Proposals for NORMAN Joint Programme of Activities 2025

Title	**Integration of computational toxicity driver prioritization tools to support non-target screening workflows in HT-EDA – part II: Designing endpoint-specific fractionation methods based on predicted toxicity.**
Type of activity	Pilot study
Leaders	UFZ (Iker Alvarez Mora) / VU (Frederic Béen)
Topic / activities	***Background / Justification for the proposed activity:*** As demonstrated in the previous activities of WG3, toxicity prediction tools as MLinvitroTox represent a significant advancement towards HT-EDA. Using these tools, HRMS analysis of a sample provides a preliminary insight into the distribution of potentially active compounds within our 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. With this JPA, our objective is to test MLinvitroTox to obtain preliminary information that can guide the design of tailored, endpoint-specific chromatographic conditions for fractionation in HT-EDA. ***Description of the proposed activity and expected outcomes for 2025:*** *Activity 1:* Identify the endpoints of interest (primarily those being tested within the ongoing PARC 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, which will also include MS/MS diagnostic information prediction (Codrean et al., 2023) 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. *Expected outcomes:* 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. ***Added value / Link with other NORMAN activities and / or other projects*** This activity builds upon the efforts initiated with the previous 2024 JPA to develop efficient workflows that elevate the identification of effect drivers in complex mixtures through HT-EDA 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 new agreement between NORMAN and PARC, this activity will complement an ongoing project in PARC with new ideas emerging from NORMAN, fostering mutual benefit.
Participants	UFZ, VU, UPV/EHU, INERIS, more to be confirmed…
Proposed in-kind contribution	All practical work for chemical analysis will be done as part of the PARC Task 4.3. E01. Support is needed to cover the consumables for fractionation experiments and bioassays, and sample shipment for the Activity 3.
Contribution needed from NORMAN Association[1]	Total: 8000 € (3000 € for sample preparation and logistics, 5000 € for bioassay costs)

[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.)