Effect-based monitoring of the Danube River using mobile passive sampling

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Abstract

Many aquatic pollutants can be present at low concentrations, but their mixtures can still affect health or behavior of exposed organisms. In this study, toxicological and chemical analyses were combined for spatial contamination profiling using an innovative passive sampling approach. A novel Dynamic

Passive Sampler (DPS) was employed as a mobile sampler from a ship cruising along 2130 km of the Danube river during the Joint Danube Survey 3 (JDS3). The sampling was performed in eight subsequent river stretches with two types of complementary passive samplers: silicone rubber sheets (SR) used for non-polar chemicals and SDB-RPS Empore™ disks (ED) for more hydrophilic compounds. Besides extensive chemical analyses, the bioactivity of samples was characterized by a battery of reporter gene bioassays. Cross-calibration of the employed passive samplers enabled robust estimation of water concentrations applicable for compounds with a wide range of physicochemical properties. DPS was suitable for sampling of water contaminants even at pg L^{-1} levels, with 209 of 267 analyzed compounds detected in the samples. Biological effects were detected in both ED and SR extracts across all river stretches by bioassays focused on xenobiotic metabolism mediated by the aryl hydrocarbon and pregnane X receptors, endocrine disruptive potential mediated by estrogen and androgen receptors and the oxidative stress response. The bioassay responses expressed as bioanalytical equivalent concentrations (BEQ_{bio}) were comparable with data obtained from large volume active sampling. The extracts of the ED samplers were more biologically active than extracts of SR samplers. Except of estrogenicity, where the analyzed chemicals explained on average 62 % of the effects in ED samples, the detected chemicals explained less than 8 % of BEQ_{bio} values. The study shows the utility of the combination of the innovative passive sampling approach with effect-based tools for efficient and fast monitoring even in water bodies with relatively low levels of contamination.

1. Introduction

Contamination of river water with complex mixtures of organic micropollutants poses a challenge for current pollution monitoring. Monitoring programs are typically driven by a need to protect aquatic organisms and, in an indirect way, also human populations from potentially toxic effects of environmental pollutants. This is complicated by the fact that pollutants are mostly present in very complex mixtures and their biological effects are the outcome of the integrated effects of many individual chemicals (Escher and Leusch, 2012). Because these mixtures can consist of thousands of chemicals whose toxic properties are often not known, targeted chemical analysis of individual compounds can sufficiently describe neither their composition nor toxic properties (Neale et al., 2015a). It has been previously shown that this problem could be addressed using a bioanalytical approach such as *in vitro* bioassays (Escher and Leusch, 2012; Giesy et al., 2002). These bioassays present rapid, sensitive and relatively inexpensive detection tools, which provide complementary information to data from instrumental chemical analysis. They enable estimation of the overall

biological activities of compounds present in environmental mixtures covering potential interactions among chemicals.

In order to cover a wide range of possible adverse effects, the bioassay battery should include endpoints reflecting a diverse set of possible toxic mechanisms, such as initiation of xenobiotic metabolism, endocrine disruptive potential and adaptive stress responses (Escher et al., 2014). To describe the overall bioactive potential of mixtures of chemicals, the concept of bioanalytical equivalent concentration (BEQ) was developed (Villeneuve et al., 2000; Baston and Denison, 2011). In this concept, the bioassay-detected biological potential of a complex mixture of chemicals is expressed as a concentration of a reference compound that would elicit the same effect as the mixture (BEQ_{bio}). To estimate the impact of detected chemicals, a comparable equivalent (BEQ_{chem}) can be modeled based on measured concentrations of individual chemicals using the concentration addition concept. Thus, by comparison of BEQ_{chem} with the equivalents detected in bioassays (BEQ_{bio}), it is possible to identify the chemicals most significantly contributing to the effect detected by bioassays (König et al., 2016; Neale et al., 2015a, 2017a).

Another challenge in aquatic pollutant monitoring is related to the requirement of a representative sampling in temporally and spatially variable water streams. Since concentrations of pollutants tend to fluctuate, frequent spot sampling or continuous sampling is required to provide a representative sample. This type of sampling is often laborious and resource-intensive. Many of the potentially toxic chemicals are present at very low concentrations and the effective pre-concentration step is thus needed prior to their instrumental analysis.

These problems can be effectively addressed using integrative passive sampling. In this approach, samplers spontaneously absorb or adsorb chemicals from water. The integrative concentration of chemicals in a passive sampler throughout the sampling period decreases their limit of detection. It also allows estimation of their time-weighted average concentrations, which include residues from episodic pollution events often not detectable even using frequent spot sampling. As the uptake of pollutants by the passive samplers is affected by environmental variables such as temperature and flow velocity, accurate in situ assessment of water volume sampled during exposure is required. For individual compounds, estimation of sampled water volume can be performed through the application of performance reference compounds (PRCs) and models that relate the sampling rate to properties that control the compound mass transfer (Booij et al., 2007).

Passive sampling as well as effect-based approaches are being considered as potentially suitable tools that could be employed for monitoring of European water bodies in the implementation strategy of the EU Water Framework Directive (European Comission, 2015). While both approaches are often

employed independently, the utility of their combination has been demonstrated previously in studies focusing on small water streams and wastewater treatment plant effluents and affected rivers (Creusot et al., 2013; Jalova et al., 2013; Jarosova et al., 2012). However, these approaches have not been validated for large rivers with moderate or low levels of contamination.

This study investigates the applicability of the newly developed dynamic passive sampling (DPS), which speeds up the uptake of compounds, for pollution and toxicity profiling of large rivers. The DPS was employed in the Joint Danube Survey 3 (JDS3) as a mobile sampler from a ship cruising downstream along 2130 km of the Danube river, one of the largest rivers in Europe. The relatively low contamination level makes the Danube suitable for testing of sampling techniques for monitoring of less concentrated pollutants. The sampling was performed in eight subsequent river stretches with two types of complementary passive samplers: partitioning silicone rubber sheets (SR) focused on non-polar chemicals, which allow quantification of sampling rate, and adsorption SDB-RPS Empore™ disks (ED) for non-polar as well as more hydrophilic compounds. Besides extensive chemical analyses, sample extracts were characterized by a battery of *in vitro* bioassays covering a range of endpoint types including endocrine disruption, xenobiotic metabolism, and adaptive stress responses. BEQ modeling was used to estimate the portion of biological effects of the samples that can be explained by detected chemicals. Since the same bioassays were used also for the assessment of samples from large volume active sampling during the JDS3 ship cruise (Neale et al., 2015a), it allowed for a mutual comparison of passive and active sampling approaches for toxicity profiling.

2. Material and methods

2.1. Passive sampling

Sample collection was performed during the JDS3 (Liska et al., 2015) on a sampling cruise in August and September 2013 using a dynamic passive sampling (DPS) system described by Vrana et al. (submitted). Briefly, the DPS device consisted of a stainless-steel chamber equipped with a submersible pump (approx. 9 m^3 h^{-1}) that provided a forced flow of sampled water through the sampling chamber with a current velocity of 1–2 m s^{-1}. For mobile sampling, the device was immersed in a flow-through tank located on the frontal deck of the JDS3 expedition ship (Vrana et al., submitted). Each individual sampling period lasted approximately five days, which resulted in a total of eight samples collected from each sampler type representing eight stretches of the Danube River (Tab. S1). Two parallel DPS devices were in operation during each sampler deployment. The samplers exposed in one of the devices were dedicated to chemical analyses and the samplers from the other device were used for

bioanalyses. Besides sampling from the ship, stationary DPS was also performed at a site located at the Danube river kilometer 1852, approximately 15 km downstream of Bratislava (Fig. 1) and the sampling dates coincided with time periods when the JDS3 expedition ship moved by the stationary site.

Two types of passive samplers were utilized for bioanalysis: silicone rubber (SR) AlteSil™ sheets, and SDB-RPS Empore™ disks (ED) based on styrene-divinylbenzene sorbent modified with sulfonic acid groups.

AlteSil™ translucent SR sheets 0.5 mm thick were purchased from Altec, UK. The sampler consisted of a single AlteSil™ SR sheet with dimensions 14×28 cm. SR samplers in the DPS device dedicated for chemical analyses were spiked prior to exposure with a set of PRCs. The ED sampler consisted of ten solid phase Empore™ SDB-RPS extraction disks with 47 mm diameter (Sigma Aldrich, Czech Republic). ED samplers were not spiked with any PRCs.

Besides SR and ED samplers, the DPS devices were equipped with a reference passive sampler that served to compare the sampling performance of the two DPS devices operating in parallel. It consisted of a strip of low-density polyethylene (LDPE; 4×28 cm and 70 µm thickness; Brentwood Plastics Inc, USA). The LDPE samplers mounted in both DPS devices were spiked with another set of PRCs. The LDPE samplers were located sideways from the SR and ED samplers to minimize cross-contamination of the samplers by PRCs, for further detailed information on sampling, list of PRCs and sample processing see SI1 and Vrana et al. (submitted).

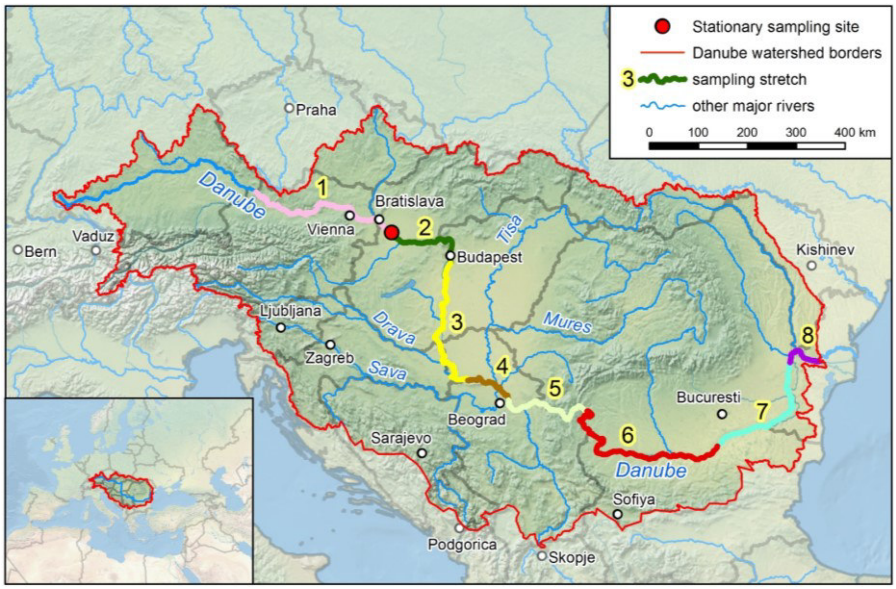


Fig. 1 Map of the sampling cruise with the stretches and stationary exposure site (red dot)

2.2. Chemical analysis

SR and ED were processed as described in the SI (1.1.1-1.1.2). SR extracts were analyzed for 81 hydrophobic compounds from the following groups: polycyclic aromatic hydrocarbons (PAHs; 29 individual chemicals), PCBs (7), organochlorine pesticides and their degradation products (OCPs; 12), brominated diphenyl ethers (PBDEs; 9) and novel brominated flame retardants (15), alkylphenols (3), alkyl- and aryl- phosphates (14), and synthetic musks (6). ED extracts were analyzed for 204 chemicals from the following groups: currently used pesticides (CUPs; 40), steroids (5), phytosterols (11), flavonoids (8), alkylphenols (3), pharmaceuticals (108) and PAHs (29). For a comprehensive list of analyzed compounds see SI (Tab.S2, S3).

PAHs were analyzed by gas chromatography/mass spectrometry (GC/MS) using GC 7890/MS5975 (Agilent, USA) equipped with J&W Scientific fused silica column DB-5MS (0.25 mm I.D., film thickness: 0.25 µm; Agilent). For PCBs and OCPs, the analysis was performed using GC 7890/MS-MS Triple Quadrupole 7000B (Agilent), equipped with HT8 SGE Analytical Science column. PBDEs were analyzed by GC 7890A (Agilent) equipped with 15m × 0.25mm × 0.10 µm RTX-1614 column (Restek, USA), HRMS (AutoSpec Premier) was operated in EI+ mode at the resolution of >10,000. Alkylphenols were derivatized with dansyl chloride, separated using an Agilent 1200 Infinity Series liquid chromatograph equipped with an ACE 5 C18 column (ACE, UK) and detected by MS/MS Agilent 6410 Triple Quadrupole (Pernica et al., 2015). Novel brominated flame retardants were analyzed using GC 7890A (Agilent) equipped with 15 m × 0.25 mm × 0.10 µm DB5 column coupled to an AutoSpec Premier MS (Waters, Micromass, UK; Lohmann et al., 2013). Analyses of alkyl phosphates and polycyclic musks were performed using GC 6890 (Agilent) coupled to MSD 5975 mass spectrometer (Agilent). For more details see SI 1.2.

Currently used pesticides (CUPs) were separated using Agilent 1290 series HPLC (Agilent) and detected with mass spectrometer AB Sciex Qtrap 5500 (AB Sciex, Canada; Brumovský et al., 2016).

Steroid analysis was performed by liquid chromatography (HPLC Agilent 1200 Series) with mass spectrometry (MS-MS Agilent 6410 Triple Quad) after precolumn derivatization with dansyl chloride as described previously (Lin et al., 2007; Sadílek et al., 2016).

For the detection of flavonoids and phytosterols, the HPLC-MS/MS method previously described by Bláhová et al. (2016) was employed.

Pharmaceuticals were analyzed using a triple stage quadrupole MS/MS TSQ Quantum Ultra mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) coupled with an Accela 1250 LC pump (Thermo Fisher Scientific) and an HTS XT-CTC autosampler (CTC Analytics AG, Switzerland) as described previously by Grabic et al., (2012).

LDPE extracts were analyzed for PAHs, PCBs, and HCB according to Allan et al. (2013). For further details on chemical analyses see SI 1.2.

2.3. Bioanalysis

Specific toxic potentials were assessed using a battery of bioassays (Table 1). A detailed description of the bioassays can be found in SI 1.3.1 and Neale et al. (2015a). The sample concentrations in bioassays were expressed as relative enrichment factor (REF), which expresses the water sample enrichment by passive sampling and sample processing together with the dilution in the bioassays. The effect in the sample was expressed as either the concentration causing 20% effect (EC_{20}), the concentration causing 20% inhibition (IC_{20}), or the effect concentration causing an induction ratio (IR) of 1.5 ($EC_{IR1.5}$) (Table 1). Log-logistic dose-response and linear regression models were used for the calculation of EC_{20} and $EC_{IR1.5}$, respectively. Cell viability was assessed in parallel and cytotoxic sample concentrations were excluded from further calculations.

2.4. Data analysis

2.4.1. Estimation of water volume extracted by passive samplers

The calculated concentrations of chemicals in water (C_w) derived from the passive sampling correspond to the freely dissolved concentration C_{free} (Mayer et al., 2003). Sampling rate $R_{S,SR}$ of individual compounds to SR passive samplers was modeled as a function of the molar mass M by the water boundary layer-controlled uptake model from Rusina et al. (2010):

$$R_{s,SR} = AB \times M^{-0.47} \qquad \text{Equation 1}$$

with an exposure-specific parameter AB. The parameter was estimated from the dissipation of PRCs from samplers during exposure using a nonlinear least squares method by Booij and Smedes (2010), considering the fractions of individual PRCs that are retained in the sampler after exposure as a continuous function of their sampler-water partition coefficient $K_{SR,w}$. The models applied for SR samplers are described in detail in Vrana et al. (submitted).

C_w of individual compounds were calculated from the mass absorbed by the SR samplers N_{SR}, the degree of equilibrium *DEQ* that the compound attained during sampler exposure, the mass of sampler m_{SR} and their sampler-water partition coefficients $K_{SR,w}$ as described in Booij et al. (2007).

$$C_w = \frac{N_{SR}}{K_{SR,w}\, m_{SR}\, DEQ} \qquad \text{Equation 2}$$

The *DEQ* was calculated as:

$$DEQ = \left(1 - \exp\left(-\frac{R_{S,SR}\, t}{K_{SR,w}\, m_{SR}}\right)\right) \qquad \text{Equation 3}$$

Since the compounds that caused effects detected in passive sampler extracts were unknown they were expressed as BEQ per sampler ($BEQ_{SR,ED}$). A conversion to concentration in water (BEQ_w) was approximated using a sampling rate $R_{S,SR}$ of a compound with an intermediate molar mass of 300 $g.mol^{-1}$, assuming a fully integrative sampling during the entire sampler exposure. In such situations, when *DEQ* is <<1, Equation 3 can be simplified to $DEQ = R_{S,SR}\, t/(K_{SR,w} \times m_{SR})$ and

$$C_w = \frac{N_{SR}}{R_{S,SR}\, t} \qquad \text{Equation 4}$$

The BEQ_w for SR was calculated as:

$$BEQ_w = \frac{BEQ_{SR}}{R_{S,SR}\, t} = \frac{BEQ_{SR}}{AB \times 300^{-0.47}\, t} \qquad \text{Equation 5}$$

For ED samplers, the PRC approach was not applied since its application in adsorption-based samplers is questionable. The sampling rates of ED samplers were derived from a correlation of uptake of PAHs and 4-nonylphenol to ED and SR samplers as is described in detail in SI 1.3 and Vrana et al. (submitted).

Sampling rate values of the ED samplers $R_{S,ED}$ were estimated from sampling rates derived for SR samplers ($R_{S,SR}$), the calculated overall median $F_{ED/SR}$ factor for 10 PAH individuals and 4-nonylphenol, and the surface areas of both samplers A_{ED}, A_{SR} as has been explained in detail by Vrana et al. (submitted):

$$R_{S,ED} = F_{ED/SR} \times \frac{A_{ED}}{A_{SR}} \times R_{S,SR} = 0.83 \times \frac{173}{392} \times R_{S,SR} = 0.366 \times R_{S,SR} \qquad \text{Equation 6}$$

The BEQ_w for ED was then calculated as:

$$\mathrm{BEQ_w} = \frac{BEQ_{ED}}{R_{S,ED}t} = \frac{BEQ_{ED}}{0.366 \times R_{S,SR}t} \quad \text{Equation 7}$$

2.4.1. Bioanalytical Equivalent Concentration

The EC and IC values from the different bioassays (Table 1) were converted to BEQ_{bio} using Equation 6, with the EC_{20} or $EC_{IR1.5}$ value of the reference compound (rc) and the matching EC_{20} or $EC_{IR1.5}$ value of the extract.

$$\mathrm{BEQ_{bio}} = \frac{\mathrm{EC_{20}\ (rc)}}{\mathrm{EC_{20}\ (extract)}} \text{ or } \frac{\mathrm{IC_{20}\ (rc)}}{\mathrm{IC_{20}\ (extract)}} \text{ or } \frac{\mathrm{EC_{IR1.5}\ (rc)}}{\mathrm{EC_{IR1.5}\ (extract)}} \quad \text{Equation 8}$$

To calculate BEQ_{chem}, relative effect potency (REP_i) of the detected chemicals was calculated from measured data, complemented by information from the literature or calculated based on data from the US EPA ToxCast database (US EPA, 2015; Tab. S5). REP_i was calculated using Equation 10, with EC_{10}, IC_{20} or $EC_{IR1.5}$ value of the reference compound and the matching $EC_{10,}$ IC_{20} or $EC_{IR1.5}$ value of detected chemical i. REPs derived from the literature data and ToxCast database were based on EC levels indicated in Tab. S5. Limit of quantification (LOQ) was calculated using equation 6; the extract effective concentration was replaced by the highest non-cytotoxic sample concentration tested.

$$\mathrm{REP_i} = \frac{\mathrm{EC_{10}\ (rc)}}{\mathrm{EC_{10}\ (i)}} \text{ or } \frac{\mathrm{IC_{20}(rc)}}{\mathrm{IC_{20}\ (i)}} \text{ or } \frac{\mathrm{EC_{IR1.5}\ (rc)}}{\mathrm{EC_{IR1.5}\ (i)}} \quad \text{Equation 9}$$

BEQ_{chem} was calculated from REP and the estimated concentration of each chemical in water in molar units ($c_{w,i}$) using Equation 10.

$$\mathrm{BEQ_{chem}} = \sum_{i=1}^{n} \mathrm{REP_i} \cdot \mathrm{C_i} \quad \text{Equation 10}$$

3. Results and Discussion

3.1. Passive sampling

The Danube river watershed covers a significant part of the European continent reaching into the territory of nineteen countries of different developmental stages. The river is affected by a range of pollution sources discharging a wide spectrum of contaminants. On the other hand, being a large river, the Danube has a great dilution capacity and so the pollutant concentrations in the water may be relatively low compared to some rivers with a smaller dilution capacity (Keller et al., 2014). To representatively characterize the river pollution, the current study employed mobile passive sampling during the cruise of the expedition ship along defined stretches of the Danube. The overall sampled

river section covered by eight mobile-sampled river stretches spanned through nine countries (Fig. 1). Employment of two sampler types enabled sampling of chemicals with a wide range of physical-chemical properties. Partitioning-based silicon rubber (SR) samplers have been shown previously to effectively sorb non-polar chemicals (Rusina et al., 2010; Smedes and Booij, 2012). In order to facilitate the collection of more polar chemicals, we employed Empore™ SDB-RPS disc adsorption samplers (ED). The modification of poly(styrene-divinylbenzene) copolymer with sulfonic acid groups in ED was selected because it provides improved sorption capacity for polar chemicals with lower log K_{ow} such as pharmaceuticals and their metabolites, steroids, pesticides and their metabolites, explosives, or amine-containing analytes (Vrana et al., submitted).

Since the uptake principle is identical in both DPS and classical passive sampling, results may be evaluated using available passive sampler calibration parameters and models. The sampling rates for SR were determined using dissipation of PRCs from the samplers during their exposure, while for the ED samplers, the sampling rates were estimated from the relation of surface specific uptake of PAHs and 4-nonylphenol between SR and ED. The good correlation for these chemicals, which were integratively accumulated in SR, provided evidence that sampling rates under water-boundary layer control in ED are proportional to the sampling rates of SR. The proportionality factor of sampling rates is roughly given by the ratio of surface areas of the two samplers (details in SI 1.3; Fig. S1; Vrana et al., submitted).

The increase of the sampling rate achieved by means of DPS was significant: in comparison with data from the passive samplers with the same dimensions subjected to stationary exposure without forced water exchange, DPS sampling was approximately five times faster (Vrana et al., submitted). This allowed us to collect and detect many chemicals with concentrations in the pg/L range during less than two days of sampler exposure (Tab. S2).

The LDPE samplers were used for checking whether the sampled water volume for the DPS device dedicated to toxicological analyses was equal to the device intended for chemical analysis. PRC release was monitored for LDPE samplers in both DPS devices per location since they had the same configuration and were exposed side by side. The comparison was relevant for the situations where the released PRC fraction f_{PRC} was quantifiable or lower than 80 % (Fig. S2; i.e. d_{10}-FLT and d_{12}-CHR). Their sampling rate ratio was 1.00±0.11 and 0.93±0.14, respectively, as average across the eight sampling stretches. The corresponding sampling rates obtained demonstrate that the sampling performance of the two DPS devices deployed in parallel was equivalent. PRCs were not spiked to SR and ED samplers for toxicological analysis, but based on the equivalence of LDPE sampling rates in the

two devices, sampling rates in SR samplers for chemical analysis can be applied as a good estimate of sampling rates in SR samplers applied for toxicological analysis (details in SI 1.3.3, Fig. S2).

3.2. Chemical analysis

From a total of 267 analyzed chemicals, 209 were detected at least in one sample. A number of detected chemicals ranged from 52 to 70 and 103 to 131 for the SR and ED samplers across stretches, respectively (Fig. 2). Among non-polar compounds analyzed in the SR samples, triisobutylphosphate, 4-nonylphenol, naphthalene and phenanthrene, and synthetic musk galaxolide were estimated to be present in the highest free dissolved concentrations in the river water (4-101 ng L^{-1}; Tab. S2). Among compounds analyzed in the ED samples, the chemicals with the highest estimated concentration in river water were the pharmaceuticals carbamazepine, irbesartan, and sulphapyridine; atrazine, bisphenol A, cholesterol and sitosterol (7-17 ng L^{-1}; Tab. S3).

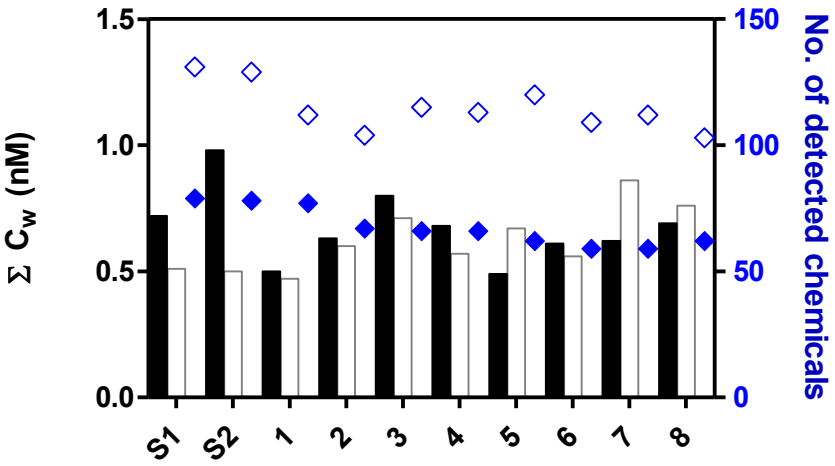


Fig. 2 The sum of freely dissolved molar concentrations of chemicals in water (C_w), estimated from their amounts analyzed in passive samplers (bar graph) and a number of chemicals detected in the samples (diamond); SR samples (black bar and blue diamond) and ED samples (white bar and empty diamond). S1-2 samples from stationary exposure of samplers downstream of Bratislava (Slovakia); 1-8 samples from a mobile sampling of river stretches.

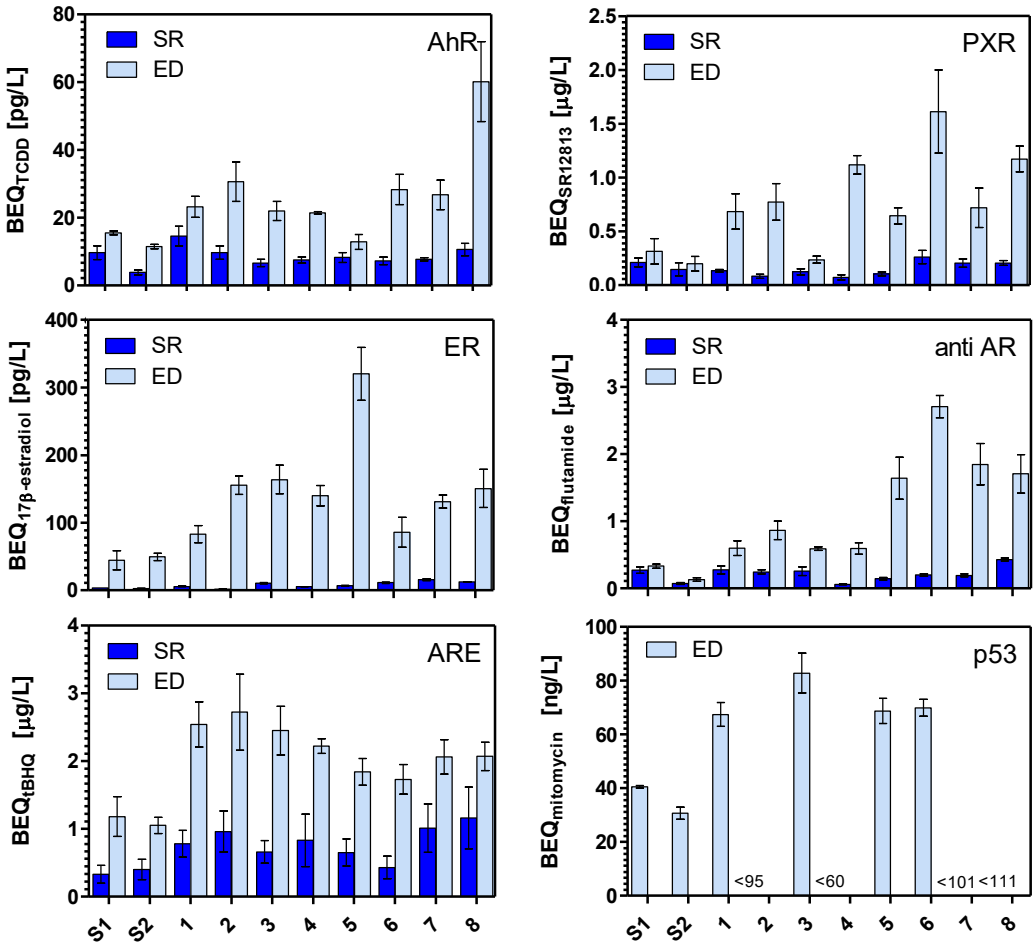
3.3. Bioanalysis

In the present study, we employed bioassays for the assessment of xenobiotic metabolism initiation by the aryl hydrocarbon receptor (AhR) and pregnane X receptor (PXR)-mediated effects. AhR-activation has been previously described to induce carcinogenicity, immunotoxicity and indirectly also

endocrine disruption (Kortenkamp et al., 2012). PXR induces xenobiotic detoxification systems and is linked, for example, with liver steatosis and is sensitive to a wide range of chemicals (Creusot et al., 2010). The assessment of endocrine disruptive potential focused on the estrogen receptor (ER) and androgen receptor (AR)-mediated effects. Effects of xenobiotics on ER and AR signaling are relatively well characterized and they are reported to affect reproduction, development and play a clear role in carcinogenicity (Janošek et al., 2006; McLachlan, 2016). While activation and inhibition of ER by chemicals in water samples has been studied intensively, effects of aquatic samples mediated by AR have obtained less attention (Brack et al., 2007; Leusch et al., 2017). Exposure to xenobiotics often causes stress to organisms and it leads to activation of rather non-specific adaptive stress responses to restore homeostasis such as activation of systems dealing with oxidative stress, genotoxicity or inflammation. Transcription of many detoxification enzymes is coordinately regulated by antioxidant response elements (ARE) and their activation can serve as a marker of exposure-related oxidative stress (Reddy, 2008). The p53-mediated response is triggered by DNA damage and it activates repair mechanisms or apoptosis. It can thus serve as an indicator of genotoxic chemicals (Duerksen-Hughes et al., 1999). NF-κB-mediated response plays an important role in inflammatory reactions (Simmons et al., 2009).

Extracts of both SR and ED samplers elicited quantifiable effects in all employed bioassays except for the assay indicative of the NF-κB response (Table 3; Fig. 3). In the case of p53-mediated response, the effects were detected only in six ED samplers with higher sampled water volume and even then, they were very close to the LOQ.

All other biological endpoints were detected across all mobile and stationary samples and both sampler types. When comparing the BEQ_{bio} of mobile sampled stretches across the assessed endpoints, spatial patterns along the river for some endpoints were revealed. For example, there was an increase in the AhR-mediated response in the ED sample at stretch 8 in the river delta in Romania (Fig. 3). There was also an increase in anti-androgenic potential from stretch 5 onward (Serbia, Romania, and Bulgaria). Similarly, an increased estrogenic potential was observed in stretch 5 (mainly Serbia and Romania), where also the highest levels of steroid estrogens, alkylphenols and musks were observed (Table 2, Tab. S4). This may be related to the fact that significant amounts of wastewaters are released to the Danube directly without treatment in this part of Europe, which leads to increased levels of chemicals with endocrine disruptive potential.



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356 **Fig. 3 Spatial profiles of biological potentials in Danube river water, derived from passive sampler**
357 **data and expressed as BEQ_{bio} of a respective reference compound; SR silicone rubber**
358 **samples, ED Empore™ disc samples; S1,2 – stationary sampling site; 1-8 sampled river**
359 **stretches (mean±SEM).**

360

361 EC values for the SR and ED extracts from both stationary samples and sampled stretches are compared
362 in Fig. 4. On average for each bioassay, the ED sample EC values were 3.5 to 12.3 times lower than the
363 SR samplers. This indicates that ED samplers were correspondingly more effective in the collection of
364 bioactive chemicals across all bioassays which detected significantly more biological potential. Thus,
365 the assessed endpoints were most likely elicited mainly by polar chemicals, for which the uptake
366 capacity in SR samplers is low. While this is not surprising for chemicals that act as inducers of oxidative
367 stress, endocrine disruptors or PXR-activators, the AhR-mediated effect has been often attributed to
368 more hydrophobic compounds, such as polychlorinated dibenzo-*p*-dioxins and furans or some PCB
369 congeners and PAHs. However, it has been shown that a considerable part of the AhR-mediated

activity is elicited by polar chemicals in water and sediment extracts (Jalova et al., 2013; Liu et al., 2014). Anyway, the greatest difference between BEQ_{bio} of SR and ED samplers was observed for estrogenicity where EC values of ED samples were twelve times lower on average. Estrogenicity was thus the endpoint where non-polar compounds contributed the least to the overall biological potential of river water.

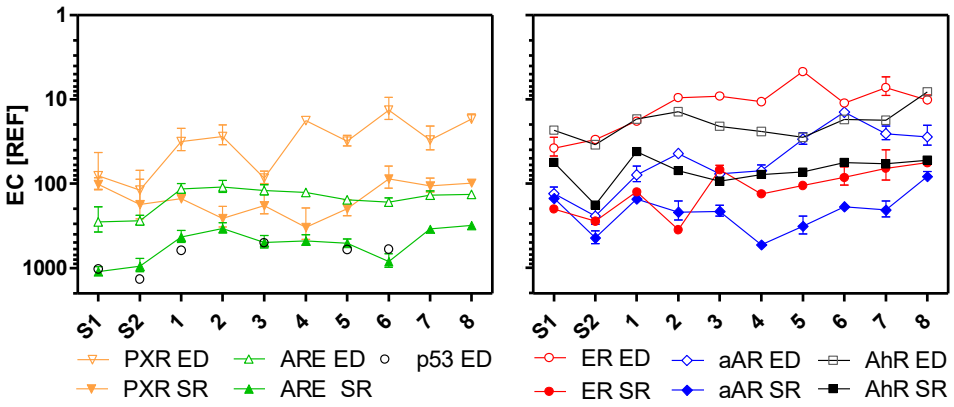


Fig. 4 Effective concentration (EC) values of samples from dynamic passive sampling expressed as relative enrichment factors of the samples (REF). EC_{20} of PXR, ER, AhR-mediated effects, anti-androgenic effect (aAR) and $EC_{IR1.5}$ of ARE and p53-mediated effects; S1-2 samples from stationary exposure of the samplers downstream of Bratislava (Slovakia); 1-8 samples from a mobile sampling of river stretches; SR silicone rubber sampler, ED Empore™ disc sampler.

BEQ_{bio} values detected at samples collected during stationary exposure of the DPS device in a sampling site, which was located at the interface of the mobile sampled stretches 1 and 2, were among the lowest observed values in the study for all assessed endpoints in the case of ED (Fig. 3). Even when comparing data from stationary sampling site and stretch 1, which was directly upstream, the BEQ_{bio} values of the ED sample were lower for the stationary samples. This might indicate that mobile sampling along the river stretches was more effective at sampling bioactive chemicals. On the other hand, sampling at the stationary site took more than twice as long as in the mobile sampling and it is possible that due to the longer exposure time at least some of bioactive chemicals reached equilibrium between the ED samplers and sampled water. Thus, the calculated sampled water volume would be overestimated for such chemicals and so the BEQ_{bio} could be underestimated in the stationary exposure samples. On the other hand, due to the longer sampling period, some non-polar chemicals such as benzo(*k*)fluoranthene, benzo(*ghi*)perylene or some brominated diphenyl ethers, whose

concentrations were below method LOQ in samples from the mobile sampling, were quantified in the stationary sampling site (Tab. S2, S3).

The AhR-mediated BEQ_{bio} levels in the samples were 3.8 –14.6 and 11.5–60.1 pg L^{-1} for SR and ED, respectively (Table 3). For this endpoint, van der Oost et al. (2017) has proposed an effect-based trigger value (EBT; i.e. the BEQ_{bio} level with a potential ecological health risk, of 150 ng L^{-1} of benzo(*a*)pyrene equivalent). When transformed to the corresponding data format using the REP value of benzo(*a*)pyrene (Tab. S5), our data are relatively close to the proposed EBT limit and even exceed the value in ED sample from the stretch 8 (20–76 and 60–300 ng L^{-1} of benzo(*a*)pyrene -equivalent for SR and ED, respectively).

The observed estrogenicity BEQ_{bio} ranged 2–15 and 44–320 pg L^{-1} in SR and ED samples, respectively. The concentrations in ED are relatively close to an annual average environmental quality standard of 400 pg L^{-1} for 17β-estradiol proposed by the European Commission (European Comission, 2015) as well as the previously proposed EBT values (Jarošová et al., 2014; van der Oost et al., 2017). Jarošová et al., who derived a safe BEQ_{bio} level for water burdened by wastewater treatment plants effluents using *in vivo* estrogenic PNECs, proposed the long-time exposure EBT of 200 pg L^{-1} specifically for the MELN bioassay employed in the current study. Van der Oost et al. (2017) derived an EBT of 500 pg L^{-1} BEQ_{bio} from LOEC values for the ERα CALUX bioassay. Thus, our results show that estrogenic activity in the Danube river did not exceed the proposed EBT values, with the exception of stretch 5 in the case of the BEQ value proposed by Jarošová et al. (2014). On the other hand, even the Danube with its great dilution capacity contains estrogenic BEQ_{bio} levels that are close to or, in case of stretch 5 (Romania, Serbia), exceed proposed EBT values.

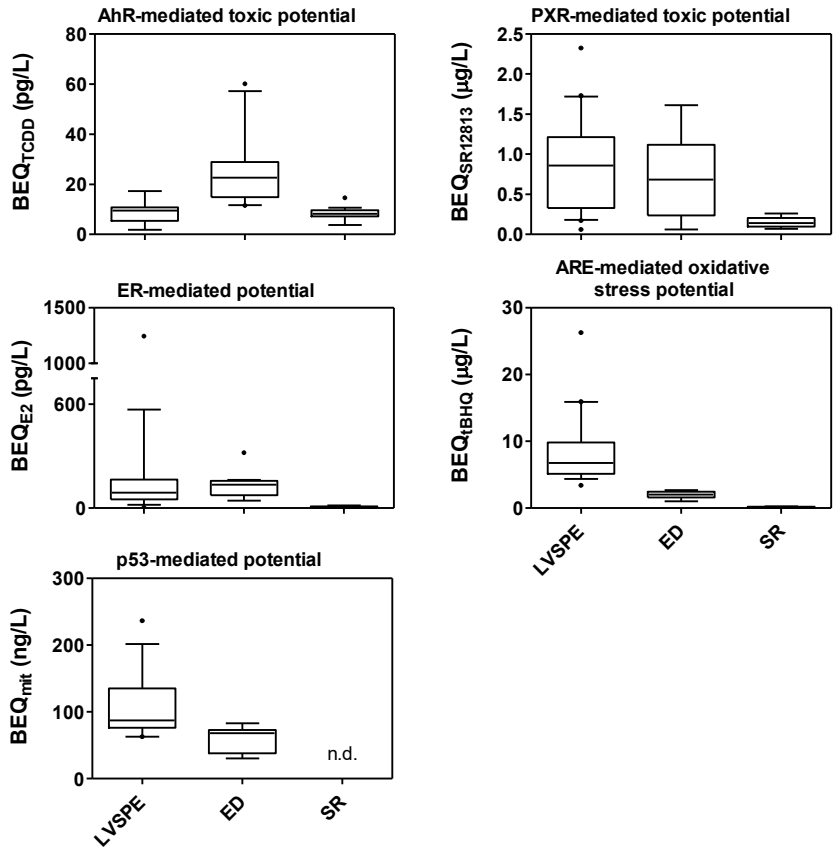
The AR-mediated effect was assessed both in agonistic and antagonistic mode (see SI 1.4), but no androgenic effects were detected (data not shown) by passive nor large volume solid-phase extraction (LVSPE) sampling during JDS3 (Liska et al., 2015). In fact, androgenic effects in surface water were described to be associated with wastewater effluents, while anti-androgenic effects mostly prevail in the less-impacted surface waters (Jalova et al., 2013; König et al., 2016; Neale et al., 2017b). Antiandrogenic flutamide BEQ_{bio} was 63 –432 and 132–2,707 ng L^{-1} for SR and ED samples, respectively. Proposed EBT value for anti-androgenicity based on the AR CALUX assay is 25,000 ng L^{-1} (van der Oost et al., 2017), so the detected levels should be safely below the EBT value, even though there might be some difference in sensitivity between the assay used for the EBT value estimation and MDA-kb2 assay employed in the present study.

3.3.1. Comparison of BEQ_{bio} from passive and active sampling

Besides passive sampling by DPS, the LVSPE approach for spot sampling was employed during the JDS3 expedition cruise on the Danube River (Fig. S3; Neale et al., 2015a). In this method, suspended particles were first removed by flow-through centrifugation before extraction of water and so, similarly to passive sampling, the active sampling method collected mainly the dissolved fraction of chemicals that can serve as an estimate of C_{free} for chemicals with log K_{ow} lower than five (Prokeš et al., 2012). The LVSPE samples were assessed using a similar bioassay battery as in the current study. Thus, it was possible to compare BEQ_{bio} of samples obtained by both sampling methods. Nevertheless, DPS samples reflect pollutants integrated along river stretches, while LVSPE was based on spot sampling, which did not representatively cover the stretches, thus individual samples could not be compared side by side. Therefore, it was only possible to compare the two sampling approaches using aggregated data from both studies. According to this comparison, the determined endocrine-disruptive and xenobiotic metabolism initiating potential of mixtures of compounds from passive sampling corresponded very well with LVSPE data considering the difference in sampling approaches (Fig. 5). The comparison of data from passive and active LVSPE sampling independently confirm that the calculation of sampled water volume by passive samplers was sufficiently accurate and representative to characterize the toxic potential of most bioactive chemicals contributing to these effects. Overall, BEQ_{bio} of samples from LVSPE correspond much better with ED samplers than SR samples. It is not surprising since the SR sampler is designed mainly for non-polar chemicals, and the assessed biological endpoints are triggered rather by polar chemicals. On the other hand, non-polar chemicals tend to bioaccumulate, which can increase their potential to elicit chronic toxic effects in organisms *in situ*. Anyway, for active LVSPE and passive ED sampling, the ranges and medians of the BEQ_{bio} for estrogenic and PXR-mediated potentials were in very good agreement and so both sampling methods seem to be similarly efficient for sampling of chemicals with these modes of action. AhR-mediated potential in SR samples corresponded very well to LVSPE samples, while it was on average more than two times higher in ED samples. No such effect was observed for the other endpoints that are more sensitive to polar chemicals.

ARE-mediated oxidative stress potential was on average three-times lower in the ED samples compared to LVSPE. The difference can be at least partly caused by the fact that LVSPE sample extracts were assessed for ARE-mediated potential using ARE-bla bioassay, which has differing sensitivity compared to the AREc32 assay employed in the current study (Stalter et al., 2016). Another adaptive stress response bioassay detected the p53-mediated effect in six out of ten ED samples with significantly higher $EC_{IR1.5}$ than in the LVSPE samples (median $EC_{IR1.5}$ of 609 and 173 REF, respectively), while no response was detected for the SR extracts. Thus, neither ED nor SR were effective samplers

for chemicals affecting the p53 activity. The NF-kB bioassay detected quantifiable effects only in LVSPE samples, but not in the passive samples. While the LVSPE water samples needed relatively low pre-concentration to elicit quantifiable effect, the passive samples were without any effect even at much higher pre-concentration levels. The low ARE and p53-mediated potentials and non-quantifiable NFκB-associated effects in the ED extracts indicate the differences in the spectrum of sampled chemicals between passive and LVSPE sampling. Apparently, the applied passive samplers are less suitable for sampling compounds causing adaptive stress responses. For LVSPE sampling, a combination of several adsorbents was used to quantitatively retain compounds with a very broad range of polarity, as well as neutral and charged chemicals (Neale et al., 2015a). In contrast, EDs consisted of the SDB-RPS sorbent material that retains mainly hydrophobic compounds, non-ionized polar compounds and organic cations. Since the SDB-RPS phase contains sulfonic acid functional groups, secondary sorption interactions can occur especially with basic compounds that contain amine functional groups. On the other hand, this sorbent is known to have a limited capacity for anionic compounds or dissociating compounds present dominantly in anionic form (Kaserzon et al., 2014) at neutral or slightly alkaline pH such as in the Danube river. The compounds with a low uptake capacity from neutral water include acidic compounds, such as most carboxylic acids (e.g. acidic herbicides or pharmaceuticals, but also naturally occurring carboxylic acids), some phenols, sulfinic and sulfonic acids and anions in general.



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479 **Fig. 5 Comparison of bioanalytical equivalent concentrations in river water (BEQ_{bio}) calculated**
480 **from passive and active sampling throughout all sampling sites and stretches; LVSPE active**
481 **sampling (large volume solid phase extraction), SR silicone rubber passive samplers, ED**
482 **Empore™ discs passive samplers; n.d.- not detected; data on active sampling taken from Neale**
483 **et al. (2015a); the box in the graph consists of two quartiles divided by median, the ends of the**
484 **whiskers represent the 10th and the 90th percentile and the dots individual outliers.**

485

486 ### 3.3.2. Contribution of detected chemicals to the biological potentials

487 To compare data from chemical and biological analyses, levels of detected chemicals were converted
488 to BEQ_{chem} using the concentration addition concept. We used REP values either from our own
489 experiments, from the peer reviewed literature, or the ToxCast database (Tab. S5). For some
490 chemicals, we were not able to find REP values assessed with the same bioassay as in the current study.
491 In these cases, use of REP values from an alternative analogous bioassay is indicated in Tab. S5 together
492 with EC values that were applied for REP calculation because these values differed among literature
493 sources.

Unfortunately, information on toxicological properties of many of the detected chemicals and their bioactivity in the studied endpoints is not available (Fig. S4), which limited the assessment of their potential contribution to the overall biological response. As soon as possible, the most widespread and abundant pollutants need to be prioritized for the characterization of their bioactivities to improve the mass-balance calculations and explanation of the observed effects.

Between 0.7 to 7.9 and 0.3 to 2% of BEQ_{bio} for SR and ED samples, respectively, was explained by detected chemicals for the AhR-mediated response (Table 3). The main portion of BEQ_{chem} was contributed by PAHs (namely benzo(*k*)fluoranthene, benzo(*b*)fluoranthene, benzo(*j*)fluoranthene and chrysene) in the SR samples and benzo(*k*)fluoranthene, terbuthylazine, propiconazole and 4-nonylphenol in ED (Fig. 6). A higher portion of the BEQ_{bio} was explained in samples from the stationary exposure because of benzo(*k*)fluoranthene, whose concentration was below LOQ in the samples from the mobile sampling. The detection of this chemical in the stationary samples is likely caused by the longer integrative sampling at the stationary site which allowed sampling of its quantifiable level.

Less than 0.2% of PXR-mediated BEQ_{bio} was explained in the SR samples, with 4-nonylphenol as the main contributor (Fig. S5). In the ED samples, less than 0.4% of the BEQ_{bio} was explained, with 4-t-octylphenol, estrone, clotrimazole and metolachlor the main contributors of toxicity. Neale et al. (2015a) observed a similar level of explicability with samples from the parallel active sampling and also identified metolachlor as the main driver. Thus, either the more significant PXR activators were not identified yet or PXR is not very specific and it is activated by a large number of weak agonists. It might be also possible that synergistic interaction plays a role in the low explicability of this endpoint because it has been described that the ligand-binding domain of PXR can accommodate more than one weak agonist at the same time (Delfosse et al., 2015).

While estrogenic BEQ_{bio} was explained by 0.2–3 % with bisphenol A, 4-nonylphenol, and 4-t-octylphenol as significant contributors in SR, 25 to 104% of estrogenicity was explained in the ED with estrone, 17β-estradiol and estriol, which showed very limited accumulation in SR (unpublished data). Other chemicals played a minor role. This supports the importance of steroids as water contaminants that play the most significant role in estrogenicity of waste and surface waters (Miège et al., 2009).

Anti-androgenicity was explained mostly by less than 4% and 6% in the SR and ED sampler extracts, respectively. The main contributors were tris(1,3-Dichloro-2-propyl)phosphate, bisphenol A, 4-t-octylphenol and 4-nonylphenol in SR extracts. The main identified contributors in ED extracts were clotrimazole, 4-t-octylphenol and ketoconazole. The rest of 19 identified anti-androgens did not contribute significantly.

ARE-mediated oxidative stress BEQ_{bio} was explained by less than 0.3 % with benzo(*b*)fluoranthene, 4-nonylphenol and benzo(*a)*pyrene in SR. In ED samples, less than 0.05 % of the BEQ_{bio} was explained and the main contributors were bisphenol A, 4-nonylphenol, propiconazole and atrazine in case of extracts from mobile sampling together with benzo(*b*)fluoranthene and benzo(*a)*pyrene in case of samples from stationary exposures. Similarly limited fraction of oxidative stress-mediated effects explained by detected chemicals was reported in the parallel active sampling study using an analogous ARE-bla bioassay (Neale et al., 2015a). Nevertheless, oxidative stress is probably elicited by a large number of diverse chemicals so it is not likely that there would be only a few drivers of this effect explaining a considerable portion of the BEQ_{bio} in most environmental chemical mixtures. While p53-response was below LOQ in SR samples and it was quantified in six of ten ED samples (Table 3), the detected chemicals allowed calculation of BEQ_{chem} (Fig. S6). The calculated BEQ_{chem} levels were lower than 0.004 and 0.015 ng L^{-1} for SR and ED samplers, respectively, which was below LOQ of the bioassay (median value 31 and 59 ng L^{-1}, respectively). BEQ_{chem} explained less than 0.02% of the BEQ_{bio} levels in the ED samples with the quantifiable response. This was comparable with data from LVSPE sampling where BEQ_{chem} explained 0.004 – 0.07 % of BEQ_{bio} (Neale et al., 2015a).

Our data document that employed passive sampling worked well for capturing chemicals responsible for the common studied endpoints such as AhR-, ER and AR-mediated activity, which are known to be associated with numerous known pollutants. On the other hand, in case of adaptive stress endpoints, namely p53- and NF-kB-mediated response, the effectiveness of the passive sampling was lower compared to the LVSPE sampling. Nevertheless, the drivers of these responses in surface waters are mostly unknown. For example, none of the 272 analyzed compounds in LVSPE samples were known to induce NF-kB according to the ToxCast database (Neale et al., 2015a). This was also documented by the low explicability of the observed BEQ_{bio} by the detected chemicals in case of p53-mediated genotoxic potential and ARE-mediated response despite the relatively large number of chemicals that were taken into account. Considering the selectivity of the used ED sampler, the chemicals present in water in an anionic form could contribute to the effects selectively detected in LVSPE samples. To better capture these types of chemicals, it is possible to broaden the applicability range of Empore™ disk-based passive samplers to increase their capacity for binding anionic compounds. There is a variant of Empore™ disk, Empore™ Anion-SR available that is, similar to SDB-RPS, also based on polystyrene divinylbenzene particles, but contains additional strong anion exchange (SAX) functional groups. This disk variant was specifically designed for the extraction of acidic compounds (e.g., carboxylic acids) from water samples. For sampling even broader range of compounds, including the dissociating acids and bases, SDB-RPS and Anion-SR Empore™ disks could be exposed side by side and their extracts combined to make a composite sample.

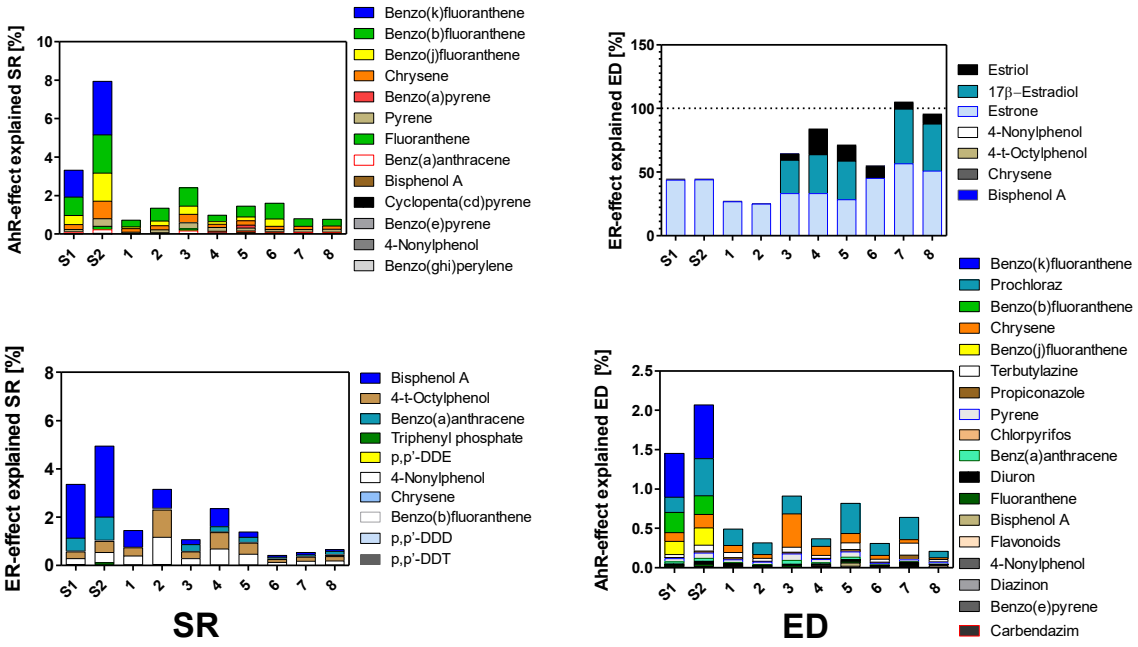

Fig. 6 Percentage of the bioanalytical equivalents (BEQ_{bio}) of ER and AhR- mediated effects explained by the detected chemicals (BEQ_{chem}) in samples from a dynamic passive sampling of the Danube river; data from other bioassays are in Fig. S5 and S6; SR silicone rubber samples, ED Empore™ discs samples; S1,2 – stationary sampling site; 1-8 sampled river stretches.

4. Conclusions

The DPS system provided a representative picture of the pollution situation at the studied site and along the defined river stretches. The system could thus be suitable for sampling of transects of large water bodies including rivers, lakes or seas. DPS device has effectively increased the sampling rate of the passive samplers and this approach allowed toxicological profiling and detection of many chemicals present in the water down to pg L^{-1} range in a short sampling time of several days. An integrated approach combining passive sampling with chemical and biological analyses was shown suitable for spatial profiling of a relatively less polluted river. Our data show that passive sampling should not be limited to only one type of sampler. The complementarity of the samplers for hydrophilic and hydrophobic compounds has been clearly demonstrated. While the data from bioanalysis, which provides information on the sampled pollutant mixtures, indicated that most of the detected biological

effects were elicited by hydrophilic chemicals, the hydrophobic compounds have a higher bioaccumulative potential that could increase their relevancy for chronic impact on the exposed river ecosystem. However, besides estrogenicity only small portion of the biological effects could be explained by analyzed chemicals. This finding is also influenced by the lack of data on the biological potencies of detected chemicals. Comparison of the data from passive and active sampling indicates that the sampling rate of passive samplers for compounds contributing to endocrine-disruptive and xenobiotic metabolism potential can be relatively accurately estimated and confirms that both sampling approaches provide efficient means to monitor these chemicals. While LVSPE provides site-specific information, DPS enables integrative characterization of the pollution situation over space and time depending on the design of its employment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at:

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Table 1 Overview of bioassays in the study

Bioassay	Endpoint	Positive reference compound	Method reference	Data evaluation method	EC value
CAFLUX-H4G1.1c2	Activation of AhR	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)	(Nagy et al., 2002)	Log-logistic dose-response model	EC_{20}
MDA-kb2	Activation / Inhibition of AR	Dihydrotestosterone / Flutamide	(Wilson et al., 2002)	Log-logistic dose-response model	IC_{20}
HG5LN-hPXR	Activation of PXR	SR 12813[1]	(Lemaire et al., 2006)	Log-logistic dose-response model	EC_{20}
MELN	Activation of ER	17β-Estradiol	(Balaguer et al., 1999)	Log-logistic dose-response model	EC_{20}
AREc32	Oxidative stress response	tert-Butylhydroquinone (tBHQ)	(Escher et al., 2012; Wang et al., 2006)	Linear concentration-effect curve	$EC_{IR1.5}$
p53RE-bla	p53 response	Mitomycin	(Neale et al., 2015b)	Linear concentration-effect curve	$EC_{IR1.5}$
NF-κB-bla	NF-κB response	Tumor necrosis factor alpha (TNFα)	(Jin et al., 2015)	Linear concentration-effect curve	$EC_{IR1.5}$

[1]Tetraethyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)ethenyl-1,1-bisphosphonate

Table 2 Sum concentrations (pg L^{-1}) of groups of chemicals detected in samples from dynamic passive sampling; 1-8 samples from mobile sampling of river stretches; SR silicone rubber sampler, ED Empore™ disc sampler; No. number of analyzed chemicals in the category; <LOD concentrations below the limit of detection; for molar concentrations or individual chemical levels see SI; CUPs-current use pesticides, PAHs- polycyclic aromatic hydrocarbons, PCBs- polychlorinated biphenyls, OCPs- organochlorinated pesticides, BDEs- brominated diphenyl ethers, NBFRs- novel brominated flame retardants

Sampler	Chemicals	No.	S1	S2	1	2	3	4	5	6	7	8
ED	ATBs	31	12,670	12,230	11,476	23,922	23,126	17,393	21,699	12,656	33,195	29,262
	Cardiovascular	15	14,183	14,964	11,748	19,693	21,649	11,367	11,730	7,262	10,764	11,026
	Psychoactive	30	25,439	21,421	17,860	28,957	26,290	18,019	24,001	24,855	33,596	28,750
	Antihistamins	8	255	91	275	<LOD	112	348	242	<LOD	583	<LOD
	Antifungals	8	3,260	4,898	4,121	8,291	8,146	8,476	10,560	10,466	10,582	9,297
	Antidiabetics	4	31	4	32	42	98	35	20	21	97	27
	Statins	4	289	318	286	400	1,259	1,031	1,219	418	590	507
	Other pharm.[1]	8	290	248	<LOD	<LOD	122	533	<LOD	295	<LOD	<LOD
	CUPs	40	17,543	28,746	33,152	20,492	32,509	17,067	27,033	28,373	67,140	41,961
	Alkylphenols	3	7,630	7,790	12,225	12,484	15,209	26,311	34,513	20,982	22,399	19,817
	PAHs	29	6,274	7,541	9,633	12,134	22,411	17,384	8,111	8,007	6,981	12,382
	Steroids	5	176	198	201	351	582	626	1,151	398	770	816
	Phytosterols	11	72,010	52,776	29,885	45,964	49,994	34,753	47,458	46,209	48,996	50,809
	Flavonoids	8	798	955	954	636	806	2,916	1,990	693	3,211	10,329
SR	PAHs	29	17,342	23,000	18,215	21,063	51,067	37,296	15,975	12,002	13,965	20,704
	PCBs	7	217	244	171	291	167	172	369	158	307	295
	OCPs	12	188	223	156	244	372	313	358	465	808	1,872
	BDEs	9	4	6	22	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	NBFRs	15	7	13	23	25	15	9	17	22	5	12
	Musks	6	18,582	27,839	11,240	11,894	18,317	17,915	25,395	10,060	13,471	9,732
	Alkylphenols	3	2,932	3,526	5,749	6,270	7,959	10,012	8,511	3,525	7,278	6,236
	Alkylphosphates	14	186,725	213,440	98,815	121,763	111,142	79,046	104,446	147,234	139,681	148,697

[1] furosemide, alfuzosin, naloxone, ranitidine

Table 3 Toxic potentials of samples assessed with bioassays expressed as bioanalytical equivalents of respective reference compound (BEQ_{bio} ± SD) and bioanalytical equivalents predicted from chemical analyses (BEQ_{chem}) with percent of BEQ_{bio} explained by chemical analyses in brackets. AhR – aryl hydrocarbon receptor–mediated response (tetrachlorodibenzo-*p*-dioxin); ER – estrogenicity (17β–estradiol); anti-AR – antiandrogenicity (flutamide); PXR – pregnane X receptor-mediated response (SR12813).

		sample No.	S1	S2	1	2	3	4	5	6	7	8
endpoint	sample		Static exposure 1	Static exposure 2	Passau–Bratislava	Bratislava–Budapest	Budapest–Vukovar	Vukovar–Belgrade	Belgrade–Turnu-Severin	Turnu-Severin–Ruse	Ruse–Braila	Braila–Tulcea
AhR [pg L^{-1}]	SR	BEQ_{bio}	9.7 ± 3.5	3.8 ± 1.1	14.6 ± 5	9.7 ± 3.4	6.6 ± 1.9	7.5 ± 1.5	8.2 ± 2.5	7.2 ± 1.9	7.7 ± 0.8	10.6 ± 3.1
		BEQ_{chem}	0.32 (3.3 %)	0.3 (7.9 %)	0.1 (0.7 %)	0.13 (1.3 %)	0.16 (2.4 %)	0.07 (0.9 %)	0.11 (1.4 %)	0.11 (1.6 %)	0.06 (0.8 %)	0.08 (0.7 %)
	ED	BEQ_{bio}	15.5 ± 1	11.5 ± 1.1	23.2 ± 5.4	30.6 ± 10.2	22 ± 4.9	21.4 ± 0.6	12.9 ± 3.9	28.3 ± 7.7	26.7 ± 7.6	60.1 ± 20.4
		BEQ_{chem}	0.249 (1.6 %)	0.265 (2.3 %)	0.141 (0.6 %)	0.131 (0.4 %)	0.267 (1.2 %)	0.11 (0.5 %)	0.135 (1 %)	0.107 (0.4 %)	0.195 (0.7 %)	0.163 (0.3 %)
PXR [ng L^{-1}]	SR	BEQ_{bio}	211 ± 73	148 ± 103	134 ± 23	83 ± 32	123 ± 48	72 ± 41	104 ± 32	261 ± 108	205 ± 68	206 ± 36
		BEQ_{chem}	0.04 (0.02 %)	0.07 (0.05 %)	0.09 (0.07 %)	0.09 (0.11 %)	0.12 (0.09 %)	0.14 (0.2 %)	0.12 (0.11 %)	0.05 (0.02 %)	0.11 (0.05 %)	0.1 (0.05 %)
	ED	BEQ_{bio}	314 ± 206	200 ± 115	685 ± 281	773 ± 292	237 ± 56	1,118 ± 148	644 ± 130	1,613 ± 670	720 ± 318	1,173 ± 207
		BEQ_{chem}	0.39 (0.2 %)	0.46 (0.4 %)	0.49 (0.1 %)	0.61 (0.1 %)	0.85 (0.4 %)	0.98 (0.1 %)	1.52 (0.2 %)	0.84 (0.05 %)	1.23 (0.2 %)	1.03 (0.1 %)
ER [pg L^{-1}]	SR	BEQ_{bio}	3.6 ± 0.2	2.6 ± 0.4	5.7 ± 0.7	2 ± 0.1	10.6 ± 1.9	5.4 ± 0.2	6.8 ± 0.8	11.2 ± 1.5	15.6 ± 2.4	12.6 ± 0.2
		BEQ_{chem}	0.12 (3.3 %)	0.12 (4.8 %)	0.08 (1.4 %)	0.08 (3.7 %)	0.13 (1.2 %)	0.15 (2.7 %)	0.11 (1.7 %)	0.03 (0.3 %)	0.08 (0.5 %)	0.09 (0.7 %)
	ED	BEQ_{bio}	44.4 ± 24	49.7 ± 9	83.2 ± 22	155.5 ± 24	163.9 ± 37	140.1 ± 26	320.3 ± 67	86 ± 38	131.4 ± 17	150.6 ± 49
		BEQ_{chem}	19.7 (44 %)	22.2 (45 %)	22.6 (27 %)	39 (25 %)	105.3 (64 %)	117.9 (84 %)	228.6 (71 %)	47.4 (55 %)	138.3 (105 %)	144.2 (96 %)
anti-AR [ng L^{-1}]	SR	BEQ_{bio}	273 ± 84	74 ± 24	274 ± 100	246 ± 51	258 ± 111	63 ± 11	146 ± 33	202 ± 30	194 ± 36	432 ± 36
		BEQ_{chem}	1.96 (0.7 %)	3.29 (4.5 %)	1.79 (0.7 %)	1.13 (0.5 %)	1.65 (0.6 %)	1.17 (1.8 %)	1.04 (0.7 %)	0.85 (0.4 %)	1.23 (0.6 %)	1.1 (0.3 %)
	ED	BEQ_{bio}	334 ± 51	132 ± 46	599 ± 189	865 ± 234	592 ± 45	595 ± 148	1,640 ± 542	2,707 ± 289	1,849 ± 531	1,708 ± 495
		BEQ_{chem}	7.29 (2.2 %)	8.38 (6.4 %)	9.33 (1.6 %)	15.25 (1.8 %)	17.79 (3 %)	16.41 (2.8 %)	21.29 (1.3 %)	19.42 (0.7 %)	21.48 (1.2 %)	15.23 (0.9 %)

ARE [ng L^{-1}]	SR	BEQ_{bio}	327 ± 230	395 ± 262	782 ± 344	962 ± 520	659 ± 288	827 ± 672	647 ± 351	433 ± 289	1,012 ± 623	1,164 ± 787
		BEQ_{chem}	1.324 (0.41 %)	1.25 (0.32 %)	0.792 (0.1 %)	0.83 (0.09 %)	1.09 (0.17 %)	0.62 (0.07 %)	1.15 (0.18 %)	0.7 (0.16 %)	0.51 (0.05 %)	0.58 (0.05 %)
	ED	BEQ_{bio}	1,181 ± 511	1,054 ± 214	2,540 ± 577	2,724 ± 971	2,449 ± 622	2,219 ± 193	1,838 ± 339	1,726 ± 378	2,063 ± 439	2,071 ± 361
		BEQ_{chem}	0.84 (0.07 %)	1.07 (0.1 %)	0.75 (0.03 %)	0.65 (0.02 %)	0.99 (0.04 %)	0.81 (0.04 %)	1.06 (0.06 %)	1.3 (0.08 %)	1.93 (0.09 %)	1.96 (0.09 %)
p53 [ng L^{-1}]	SR	BEQ_{bio}	<17.5	<16.1	<25.3	<50.9	<30.7	<32	<30.7	<33	<54	<59.6
		BEQ_{chem}	0.0016	0.0045	0.0005	0.0005	0.0005	0.0004	0.004	0.0003	0.0003	0.0004
	ED	BEQ_{bio}	50 ± 1	38 ± 5	83 ± 9	<95	102 ± 16	<60	85 ± 10	86 ± 7	<101	<112
		BEQ_{chem}	0.004 (0.008 %)	0.005 (0.012 %)	0.004 (0.005 %)	0.003	0.005 (0.005 %)	0.01	0.014 (0.017 %)	0.007 (0.008 %)	0.008	0.008

Supporting Information

Effect-based monitoring of Danube river using mobile passive sampling approach

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1 Passive sampling

The DPS devices were deployed on the frontal deck of the expedition ship. For sampling, the device was immersed in a flow-through system that consisted of a 600 L stainless steel tank. The river water in the tank was exchanged at a rate of approx. 3 m^3 h^{-1} by a high-performance pump. The water intake to the chamber was provided by a vertical steel pipe positioned in front of the ship. The water sampling depth was approx. 0.5 m below the water level. Two sets of DPS samplers were employed in stationary sampling site Čunovo (Slovakia) located at the interface of stretches 1 and 2.

The silicone rubber (SR) sampler consisted of a single sheet of Altesil® silicone rubber with dimensions 14×28 cm and 0.5 mm thickness. The mass of a sampler was approx. 23 g and the surface area exposed to water 392 cm^2 (one side of the sheet). Prior to use all SR samplers were Soxhlet-extracted in ethyl acetate for 72 h to remove non-polymerized residues. SR samplers for chemical analyses were spiked prior to exposure with Performance Reference Compounds (PRCs) that were partially released during exposure. The employed PRC mixture contained perdeuterated biphenyl and 13 polychlorinated biphenyl (PCB) congeners that do not occur in technical mixtures (PCB 1, PCB 2, PCB 3, PCB 10, PCB 14, PCB 21, PCB 30, PCB 50, PCB 55, PCB 78, PCB 104, PCB 145, PCB 204). The residual concentration of PRCs was compared with the initial amount of PRCs analyzed in samplers that have not been exposed.

The ED sampler consisted of 10 solid phase extraction disks Empore® SDB-RPS with 47 mm diameter. The mass of a sampler was approx. 3.2 g and the surface area exposed to water was 173 cm^2. Before exposure, Empore discs were washed and conditioned by subsequent immersing in 1) 100 ml acetone; 2) 100 ml isopropanol; 3) 100 ml methanol, 4) 2x 100 ml ultrapure water and kept immersed in ultrapure water until exposure. For this sampler type, PRCs were not used. ED sampling rate was determined indirectly from SR sampling rate and levels of PAHs and 4-nonylphenol that were detected both in SR and ED samples as described in detail in chapter S1.3 and Vrana et al.(submitted).

The sampling was performed only during cruising of the ship or when the ship was anchored outside harbors in areas without visible pollution point sources e.g. wastewater discharges, industrial areas next to the river or sites with visible oil film on the surface of the water. The sampling device was always switched off before entering harbors and resumed upon leaving the harbors. The samplers were mounted into the DPS device directly before sampling and recovered immediately after finishing the sampling. The recovered samplers were placed back into their respective storage containers, stored in a refrigerator at 4°C on board of the ship and transported to the processing laboratory once per week, where they were stored in a freezer at -20°C.

1.1 Sample processing

1.1.1 Silicone rubber (SR) sheets

Before extraction, SR samplers for chemical analyses were spiked with recovery internal standards (RIS; d_8-naphthalene, d_{10}-phenanthrene, d_{12}-perylene, PCB 4, PCB 29, PCB 185, a mixture of ^{13}C BDEs, C_{13} caffeine, d_{13}-alachlor, d_6-diuron, d_{10}-simazine, 4-n-nonylphenol). The SR samplers were extracted for 8 hours in methanol using Soxhlet extraction. The volume of the extract was reduced using Kuderna-Danish (K-D) apparatus and under nitrogen flow to 2 ml. Extracts of samplers intended for bioanalysis were processed in the same way but without spiking any standards. After processing, they were split to aliquots for analysis by the different bioassays. 20 % aliquot of the sample for chemical analysis was used for instrumental analysis by LC/MS methods. The remaining 80 % aliquot of samples for chemical analysis was azeotropically transferred to hexane using K-D apparatus. Aliquots of the extract were divided into vials for different types of GC/MS analysis. The extract aliquots for analysis of PAHs were further cleaned-up by a silica gel column using diethyl ether/acetone elution. The extract aliquots for analysis of organochlorine compounds (OCs), polybrominated diphenyl ethers (PBDEs) and PRCs were purified by a clean-up using activated silica gel modified with sulfuric acid. Following clean-up, addition of internal standards and volume reduction using a K-D apparatus, samples were analyzed using a GC-MS/MS method for indicator PCBs, PBDEs, organochlorinated pesticides (OCPs) and PRCs.

1.1.2 Empore disks

Unlike ED samplers for bioanalysis, the samplers for chemical analysis were spiked with recovery internal standards (c_{13}-caffeine, c_{13}-triclosan, m_8-PFOA, n_8-PFOS, c_{13}-alachlor, d_6-diuron, d_{10}-simazine, deuterated EE2, n-nonylphenol). All samplers where then freeze-dried for 24 hours in the original containers that were used for sample storage and transport. The disks were extracted three times by overnight (12 h) slow shaking at room temperature with 70 ml acetone each. The combined extracts were reduced by vacuum rotary evaporation to 10 mL. After removal of particles by filtration through

a layer of anhydrous Na_2SO_4 the extract was further reduced in volume to approx. 1 mL. The acetone extract was transferred to methanol by addition of methanol (20 mL) and subsequent evaporation and a nitrogen flow to further reduce in volume to 2 mL. The extract was split to aliquots for different types of analysis.

1.1.3 Low-density polyethylene (LDPE) sheets

LDPE samplers from both parallel DPS sampling devices were extracted twice by soaking overnight with *n*-pentane (100 mL). Recovery standards (for PAHs, PCBs and OCPs) were added to the extraction jar during the first extraction. Recovery standards were d_8-naphthalene; d_{10}-biphenyl; d_8-acenaphthene; d_{10}-dibenzothiophene, d_{10}-pyrene, d_{12}-benz[a]anthracene, d_{12}-perylene, PCB 30, PCB 53, and PCB 204. The sample volume was reduced to 2 mL by a gentle stream of nitrogen at room temperature. Extracts were first split into two equal fractions by volume. One fraction was stored as a reserve and the other received a general clean-up using gel permeation chromatography (GPC). This post-GPC sample was again split into two equal fractions by volume; the first of these was reduced in volume using nitrogen and analyzed for PAHs; the second received treatment with 2×1 mL concentrated sulfuric acid, was reduced in volume and analyzed for PCBs and OCPs. Details of the procedure are described in Allan et al. (2013).

1.2 Chemical analysis

SR sampler extracts were analyzed using GC-MS/MS (GC 7890 / MS-MS Triple Quadrupole 7000B (Agilent), equipped with HT8 SGE Analytical Science column for PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180, and OCPs: α-hexachlorocyclohexane (HCH), β-HCH, γ-HCH, δ-HCH, p,p´-DDE, p,p´-DDD, o,p´-DDT and p,p´-DDT, hexachlorobenzene (HCB), and pentachlorobenzene (PeCB). Polycyclic aromatic hydrocarbons were analyzed using GC 7890 / MS5975 (Agilent) equipped with J&W Scientific fused silica DB-5MS column. Detection was performed in single ion monitoring mode, the temperature of the ionic source was 320°C and quadrupole temperature 150°C. One µL sample was injected in splitless mode at 280°C. Helium (purity 5.5) was used as carrier gas at a flow of 1.5 mL min^{-1}. The GC instrument was operated with an initial oven temperature of 80°C (1 min hold), then ramped at 15°C min^{-1} to 180°C and at 5°C min^{-1} to 310°C which was held for 20 min.

PBDEs were analyzed by GC equipped with 15m × 0.25 mm × 0.10 µm RTX-1614 column (Restek, USA). HRMS (AutoSpec Premier) was operated in EI+ mode at the resolution of >10 000.

SR and ED sampler extracts were analyzed for alkylphenols using dansyl chloride derivatization and LC-MS/MS detection according to Pernica et al. (2015). 4 -nonylphenol served as an internal standard. Chromatographic separation was performed using LC Agilent 1200 Infinity Series, equipped with chromatographic column ACE 5 C18, 150 × 4.6 mm i.d., 5 µm particle size (ACE, Scotland, UK). Water containing 7 mmol L^{-1} formic acid (A) and methanol (B) was used as a mobile phase. The isocratic elution of 10 % (A) and 90 % (B) was used at a flow rate of 0.5 mL min^{-1}. The Agilent 6410 Triple Quadrupole (Agilent Technologies, Santa Clara, CA, USA) was used for MS/MS analysis. The instrument was operated in the ESI-positive MRM mode. Two MS/MS transitions were used for analyses.

Chromatographic analyses of alkyl phosphates and polycyclic musks were performed using GC 6890 (Agilent, USA) coupled to MSD 5975 mass spectrometer (Agilent, USA) operated in EI+ mode. Compounds were separated on the column HP-5MS (30m x 0.25mm x 0.25µm film) in selected ion mode (SIM). Helium was used as mobile phase at 1.2 mL min^{-1} at constant pressure. One or two µL of extract were injected in pulsed split-less mode at 280 °C. GC temperature program started at 70 °C

(hold for 2 min), ramped 25 °C min^{-1} to 150 °C (hold for 0 min), ramped 3 °C min^{-1} to 200 °C (hold for 0 min), ramped 8 °C min^{-1} to 280 °C (hold for 10 min).

1.3 Estimation of sampling rates

1.3.1 Silicone rubber samplers

As described in detail in Vrana et al.(submitted), concentrations of chemicals dissolved in water were calculated from amounts of analytes accumulated in SR samplers. Amounts of analytes absorbed by the samplers follow a first-order rate law to equilibrium. Aqueous concentrations were calculated from the mass absorbed by the samplers, the in situ sampling rate (*R*s) of the compounds and their sampler-water partition coefficients (Smedes et al., 2009) as described in Smedes and Booij (2012). Sampling rates were estimated from dissipation of PRCs from SR samplers during exposure using nonlinear least squares method by Booij and Smedes (2010), considering the fraction of individual PRCs that remained in the SR after the exposure as a continuous function of their sampler-water partition coefficient and their molecular mass, with adjustable parameter *B. R*s for a compound accumulated under water-boundary layer control was calculated as a function of its molecular mass $Rs=B\times M^{-0.47}$ (Rusina et al., 2010).

1.3.2 Empore disc samplers

For ED samplers, calibration is based on levels of 4-nonylphenol and 10 PAHs that were detected in both ED and SR samplers throughout all sampled river stretches. Surface specific sampling rates appeared to be well correlated and their values were close to equal. The *Rs* for ED sampler was calculated from *Rs* of SR and their respective sampling areas using equation S1.

$$\frac{R_S\,(ED)}{A\,(ED)} = 0.83\,\frac{R_S\,(SR)}{A\,(SR)} \qquad \text{Equation S1}$$

Thus, the overall ED sampling rate was calculated from SR sampling rate by comparing the levels of PAHs and 4-nonylphenol in SR and ED samplers as the median of sampling rates of the individual chemicals used for the calibration throughout the river stretches (Fig. S; for more details see Vrana et al., submitted).

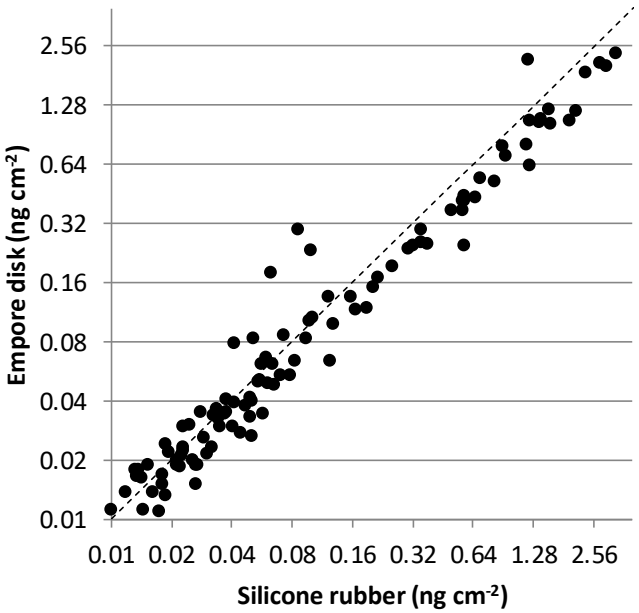

Fig. S1 Comparison of levels of PAHs and 4-nonylphenol per sampling surface of SR and ED samplers from 8 mobile and 2 stationary DPS samples.

1.3.3 Mutual comparison of two co-deployed DPS devices

The two co-deployed DPS devices may exhibit some differences if their pumps differed in volume flow rate. Their mutual comparison was done using a LDPE stripe mounted into each device during each exposure. Sampling performance of the LDPE samplers was assessed by monitoring release of 6 deuterated PAH PRCs during exposure. Since the exposure time t and mass m_x of LDPE stripes deployed in both DPS devices was the same (0.71±0.02 g), from Equation S2 the ratio of sampling rates of a PRC in both devices is related to the ratio of its retained fractions after exposure:

$$\frac{R_{s,TOX}}{R_{s,CHEM}} = \frac{\ln(f_{PRC,TOX})}{\ln(f_{PRC,CHEM})} \qquad \text{Equation S2}$$

where the subscripts 'TOX' and 'CHEM' denote the two co-deployed DPS devices containing samplers intended for toxicological and chemical analysis, respectively. The comparison is relevant only for PRCs for which f_{PRC} was quantifiable and lower than 80 % (Fig. S2).

Those PRCs included d_{10}-FLT and d_{12}-CHR. d_{10}-ACE, d_{10}-FLU, and d_{10}-PHE were released from the samplers to concentrations below their limit of quantification, whereas more than 80 % of d_{12}-BeP remained in the samplers. For d_{10}-FLT and d_{12}-CHR the calculated $R_{s,TOX}/R_{s,CHEM}$ ratio in the eight sampling stretches was 1.00±0.11 and 0.93±0.14, respectively.

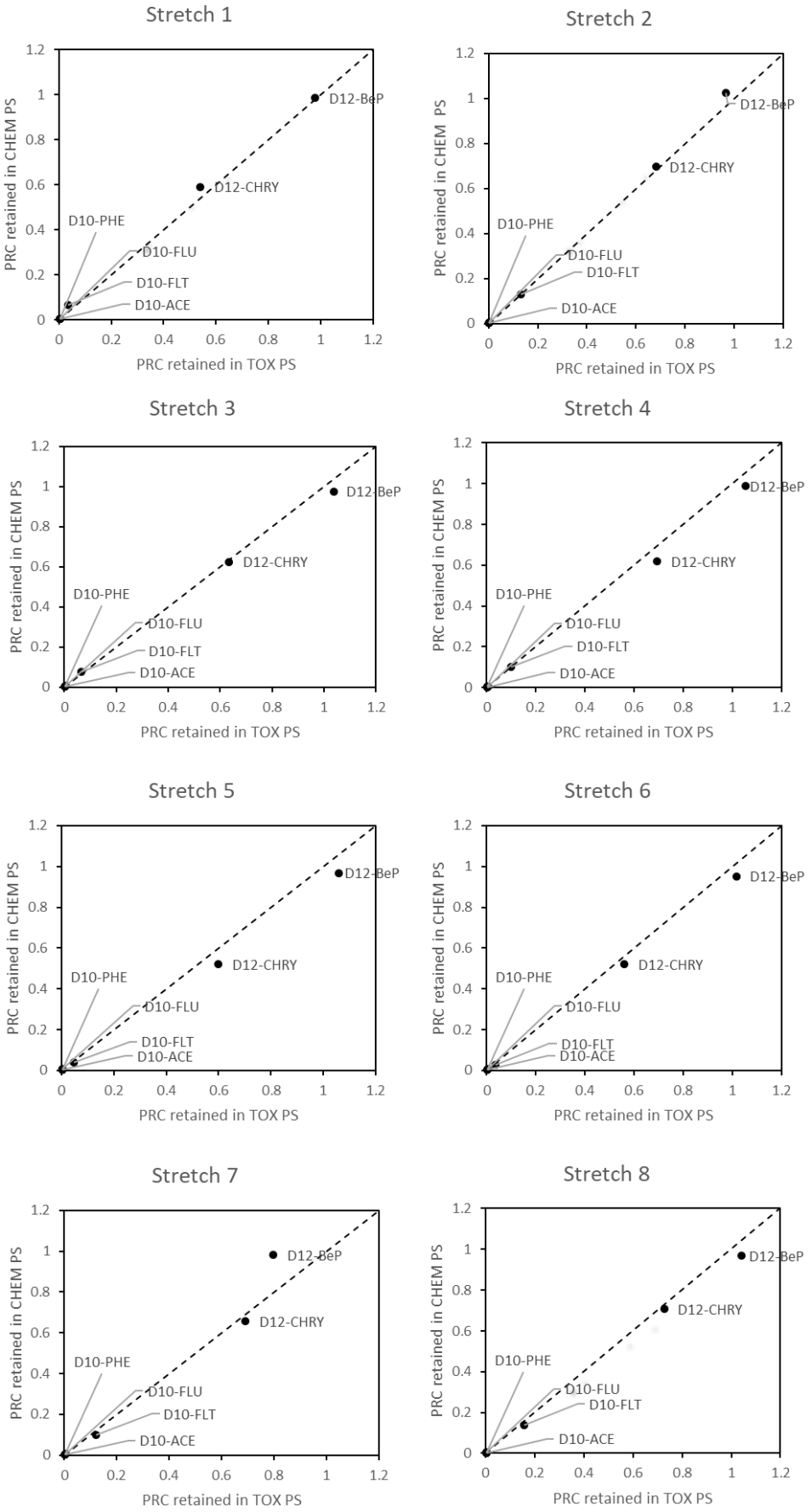

Fig. S2 Comparison of PRC fractions retained in LDPE sheets in two co-deployed DPS devices (TOX PS and CHEM PS) in parallel in 8 Danube stretches

1.4 Bioanalysis

AhR-mediated response (CAFLUX)

This type of toxicity was assessed using the rat hepatoma cell line H4G1.1c2 stably transfected by EGFP under control of dioxin response element. The assay was performed as described in Nagy et al. (2002). Cells were seeded to black clear-bottom 96 well plate in 100 µL of DMEM supplemented with 10% fetal bovine serum (FBS) in density 2×10^5 cells/mL. After 24h incubation at 37°C, dilution series of extracts were added in 100 µL of the cultivation medium with DMSO as a solvent (final concentration 0.5% v/v). After 24 h incubation, the medium was replaced and fluorescence measured. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) served as the positive reference compound. The data were expressed as EC20 using a log-logistic dose-response model. Cytotoxic concentrations of extracts were excluded from the calculation based on viability data assessed with neutral red uptake assay (Freyberger and Schmuck, 2005).

PXR-mediated response (HG5LN-hPXR)

The HG5LN-hPXR assay based on human HeLa cell line served for the assessment of the activation of the human pregnane X receptor (PXR) and was performed according to Creusot et al. (2010) with minor modifications. Briefly, HG5LN-hPXR cells in DMEM without phenol red supplemented with 5% fetal calf serum (FCS) were seeded in a white 96-well plate in 100 µL of cell suspension at a density of 6×10^5 cells/mL and incubated for 24 h at 37°C. The sampler extracts diluted in phenol red-free DMEM supplemented with 3% dextran-coated charcoal-treated fetal calf serum (DCC-FCS) were added and the exposed cells were incubated for a further 16 h. Before luminescence measurement, exposure medium was replaced with 50 µL of medium supplemented with 0.3 mM luciferin. SR 12813 (Tetraethyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)ethenyl-1,1-bisphosphonate) was used as the positive reference compound. The data was expressed as EC_{20} using a log-logistic dose-response model.

(Anti-)androgenicity (MDA-kb2)

Human mammary carcinoma-derived cell line MDA-kb2 was used for the assessment of androgen receptor-mediated responses (androgenicity and anti-androgenicity). The cells were grown in Leibovitz's L-15 medium supplemented with 10 % FBS at 37 °C and normal air with high relative humidity. The experiment was performed in 96-well plates in L-15 medium supplemented with 10% dextran-charcoal-treated FBS. Androgen dihydrotestosterone (DHT) was used as a positive reference compound. For the assessment of androgenic effect, the cells were exposed to samples alone. To assess the anti-androgenic effect, cells were exposed to extracts in combination with the physiological ligand of the AR DHT (0.1 nM). The anti-androgenic effect was quantified using flutamide as model anti-androgen. The androgenic and anti-androgenic potential was expressed as EC_{20} and IC_{20}, respectively, using a log-logistic dose-response model. The viability of the cells in the assay was evaluated using neutral red uptake assay (Freyberger and Schmuck, 2005) and cytotoxic concentrations of the samples were excluded from data evaluation.

Estrogenicity (MELN)

The MELN assay, based on human cell line MCF-7 transfected with the luciferase gene under control of estrogenic response element, was used for the assessment of estrogenicity according to the previously described procedure (Kinani et al., 2010). Briefly, 100 µL of MELN cells suspension with a density of 8×10^4 cells/mL was seeded to a white 96-well plate in phenol red-free DMEM supplemented

with 3% DCC-FCS. After plating at 37°C for 24 h, the cells were exposed to the sampler extracts and incubated for a further 16 h. Before luminescence measurement, exposure medium was replaced with 50 µL of medium supplemented with 0.3 mM luciferin. 17β-estradiol was used as the positive reference compound. The data were expressed as EC_{20} using a log-logistic dose-response model. Cell viability was assessed using the MTT assay.

ARE-mediated oxidative stress response (AREc32)

The MCF7 human breast carcinoma cell line-derived AREc32 assay was applied to assess the activation of the Nrf2 pathway (Wang et al., 2006), with the assay conducted according to Escher et al. (2012). The sampler extracts were blown to dryness, then re-suspended in DMEM with GlutaMAX™ with 10% FBS and serially diluted tert-Butylhydroquinone (tBHQ) was used as the positive reference compound. Cells were exposed in 96-well plates for 24 hours and then luminescence was detected using Luciferase Assay System (Promega E1500) according to the Promega protocol. Using a linear concentration-effect curve, the data was expressed as ECIR1.5 in units of REF, while the effect on cell viability detected with the MTS assay (Mosmann, 1983) expressed as EC10 was determined from a log-logistic concentration-effect curve. $EC_{IR1.5}$ was calculated using equation S3.

$$\mathbf{EC_{IR1.5}} = \frac{\mathbf{0.5}}{\boldsymbol{slope}} \qquad \text{Equation S1}$$

p53-mediated response (p53RE-bla)

The CellSensor p53RE-bla HCT-116 assay (Life Technology, Australia) based on human colorectal carcinoma cell line HCT-116 was used to assess activation of p53 and was conducted according to Neale et al. (2015b). Briefly, the extracts were blown to dryness, then re-suspended in Opti-MEM with 0.5% dialyzed FBS and serially diluted. Eight microliters of the serially diluted extract were added to 32 µL of cells seeded at a density of 9.4×10^5 cells/mL in the black clear bottom 384-well plate. After incubating for 40 h at 37°C, 8 µL of FRET reagent with resazurin was added to each well and fluorescence was measured after incubating for 2.5 h at room temperature. Mitomycin was used as the positive reference compound with $EC_{IR1.5}$ derived from a linear concentration-effect curve and cell viability EC_{10} derived from a log-logistic concentration-effect curve.

NF-κB-mediated response (NF-κB-bla)

The NF-κB-bla assay (Life Technology, Australia) based on the human monocyte THP-1 cell line, was used to assess the activation of NF-κB, which is indicative of inflammation response. The assay was performed as in Jin et al. (2015). The extracts were blown to dryness, then re-suspended in RPMI 1640 media with 10% dialyzed FBS and serially diluted. Eight microliters of the serially diluted extract were added to 32 µL of cells seeded at a density of 6.3×10^5 cells/mL in the black clear bottom 384-well plate. After incubating for 24 h at 37°C, 8 µL of FRET reagent with resazurin was added to each well and fluorescence was measured after incubating for 2 h at room temperature. Tumor Necrosis Factor-alpha (TNFα) was used as the positive reference compound. $EC_{IR1.5}$ values were derived from the linear concentration-effect curve, while the cell viability EC_{10} was derived from the log-logistic concentration-effect curve.

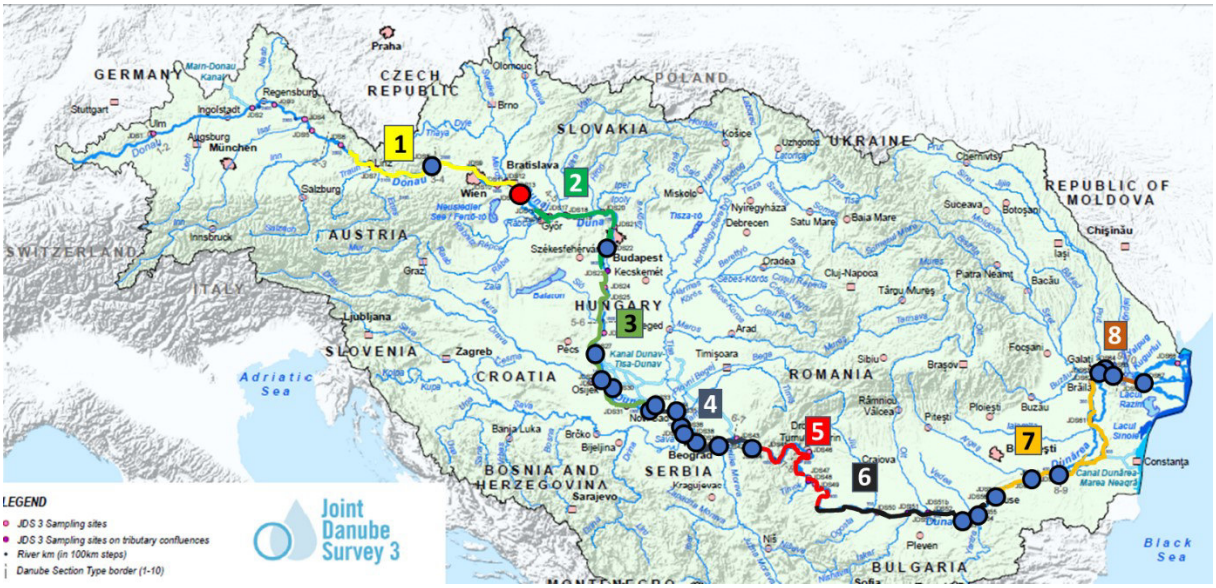

Fig. S1 Map of the sampling cruise with the stretches and stationary exposure site (red dot); blue dots indicate active LVSPE sampling sites from Neale et al. (2015a).

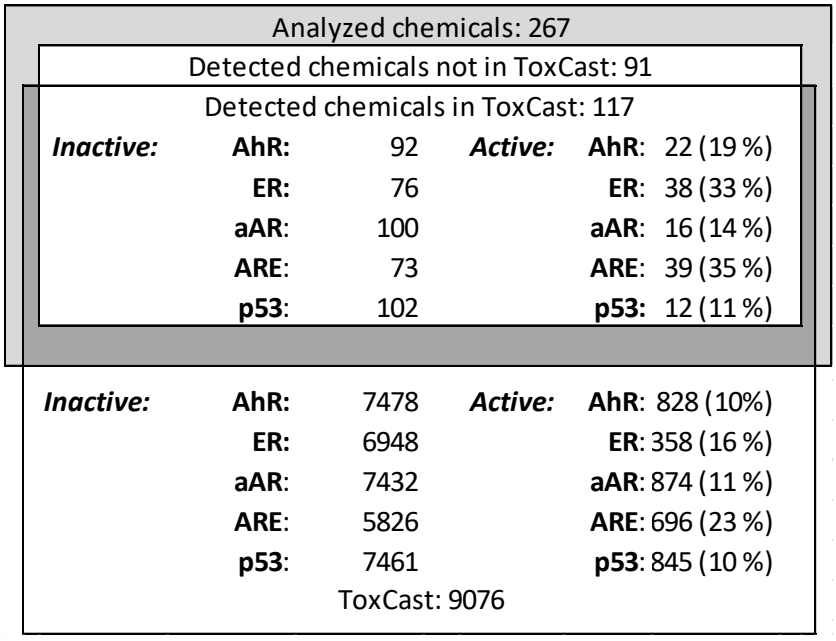

Fig. S2 Overview of detected chemicals described in ToxCast database to be in/-active in eliciting the indicated effects.

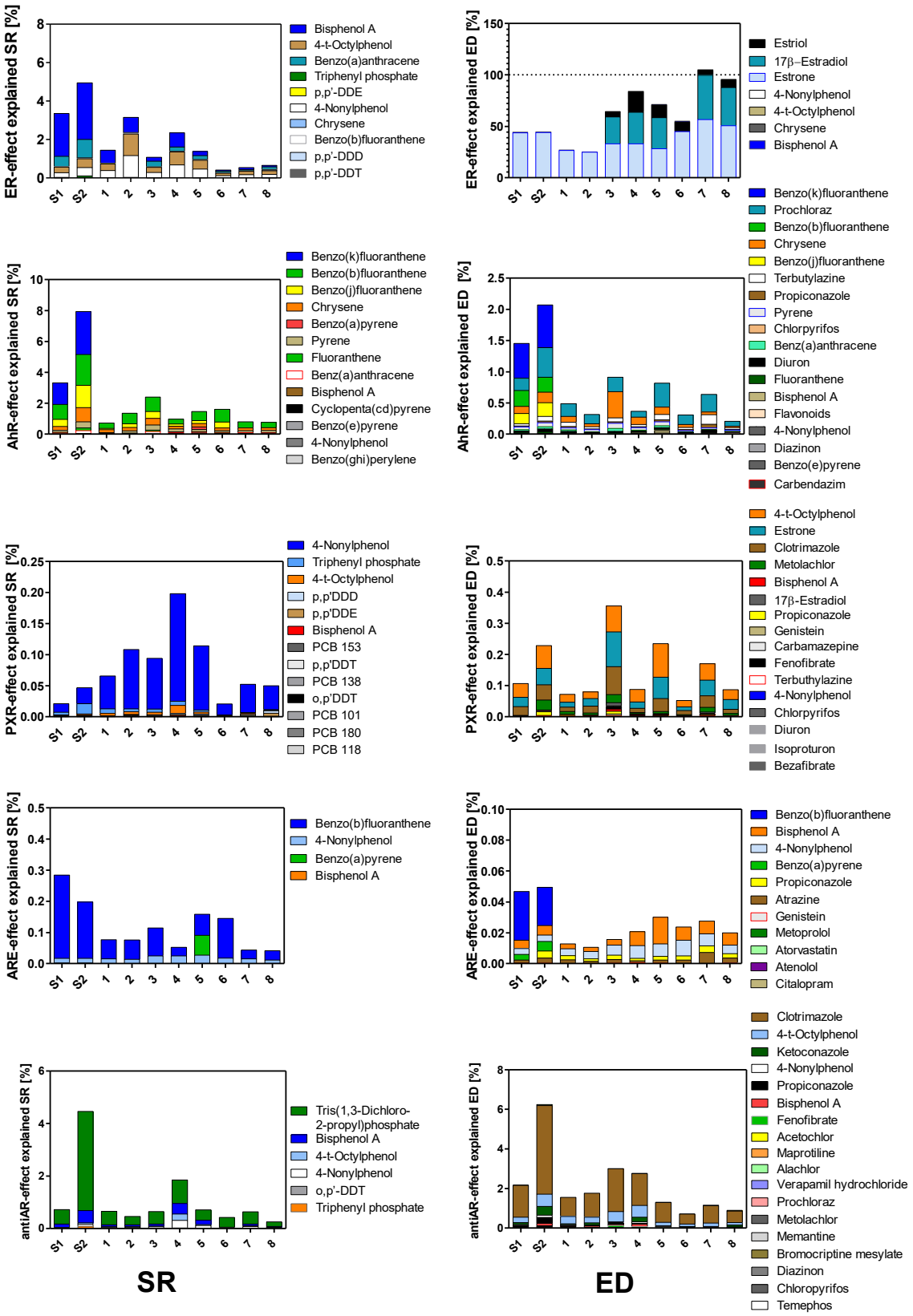

Fig. S3 Percentage of the bioanalytical equivalents (BEQ_{bio}) explained by the detected chemicals (BEQ_{chem}) in samples from a dynamic passive sampling of the Danube river; SR silicone rubber samples, ED Empore discs samples; S1, 2 – stationary sampling site; 1-8 sampled

river stretches; for ARE in ED extracts, 18 main identified drivers of the effect are given (total 42 quantified active chemicals are provided in Tab. S4)

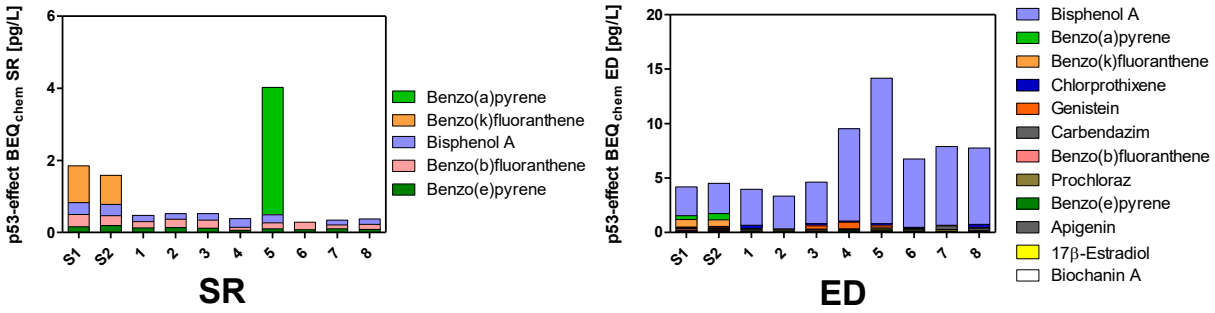

Fig. S4 p53-mediated mitomycin bioanalytical equivalents calculated from detected chemicals levels (BEQ_{chem}) in samples from a dynamic passive sampling of the Danube river; SR silicone rubber samples, ED Empore discs samples; S1, 2 – stationary sampling site; 1-8 sampled river stretches

Tab. S1 River stretches and stationary exposure localities sampled with passive samplers

Stretch/ sample number	Stretch start and end/stationary exposure site	River km	Sampling time span	Mean water temperature [°C]	Exposure time [d]	Parameter AB[b]	Water volume sampled by SR [L]a	Water volume sampled by ED [L][b]
S1	Stationary exposure 1	1,852	19.8.-23.8.	21.3	4	904	245	90
S2	Stationary exposure 2	1,852	23.8.-28.8.	21.3	5	772.6	264	97
1	Passau–Bratislava	2,203-1,852	17.8.-22.8.	21.3	2	1208.8	236	62
2	Bratislava–Budapest	1,852-1,632	22.8.-26.8.	22	1.2	1055	98	31
3	Budapest–Vukovar	1,648-1,297	26.8.-2.9.	21.9	1.7	1179	151	51
4	Vukovar–Belgrade	1,297-1,154	2.9.-6.9.	22.8	1.6	1223.8	117	49
5	Belgrade–Turnu-Severin	1,154-930	6.9.-10.9.	22.1	2	1041.2	112	51
6	Turnu-Severin–Ruse	930-495	11.9.-17.9.	21.9	2	945.1	122	47
7	Ruse–Braila	495-170	17.9.-21.9.	19.2	1.4	821.2	90	29
8	Braila–Tulcea	170-71	21.9.-26.9.	18.7	1.3	791.2	73	26

[a] Volume of water extracted by the SR sampler during exposure; volume is calculated from PRCs levels for a model compound with a molecular mass of 300. [b] Volume of water extracted by ED sampler during sampling; volume estimate based on a comparison of levels of 10 PAHs in SR and samplers as described in 1.3.2.

[b] Exposure specific parameter for calculation of R_s in equations 1; for further details see Vrana et al.(submitted)

Tab. S2 Concentrations [pg/L] of chemicals detected in SR sampler extracts; PAHs - polycyclic aromatic hydrocarbons; BDE - brominate diphenyl ethers

			Stationary exposure		River stretch							
	Analyte [pg/L]	CAS Number	S1	S2	1	2	3	4	5	6	7	8
PAHs	Naphthalene	91-20-3	3,418	4,267	3,260	7,088	8,306	5,157	2,667	2,805	4,968	6,298
	Biphenyl	92-52-4	485	1,264	423	504	4,440	1,236	688	549	616	740
	Acenaphthylene	208-96-8	1,559	1,792	2,712	1,239	2,045	3,069	1,231	315	877	1,603
	Acenaphthene	83-32-9	770	1,173	838	619	11,283	1,942	835	286	279	639
	Fluorene	86-73-7	954	1,349	1,200	1,195	8,728	4,834	1,468	914	930	1,468
	Phenantrene	85-01-8	1,917	2,800	3,086	2,441	5,527	14,755	2,717	2,178	1,975	3,502
	Anthracene	120-12-7	185	206	346	307	571	388	222	144	158	267
	Fluoranthene	206-44-0	3,527	4,440	2,672	3,516	4,659	2,464	2,654	2,371	1,463	2,386
	Pyrene	129-00-0	1,767	2,399	1,609	2,151	3,292	2,192	1,847	1,244	1,470	2,231
	Retene	483-65-8	996	1,155	617	599	358	243	258	119	137	187
	Benzo(*b*)fluorene	243-17-4	223	251	192	181	267	154	170	97	105	163
	Benzonaphthothiophene	205-43-6	82	130	132	71	89	68	61	35	64	83
	Benzo(*ghi*)fluoranthene	203-12-3	144	176	112	117	123	77	105	93	89	119
	Cyclopenta(cd)pyrene	27208-37-3	146	188	135	151	233	90	106	69	81	130
	Benzo(*a*)anthracene	56-55-3	146	188	<6	<6	234	91	106	69	81	131
	Triphenylene	217-59-4	360	442	403	403	371	295	402	384	392	425
	Chrysene	218-01-9	266	375	283	238	307	140	187	136	149	202
	Benzo(*b*)fluoranthene	205-99-2	158	129	87	109	106	41	79	100	51	63
	Benzo(*j*)fluoranthene	205-82-3	64	79	17	33	41	15	24	39	<15	<15
	Benzo(*k*)fluoranthene	207-08-9	34	27	<23	<23	<23	<23	<23	<23	<23	<23
	Benzo(*e*)pyrene	192-97-2	120	146	92	103	88	44	79	55	79	65
	Benzo(*a*)pyrene	50-32-8	<46	<46	<46	<46	<46	<46	70	<46	<46	<46
	Perylene	198-55-0	82	<66	<66	<66	<66	<66	<66	<66	<66	<66
	Indeno(*123cd*)pyrene	193-39-5	<21	<21	<21	<21	<21	<21	<21	<21	<21	<21
	Dibenzo(*ah*)anthracene	53-70-3	<13	<13	<13	<13	<13	<13	<13	<13	<13	<13

	Dibenzo(*ac*)antracene	215-58-7	<18	<18	<18	<18	<18	<18	<18	<18	<18	<18
	Benzo(*ghi*)perylene	191-24-2	21	25	<17	<17	<17	<17	<17	<17	<17	<17
	Anthanthrene	191-26-4	<49	<49	<49	<49	<49	<49	<49	<49	<49	<49
	Coronene	191-07-1	<62	<62	<62	<62	<62	<62	<62	<62	<62	<62
	ΣPAHs 16 US EPA		14,721	19,169	16,092	18,902	45,058	35,073	14,083	10,562	12,402	18,790
	ΣPAHs		17,342	23,000	18,215	21,063	51,067	37,296	15,975	12,002	13,965	20,704
OCs	PCB 28	7012-37-5	49	56	39	91	64	44	190	80	173	141
	PCB 52	35693-99-3	82	94	69	121	59	53	121	16	102	98
	PCB 101	37680-73-2	21	23	16	20	11	22	17	3.3	6.7	24
	PCB 118	31508-00-6	4.2	4.5	3.0	3.9	2.3	2.7	3.5	4.0	2.5	2.9
	PCB 153	35065-27-1	31	33	22	28	16	25	19	30	12	14
	PCB 138	35065-28-2	22	24	16	21	12	19	14	19	8.5	10
	PCB 180	35065-29-3	7.8	8.2	5.5	6.9	3.3	5.0	4.0	5.1	2.6	4.6
	Σ PCB		217	244	171	291	167	172	369	158	307	295
	PeCB	608-93-5	16	20	14	22	96	84	56	23	31	29
	HCB	118-74-1	78	90	62	81	75	41	54	48	97	97
	a-HCH	319-84-6	5.2	6.0	3.7	2.2	3.7	1.6	2.2	7.4	5.0	12.2
	b-HCH	319-85-7	3.6	3.2	8.9	10	9.1	12	14	101	146	259
	d-HCH	58-89-9	2.7	2.4	7.1	9.2	7.6	10	12	84	132	235
	o,p'-DDE	3424-82-6	1.8	1.0	0.9	2.5	2.6	2.8	3.7	3.3	6.6	24.2
	p,p'-DDE	72-55-9	34	33	18	58	54	54	72	71	165	512
	o,p'-DDD	53-19-0	16	22	13	25	49	51	73	54	88	334
	p,p'-DDD	72-54-8	22	39	21	30	60	49	66	69	125	313
	o,p'-DDT	789-02-6	2.3	1.7	1.9	1.4	5.2	2.8	2.2	1.2	2.9	17.9
	p,p'DDT	50-29-3	3.8	3.7	3.3	1.3	4.6	2.7	2.4	2.0	5.5	21.2
	Σ DDT		83	103	61	119	181	165	220	202	396	1,240
	Σ OCPs		188	223	156	244	372	313	358	465	808	1,872
PBDEs	BDE 28	41318-75-6	0.8	0.9	0.5	0.7	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
	BDE 47	5436-43-1	0.8	0.7	6.8	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
	BDE 66	189084-61-5	<0.03	0.04	0.3	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

	BDE 100	189084-64-8	0.07	0.12	0.7	0.8	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
	BDE 99	60348-60-9	0.12	0.31	2.0	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	BDE 85	182346-21-0	0.04	0.03	0.4	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	BDE 154	207122-15-4	0.22	0.42	1.2	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6
	BDE 153	68631-49-2	<0.09	0.21	0.5	1.0	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
	BDE 183	207122-16-5	1.84	3.30	10	<4.3	<4.3	<4.3	<4.3	<4.3	<4.3	<4.3
	Σ WFD BDEs		1.99	2.61	11.6	2.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Σ BDEs		3.86	5.98	22.2	2.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Alkylphenols	Bisphenol A	80-05-7	674	644	338	309	370	494	446	<0.2	251	310
	4-t-octylphenol	140-66-9	93	107	184	145	198	362	138	72	137	85
	4-nonylphenol	104-40-5	2,165	2,775	5,226	5,816	7,391	9,156	7,928	3,453	6,890	5,841
	Σ Alkylphenols		2,932	3,526	5,749	6,270	7,959	10,012	8,511	3,525	7,278	6,236
Musks	Cashmeran	33704-61-9	1,526	1,991	658	977	663	434	<341	<341	<341	<341
	Celestolide	13171-00-1	40	62	<34	<34	<34	54	<34	<34	<34	<34
	Phantolide	15323-35-0	<31	<31	<31	<31	<31	<31	<31	<31	<31	<31
	Traseolide	68140-48-7	87	129	68	70	75	81	74	39	69	<21
	Galaxolide	1222-05-5	17,021	25,063	10,104	10,848	16,722	15,831	22,312	9,183	12,096	8,864
	Tonalide	1506-02-1	1,473	2,648	1,069	976	1,521	2,003	3,008	837	1,307	868
	Σ Musks		18,582	27,839	11,240	11,894	18,317	17,915	25,395	10,060	13,471	9,732
NBFRs	TBECH	3322-93-8	0.941	1.488	1.275	0.756	0.807	0.604	1.243	0.323	0.225	0.230
	p-TBX	23488-38-2	0.018	0.012	0.030	0.039	0.056	0.015	<0.007	<0.007	<0.012	0.056
	BATE	3728-89-5	0.655	0.272	0.418	0.242	0.054	0.083	0.163	0.035	0.054	0.100
	TBCO	3194-57-8	<0.021	<0.019	<0.03	<0.061	<0.037	<0.039	<0.037	<0.04	<0.065	<0.072
	PBBZ	608-90-2	0.060	0.030	0.325	0.083	0.097	0.065	0.056	0.066	0.078	0.156
	TBCT	39569-21-6	0.006	<0.005	0.017	<0.016	<0.01	<0.01	<0.01	<0.011	<0.017	0.110
	PBT	87-83-2	0.090	0.071	0.127	0.136	1.299	0.364	0.184	0.170	0.381	0.565
	PBEB	85-22-3	0.04	<0.0016	<0.0024	<0.0049	0.1	<0.0031	<0.0029	<0.0032	<0.0052	0.2
	DPTE	35109-60-5	3.73	8.61	9.4	2.0	<0.17	<0.18	<0.17	<0.18	<0.3	<0.33
	HBB	87-82-1	0.14	0.13	0.19	<0.028	0.25	0.30	<0.017	<0.018	0.52	0.67
	PBBA	59947-55-1	<0.037	<0.034	<0.053	<0.107	<0.064	<0.067	<0.064	<0.069	<0.113	<0.125

	HCDBCO	51936-55-1	<0.023	<0.021	<0.033	<0.067	<0.041	<0.042	<0.04	<0.044	<0.071	<0.079
	BTBPE	37853-59-1	0.26	0.12	0.28	<0.03	<0.018	<0.019	<0.018	<0.019	<0.032	<0.035
	s-DP	13560-89-9	1.08	1.49	9.0	15.2	10.8	6.3	13.0	17.9	2.4	5.3
	a-DP	13560-89-9	0.47	0.80	2.5	6.3	2.0	1.6	2.2	3.2	1.8	4.4
	Σ NBFRs		7.49	13.02	23.5	24.7	15.4	9.3	16.8	21.6	5.4	11.8
Alkyl phosphates	Triisobutyl phosphate	126-71-6	57,464	81,680	38,251	59,746	52,243	43,502	57,037	94,150	97,702	101,181
	Tributyl phosphate	126-73-8	5,067	8,584	3,289	15,329	4,591	4,048	6,574	8,745	6,950	9,694
	Tris(2-Chloroethyl)phosphate	115-96-8	<396	<360	<420	<418	<390	<397	<390	<433	<380	<392
	Tris(1-Chloro-2-propyl)phosphate	13674-84-5	56,811	56,211	31,360	35,714	37,372	22,414	31,220	37,939	28,631	32,109
	Dibutyl phenyl phosphate	2528-36-1	254	311	326	151	141	94	146	108	114	123
	Butyl diphenyl phosphate	2752-95-6	<27	110	92	<77	<46	<48	<46	<50	<81	<90
	Tris(1,3-Dichloro-2-propyl)phosphate	13674-87-8	7,209	13,399	6,752	3,738	5,814	2,700	2,803	3,585	4,325	3,729
	Tris(2-butoxyethyl)phosphate	78-51-3	14,211	23,996	17,628	6,554	10,276	5,738	6,299	2,447	1,678	1,604
	Tris(2-ethylhexyl)phosphate	78-42-2	<113	<105	<164	<329	<199	<207	<199	<214	<349	<386
	Triphenyl phosphate	115-86-6	811	2,208	913	344	567	427	275	181	255	202
	2-Ethylhexyldiphenyl phosphate	1241-94-7	45	142	108	52	82	60	65	<8	<13	<15
	Tri-o-tolyl phosphate	78-30-8	17	<4	20	57	14	31	15	67	27	37
	Tri-m-tolyl phosphate	228-312-4	34	83	63	47	29	20	12	11	<9	17
	Tri-p-tolyl phosphate	1038-95-5	<4	<4	15	32	12	11	<7	<7	<12	<13
	Σ Alkylphosphates		141,923	186,725	98,815	121,763	111,142	79,046	104,446	147,234	139,681	148,697

Tab. S3 Concentrations [pg/L] of chemicals detected in ED sampler extracts

			Stationary exposure		River stretch							
	Analyte	CAS Number	S1	S2	1	2	3	4	5	6	7	8
Pharmaceuticals	Ampicillin	69-52-3	89	< 60	< 100	< 190	136	172	216	< 120	238	< 260
ATBs	Azithromycin	104491-80-7	< 20	< 20	< 30	< 60	< 40	< 40	< 40	< 40	< 70	< 70
	Ciprofloxacin	85721-33-1	21	152	26	46	53	92	88	143	128	< 30
	Clarithromycin	116836-41-0	< 160	< 170	< 240	< 520	< 390	< 310	< 310	< 400	< 590	< 570
	Clindamycine	18323-44-9	737	1,249	776	1,855	1,375	983	1,492	1,414	1,139	914
	Difloxacin	91296-86-5	< 10	< 10	< 20	< 40	< 20	< 20	< 30	< 20	< 40	< 50
	Enoxacin	206873-63-4	103	24	42	75	41	66	< 30	116	69	107
	Enrofloxacin	112732-17-9	< 10	< 10	< 20	< 40	< 30	< 30	< 30	< 30	< 50	< 50
	Erythromycin	114-07-8	< 150	< 150	< 230	< 490	< 370	< 290	< 290	< 380	< 550	< 530
	Flumequine	143984-63-8	22.33	8.26	< 10	< 40	< 20	< 20	< 20	< 30	< 50	64.8
	Levofloxacin	100986-85-4	< 10	< 10	< 20	< 40	< 20	< 20	< 30	< 20	< 40	< 50
	Lomefloxacin	98079-51-7	< 10	< 10	< 20	< 40	< 30	< 30	< 30	< 30	< 50	< 50
	Norfloxacin	68077-27-0	< 10	< 10	< 10	< 30	< 20	< 20	27	< 20	< 30	< 30
	Oxolinic acid	14698-29-4	44	61	18	114	49	20	51	127	148	103
	Oxytetracycline	79-57-2	< 130	< 130	< 190	< 360	< 240	< 250	< 260	< 230	< 450	< 460
	Penicillin_V	132-98-9	< 20	< 10	< 20	< 50	< 30	< 30	< 30	< 40	< 70	< 60
	Roxithromycin	80214-83-1	< 90	< 90	< 130	< 240	< 140	< 140	< 150	< 170	< 280	< 280
	Sulfadiazine	116-44-9	402	754	485	749	1,061	512	844	< 360	828	< 760
	Sulfadimethoxine	1037-50-9	33	20	< 10	36	26	25	55	55	< 50	80
	Sulfamerazine	127-79-7	279	320	< 360	< 680	963	< 430	844	< 440	< 760	< 910
	Sulfamethazine	1981-58-4	458	651	840	3,060	3,909	2,090	2,611	2,553	18,326	12,078
	Sulfamethizole	144-82-1	301	217	533	< 420	< 240	328	393	717	< 480	1,448
	Sulfamethoxazole	129378-89-8	922	539	1,297	2,015	1,190	1,131	1,637	1,386	2,454	2,542
	Sulfamethoxypyridazine	80-35-3	580	< 200	517	684	727	< 390	1,748	464	< 690	2,324
	Sulfamoxole	729-99-7	458	372	< 290	1,725	884	410	805	443	2,071	1,067

	Sulfaphenazole	526-08-9	15	26	13	52	26	51	24	< 30	< 50	50
	Sulfapyridine	144-83-2	8,059	7,796	6,465	12,304	12,572	11,453	10,780	5,127	7,593	8,154
	Sulfaquinoxaline	59-40-5	23	30	32	49	65	< 20	47	< 20	159	< 40
	Sulfasalazine	599-79-1	123	13	60	182	51	59	35	112	41	331
	Sulfathiazole	158269-46-6	< 250	< 200	< 320	< 620	< 350	< 390	< 390	< 400	< 690	< 840
	Trimethoprim	738-70-5	< 250	< 200	372	977	< 350	< 390	< 390	< 400	< 690	< 840
Cardiovascular	Amiodarone	1951-25-3	< 150	< 190	< 240	< 490	< 390	< 330	< 310	< 420	< 660	< 720
	Atenolol	29122-68-7	< 180	217	< 240	846	< 260	< 290	353	< 300	< 520	< 610
	Bisoprolol	66722-44-9	357	341	< 230	< 420	< 260	< 270	< 270	< 270	< 480	< 570
	Cilazapril	88768-40-5	< 10	< 10	< 10	< 20	< 10	< 10	< 10	< 20	< 30	< 30
	Diltiazem	144604-00-2	246	258	259	< 460	432	287	353	< 360	725	610
	Dipyridamole	58-32-2	301	465	< 370	< 780	< 570	< 470	609	738	< 860	< 840
	Disopyramide	3737-09-5	< 10	< 10	< 20	< 30	< 20	< 20	< 20	< 20	< 40	< 40
	Eprosartan	133040-01-4	51	145	115	260	139	84	55	63	207	324
	Flecainide	1403764-72-6	< 10	< 10	< 10	< 30	< 20	< 20	< 20	< 20	< 30	< 30
	Irbesartan	138402-11-6	10,002	10,656	9,907	14,583	16,442	8,216	7,952	4,241	6,695	4,839
	Metoprolol	13484-40-7	1,161	558	291	1,172	1,355	1,106	942	< 270	897	3,200
	Sotalol	27948-47-6	< 180	< 140	< 240	< 460	< 280	< 310	< 290	< 300	< 520	< 610
	Telmisartan	144701-48-4	1,708	2,241	1,018	2,669	2,986	1,578	1,335	2,152	1,967	1,943
	Valsartan	137862-53-4	190	85	158	163	295	96	130	68	273	110
	Verapamil	152-11-4	167	< 120	< 190	< 420	< 290	< 250	< 240	< 320	< 450	< 420
Psychoactive	Alprazolam	28981-97-7	647	743	323	1,660	1,709	1,045	1,178	2,110	2,830	1,753
	Amitriptyline	337376-15-5	< 130	176	< 210	< 460	334	< 270	< 270	< 340	< 480	< 500
	Bupropion	31677-93-7	659	475	372	< 420	334	< 270	< 260	< 270	< 480	< 570
	Carbamazepine	298-46-4	13,211	11,300	11,017	18,622	16,482	10,950	16,263	17,561	23,028	20,456
	Citalopram	128196-01-0	< 10	18	13	39	26	< 20	< 20	34	48	< 30
	Clonazepam	106955-87-7	15	11	53	117	22	47	47	76	66	110
	Codeine	70982-46-6	357	237	404	911	< 260	881	275	< 270	725	< 570
	Donepezil	120011-70-3	17	< 10	< 10	26	22	< 10	< 10	25	< 30	< 30
	Fluoxetine	100568-03-4	< 50	< 50	< 70	< 120	< 80	< 80	< 80	< 90	< 130	< 150

	Flupentixol	2709-56-0	27	27	37	78	114	< 30	51	91	197	91
	Fluphenazine	5002-47-1	268	114	66	133	< 60	82	334	165	190	278
	Haloperidol	337376-15-5	10	< 10	11	< 30	< 20	< 20	< 20	< 20	< 40	< 40
	Chlorprothixene	113-59-7	56	58	226	< 130	165	88	161	97	< 150	236
	Levomepromazine	60-99-1	< 100	< 100	< 150	< 320	< 240	195	< 190	< 250	< 350	< 340
	Maprotiline	10262-69-8	< 90	134	< 110	299	275	< 140	< 140	274	266	331
	Meclozine	1104-22-9	< 20	< 20	36	< 50	65	< 40	37	219	166	152
	Memantine	19982-08-2	13	8	< 0	< 10	12	< 10	12	< 10	< 20	< 20
	Mianserin	21535-47-7	< 130	< 130	259	< 420	< 310	< 250	< 260	< 340	< 480	< 460
	Mirtazapine	61337-67-5	< 10	< 10	< 20	< 30	< 20	< 20	< 20	< 20	< 40	< 40
	Nefazodone	337376-15-5	< 70	< 60	< 90	< 170	< 110	< 110	< 110	< 120	< 180	< 200
	Oxazepam	35295-88-6	9,310	7,641	4,477	6,543	6,306	4,016	5,144	3,966	5,142	5,144
	Paroxetine	110429-35-1	32	8	< 10	55	29	20	59	89	114	95
	Perphenazine	58-39-9	268	165	323	< 170	< 100	225	161	< 120	< 200	< 200
	Risperidone	106266-06-2	< 20	< 20	< 20	< 40	< 30	< 30	< 30	< 30	< 50	< 50
	Ropinirole	337376-15-5	< 160	< 120	< 210	< 390	< 240	< 250	< 260	< 250	< 450	< 530
	Sertaline	79617-96-2	290	77	91	192	171	328	120	< 110	656	< 180
	Tetracycline	60-54-8	< 190	< 190	< 260	< 490	< 310	< 350	< 330	< 320	< 590	< 610
	Tramadol	123154-38-1	179	176	115	215	161	107	118	103	124	103
	Trihexyphenidyl	144-11-6	< 80	< 70	< 100	< 190	< 120	< 120	< 130	< 140	< 200	< 230
	Venlafaxine	93413-44-6	80	53	37	65	65	35	41	44	45	< 30
Antihistamins	Azelastine	58581-89-8	< 70	< 60	< 80	< 160	< 100	< 100	< 110	< 110	< 170	< 190
	Clemastine	14976-57-9	< 130	< 120	< 180	< 360	< 200	< 200	< 220	< 250	< 410	< 420
	Cyproheptadine	129-03-3	123	< 110	< 180	< 360	< 280	348	242	< 270	583	< 380
	Desloratadine	100643-71-8	< 10	< 10	< 10	< 30	< 20	< 20	< 20	< 20	< 30	< 30
	Diphenhydramine	147-24-0	9	< 0	< 0	< 10	< 10	< 10	< 10	< 10	< 20	< 20
	Hydroxyzine	10246-75-0	< 70	91	116	< 170	112	< 110	< 120	< 120	< 180	< 210
	Loperamide	34552-83-5	< 120	< 120	< 180	< 390	< 290	< 230	< 240	< 300	< 450	< 420
	Orphenadrine	337376-15-5	123	< 100	158	< 330	< 240	< 190	< 200	< 250	< 350	< 350
Antifungals	Clotrimazole	117829-71-7	3,014	3,304	3,249	5,859	7,190	5,368	9,130	7,595	9,146	5,486

	Econazole	1069-66-5	47	30	44	62	59	< 30	< 30	< 30	93	213
	Fluconazole	123631-92-5	324	310	242	423	413	389	452	401	725	648
	Itraconazole	84625-61-6	< 70	< 90	< 110	< 220	< 180	< 160	< 150	< 200	< 310	< 340
	Ketoconazole	142128-59-4	625	1,136	485	1,823	373	2,623	746	2,131	< 480	2,858
	Miconazole	22832-87-7	50	68	57	124	47	59	71	196	466	91
	Sulconazole	61318-90-9	39	51	45	< 50	65	37	161	143	152	< 80
	Terbutaline	23031-25-6	< 170	< 130	< 230	< 420	< 260	< 270	< 270	< 270	< 480	< 570
Antidiabetics	Bromocriptine	22260-51-1	6.70	4.13	< 0	42.3	11.8	34.8	19.6	21.1	24.2	26.7
	Glibenclamide	10238-21-8	25	< 20	32	< 50	86	< 30	< 30	< 40	72	< 70
	Glimepiride	29094-61-9	< 30	< 30	< 40	< 90	< 70	< 60	< 60	< 80	< 120	< 130
	Repaglinide	135062-02-1	< 110	< 100	< 160	< 290	< 170	< 180	< 180	< 200	< 340	< 340
Statins	Atorvastatin	110862-48-1	56	< 40	< 60	< 110	< 70	< 70	< 70	84	< 120	< 130
	Bezafibrate	41859-67-0	37	102	39	49	53	156	310	95	211	217
	Fenofibrate	49562-28-9	99	207	226	273	1,179	820	864	186	380	< 220
	Rosuvastatin	1094100-06-7	97	9	21	78	28	55	45	53	< 20	290
Cancer treatment	Flutamide	13311-84-7	< 90	< 110	< 150	< 290	< 240	< 200	< 190	< 250	< 410	< 420
	Fulvestrant	129453-61-8	< 40	< 50	< 60	< 130	< 100	< 90	< 80	< 110	< 180	< 190
	Tamoxifen	10540-29-1	< 10	< 20	< 20	< 50	< 40	< 30	< 30	< 40	< 70	< 70
NSAID	Diclofenac	15307-86-5	< 30	< 40	< 50	< 110	< 90	< 70	< 70	< 90	< 150	< 160
Diuretic	Furosemide	106391-48-4	< 50	< 30	< 50	< 140	122	< 100	< 90	< 110	< 200	< 200
Other	Alfuzosin	337376-15-5	< 160	< 120	< 210	< 420	< 240	< 270	< 260	< 270	< 450	< 530
	Naloxone	465-65-6	290	248	< 230	< 460	< 260	533	< 270	295	< 520	< 610
	Ranitidine	66357-35-5	< 160	< 120	< 210	< 390	< 240	< 250	< 260	< 250	< 450	< 530
	Σ Pharmaceuticals		57,256	54,175	45,799	81,306	80,803	57,203	69,470	55,973	89,406	78,869
CUPs	Acetochlor	34256-82-1	11	45	< 3.2	< 6.5	57	42	138	498	592	965
	Alachlor	15972-60-8	21	45	< 16.3	< 66.1	< 19.8	< 20.7	29	< 42.6	101	56
	Atrazine	1912-24-9	3,025	4,339	7,349	4,703	6,995	3,400	4,817	4,767	18,295	8,482
	Azinphos methyl	86-50-0	< 2.2	< 2.1	< 3.2	< 6.5	< 3.9	< 4.1	< 3.9	< 4.2	< 6.9	< 7.6
	Carbaryl	63-25-2	< 1.1	< 1	< 1.6	< 3.3	< 2	< 2	< 2	< 2.1	< 3.5	< 3.8
	Carbendazim	10605-21-7	11	15	20	12	25	17	25	22	82	40

Chlorpyrifos	2921-88-2	179	257	289	117	351	188	415	329	1,482	608
Chlorsulfuron	64902-72-3	< 2.2	< 2.1	< 3.2	< 6.5	< 3.9	< 4.1	< 3.9	< 4.2	14	< 7.6
Chlortoluron	15545-48-9	1,815	1,717	3,463	2,118	2,439	603	687	673	1,992	780
Clopyralid	1702-17-6	< 22.2	< 20.8	< 32.5	< 66.1	< 39.5	< 41.2	< 39.7	< 42.6	< 69.7	< 76.2
Diazinon	333-41-5	141	165	116	144	245	299	677	608	1,415	1,363
Dimethachlor	50563-36-5	22	242	36	44	130	73	120	20	116	91
Dimethoate	60-51-5	24	33	77	32	34	18	37	24	92	43
Disulfoton	298-04-4	< 1.1	< 1	< 1.6	< 3.3	< 2	< 2	< 2	< 2.1	< 3.5	< 3.8
Diuron	330-54-1	1,228	1,825	2,409	1,238	1,770	804	1,266	1,079	3,533	1,504
Fenitrothion	122-14-5	< 66.6	< 62.2	< 97.6	< 198.6	< 118.6	< 123.8	< 119	< 127.9	< 208.8	< 228.2
Fenoxaprop ethyl	66441-23-4	< 1.1	< 0.4	< 0.6	< 1.3	< 2	< 0.8	< 2	< 2.1	3	< 3.8
Fenpropimorph	67564-91-4	10	13	15	< 1.3	5	3	2	< 0.8	< 3.5	< 1.5
Florasulam	145701-23-1	5.13	5.99	9.1	7.2	9.8	2.0	3.5	5.9	20.0	4.6
Fluroxypyr	69377-81-7	< 5.6	< 5.2	< 8.1	< 16.6	< 9.8	238	428	< 10.8	< 17.3	< 19.1
Fonofos	944-22-9	< 2.2	< 2.1	< 3.2	< 6.5	< 3.9	< 4.1	< 3.9	< 4.2	< 6.9	< 7.6
Isoproturon	34123-59-6	632	816	1,416	783	977	436	723	569	1,856	728
Malathion	121-75-5	< 5.6	14	23	18	16	47	214	31	89	35
Metamitron	41394-05-2	< 5.6	< 5.2	40	< 16.6	75	11	10	21	61	< 19.1
Metazachlor	67129-08-2	149	483	374	204	418	213	327	134	487	1,026
Metolachlor	51218-45-2	1,238	1,889	1,988	1,470	1,882	1,218	1,397	1,530	3,210	2,129
Metribuzin	21087-64-9	142	218	< 3.2	26	205	193	81	156	224	146
Parathion methyl	298-00-0	< 44.4	2,563	2,361	256	2,483	1,592	3,797	4,756	5,479	7,795
Pendimethalin	40487-42-1	95	172	162	113	200	88	95	69	97	53
Phosmet	732-11-6	< 2.2	< 5.2	17.1	8.5	16.9	6.1	5.9	12.7	11.7	< 19.1
Pirimicarb	23103-98-2	31	41	74	46	72	36	32	20	45	11
Prochloraz	67747-09-5	78	141	124	118	130	52	128	111	197	126
Propiconazole	60207-90-1	3,745	6,719	5,250	3,208	5,462	2,793	3,192	3,484	6,508	4,284
Pyrazon	216-920-2	67	87	175	195	296	120	191	208	831	412
Simazine	122-34-9	451	651	968	488	739	309	498	575	2,015	1,362
Tebuconazole	107534-96-3	3,114	4,490	3,331	2,864	3,911	2,458	3,508	4,169	7,473	5,174

	Temephos	3383-96-8	3.80	1.65	< 1	< 2	3.9	< 1.2	3.1	1.3	2.8	3.0
	Terbufos	13071-79-9	272	270	313	386	1,125	478	2,296	2,676	3,519	1,532
	Terbuthylazin	5915-41-3	1,029	1,481	2,708	1,885	2,409	1,320	1,873	1,818	7,241	3,198
	Tribenuron-methyl	101200-48-0	3.13	8.47	41.7	8.5	29.1	8.2	17.7	7.2	56.6	11.4
	Σ CUPs		17,543	28,746	33,152	20,492	32,509	17,067	27,033	28,373	67,140	41,961
Alkylphenols	Bisphenol A	80-05-7	5,426	5,771	6,821	6,207	7,862	17,471	27,537	12,921	14,894	14,449
	4-t-octylphenol	140-66-9	83	101	191	334	178	485	143	98	169	94
	4-nonylphenol	104-40-5	2,121	1,918	5,214	5,943	7,169	8,355	6,833	7,963	7,336	5,274
	Σ Alkylphenols		7,630	7,790	12,225	12,484	15,209	26,311	34,513	20,982	22,399	19,817
Steroids	Estriol	50-27-1	<8.93	<8.26	<25.86	<52.08	47	161	234	46	38	66
	17α-estradiol	57-91-0	<17.86	<16.52	<25.86	<52.08	<31.43	<32.78	<31.42	<33.76	<27.61	<30.48
	17β-estradiol	50-28-2	<17.86	<16.52	<25.86	<52.08	43	43	97	<33.76	57	56
	17α-ethinyl-estradiol	57-63-6	<25.86	<25.86	<25.86	<25.86	<25.86	<25.86	<25.86	<25.86	<25.86	<25.86
	Estrone	53-16-7	176	198	201	351	492	422	820	352	675	694
	Σ Steroids		176	198	201	351	582	626	1,151	398	770	816
PAHs	Naphthalene	91-20-3	129	426	1,152	3,531	<3	875	790	1,963	1,725	2,646
	Biphenyl	92-52-4	92	156	204	299	981	172	195	148	112	209
	Acenaphthylene	208-96-8	59	79	164	115	661	92	94	59	47	103
	Acenaphthene	83-32-9	72	129	175	140	2,445	195	125	72	30	155
	Fluorene	86-73-7	224	308	379	403	3,363	1,395	424	259	236	585
	Phenanthrene	85-01-8	1,121	1,507	2,601	2,048	5,136	10,235	2,098	1,948	1,458	3,403
	Anthracene	120-12-7	79	114	375	277	788	294	161	108	105	294
	Fluoranthene	206-44-0	1,972	2,134	1,967	2,504	3,633	1,542	1,832	1,515	1,152	1,953
	Pyrene	129-00-0	1,005	1,125	1,162	1,430	2,712	1,311	1,258	874	1,005	1,630
	Retene	483-65-8	482	439	328	272	605	98	128	127	179	123
	Benzo(*b*)fluorene	243-17-4	104	114	115	127	<12	119	116	80	51	99
	Benzonapthothiophene	205-43-6	63	71	101	51	<7	58	64	40	51	65
	Benzo(*ghi*)fluoranthene	203-12-3	95	97	82	105	<5	75	89	85	81	91
	Cyclopenta(*cd*)pyrene	27208-37-3	<3	<2	<4	<7	<4	<5	<4	<5	<8	<9
	Benzo(*a*)anthracene	56-55-3	99	114	114	95	281	86	101	61	58	118

	Triphenylene	217-59-4	264	273	380	347	795	304	358	373	366	435
	Chrysene	218-01-9	189	211	231	166	1,012	278	169	143	120	142
	Benzo(*b*)fluoranthene	205-99-2	67	47	<12	<24	<14	<15	<14	<15	<25	<28
	Benzo(*j*)fluoranthene	205-82-3	36	36	<12	<24	<14	<15	<14	<16	<25	<28
	Benzo(*k*)fluoranthene	207-08-9	22	20	<13	<26	<16	<16	<16	<17	<27	<30
	Benzo(*e*)pyrene	192-97-2	64	62	64	74	<12	63	64	54	<21	<24
	Benzo(*a*)pyrene	50-32-8	7	11	<14	<28	<17	<18	<17	<18	<29	<33
	Perylene	198-55-0	18	19	<13	<26	<16	<17	<16	52	117	194
	Indeno(*123cd*)pyrene	193-39-5	<8	<7	<11	<22	<13	<14	<13	<14	<23	<26
	Dibenzo(*ah*)anthracene	53-70-3	<9	<8	<13	<26	<16	<16	<16	<17	<27	<30
	Dibenzo(*ac*)anthracene	215-58-7	<9	<8	<13	<26	<16	<16	<16	<17	<27	<30
	Benzo(*ghi*)perylene	191-24-2	8	7	<7	<15	<9	<9	<9	<9	<15	<17
	Anthanthrene	191-26-4	<12	<11	<18	<36	<22	<23	<22	<23	<38	<42
	Coronene	191-07-1	<14	<13	<21	<42	<26	<27	<26	<27	<45	<49
	Σ PAHs		6,271	7,498	9,594	11,984	22,411	17,190	8,065	7,962	6,893	12,245
Sterols	Brassicasterol	474-67-9	5,465	3,631	1,253	1,927	3,042	2,293	2,479	3,925	1,519	1,166
	Campesterol	474-62-4	5,882	5,829	3,172	5,128	<196.44	3,988	5,480	4,972	7,294	6,127
	Desmosterol	313-04-2	977	1,135	1,042	<325.51	964	302	618	603	1,160	741
	Ergosterol	57-87-4	<66.98	324	<96.97	<195.31	<117.86	<122.93	<117.81	<126.58	<207.08	<228.6
	Fucosterol	17605-67-3	2,044	1,204	1,422	<325.51	1,177	1,120	1,034	1,301	1,260	1,655
	Cholestanol	80-97-7	2,114	2,284	<161.62	<325.51	2,281	<204.89	<196.35	<210.97	<345.13	2,915
	Cholesterol	57-88-5	20,035	23,144	10,522	15,737	18,385	11,067	22,500	17,751	19,607	14,364
	Sitosterol	83-46-5	16,120	<20.65	5,990	10,776	9,077	6,421	6,775	8,936	7,298	12,106
	Spinasterol	481-18-5	9,786	7,531	2,196	7,967	7,497	3,159	2,577	6,274	5,788	<381.01
	Stigmastanol	19466-47-8	805	905	<161.62	<325.51	<196.44	889	<196.35	627	<345.13	<381.01
	Stigmasterol	83-48-7	8,782	6,791	4,286	4,429	7,572	5,514	5,995	1,821	5,072	11,735
	Σ Sterols		72,010	52,776	29,885	45,964	49,994	34,753	47,458	46,209	48,996	50,809
Flavonoids	Apigenin	520-36-5	18.98	18.07	12.9	14.6	24.6	34.8	20.6	21.1	19.0	19.1
	Biochanin A	491-80-5	30.70	35.11	22.6	9.8	11.8	11.3	3.9	<0.04	3.5	3.8
	Coumestrol	479-13-0	<0.22	<0.21	<0.32	<0.65	<0.39	<0.41	<0.39	<0.42	<0.69	<0.76

Daidzein	486-66-8	73	108	93	76	88	488	595	162	255	404
Equol	94105-90-5	207	250	318	150	138	1,332	821	268	2,571	9,449
Formononetin	485-72-3	268	302	328	270	177	163	231	141	178	389
Genistein	446-72-0	33	53	47	21	268	585	159	28	47	65
Naringenin	266-769-1	166	188	132	94	98	302	160	72	138	<0.08
Σ Flavonoids		798	955	954	636	806	2,916	1,990	693	3,211	10,329

Tab. S4 Sum molar concentrations of groups of chemicals detected in samples from dynamic passive sampling

1-8 samples from mobile sampling of river stretches; SR silicone rubber sampler, ED Empore™ disc sampler; No. number of analyzed chemicals in the category; <LOD concentrations below the limit of detection; for individual chemical identification see SI.

Sampler	Chemicals (pmol L^{-1})	No.	S1	S2	1	2	3	4	5	6	7	8
ED	ATBs	31	48.34	46.17	43.51	89.63	87.56	66.47	81.53	46.24	122.21	108.15
	Cardiovascular	15	31.57	33.97	26.78	45.09	48.20	24.97	26.34	15.84	23.62	22.23
	Psychoactive	30	98.12	82.94	69.93	114.01	103.54	70.08	94.72	98.63	132.72	114.32
	Antihistamins	8	0.92	0.24	0.90	<LOD	0.30	1.21	0.84	<LOD	2.03	<LOD
	Antifungals	8	11.32	13.10	11.49	22.26	23.33	22.01	29.93	28.18	30.63	24.18
	Antidiabetics	4	0.06	0.01	0.07	0.06	0.19	0.05	0.03	0.03	0.18	0.04
	Statins	4	0.57	0.86	0.76	0.97	3.44	2.76	3.30	0.98	1.63	0.89
	Other pharm.[1]	8	0.89	0.76	<LOD	<LOD	0.37	1.63	<LOD	0.90	<LOD	<LOD
	CUPs	40	67.55	107.47	132.15	81.52	126.65	65.13	102.24	106.26	266.67	160.25
	Alkylphenols	3	0.65	0.73	0.74	1.30	2.14	2.28	4.20	1.46	2.84	3.00
	PAHs	29	33.80	34.47	54.47	55.78	67.84	116.80	152.33	93.21	99.35	87.69
	Steroids	5	31.78	39.30	53.14	71.50	123.13	96.11	44.11	46.47	39.98	71.16
	Phytosterols	11	178.93	132.94	74.58	117.35	124.81	86.46	119.31	115.55	122.70	126.43
	Flavonoids	8	3.05	3.66	3.69	2.44	3.06	11.47	7.85	2.72	13.04	42.36
SR	PAHs	29	100.66	133.97	108.06	130.08	315.96	223.61	93.50	70.97	87.78	128.33
	PCBs	7	0.72	0.80	0.56	0.99	0.58	0.57	1.32	0.54	1.11	1.05
	OCPs	12	0.63	0.75	0.53	0.82	1.28	1.07	1.20	1.55	2.68	6.06
	BDEs	9	0.01	0.02	0.06	0.01	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	NBFRs	15	0.01	0.02	0.04	0.04	0.03	0.02	0.03	0.03	0.01	0.02
	Musks	6	144.03	215.80	46.69	50.77	74.10	138.94	<LOD	<LOD	<LOD	<LOD
	Alkylphenols	3	13.23	15.93	26.09	28.45	36.12	45.47	38.60	16.02	33.03	28.28
	Alkylphosphates	14	464.26	610.68	316.42	418.14	369.38	269.61	358.08	517.79	495.86	528.22

[1] furosemide, alfuzosin, naloxone, ranitidine

Tab. S5 Relative effect potencies (REPs) of detected compounds

REPs that were used for calculation of chemical data-based bioanalytical equivalents (BEQ_{chem}) were obtained either from experiments from the current study or from literature. Literature was preferentially searched for REP values specific for the bioassays employed in the current study. Where no such REPs were available, we used data from analogical bioassays as indicated. When there was more than one relevant literature source available, geometrical mean of REP value was calculated.

Assay	**Activation of AhR**		**Activation of PXR**			**Activation of ER**			**Inhibition of AR**			**Oxidative stress response**			**Activation of p53**		
No. chemicals	23		27			26			22			11			12		
Reference compound	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)		SR 12813			17β-Estradiol			Flutamide			tert-Butylhydroquinone (tBHQ)			Mitomycin		
Chemical	REP	based on	Chemical	REP	based on	Chemical	REP	based on	Chemical	REP	based on	Chemical	REP	based on	Chemical	REP	based on
4-Nonylphenol	1.0×10^{-8}	$EC_{10}^{1,4,\ a}$	17β-Estradiol	3.0×10^{-3}	$EC_{20}^{3,13,\ s}$	17β-Estradiol	$1.0\times10^{+0}$	$EC_{25}^{3,10,\ i}$	4-Nonylphenol	0.02	$IC_{20}^{3,12,\ i}$	4-Nonylphenol	2.9×10^{-2}	$EC_{IR1.5}^{3,14,\ a}$	17β-Estradiol	9.8×10^{-4}	$EC_{IR1.5}^{3,15,\ v}$
Benzo(*a*)anthracene	2.6×10^{-5}	$EC_{25}^{1,5,\ b,\ c,\ d}$	4-Nonylphenol	3.0×10^{-2}	$EC_{20}^{3,13,\ s}$	2,4'-DDE	6.3×10^{-6}	$EC_{50}^{3,10,\ j}$	4-t-Octylphenol	0.34	$IC_{20}^{3,12,\ i}$	Atenolol	1.5×10^{-3}	$EC_{IR1.5}^{3,14,\ u}$	Apigenin	3.2×10^{-3}	$EC_{IR1.5}^{3,15,\ r}$
Benzo(*a*)pyrene	1.5×10^{-4}	$EC_{20}^{1,6,\ e}$	4-t-Octylphenol	5.8×10^{-2}	$EC_{20}^{3,13,\ s}$	2,4'-DDT	1.7×10^{-5}	$EC_{25}^{3,10,\ i}$	Acetochlor	0.62	$IC_{20}^{3,12,\ r}$	Atorvastatin	3.5×10^{-2}	$EC_{IR1.5}^{3,14,\ u}$	Benzo(a)pyrene	6.6×10^{-2}	$EC_{IR1.5}^{3,15,\ r}$
Benzo(*b*)fluoranthene	4.6×10^{-4}	$EC_{25}^{1,5,\ b,\ c,\ d}$	Bezafibrate	6.2×10^{-4}	$EC_{IR1.5}^{3,13,\ o}$	4,4'-DDD	6.8×10^{-7}	$EC_{20}^{3,10,\ k}$	Alachlor	0.83	$IC_{20}^{3,12,\ r}$	Atrazine	1.0×10^{-2}	$EC_{IR1.5}^{3,14,\ u}$	Benzo(b)fluoranthene	2.8×10^{-3}	$EC_{IR1.5}^{3,15,\ r}$
Benzo(*e*)pyrene	6.6×10^{-7}	$EC_{25}^{1,5,\ b,\ c}$	Bisphenol A	5.8×10^{-3}	$EC_{20}^{3,13,\ s}$	4,4'-DDE	7.0×10^{-7}	$EC_{50}^{3,10,\ j}$	Benzo(a)pyrene	0.16	$IC_{20}^{3,12,\ a}$	Benzo(a)pyrene	$4.8\times10^{+0}$	$EC_{IR1.5}^{3,14,\ a}$	Benzo(e)pyrene	1.7×10^{-3}	$EC_{IR1.5}^{3,15,\ r}$
Benzo(*ghi*)perylene	2.6×10^{-6}	$EC_{25}^{1,5,\ b}$	Carbamazepine	4.6×10^{-4}	$EC_{IR1.5}^{3,13,\ o}$	4,4'-DDT	1.3×10^{-6}	$EC_{50}^{3,10,\ l}$	Bisphenol A	1.20	$IC_{20}^{3,12,\ a}$	Benzo(b)fluoranthene	$8.4\times10^{+0}$	$EC_{IR1.5}^{3,14,\ a}$	Benzo(k)fluoranthene	4.0×10^{-2}	$EC_{IR1.5}^{3,15,\ r}$
Benzo(*j*)fluoranthene	5.5×10^{-4}	$EC_{25}^{1,5,\ b,\ c}$	Chlorpyrifos	3.1×10^{-3}	$EC_{10}^{3,13,\ a}$	4-Nonylphenol	3.3×10^{-6}	$EC_{20}^{3,10,\ k}$	Bromocriptine mesylate	1.68	$IC_{20}^{3,12,\ r}$	Bisphenol A	1.5×10^{-2}	$EC_{IR1.5}^{3,14,\ a}$	Biochanin A	5.6×10^{-4}	$EC_{IR1.5}^{3,15,\ r}$
Benzo(*k*)fluoranthene	3.1×10^{-3}	$EC_{25}^{1,5,\ b,\ c,\ d}$	Clotrimazole	4.3×10^{-2}	$EC_{20}^{3,13,\ t}$	4-t-Octylphenol	1.1×10^{-4}	$EC_{25}^{3,10,\ i}$	Chloropyrifos	0.01	$IC_{20}^{3,12,\ a}$	Citalopram	3.0×10^{-2}	$EC_{IR1.5}^{3,14,\ u}$	Bisphenol A	7.1×10^{-4}	$EC_{IR1.5}^{3,15,\ r}$
Bisphenol A	1.4×10^{-7}	$EC_{10}^{1,6,\ e}$	Diazinon	1.1×10^{-2}	$EC_{10}^{3,13,\ a}$	Benzo(*a*)anthracene	7.9×10^{-7}	$EC_{25}^{3,10,\ n}$	Clotrimazole	2.23	$IC_{20}^{3,12,\ r}$	Genistein	2.7×10^{-2}	$EC_{IR1.5}^{3,14,\ a}$	Carbendazim	7.3×10^{-3}	$EC_{IR1.5}^{3,15,\ o}$
Carbendazim	1.2×10^{-8}	$EC_{10}^{1,6,\ e}$	Diuron	4.3×10^{-4}	$EC_{10}^{3,13,\ a}$	Benzo(*a*)pyrene	4.7×10^{-6}	$EC_{10}^{3,10,\ a}$	Diazinon	0.02	$IC_{20}^{3,12,\ e}$	Metoprolol	3.0×10^{-3}	$EC_{IR1.5}^{3,14,\ u}$	Chlorprothixene	1.2×10^{-3}	$EC_{IR1.5}^{3,15,\ r}$
Chlorpyrifos	1.1×10^{-7}	$EC_{10}^{1,4,\ a}$	Estrone	4.1×10^{-3}	$EC_{20}^{3,13,\ s}$	Benzo(*b*)fluoranthene	3.4×10^{-6}	$EC_{10}^{3,10,\ a}$	Fenofibrate	0.59	$IC_{20}^{3,12,\ r}$	Propiconazole	2.7×10^{-2}	$EC_{IR1.5}^{3,14,\ u}$	Genistein	1.2×10^{-3}	$EC_{IR1.5}^{3,15,\ o}$
Chrysene	6.5×10^{-5}	$EC_{25}^{1,5,\ b,c}$	Fenofibrate	2.5×10^{-2}	$EC_{20}^{3,13,\ t}$	Biochanin A	8.8×10^{-5}	$EC_{50}^{3,11,\ m}$	Ketoconazole	0.98	$IC_{20}^{3,12,\ r}$				Prochloraz	7.2×10^{-4}	$EC_{IR1.5}^{3,15,\ r}$
Cyclopenta(*cd*)pyrene	6.5×10^{-7}	$EC_{25}^{1,5,\ c}$	Genistein	1.2×10^{-2}	$EC_{10}^{3,13,\ a}$	Bisphenol A	4.4×10^{-5}	$EC_{20}^{3,10,\ k}$	Maprotiline	0.51	$IC_{20}^{3,12,\ r}$						
Daidzein	1.1×10^{-6}	$LOEC^{2,8,\ f}$	Isoproturon	1.6×10^{-3}	$EC_{IR1.5}^{3,13,\ o}$	Chlorpyrifos	2.8×10^{-7}	$EC_{10}^{3,10,\ a}$	Memantine	0.58	$IC_{20}^{3,12,\ r}$						
Diazinon	1.0×10^{-8}	$EC_{20}^{1,6,\ a}$	Metolachlor	5.9×10^{-2}	$EC_{10}^{3,13,\ a}$	Chrysene	2.2×10^{-6}	$EC_{25}^{3,10,\ q}$	Metolachlor	0.02	$IC_{20}^{3,12,\ e}$						
Diuron	1.8×10^{-6}	$EC_{20}^{2,9,\ h}$	o,p'-DDT	1.7×10^{-2}	$EC_{20}^{3,13,\ s}$	Daidzein	6.4×10^{-5}	$EC_{50}^{3,10,\ o}$	o,p-DDD	0.31	$IC_{20}^{3,12,\ r}$						
Equol	1.0×10^{-6}	$LOEC^{2,8,\ f}$	p,p'-DDD	4.1×10^{-2}	$EC_{20}^{3,13,\ t}$	Diazinon	2.9×10^{-7}	$EC_{10}^{3,10,\ a}$	Prochloraz	0.43	$IC_{20}^{3,12,\ r}$						
Fluoranthene	9.3×10^{-7}	$EC_{25}^{1,5,\ c}$	p,p'-DDE	3.0×10^{-2}	$EC_{20}^{3,13,\ t}$	Donepezil hydrochloride	6.3×10^{-5}	$EC_{10}^{3,11,\ p}$	Propiconazole	0.07	$IC_{20}^{3,12,\ a}$						
Genistein	7.9×10^{-8}	$EC_{10}^{1,6,\ a}$	p,p'-DDT	2.9×10^{-2}	$EC_{20}^{3,13,\ t}$	Equol	2.8×10^{-4}	$EC_{50}^{3,10,\ i}$	Triphenyl phosphate	0.03	$IC_{20}^{3,12,\ a}$						
Prochloraz	2.3×10^{-6}	$EC_{20}^{2,9,\ g}$	PCB 101	3.4×10^{-3}	$EC_{20}^{3,13,\ s}$	Estriol	1.7×10^{-1}	$EC_{25}^{3,10,\ i}$	Temephos	0.41	$IC_{20}^{3,12,\ r}$						

Propiconazole	1.9×10^{-5}	EC_{50}[2,8, h]	PCB 118	7.0×10^{-3}	EC_{20}[3,13, t]	Estrone	1.1×10^{-1}	$EC_{IR1.5}$[3,10, a]	TDCPP[w]	0.33	IC_{20}[3,12, r]
Pyrene	4.1×10^{-6}	EC_{25}[1,5, b,c]	PCB 138	5.3×10^{-3}	EC_{20}[3,13, s]	Formononetin	2.3×10^{-4}	EC_{50}[3,11, m]	Verapamil hydrochloride	0.27	IC_{20}[3,12, r]
Terbuthylazine	1.3×10^{-5}	EC_{50}[2,8, h]	PCB 153	6.0×10^{-3}	EC_{20}[3,13, s]	Genistein	2.0×10^{-4}	EC_{10}[3,10, a]			
			PCB 180	6.0×10^{-3}	EC_{20}[3,13, s]	Naringenin	2.2×10^{-5}	EC_{50}[3,11, m]			
			Propiconazole	5.4×10^{-3}	EC_{10}[3,13, a]	Terbuthylazine	1.6×10^{-7}	EC_{10}[3,10, a]			
			Terbuthylazine	4.1×10^{-3}	EC_{10}[3,13, s]	Triphenyl phosphate	1.4×10^{-6}	EC_{10}[3,10, a]			
			Triphenyl phosphate	1.7×10^{-2}	EC_{10}[3,13, a]						

[1] rat-based cell line
[2] mouse-based cell line
[3] human-based cell line
[4] DR-CALUX
[5] H4EII-*luc*
[6] H4G1.1c2
[7] H4L1.1c4
[8] Hepa1.12cR
[9] Hepa1c1c7
[10] MELN
[11] HeLa9903
[12] MDA-kb2
[13] HG5LN-hPXR
[14] AREc32
[15] p53RE-bla

[a] (Neale et al., 2017)
[b] (Larsson et al., 2012)
[c] (M Machala et al., 2001)
[d] (Behnisch et al., 2003)
[e] (current study)
[f] (Denison et al., 1998)
[g] (Takeuchi et al., 2008)
[h] (Ghisari et al., 2015)
[i] (Kinani et al., 2010)
[j] (Pillon et al., 2005)
[k] (Creusot et al., 2013)
[l] (Leusch et al., 2010)
[m] (Procházková et al., 2017)
[n] (Machala et al., 2001)
[o] (Neale et al., 2015a)
[p] (Ceger et al., 2015)
[q] (Lam et al., 2017)
[r] (US EPA ToxCast)
[s] (Creusot et al., 2010)
[t] (Creusot, 2011)
[u] (Escher et al., 2013)
[v] (König et al., 2016)
[w] Tris(1,3-Dichloro-2-propyl)phosphate
[x] Tris(2-butoxyethyl) phosphate

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