# Tracking down use of new psychoactive substances using sewage-based epidemiology: detection and identification of transformation products of methylone and methylenedioxypyrovalerone in sewage using accurate-mass mass spectrometry

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## INTRODUCTION

- Sewage-based epidemiology (SBE) is the analysis of excretion products of (illicit) drugs in sewage with the purpose of estimating community drug use.
- Concentrations of illicit drugs and metabolites in influent sewage are used to back-calculate amounts of these substances used by a community.
- New psychoactive substances (NPS) are compounds that mimic effects of illicit drugs and are produced by introducing slight modifications to chemical structures of controlled illicit drugs to by-pass law enforcement.
- SBE studies[1-3] have shown that NPS levels in sewage are generally very low.
- Parent compounds may be subject to transformation during their in-sewer transport[4]. Which in-turn would affect the levels present.
- It is important to explore possible transformation products (TPs) formed in sewer as potential biomarkers for NPS.

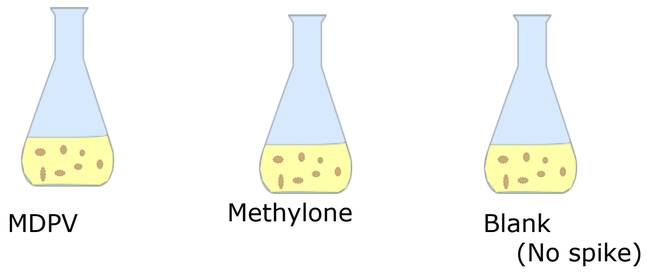
## OBJECTIVES

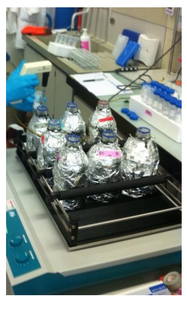
- To study **stability** of two NPS- methylone and MDPV in sewage **in the presence of biofilm** under aerobic conditions
- To investigate the **formation of transformation products** (TPs) for these compounds over a 24h period using HRMS

## METHODOLOGY

### *I. Incubation reactors*

- 0.5 L grab sewage + ≈ 30 g biofilm scraped from real sewer pipe
- Spike : 500 ug/L of MDPV and methylone in different reactors
- Monitored: COD sol, NH4, pH, °C, DO
- Time points: -15 min, 0h, 2h, 8h, 12h, 24h collected 20ml



### *II. Instrumentation*

| Instrument | Agilent 1290 Infinity LC, Agilent 6530 QTOFMS |
|---|---|
| Column | Phenomenex Biphenyl (100 x 2.1 mm, 2.6 µm) |
| Mobile Phase | A: 0.04 % formic acid in $H_2O$ B: 0.04 % formic acid in (80:20 v/v) acetonitrile/$H_2O$ |
| Flow rate | 0.4 ml/ min; run time 33min. Injection vol= 10 µL |
| Acquisition | 2.5 spectra/s; scans: 0, 15 and 35 eV with fragmentor at 100 V |

### *III. Data processing*

- Suspect lists- Eawag pathway prediction system and literature [5-8]
- Identified using ACD/MS Workbook Suite 2015 software
- ChemBioDraw 14.0 for drawing proposed TPs

## RESULTS

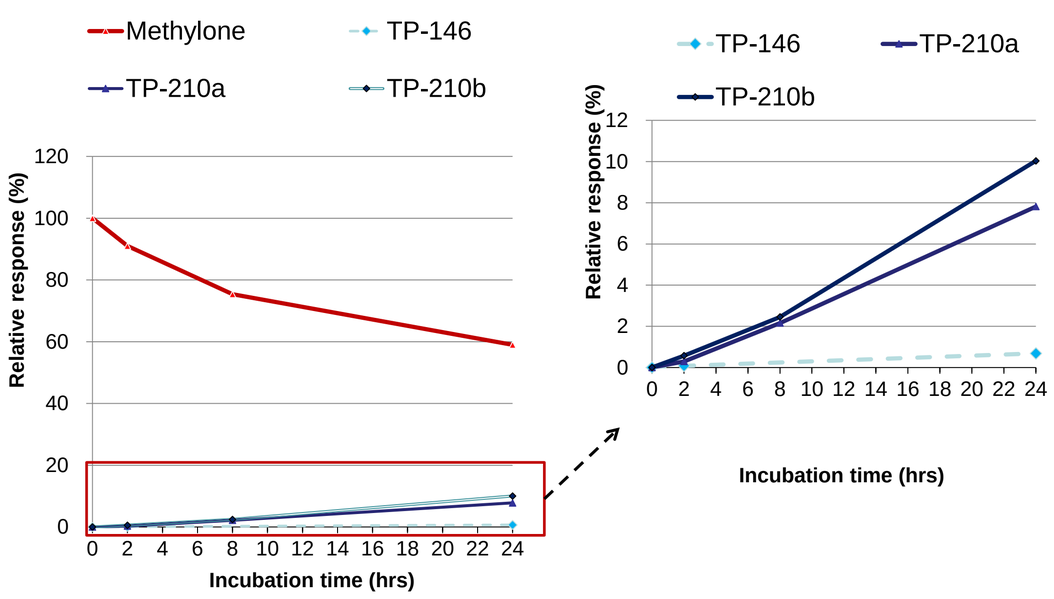



Fig 1: Estimated formation of methylone TPs in sewage + biofilm over 24h

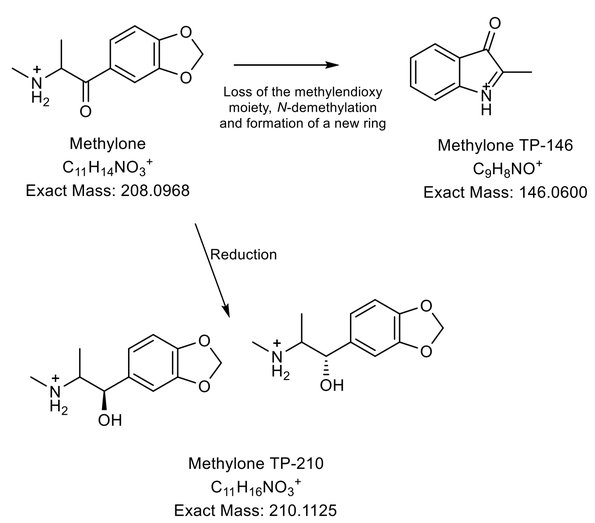



Fig 2: Proposed pathway for biotransformation of methylone in sewage in the presence of biofilm

- Overall methylone and MDPV show medium stability (20 - 60% transformation)[4] under aerobic conditions.
- 3 TPs for methylone, main TP (TP-210b) formed from reduction of β-keto group within 2h of incubation.
- TP-210 (dihydromethylone) also formed in vitro as minor metabolite[8].
- 4 TPs for MDPV, main TP (TP-292) formed from hydroxylation and ring opening immediately after spiking MDPV.
- TP-292 has several isoforms as found in previous studies[5,6].

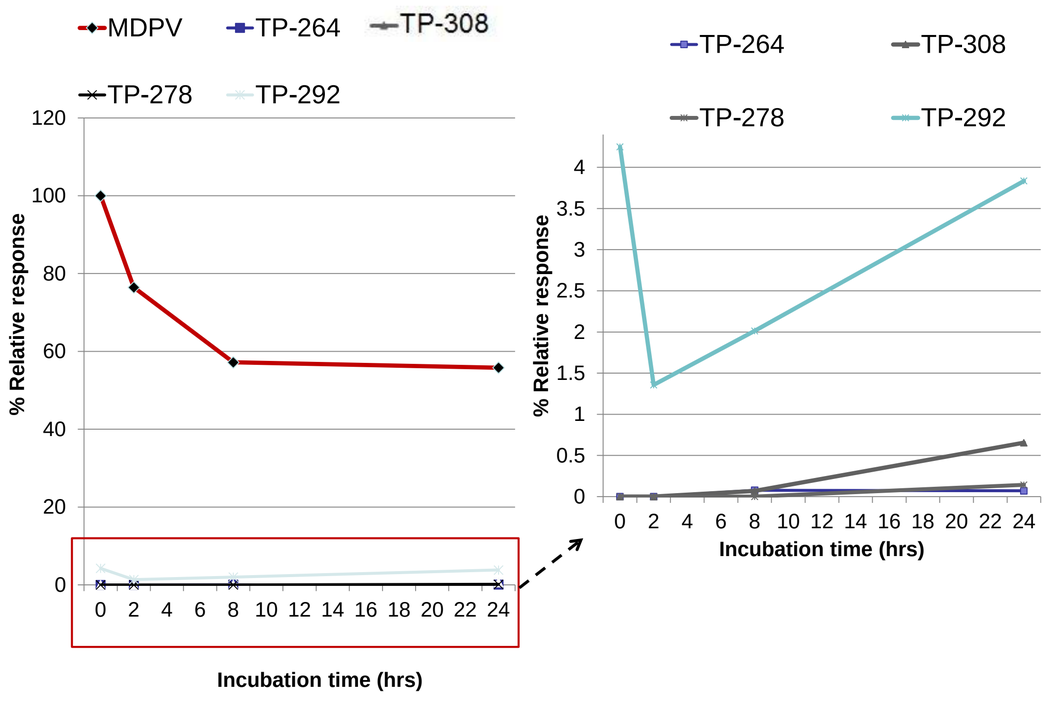



Fig 3 : Estimated formation of MDPV TPs in sewage + biofilm over 24h

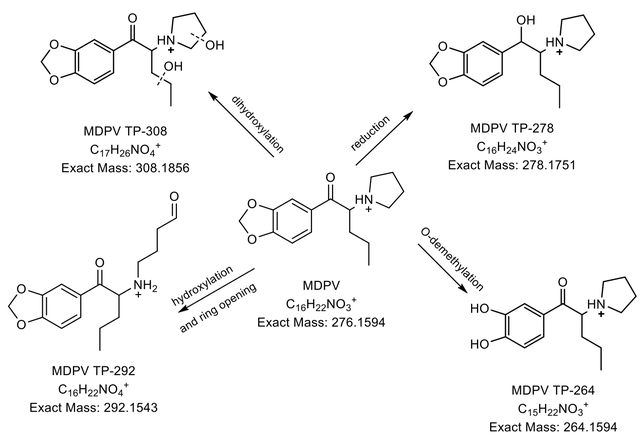



Fig 4: Proposed pathway for biotransformation of MDPV in sewage in the presence of biofilm

## Conclusions:

- Overall TPs increase but remain at low levels (<20% relative response ) within 24h .
- TPs should also be detectable in influent sewage depending on their levels.

## Future Directions:

- Determine the specific isoforms of the TPs formed here and those proposed in literature and *in silico*.
- Add the TPs' information to screening methods for SBE analysis.

## Acknowledgements

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