractionated filtration) will be performed by UBA Germany and Eurofins Hungary repeatedly (3x) in samples from five sites.

Samples will undergo, *i.a.*, a thorough eDNA screening for holistic analysis of aquatic biodiversity, including all BQEs, microbiology, and antibiotic resistance genes/bacteria (ARGs/ARBs) analyses. Additionally, samples from:

- 10 WWTPs (10 countries; influents/effluents; 7-days composite samples; Combined Sewage Overflow (CSO samples) and possibly sewage sludge samples)
- 7 ground water sites used for production of drinking water (impacted by the Danube River water via bank filtration)
- 10 (batteries of) passive samplers will be included. Groundwater samples will also be screened for eDNA and the wells will be sampled for presence of groundwater fauna.

Altogether 49 river water, 98 biota (49 fish and 49 molluscs/crustaceans; freeze dried), 7 groundwater and 10 wastewater influent/effluent samples will be available for analyses by NORMAN members in case of interest. Additionally, 15 SPM samples (from microplastics studies) and 49 sediments (<63 um fraction; from radiology studies) could be provided. Tissues from benthic invertebrates from 25 sites used for microplastics studies might be provided as well. Extracts for GC- and LC-HRMS analyses from 10 passive samplers will be available.

NORMAN members interested in analysis of these extracts by in vitro bioassays are welcome to express their interest by the end of January 2025.

As planned in the JPA 2024, NORMAN – ICPDR JDS5 Coordination Workshop involving experts from the ICPDR and NORMAN network took place in Bratislava on 23-24 September 2024. Areas of common interest were identified and

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translated into several NORMAN JPA 2025 proposals or Standard Operational Procedures (SOPs) collected by the ICPDR. A brief overview of the proposals/SOPs received so far is provided here.

### A preliminary distribution of analyses is as follow:

Task 1: All surface water, groundwater, SPM, whole fish, molluscs/crustaceans, benthic invertebrates (lyophilised) samples subjected to wide-scope target (>2,500 substances, including WFD PS, WL 4/5 and RBSPs) and suspect screening (>95,000 substances) using LC-HRMS, GC-APCI-HRMS and GC-EI-MS/MS by NKUA/EI. The wastewater influents/effluents, CSO and sewage sludge samples subjected only to suspect screening (see Task 13 below).

Task 2: Analysis of surface water, groundwater and wastewater effluent samples by novel GC-(simultaneous) EI/CI-HRMS by BOKU.

Task 3: Analysis of surface water, groundwater and wastewater influent/effluent samples by direct injection LC-HRMS (NLZOH, Slovenia).

Task 4: Collaborative Trial for suspect screening approaches using wastewater effluent sample (lead EI; preliminary participants UFZ, LfU, NKUA, UBA Austria, BOKU, DTU, NLZOH...).

Task 5: Analysis of PFAS compounds in fish samples by Top Assay and LC-HRMS by UBA Germany (10 samples).

Task 6: Analysis of 118 pharmaceuticals in surface water and groundwater samples by University of Pannonia (UoP), Hungary.

Task 7: Analysis of 19 tire-derived compounds in surface water and wastewater effluents by University of Vienna (UoV) in close cooperation with UFZ.

Task 8: Analysis of 21 PMT substances in surface water and groundwater samples using IC-HRMS by LW Langenau (20 samples). Possibly including suspect/non-target screening in 2025.

Task 9: Analysis of metals and REE in surface water and ground water by BOKU. Determination of metals in both the truly dissolved phase and unfiltered samples.

Task 10: Analysis of REE in surface water, groundwater, wastewater effluents, molluscs and possibly drinking water by University of Lorraine (UoL).

Task 11: Analysis of illicit drugs in surface water by WRI Prague (9 samples).

Task 12: Analysis of isotopes of H, O, N, P and C in surface water samples by BOKU.

Task 13: Wide-scope target, suspect and non-target screening in wastewater influents and effluents by LC- and GC-HRMS; analysis of PMT and PFAS compounds by SFC-HRMS; analysis of pharmaceuticals and plastic additives by UFZ (cf. specific JPA proposal).

Task 14: Biological analysis of wastewater effluents by a battery of bioassays:

Algae (S. vacuolatus), Acute daphnia, Neurite outgrowth, MitoOxTox, CAFLUX AhR, CALUX assays (ER, AR, anti-ER, anti-AR, PFAS, GR, PR) and metaproteome by UFZ and Goethe-University Frankfurt (cf. specific JPA proposal).

Task 15: Analysis of surface water samples by a battery of in vivo and in vitro bioassays for genotoxicity and embryotoxicity by IBISS Belgrade and University of Ljubljana (cf. specific JPA proposal).

Task 16: Passive sampling of surface water at 10 sites (identical to those from JDS4) by RECETOX. Three types of PS to be used (hydrophobic, polar, PFAS).

Task 17: Passive sampling of surface water at 10 sites using novel passive sampling device (UFZ; cf. specific JPA proposal).

Task 18: Compilation of all target and suspect screening data and prioritisation of RBSPs in surface water, biota and groundwater matrices; lead by INERIS, France (cf. also JPA 2025 WG-1 proposal).

There is an on-going discussion on the involvement of EC JRC in JDS5. Preliminarily, an interest has been expressed in the analysis of wastewater effluents for microbiology/AMR parameters and two bioassays; collection of large volume surface water samples by Mariani box and follow up analysis of selected WFD PS, Watch list substances and RBSPs (to be specified). Additionally, there is a preliminary offer to use EC JRC floating litter app by the National teams in the DRB.

Each of the above activities is being discussed between EI and responsible partners to avoid overlaps and increase synergies.

There is still a possibility to come with additional in-kind proposals/requests for samples by the end of January 2025.



The samples would be made available by EI to any interested NORMAN partner.

Expected outcomes for 2025:

All data obtained from analyses of JDS5 samples will be archived in the NORMAN Database System ready for further evaluation in 2026 together with a design of plan of publications (Workshop NORMAN – ICPDR 2026).

Added value / Link with other NORMAN activities and / or other projects

Proof-of-concept application of the new analytical techniques (e.g., wide-scope target and suspect screening, passive sampling, AMR, microplastics) in the regulatory framework with focus on the WFD, updated EQSD and updated UWWTD.

Target and wide-scope target analyses data uploaded in the NORMAN Database System (EMPODAT).

NTS data uploaded into DSFP; extracted suspect screening data into EMPODAT-SUSPECT.

NTS data obtained within the Collaborative Trial with wastewater sample by multiple LC-HRMS and GC-HRMS techniques uploaded in DSFP for critical comparison.

Bioassays monitoring data uploaded in the NORMAN Database System.

Passive sampling data uploaded in the NORMAN Database System.

Validation of the NTS-based prioritisation framework at the river basin scale (for details, see a separate JPA WG-1 proposal).

## Joint Danube Survey 5 (JDS5): The WWTP project - An integrated assessment

Joint Danube Survey (JDS) 5: The WWTP project - An integrated assessment (Activity coordinated by UFZ charlotte.henkel@ufz.de)

Increasing chemical pollution is a threat to sustainable water resources worldwide. Waste water treatment plants (WWTPs) are often suspected to add to this chemical burden. This is because increasing complexity of chemical pollution mixtures entering wastewater treatment plants pose challenges to common treatment technologies. As a consequence, effluents containing an unknown number of various contaminants might impact surface water bodies, with unknown effects for aquatic organisms and, ultimately, for humans. Elucidating the chemical complexity of contaminants in wastewater (both influents and effluents) is required to understand the contribution of wastewater treatment plants to chemical pollution of the aquatic environments and to determine the associated risks.

The Danube River is the second largest river in Europe and an important water resource for many European countries. However, the fact that many large cities and wastewater treatment plants are located in the (catchment) area of the Danube River, makes it susceptible to the entry of a large number of domestic and industrial pollutants. The Joint Danube Survey (JDS)3, JDS4, and many other studies have assessed different types of stressors in wastewater individually, but a comprehensive characterisation and harmonised evaluation of mixtures of all types of chemicals emitted via WWTP effluents to the Danube catchment are missing. The proposed JPA aims at narrowing this knowledge gap by integrating the analysis of a broad range of organic and inorganic contaminants including metals, rare earth elements, hydrophilic and hydrophobic organic micropollutants, micro- and nanoplastics assessed for example through chemical analysis, bioassays and antibiotic resistance genes.

In the framework of the Joint Danube Survey 5, wastewater influent, effluent, sludge and combined sewer overflow samples will be taken in ten wastewater treatment plants in the Danube catchment. In this JPA, the samples will be analyzed by international experts using complementary tools, combining chemical analysis and bioassays, with the overarching goal being a comprehensive integrated assessment.

Description of the proposed activity and expected outcomes for 2025:

#### Activity 1: Scientific coordination of the project

An important part of this JPA is to bring together experts from different disciplines, aligning the analyses and ensuring a complementary and holistic assessment of the wastewater samples. This includes the compilation of data and evaluation of results from different analyses in a comprehensive and integrated way. Results will be presented to the scientific community and public, and published in internationally recognised journals. Further, this project is committed to giving advice to policy makers.  $\rightarrow$  UFZ

Activity 2: Sample preparation and distribution for chemical analyses and bioassays



From the daily taken influent and effluent samples, 7-day composite samples will be prepared. These will be filtered and extracted using solid phase extraction (SPE). Extracts will be concentrated under N2 steam and reconstituted in methanol. These extracts (enrichment factor 1000) will be the basis for the chemical analyses and bioassays enabling the knowledge transfer between the different analyses.  $\rightarrow$  UFZ

## Activity 3: Target, suspect and non-target screening of organic substances

Analysis of a broad range of target compounds (~580 chemicals) including pesticides, industrial chemicals, polymer additives, surfactants and pharmaceuticals using high-performance liquid chromatography (HPLC) coupled to high-resolution mass spectrometry (HRMS). Results will be supported by findings from suspect and non-target screening.  $\rightarrow$  UFZ

## Activity 4: Target analysis of the samples with a focus on selected WWTP contaminants of particular concern

- ~ 150 persistent, mobile (PM) and toxic (Ts) compounds and PFAS (including ultra short chain PFAS) will be analysed using super critical fluid chromatography (SFC)- HRMS and LC-HRMS → UFZ
- Tire-derived compounds will be analysed using LC-MS/MS  $\rightarrow$  University of Vienna
- Priority substances  $\rightarrow$  UFZ
- Drugs of abuse  $\rightarrow$  IDAEA-CSIC Barcelona

## Activity 5: Analysis of micro-and nanoplastics

- nanoplastics → UFZ
- microplastics  $\rightarrow$  tbc

## Activity 6: Analysis of inorganic contaminants

- Rare Earth Elements → University of Lorraine
- Metals → University of Vienna

## Activity 7: Battery of bioassays

Besides the chemical analysis of the influent and effluent samples, a batterie of bioassays will be conducted targeting a range of specific endpoints, including endocrine, dioxin-like and neurotoxic effects. Findings from the chemical analysis will complement findings from the bioassays and help to explain mixture effects and identify potential drivers of the effects observed in the bioassays.

- Neurite Outgrowth inhibition in SH-SY5Y cells → to test developmental neurotoxicity
- Multiplex Assay MitoOxTox using AREc32 cells → to quantify cytotoxicity, oxidative stress response and mitochondrial toxicity using the reporter cell line AREc32
- CAFLUX AhR using H4G1.1c2 cells  $\rightarrow$  for screening of dioxin-like compounds
- Algae test with S. vacuolatus → to assess chemicals affecting photosynthesis activity → UFZ
- CALUX bioassay battery using U2OS cells → to assess the activation and inhibition of (anti-) estrogens, (anti-) androgens, progestins and glucocorticoids. The PFAS-Calux assay will be conducted, which is a TRR binding test in combination with the TRβ CALUX assay. → Goethe-University Frankfurt

## Activity 8: Metaproteomics to explore the microbial community in sewage sludge

Proteins extracted from sewage sludge are enzymatically digested into peptides and then analyzed with nano-liquid chromatography coupled to a mass spectrometer (MS). Results will be used to better understand biological processes in wastewater treatment plants including the degradation of pollutants.  $\rightarrow$  UFZ

#### Activity 9: Target analysis of more hydrophobic compounds using passive samplers

In addition to the chemical analysis of the water samples, the WWTP effluents will be analysed using passive samplers.  $\rightarrow$  RECETOX, UFZ (tbc)

#### Added value / Link with other NORMAN activities and / or other projects

An integrated assessment of wastewater influents and effluents is highly needed in order to determine the impact of WWTPs on the pollution of the Danube River with the overarching goal to contribute to maintain the Danube River a safe and clean water resource in the future. The results from this integrative JPA can help to guide the monitoring and prioritisation of water pollution, and identify and improve strategies for removal. Thereby this JPA serves as a best practice analysis for the implementation of the EU Urban Wastewater Directive (UWWTD).



Regarding other NORMAN activities, results from chemical analyses and bioassays of the wastewater influent and effluent samples conducted within this study can be used to support findings from other JDS5 activities such as the analysis of the surface water and bank filtrate along the Danube River and provide insights on possible entry pathways of contaminants. In addition, findings from the passive sampler project in JDS-5 conducted near selected wastewater treatment plants will provide valuable time-integrated assessments of chemicals which might be below the detection limit and thus overlooked by the analysis conducted in the WWTP study. The here presented JPA is strongly connected to other projects within JDS5 and contributes to a holistic assessment of the Danube River.

# Interlaboratory studies (ILS) on trifluoroacetic acid, benzotriazoles and artificial sweeteners

Interlaboratory studies on trifluoroacetic acid, benzotriazoles and artificial sweeteners (Leader: IWW, Gerhard Schertzinger <u>g.schertzinger@iww-online.de</u>)

There are increasing reports about the occurrence of these substances in the aqueous environment: in surface water, groundwater and for some of them even in drinking water.

Reliable analytical methods are needed to better assess the current situation and to investigate the effectiveness of several measures (such as advanced wastewater treatment) to reduce emission of these substances into surface waters.

However, for these substance groups there are no European or internationally harmonised or standardised analytical methods available so far, and a thorough assessment of the suitability of different analytical methods used is still lacking.

Together with AQS BW, IWW Water Centre will organise interlaboratory studies on these compounds in drinking water.

- The ILS on trifluoroacetic acid (TFA) will be carried out during the 2nd quarter 2025
- The ILS on benzotriazoles and artificial sweeteners is scheduled for autumn 2025. Parameters will be: 1Hbenzotriazole, 4-methyl-1H-benzotriazole, 5-methyl-1H-benzotriazole, acesulfam-K, cyclamate, saccharin, and sucralose.

More technical details and the dispatch dates can be found at www.iswa.uni-stuttgart.de/ch/aqs/index.en.html

The studies will combine proficiency testing of laboratories and evaluation of the suitability of methods used (V3 level).

As a result of this activity a comprehensive report on the outcome of the interlaboratory studies will be delivered with conclusions on:

- the proficiency levels or European analytical laboratories;
- the suitability of analytical methods for analysis of these two compound classes in water samples.

Dissemination of information about the ILS (announcement/invitation, registration form etc.) will be done through the NORMAN website.

Further technical details, registration deadline and the shipment dates can be found at <a href="https://www.iswa.uni-stuttgart.de/institute/central-services/lflaqs/aqs/pt/">https://www.iswa.uni-stuttgart.de/institute/central-services/lflaqs/aqs/pt/</a>

The proposed budget for this JPA may be revised by the Steering Committee in May 2025. All approved scientific activities will be implemented, independently of the revision of the budget.