

Proposals for NORMAN Joint Programme of Activities 2025

Title	NORMAN – ICPDR Joint Danube Survey 5 (JDS5)
Type of activity	Field study
Leader	EI, ICPDR, UFZ
Topic / activities	<p>Background / Justification for the proposed activity:</p> <p>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>i.a.</i>, for prioritisation of the 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.</p> <p>The key objectives of JDS5 are:</p> <ul style="list-style-type: none"> • 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. <p>The activities of NORMAN members in JDS5 aim (not exhaustively) at:</p> <ul style="list-style-type: none"> • 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). <p>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.</p> <p>Description of the proposed activity:</p> <p>Main bulk of JDS5 activities is planned to be carried out in July – 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.</p> <p>Samples will undergo, <i>i.a.</i>, 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:</p> <ul style="list-style-type: none"> • 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 µm 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 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).

	<p>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).</p> <p>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.</p> <p>Each of the above activities is being discussed between EI and responsible partners to avoid overlaps and increase synergies.</p> <p>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.</p> <p>Expected outcomes for 2025:</p> <p>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).</p> <p>Added value / Link with other NORMAN activities and / or other projects</p> <p>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.</p> <p>Target and wide-scope target analyses data uploaded in the NORMAN Database System (EMPODAT).</p> <p>NTS data uploaded into DSFP; extracted suspect screening data into EMPODAT-SUSPECT.</p> <p>NTS data obtained within the Collaborative Trial with wastewater sample by multiple LC-HRMS and GC-HRMS techniques uploaded in DSFP for critical comparison.</p> <p>Bioassays monitoring data uploaded in the NORMAN Database System.</p> <p>Passive sampling data uploaded in the NORMAN Database System.</p> <p>Validation of the NTS-based prioritisation framework at the river basin scale (<i>for details, see a separate JPA WG-1 proposal</i>).</p>
Participants	<p>EI – overall coordination</p> <p>NORMAN partners – UFZ, INERIS, NKUA, BOKU, RECETOX, UoF, LfU, WRI Prague, UoL, UBA Austria, UBA Germany, DTU...</p> <p>ICPDR partners (not exhaustive) – MU Vienna, MU Graz, UoV, LW Langenau, IBISS, University of Ljubljana, NZOH, Eurofins Hungary...</p>
Proposed in-kind contribution	<p>It is estimated that an overall market value of all activities in this proposal is several hundreds of thousands of EUR. Each involved partner proposed a generous in-kind contribution.</p> <p>Additional in-kind contributions by other NORMAN partners are very much welcome.</p>
Contribution needed from NORMAN Association ¹	<p>Similar to JDS4, ICPDR will cover all expenses related to the collection of samples, their distribution to interested laboratories, related consumables/materials both in the field and in the laboratory. The distribution of the ICPDR contribution to individual JDS5 NORMAN partners will be decided by the NORMAN Steering Committee in February 2025. It is expected that no contribution will be needed from NORMAN Association to support JDS5 in 2025.</p>

¹ Please, provide here a transparent justification of the requested resources and of the in-kind contribution, thereby distinguishing between the costs associated with “person-months” for the organisation, the “travelling costs” for invited speakers and the costs for the logistics (e.g. meals, room rental etc.)