Cost-effective and effect-based evaluation of the wastewater quality using a battery of *in vitro* bioassays

Kelsey Ng [1,2*], Nikiforos Alygizakis [1,3], Niki Maragou [3], Sylvana Alirai [3], Peter Behnisch [4], Harrie Besselink [4], Peter Oswald [1], Ľuboš Čirka [1,5], Nikolaos S. Thomaidis [3] and Jaroslav Slobodnik [1]

[1] Environmental Institute, Okružná 784/42, 97241 Koš, Slovakia

[2] Faculty of Science, Masaryk University, RECETOX, Kamenice 753/5, Building D29, 62500 Brno, Czech Republic

[3] Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, 15771 Athens, Greece

[4] BioDetection Systems b.v., Science Park 406, 1098 XH Amsterdam, The Netherlands

[5] Faculty of Chemical and Food Technology, Institute of Information Engineering, Automation, and Mathematics, Slovak University of Technology (STU), Radlinského 9, 81237 Bratislava, Slovakia

***Presenting author – contact: ng@ei.sk / 527416@muni.cz**

Background

Effluent wastewater is a major point source for contaminants of emerging concern (CECs) in surface water in regions including the Danube River Basin (DRB). Chemical screening is the conventional monitoring approach for wastewater effluents, which provides holistic chemical profiles of the wastewater. However, a highly standardized laboratory with advanced analytical instruments is required for such analysis. Bioassays with reference to effect-based trigger values (EBTs) could assess the mixture toxicity effect of wastewater. It is a complementary analytical tool for evaluating wastewater quality, which acts as an effect-based and cost-effective early warning system for wastewater management. This is a feasibility study of such approach on wastewater treatment plant (WWTP) effluents collected in 11 DRB countries.

Objectives

- **To evaluate: mixture toxicity effects of effluent wastewater of 11 DRB countries collected in the Joint Danube Survey 4**
- **To propose: action plans at WWTP operator level for prioritized cases of effect-based risk assessment**
- **To provide: future outlook for the application of effect-based bioanalytical monitoring for wastewater management**

Effect-based assessment with bioassays

Seven CALUX® bioassays (incorporated with firefly luciferase gene) previously proposed by the NORMAN Association and Water Europe were applied to effluent wastewater samples which underwent optimized solid-phase extraction. Extent of exceedance (EoE) to EBTs was established based on the measured signals and bioanalytical equivalents of reference compounds of the assay. Based on the EoE, risk assessment was performed and action plans were proposed.

Table 1. Performance indicator bioassays and their effect-based trigger values

Activity	Estrogenic (ER_α)	Antiandrogenic (anti-AR)	Glucocorticoid (GR)	PPARγ receptor ($PPAR_\gamma$)	PAH activity (PAH)	Oxidative stress (Nrf_2)	Pregnane X receptor (PXR)
EBT value (bioanalytical equivalents)	0.1 ng 17ß-Estradiol-eq/L	14 µg Flutamide-eq/L	100 ng Dexamethasone-eq/L	10 ng Rosiglitazone-eq/L	6.2 ng B[a]P-eq/L	10 µg Curcumine-eq/L	3 µg Nicardipine-eq/L

Effect-based risk assessment of DRB wastewater

Table 2. Extent of exceedance (EoE) of EBTs based on the *in vitro* bioassays

Effluent Wastewater Sampling Site	PAH	ER_α	Nrf_2	PXR	Anti-AR	$PPAR_\gamma$	GR
Asten, AT	17.7	13.0	6.8	<LOD	1.6	<LOD	0.4
Vratsa, BG	11.0	29.0	<LOD	<LOD	1.9	<LOD	<LOD
Hodonín, CZ	14.8	24.0	<LOD	85.7	1.6	<LOD	<LOD
Donauwörth, DE	5000	15.0	29.0	41.7	2.2	63.0	1.2
Županja, HR	14.2	15.0	18.0	49.3	<LOD	<LOD	<LOD
Győr, HU	13.9	8.5	16.0	23.0	0.9	82.0	<LOD
Giurgiu, RO	5.6	22.0	3.9	25.0	<LOD	<LOD	0.6
Šabac, RS	11.8	16.0	2.4	34.3	0.8	<LOD	<LOD
Novo Mesto, SI	9.0	2.5	2.9	21.0	<LOD	<LOD	<LOD
Bratislava, SK	27.4	6.2	3.1	28.7	<LOD	<LOD	<LOD
Uzhgorod, UA	16.1	19.0	3.3	22.7	0.8	<LOD	<LOD
Samples with EoE >1	11	11	9	9	4	2	1
Mean EoE value	467	15	8	30	1	13	0.2
Standard deviation	1503	8	9	24	1	30	NA

Proposed action plans for WWTP operators

Table 3. Proposed actions in case of exceedance of EBTs.

Extent of exceedance	Mitigation Plans Proposed for WWTP Operators
Below 1	-No further action
Between 1 and 3	-Perform quality check of data; -Monitor every 3 months for 1 year until EoE is below 1.
Between 3 and 10	-All actions of the category above; -Resample and reanalyze immediately to confirm EBT exceedance; -Quantify toxicity drivers.
Between 10 and 100	-All actions of the category above; -Enhance program for source identification; -Monitor the distribution system closer to the point of exposure to confirm attenuation of CECs and to confirm the magnitude of assumed safety factors associated with removal efficiency, dilution, and post treatment.
More than 100	-All actions of the category above; -Consult the local environmental authorities immediately to determine the required response action; -Confirm plant corrective actions through additional monitoring to establish an EoE at least below 100.

Future outlook for wider application

Regular application – complementary to chemical analysis

- Bridge chemical data to effect data to identify toxicity driver
- Establish relative effect potency values of the detected CECs

Evaluation of removal efficiency at WWTPs

- Measure toxicity endpoints at WWTP effluents and sludge

Inclusion of *in vivo* bioassays

- Include *in vitro* and *in vivo* assays to target more endpoints
- Address wisely selected apical effects

Discussion & Conclusion

- **Feasibility study** on wastewater effluents collected in 11 DRB countries demonstrated the applicability of the proposed battery of 7 *in vitro* bioassays for **effect-based assessment for wastewater management**.
- Risk assessment revealed that CECs causing **PAH activity, xenobiotic metabolism, estrogenicity and oxidative stress** are present in WWTP effluents in the DRB, with highest EoE observed for PAH activity.
- A regular application (once every 6 months) of the present battery on WWTP effluent is recommended as a time- and cost-effective **complement to chemical analysis**, which provides a **'safety net' for the aquatic ecosystems**.

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Reference: Alygizakis, N., Ng, K., Maragou, N., et al., *Battery of In Vitro Bioassays: A Case Study for the Cost-Effective and Effect-Based Evaluation of Wastewater Effluent Quality.* Water, 2023. **15**(4).