Advancements in effect-based water quality assessment

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CHAPTER 3

ACTIVE CHOICES IN PASSIVE SAMPLING: THE INFLUENCE OF SAMPLER HOUSING AND SORBENT TYPE ON BIOASSAY RESPONSES TO POLAR PASSIVE SAMPLER EXTRACTS

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ABSTRACT

The combination of integrative passive sampling and bioassays is a promising approach for monitoring the toxicity of polar organic contaminants in aquatic environments. However, the design of integrative passive samplers can affect the accumulation of compounds and therewith the bioassay responses. The present study aimed to determine the effects of sampler housing and sorbent type on bioassay responses to polar passive sampler extracts. To this end, four integrative passive sampler configurations, resulting from the combination of polar organic chemical integrative sampler (POCIS) and Speedisk housings with hydrophilic-lipophilic balance and hydrophilic divinylbenzene sorbents, were simultaneously exposed at reference and contaminated surface water locations. To measure the toxicity of the accumulated polar organic compounds, a battery of five bioassays was exposed to the extracts. Extracts from POCIS caused higher bioassay responses in 91% of cases, while extracts from the two sorbents caused equally frequent but different bioassay responses. Hence, the passive sampler design critically affected the toxicity detection of polar organic contaminants, highlighting the importance of active choices in passive sampling for effect-based water quality assessment.

INTRODUCTION

Aquatic ecosystems are under threat from an ever-increasing diversity of contaminants that are released into the environment.[3,7] These contaminants of emerging concern (CECs) are generally (highly) polar and mobile in water, challenging water treatment as well as monitoring technologies.[79,83–85] Therefore, there is a need for monitoring methods that enable the sampling and toxicity assessment of polar CECs in the aquatic environment. However, conventional toxicity assessment of CECs on an analyte-by-analyte basis is problematic since i) many of the compounds are unknown, ii) if known, toxicity data for these new compounds are very scarce, and iii) mixture toxicity data are even less available. To overcome the drawbacks of traditional water quality monitoring frameworks that are based on a limited number of target pollutants, effect-based methods can be applied to identify the ecotoxicological risks associated with mixtures of (un)known CECs present in the water.[86] Therefore, bioassay batteries are increasingly applied in water quality assessment, representing a wide range of toxicological endpoints relevant to aquatic ecosystem health.[87] This allows the ranking of sites based on ecotoxicological risks rather than on the presence and absence of contaminants.[14,15,42,88] However, sampling moments and methods critically affect the detection of the activity of micropollutants in bioassay batteries. Since the environmental concentrations of polar organic micropollutants typically vary over time and are, by definition, very low (ng to µg L^{-1}),[13,89] traditional discrete spot sampling methods provide only snapshots in time of contaminant concentrations and require additional sample enrichment of large water volumes to detect trace level pollutants.[19] Passive sampling can overcome these limitations of spot sampling by providing a time-integrative representation of contaminant concentrations in the water while simultaneously allowing the *in situ* pre-concentration of compounds from the surface water.[13,19] The advantages of passive sampling – a time-integrative representation of contaminant concentrations – and bioassays – the identification of the ecotoxicological risks associated with mixtures of all (un)known CECs – over conventional methods make their combination especially appropriate for the toxicity assessment of the wide variety of polar organic CECs that are present at low and fluctuating concentrations in surface waters.

Integrative (kinetic) passive samplers, here defined as a sampler body housing a sorbent that serves as the receiving phase in which the sampled compounds accumulate, are increasingly used to provide time-integrated measurements of polar organic contaminants in surface waters.[13] The accumulation of polar compounds into integrative passive samplers is governed by the diffusion of the freely dissolved analytes from the surface water across three spatial stages that are inherent to the use of adsorption-based passive samplers in surface water.[52] The first stage is a viscous layer of water at the surface of the sampler, the so-called water boundary layer (WBL), also referred to as the aquatic or diffusive boundary layer. The second stage is the (membrane) filter used to limit the speed of diffusion of compounds into the sampler and to keep the receiving sorbent phase in place. The final stage is the sorbent itself, to which the analytes ultimately adsorb. The sampler design affects the hydrodynamic conditions in and around the sampler housing, determining the uptake of chemicals into integrative passive

samplers.[90] The sorbent that is applied determines which polar organic compounds can be retained throughout the exposure in the environment.[91] These sampler characteristics determine the accumulation of compounds in passive samplers and, in turn, dictate bioassay responses to the passive sampler extracts.[89] The choice of the sampler can thus critically affect the outcome of effect-based water quality assessments.

The most widely used type of integrative passive sampler is the polar organic chemical integrative sampler (POCIS),[52,92] but a variety of alternative devices is available and new sampler configurations are frequently developed. Most recently, the commercially available Speedisk® solid-phase extraction (SPE) columns were proposed as a promising passive sampling device.[93] Speedisk contain the polymeric sorbent hydrophilic divinylbenzene (H-DVB), which was suggested as a favorable alternative to the commonly used hydrophilic-lipophilic balance (HLB) sorbent for the sorption of organic CECs in passive samplers.[93] The robust plastic housing of the Speedisk makes them resistant to damage during field deployment and readily applicable as passive samplers in surface waters. This raises the question of whether this alternative sampler housing and sorbent may be more fit than the well-established POCIS for the monitoring of polar organic CECs in aquatic environments. Although separate comparisons of sampler designs [e.g. Ahrens *et al.* (2015)[94]] and sorbents [e.g. Bäuerlein *et al.* (2012)[91]] on the uptake of polar organic compounds in passive samplers are available, a full-factorial study that allows the simultaneous comparison of multiple sampler housings and sorbents in field-exposed integrative passive samplers has, until now, not been performed. The present study aimed to determine the effects of sampler housing and sorbent type on bioassay responses to polar passive sampler extracts. To this end, four integrative passive sampler configurations, resulting from the combination of the POCIS and Speedisk housings with the HLB and H-DVB sorbents, were simultaneously exposed at reference and contaminated surface water locations. To measure the toxicity of the accumulated polar organic compounds, a battery of bioassays for bacterial inhibition, cytotoxicity and three reporter-gene bioassays for endocrine disruption was exposed to the sampler extracts. The outcomes of this study provide insight into the influence of sampler design on the toxicity detection of polar CECs, thereby supporting active choices of passive sampler characteristics for application in effect-based surface water quality assessment.

MATERIALS AND METHODS

Sampler and sorbent types

The four types of passive samplers used in the present study resulted from the combinations of two types of sampler housing, POCIS and Speedisk (Figure 3.1), and two types of sorbent, HLB and H-DVB. The POCIS consists of two stainless steel rings, with an inner diameter of 5.4 cm, that retain the sorbent between two membranes, leaving approximately 46 cm^2 of surface area exposed to the surrounding water (Figure 3.1). Speedisk were originally designed as SPE columns, which can be modified to render them suitable for deployment as passive samplers. The Speedisk consists of a plastic housing retaining a sorbent between two glass fiber filters by two plastic screens and a retaining ring (Figure 3.1). The bottom side of the Speedisk is sealed,

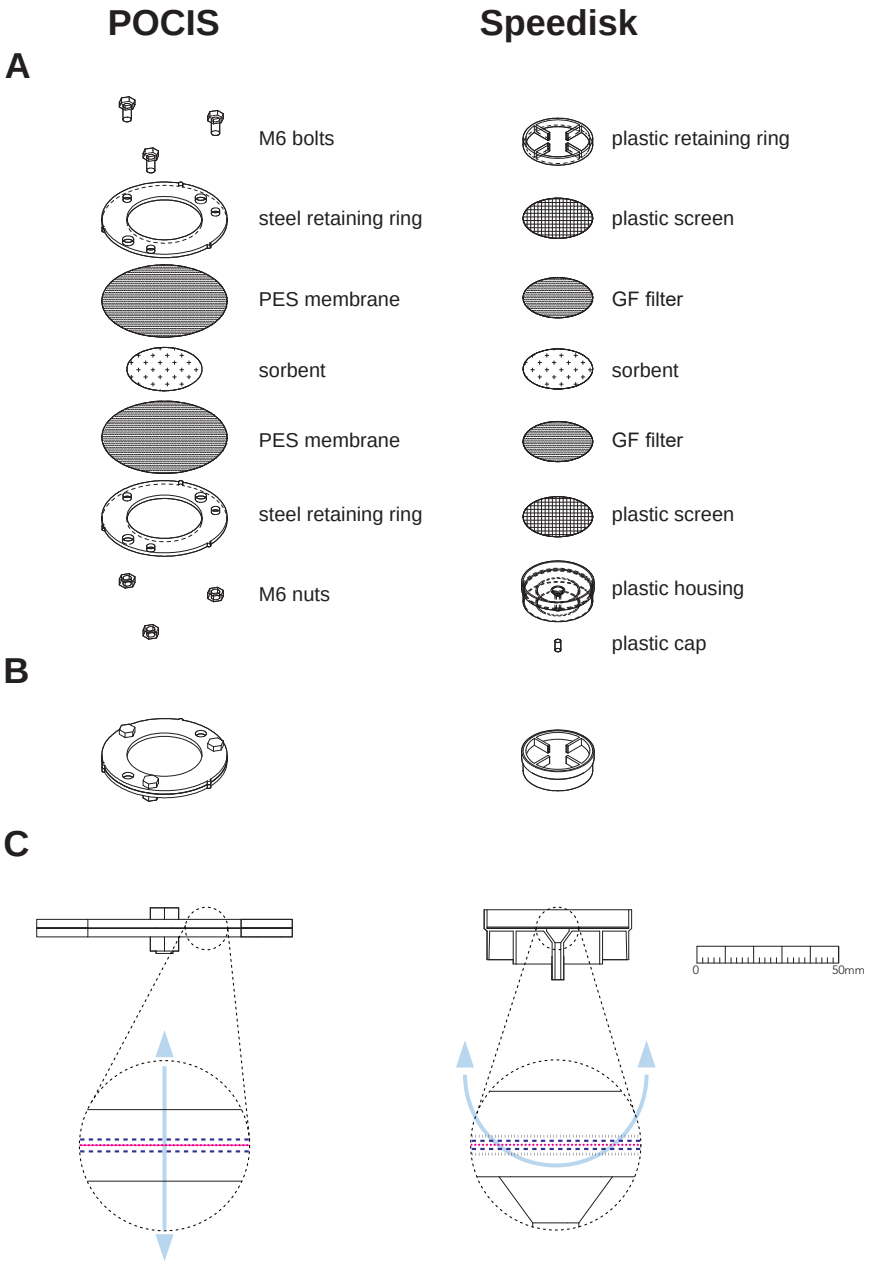

Figure 3.1. Technical drawing of polar organic chemical integrative sampler (POCIS) and Speedisk integrative passive sampling devices depicting A: a disassembled 3D view listing the separate passive sampler components (PES = polyethersulfone, GF = glass fiber), B: the 3D assembled configurations, and C: sections with expanded detailed hydrodynamic flow diagrams (dark blue dashed lines represent the membranes/ filters, magenta dotted lines represent the sorbent, light blue arrows illustrate the water movement through the passive samplers).

allowing exchange with the surrounding water from only one side of the sampler with an inner diameter of 5.1 cm, leaving an exposure area of approximately 20 cm^2. In the original POCIS design, the receiving phase consists of 200 mg of HLB sorbent (Oasis, Waters, MA, USA), while the original Speedisk contains 400 mg of H-DVB sorbent (Bakerbond, Avantor, Deventer, The Netherlands). The two sorbents were applied in the two sampler housings resulting in four sampler types.

Sampler preparation

The sorbents were conditioned by eluting with a sequence of organic solvents (Biosolve, The Netherlands; all chromatography grade) and dried under vacuum. For POCIS this was done before sampler assembly using acetone, dichloromethane, and methanol (Supporting Information 1). For Speedisk this was done after the preparation of the samplers (see below).

For the construction of the POCIS, stainless steel rings (Exposmeter, Sweden), nuts and bolts, as well as all used tools were cleaned in acetone before the assembly of the samplers. Polyethersulfone (PES) diffusion limiting membrane filters (Pall Corporation, NY, USA; 0.1 µm pore size, 90 mm diameter) were used to enclose 200 mg of either HLB or H-DVB sorbent. The PES membranes were cleaned before the assembly of the POCIS in LC grade methanol:ultra-pure water (50:50, v:v) followed by rinsing in ultra-pure water. After the final assembly, the POCIS were stored at 4°C in food-grade Mylar zip lock bags until deployment.

For the modification of the Speedisk, the upper half of the Speedisk housings were trimmed to limit the formation of a WBL between the sampler and the surrounding water, to improve the exchange of compounds between the water and the sorbent. Four holes were made in the sorbent-free bottom part of the housing to allow the attachment of the samplers during field deployment. Original Speedisk were advertised to contain 600 mg of H-DVB sorbent. Therefore, to assess the performance of the HLB sorbent in the Speedisk, these were disassembled and the original sorbent was replaced with 600 mg of HLB, followed by reassembly. However, upon disassembly Speedisk appeared to contain only 400 mg of H-DVB sorbent per column. Nevertheless, the amount of sorbent in both Speedisk configurations was sufficient to ensure a surface area per mass of sorbent ratio greater than that of the POCIS.[92] All Speedisk were sequentially eluted with dichloromethane, acetone, and ultrapure water (Supporting Information 1) over a vacuum manifold and the bottoms of the columns were closed with syringe caps to ensure that compound accumulation in the Speedisk during field deployment occurred only by diffusion from the top of the sampler (Figure 3.1). The Speedisk were then placed in a jar filled with ultrapure water and stored at 4°C until deployment.

Sampling locations and sampler deployment

Sampling locations were selected in collaboration with Dutch regional water authorities. This resulted in a set of eight lowland streams and drainage ditches in The Netherlands. The locations were categorized into three location types (Supporting Information 2), either surrounded by ornamental flower bulb horticulture (agriculture; n=3), directly receiving WWTP effluent

(WWTP; n=2), or reference locations with no known contamination sources (reference; n=3). Sampling was conducted between August 20th and October 5th, 2018. The four sampler types were deployed simultaneously at each sampling location, attached to stainless steel cages. Cages with samplers were installed in the middle of the water column to ensure permanent inundation and a continuous flow of water around the samplers, while avoiding direct diffusion of compounds from the sediment to the samplers. Per location, samplers were exposed in quadruplicate for a period of six weeks. After exposure, the samplers were cleaned in the field with local water and a scrubbing sponge to remove biofouling, transported to the laboratory on ice and stored at -20 °C until extraction.

Extraction of organic compounds from the passive samplers

The extraction of organic compounds from the passive samplers was performed according to the general protocol described below. The POCIS extractions were performed at the laboratory of the University of Amsterdam (The Netherlands) and Speedisk extractions at the laboratory of TNO (Utrecht, The Netherlands). Slight differences in the extraction procedures due to sampler type-specific characteristics and differences in laboratory equipment are outlined in Supporting Information 1. Frozen samplers were freeze-dried overnight. All glassware used in the subsequent extraction procedure was cleaned and dried. Each sampler was disassembled and the dry sorbents of the quadruplicates per sampler type per location were pooled and transferred to an empty 6 mL glass Supelco SPE column with Teflon frit (Sigma-Aldrich, The Netherlands) by a glass funnel. The mass of the recovered sorbent per location was recorded with an analytical balance. The SPE columns were placed on an SPE manifold and eluted with LC grade acetonitrile under vacuum. The LC grade acetonitrile that was used in all extractions in both laboratories originated from the same bottle to rule out any confounding influence of the batch of solvent used. Finally, the extracts were topped up to exactly 10 mL with acetonitrile by weight and stored at -20°C until analyses. Blanks for all sampler types were obtained by extracting unexposed dry samplers following the same procedure as their exposed counterparts and were included in the subsequent analyses.

Toxicity of the passive sampler extracts

The toxicity of the passive sampler extracts was assessed with a battery of five bioassays that were previously shown to be responsive to polar passive sampler extracts:[88] i.e. a bacterial bioluminescence inhibition bioassay and *in vitro* chemical activated luciferase expression (CALUX®) bioassays for estrogenic (ERα), *anti*-androgenic (*anti*-AR) and *anti*-progestogenic (*anti*-PR) activities, and cytotoxicity. Results from the latter test were also used to rule out confounding influences of cytotoxicity by the passive sampler extracts on test outcomes of the other three CALUX assays. Before application in the bioassays, the acetonitrile extracts were transferred to dimethyl sulfoxide (DMSO). A 1 mL aliquot of each sampler extract was used for the bacterial bioluminescence inhibition assay and a 2 mL aliquot for the four CALUX assays. The extracts were dried under constant N_2 flow at room temperature and redissolved in DMSO.

Bioassays with the extracts of the four passive sampler types were performed at a 0.1-1% DMSO concentration to improve the compound solubility in the exposure media, always including a control to confirm the non-toxicity of the solvent.

The bacterial bioluminescence inhibition assay, using the marine bacterium *Aliivibrio fischeri*, was based on the Microtox® bioassay and was performed in a miniaturized setup according to Hamers *et al.* (2001).[95] This bioassay is further referred to as the 'bacterial bioluminescence assay'. Luminescence inhibition was measured after 15 min of exposure to a dilution series of the passive sampler extracts. The *in vitro* cytotoxicity, ERα, *anti*-AR and *anti*-PR CALUX bioassays were performed according to previously described protocols at the BioDetection Systems laboratory (Amsterdam, The Netherlands).[96]

2.6. Data analyses

Toxicity in the bacterial bioluminescence assay was expressed as toxic units (TU), wherein one TU represented the dilution at which the extracts caused a 50% effect (EC_{50}). EC_{50} values were determined by nonlinear regression analysis with the built-in log-logistic model in GraphPad Prism® (GraphPad Software Inc., v. 5.00, San Diego, CA, USA). Responses in the *in vitro* CALUX assays were expressed as bioanalytical equivalent (BEQ) concentrations of the reference compounds. Responses in the ERα assay were expressed as ng 17β-estradiol eq. per mL extract (ng EEQ mL^{-1}), in the *anti*-AR assay as µg flutamide eq. per mL extract (µg FEQ mL^{-1}), in the *anti*-PR assay as ng RU486 eq. per mL extract (ng REQ mL^{-1}), and cytotoxicity as µg tributyltin eq. per mL extract (µg TEQ mL^{-1}). Bioanalytical responses were corrected for the recovered fraction of the sorbent to account for sorbent loss during the extraction procedure and normalized for the exposure area of the samplers (POCIS 46 cm^2; and Speedisk 20 cm^2). The normalized responses were then compared between the two types of sampler housing and the two types of sorbent. In this comparison, responses were considered higher if they exceeded those from the alternative housing or sorbent, respectively, by >20%. Responses were also considered higher if the alternative housing or sorbent caused no response at all in the bioassays.

RESULTS AND DISCUSSION

Bioassay validity and effect expression

The passive samplers were successfully retrieved from the field and extracted in the laboratory, except two of the four POCIS H-DVB samplers at location 'WWTP 1', of which the PES membranes were damaged during deployment and which were therefore not included in the subsequent analyses. The five bioassays were successfully performed with all extracts and all assays met their respective validity criteria.[15,96] Responses were observed in all bioassays and for all sampler types (Figure 3.2). Responses in the bacterial bioluminescence inhibition assay were corrected for responses to the blank extracts, while no responses to the blank extracts were observed in the CALUX assays.

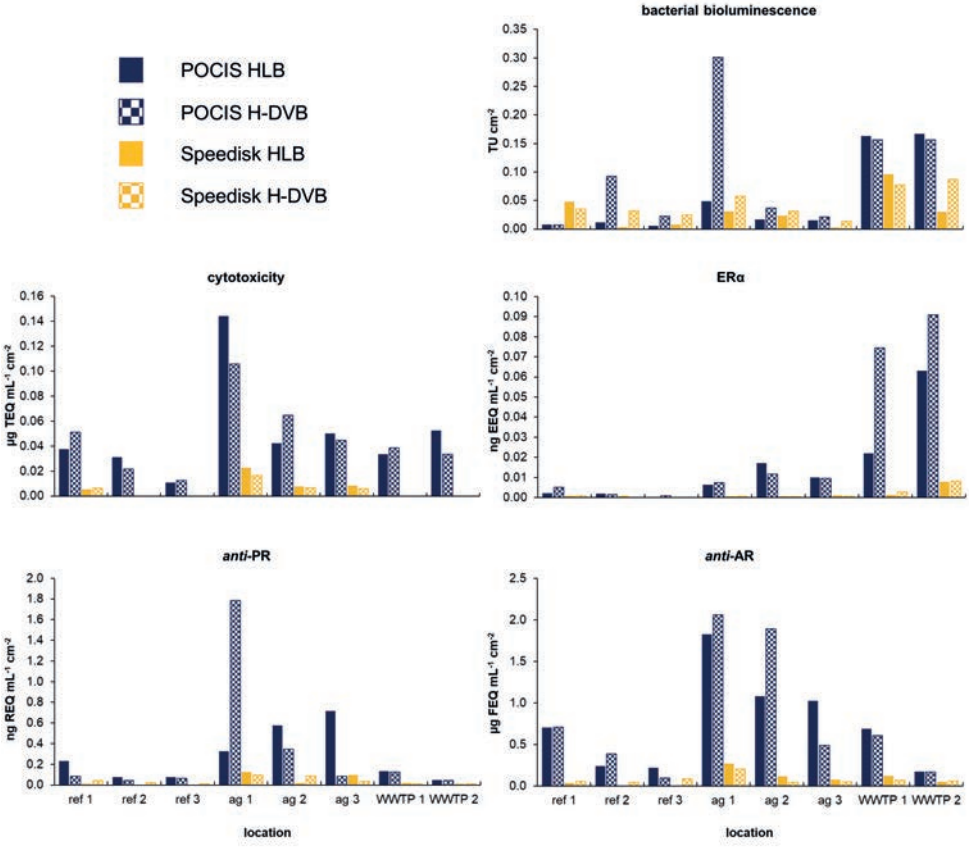

Figure 3.2. Bioanalytical responses to extracts from four passive sampler configurations with different housings (POCIS vs. Speedisk) and sorbents (HLB vs. H-DVB) for the bacterial bioluminescence inhibition assay and four CALUX bioassays. Responses were normalized for sampler exposure area and sorbent recovery after extraction. TU = toxic unit, EEQ = 17β-estradiol eq., TEQ = tributyltin eq., REQ = Ru486 eq., FEQ = flutamide eq., ref = reference, ag = agriculture, WWTP = wastewater treatment plant.

The influence of sampler housing on bioassay responses

The comparison of the bioassay responses between the POCIS and Speedisk housings, independent of the applied sorbents, clearly illustrates the substantially higher responses caused by the POCIS extracts in most bioassays for almost all locations (Figure 3.2). Indeed, when quantifying the differences in responses (applying a 20% cutoff value) between the sampler housings, POCIS caused higher responses in 91% of extract x bioassay combinations (Table 3.1). This was observed for the bacterial bioluminescence assay but was especially pronounced for the CALUX *in vitro* assays, in which the Speedisk extracts never caused a response higher than the POCIS extracts. Evidently, the POCIS in the majority of cases accumulated higher amounts of compounds that elicit responses in all of the applied bioassays. Since the responses were corrected for the exposure area of the samplers, this cannot be attributed to the higher exchange surface area of the POCIS, but rather is a result of the design

Table 3.1. Comparison of bioanalytical responses to extracts from four types of passive samplers with different housings (POCIS vs. Speedisk) and sorbents (HLB vs. H-DVB). Bioanalytical responses were considered higher (+) when they exceeded responses to extracts from the alternative housing or sorbent, respectively, by >20%.

		CALUX					
bioassay response	bacterial bioluminescence	cytotoxicity	ERα	*anti*-AR	*anti*-PR	TOTAL	%
housing							
equal	2	0	0	1	0	3	**4**
POCIS +	10	16	16	15	16	73	**91**
Speedisk +	4	0	0	0	0	4	**5**
sorbent							
equal	3	5	5	5	3	21	**26**
HLB +	2	9	6	6	8	31	**39**
H-DVB +	11	2	5	5	5	28	**35**

of the sampler. Apparently, the POCIS housing allows the accumulation of substantially higher amounts of toxic compounds from the surrounding surface waters than the Speedisk housing does.

The higher bioassay responses caused by the POCIS extracts cannot be attributed to differences in the used sorbents, since both types of sorbent were applied in both sampler housings (the influence of the sorbent type on bioassay responses is discussed in section 3.3). Hence, the differences in the bioassay responses between POCIS and Speedisk are attributable to differences in either the thickness of the WBL or the diffusion across the (membrane) filter or both. The thickness of the WBL is partly dictated by water turbulence,[52] which was identical for all sampler types since they were simultaneously exposed at the same locations. For the other part, the WBL thickness depends on hydrodynamic conditions in the vicinity of the membrane, which are significantly affected by the sampler housing geometry. The depth of the sampler body (i.e. the distance between the outer housing and the surface of the filter) influences the rate of the convective transport of analytes to the filter, where a deeper sampler body effectively reduces passive sampling rates.[97] The shallower depth of the POCIS (3 mm) compared to the Speedisk (6 mm) may thus very well have resulted in higher sampling rates for POCIS. Similar to sampler depth, obstructions to water movement in the sampler housing can also negatively affect sampling rates. Where the POCIS membrane is in direct contact with the WBL, the Speedisk filter and sorbent are held in place by a plastic screen and a retaining ring (Figure 3.1). These physical obstructions are also expected to decrease the convective transport of analytes to the Speedisk filter, further limiting Speedisk sampling rates. These observations suggest that the hydrodynamic conditions in the sampler housings appear to be more favorable for the diffusion of compounds into the POCIS and may thus, at least partly, explain the higher bioassay responses caused by the POCIS extracts.

Once compounds have reached the (membrane) filter of the sampler, permeation through the filter can occur in two ways, either through the water-filled pores or via the filter material itself.[52] Filters are applied in passive samplers to retain and protect the sorbent, but also to regulate the uptake rate of compounds.[98] Hence, the filters intentionally limit the diffusion of compounds to the sorbent to extend the linear uptake phase of compounds into the sampler. Polymeric filters like PES membranes have been shown to substantially limit the uptake rates of compounds in passive samplers, especially for compounds with a $\log K_{OW}$ >2.[98,99] This is attributable to the accumulation of hydrophobic compounds in the PES membrane, which leads to a lag in the transfer of these compounds to the sorbent.[99] The undesirable sorption of compounds to the filters can be avoided by the use of alternative inert membrane materials, like PTFE.[98] The glass fiber filters used in the Speedisk are also expected to exhibit a very low affinity towards compounds, and should thus result in faster transport of analytes through the filter. This would result in improved sampling rates, especially for hydrophobic chemicals, compared to samplers in which sorptive polymeric filters are used. Nevertheless, it appears that any advantage of the use of glass fiber filters in the Speedisk for the passive sampling of polar compounds from surface waters was offset by the decreased hydrodynamics resulting from the design of the Speedisk housing.

A proposed advantage of the Speedisk is its commercial availability in a robust housing, which simplifies its application as a passive sampler.[93] The robustness makes the loss of samplers resulting from damage less likely than with POCIS, in which puncturing or rupturing of the PES membrane sometimes occurs, as was also observed in the present study. Additionally, the Speedisk design as an SPE column can simplify the extraction procedure after deployment. However, biofouling is likely to occur on the surface of passive samplers during extended field deployments. This is an issue if the intact Speedisk sampler is extracted after field exposure since the co-extraction of compounds accumulated in the biofilm will occur. To avoid this, the samplers should be partly disassembled to remove the biofouled parts of the sampler housing before extraction. The greater robustness of the Speedisk housing alone does not offer a convincing advantage over the use of the POCIS housing, since the Speedisk housing geometry limits the sampling rate, resulting in less frequent and less intense bioassay responses. Following these observations, it is concluded that the use of the POCIS housing results in much more frequent detections of potentially toxic polar organic compounds in surface waters compared to Speedisk when using a combination of passive sampling and bioassays.

The influence of sorbent type on bioassay responses

The comparison of the bioassay responses between the HLB and H-DVB sorbents, independent of the applied housings, elucidated that the two sorbents caused equal bioassay responses in only 26% of cases (Table 3.1). However, there was not one sorbent that clearly outperformed the other, as extracts from samplers containing HLB caused higher responses in 39% of cases and extracts from samplers containing H-DVB caused higher responses in 35% of cases

(Table 3.1). Apparently, both sorbents effectively adsorb a partly different suite of compounds, leading to differences in bioassay responses.

The efficacy of H-DVB in the sorption of organic CECs from water was recently elaborately investigated and compared to that of HLB.[93] This revealed a higher degree of cross-linkage and functionalization for H-DVB compared to HLB. These findings are in line with the reported higher extraction efficiencies for a range of nonpolar organic CECs of H-DVB compared to HLB.[100,101] Indeed, this higher degree of polymer functionalization for H-DVB is expected to provide an improved sorption capacity, especially for nonpolar compounds.[93] However, the present results illustrate that the higher sorption capacity of H-DVB did not result in higher bioassay responses *per se*. Apparently, the HLB sorbent was able to adsorb other – or higher quantities of the same – toxic compounds from the same surrounding surface water than H-DVB in 39% of cases, as reflected in the higher bioassay responses caused by the HLB extracts. Given the stronger sorption of nonpolar compounds to H-DVB, the higher responses to the HLB extracts may be attributable to its superior sorption of polar compounds. However, this hypothesis should be tested in laboratory-based experiments that quantify the sorption capacity of H-DVB and HLB for (highly) polar compounds.

The present study illustrated that the choice of sorbent can strongly affect the observed toxicity detected in surface water quality assessment strategies that apply passive sampling. In such strategies, a passive sampler, or a combination of multiple types of samplers, ideally accumulates all potentially toxic substances from the water so that false-negative toxicity detections are avoided. In the present study, false-negatives may have occurred for location 'ag 1', where only the POCIS H-DVB sampler extract caused relatively high responses in the bacterial bioluminescence assay and the *anti*-PR CALUX assay. At this specific location, the other three sampler types appear to have underestimated the presence of toxic levels of (a) certain compound (groups) that caused a toxic response in these bioassays. An elegant solution to the potential occurrence of false-negatives is the application of multiphasic sampler configurations that apply multiple sorbents with specific characteristics, which have been developed in particular for POCIS.[52] In some cases these configurations can indeed result in improved uptake and recovery for certain classes of compounds [e.g. Alvarez *et al.* (2004)[92]]. Given the anticipated shift in the characteristics of CECs to more highly mobile polar and ionizable compounds,[85,102] future-proof sampler configurations can be developed that house (mixtures of) novel adsorbents with ion exchanging or extremely polar properties.[103] Nonetheless, both HLB and H-DVB can adsorb a wide range of highly polar to moderately nonpolar organic compounds from aquatic matrices,[93] and their use as non-selective sorbents in polar passive samplers in surface waters is justified.

Pollution source-specific ecotoxicological profiling

Since the extracts from the samplers with POCIS housing caused more frequent and higher bioassay responses, the results from the POCIS samplers were used for the pollution source-specific profiling of the ecotoxicological responses. To cover as wide a range of potentially toxic

compounds as possible, responses of both the HLB and the H-DVB POCIS configurations were considered.

The identification of ecotoxicological risks to aquatic ecosystems can follow from the comparison of bioassay responses to so-called effect-based trigger values (EBTs).[31] However, the currently available EBTs are expressed as BEQ concentrations per volume of surface water and thus require the estimation of sampled water volumes for passive sampler extracts. Yet, the uncertainty in sampling rate estimations can negatively affect the data quality for passive samplers.[13] Especially considering the paucity of data on the sampling rates for Speedisk samplers, but also the uncertainties in the sampling rates for non-polar compounds arising from the use of PES membranes in the POCIS, this could affect the accuracy of the comparison between the samplers. This is undesirable since the aim of the present study was an impartial comparison of multiple sampler configurations. Hence, it was decided to refrain from the estimation of sampled water volumes and the subsequent comparison of bioassay responses to EBTs. However, it must be noted that differences in the flow velocities may have affected passive sampling rates during field exposure. This did not affect the comparison between the different sampler configurations, as they were simultaneously exposed to the same environmental conditions, but may have caused differences in the intensity of the observed bioassay responses between the investigated locations. Therefore, the differences in the ecotoxicological profiles that were observed in the present study are discussed only in terms of the occurrences of bioassay responses, without the subsequent interpretation of potential ecotoxicological risks.

For the bacterial bioluminescence assay, higher responses were observed for the two WWTP locations than for all other locations, except for the strikingly high response to the POCIS H-DVB extract from location 'ag 1' (Figure 3.2). The highest response in the CALUX cytotoxicity assay was also observed for location 'ag 1', for both POCIS configurations, while responses in this bioassay were otherwise rather uniformly caused by extracts from all the other locations. The ERα CALUX assay was particularly responsive to extracts from WWTP locations, while lower activities were caused by extracts from the agricultural locations. The *anti*-PR and *anti*-AR CALUX assays both showed the highest responses to extracts from agricultural locations, and lower activities for reference and WWTP locations, which were most notable in the *anti*-AR assay. A particularly high response in the *anti*-PR assay was caused by the POCIS H-DVB extract from location 'ag 1', in line with the bacterial bioluminescence assay results.

The results of the present study showed that extracts from reference locations typically caused relatively low bioassay responses, indicating limited, if any, toxicity compared to agricultural and WWTP locations. Agricultural locations were characterized by the highest responses in the *anti*-PR and *anti*-AR CALUX assays, and to a lesser extent by responses in the ERα CALUX assay. Responses in those assays can be caused by the endocrine-disrupting activity of pesticides and their metabolites,[104–106] and the elevated responses observed here are likely attributable to the use of pesticides on the agricultural fields surrounding these locations. The WWTP locations, contrastingly, were characterized by elevated bacterial bioluminescence assay and ERα activities. The bacterial bioluminescence assay is indicative of baseline toxicity and is hence a good indicator for the general presence of toxic compounds as well as a selection

of antimicrobial compounds in surface waters.[107] As a result, bacterial bioluminescence assay activity is commonly found in WWTP effluents, caused by a wide variety of micropollutants.[108] Estrogenic activity is often caused by a combination of natural and synthetic estrogens and industrial compounds,[42,109] and is typical for WWTP effluent impacted surface waters.[24,96] The implementation of advanced treatment steps in WWTPs can lead to a reduction of estrogenic activity in the effluent and the receiving surface water, which is often concurrent with reductions in concentrations of nutrients, pathogens, and micropollutants.[110,111] As such, ERα activity is a useful proxy for the pollutant burden that WWTP effluents exert on the receiving surface waters and can thus be used to evaluate WWTP performance, for example after the implementation of technological improvements.

In 2014, the Swiss government implemented a nationwide strategy to upgrade municipal WWTPs to effectively reduce the micropollutant load and toxicity in effluent over 20 years.[110] This visionary amendment to water quality legislation is expected to substantially improve surface water quality on a countrywide scale. Monitoring to assess the effectiveness of the implemented measures should be an integral part of the implementation of technical measures for water quality improvement. Given the low infrastructural demands and high throughput capacity of *in vitro* bioassays like the ERα CALUX, and its representativeness of the pollutant burden of WWTP effluent, there is high applicability of bioassays in such monitoring efforts. The anticipated improvements can readily be monitored using bioassays for estrogenic activity, allowing the identification of locations or WWTPs that require additional measurements or technical improvements. This suggested application is exemplary for the potential of bioassay response-driven mitigation measures for surface water quality improvement, highlighting the benefit of bioanalytical tools in the protection of water resources.

The future of passive samplers and bioassays in environmental monitoring

The present study demonstrated that passive sampling is a promising approach for the monitoring of polar organic micropollutants in surface waters. The sampler configuration determines the efficacy of the passive sampling device for the accumulation and sequestration of compounds, and hence the detection of potentially toxic elements in the environment. Improvements to sampler designs can be made to enable the integrative sampling of as wide a range of compounds as possible, which is necessary given the changing nature of anthropogenic chemical use. Furthermore, improved accuracy of sampled volume estimations for integrative passive samplers will strengthen ecotoxicological risk interpretations. Nonetheless, a substantial body of literature confirms the superiority of passive sampling approaches over conventional spot sampling and supports the application of passive samplers in chemical and effect-based environmental monitoring.[13–15,19,52,88] The combination with bioassays allows for the detection of the toxicity of a wide range of polar pollutants, with specific ecotoxicological response profiles that are related to the origin of the pollution. This, in turn, highlights the applicability of passive sampling and bioassays in water quality monitoring and their use for targeted mitigation measures to protect aquatic ecosystems from the increasing use of chemicals by society.

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SUPPORTING INFORMATION

Table S1. Technical details on construction, preparation and extraction of four passive sampler configurations resulting from the combinations of polar organic chemical integrative sampler (POCIS) and Speedisk housings and hydrophilic-lipophilic balance (HLB) and hydrophilic divinylbenzene (H-DVB) sorbents.

	POCIS		Speedisk	
Sorbent type	**HLB**	**H-DVB**	**HLB**	**H-DVB**
Sorbent mass per sampler	200 mg		600 mg	400 mg
Conditioning	Before assembly in original column: 30 mL acetone 30 mL dichloromethane 30 mL methanol		After Speedisk assembly: 15 mL dichloromethane 10 mL acetone 20 mL ultrapure water	
Freeze-drying	At −53°C in a Scanvac CoolSafe freeze-dryer		At −55°C in a Heto Powerdry LL3000 (Thermo Scientific) or IlShin Biobase (Scala Scientific) freeze-dryer	
Glassware cleaning	With acetone and LC grade acetonitrile and dried in an oven at 50°C		In a laboratory dishwasher and fired out at 350°C in an oven to dry	
Elution (LC grade acetonitrile)	3 x 3 mL		4 x 5 mL (Sorbent divided over two glass SPE columns)	3 x 5 mL
Extract concentration	n/a		On a Rotavapor system at 45°C and 117 mbar to approximately 3 mL	
Final extract	Topped up to exactly 10 mL with acetonitrile by weight			

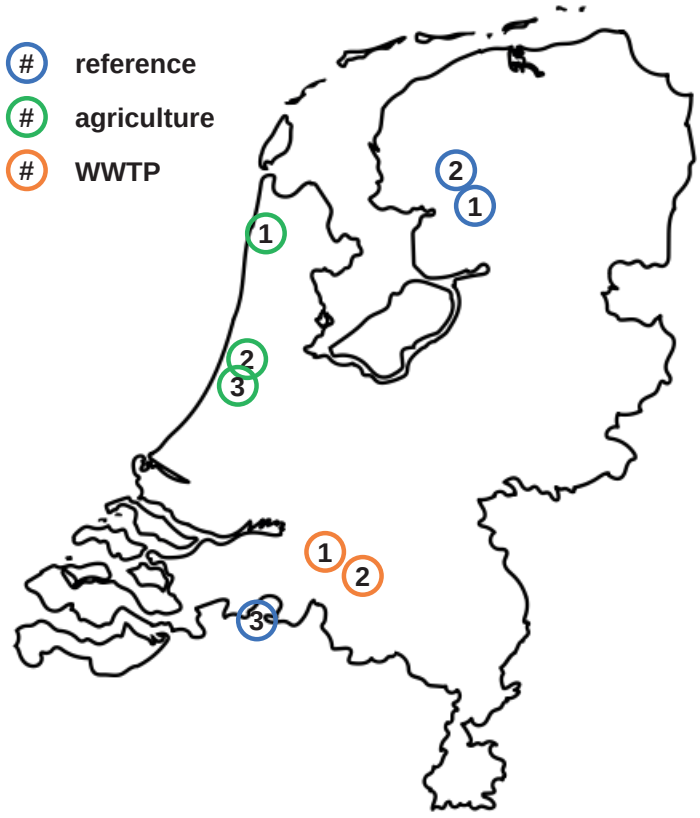

Figure S1. Surface water sampling locations in The Netherlands. WWTP = wastewater treatment plant.

Table S2. GPS coordinates and general field parameters (taken once during the sampling period along 25 m stretches) for surface water locations in The Netherlands. Location ID acronyms: ref = reference, ag = agriculture, WWTP = wastewater treatment plant.

location ID	latitude	longitude	depth (m)	width (m)	flow velocity (cm/s)
ref 1	52°49'22.7"N	5°54'26.5"E	1.0	6.0	0.9
ref 2	53°00'22.3"N	5°48'43.4"E	0.7	2.5	2.0
ref 3	51°25'40.9"N	4°46'46.8"E	1.0	3.0	1.3
ag 1	52°45'51.4"N	4°40'52.0"E	1.2	6.0	11.6
ag 2	52°17'23.2"N	4°30'37.7"E	0.6	4.0	1.3
ag 3	52°17'05.3"N	4°29'54.7"E	1.2	5.5	1.0
WWTP 1	51°36'08.3"N	5°04'32.9"E	0.4	5.0	25.5
WWTP 2	51°30'15.0"N	5°10'19.9"E	0.4	4.0	14.7