Time integrative sampling properties of Speedisk and silicone rubber passive samplers determined by chemical analysis and \textit{in vitro} bioassay testing

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**HIGHLIGHTS**

- Speedisks are good time integrative samplers for individual compounds.
- Linear uptake of compounds by Speedisks is reflected by \textit{in vitro} bioassay responses.
- Linear uptake by silicone rubber is poor for compounds active in \textit{in vitro} bioassays.
- DR-LUC bioassay responses cannot only be attributed to hydrophobic compounds.

**ABSTRACT**

Compared to grab samples, passive samplers have the advantage that they sample over a longer time period and can detect lower compound concentrations in water quality monitoring campaigns. To allow the determination of time-weighted average concentrations, however, sampler uptake should remain linear in time over the entire sampling period. Therefore, the time integrative or linear uptake properties of adsorption-based Speedisks and partitioning-based silicone rubber samplers were assessed with respect to chemically analyzed single compounds and measured bioactivity in \textit{in vitro} bioassays. Both sampler types were deployed in consecutive and overlapping time series in a WTTP effluent and in the river Meuse up to 105 days. Extracts were chemically analyzed for PCBs, PAHs and pesticides and tested in the \textit{Aliivibrio fischeri} and DR-LUC bioassays. Speedisks showed time integrative sampling for the detected pesticides as well as for bioassay responses at both sampling locations for the entire sampling period. The silicone rubber samplers showed poor linear uptake in time for the unknown compounds causing bioassay responses. The bioassay results indicate that conversion of a bioassay response to a passive sampler extract into a time-weighted average bioactivity per liter water seems justified for Speedisks, confirming that concentrations in the samplers correspond to a single volume of sampled water for all compounds. The bioassay results also indicate that a similar conversion for silicone rubber extracts should be interpreted with caution. In principle, it is actually impossible, because the concentration of each compound contributing to the bioassay response corresponds to a different sampled water volume.

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1. Introduction

Worldwide, man-made organic compounds like pesticides, pharmaceuticals, flame retardants, plasticizers and polychlorinated
biphenyls (PCBs) are found in the aquatic environment (Luo et al., 2014; Petrie et al., 2015). In the European Union, legislative monitoring is in force to obtain information of the contaminants of concern and the trends in concentrations of such compounds (EU, 2008). This monitoring is based on grab sampling of the water column followed by chemical analysis of target contaminants and a compound-by-compound compliance check between the observed concentration in water \( C_w \) versus the environmental quality standard (EQS). This monitoring strategy, however, has several drawbacks, i.e. (1) the grab sampling only reflects the concentration at the moment of sampling while concentrations of contaminants can fluctuate over time due to variations in flow or irregular emission patterns; (2) testing measured \( C_w \) of only targeted contaminants ignores the presence of other contaminants including those present below the limit of detection (<LOD); (3) such an approach ignores the possible mixture toxicity of multiple contaminants present in the environment (Silva et al., 2002; Walter et al., 2002).

To overcome these drawbacks, new monitoring strategies have been proposed. One of these strategies is based on time integrative passive sampling followed by toxicity profiling with bioassays (TIPTOP) (Hamers et al., 2018). Passive sampling is based on the deployment of adsorption or partitioning material in the aquatic environment for typically a couple of weeks. During this deployment freely dissolved contaminants accumulate into the sampler material at a rate controlled by their diffusion through the water boundary layer (WBL) at the surface of the sampler. This passive accumulation of compounds in the sampler makes passive sampling a promising tool for monitoring low concentrations of substances with polar functional groups in water. Moreover, temporal variability in \( C_w \) will be time integrated during the deployment period (Greenwood et al., 2007; Vrana et al., 2014). Passive sampling is traditionally used in combination with chemical target analysis. To account for non-analyzed compounds and for possible mixture toxicity, however, passive sampler extracts can also be tested in a battery of bioassays that can estimate the overall toxicity of the complex mixture (Hamers et al., 2010, 2013; 2018; Van der Oost et al., 2017b; Escher et al., 2018). Given the benefits of passive sampling versus grab sampling, and of effect-based monitoring versus chemical monitoring, the combination of passive sampling and bioassays has been frequently suggested for monitoring chemical water quality (Emelougu et al., 2013; Jahnke et al., 2016; Van der Oost et al., 2017a,b; Hamers et al., 2018, De Baat et al., 2019).

Several types of passive samplers are currently in use, which can be divided into two groups according to their main working principle, i.e. adsorption-based samplers and partitioning-based passive samplers. Adsorption-based samplers bind the substances at the surface of the adsorption material even if only a part of the molecule has the appropriate affinity. These types of samplers were introduced especially for substances with polar functional groups that avoid absorption by partitioning-based samplers. If sufficient binding capacity is present, adsorption-based samplers are assumed not to reach equilibrium and show contaminant uptake that is linear over time, reflecting the time-weighted average concentration for all compounds present in the aqueous phase over the entire sampling period (Supplementary Information Fig. S1).

Partitioning-based samplers mainly absorb hydrophobic substances from the aqueous phase because of their much better solubility in the sampler material (mostly polymeric) compared to the water. If exposed long enough, contaminant concentrations in the sampler \( C_p \) will ultimately attain equilibrium with \( C_w \) in a ratio equal to the sampler–water partition coefficient, \( K_{pw} = C_p/C_w \) (Fig. S1). For partitioning-based samplers, the time required for a compound to attain equilibrium increases proportional with the compound’s \( K_{pw} \) (Huckins et al., 2006; Booij et al., 2007).

Hydrophilic compounds have a lower \( K_{pw} \) compared to hydrophobic compounds and therewith a shorter equilibrium time. Time to reach equilibrium is also shortened when water turbulence is higher, causing a thinner WBL and therefore faster uptake (Booij et al., 1998; Vrana and Schüürmann, 2002). Because individual compounds reach equilibrium in different times, the sampled volume per compound is different as well. For compounds with known \( K_{pw} \) values and molecular weight, the sampled water volume corrected for variation in field conditions can be determined based on the release of Performance Reference Compound (PRC) dosed to the sampler prior to deployment (Rusina et al., 2010; Booij and Smedes, 2010). So, the time integrative sampling properties of these samplers are well-known regarding individual known chemical compounds and it can be determined whether the compounds are in the linear uptake phase or have reached equilibrium (Huckins et al., 2006; Booij et al., 2007). In principle, quantification of sampled water volumes is not possible for unknown compounds or mixtures because the \( K_{pw} \) and molecular weight are of course unknown as well. Similarly, the time integrative sampling properties are not known regarding bioassay responses to complex mixtures sampled by silicone rubber samplers in the field situation and consisting of compounds in the linear uptake phase, in equilibrium, or in between.

In principle, time-integrative bioassay responses to passive sampler extracts indicate that the compounds responsible for the bioassay response were sampled from the same aqueous volume in a time-integrative way. This is a requirement when bioassay responses to a passive sampler extracts are converted into time-weighted average bioactivities per liter water. Therefore, the aim of this study was to investigate the time integrative sampling properties of adsorption-based and partitioning-based passive samplers for known compounds by chemical analysis and for unknown mixtures by bioassay testing.

Speedisk was chosen as adsorption-based sampler. Although Speedisk was originally designed as SPE column, it was made fit for passive sampler use after some modifications. Speedisk has a higher sorption than frequently used POCIS (Polar Organic Chemical Integrative Sampler) (Alvarez et al., 2004) and Chemcatcher (Kingston et al., 2000) due to the larger amount of adsorption material. In contrast to the polyether sulfone membrane of the POCIS (Vermeiren et al., 2012), the glass fibre filter in Speedisks has a permeability only slightly lower than water with little variation between substances, whereas no sorption of compounds takes place (Fig. S2). The higher adsorption capacity and the glass fibre filter properties are the main elements that give the Speedisk the potential for a long time integrative period. Previous applications have demonstrated that Speedisk is a robust sampler for environmental monitoring of chemical compounds (Foekema, 2012; Hamers et al., 2018; Van Hoorn and De Weert, 2012). So far, time integrative sampling properties of adsorption-based samplers have been studied over a period of 1 month at maximum (Harman et al., 2012). In some situations, however, a longer exposure of the samplers in the field may be required, e.g. while sampling in remote areas or at locations far apart from each other. For such situations, it is necessary to confirm that the uptake is time-integrative indeed. Therefore, we designed a long-term field exposure experiment, which – to our knowledge – has not been performed with adsorption-based samplers before. Samplers were deployed for consecutive short periods and for a parallel single deployment over the total sampling period ranging from 2 to 15 weeks. Extracts were analyzed for amount of compounds and for two types of bioassay responses, i.e. the inhibition of bioluminescence in Aliivibrio fisheri bacteria by compounds ranging from hydrophilic to hydrophobic, and the activation of the arylhydrocarbon receptor (AhR) in the DR-LUC bioassay, supposedly by hydrophobic compounds. Time
integrative sampling properties were determined by comparing the cumulative results of short consecutive deployments to the results for a parallel long deployment. Results for the adsorption-based Speedisk samplers were compared with results for partitioning-based samplers. Silicone rubber was chosen as partitioning-based sampler material given the high absorption capacity, the well-investigated uptake model (Rusina et al., 2010), and the possibility to determine the degree to which equilibrium is attained from the release of PRCs (Booij and Smedes, 2010).

2. Materials and methods

2.1. Passive sampler preparation

A Speedisk (BAKERBOND® Speedisk®) consists of an ultra-clean polypropylene cylinder-shaped container filled with ~600 mg of H2O-Phlic DiVinylBenzene (DVB) sorbent placed on the bottom as a uniform layer covered with a fine nylon mesh and a 0.5 mm glass fibre filter held in place by a 2 mm nylon mesh. The 0.5 mm glass fibre filter may act as a fixed water boundary layer largely regulating the diffusive uptake of compounds.

To prepare the Speedisks as passive samplers, the upper half (filling side) of the Speedisk container was removed to improve the contact with the surface water and holes were made at the elution side (bottom) of the container to enable connection to a sampling frame. More details about the deployment has been described in Hamers et al. (2018) (supplemental material). Prior to deployment the Speedisk adsorbent was cleaned by elution with 3 times 5 mL dichloromethane, acetone and milli-Q water, respectively. The wet Speedisks were immersed in water in a 800 mL glass jar to keep the sorption material wet and stored at 4 °C until deployment. Per sampling site and sampling occasion three Speedisks were deployed considered as one sampler, of which the combined extracts were used for bioassay testing and chemical analysis.

For the silicone rubber samplers, translucent silicone rubber sheets (Altecweb.com (UK); 0.5 mm thick) were cut in pieces of 9.5 × 5.5 cm containing two punched holes allowing attachment to the sampling frame. To remove impurities and oligomers, the sheets were pre-extracted in a soxhlet with ethylacetate (Boom BV, Netherlands) for a week. Silicone rubber sampler sheets meant for bioassay testing and chemical analysis of the unspiked samplers deployed in the present study. The extraction and processing of the extracts has been described in detail by Hamers et al. (2018) (supplemental material). In brief, the sorption material of the Speedisk sampler was removed from the holder and dried under a flow of dry nitrogen gas at room temperature. Then the adsorption material was extracted with 250 mL dichloromethane by gently shaking for 15 days. The dichloromethane was concentrated to ~5 mL, of which a 25% aliquot (based on weight) was used for chemical analysis and the remaining 75% for the in vitro bioassays and vapor pressure osmometry to measure the molar amount of the mixture of compounds in the extracts. The extract for chemical analysis was spiked with an internal standard. Half of the extract was used for GC-MSMS or GC-MS-EI analysis and after addition of 100 mL acetonitrile to the other half of the extract, dichloromethane was removed azeotropically by Kuderna Danish evaporation, further concentrated and used for LC-MSMS analysis.

The silicone rubber samplers intended for bioassay testing and chemical analysis were extracted twice by gently shaking for three days with 75 mL acetonitrile. The combined extracts were concentrated to about 5 mL acetonitrile, which was split based on weight keeping ~25% for chemical analysis and ~75% for in vitro bioassays and vapor pressure osmometry. To the 75% portion 100 mL hexane was added and acetonitrile was removed azeotropically by Kuderna Danish evaporation. The 25% portion was concentrated to 1 mL, spiked with an internal standard, and split into two equal parts. One part was used for LC-MSMS analysis, and the other part was transferred to hexane by azeotropic evaporation and used for GC-MSMS or GC-MS-EI analysis. The PVC spiked silicone rubber samplers were extracted similarly as the non-spiked silicone rubber samplers. Internal standard addition, extract split, and chemical analysis were also performed similarly. The PVC concentrations were determined by GC-MSMS.

Prior to bioassay testing, Speedisk extracts in dichloromethane and silicone rubber extracts in hexane were evaporated under a gentle nitrogen stream and transferred to DMSO.

2.2. Sampling locations and deployment scheme

Samples for bioassay testing and chemical analysis were exposed in the effluent of a wastewater treatment plant (WWTP) at Amersfoort and in the river Meuse at Eijsden (The Netherlands) in summer and fall of 2014 (Fig. S3). The samplers were deployed in time series following the timeline depicted in Fig. 1. In these time series, three consecutive sampling periods of two weeks (i.e. week 1–2, 3–4, and 5–6) were performed in parallel to a single sampling period of six weeks (week 1–6). The six-week sampling period was followed by a nine-week sampling period (i.e. week 7–15) which formed again a consecutive set with a parallel fifteen-week sampling period (week 1–15). This setup allowed to assess the time integrative sampling properties of the two sampler types, even if the aqueous concentrations in the field are not constant.

Simultaneously to the 1–6 week sampling period, a duplicate Speedisk sampler was deployed for chemical analysis and bioassay testing and a duplicate unspiked silicone rubber sampler was deployed during week 1–6 for bioassay testing. Both were used within the context of a parallel study on the toxicity profiling of six-week passive samplers at multiple sampling sites (Hamers et al., 2018). Finally, additional PRC-spiked silicone rubber samplers were deployed during week 1–6 and 7–15 to allow for sampling rate (L/d) estimation, which is used to derive a time-weighted average CW of the chemically analyzed compounds. Extracts from the latter silicone rubber samplers were also used for duplicate chemical analysis of the unspiked samplers deployed in the present study.

2.3. Extraction and processing of the samples

The extraction and processing of the extracts has been described in detail by Hamers et al. (2018) (supplemental material). In brief, the sorption material of the Speedisk sampler was removed from the holder and dried under a flow of dry nitrogen gas at room temperature. Then the adsorption material was extracted with 250 mL dichloromethane by gently shaking for 15 days. The dichloromethane was concentrated to ~5 mL, of which a 25% aliquot (based on weight) was used for chemical analysis and the remaining 75% for the in vitro bioassays and vapor pressure osmometry to measure the molar amount of the mixture of compounds in the extracts. The extract for chemical analysis was spiked with an internal standard. Half of the extract was used for GC-MSMS or GC-MS-EI analysis and after addition of 100 mL acetonitrile to the other half of the extract, dichloromethane was removed azeotropically by Kuderna Danish evaporation, further concentrated and used for LC-MSMS analysis.

The silicone rubber samplers intended for bioassay testing and chemical analysis were extracted twice by gently shaking for three days with 75 mL acetonitrile. The combined extracts were concentrated to about 5 mL acetonitrile, which was split based on weight keeping ~25% for chemical analysis and ~75% for in vitro bioassays and vapor pressure osmometry. To the 75% portion 100 mL hexane was added and acetonitrile was removed azeotropically by Kuderna Danish evaporation. The 25% portion was concentrated to 1 mL, spiked with an internal standard, and split into two equal parts. One part was used for LC-MSMS analysis, and the other part was transferred to hexane by azeotropic evaporation and used for GC-MSMS or GC-MS-EI analysis. The PVC spiked silicone rubber samplers were extracted similarly as the non-spiked silicone rubber samplers. Internal standard addition, extract split, and chemical analysis were also performed similarly. The PVC concentrations were determined by GC-MSMS.

Prior to bioassay testing, Speedisk extracts in dichloromethane and silicone rubber extracts in hexane were evaporated under a gentle nitrogen stream and transferred to DMSO.

2.4. Chemical analysis

Passive sampler extracts were analyzed for polychlorinated biphenyls (PCBs) including the PRCs, polycyclic aromatic hydrocarbons (PAHs) and about 120 pesticides using GC-MSMS, GC-MS-EI and LC-MSMS using the same method as described by Hamers et al. (2018) (supplemental material).
2.5. Vapor pressure osmometry

To measure the total molar concentration of the mixture of compounds in the extracts of the different unspiked samplers, vapor pressure osmometry was performed in aliquots of the extracts using the Gonotec Osmomat 070 vapor pressure osmometer with Control Unit B (Gonotec GmbH, Berlin, Germany). The procedure has been described in detail by Hamers et al. (2018) (supplemental material). Molar concentrations could only be determined in the silicone rubber extracts in hexane. DCM turned out to be too polar for vapor pressure osmometric analysis of the Speedisk extracts. Stepwise transfer of the DCM extracts to hexane using a Rotavapor distillation device resulted in molar background concentrations in blank Speedisk extracts, which could not be distinguished from molar concentrations in the extracts of the exposed Speedisk samplers.

2.6. Aliivibrio fischeri bioassay

The Aliivibrio fischeri bioluminescence bioassay was selected because it responds to a wide variety of compounds with different hydrophilic properties. A. fischeri are luminescent marine bacteria that use part of the energy released from the metabolic citric acid cycle (Krebs cycle) for light emission. Any disruption in this complex metabolic pathway as a result of toxicity will result in a reduction of the amount of light emitted. The A. fischeri bacteria were exposed to a range of serial sample dilutions of the passive sampler extracts in DMSO in a final volume of 200 μL containing 2% NaCl and 0.5% DMSO. Light emission was measured after 15 min, according to Hamers et al. (2001). In all experiments, CuSO4 was tested as a positive reference compound.

2.7. DR-LUC bioassay

The DR-LUC bioassay typically responds to compounds with a high $K_{ow}$, such as PAHs, PCBs and polyhalogenated dibenzo-p-dioxins. For silicone rubber samplers, such compounds are expected to be in the time integrative sampling phase (i.e. not in equilibrium) during the sampling period and therefore this test was chosen. The agonistic potencies towards the arylhydrocarbon receptor (AhR, also known as dioxin-receptor (DR)) was tested in the H4L1.1c4 reporter gene bioassay (DR-LUC; Greenwood et al., 2007). A confluent monolayer of cells was exposed in a 96-wells plate towards serial dilutions of the extracts and of the reference compound 2,3,7,8-tetrachloro-p-dioxin (TCDD) in DMSO (final concentration 0.4%) in a final volume of 200 μL. After 24 h of exposure, luciferase activity was measured by lysis of the cells followed by addition of the substrate luciferin. The produced light is a direct measure for the presence of AhR-activating compounds in the exposure medium. By interpolating the light production in a calibration curve of TCDD, bioassay responses towards the extracts could be expressed as bioassay TCDD equivalent (bioTEQ) concentrations (i.e. the concentration of the reference compound giving similar AhR activating potency as the sample extract).

2.8. Sampling rate calculation for silicone rubber samplers

Following Rusina et al. (2010), the substance specific sampling rate ($R_{SR}$, L/d) of the silicone rubber was calculated as:

$$R_{SR} = \frac{B}{M^{0.47}}$$  

Eq.1

where $M$ is the molecular weight (g/mol) of the substance and $B$ is a proportionality constant accounting for the geometry of the sampler, the water turbulence and unit conversion. The fraction of a PRC that is retained in the silicone rubber sampler after deployment ($f_{PRC}$) is then given by:
where $t$ is the deployment time (d) of the sampler in the field, $m_p$ the mass of the sampler (kg), and $K_{pw}$ the sampler water partition coefficient (L/kg) (Smedes et al., 2009). The proportionality factor $B$ was calibrated in situ from the release of the PRCs by fitting the measured $f_{PRC}$ values in the deployed samplers to the $f_{PRC}$ values modelled by Eq. (2) using non-linear regression (Booij and Smedes, 2010). Next, the amount of each compound in the sampler ($N_{i,SR}$) and the obtained $f$ values for $B$ were used to estimate its concentration in the aqueous phase ($C_w$) using (Smedes and Booij, 2012):

$$C_w = \frac{N_{i,SR} \cdot m_p \cdot K_{pw}}{m_p \cdot K_{pw} \cdot \left(1 - \exp \left(-\frac{B \cdot C_w \cdot t}{m_p \cdot K_{pw}} \right) \right)}$$

(3)

The time window ($\tau, d$) in which time integrative sampling takes place was calculated by (Smedes and Booij, 2012):

$$\tau = \frac{K_{pw} \cdot m_p}{R_{SR}}$$

(4)

PRC spiked samplers were only deployed in two periods, i.e. week 1–6 and 7–15. Freely dissolved aqueous phase compound concentrations for the other periods were calculated assuming that the sampling rate was constant during the sampling of week 1–6 and 7–15. $K_{pw}$ values for PRCs, PCBs and PAHs were taken from Smedes et al. (2009). For substances with no determined $K_{pw}$ values, $K_{ow}$ values were used instead.

2.9. Contribution of analyzed compounds to the bioassay responses

The contribution of each compound analyzed in the passive sampler extract to the observed bioassay response to that particular extract was calculated according to the principle of concentration addition. The measured A. fischeri bioassay response to the extract was converted into toxic units (TU) by taking the inverse of the IC50 (expressed in SD/L or in g SR/L). The concentrations of the individual compounds in the sampler (ng/SD or ng/g SR) were converted into TU by dividing them by their IC50 (ng/L) in the bioassay as reported in an in house database. The TU calculated for the individual compounds were summarized and compared with the TU measured for the passive sampler extract in the A. fischeri bioassay. Since the DR-LUC bioassay was expected to be mainly caused by PAHs, concentrations of 12 individual PAHs analyzed in the samplers were multiplied with their relative potency (REP) towards 2,3,7,8-TCDD as reported by Machala et al. (2001), resulting in a bioTEQ concentration per PAH (pg bioTEQ/SD or pg bioTEQ/g SR). The 12 bioTEQ concentrations were summarized and compared with the overall bioTEQ concentration measured for the passive sampler extract in the DR-LUC bioassay.

3. Results and discussion

3.1. Speedisk samplers – Time integrative sampling properties

In Speedisk samplers, 14 out of the 120 analyzed pesticides accumulated in concentrations above LOQ at WWTP Amersfoort and the single 1–6 weeks deployment. Similarly, an average ratio of 0.96 (±0.26) was found for 47 substances between the sum uptake of 1–6 and 7–15 weeks deployment and the uptake during the 1–15 weeks deployment. Both average ratios are close to 1, indicating that the overall uptake was time integrative.

At the WWTP of Amersfoort, for terbutylazin, terbutryn, DEET and 2-aminoacetophenone, the cumulative amounts in the consecutive samplers from week 1–2, 3–4, and 5–6 were slightly higher than in the sampler constantly deployed during this period (i.e. week 1–6; Figs. S4A and S4B). Similarly, amounts of terbutylazin and diuron (Fig. S4A) and BAM (Fig. S4B), collected in week 1–6 and 7–15 were higher compared with the sampler deployed during week 1–15. This can be caused by measurement error of concentrations close to the limit of detection, as for terbutryn.

Possibly, the Speedisk may also have a slightly faster uptake of some compounds at the start of its deployment, resulting in a higher cumulative amount of compounds for multiple Speedisks deployed in consecutive periods than in a single Speedisk constantly deployed in parallel. This can also be seen in the larger variation of the average ratio between the sum uptake from the three 1–2, 3–4, and 5–6 weeks deployments compared with the single 1–15 weeks deployment. For 39 substances, an overall evaluation of the time integrative sampling by Speedisk yielded an average ratio of 1.10 (±0.38) between the sum of uptake from the three 1–2, 3–4, and 5–6 weeks deployments.
Fig. 2. Uptake of anthraquinone and carbenzadim in consecutively and in parallel exposed Speedisk samplers at the WWTP of Amersfoort (left panels) and in the river Meuse (right panels) shown as the amounts on the samplers (ng/SD) sampled during the weeks indicated at the x-axis. In green the duplicate sampler exposed in parallel (Hamers et al., 2018). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 3. Uptake of isoproturon (ng) in consecutively and in parallel exposed Speedisk (left) and silicone rubber samplers (right) in the river Meuse. The green bars represent the data of the duplicate sampler exposed in parallel (Hamers et al., 2018). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
extracts (Table S3). These findings suggest that the overall toxic potency in the water was stable in time, while concentration changes of the compounds contributing to the bioassay response seemed to be averaged out in time.

The observed correspondence between the cumulative response in consecutively deployed samplers and in the corresponding sampler constantly deployed in parallel, indicate that Speedisks are well applicable for time integrative sampling of individual chemicals and their integrated toxic potency. Similar as for other adsorption-based samplers, a disadvantage of Speedisk samplers is that the use of PRCs to determine the sampling rate as currently applied for partitioning-based silicone rubber samplers is not possible. Although the 0.5 mm glass fiber filter is an advantage for regulating the uptake, further studies on the uptake mechanism are required to enable calculation of the freely dissolved compound concentrations in water. These can be similar studies as performed for POCIS samplers under laboratory and controlled field conditions in which sampling rates for different compounds like pesticides were derived in flow through and static stirring systems (Harman et al., 2012, Morin et al., 2013; Charlestra et al., 2012; Al Ashi et al. (2018)). However, flow regimes may change under field conditions, resulting in changing sampling rates over time and no methods are currently available to directly determine the sampling rate of adsorption-based samplers. Previously reported indirect overall sampling rate estimations for the Speedisks (Hamers et al., 2018) indicated sampling rates ranging from 0.031 to 0.47 L/d per sampler depending on the flow regimes at the different sampling locations. These estimates were made by comparing compound levels in Speedisk with their aqueous concentrations based on silicone rubber sampling volumes determined by PRC-release. These estimated Speedisk sampling rates were 1–2 orders of magnitude lower than sampling rates determined for silicone rubber samplers by the release of the PRCs (for MW = 300) deployed in parallel, which ranged from 1.7 to 13.3 L/d for the different sampling locations (Hamers et al., 2018).

As far as we know, no similar field studies have investigated the time integrative sampling properties of other adsorption-based passive samplers by deploying for instance Chemcatcher or POCIS for consecutive short sampling periods and parallel longer sampling periods covering a time span up to 15 weeks (105 days). Few laboratory studies showed linear uptake for example for pesticides (Shaw et al., 2009; Morin et al., 2013, Kazerson et al., 2014), although some reached equilibrium, like ionic pesticides. These experiments were performed for a period of 30 days and it is unclear whether the time integrative sampling properties would continue for prolonged exposure times. For the DVB adsorbent used in the Speedisks, Huysman et al. (2019) found equilibrium for all the tested organic compounds (like pharmaceuticals, pesticides and personal care products) after 12–24 h of static exposure in a 800 mL container with 25 mg/L DVB in artificial seawater under intensive stirring. Yet, the high equilibrium rate by Huysman et al. (2019) exhausted the small water volume used and does not apply to field conditions where an infinite amount of water is available and concentrations do not decrease by sampling (Bouij and Tucca, 2015). In the Speedisk sampler, however, the interaction between the sorbent material and the water is much less intensive than in the stirring experiments, since compounds have to migrate through a layer of sorbent instead of adsorbing under optimal mixed conditions to individual particles. The time integrative sampling of the Speedisk, which lasted for at least 15 weeks in the present study, is attributed to the sampler construction, which leads to lower sampling rates. Huysman et al. (2019) determined a partition coefficient between DVB and water of logfDVB-W = 5. For the given range of sampling rates (0.031–0.47 L/d) and a DVB mass of 0.6 g per Speedisk, the linear uptake time window (τ) of the Speedisk sampler according to Eq. (4) is expected to range from 128 days under very turbulent water-flow conditions to 1935 days under almost static conditions. For the particular sampling rates determined by Hamers et al. (2018) at the WWTP of Amersfoort (0.064 L/d) and the river Meuse (0.087 L/d), this corresponds to τ = 938 and τ = 690 days, respectively. Because these calculated linear uptake windows exceed the maximum sampling period of 105 days in the present study, they confirm that Speedisk remained in the linear uptake phase, as was demonstrated for the chemically analyzed individual compounds as well as for the aggregated bioactivity determined in in vitro bioassays.
Altogether, Speedisk seems to be a suitable sampler for time integrative passive sampling followed by toxicity profiling using bioassays (TIPTOP approach; Hamers et al., 2018). The sampler is assumed to passively extract a more or less equal volume of water for all substances as argued by Hamers et al., (2018) (supplemental material), with some variations due to differences in diffusion rates, thereby keeping the same concentration profile in the final concentrated extract as in the sampled water. If this assumption is correct and the influence of the differences in diffusion rates are limited, further laboratory and field tests may derive a single Speedisk sampling rate for all compounds. This sampling rate can then be used to convert the bioassay response to a Speedisk extract into a bioactivity per liter water.

3.2. Silicone rubber samplers — Time integrative sampling properties

The absorption (partitioning) based silicone rubber samplers showed good time integrative sampling properties for substances with a high hydrophobicity like PCB-153, which has a logKow of 7.75 and an estimated equilibrium time of 3–4 years (Fig. S6). This in agreement with previous studies by e.g. Rusina et al. (2010) and Ter Laak et al. (2008). For PCB-153 the sum of the sampled amounts in consecutive samplers was comparable to the amount sampled in the sampler constantly exposed in parallel. Lower PCB-153 amounts in the sampler exposed during week 7–15 indicated a decrease in aqueous concentrations from week 7 on. Linear uptake was to be expected, given the fact that the uptake phase of such compounds is far from equilibrium (Fig. S1) (Booij et al., 2007; Rusina et al., 2010). However, most pesticides that were chemically compounds is far from equilibrium (Fig. S1) (Booij et al., 2007; Rusina et al., 2010). The absorption (partitioning) based silicone rubber samplers showed good time integrative sampling properties for substances with a high hydrophobicity like PCB-153, which has a logKow of 7.75 and an estimated equilibrium time of 3–4 years (Fig. S6). This in agreement with previous studies by e.g. Rusina et al. (2010) and Ter Laak et al. (2008). For PCB-153 the sum of the sampled amounts in consecutive samplers was comparable to the amount sampled in the sampler constantly exposed in parallel. Lower PCB-153 amounts in the sampler exposed during week 7–15 indicated a decrease in aqueous concentrations from week 7 on. Linear uptake was to be expected, given the fact that the uptake phase of such compounds is far from equilibrium (Fig. S1) (Booij et al., 2007; Rusina et al., 2010). However, most pesticides that were chemically analyzed had low affinity to silicone rubber and attained equilibrium during the test period, confirming poor time integrative sampling properties for these rather polar compounds. This is demonstrated for anthraquinone (logKow = 3.39; Fig. S6), for which sampling rates at the WWTP of Amersfoort were calculated (Eq. (2)) of R0 = 44 and R0 = 46 L/d during the 1–6 and 7–15 week sampling period, respectively. The corresponding linear uptake window (τ) was only 1.0 day (Eq. (4)). The sum of anthraquinone in weeks 1–2, 3–4 and 5–6 was higher compared with the amount sampled from 1 to 6 weeks, with more or less comparable amounts in the samplers during week 5–6 and week 1–6. This observation confirms that anthraquinone reached fast equilibrium and that the amount in the samplers reflected the compound concentrations in the water during the last day of each sampling period. The amounts in week 1–2 and 3–4 were also higher than the amounts on the samplers from week 7–15 and 1–15. Apparently, the aqueous concentration was higher in the first 4 to 5 weeks and remained constant in the 10 to 11 weeks thereafter. The measured amounts of all compounds detected on the silicone rubber samplers are given in Table S2.

The molarity measurements also indicated that the majority of the compounds quickly reached equilibrium in the silicone rubber samplers and apparently had polar characteristics, while the minority of hydrophobic compounds remained in the linear uptake phase (Fig. 5). The steep part of the uptake curve, which was more apparent for the WWTP, indicates a fast uptake/equilibrium of more polar substances. The following slower increase in molarity during the next weeks represents the linear uptake of the more hydrophobic substances.

The results of the A. fischeri bioluminescence bioassay indicate that the silicone rubber samplers have poor time integrative sampling properties for the substances causing a response in this bioassay (Fig. S7). Cumulative bioassay responses for the consecutive short exposure periods exceeded the bioassay response for the sampler constantly exposed in parallel. This may indicate that the more hydrophilic and more reactive or specifically acting substances that quickly reached equilibrium with the sampler are causing the response in this test and not the hydrophobic substances that are still in the linear uptake phase. This indication was confirmed for the silicone rubber extract from the WWTP of Amersfoort, for which 27–54% of the observed inhibition in bioluminescence could be explained by the compounds analyzed in the samplers (Table S3). The majority of this inhibition could be attributed to phenanthrene, acenaphthylene, and/or diazinon, which all three were calculated to be in equilibrium with the silicone rubber sampler within 14 days (i.e. after 5, <1, and 12 days, respectively). This confirms that the corresponding response in the A. fischeri bioassay was also not time integrative. For the silicone

Fig. 5. The molarity (µmol/g SR) versus time in the silicone rubber samplers for the WTTP of Amersfoort (left) and the river Meuse (right) measured by vapor pressure osmometry. The points at time point zero are the blank values (not subtracted). The green dots are the duplicate samplers. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
rubber extract from river Meuse, the analyzed compounds only explained 1–2% of the observed _A. fischeri_ response (Table S3).

The results from the DR-LUC bioassay on silicone rubber extracts from the river Meuse indicate relatively good time integrative properties, with uptake following the linear part of a first order uptake curve that only in the last deployment weeks tends to equilibrium (Fig. 6). The silicone rubber sampler from the WWTP site, however, showed poor time integrative sampler properties, similar to the _A. fischeri_ bioluminescence results. Although the DR-LUC response was expressed in terms of TCDD-equivalent concentrations, the observed response was most likely not caused by persistent dioxin-like compounds. Because no further cleanup was performed on the extracts, the observed DR-LUC response is most likely due to less persistent compounds in the extracts. PAHs are often considered as a relevant class of non-persistent compounds responsible for the DR-LUC response to environmental extracts. Using the relative potencies of PAHs towards the dioxin-receptor AhR (Machala et al., 2001), the summarized potency of 12 PAHs analyzed in the sampler explained a larger part of the observed DR-LUC response for river Meuse (8–15%) than for the WWTP of Amersfoort (2–4%; Table S3). Moreover, the contribution of PAHs to the DR-LUC response for river Meuse decreased in time, i.e. 10–15% for 2-week, 9% for 6-week, and 8% for 9-week and 15-week sampling periods (Table S3). These findings suggest that the time integrative DR-LUC response observed for river Meuse could be partly attributed to PAH-like compounds (i.e. most likely more than the 12 analyzed PAHS for which relative potencies were available), and - for longer deployment periods - also to AhR activating compounds that are more hydrophobic than PAHs, like the dioxin-like PCBs. At the WWTP of Amersfoort, the poor time integrative response for the DR-LUC bioassay corresponded to a lower contribution of PAHs, suggesting that more polar compounds that had...
reached equilibrium with the silicone rubber sampler were responsible for the observed DR-LUC response. Possibly, these were commonly used pharmaceuticals like lefluonamide, flutamide, nimodipine, or mexiteline that are known to have AHr-active capacities (Jin et al., 2012) and logK_{ow} values in the range from 2 to 4. To improve the time integrative properties of silicone rubber samplers for more hydrophilic compounds, extension of τ (Eq. (4)) is necessary. This can be achieved by using thicker sheets and thereby increasing m_{p}\cdot K_{pw} (Eq. (4)), so basically the capacity of the sampler. With the same sampling area, the sampling rate remains the same, but due to the larger capacity it will take longer before the sampler reaches equilibrium and thereby the sampler will remain longer in the linear uptake phase. Alternatively, the sampling rate can be decreased by reducing the flow effect or by covering the sampler with a glass fiber paper.

Chemical analysis of compounds reaching equilibrium during sampler deployment is not a disadvantage per se, as results from equilibrated samplers deployed at different stations allow direct mutual comparison of compound levels without knowledge on site specific uptake kinetics and/or partition coefficients for compounds that reach equilibrium (Webster et al., 1999). Moreover, results from equilibrated samplers reflect the internal concentrations in exposed feral organisms better than results from samplers in the kinetic uptake phase (Rusina et al., 2017). Uptake kinetics and partition coefficients are only required if sampler concentrations need conversion to aqueous concentrations, e.g. to check compliance with environmental quality standards. Equilibrium, however, is a disadvantage when aiming to obtain time weighted average concentrations over the complete sampling period as uptake until equilibrium represents a shorter time period. Still, this period reflects the water quality during a much longer period than in the case of grab sampling.

The unknown compounds in a silicone rubber sampler extract each have a different sampled volume depending on their logK_{ow}. Some will be in the linear uptake phase and their sampled volume is determined by R_{f}τ and others reach equilibrium with a sampled volume of m_{p}\cdot K_{pw}. Given the unknown identity of the compounds in the mixture responsible for the bioassay response, and the fact that these compounds each have different sampled volumes, it is in principle impossible to determine a single sampled volume for the complex mixture as a whole. Therefore, conversion of measured bioactivities in silicone sampler extracts to bioactivities in a water body by using a single estimated sampled volume should be considered with great precaution.

4. Conclusions

Passive sampling is often recommended as a method to estimate time integrative aqueous concentrations and specifically freely dissolved concentrations of compounds. This study demonstrated that this paradigm indeed holds for the adsorption-based Speedisk samplers. This sampler is working very well as time integrative sampler for the monitoring of individual chemical compounds and for bioassay responses to unknown mixtures. For partitioning-based silicone rubber passive samplers, however, compounds with K_{ow}<10^{3} have a restricted and compound-specific period of time integrative sampling, depending on their sampler–water partition coefficient, the sampler dimensions, and the field conditions.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.127498.

References

Al Ashi, A., Kouziaya, A., Rifai, A., Abdel Rahman, R., Budzinski, H., Jaber, F., 2018. Estimation of in–lab sampling rates and uptake kinetics for 24 polar organic micro pollutants by polar organic chemical integrative sampler “POCIS”. MOJ Ecol. Environ. Sci. 3, 72–80.
Alvarez, D.A., Petty, J.D., Huckins, J.N., Jones-Lepp, T., Getting, D.T., Goddard, J., Manahan, S.E., 2004. Development of a passive, in situ, integrative sampler for hydrophobic organic contaminants in aquatic environments. Environ. Toxicol. Chem. 23, 1640–1648.
Booij, K., Tucca, F., 2015. Passive samplers of hydrophobic organic chemicals reach equilibrium faster in the laboratory than in the field. Mar. Pollut. Bull. 98, 365–367. https://doi.org/10.1016/j.marpollbul.2015.07.007.
Booij, K., Sleiderink, H.M., Smedes, F., 1998. Calibrating the uptake kinetics of permselective membrane devices using exposure standards. Environ. Toxicol. Chem. 17, 1236–1245, 1998.
Booij, K., Vrana, B., Huckins, J.N., 2007. Theory, modelling and calibration of passive samplers used in water monitoring. In: Greenwood, R., Mills, G.A., Vrana, B. (Eds.), Passive Sampling Techniques in Environmental Monitoring. Elsevier, Amsterdam, pp. 141–169.
Booij, K., Smedes, F., 2010. An improved method for estimating in situ sampling rates of nonpolar passive samplers. Environ. Sci. Technol. 44, 6789–6794.
Charlestra, L., Amirabrahim, A., Countemanch, D.L., Alvarez, D.A., Patterson, H., 2012. Estimating pesticide sampling rates by the polar organic chemical integrative sampler (POCIS) in the presence of natural organic matter and varying hydrodynamic conditions. Environ. Pol. 168, 98–104.
De Baat, M.L., Kraak, M.H.S., Van der Oost, R., De Vos, P., Vandenschot, P.M.J., 2019. Effect-based nationwide surface water quality assessment to identify ecotoxicological risks. Water Res. 159, 434–442.
Escher, B.J., Ait-Aissa, S., Behnisch, P.A., Brack, W., Brion, F., Brouwer, A., Buchinger, S., Crawford, S.E., Du Pasquier, D., Hamers, T., Hettwer, K., Hilscherova, K., Hollert, H., Kase, K., Kienle, C., Tindall, A.J., Tuerk, J., Van der Oost, R., Vermeiren, E., Neale, P.A., 2018. Effect-based trigger values for in vitro and in vivo bioassays performed on surface water samples supporting the environmental quality standards (EQS) of the European Water Framework Directive. Sci. Total Environ. 628–629, 748–765.
EU, 2008, Directive 2008/105/EC of the European parliament and of the council of 16 December 2008 on environmental quality standards in the field of water policy. O.J. L348, 84–97.
Emelogu, E.S., Pollard, P., Robinson, C.D., Smedes, F., Websterian, L., Oliver, I.W., McKenzie, C., Seiler, T.B., Hollert, H., Moffata, C.J., 2013. Investigating the significance of dissolved organic contaminants in aquatic environments: coupling passive sampling with in vitro bioassays. Chemosphere 90, 210–219.
Foekema, E.M. (Ed.), 2012. De invloed van moerassystemen op de milieukwaliteit. Boekverslaving. Stichting De Pers, Amsterdam, pp. 141–169.

J. de Weert et al. / Chemosphere 259 (2020) 127498
12 (in Dutch). http://edepot.wur.nl/193743.
Greenwood, R., Mills, G., Vrana, B., 2007. Passive sampling techniques in environ-
mental monitoring. In: Barcelo, D. (Ed.), Comprehensive Analytical Chemistry
Series, vol. 48. Elsevier, Amsterdam.
Hamers, T., Legrady, J., Zwart, N., Smedes, F., De Weert, J., Van den Brandhof, E.-J.,
Van de Meent, D., De Zwart, D., 2018. Time Integrative Passive sampling com-
bined with TOXicity Profiling (TIPTOP): an effect-based strategy for cost-
effective chemical water quality assessment. Environ. Toxicol. Pharmacol. 64, 48–59.
Hamers, T., Legler, J., Blaha, L., Hylland, K., Marigomez, I., Schipper, C.A., Segner, H.,
Verhaak, A.D., Witters, H., De Zwart, D., Leonards, P.E.G., 2013. Expert opinion on
toxicity profiling — report from a NORMAN expert group meeting. Integrated Environ.
Assess. Manag. 9, 185–191.
Hamers, T., Leonards, P.E.G., Legler, J., Verhaak, A.D., Schipper, C.A., 2010. Toxicity
profiling: an integrated effect-based tool for site-specific sediment quality assessment.
Integrated Environ. Assess. Manag. 6, 761–773.
Hamers, T., Smit, M.G.D., Murk, A.J., Koehn, J.H., 2001. Biological and chemical analysis
of the toxic potency of pesticides in rainwater. Chemosphere 45, 609–624.
Harman, C., Allen, I.J., Vermeirssen, E.L.M., 2012. Calibration and use of the polar
organic chemical integrative sampler: A critical review. Environ. Toxicol. Chem.
31, 2724–2738.
Huckins, J.N., Petty, J.D., Booij, K., 2006. Monitors of Organic Chemicals in the
Environment and their fate and removal during waste water treatment. Sci. Total En-
viron. 473, 2014. A review on the occurrence of micropollutants in the aquatic environ-
ment. Monit. 2, 487
Huysman, S., Vanryckeghem, F., De Paepe, E., Smedes, F., Haughey, S.A., Elliott, C.T.,
Harman, C., Allen, I.J., Vermeirssen, E.L.M., 2012. Calibration and use of the polar
organic chemical integrative sampler: A critical review. Environ. Toxicol. Chem.
31, 2724–2738.
Huckins, J.N., Petty, J.D., Booij, K., 2006. Monitors of Organic Chemicals in the
Environment: Semipermeable Membrane Devices. Springer, New York, USA.
Huysman, S., Vannycykeghm, F., De Paepe, E., Smedes, F., Haughey, S.A., Elliott, C.T.,
Demeestere, K., Vanhaeck, L., 2019. Hydrophilic divinylbenzene for equilibrium
sorption of emerging organic contaminants in aquatic matrices. Environ. Sci.
Technol. 53, 10803–10812.
Jahnke, A., Mayer, P., Schafer, S., Witt, G., Haase, N., Escher, B.I., 2016. Strategies for
transferring mixtures of organic contaminants from aquatic environments into
bioassays. Environ. Sci. Technol. 50, 5424–5431.
Jin, U.H., Lee, S., Safe, S., 2012. Aryl hydrocarbon receptor (AHR)-Active pharma-
ceuticals are selective AHR modulators in MDA-MB-468 and BT474 breast
cancer cells. J. Pharmacol. Exp. Therapeut. 343, 333–341.
Kaserzon, S.L., Hawkcr, D.W., Kennedy, K., Barklow, M., Carter, S., Booij, K.,
Mueller, J.F., 2014. Characterisation and comparison of the uptake of ionizable and
polar pesticides, pharmaceuticals and personal care products by POCIS and
Chemcatchers. Environ. Sci.: Processes Impacts. 16, 2517–2526.
Kingston, J.K., Greenwood, R., Mills, G.A., Morrison, G.M., Persson, L.B., 2000.
Development of a novel passive sampling system for the time-averaged mea-
surement of a range of organic pollutants in aquatic environments. J. Environ.
Monit. 2, 487–495.
Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.L., Zhang, J., Liang, S., Wang, X.C.,
2014. A review on the occurrence of micropollutants in the aquatic environ-
ment and their fate and removal during waste water treatment. Sci. Total En-
viron. 473–474, 619–641.
Machala, M., Vondracek, J., Blaha, L., Ciganek, M., Necu, J., 2001. Aryl hydrocarbon
receptor- mediated activity of mutagenic poly cyclic aromatic hydrocarbons
determined using in vitro reporter gene assay. Mutat. Res. 497, 49–62.
Morin, N., Camilleri, J., Cren-Olive, C., Coguer, M., Mige, C., 2013. Determination of
uptake kinetics and sampling rates for 56 organic micropollutants using
“pharmaceutical” POCIS. Talanta 101, 61–73.
Petrie, B., Barden, R., Kasprzyk-Hordern, B., 2015. A review on emerging contami-
nants in waste waters and the environment; Current knowledge, understudied
areas and recommendations for future monitoring. Water Res. 72, 3–27.
Rusina, T.P., Carlson, P., Vrana, B., Smedes, F., 2017. Equilibrium passive sampling of
POP in lipid-rich and lean fish tissue; quality control using performance
reference compounds. Environ. Sci. Technol. 521, 111250–111257.
Rusina, T.P., Smedes, F., Koblizkova, M., Klanova, J., 2010. Calibration of silicone
rubber passive samplers: experimental and modeled relations between sam-
ping rate and compound properties. Environ. Sci. Technol. 44, 362–367.
Shaw, M., Eaglesham, G., Mueller, J.F., 2009. Uptake and release of polar compounds
in SDB-RPS EmporeTM disks: implications for their use as passive samplers.
Chemosphere 75, 1–7.
Silva, E., Rajalakse, N., Kortenkamp, A., 2002. Something from “nothing” — eight
weak estrogenic chemicals combined at concentrations below NOECs produce
significant mixture effects. Environ. Sci. 36, 1751–1756.
Smedes, F., Geertsma, R.W., Van der Zande, T., Booij, K., 2009. Polymer-water
partition coefficients of hydrophobic compounds for passive sampling: appli-
cation of cosolvent models for validation. Environ. Sci. Technol. 43, 7047–7054.
Smedes, F., Booij, K., 2012. Guidelines for passive sampling of hydrophobic con-
taminants in water using silicone rubber samplers. IJES Tech. Mar. Environ. Sci.
No. 52 Copenhagen DK http://www.ispassivesampling.net/?PageGuidanceTimes52.
df.
Ter Laak, T.L., Busser, F.J.M., Hermens, J.L.M., 2008. Poly(dimethylsiloxane) as passive
sampler material for hydrophobic chemicals: effect of chemical properties and
sample characteristics on partitioning and equilibration times. Anal. Chem. 80,
3859–3866.
Van der Oost, R., Sileno, G., Suarez-Munoz, M., Nguyen, M.T., Besselink, H.,
Brouwer, A., 2017. Simoni (smart integrated monitoring) as a novel bioanalytical
strategy for water quality assessment: Part I-model design and effect-based
trigger values. Environ. Toxicol. Chem. 36, 2389–2399.
Van der Oost, R., Sileno, G., Janse, T., Nguyen, M.T., Besselink, H., Brouwer, A., 2017.
Simoni (Smart Integrated Monitoring) as a novel bioanalytical strategy for
water quality assessment; part II- Field feasibility survey. Environ. Toxicol.
Chem. 36, 2400–2416.
Van Hoon, M., De Weert, J., 2012. Passieve monitornage om de effectiviteit van
natuurrivendelijke oevers en bufferstroken te meten. H2O 45, 34–36 (in Dutch).
Vermeirssen, E.L.M., Dietschweiler, C., Escher, B.I., Van der Voet, J., Hollender, J.,
2012. Transfer kinetics of polar organic compounds over polyethersulfone
membranes in the passive samplers Pocis and Chemcatcher. Environ. Sci.
Technol. 46, 6759–6766.
Vrana, B., Schuurmann, G., 2002. Calibrating the uptake kinetics of semipermeable
membrane devices in water: impact of hydrodynamics. Environ. Sci. Technol.
36, 290–296.
Vrana, B., Klucarova, V., Benicka, E., Abou-Mrad, N., Amday, R., Horakova, S.,
Draxler, A., Humer, F., Gans, O., 2014. Passive sampling: an effective method for
monitoring seasonal and spatial variability of dissolved hydrophobic organic
contaminants and metals in the Danube river. Environ. Pol. 184, 101–112.
Walter, H., Consolaro, F., Gramatica, P., Scholze, M., Altenburger, R., 2002. Mixture
toxicity of priority pollutants at no observed effect concentrations (NOECs).
Ecotoxicology 11, 299–310.
Webster, E., Mackay, D., Qiang, K., 1999. Equilibrium lipid partitioning concentra-
tions as a multi-media synoptic indicator of contaminant levels and trends in
aquatic ecosystems. J. Great Lake. Res. 25, 318–329.
Supplementary information

Time integrative sampling properties of Speedisk and silicone rubber passive samplers
determined by chemical analysis and in vitro bioassay testing

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$K_{pw} = \frac{C_p}{C_w} = \frac{N^t}{m_p} C_w / C_w$

$N^t = K_{pw} m_p C_w (1 - e^{-\frac{R_s t}{m_p K_{pw}}})$

Also uptake principle for adsorption samplers

Based on Huckins et al (2006) and Booij et al (2007)
Figure S2: Diffusion of different compounds through four different types of membranes with modeled sampling rates. From Poster at SETAC Glasgow, 2013. (Smedes, F., Beeltje, H., Kotte, M., Jonker, M.T.O. Sorption of various polar chemicals to different sorbent and membrane materials applied in passive sampling).
Figure S3: Sampling locations in the Netherlands where samplers were deployed, i.e. in the effluent of the WWTP of Amersfoort and in the river Meuse at Eijsden.
Figure S4A: Uptake of terbutylazin, terbutryn and diuron in consecutively and in parallel exposed Speedisk samplers at the WWTP of Amersfoort (left panels) and in the river Meuse (right panels) shown as amounts on the samplers (ng/SD) sampled within the weeks indicated at the x-axis. In green is the duplicate exposed sampler.
Figure S4B: Uptake of BAM, DEET and 2-aminoacetophenone in consecutively and in parallel exposed Speedisk samplers at the WWTP of Amersfoort (left panels) and in the river Meuse (right panels) shown as the amounts on the samplers (ng/SD) sampled within the weeks indicated at the x-axis. In green is the duplicate exposed sample.
Figure S5: Aliivibrio fischeri (left panel) and DR-LUC bioassay response (right panel) to consecutively and in parallel exposed Speedisk samplers in the river Meuse expressed in bioassay TCDD-Equivalent (bioTEQ) concentrations, corresponding to the sampling weeks indicated at the x-axis. The green bar is from the duplicate sampler. Error bars indicate propagated standard deviation for direct measurements and propagated standard deviation for cumulative results.
Figure S6: Uptake of anthraquinone (upper panels) and PCB-153 (lower panels) in consecutively and in parallel exposed silicone rubber samplers at the WTTP of Amersfoort. The left panels show the amounts (ng) sampled within the weeks indicated at the x-axis. The green bars were from data from the samplers loaded with PRCs. In the right panels aqueous concentrations derived from the uptake are plotted versus time. The length and horizontal position of the line represents the exposure period.
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Table S1 Detected amounts on Speedisk samplers. Empty cell; compound is not detected above the limit of quantification

| Exposure time (days) | amount on SD of ng/SD | WWTP Amersfoort |
|---------------------|-----------------------|------------------|
|                     | Meuse, Eijsden        |                  |
| 14                  | 14                    | 14               |
| 14-28               | 42                    | 63               |
| 28-42               | 42-105                | 0-105            |
| 0-14                | 14                    | 14               |
| 14-28               | 42                    | 63               |
| 28-42               | 42-105                | 0-105            |

| Compound            | Cas-number            |
|---------------------|-----------------------|
| 2,4,5-T             | 93-76-5               |
| 2,4-D               | 94-75-7               |
| 2,4-dinitrophenol   | 51-28-5               |
| 2,4-DP              | 120-36-5              |
| 2-aminoacetophenon  | 551-93-9              |
|acenafine            | 83-32-9               |
|acenafylene          | 208-96-8              |
|aclonifen            | 74070-46-5            |
|anthraquinone        | 84-65-1               |
|antracene            | 120-12-7              |
|atrazine             | 1912-24-9             |
|atrazine-desethyl    | 6190-65-4             |
|azaconazole          | 60207-31-0            |
|azoxyystrobine       | 131860-33-8           |
|BAM                  | 2008-58-4             |
|bentazon             | 25057-89-0            |
|benzo(a)antracene    | 56-55-3               |
|benzo(a)pyrene       | 50-32-8               |
|benzo(b)fluorantene  | 205-99-2              |
|benzo(ghi)perylen    | 190-86-3              |
|benzo(k)fluorantene  | 207-08-9              |
|bifenox              | 42576-02-3            |
|bifenthrin           | 82657-04-3            |

0-14-28 28-42 0-42 42-105 0-105 0-14 14-28 28-42 0-42 42-105 0-105
| Compound               | Meuse, Eijsden | WWTP Amersfoort |
|-----------------------|----------------|-----------------|
|                       | Exposure time (days) |                 |                 |
|                       | 14 | 14 | 14 | 42 | 63 | 105 | 14 | 14 | 14 | 42 | 63 | 105 |
|                       | 0-14 | 14-28 | 28-42 | 0-42 | 42-105 | 0-105 | 0-14 | 14-28 | 28-42 | 0-42 | 42-105 | 0-105 |
|                       | 3.5 | 5.3 | 3.3 | 0.83 | 1.1 | 6.1 | 2.3 |
|                       | 1.1 | 0.93 | 2.7 | 5 | 3.6 | 18 | 18 |
|                       | 1.7 | 2.1 | 1.7 | 2.5 | 5.7 | 5.3 | 462 |
|                       | 1.7 | 2.1 | 1.7 | 2.5 | 5.7 | 5.3 | 462 |
|                       | 1.6 | 2.5 | 2.3 | 4.4 | 5.2 | 8.5 | 0.96 | 1.6 | 1.7 | 2.1 | 1.7 | 2.5 |
|                       | 4.8 | 4.8 | 4.8 | 4.8 | 4.8 | 4.8 | 4.8 | 4.8 | 4.8 | 4.8 | 4.8 | 4.8 |
|                       | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 |
|                       | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 |
|                       | 33 | 27 | 22 | 146 | 76 | 278 | 1120 | 384 | 219 | 1250 | 550 | 1660 |
|                       | 52 | 52 | 52 | 52 | 52 | 52 | 52 | 52 | 52 | 52 | 52 | 52 |
|                       | 3.2 | 3.2 | 3.2 | 3.2 | 3.2 | 3.2 | 3.2 | 3.2 | 3.2 | 3.2 | 3.2 | 3.2 |
|                       | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 |
|                       | 5.7 | 5.7 | 5.7 | 5.7 | 5.7 | 5.7 | 5.7 | 5.7 | 5.7 | 5.7 | 5.7 | 5.7 |
| Compound               | Cas-number         | Meuse, Eijsden | WWTP Amersfoort |
|------------------------|--------------------|----------------|-----------------|
| Fluorantenn            |                    |                |                 |
| Fenpropimide           |                    |                |                 |
| Fenoxycarb             |                    |                |                 |
| Phenantrene            |                    |                |                 |
| Fenamiphos             |                    |                |                 |
| Ethifumesate           |                    |                |                 |
| Ethoprophos            |                    |                |                 |
| Fenamiphos             |                    |                |                 |
| Phenanthrene           |                    |                |                 |
| Fenarimol              |                    |                |                 |
| Fenbutatin oxide       |                    |                |                 |
| Fenhexamid             |                    |                |                 |
| Fenoxycarb             |                    |                |                 |
| Fenpropimor            |                    |                |                 |
| Fluorantenn            |                    |                |                 |
| Fluorene               |                    |                |                 |
| Flutolanil             |                    |                |                 |
| Imidacloprid           |                    |                |                 |
| Amount on SD*          | ng/SD              |                |                 |
| Exposure time (days)   | 14                 | 14             | 14              |
|                        | 0-14               | 14-28          | 28-42           |
| Exposure period (days) | 14                 | 14-28          | 28-42           |
|                        | 0-14               | 14-28          | 28-42           |
| Compound               | Cas-number       | Meuse, Eijsden | WWTP Amersfoort |
|------------------------|------------------|----------------|-----------------|
|                        | amount on SD*    | ng/SD          |                 |
|                        | Exposure time (days) |             |                 |
|                        | 14               | 14            | 14              |
|                        | 0-14             | 14-28         | 28-42           |
|                        | 42               | 63            | 105             |
|                        | 0-42             | 42-105        | 0-105           |
|                        | Exposure period (days) |             |                 |
|                        | 0-14             | 14-28         | 28-42           |
|                        | 42               | 63            | 105             |
|                        | 0-42             | 42-105        | 0-105           |
| indeno(123-cd)pyrene   | 193-39-5         | 0.85          | 0.85            |
|                        |                  | 1.1           |                 |
| iprodion               | 36734-19-7       | 4.3           | 14              |
|                        |                  | 4.6           | 11              |
|                        |                  |               | 14              |
| isoproturon            | 34123-59-6       | 4.1           | 161             |
|                        |                  | 218           | 2.1             |
|                        |                  |               | 5               |
| lenacil                | 96639            | 5.3           | 2.3             |
|                        |                  |               | 5.5             |
| lindane               | 58-89-9          | 3.2           | 2.3             |
|                        |                  |               | 8.1             |
| linuron                | 330-55-2         | 2.4           | 2.9             |
|                        |                  |               | 5.1             |
| malathion             | 121-75-5         | 4.3           |                 |
|                        |                  |               | 8.6             |
|                        |                  |               | 5.7             |
| MCPA                   | 94-74-6          | 11            | 11              |
|                        |                  |               | 35              |
|                        |                  |               | 21              |
|                        |                  |               | 34              |
| MCPB                   | 93-65-2          | 2.1           | 4.1             |
|                        |                  |               | 28              |
|                        |                  |               | 23              |
|                        |                  |               | 4.2             |
|                        |                  |               | 79              |
|                        |                  |               | 59              |
|                        |                  |               | 111             |
| metalaxyl             | 57837-19-1       | 1.7           | 1.6             |
|                        |                  |               | 4.4             |
|                        |                  |               | 4.5             |
|                        |                  |               | 5.7             |
| metamitron            | 41394-05-2       | 4             | 4.1             |
|                        |                  |               | 28              |
|                        |                  |               | 23              |
|                        |                  |               | 4.2             |
|                        |                  |               | 79              |
|                        |                  |               | 59              |
|                        |                  |               | 111             |
| metazachlor           | 67129-08-2       | 4             | 135             |
|                        |                  |               | 185             |
|                        |                  |               | 4.4             |
|                        |                  |               | 6.7             |
|                        |                  |               | 13              |
| methiocarb            | 2032-65-7        | 5.2           | 2.4             |
|                        |                  |               | 4.1             |
|                        |                  |               | 17              |
|                        |                  |               | 8.4             |
|                        |                  |               | 35              |
| metolachlor (-S)      | 51218-45-2       | 4             |                 |
|                        |                  |               | 3.5             |
| metribuzin            | 21087-64-9       | 5.5           | 4.1             |
|                        |                  |               | 4.7             |
|                        |                  |               | 5               |
| monocrotoxin         | 150-68-5         | 6.8           | 5.6             |
|                        |                  |               | 5.9             |
|                        |                  |               | 10              |
| naphtalene            | 91-20-3          | 9.5           | 13              |
|                        |                  |               | 9.6             |
|                        |                  |               | 15              |
|                        |                  |               | 18              |
|                        |                  |               | 19              |
|                        |                  |               | 7.7             |
|                        |                  |               | 8               |
|                        |                  |               | 12              |
|                        |                  |               | 9.3             |
|                        |                  |               | 16              |
|                        |                  |               | 19              |
| nicosulfuron          | 111991-09-4      | 7.2           | 16              |
| oxydemeton-methyl     | 301-12-2         | 5.5           | 4.1             |
|                        |                  |               | 4.7             |
|                        |                  |               | 5               |
| parathion-methyl      | 298-00-0         | 6.8           | 5.6             |
|                        |                  |               | 5.9             |
|                        |                  |               | 10              |
| penconazole           | 66246-88-6       | 6.8           | 5.6             |
|                        |                  |               | 5.9             |
|                        |                  |               | 10              |
| pencycuron            | 66063-05-6       | 6.8           | 5.6             |
|                        |                  |               | 5.9             |
|                        |                  |               | 10              |
| pendimethalin         | 40487-42-1       | 6.8           | 5.6             |
|                        |                  |               | 5.9             |
|                        |                  |               | 10              |
| Compound                  | Cas-number | Meuse, Eijsden | WWTP Amersfoort |
|---------------------------|------------|----------------|-----------------|
|                           |            | ng/SD          | ng/SD           |
| **Exposure time (days)**  |            |                |                 |
| 14                        | 14         | 14             | 14              |
| 14                        | 14         | 42             | 14              |
| 14                        | 14         | 63             | 14              |
| 14                        | 14         | 105            | 14              |
| 0-14                      | 14-28      | 28-42          | 0-42            |
| 0-42                      | 42-105     | 0-105          | 0-14            |
| **Exposure period (days)**|            |                |                 |
| 0-14                      | 14-28      | 28-42          | 0-42            |
| 0-42                      | 42-105     | 0-105          | 0-14            |
| **Compound**              |            |                |                 |
| permethrin                | 52645-53-1 |                |                 |
| pirimicarb                | 23103-98-2 | 1.5            | 0.34            |
| pirimiphos-methyl         | 29232-93-7 |                | 4.4             |
| prochloraz                | 67747-09-5 |                | 5.2             |
| procyomidone              | 32809-16-8 | 1.9            | 2.4             |
| propiconazole             | 60207-90-1 | 5.9            | 9.7             |
| propoxur                  | 114-26-1   | 6.5            | 7.4             |
| propyzamide               | 23950-58-5 | 6.9            | 12              |
| prosulfocarb              | 52888-80-9 | 5.3            | 3.8             |
| pyrene                    | 129-00-0   | 7.4            | 4.6             |
| pyridate                  | 55512-33-9 | 2.9            | 9.6             |
| pyrimethanil              | 53112-28-0 | 2.6            | 4.6             |
| simazine                  | 122-34-9   | 2.6            | 9.2             |
| sulcotrion                | 99105-77-8 | 2.6            | 9.2             |
| tebuconazole              | 107534-96-3| 2.6            | 3.9             |
| tebufenpyrad              | 119168-77-3| 2.6            | 2.2             |
| teflubenzuron             | 83121-18-0 | 2.6            |                 |
| terbutryn                 | 886-50-0   | 2.6            |                 |
| terbutylazine             | 5915-41-3  | 2.6            |                 |
| tetramethrin              | 7696-12-0  | 2.6            |                 |
| thiaifendazole            | 148-79-8   | 2.6            |                 |
| thiacloprid               | 111988-49-9| 2.6            |                 |
| tolclofos-methyl          | 57018-04-9 | 2.6            |                 |
| Compound      | Meuse, Eijsden | WWTP Amersfoort |
|--------------|----------------|-----------------|
|              | Exposure time (days) | Exposure period (days) | amount on SD* (ng/SD) | |
|              | 14 | 14 | 14 | 42 | 63 | 105 | 14 | 14 | 14 | 42 | 63 | 105 |
| tolyfluanide | 0-14 | 14-28 | 28-42 | 0-42 | 42-105 | 0-105 | 0-14 | 14-28 | 28-42 | 0-42 | 42-105 | 0-105 |
| triadimenol  | 196-27-1 | 55219-65-3 | 5429-3 | 586-5 | 745-2 | 753-5 | 105 | 14 | 14 | 42 | 63 | 105 |
| tri-allate    | 2303-17-5 | 2303-17-5 | 2303-17-5 | 2303-17-5 | 2303-17-5 | 2303-17-5 | 2303-17-5 | 2303-17-5 | 2303-17-5 | 2303-17-5 | 2303-17-5 | 2303-17-5 |
| triazamate    | 112143-82-5 | 112143-82-5 | 112143-82-5 | 112143-82-5 | 112143-82-5 | 112143-82-5 | 112143-82-5 | 112143-82-5 | 112143-82-5 | 112143-82-5 | 112143-82-5 | 112143-82-5 |
| trifloxystrobin | 141517-21-7 | 141517-21-7 | 141517-21-7 | 141517-21-7 | 141517-21-7 | 141517-21-7 | 141517-21-7 | 141517-21-7 | 141517-21-7 | 141517-21-7 | 141517-21-7 | 141517-21-7 |
| Compound          | Cas-number | Meuse, Eijsden | WWTP Amersfoort |
|-------------------|------------|----------------|-----------------|
|                   | amount on silicone rubber$^b$ | ng/g SR |               |               |
| Exposure time (days) | 14 14 14 42 63 105 | 14 14 14 42 63 105 |               |               |
| Exposure period (days) | 0-14 14-28 28-42 0-42 42-105 0-105 | 0-14 14-28 28-42 0-42 42-105 0-105 |               |               |
| PCB-28            | 7012-37-5  | 8.2 10.3 9.1 16 15 | 20 40 43 44 81 84 |               |
| PCB-52            | 35693-99-3 | 0.7 0.7 0.7 0.8 1.0 | 1.0 0.2 0.3 0.3 0.3 0.3 |               |
| PCB-101           | 37680-73-2 | 0.6 0.6 0.6 0.7 0.8 | 0.8 0.3 0.3 0.3 0.3 0.3 |               |
| PCB-118           | 31508-00-6 | 0.3 0.3 0.3 0.4 0.3 | 0.3 0.6 0.5 0.6 0.6 0.6 |               |
| PCB-153           | 35065-27-1 | 1.6 1.6 1.7 1.7 1.9 | 1.6 0.5 0.5 0.4 0.4 0.4 |               |
| PCB-138           | 35065-28-2 | 0.8 0.8 0.8 0.9 0.8 | 0.9 1.2 1.4 1.2 1.4 1.4 |               |
| PCB-180           | 35065-29-3 | 0.3 0.3 0.3 0.3 0.3 | 0.3 0.1 0.1 0.1 0.1 0.1 |               |
| 2,4,5-T           | 93-76-5    |               | 0.5            |               |
| 2,4-D             | 94-75-7    |               |               |               |
| 2,4-dinitrophenol | 51-28-5    |               | 4 5.3         |               |
| 2,4-DP            | 120-36-5   |               |               |               |
| 2-aminoacetophenon | 551-93-9   | 0.61 0.67 0.78 0.66 1 | 0.74 |               |
| acenafene         | 83-32-9    | 12 19 12 7.4 15 | 11 29 25 26 57 27 | 27 |
| acenaftylene      | 208-96-8   | 5.5 11 5.1 4.5 4.3 4.1 | 4.7 6.3 7.5 4.8 6.7 3.6 |
| aclonifen         | 74070-46-5 | 1.3 | 1.5 1.3 2.2 | 2.1 3.7 |
| anthraquinone     | 84-65-1    | 13 16 12 9.9 15 | 13 36 36 29 21 22 | 19 |
| antracene         | 120-12-7   | 8.6 11 8.6 3.7 2.3 3.1 | 2.5 1.8 2.2 1.4 2.3 1.5 |
| atrazine          | 1912-24-9  | 3.8 4.5 3.4 2.7 3.2 2.5 | 0.15 | 0.19 |
| atrazine-desethyl | 6190-65-4  |               |               |               |
| azaconazole       | 60207-31-0 | 0.31 | 0.6 | 6.2 6 6.6 21 8.1 17 |
| azoxyostrobin     | 131860-33-8 | 2.4 1.6 2.4 3 | 2.5 2.2 | 2.1 2.4 3.4 6.3 3.3 4.5 |
| BAM               | 2008-58-4  |               |               |               |
| Compound                  | Cas-number    | Meuse, Eijsden | WWTP Amersfoort |
|---------------------------|---------------|----------------|------------------|
|                           | amount on silicone rubber* | ng/g SR |                   |
|                           |               | 14 | 14 | 14 | 42 | 63 | 105 | 14 | 14 | 14 | 42 | 63 | 105 | 14 | 14 | 14 | 42 | 63 | 105 |
| Exposure time (days)      |               | 0-14 | 14-28 | 28-42 | 0-42 | 42-105 | 0-105 | 0-14 | 14-28 | 28-42 | 0-42 | 42-105 | 0-105 |
| Exposure period (days)    |               | 0-14 | 14-28 | 28-42 | 0-42 | 42-105 | 0-105 | 0-14 | 14-28 | 28-42 | 0-42 | 42-105 | 0-105 |
| bentazon                 | 25057-89-0     | 20 | 26 | 29 | 35 | 42 | 41 | 13 | 8.8 | 10 | 20 | 16 | 18 |       |
| benzo(a)anthracene       | 56-55-3         | 5.7 | 7.3 | 6.8 | 11 | 16 | 16 | 3.5 | 2.7 | 2.3 | 7.7 | 5.8 | 8.3 |       |
| benzo(a)pyrene           | 50-32-8         | 205-99-2 | 23 | 29 | 26 | 44 | 57 | 75 | 11 | 9.1 | 7.5 | 23 | 17 | 24 |       |
| benzo(b)fluoranthene     | 190-86-3        | 3.4 | 3.9 | 3.8 | 7.2 | 9.5 | 12 | 1 | 0.81 | 0.76 | 2.5 | 2 | 3 |       |
| benzo(k)fluoranthene     | 207-08-9        | 8.1 | 11 | 8.9 | 17 | 19 | 23 | 3.6 | 2.5 | 2.8 | 8.7 | 6.6 | 7.8 |       |
| bifenthrin               | 82657-04-3      | 1.1 |     |     |     |     |     | 1.1 | 1.2 | 0.99 | 1 | 0.73 |       |       |
| bitertanol               | 55179-31-2      | 1.1 | 0.65 |     |     |     |     | 0.68 |     |     |     |     |     |       |       |
| broompropylate           | 18181-80-1      |     |     |     |     |     |     | 1.8 |     |     |     |     |     |       |       |
| bupirimate               | 41483-43-6      |     |     |     |     |     |     |     |     |     |     |     |     |       |       |
| carbaryl                 | 63-25-2         | 0.57 | 0.45 | 0.56 | 0.48 | 0.52 | 0.84 | 1.2 | 1.1 | 0.66 | 1.6 | 1.6 | 1.6 |       |
| carbendazim              | 10605-21-7      | 0.38 | 0.81 | 0.44 | 0.65 | 0.49 | 0.64 | 0.87 | 0.72 | 0.56 | 0.83 | 1 | 0.71 |       |
| chlorpropham             | 101-21-3        | 1.3 | 4.1 | 1.9 | 1.1 | 2 | 1.5 |     |     |     |     |     |     |       |       |
| chlorpyrifos-ethyl       | 2921-88-2       | 43 | 31 | 25 | 35 | 41 | 33 | 5.2 | 6.2 | 3.9 | 6.8 | 6 | 6.3 |       |
| chlorotoluon             | 15545-48-9      | 0.51 |     |     |     | 3.7 | 3 |     |     |     |     |     |     |       |       |
| chloridazon              | 1698-60-8       |     |     |     |     |     |     |     |     |     |     |     |     |       |       |
| chrysene                 | 218-01-9        | 65 | 74 | 76 | 96 | 100 | 95 | 24 | 23 | 20 | 37 | 37 | 32 |       |
| cinidon-ethyl            | 142891-20-1     | 0.57 |     |     |     |     |     |     |     |     |     |     |     |       |       |
| clomazone                | 81777-89-1      | 0.62 | 0.68 | 1 | 0.63 | 1.4 | 0.96 |     |     |     |     |     |     |       |       |
| clopyralid               | 1702-17-6       |     |     |     |     |     |     |     |     |     |     |     |     |       |       |
| cycloxidim               | 101205-02-1     |     |     |     |     |     |     |     |     |     |     |     |     |       |       |
| cyhalothrin-lambda       | 91465-08-6      |     |     |     |     |     | 1.7 |     |     |     |     |     |     |       |       |
| Compound                        | Cas-number | Exposure time (days) | Meuse, Eijsden | WWTP Amersfoort |
|--------------------------------|------------|----------------------|----------------|-----------------|
| cypermethrin                   | 52315-07-8 | 14, 14, 14, 14, 42, 63, 105 | 14, 14, 14, 42, 63, 105 |
| cyproconazole                  | 94361-06-5 | 0.4, 0.76, 0.6, 8.5, 7.4, 17 | 0.69, 0.69 |
| cyprodinil                     | 121552-61-2 | 22, 17, 14, 8.5, 7.4, 17 | 16, 9.7, 15, 8.7 |
| DEET                           | 134-62-3   | 52, 48, 30, 26, 14, 12 | 468, 178, 78, 59, 94, 61 |
| deltamethrin                   | 52918-63-5 | 87, 67, 46, 59, 378, 28 | 0.61 |
| diazinon                       | 333-41-5   | 2.4, 3.8, 3.5, 3.3, 5.9 | 4.5 | 85, 77, 66, 90, 81, 76 |
| dibenzo(ah)anthracene          | 53-70-3    | 1.2, 1.2, 1.1, 1.8 | 3.1, 4.1 | 0.33, 0.22, 0.21, 0.83, 0.65, 1.1 |
| dichlobenil                    | 1194-65-6  | 0.29 | |
| dichlorphos                    | 62-73-7    | 0.17, 0.056, 0.095 | 0.059, 0.12 | 0.92, 0.52, 0.39, 0.54, 0.14, 0.21 |
| difenoconazole                 | 119446-68-3 | 5.9, 9.6, 9 | 12, 9.8 | 14 | 0.5, 0.64, 1.1, 0.7, 1.3 |
| diflubenzuron                  | 35367-38-5 | 0.69 | 0.57 |
| difufenican                    | 83164-33-4 | 378, 365, 361 | 420, 343 | 313, 8.4, 9.3, 5.5, 9.1, 10, 7.7 |
| dimethenamid (-P)              | 87674-68-8 | 1.5, 1.4, 1.8 | 1.3, 2.4 | 1.8, 3.7 | 0.66 |
| dinoterb                       | 1420-07-1  | 1.2 | 2.4 |
| diuron                         | 330-54-1   | 6.1, 5.7, 5.6 | 3.8, 1.7 | 1.6 | 2.6, 2.3, 1.9, 1.4, 1.6, 1.1 |
| dodemorf                       | 1593-77-7  | 1.8, 0.95 | 0.64, 4.2 | 3.1, 5.5 |
| endosulfan-alfa                | 959-98-8   | 0.46, 0.57 | |
| ethofumesate                   | 26225-79-6 | 2.8, 2.2 | 1.9, 1.5 | |
| ethoprophen                    | 13194-48-4 | 2.2 | 1.6, 1.7 |
| fenamiphos                     | 22224-92-6 | 0.66, 0.78 | 0.33, 0.20 | 0.30, 0.21 | 0.13, 0.11, 0.14, 0.11, 0.14, 0.10 |
| phenantrene                    | 85-01-8    | 0.72, 0.76 | 0.72 | 0.42, 3, 1.4, 1.7 |
| fenarimol                      | 60168-88-9 | 0.72 | 0.72, 0.72 | 0.42, 3, 1.4, 1.7 |
| fenbutatinoxide                | 13356-08-6 | 0.55, 0.99 | |

amount on silicone rubber
ng/g SR

Exposure period (days)
0-14, 14-28, 28-42, 0-24, 42-105, 0-105
| Compound            | Cas-number | Meuse, Eijsden | WWTP Amersfoort |
|---------------------|------------|----------------|-----------------|
|                     |            | 14 14 14 42 63 | 14 14 14 42 63 |
| Exposure time (days)|            | 0-14 14-28 28-42 | 0-14 14-28 28-42 |
| Exposure period (days) |          | 0-42 42-105 0-105 | 0-42 42-105 0-105 |
| fenhexamid          | 126833-17-8| 0.18 1.5 1.6 1.3 2 | 2 2.1 1.6 2.8 4 |
| fenoxycarb          | 72490-01-8 | 1.3            | 5              |
| fenpropimorf        | 67564-91-4 | 0.67 1.3 2.8 3.2 7.3 8.1 | 2 2.1 1.6 2.8 4 |
| fluorantene         | 206-44-0   | 153 147 113 94 86 67 | 67 51 66 50 82 42 |
| fluorene            | 86-73-7    | 19 34 16 11 21 15 | 3.5 4.3 5.3 4.1 5.5 4.6 |
| flutolanil          | 66332-96-5 | 1.7 2 1.9 1 0.32 |               |
| imazalil            | 35554-44-0 | 2.6 3.4 3.5 6.7 9.7 15 | 8.1 21 26 72 42 74 |
| imidacloprid        | 138261-41-3|               |               |
| indeno(123-cd)pyrene| 193-39-5   | 2.9 2.9 2.7 5.3 7 9.1 | 0.9 0.62 0.54 2.3 1.4 2.2 |
| iprodion            | 36734-19-7 | 0.66 1.1 | 1.2 | 3.8 4.1 3.3 6.1 2.8 3 |
| isoproturon         | 34123-59-6 | 0.73 0.66 0.85 0.58 23 18 | 0.23 0.59 0.29 0.36 |
| lenacil             | 96639      |               |               |
| lindane             | 58-89-9    | 0.42 0.67 0.32 | 0.74 0.68 2.3 0.45 2.6 0.52 |
| linuron             | 330-55-2   | 1.4 1.1 1.4 0.88 |               |
| malathion           | 121-75-5   |               |               |
| MCPA                | 94-74-6    |               |               |
| MCPP                | 93-65-2    |               |               |
| metalaxyl           | 57837-19-1 |               | 0.31           |
| metamitron          | 41394-05-2 |               |               |
| metazachlor         | 67129-08-2 | 0.99 2 5.3 4.4 18 15 | 3 3.3 0.98 0.99 1 1.1 |
| methiocarb          | 2032-65-7  |               |               |
| metolachlor (-S)    | 51218-45-2 | 20 15 13 9.4 5.5 4.8 | 1.6 1.3 2.2 1.8 |
| metribuzin          | 21087-64-9 |               |               |
### Compound

| Compound        | Cas-number       | Meuse, Eijsden | WWTP Amersfoort |
|-----------------|------------------|----------------|-----------------|
| monuron         | 150-68-5         | 14             | 14              |
| naphtalene      | 91-20-3          | 14             | 14              |
| nicosulfuron    | 111991-09-4      | 42             | 63              |
| oxydemeton-methyl | 301-12-2     | 105            | 105             |
| parathion-methyl | 298-00-0       | 0-14           | 14              |
| penconazole     | 66246-88-6       | 0-14           | 14              |
| penicycuron     | 66063-05-6       | 14-28          | 14              |
| pendimethalin   | 40487-42-1       | 28-42          | 14              |
| permethrin      | 52645-53-1       | 0-28-42        | 63              |
| pirimicarb      | 23103-98-2       | 0-105          | 105             |
| pirimiphos-methyl | 29232-93-7   | 11             | 11              |
| prochloraz      | 67747-09-5       | 14             | 14              |
| procymidone     | 32809-16-8       | 14             | 14              |
| propiconazole   | 60207-90-1       | 14             | 14              |
| propoxur        | 114-26-1         | 14             | 14              |
| propyzamide     | 23950-58-5       | 14             | 14              |
| prosulfocarb    | 52888-80-9       | 14             | 14              |
| pyrene          | 129-00-0         | 14             | 14              |
| pyridate        | 55512-33-9       | 14             | 14              |
| pyrimethanil    | 53112-28-0       | 14             | 14              |
| simazine        | 122-34-9         | 14             | 14              |
| sulcotrin       | 99105-77-8       | 14             | 14              |
| tebuconazole    | 107534-96-3      | 14             | 14              |

*amount on silicone rubber* (ng/g SR)

| Exposure time (days) | 14 | 14 | 14 | 42 | 63 | 105 | 14 | 14 | 14 | 42 | 63 | 105 |
|----------------------|----|----|----|----|----|-----|----|----|----|----|----|-----|
| Exposure period (days)| 0-14| 14-28 | 28-42 | 0-28 | 42-105 | 0-105 | 0-14 | 14-28 | 28-42 | 0-28 | 42-105 | 0-105 |
| Compound       | Cas-number          | Meuse, Eijsden (ng/g SR) | WWTP Amersfoort (ng/g SR) |
|---------------|---------------------|--------------------------|---------------------------|
|               | Exposure time (days)| 0-14 14-28 28-42 0-42 42-105 0-105 | 0-14 14-28 28-42 0-42 42-105 0-105 |
|               | Exposure period (days)|                         |                           |
| tebufenpyrad  | 119168-77-3         | 0.17 0.47 0.43 3.6 4.8 1.3 2.1 5.7 4.4 |                           |
| teflubenzuron | 83121-18-0          | 32 36 30 29 26 21 32 26 44 23 42 19 |                           |
| terbutryn     | 886-50-0            | 47 35 36 28 13 12 15 7.4 5.2 2.9 6.9 2.7 |                           |
| terbutylazine | 5915-41-3           | 7696-12-0               | 1.1 1.2 0.56 3.0 2.2 3.5 3.4 3.3 |                           |
| tetramethrin  | 148-79-8            | 4.1 18 2.2 8.1 15 13 3 2.2 2.1 3.5 3.4 3.3 |                           |
| thiabendazole | 111988-49-9         | 57018-04-9              | 0.89 1.4 1.4 0.45 1.3 0.31 |                           |
| thiacloprid   | 731-27-1            | 55219-65-3              | 1.1 1.4 1.7 3.9 2.5 3.7   |                           |
| tolclofos-methyl | 2303-17-5          | 4 4.1 8.1 7.3 27 21 0.94 1.1 1 9.5 1.4 8.2 |                           |
| tolyfluamide  | 112143-82-5         | 141517-21-7             |                           | 1.4                       |
Table S3: Percentage of the bioassay response explained by the PAHs (DR-LUC bioassay) or by all compounds (A. fischeri bioluminescence assay) analyzed in the passive samplers (see Table S1)

| Parameter                                                               | Unit          | Weeks       | Meuse, Eijsden | WWTP Amersfoort |
|------------------------------------------------------------------------|---------------|-------------|----------------|-----------------|
|                                                                        |               | 1-2 3-4 5-6| 1-6 7-15 1-15 | 1-2 3-4 5-6 1-6 7-15 1-15 |
| PAHs with DR-LUC REP values<sup>a</sup>                                 | #             | 12 12 12 12| 12 12 12      | 12 12 12 12 12 12 12 12 |
| calculated DR-LUC response by PAHs                                     | pg TCDD-EQ/SD | 0.26 0.73 0.39| 1.0 1.2 1.9 | 0.15 0.24 0.41 0.32 0.27 2.6 |
| measured DR-LUC response in extract                                   | pg TCDD-EQ/SD | 31 35 32 90 | 116 240       | 102 104 88 449 464 700 |
| DR-LUC response explained by PAHs                                      | %             | 0.83% 2.1% 1.2%| 1.1% 1.0% 0.78% | 0.15% 0.23% 0.47% 0.071% 0.058% 0.37% |
| compounds with A. fischeri IC<sub>50</sub> values<sup>b</sup>           | #             | 9 8 8 10 11 13 | 14 8 7 12 13 14 |
| calculated toxic units (TU)<sup>c</sup>                                | mL/SD         | 0.16 0.15 0.12| 0.26 0.22 0.35 | 0.47 0.13 0.14 1.39 1.97 3.11 |
| measured toxic units (TU)<sup>d</sup>                                  | mL/SD         | 9.6 7.7 16.5| 26.5 32.0 46.8 | 30.8 28.4 32.1 79.3 94.5 141.2 |
| bioluminescence inhibition explained                                  | %             | 1.6% 2.0% 0.73%| 1.0% 0.70% 0.76% | 1.5% 0.47% 0.43% 1.7% 2.1% 2.2% |

For Silicone rubber:

| PAHs with DR-LUC REP values<sup>a</sup>                                 | #             | 12 12 12 12| 12 12 12      | 12 12 12 12 12 12 12 12 |
| calculated DR-LUC response by PAHs                                     | pg TCDD-EQ/SD | 31 38 34 57| 66 77         | 13 10 10 28 22 25 |
| measured DR-LUC response in extract                                   | pg TCDD-EQ/SD | 298 256 262| 639 832 931   | 334 463 319 788 740 579 |
| DR-LUC response explained by PAHs                                      | %             | 10% 15% 13%| 9.0% 7.9% 8.3% | 3.9% 2.2% 3.2% 3.5% 3.0% 4.4% |
| compounds with A. fischeri IC<sub>50</sub> values<sup>b</sup>           | #             | 12 12 11 11| 13 12         | 14 13 13 13 14 13 |
| calculated toxic units (TU)<sup>c</sup>                                | mL/g SR       | 1.73 2.18 1.19| 0.92 1.44 1.10 | 9.06 8.15 7.16 9.55 8.72 8.06 |
| measured toxic units (TU)<sup>d</sup>                                  | mL/g SR       | 60.5 45.5 50.8| 96.0 172.4 73.6 | 33.9 15.6 16.7 32.8 30.6 24.9 |
| bioluminescence inhibition explained                                  | %             | 2.9% 4.8% 2.3%| 1.0% 0.84% 1.5% | 27% 52% 43% 29% 29% 32% |

<sup>a</sup>: REP values were obtained for the 12 US-EPA PAHs from Machala et al. (2001) Mutation Research 497: 49–62. No REP values were available for the light PAHs acenaphthene, acenaphthylene, phenanthrene, and naphthalene, which were analyzed in this study, but are supposed to have no or very low AhR-activating potency.

<sup>b</sup>: IC<sub>50</sub> values were obtained from an in-house database collected by Dick de Zwart.

<sup>c</sup>: calculated TU were calculated by dividing the concentration in the sampler (ng/SD or ng/g SR) by the IC<sub>50</sub> value (ng/L).

<sup>d</sup>: measured TU were calculated as the inverse of the measured IC<sub>50</sub> (SD/L or g SR/L).