

Standard Operating Procedure (SOP) for the extraction and GC-MS analysis of the vehicle tyre wear chemical 6PPD in riverine sediments

Sample Preparation

Field samples were stored frozen at -20 °C in cleaned glass jars with foil lining the lids (following the same washing and contamination control procedures for equipment as for the microplastic μ -FTIR analysis protocol) to protect the sample from contamination and defrosted only as required for the analysis. Defrosted samples were mixed thoroughly using a metal spatula, visually ensuring agitation of sediment in contact with the vessel and down from the top surface, to homogenise. Approximately 4 g of sediment was decanted into a clean beaker and the weight recorded accurately. The samples were then dried with sodium sulphate before being transferred to the microwave vessels. Each sample was spiked with labelled recovery standard (D10-Fluoranthene; Spex) and microwave extracted in dichloromethane:acetone (DCM:Acetone) for 0.5 h at 80°C. The extract was concentrated in DCM only to 1.5 mL and cleaned using automated size exclusion chromatography (Agilent HPLC 1200). Post clean-up extracts in DCM were concentrated to 1 mL and transferred to GC vials for analysis.

GC-MS Analysis

1.7 μ L of sample extract was injected into a GC-MS (Agilent, 6890N, 5975B) with splitless injection at 250 °C. The GC-MS had a 30 m HP5-MS column (0.25 mm diameter, 0.25 μ m internal diameter, Agilent, Santa Clara, CA) and the carrier gas was helium (1.5 mL min⁻¹). On each run, the GC oven was programmed from an initial temperature of 50 °C (1.5 min) followed by a 30 °C min⁻¹ increase to a final temperature of 260 °C, which was held for 10 minutes. 6PPD was quantified using a calibration curve of the 6PPD (Sigma Aldrich) and the samples were recovery corrected using D10-Fluoranthene. The mean recovery of the D10 was 95.8% (78% to 119% (n=24, s.d = 10.73, CoV = 11.2%) and the limit of detection (LOD) ranged from 0.26 – 0.4 μ g kg⁻¹ d.w.

Quality Assurance

Blank assessment and limits of detection

As with the microplastic extraction and analysis and for quality control and assurance purposes, a solvent blank sample was included in each batch which followed the entire extraction protocol. The performance of the method was assessed in terms of the limit of detection (LOD), which was calculated based on the lowest standard we were able to quantify above the noise. This resulted in excellent and consistent mean instrument method limits of detection equivalent to 0.34 μ g kg⁻¹ (CoV 15.7%) dry weight of sediment.

Recovery correction through spiked analogous chemical tracer

For identification and quantification purposes, each batch had its own set of calibration standards. Every sample, including blanks were spiked with an analogous chemical recovery standard (D10-Fluoranthene) which was used to assess and adjust for the recovery efficiency of the process. Recovery was excellent, with an overall mean recovery across samples of 95.8% (SD 10.73%, CoV 11.2%). All 6PPD concentration values were corrected for any loss in recovery from the D10 reference standard to give a 100% result in the sediment. Thus, all variability between replicates and sites is assumed to arise from heterogeneity in the samples, as any analytical or instrument variability is controlled for through this recovery correction procedure. In this way, the sampling and homogenisation method in the lab is evaluated through assessment of the variability of 6PPD to see whether further improvements through drying and sieving for example to homogenise before extraction could further improve repeatability of the method.