Refining wastewater-based epidemiology for cannabis consumption monitoring: relevance of analysing both the aqueous phase and suspended solids of influent wastewater

Natan Van Wichelen¹, Marina Celia Campos-Mañas², Claudia Simarro-Gimeno³, Adrian Covaci⁴, Alexander L.N. van Nuijs¹, Christoph Ort⁵, Sara Castiglioni⁶, Frederic Béen⁷, Félix Hernández⁸, Lubertus Bijlsma²

¹Toxicological Centre, University of Antwerp, Antwerp, Belgium ²Research Institute for Pesticides and Water Quality (IUPA), University of Antwerp, Antwerp, Belgium ³State Federal Institute of Aquatic Science and Technology (Bogor), Bogor, Indonesia ⁴Institute of Forensic Pathology, Marburg, Germany ⁵IRCCS, Department of Environmental Health Sciences, Milan, Italy ⁶Eawag Research Institute for Water Science, Dübendorf, Switzerland ⁷Research Centre for Environment, Health and Sustainability, Utrecht, The Netherlands ⁸Generalitat Valenciana, Spain

Wastewater-based epidemiology & Cannabis use

- **Wastewater-based epidemiology (WBE)** employs the analysis of human metabolic excretion products of xenobiotics in influent wastewater (IWW) with the aim of estimating their community use.
- **Cannabis** is worldwide the most commonly consumed illicit drug.
- **Δ9-tetrahydrocannabinol** (THC) is the major psychoactive ingredient of cannabis, further metabolised (microsomal hydroxylation) to 11-hydroxy-Δ9-tetrahydrocannabinol (THC-OH) and its main human metabolite 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH).
- Careful monitoring of THC use is necessary to detect changes in consumption patterns and to understand shifts in the drug markets.

**THC-COOH detection** in the liquid phase of IWW in the context of WBE is complex as no problems were faced with other illegal substances (e.g., cocaine) and with THC-COOH detection in methanol (interlab study, SCORE, 2018).

- Relatively high lipophilicity of cannabis biomarkers ➔ Consider partition between liquid phase and suspended solids (SS) in IWW?

**Objective**: Improve the estimation of cannabis use through WBE

**Methods**

- Conventional method to measure cannabis biomarkers: 
  **solid-phase extraction (SPE)**
  - Filtration is necessary ➔ suspended solids are not considered.
  - By applying liquid-liquid extraction (LLE), both liquid and suspended solids are considered.
  - By applying solid-liquid extraction (SLE), only the suspended solids are considered.

- Seven daily IWW samples (24-h composite, time proportional with a time interval of 10 min) were collected from wastewater treatment plant (WWTP) of Castellon, Spain.

- The different validated methods were applied to different fractions of the IWW samples, as illustrated in Figures 1 and 2.

- UPLC-MS/MS sample analysis was performed with a Xevo TQD (Waters) for THC, THC-OH and THC-COOH.

**Results and Discussion**

- **Liquid phase only**: no substantial difference between SPE (recovers ≤ 97%) and LLE (recovers ≤ 97%) for THC-COOH analysis.
  - Deviations <30% in 6 out of 7 samples analysed (Table 1).
  - LLE was considered as a good alternative taking into account the higher cost and time consumption of SPE.

- **Suspended solids (SS)**: substantial amounts of cannabis biomarkers detected.
  - In agreement with the polarity characteristics of the three compounds (higher lipophilicity favours presence in SS)

- **Raw IWW**: Results support the hypothesis that data obtained analysing the raw IWW by LLE without previous removal of suspended solids is similar to the sum of biomarkers in the suspended solids (SLE) and the liquid phase (LLE or SPE) (Fig. 4).

**Conclusions**

- The suspended solids of IWW contain a high percentage (+/- 42% for THC-COOH) of cannabis biomarkers.
  - SPE without correction factor for the amount of biomarkers in the suspended solids would lead to an underestimation since it only considers the liquid phase of IWW.
  - The analysis of the IWW without separation of the solid phase offers more realistic information on the biomarker concentration in IWW.

- More research is required to calculate consumption estimates.

**Future research**

- Sampling uncertainty related to solids.
- Partition of the cannabis biomarkers between liquid and solid phases.
- Extrapolating to WBE applications.