

Supporting information

Microplastics as a vector for exposure to hydrophobic organic chemicals in fish: a comparison of two polymers and silica particles spiked with three model compounds

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Table S1 Information about experimental particles

Table S2 Information about model compounds that were used for spiking particles

Table S3 Primer sequences, concentrations and reaction temperatures used in the qPCR analyses

Figure S1 PCA analysis of fish biomarker responses. Differential grouping between virgin (PS, PE, SG and control) and chemically-spiked particles (PS_{mix}, PE_{mix}, SG_{mix} and chemical mixture) is demonstrated.

Table S4 The statistical output from two-ANOVA (fixed factors: particle type and chemical spiking).

Microparticle characterization

Morphometric particle characterization revealed that PE MPs (200-250 μm) and SG (200-320 μm) were very alike, in terms of shape and surface topography; particles were rather symmetrical and spherical (coefficients: 0.99 and 0.96; 0.97 and 0.95, respectively) and had smooth and homogenous surface (with high resemblance to primary MPs) (Figure 1). Quantitative morphometric analysis identified high particle abundance in the lowest micro-size ranges ($< 50 \mu\text{m}$) for these particles (Figure 1, Table S1). Even though PE and SG were comparatively very similar (spherical shape and had overlapping particle size distributions), they did differ in terms abundance of small microparticles ($>50 \mu\text{m}$) and corresponding surface area. On the other hand, PS MPs (50-250 μm) were morphologically less spherical (0.75), had high degree of surface irregularities and appeared brittle (and with high resemblance of weathered MPs) (Figure 1, Table S1). PS MPs size distribution revealed that the most abundant particle population were below 200 μm .

Given these inherent differences between particle size distributions and surface topography, estimations of particle numbers (per g particles) and surface area (cm^2) were complementarily performed to provide additional metrics for studying chemical sorption and desorption (Table S1). For surface area and particle volume, estimations were based on spherical approximations. Particle number (and daily particle intake (Table S1)) estimations were performed using mass-data according to a previously used approach ¹.

Table S1 Information about experimental particles, including particle number and surface area estimates. The density of experimental particles was provided by supplier, with an exception of PS, which was obtained from literature. Sphericity and symmetry values, are expressed as a coefficient (0-1), where 1 represents ideal sphere and symmetry (data is provided for the most abundant size classes).

| Particle | Type | Density (g/cm ³) | Sphericity | Symmetry | Number of particles (particles/g) | Daily intake (particles/dietary serving/fish) | BET-surface area (m ² /g) | BET-total volume in pores (Å) | Surface area (m ² /g) | Particle volume (cm ³) |
|-------------------|---------------------|------------------------------|------------|------------|-----------------------------------|---|--------------------------------------|-------------------------------|----------------------------------|------------------------------------|
| Polystyrene (PS) | Glassy polymer | 1.04 | 0.75±0.06 | 0.84±0.04 | 3.44*10 ⁶ | 5154 | 2.5 | 18.59 | 0.24 | 8.53 |
| Polyethylene (PE) | Rubbery polymer | 1.12 | 0.89±0.47 | 0.92±0.035 | 5.86*10 ⁵ | 878 | 1.8 | 20.02 | 0.06 | 2.26 |
| Silica glass (SG) | Reference particles | 2.53 | 0.91±0.09 | 0.93±0.04 | 2.68*10 ⁵ | 401 | 0.6 | 23.41 | 0.07 | 6.82 |

Table S2 Information sheet about model compounds that were used for spiking particles

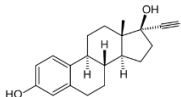
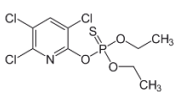
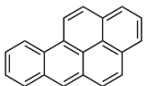
| Chemical compound | Structural formula | Biological mode of action | Log K _{ow} | MW (g mol ⁻¹) | Solvent | CAS-number |
|------------------------|---|--|---------------------|---------------------------|------------------------------|------------|
| Ethinylestradiol (EE2) |  | Synthetic estrogen, ligand for estrogen receptor (ER) upregulating egg yolk protein - vitellogenin (VTG) | 3.87 | 296.40 | Methanol | 57-63-6 |
| Chlorpyrifos (CPS) |  | Organophosphate pesticide, acetylcholine (AChE) inhibitor | 4.66 | 350.59 | Methanol | 2921-88-2 |
| Benzo(a)pyrene (BaP) |  | Polycyclic aromatic hydrocarbon, aryl hydrocarbon (AhR) receptor agonist, promoting cytochrome P450 (CYP1a) metabolism | 5.99 | 252.31 | Acetonitrile: Methanol (1:1) | 50-32-8 |

Table S3 Primer sequences, concentrations and reaction temperatures used in the qPCR analyses

| Gene | Sequence | Primer concentration (nM) | Temperature |
|--------------------------------------|--------------------------------------|---------------------------|-------------|
| β-tubulin ² | F: AACCAGATCGGCGCAAAGT | 500 | 60 |
| | R: ACCCGATGCCTCATTGTAGTAGAC | | |
| RPL13 ³ | F: CACCTTGGTCAACTTGAACAGTG | 500 | 60 |
| | R: TCCCTCCGCCCTACGAC | | |
| HPRT ³ | F: GAC GCA GAT ATG GTT CAG ATC TC | 500 | 60 |
| | R: GTC TTA ATT GTG GAG GAT ATT ATC G | | |
| CYP1a ⁴ | F: ACGTGCAGATGTCAGACGAG | 500 | 60 |
| | R: TTGGGTTTGTTCGAGAGAAG | | |
| Estrogen receptor (ERα) ⁴ | F: CTGCCAGGCTTGCCGTCTTAG | 500 | 60 |
| | R: AGTGCGTCTCTTGTCCCGCC | | |
| Vitellogenin (VTG) ⁵ | F: GTGAGGACGCAAAGGATGAG | 700 | 60 |
| | R: TCCGTGTAAGTCAAACCAATGT | | |
| Acetylcholine esterase (AChE) | F: CTGGAAGGGGTCAAGCTGAG | 500 | 60 |
| | R: GCGTAGATGCGAGCAAAGTG | | |

Multivariate analysis of biomarker responses

PCA illustrates presence of chemical-specific effects (Figure S1). Results from the multivariate analysis is summarized in Table S4-S5.

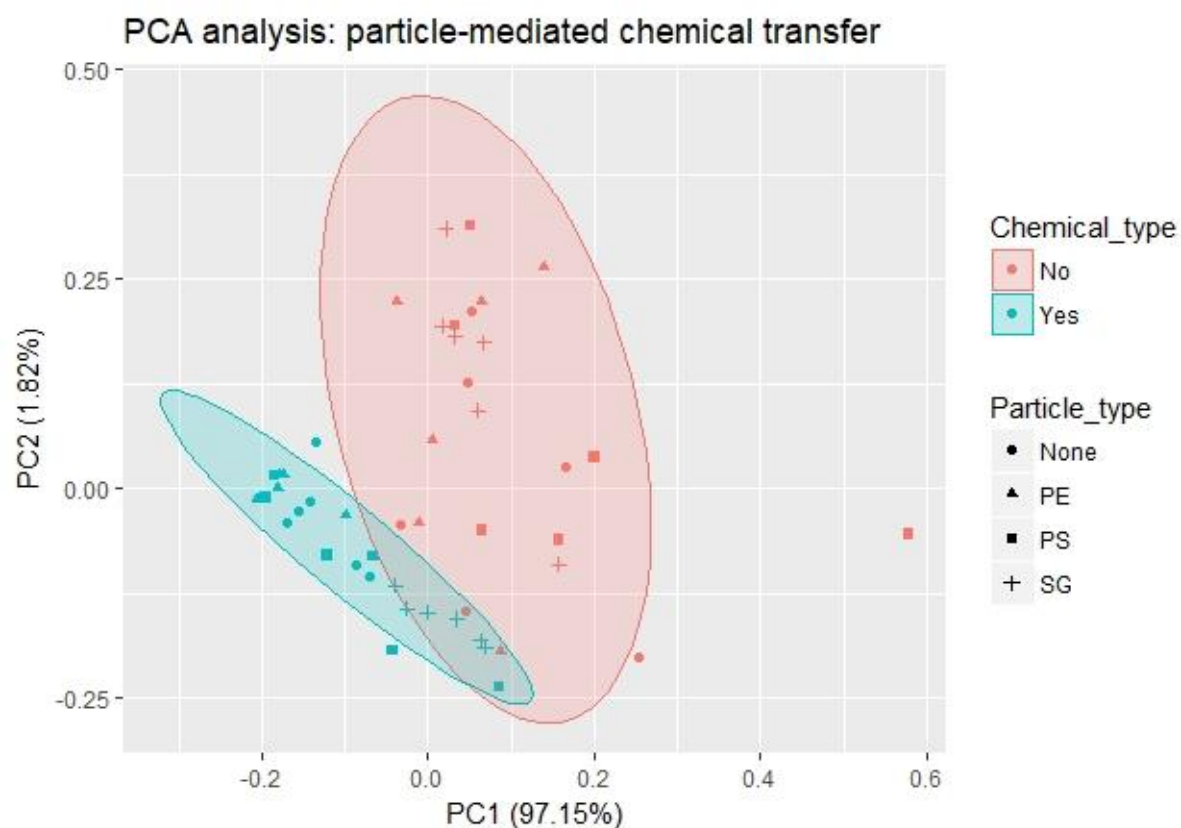


Figure S1 PCA analysis of fish biomarker responses. Differential grouping between virgin (PS, PE, SG and control) and chemically-spiked particles (PS_{mix}, PE_{mix}, SG_{mix} and chemical mixture) is demonstrated.

Table S5 The statistical output from two-ANOVA (fixed factors: particle type and chemical spiking).

Two-way ANOVA (tests of between-subjects effects)

| Source | Dependent Variable | Type III Sum of Squares | df | Mean Square | F | Sig. |
|--------------------------------------|------------------------|-------------------------|----|-------------|--------|------|
| Chemical effect | EROD activity (liver) | 3.372E-6 | 1 | 3.372E-6 | 61.750 | .000 |
| | AChE activity (brain) | 19210.435 | 1 | 19210.435 | 40.974 | .000 |
| | ACHE mRNA (intestine) | 38.391 | 1 | 38.391 | 10.580 | .002 |
| | CYP1A mRNA (intestine) | 42.876 | 1 | 42.876 | 50.412 | .000 |
| | ER mRNA (intestine) | 60.942 | 1 | 60.942 | 85.066 | .000 |
| | VTG mRNA (intestine) | 93.753 | 1 | 93.753 | 39.887 | .000 |
| | ACHE mRNA (liver) | 8.008 | 1 | 8.008 | 9.810 | .003 |
| | CYP1A mRNA (liver) | 25.008 | 1 | 25.008 | 28.463 | .000 |
| | ER_ mRNA (liver) | 67.960 | 1 | 67.960 | 32.323 | .000 |
| | VTG mRNA (liver) | 662.215 | 1 | 662.215 | 63.500 | .000 |
| Particle effect | EROD activity (liver) | 4.743E-7 | 3 | 1.581E-7 | 2.895 | .048 |
| | AChE activity (brain) | 4255.443 | 3 | 1418.481 | 3.025 | .042 |
| | ACHE mRNA (intestine) | 4.839 | 3 | 1.613 | .445 | .723 |
| | CYP1A mRNA (intestine) | 1.399 | 3 | .466 | .548 | .652 |
| | ER mRNA (intestine) | .291 | 3 | .097 | .135 | .938 |
| | VTG mRNA (intestine) | 6.376 | 3 | 2.125 | .904 | .449 |
| | ACHE mRNA (liver) | 3.385 | 3 | 1.128 | 1.382 | .264 |
| | CYP1A mRNA (liver) | 3.782 | 3 | 1.261 | 1.435 | .249 |
| | ER_ mRNA (liver) | 8.663 | 3 | 2.888 | 1.373 | .266 |
| | VTG mRNA (liver) | 21.686 | 3 | 7.229 | .693 | .562 |
| Chemical * Particle (interaction) | EROD activity (liver) | 2.474E-7 | 3 | 8.248E-8 | 1.510 | .228 |
| | AChE activity (brain) | 5174.578 | 3 | 1724.859 | 3.679 | .021 |
| | ACHE mRNA (intestine) | 2.489 | 3 | .830 | .229 | .876 |
| | CYP1A mRNA (intestine) | 5.329 | 3 | 1.776 | 2.089 | .119 |
| | ER mRNA (intestine) | 6.886 | 3 | 2.295 | 3.204 | .035 |
| | VTG mRNA (intestine) | 10.554 | 3 | 3.518 | 1.497 | .232 |
| | ACHE mRNA (liver) | 1.249 | 3 | .416 | .510 | .678 |
| | CYP1A mRNA (liver) | 3.729 | 3 | 1.243 | 1.415 | .254 |
| | ER_ mRNA (liver) | 9.530 | 3 | 3.177 | 1.511 | .228 |
| | VTG mRNA (liver) | 31.751 | 3 | 10.584 | 1.015 | .397 |

References

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