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Effects based methods (EBMs) in combination with passive sampling of refinery streams

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ABSTRACT

This report provides an overview of spot and passive sampling data of three refinery effluents after treatment that were collected for chemical and ecotoxicological effluent assessment. The report includes a discussion of the relationships between toxic units derived from spots samples and passive samplers. Further, it discusses if the observed toxicity from the passive sampler extracts can be explained by chemical analyses and makes an overall comparison between spot and passive sampling.

Return of experience on the application of passive samplers within refinery effluent assessments, including technical considerations on the use of passive vs. spot sampling are summarized. If and how passive sampling can help towards making better decisions and assessments of refinery waters is also discussed.

The report concludes that passive sampling provides information on the dissolved fraction as well as a time-weighted averaged sample over several weeks, which is not provided by spot sampling. However, there are a number of challenges when translating bioassay outcomes of the passive sampler extracts to the toxicity response of the original waters, and these challenges seem largely related to the use of partition based passive samplers. From the work conducted in this project it can be concluded that expert labs and detailed knowledge are needed to properly interpret the results from assessments that combine passive sampling studies with effect-based methods testing. Overall, this approach is not yet ready for routine monitoring but might be more suitable for targeted, location specific surveillance studies.

KEYWORDS

passive samplers; refinery effluents; effect-based methods;

INTERNET

This report is available as an Adobe pdf file on the Concawe website (www.concawe.org).

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SUMMARY

This report provides the findings of a Concawe project on the application of effects-based methods (EBM) in combination with passive sampling and chemical analysis for the assessment of three selected refinery effluent streams. A critical assessment of EBM in combination with passive samplers (PSS) is provided especially in relation to possible inclusion of EBM in coming legislation. The study aim was to investigate the added value of passive sampling, and to determine the toxicity contribution from treated effluents (outlet waters) of refineries. Passive samplers (silicon rubber sheets) were deployed for a period of 5-6 weeks in three refineries' (A, B and C) inlet and outlet waters. Furthermore, various chemical analysis, *in vivo* (*Daphnia magna, Vibrio fischeri* (Microtox®), *Hyalella azteca, Pseudokirchneriella subcapitata, Brachionus calyciflorus, Thamnocephalus platyurus* and *Danio rerio)*, and *in vitro* bioassays (AhR-activity, estrogenicity, androgenicity) were used to assess the toxicity of the water samples collected with PSS. Extracts of the PSS were tested with these assays. Toxicity (*V. fischeri*) from passive sampling was compared to toxicity (*V. fischeri)* and chemistry of weekly spot samples.

Passive samplers (PSS) were successfully deployed and retrieved from the refineries, and the PSS concentrated enough material to determine the dissolved concentrations of hydrocarbons (HCs) in refinery waters, and perform the *in vivo* and *in vitro* assays. The PSS concentrated the compounds from the water into the PSS, and the concentrations were, therefore, recalculated to original waters both for the chemical analysis as the bioassays using concentration factors. Average concentrations factors were used for the recalculation of the bioassay results due to the fact that the compounds causing the toxicity are unknown. This recalculation has larger uncertainties in the concentration factors for the bioassay than for the chemical dissolved water concentration calculations for which the compounds are known. Therefore, the recalculated bioassay results from the PSS extracts can hardly be used as a quantitative prediction of toxicity in the original samples.

Assessment of passive sampler extracts with the bioassays showed that:

1) Refinery outlet samples showed in general a higher toxicity than refinery inlet waters for most assays. This is consistent with the chemical (TPH, PAH, GCxGC, some metals) and biomimetic solid-phase microextraction (BE-SPME) analysis, which were also elevated in outlet samples. The outlet sample of refinery B had a marked higher toxicity compared to the inlet sample (for Microtox and *H. azteca*).

2) Bioassay assessment of the passive sampler extracts indicated that no acute or chronic toxicity was expected for the original water samples as the back calculated Toxic Units (TUs; using the concentration factor) were all much lower than 0.1. This with the exception for the outlet of refinery B where the TU values ranged up to 0.47 for the amphipod *H. azteca*. Acute toxicity and especially chronic toxicity are to be expected in this outlet sample as well as the TU for *D. magna, P. subcapitata*, and *D. rerio* are between 0.1 and 0.3. Note, however, that after discharge the effluent water will be diluted within the initial mixing zone.

3) Spot samples tested with *V. fischeri (*Microtox) showed toxicity for outlet samples of refinery A and C, while the *V. fischeri* assessment of the passive sampler extract showed TU<0.1, as shown above. This provided first indications that other compounds than HCs might be present in refinery waters and contribute to a large extent to the toxicity. This shows that most likely apolar HCs only marginally contributed to the toxicity.

4) The *in vitro* test battery showed elevated arylhydrocarbon receptor (AhR) activity, estrogenicity, and androgenicity activity in the outlet waters compared to the inlet waters. The *in vitro* assays are useful as early warning systems to detect specific compounds related to specific mode-of-actions. They provide a sum parameter for known and unknown chemicals with a known toxicity mechanism. Comparison of the *in vitro* assay results with proposed effect-based trigger (EBT) values for AhR-activity, estrogenic, and anti-androgenic activity showed that all inlet and outlet samples were below these values, except for one outlet sample (refinery B) which was above the proposed AhR EBTs.

5) Comparing the TUs on measured (HCs) (GCxGC) (TU_{chem}) and measured by *in vivo* assays ($TU_{bioassay}$) showed that $TU_{bioassay}$ were in general 1 to 2 orders of magnitude higher than the chemical determined toxicity. This difference could be due to the uncertainty in the calculated concentration factor for the bioassays, but could also be due that the bioassays detect more active chemicals than only the measured HCs.

6) The results indicate that the HCs could not fully explain the observed toxicity, and that other compounds seem to be causative factors for the observed toxic effects for all test species. This is also illustrated with the *V. fischeri (*Microtox) results that showed less than 5% of the observed toxicity in the refinery outlet water was due to HCs. In those cases, risk assessments based on GCxGC analyses might underestimate the 'true' risks for the receiving surface waters.

PSS provided information on the dissolved fraction which is not provided by spot sampling and TPH analysis. In addition, PSS provided a time-weighted average concentration of HCs. However, there are a number of challenges when translating the bioassay outcomes of the PSS extract to the toxicity of the original waters, and these challenges seem largely related to the use of partition-based PSS. From the work conducted in this project it can be concluded that expert labs and detailed knowledge are needed to properly interpret results from studies that combine PSS with EBM testing. This approach is not yet ready for routine monitoring studies but more suitable for targeted and location specific surveillance studies.

1. INTRODUCTION

This report provides the findings of a Concawe project to test the application of effects-based methods (EBMs) in combination with passive sampling and chemical analysis for the assessment of refinery effluent streams. As part of a re-evaluation of the Water Framework Directive (WFD) (EC, 2019), the European Commission (EC) is considering EBMs as an alternative to, or in combination with, the monitoring of individual substances, for investigating the chemical status of water bodies, and by extension may, in the future, also consider them for effluents monitoring.

Passive samplers are increasingly used in the monitoring of chemical compounds in the environment. The basis of PSS is the diffusion of such compounds from the water to the sampler. Various types of PSS are available and needed to cover the full range of chemicals (polar, apolar, metals). The absorption of the compounds initially follows a linear uptake stage, and finally equilibrium between the sampler and the water is reached. Silicon based PSS can be used to measure the free dissolved concentration of apolar organic compounds in water (e.g. Smedes et al. 2007, Mayer et al. 2014), and have the advantage that this bioavailable fraction is considered to drive the toxicity (Carls et al. 2008; Nordtug et al. 2011; Letinski et al. 2014; Redman and Parkerton 2015). Another advantage of passive sampling, compared to spot sampling, is the time integrated sampling, resulting in time-weighted average concentrations. For more details about the principal and background of passive sampling see for example Smedes et al. (2007) and Taylor et al. (2019).

The main aim of this project was to determine the added value of passive sampling of apolar compounds, in combination with EBMs to assess the impact of refining operations on effluent toxicity, and more specifically the potential toxicity contribution from apolar substances. The impact was assessed by monitoring the water quality exiting (outlet) the refinery wastewater treatment plant (WWTP) by means of passive sampler devices, plus the water quality entering (main inlet) in order to determine the background, not attributable to a refinery contribution. Note, the inlet water cannot be one-by-one related to the outlet water as different inlet sources were present at the refineries included in this study.

The project objectives were:

1) Perform time-integrated sampling using passive sampler devices, suitable for apolar compounds, deployed in refinery main inlet waters entering the refinery and final effluent waters discharged to the surrounding water (outlet waters)
2) Determine concentrations of HC blocks, total petroleum hydrocarbons (TPH), and biomimetic solid phase microextraction (BE-SPME) in PSS and compare to spot sampling of the inlet and outlet waters
3) Determine toxicity profiles in the passive sampler extracts of apolar compounds using a battery of bioassays consisting of seven small-volume *in vivo* organism-level bioassays representing four trophic levels and five mechanistic *in vitro* bioassays
4) Determine correlation between passive sampling response and BE-SPME measurements
5) Assess the added value of using passive sampling compared to spot sampling
6) Determine the effect of chemical analysis for the observed toxicity

This report provides an overview of the spot (§3.1) and passive sampler (§3.2) data, including a discussion of the outcomes, relationships between TUs and dissolved water concentrations or PSS (§3.2.3), whether the toxicity can be explained by the chemical analyses (§3.3), and finally a comparison between spot and passive sampling (§3.4). The application of PSS within refinery effluent assessments, including technical considerations on the use of passive vs. spot sampling are discussed in §5.1, and whether or not passive sampling can help or not towards making better decisions and assessments of refinery waters is discussed in §4.2.

2. MATERIALS AND METHODS

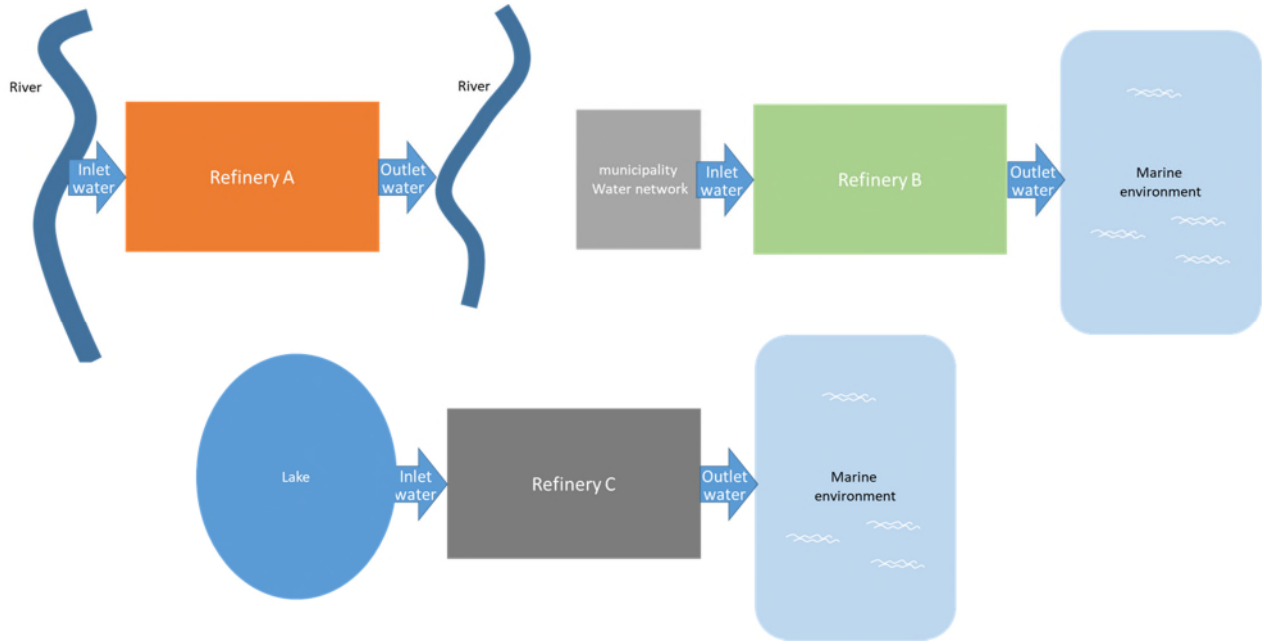
2.1. SELECTION OF REFINERIES AND SAMPLING LOCATIONS

The refineries were selected in collaboration with Concawe. Three refineries (coded as refinery A, B, and C) participated in the project. To select appropriate sampling locations based, for example, on flow rate requirements and water depth, location A was visited before passive sampler deployment by representatives of Vrije University Amsterdam (VU) and Concawe, location B by Concawe representatives. Location C was not visited upfront deployment, as Refinery C had previous experience with the application of PSS.

VU prepared the sampling protocol, PSS installation instructions, and sampling kits for sample collection and shipped all material to the refineries (see appendix A). The sampling kits contained the samplers, deployment racks, glass and plastic bottles and conservation agents. VU installed and deployed the PSS at refineries A and B and recovered the samplers after sampling. Deployment of the PSS at refinery C was performed by their own personnel. Spot samples were collected by personnel of the refineries and shipped to VU (The Netherlands) by courier.

At each refinery, two passive sampler arrays were deployed. One sampler was deployed in an appropriate inlet zone (intake water), and the second at the refinery WWTP discharge point (outlet zone, also known as final effluent water), see **Figure 1**. Note that the inlet is not necessarily representative for the outlet and receiving environment.

Figure 1: Schematic diagram of the inlet and outlet waters where spot samples were taken and passive sampler were deployed of refineries A, B and C.



Passive sampling and spot sampling

Preparation of passive sampler (PSS) sheets included various steps: sheet preparation, cleaning procedure, performance reference compounds (PRC) addition, PSS extraction, preparation of the deployment rackets, and deployment. For more details see Smedes and Booij (2012). Briefly, the silicone based PSS sheets (5.5 x 9.0 cm with an area of ca. 100 cm^2, about 3 g per sheet) were cut and pre-cleaned before use. After cleaning the PSS sheets one set of sheets was spiked with performance reference compounds (PRCs) (spiked, n=6), and one set was unspiked (n=12). The set of performance reference compounds consisted of a set of PCBs - PCB 1 (47 ng), PCB-2 (48 ng), PCB-3 (47 ng), PCB-10 (29 ng), PCB-14 (32 ng), PCB-30 (18 ng), PCB-50 (13 ng), PCB-21 (32 ng), PCB-104 (9 ng), PCB-55 (13 ng), PCB-78 (15 ng), PCB-145 (9 ng), PCB-204 (15 ng)-, and biphenyl-d10 (112 ng): in brackets the spiked amount added to each sampler sheet. The spiked sheets were used to calculate the water sampling rates (R_{SR}, for more information see §2.4). The unspiked samplers were used for the toxicity test. The sheets were obtained from Deltares (NL). PSS were deployed in accordance to the TIPTOP project (Hamers et al., 2018). In addition, Speedisk PSS (n=6), which can collect more polar compounds, were deployed as well. In this study only the silicon based PSS were used for the chemical analysis and toxicity tests. The Speedisks were stored at -20°C and remains available for further analysis if needed.

PSS were deployed in 2018 and 2019 at three refineries (refinery A, B, C) (**Table 1**). The deployments of the samplers and the spot sampling were successful and all PSS were retrieved (**Figure 2**). In refinery A and C, the samplers were exposed for ca. 5 weeks to the inlet and outlet waters, and in refinery B for ca. 6 weeks.

Table 1: Deployment of PSS and spot sampling at three refineries.

	Refinery A	**Refinery B**	**Refinery C**
Deployment period passive sampling	9 July – 14 August 2018	29 October - 11 December 2018	25 February – 1 April 2019
	Ca. 5 weeks	Ca. 6 weeks	Ca. 5 weeks
	Inlet water and outlet zone	Inlet water and outlet zone	Inlet water and outlet zone
Spot sampling	Week 1, 2, 3, 4, 5	Week 2, 3, 4, 5, 6	Week 1, 2, 3, 4, 5
	Inlet water and outlet zone	Inlet water and outlet zone	Inlet water and outlet zone

Figure 2: PSS tray before (left) after 5 weeks of sampling (right).

In parallel, spot samples were collected at weekly intervals (n=5) for benchmarking the time-integrative PSS/EBM responses. Spot samples were taken from the inlet and outlet. Water samples were conserved with a conservation agent and shipped as soon as possible to VU under room temperature. The PSS were as soon as possible transported after collection in bottles of water from the location to VU. At VU the water was removed, and the samplers frozen at -20°C.

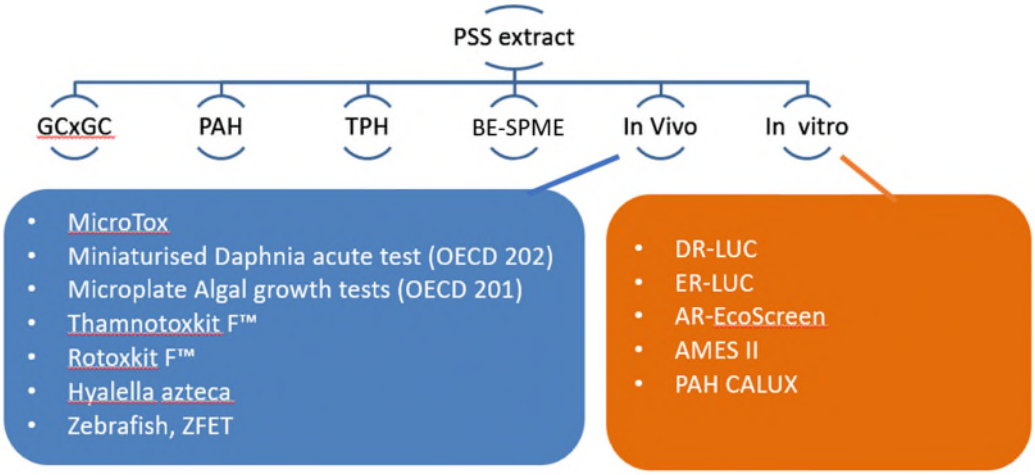
2.1.1. PSS extraction

The silicon PSS were cleaned in the laboratory with MilliQ water, dried and weighted.

The unspiked samplers, which were used for the toxicity and chemical analysis, were extracted together twice, by gentle shaking using a plate shaker for 3 days with 250 mL acetonitrile. MilliQ water (750 mL) was added to the combined acetonitrile extract, and further liquid-liquid extracted three times with 100 mL pentane. The combined pentane extracts were evaporated with Kuderna Danish (KD) at 50°C to about 5 mL, followed by evaporation with a mini KD evaporator (50°C) to about 1 mL and weighted. Each pentane extract was split for chemical and toxicity testing (about 1:3, v/v). The pentane extract for chemical analysis was divided into extracts for two-dimensional comprehensive gas chromatography (GCxGC), TPH, BE-SPME, and polycyclic aromatic hydrocarbons (PAH) analysis. The pentane extract for toxicity testing was transferred to dimethylsulfoxide (DMSO) by adding 500 µl DMSO for the outlet and 200 µl for the inlet samples, respectively. The pentane was removed by a very gentle stream of nitrogen and the HCs were transferred to the DMSO. A lower volume of DMSO was used for the inlet samples, as lower HCs levels were expected, resulting in a more concentrated sample and therefore a higher ability to find effects in the bioassays. Afterwards, evaporation using mini-KD was applied to remove the pentane, and the procedure was checked by weighing the final DMSO amount. Each DMSO extract was divided in various aliquots for *in vitro* and *in vivo* testing (**Figure 3**). Earlier studies (Concawe, 2010) showed that acceptable recoveries (70-100%) were found with KD evaporation for C9 and higher HCs. Furthermore, during the waste water treatment process in refineries more volatile HCs are removed (Hjort et al., 2021), and will therefore be less present in effluent waters.

The spiked samplers (n=6) were extracted with 2x120 mL acetonitrile. The extract was liquid-liquid extracted using 3x 50 mL pentane. The pentane extract was evaporated with KD and mini-KD to ca. 1 mL. Performance reference compounds (PRCs) were analysed in the final extract to determine the total water volume extracted by the PSS.

Figure 3: Aliquots of the PSS extract.



2.2. CHEMICAL ANALYSIS

For the **spot samples** the following parameters were determined:

- General parameters (chemical oxygen demand (COD), dissolved organic carbon (DOC), total organic carbon (TOC), total N, phenol index, total suspended solids (TSS)
- TPH
- BE-SPME
- Dissolved metals (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, Co, Se, V)

In the **PSS extracts** the following parameters were determined:

- HC speciation (GCxGC)
- TPH
- BE-SPME
- PAH (32 priority PAHs)
- PAH (16 EPA)
- Methyl-PAHs

The analysis of TPH, COD, DOC, TOC, and metals was carried out by Eurofins, Omegam laboratories (The Netherlands). VU performed the GCxGC, BE-SPME, PAH (16 EPA) and methyl-PAHs analysis. The Biochemical Institute for Environmental Carcinogens (BIU; Grosshansdorf, Germany) performed the 32 priority PAH analysis.

An overview of the different analysis and methods used are provided in **Table 2.**

Table 2: Determined chemical parameters and analytical methods used.

Parameter	Analysis method
GCxGC	Concawe, 2010
BE-SPME	Leslie and Leonards (2005)
Metals	All metals analysed by using inductively coupled plasma mass spectrometry using NEN-EN-ISO 17294-2 and NEN-EN-ISO 15587-1, except Hg which was analysed by NEN-EN-ISO 12846
TPH	NEN-EN-ISO 9377-2 (2000)
Phenol index	NEN-EN-ISO 14402
Total N	NEN 6643
DOC and TOC	NEN-EN-ISO 1484
COD	NEN 6633
TSS	VU protocol, based on filtration with Whatman ME25 filter (pore size 0.45 µm), dried and weighted
EU 32 priority PAHs	BIU protocol according to Grimmer et al., 1997
16 US-EPA PAHs, and Me-PAHs	VU protocol using GC-MS

BE-SPME were determined in the spot and PSS samples. Spot samples were directly measured. PSS extracts were redissolved in water by adding the DMSO extract to an amount of water representing the original water concentration, and DMSO concentrations were <0.1%. BE-SPME were determined using solid-phase microextraction (SPME) combined with analysis by gas chromatography (GC) coupled with a flame ionization detector (GC-FID) according to the protocol of Leslie and Leonards (2005). Briefly, SPME fibers (100 µm poly-dimethylsiloxane PDMS fibers) were exposed to 250 mL of inlet or outlet waters, with agitation using a Teflon stir bar, for 24 h in a closed glass bottle without head space. After 24 h exposure the fibers were removed from the effluent solution, dried with a tissue and then directly injected into a GC equipped with a FID. A DB-1 (210 m x 0.25 mm x 0.1 µm) GC column was used. For quantification 2,3-dimethylnaphthalene was used as external standard. The temperature programme of the GC was designed to sum the peaks as much as possible by using a fast temperature ramp. The total peak area of the chromatogram was integrated (between C_9 and C_{38}) and the molar concentration was calculated.

The HC speciation of the PSS samples was performed according to the protocol used in the Concawe effluent speciation project (Concawe, 2010). Briefly described, the PSS extract was fractionated in an aliphatic and aromatic fraction, and analysed by two-dimensional GC coupled to a FID (GCxGC-FID) to characterise and quantify the HCs. The HC blocks were reported as shown in **Table 3**.

Table 3: Blocks that were analysed are indicated with a "X". nP: normal paraffins, iP: iso-paraffins, mN: mono-naphthanics, dN: dinaphthanics, pN: polynaphthanics, MAH: mono-aromatics, nMAH: naphthenic mono-aromatics, DAH: diaromatics; nDAH: naphthenic diaromatics, PAH: polycyclic aromatics.

		n-P	i-P	mN	dN	pN	MAH	nMAH	DAH	nDAH	PAH
Block	C#i										
1	3										
2	6										
3	9	X	X	X	X		X	X	X		
4	10	X	X	X	X		X	X	X		
5	11	X	X	X	X		X	X	X		
6	12	X	X	X	X		X	X	X	X	X
7	13	X	X	X	X		X	X	X	X	X
8	14	X	X	X	X		X	X	X	X	X
9	15	X	X	X	X		X	X	X	X	X
10	16	X	X	X	X		X	X	X	X	X
11	17	X	X	X	X		X	X	X	X	X
12	18	X	X	X	X	X	X	X			X
13	19	X	X	X	X	X	X	X			X
14	20	X	X	X	X	X	X	X			X
15	21	X	X	X		X					
16	22	X	X	X		X					
17	23	X	X	X		X					
18	24	X	X	X		X					
19	25	X	X	X		X					
20	26	X	X	X		X					
21	27	X	X	X		X					
22	28	X	X	X		X					
23	29	X	X	X		X					
24	30	X	X			X					
25	>30	X	X			X					

2.3. EFFECT-BASED METHODS: *IN VITRO* AND *IN VIVO* BIOASSAYS

Time integrated effluent toxicity was evaluated using extracts from the PSS tested with both *in-vitro* and *in-vivo* bioassays as specified below. Spot samples were also evaluated for *V. fischeri* (Microtox). For each refinery only one PSS extract was obtained for both inlet and outlet. Statistical analyses could therefore not be performed. Ecofide performed the *in vivo* tests (*V. fischeri* (Microtox), *Pseudokirchneriella subcapitata*, *D. magna*, *Hyalella azteca*, *Brachionus calyciflorus* (Rotoxkit), and *Thamnocephalus platyurus* (Thamnotoxkit), and VU the *in vitro* (DR-Luc, ER-Luc, AR-EcoScreen) and zebrafish tests. BDS performed the PAH-CALUX.

2.3.1. *In vivo* tests

Extracts of the silicon sheets were prepared in DMSO, as described in Section 3.2.1. For each bioassay and PSS extract the same series of test concentrations was used. Highest test concentration consisted of a 1000-fold dilution of the original extract, by adding 1 µL extract in 1 mL of exposure media. For each further dilution the reduced amount of PSS extract (in DMSO) was compensated for by the addition of a comparable amount of pure DMSO to keep the amount of DMSO in each test concentration at a constant level of 0.1 vol%[1]. Ten different concentrations were prepared for each bioassay resulting in the following concentrations of sample extract in final exposure media (v/v): 0.1, 0.05, 0.025, 0.0125, 0.0063, 0.0031, 0.0016, 0.0008, 0.0004 and 0.0002 %. For each bioassay this dilution series was prepared in the respective standardized test medium as specified in the test guidelines (see below). For the Zebrafish a different procedure was chosen as described below.

Toxicity in spot samples was assessed with the bacteria *Vibrio fischeri* only. For this bioassay standard procedures were followed as described in the ISO 11348-3 guideline (2007), resulting in four concentrations of effluent in final exposure media (v/v): 45, 22.5, 11.25 and 5.63 %. Conductivity, pH, ammonia and nitrate concentrations were measured in each spot sample to verify that the values fall within the ranges specified for *V. fischeri* (Microtox). As this was the case, possible toxic effects in spot samples were not caused by these factors.

In addition to the extracts of the in- and outlet samples five different control tests were performed for each test organism: i) standardized test medium, ii) a concentration series of pure DMSO to check for possible toxic effects up to 1.6 vol % DMSO, iii) procedure blank, iv) blank PSS extract and v) positive control with a reference toxicant.

Toxicity tests were performed with the bacteria *Vibrio fischeri* (Microtox®-test), the algae *Pseudokirchneriella subcapitata*, the daphnid *Daphnia magna*, the amphipod *Hyalella azteca*, the rotifer *Brachionus calyciflorus* (Rotoxkit), the shrimp *Thamnocephalus platyurus* (Thamnotoxkit) by Ecofide, and the zebrafish *Danio rerio* by VU, all in open test vessels. All tests followed static exposure, being either acute or semi-chronic (algae, zebrafish) tests. More detailed information regarding the different test procedures is provided below, or can be found in the international guidelines also mentioned below.

Bacteria *Vibrio fischeri*

In the Microtox®-test, inhibition of the bioluminescence of the bacterium *V. fischeri* is used to assess possible toxic effects. Tests were performed according to ISO 11348-3 guideline (2007) in glass vials using the original Microtox-equipment and media. Bioluminescence was determined after 5, 15 and 30 minutes of exposure to a range of test concentrations. As the bacterium is a marine species, salt solutions were added to each test solution. Each test concentration was tested at 15°C in duplicate. All toxicity tests were valid based on the validity criteria described in ISO 11348-3 guidelines[2]. The twenty and fifty percent effect concentration values

[1] A maximum DMSO concentration of 0.01% is often advised for ecotoxicity studies, although such specification is not specified in the ISO-guidelines. However, lowering the amount of DMSO in the present experiments would also result in lower exposure concentrations. DMSO concentrations were therefore set at 0.1% while in addition DMSO up to 1.6% was tested as negative control.

[2] f_{kt}-value for 15 min or 30 min incubation ranges between 0.6 and 1.8 and parallel determinations do not deviate from their mean by more than 3 % for the control samples

(EC_{20} and EC_{50}) were calculated based on the decrease in bioluminescence in the samples relative to the controls.

Algae *Pseudokirchneriella subcapitata*

Growth inhibition of unicellular algae was determined with the freshwater species *P. subcapitata* and based on the test procedures specified in the international guideline ISO 8692 (2012). Tests were run in 96-wells plates for 72h. Controls, containing standardized medium and algae only, were performed using twelve wells on each plate, while five replicate wells were used for the samples. In addition each test concentration was also tested in a single well, without the addition of algae, to check for background signals. Algal growth was measured on a daily base. Tests were performed at 23˚C (±1˚C) under continuous light and periodic stirring (plate shaker). The observed growth rates were compared to the growth rates of unexposed algae. Validity of the toxicity test was confirmed by comparing the growth rate (1.5-1.9 d^{-1}) and variation coefficients (2.3-4.1%) in the controls with the criteria mentioned in the ISO 8692 guideline (growth rate >1.4 d^{-1}; variation coefficient <5%). No-observed effect concentrations (NOEC), and EC_{50} values were determined for all toxicity tests using the intrinsic growth rates after 72h. These concentration-response relationships were calculated with the ToxCalc software for Excel (version 5.0; Tidepool Scientific software).

Daphnid *Daphnia magna* and amphipod *Hyalella Azteca*

Acute toxicity to the daphnid *D. magna* and the amphipod *H. azteca* was evaluated based on existing guidelines (ISO 6341, 2018; ASTM E 1706[3], 2019 respectively), with miniaturized procedures to suit the testing of small volumes of PSS extracts. Tests were performed in 24-wells plates using 1 mL exposure solution, 10 organisms in each well and three wells for each test concentration. Elendt M4 medium (OECD 211) was used as control and in preparing the test dilutions. Juvenile organisms (daphnids <24 h old; amphipods: 7 d old) were exposed at 20 and 23 ˚C respectively, and under a light-cycle of 16h/8h (light/dark). Organisms were not fed, and exposures were performed during 48 (daphnids) and 72 (amphipods) hours. Mobility (survival) of the organisms was assessed on a daily basis. Validity of the toxicity tests was confirmed by comparing the mortality in the controls (0%; blanc as well as solvent control) with the criteria mentioned in the ISO 6341 guideline (mortality <10%). NOEC and EC_{50} values were determined using the survival rates at the end of the exposure. These concentration-response relationships were calculated with the ToxCalc software for Excel (version 5.0; Tidepool Scientific software).

Rotifer *Brachionus calyciflorus* (Rotoxkit), shrimp *Thamnocephalus platyurus* (Thamnotoxkit)

Acute toxicity to the rotifer *B. calyciflorus* and the shrimp *T. platyurus* was evaluated based on the guidelines provided by the supplier of the respective toxkits (Microbiotests; Ghent, Belgium), which have also been internationally standardized (ISO 19827, 2016; ISO 14380, 2011). Rotifer tests (Rotoxkit) were performed in special 36-wells plates using 0.3 mL exposure solution, five organisms in each well and six wells for each test concentration. The standardized medium as provided by the supplier of this toxkit was used as control and in preparing the test dilutions. Juvenile organisms (< 24 h old) were not fed and exposed for 24 hours at 25˚C in the dark after which survival (mobility) of the organisms was assessed.

[3] Refers to sediment toxicity testing, but also provides guidance for reference toxicants in a water only exposure

Crustacean tests (Thamnotoxkit) were performed in 24-wells plates using 1 mL exposure solution, 10 organisms in each well and three wells for each test concentration. The standardized medium as provided by the supplier of this toxkit was used as control and in preparing the test dilutions. Juvenile organisms (< 24 h old) were not fed and exposed for 24 hours at 23 ˚C in the dark after which survival (mobility) of the organisms was assessed.

Validity of the toxicity tests was confirmed by comparing the mortality in the controls (0-3%; blanc as well as solvent control) with the criteria mentioned in the ISO guidelines (mortality <10%). NOEC and EC_{50} values were determined using the survival rates at the end of the exposure. These concentration-response relationships were calculated with the ToxCalc software for Excel (version 5.0; Tidepool Scientific software).

Zebrafish *Danio rerio*

The freshwater fish acute toxicity tests were performed using zebrafish (Danio Rerio) in an adapted version of the OECD 236 guideline (2013). Spawning was induced by separating male and female fish overnight and joining them together in a breeding cage with a mesh net the next morning. Eggs from several clutches were mixed and fertilization and quality were assessed under a stereo microscope (M7.5, Leica, Eindhoven, The Netherlands). Within 1 h post fertilization (hpf), fertilized eggs were transferred to Petri dishes filled with Embryo Standard Water (ESW: 100 mgL^{-1} $NaHCO_3$, 20 mgL^{-1} $KHCO_3$, 180 mgL^{-1} $MgSO_4$ and 200 mgL^{-1} $CaCl_2$) at 26˚C. Dilutions were made with ESW before the start of the experiment and stored at 4 C. Zebrafish embryos were exposed from 4 hpf to 5 days post fertilization (dpf). The test was repeated twice. Exposure concentrations were for the first test 0.1%, 0.01% and 0.001% dilutions of the extract. Based on the results or the first test, for the second test inlets were tested with 0.1%, 0.075%, 0.025% and 0.01% and outlets were tested with 0.05%, 0.01%, 0.0075%, 0.0025% and 0.001%. Different DMSO concentrations were tested as well for the control groups. All exposures were performed at 26˚C in 24 well plates, using 2 mL exposure solution and 10 fertilized eggs/embryos per well in triplicates. Corresponding solvent controls and water controls were included. A positive control was not performed (we include this only if our medium or used fish change which was not the case). Apical endpoints such as mortality, hatching and other morphological abnormalities (e.g. swim bladder inflation, curved spinal cord, cardiac edema) were scored on days 5 for the first test and days 1, 2, and 5 for the second test, using a stereo microscope (M7.5, Leica, Eindhoven, The Netherlands). The embryos were not fed, nor the medium refreshed during the tests. NOEC and LOEC were determined for all toxicity tests using the survival and frequency of malformations after 5 days. At the end of the experiments, the fish were submitted to euthanasia by rapid cooling as recommended by Köhler et al. (2017). The test met the method validation criteria. The zebrafish PSS extract exposure showed not repeatable results due to the very high concentrations of HCs in the extracts and therefore cross contamination of wells.

2.3.2. *In vitro* tests

The same *in vitro* assays as in the TIPTOP project (Hamers et al., 2018) were used, and the PAH-CALUX was added. In vitro assays were performed by VU, the PAH-CALUX that was performed by BDS (Amsterdam, The Netherlands). Exposure concentrations were by a 1000-fold dilution and 5 further dilutions were made ranging from 1 to 10000 in 10-fold steps as range finding. In additional tests smaller steps (factor of 3) were tested to quantified the exact concentration. Further a procedural blank and a reference compound were tested, and quantification of the

response of the samples was performed using a calibration line of the reference compounds. Dose-response curves were fitted with Graphpad. The dilution factors are used to get the response of the in vitro assay in the correct part of the calibration curve. The assays are briefly described below, and full details can be found in the references given.

Agonistic potencies towards the activation of the arylhydrocarbon receptor (AhR), the estrogen receptor (ER) and the androgen receptor (AR) were tested in the H4L1.1c4 (DR-LUC; Garrison et al. 1996), the BG1Luc4E2 (ER-LUC; Rogers and Denison, 2000), and AR-EcoScreen (Araki et al. 2005) reporter gene assays, respectively. The DR-Luc measures the dioxin-like or PAH-like activity through the arylhydrocarbon receptor (AhR) activation. These assays were performed in 96-well plates, and all three bioassays contain a stably transfected luciferase construct that is responsive towards the activated AhR, ER, or AR, respectively, and produce light when compounds bind to these specific receptors. The amount of light is a direct measure of the amount of the compounds. For the DR-LUC the reference compound is 2,3,7,8-tetrachlorodibenzo-[p]-dioxin (TCDD), for the ER-LUC 17ß-estradiol (E2), and for the AR-Ecoscreen dihydrotestosterone (DHT) for agonist activity and flutamide for anti-androgenic activity. After exposure to the PSS extract and the luciferase production is measured by lysis of the cells followed by addition of the substrate luciferine. The produced light is a direct measure for the presence of receptor-activating compounds in the exposure medium. The light activity can be interpolated in a calibration curve of the reference compounds. The bioassay responses can be expressed as equivalent concentrations of the reference compounds (i.e. similarly toxic as the interpolated concentration of the reference compound).

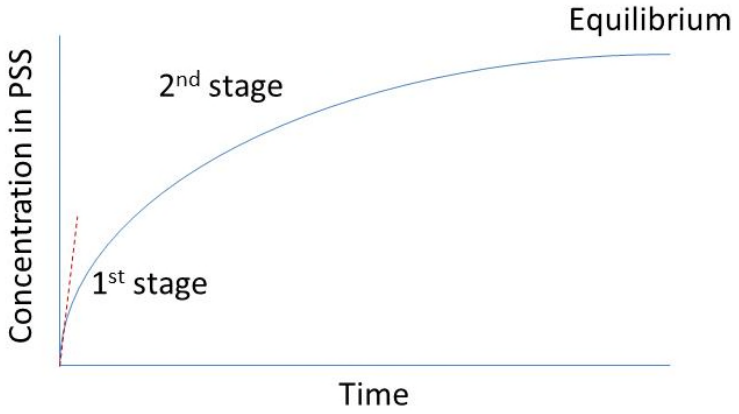
Mutagenic potency was determined with the Ames II fluctuation assay (Reifferscheid et al. 2012). The assay makes use of a *Salmonella typhimurium* TA98strain containing a mutation in its histidine gene, making the bacteria no longer autotrophic but histidine dependent. More details of the bioassays can be found in Hamers et al. 2018. The bacteria were exposed to test extracts in the presence of an Arochlor-1254 induced, exogenous, metabolic rat liver S9-system. A single concentration of the reference compound 2-aminoanthracene was taken along as positive control.

The PAH-CALUX was performed according to Pieterse et al., 2013. This assay uses benzo[a]pyrene as reference compound. The PAH-CALUX is a bioassay optimized for the detection of PAHs and contains a quadruplicate repeat of the DRE gene.

2.4. ESTIMATIONS OF FREE DISSOLVED WATER CONCENTRATIONS FROM PSS EXTRACTS

The uptake of HCs by the silicon sheet PSS follows three main phases assuming that the water concentration is constant and continuous during the exposure period (**Figure 4**). In the field situation constant exposure is not expected but this diagram shows the basic principles of passive sampling. In the first linear uptake phase HCs are only taken up by the samplers. In the second phase uptake and depuration of HCs occurs, and in the last phase the uptake and depuration of HCs is in equilibrium between the water and PSS phase.

Figure 4: Uptake of hydrocarbons (HCs) by the silicone sheet PSS.



As said, the silicone sheet PSS measure the freely dissolved concentrations (C_w), and these can be predicted using equation [1] based on Booij et al. (2007) and Huckins et al. (2006).

$$C_w = \frac{N_{t,SR}}{m_P K_{PW}\left\{1-\exp\left(-\frac{R_{SR}\ t}{m_P K_{PW}}\right)\right\}} \quad [1]$$

C_W = Predicted freely dissolved concentration of a HC block in inlet or outlet water of refinery
$N_{t,SR}$ = amount of each HC block in the sampler after exposure (µg)
M_P = mass of the silicone sheet (g)
K_{PW} = silicone water partitioning coefficient
R_{SR} = (equivalent) water sampling rate (l/d) and based on a mean MW of 250
t = deployment time (d)

R_{SR} is dependent on the water flow rates at the sample location. To correct for these differences, performance reference compounds (PRCs) were added to the silicone sheets. The PRCs migrate out of the silicone sheets during sampling and based on this depuration the water sampling rate can be predicted. The R_{SR} was fitted using the PRC fraction (f_{prc}) of each PRC that is retained in the sampler as a function of $\log(K_{pw}M^{0.47})$ according to Booij and Smedes (2010; Rusina et al. 2010), see equation [2].

$$f_{PRC} = \exp\left(-\frac{\frac{B}{M^{0.47}}\ t}{m_P K_{PW}}\right) \quad [2]$$

K_{pw}: sampler water partition coefficient of each PRC, M: molar mass, 200 was taken as an average molar mass. B: constant. The f_{PRC} values of the retaining PRCs are fitted using an unweighted non-linear least-squares estimation using equation [2]. This gives the B for R_S estimation. An example of a fitting is given in Appendix E.

R_{SR} can be found in Appendix E, and the precision of the R_{SR} varied between 18% and 32%.

Silicone water partitioning coefficient (K_{pw}) of each HC block *i* were predicted based on equation [3] which is a PAH model made by Smedes et al. (2009). K_{ow} values from the Concawe library (Concawe, 2020) were used.

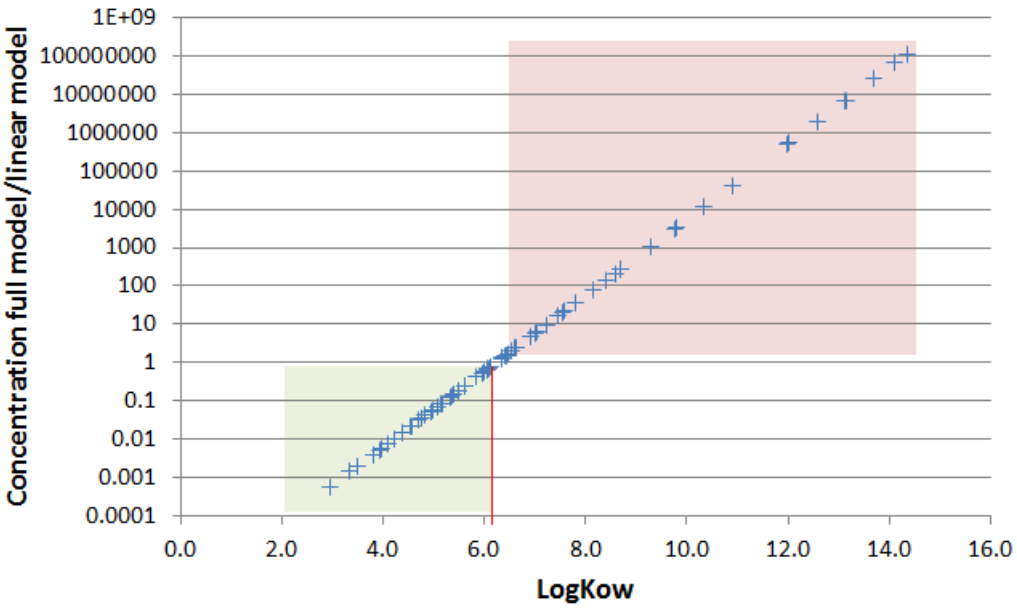
$$\text{Log}K_{PW,i} = 0.99\text{log}K_{OW}\text{ ,}i\text{- }0.42 \qquad [3]$$

In addition to the full uptake model C_W was also calculated with a linear uptake model (equation [4]) using the amount of HC block *i* found by GCxGC ($N_{t,SR,i}$) divided by the equivalent water sampling rate R_{SR}.

$$C_{w,i} = \frac{N_{t,SR}}{R_{SR}} \qquad [4]$$

A comparison between the two model outcomes shows that at the end of the exposure HCs with $\log K_{ow}<6$ are in equilibrium (**Figure 5**, green square) between the water and silicone sheet phase, and HCs with $\log K_{OW}>6$ are not in equilibrium (**Figure 4**, red square). The compounds in the red square of **Figure 3** have a lower concentration based on the linear model than the full concentration model. Therefore, C_W was predicted with the full model for HCs with $\log K_{OW}<6$, and for HCs $\log K_{OW}>6$ the linear uptake model was used.

Figure 5: Example of C_W concentration predictions based on the full uptake model (equation [1]) or a linear uptake model (equation [2] in relationship with $\log K_{OW}$). Red square illustrates compounds not in equilibrium, and green square compounds in equilibrium.



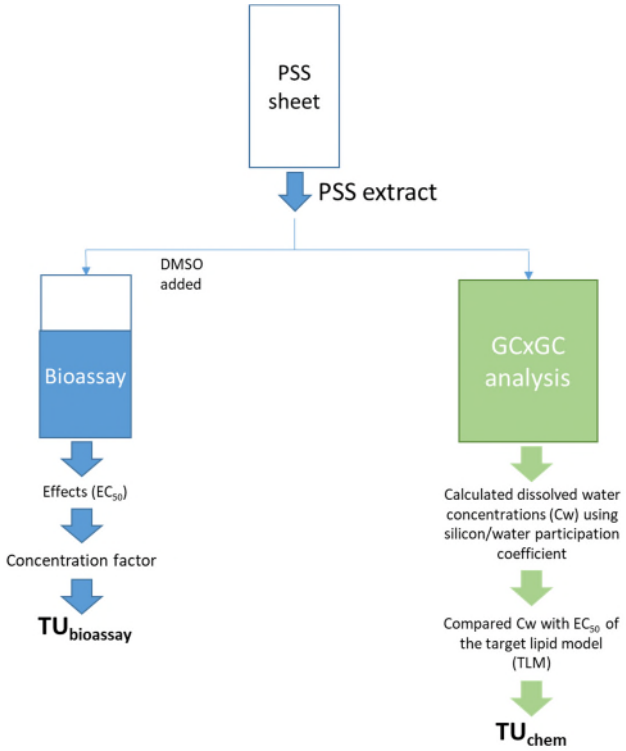
2.5. TOXIC UNITS (TU)

In this report two TU calculations are performed:

1) **$TU_{bioassay}$**: Effects (EC_{50}) found with the *in vivo* tests that were exposed to the PSS extracts. TUs were based on the effects in each PSS exposure series (dilutions of the PSS extract) and converting the effects to the original inlet and outlet waters using the water concentration factor from Appendix L, and for explanation of concentration factor see below.
2) **TU_{chem}**: Predicted acute toxicity units for *D. magna, V. fischeri, H. azteca, P. subcapitata,* and *D. rerio*[4] were based on the GCxGC HC blocks data measurements in the PSS extracts. Dissolved water concentrations (C_W) of the GCxGC data were predicted, see below, and compared to EC_{50} of the target lipid model (TLM). The TU is recalculated to the original water.

The different TUs are further described below and graphically displayed in **Figure 6**.

Figure 6: Illustration of the two different TU calculations.



$TU_{bioassay}$

TUs for the *in vivo* assay results (*$TU_{bioassay}$*) were calculated based on the measured EC_{50} values of the *in vivo* tests of the PSS extracts in DMSO (EC_{50} vol% DMSO extract) and the equivalent volume of sampled water of the DMSO extract ($V_{water\_DMSO\ extract}$) and the volume of the DMSO extract (V_{DMSO}) added to the test, see equations [5] and [6]. The ratio of the two volumes is called the concentration factor (CF, equation [6]). -

[4] Calculations could not be performed for the other organisms used as no CTLBB were available

$$TU_{bioassay} = \frac{CF * 100/EC50\ vol\%\ DMSO\ extract}{100\ or\ 45} \qquad [5]$$

** 100 for D. magna, H. Azteca, P. subcapitata, T. platyurus, and 45 for V. fischeri*

$$CF = \frac{V_{water_{DMSO\ extract}}}{V_{DMSO}} \qquad [6]$$

TU_{chem}

TU on GCxGC data were calculated based on predicted water concentrations $C_{W,i}$ (mM) and predicted $LC_{50,i}$ values for each HC block i (equation [7]). The sum of all $TU_{chem,i}$ of each block was the total TUchemc, and all $TU_{chem,i}$ were summed to get the total TU_{chem}. The target lipid model LC_{50} (TLM $LC_{50,i}$) for each of the species studied and each HC block i was predicted (equation [8]) based on the work of Di Toro et al. (2000), which is also used in the PetroTox model (Redman et al., 2012).

$$TU_{chem,i} = \frac{Cw,i}{TLM\ LC50,i} \quad \text{and } TU_{chem} = \sum_i TU_{chem,i} \qquad [7]$$

$$\log LC_{50,i} = -0.936 \log K_{OW,i} + \Delta c + \log C_l^* \qquad [8]$$

A cutoff of K_{OW} >6 was used for HCs that have a $\log K_{OW}$ >6 resulting in, $\log LC_{50,i}$ = -0.936 * 6 + Δc + $\log C_l^*$

Δc: chemical class correction
Cl_1^*: species specific critical target lipid body burden (CTLBB) for narcosis (µmol/ g octanol) from McGrath and Di Toro (2009).

TUs for acute toxicity were predicted for *D. magna*, *V. fischeri*, *H. Azteca*, and *P. subcapitata* as CTLBB were available for these species. The sum of $TU_{pred.,i}$ for all HC blocks provides information on the expected toxicity of the inlet and outlet waters. For *Thamnocephalus platyurus* (Thamnotoxkit), and the rotifer *Brachionus calyciflorus* (Rotoxkit) no CTLBB values are given in McGrath and Di Toro (2009) and therefore no TU could be predicted for these two species.

2.6. BIOANALYTICAL EQUIVALENT CONCENTRATIONS (BEQ) OF PAH ACTIVITY EXPRESSED USING DR-LUC AND PAH-CALUX ASSAYS

In vitro assays were used to obtain information on mechanistic toxicity. To obtain information on the toxicity of PAHs in the PSS extracts, the DR-Luc and PAH-Calux assays were performed, both related to the Aryl Hydrocarbon Receptor (AhR) activity. The bioassay activities can be compared to the chemical concentrations of PAHs measured in the PSS extracts. Therefore, relative potencies (REP) values are used, and refer to the activity of a compound that is tested (e.g. a specific PAH) compared to the activity of a reference compound. In case of the DR-Luc 2,3,7,8-TCDD is used as the reference compound, and for the PAH-CALUX benzo[a]pyrene is used as the reference compound. For example, a REP of 0.1 for the DR-LUC means that the activity of a specific compound is 10 times less than TCDD. To calculate the total bioanalytical equivalent concentration (BEQ) of a sample the concentrations of each PAH is multiplied by it specific REP value, see Appendix Q for all REP values used, and all these are summed.

For example, the REP of the AhR activity of the DR-Luc or PAH-CALUX for each PAH was multiplied by the predicted C_W concentration of each PAH resulting in a BEQ for AhR-activity (equation [9]). The BEQ was calculated as given in equation [9].

$$BEQ = \sum C_{W,PAHi}\ REP_{PAHi} \qquad [9]$$

The units are TCDD equivalent (pg TCDD EQ/L) or B[a]P equivalent (ng B[a]P eq./L) concentrations for respectively the DR-LUC or PAH-CALUX. For the DR-LUC the $EC_{50,}$ 6 hours REP values of PAHs from Machala et al. (2001) were used, and the REP values of Pieterse et al. (2013) for the PAH-Calux.

3. RESULTS AND DISCUSSION OF THE CHEMICAL ANALYSES, IN VIVO AND IN VITRO ASSAYS

3.1. SPOT SAMPLES

3.1.1. Chemical analyses in spot samples

The mean concentrations of the spot samples collected of various chemicals and sum parameters (e.g. TPH) from inlet and outlet zones from refinery A, B, and C can be found in **Table 4** and all data of the individual weeks in Appendix B-D.

Metal concentrations in inlet samples were in general below respective limits of quantification (LOQ). In the outlet of all refineries nickel, selenium, vanadium, and zinc were found. TPH concentrations in the outlet varied between 0.23 to 2 mg/L, and the phenol index between 6.8 and 75 µg/l. The HC composition in the outlet water differs between the refineries; in refinery A the C_{10}-C_{19} HCs are dominant while the C_{10}-C_{29} HCs were dominant in refinery B and C (**Figure 7**). The concentrations of various metals (e.g. arsenic, cobalt, nickel, selenium, vanadium), TPH, total N, COD, DOC, TOC, and phenol index were significantly higher in the outlet water than the inlet waters for all sampled refineries (T-test, data of individual weeks in Appendix B-D). This shows that the chemical contribution from the inlet water for all refineries is limited compared to the outlet water. The concentrations in the outlets were in similar ranges as found in earlier studies were 105 European refineries were studied (Concawe, 2010).

Table 4: Mean concentrations of metals, general parameters, TPH, and phenol index in the spot samples of inlet and outlet waters of refinery A, B and C. *: mean concentration significant higher in outlet than inlet water (T-test, p<0.05).

	Refinery A		Refinery B		Refinery C	
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
Arsenic (µg/l)	4.5	14*	<4	<4	<4	<4
Cadmium (µg/l)	<1	<1	<1	<1	<1	<1
Chromium (µg/l)	<5	<5	<5	<5	<5	<5
Cobalt (µg/l)	<2	3.6*	<2	<2[1]	<2	<2
Copper (µg/l)	27[4]	9.2[4]	<5	<5	<5	<5
Mercury (µg/l)	<0.05	<0.05[3]	<0.02	<0.02	<0.02	<0.02
Lead (µg/l)	<5	<5	<5	<5	<5	<5
Nickel (µg/l)	<5	33*	<5	15*	<5	6.5*
Selenium (µg/l)	<3	18*	<3	15*	<3	32*
Vanadium (µg/l)	8.4	13*	<5	13*	<5	<5
Zinc (µg/l)	<20	25*	<20	49*	<20	<20
Total N (mg N/l)	1.7	3.5*	<1	2*	<1	14*
DOC (mg C/l)	<5	46*	<5	17*	6.6	26*
TOC (mg C/l)	<5	64*	5.0[4]	23*	8.4	30*
TPH (mg/l)	<0.05	0.23*	<0.05	2*	<0.05	0.53*
Phenol index (µg/l)	<5	75*	6.8[4]	11*	<5	10*
COD (mg/l)	<10[2]	174	<10	40*	12	57*

[1]: one out of five samples 3.2 µg/l; [2]: one out of five samples 13 mg/l; [3]: out of five samples 0.08 µg/l; [4]: two out of 5 samples >LOQ.

A representative example from refinery A is provided in **Figure 8**. In refinery A the concentrations of cadmium, chromium, lead and mercury were all below respective LOQ in both the inlet and the outlet zone (except one spot sample for mercury which was just above the LOQ), see appendix B-D, and thus not shown in **Figure 8**. Concentrations of cobalt, nickel, selenium and zinc were below the LOQ in the inlet water. However, some metals (arsenic, cobalt, nickel, selenium, and vanadium) were in all weeks significantly higher in the outlet than the inlet waters of refinery A. The TPH concentration in the outlet (average 0.23 mg/l) was higher than the inlet (<0.05 mg/l). Total N, DOC, COD, TOC, phenol index were all significantly higher in the outlet than the inlet waters that enters the whole refinery.

Figure 7: TPH fractions per carbon chain group in spot samples of the outlet samples.

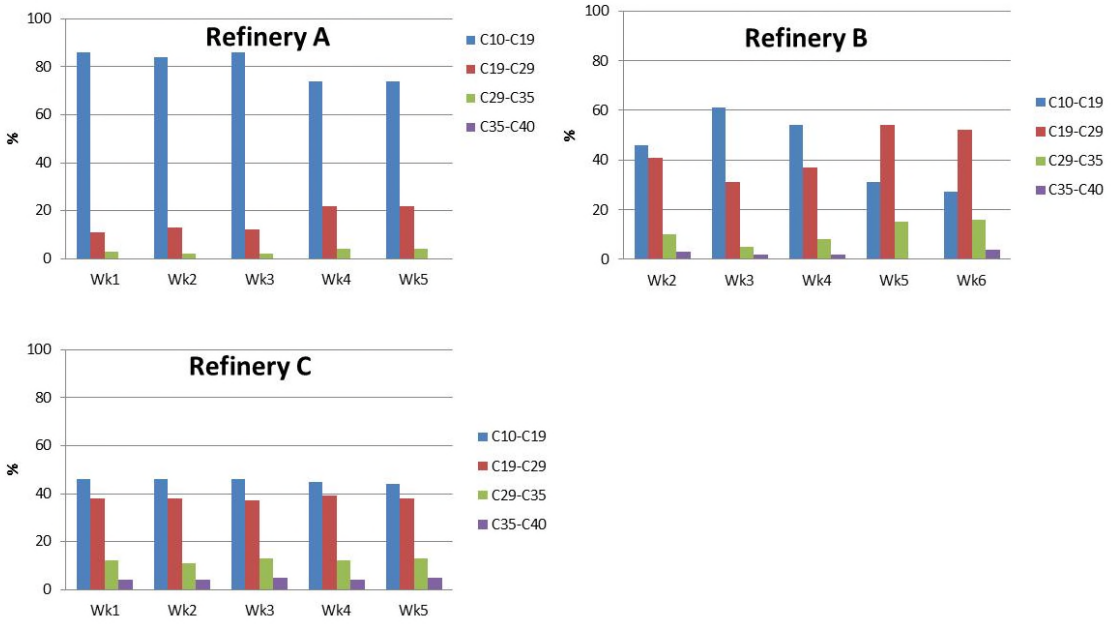
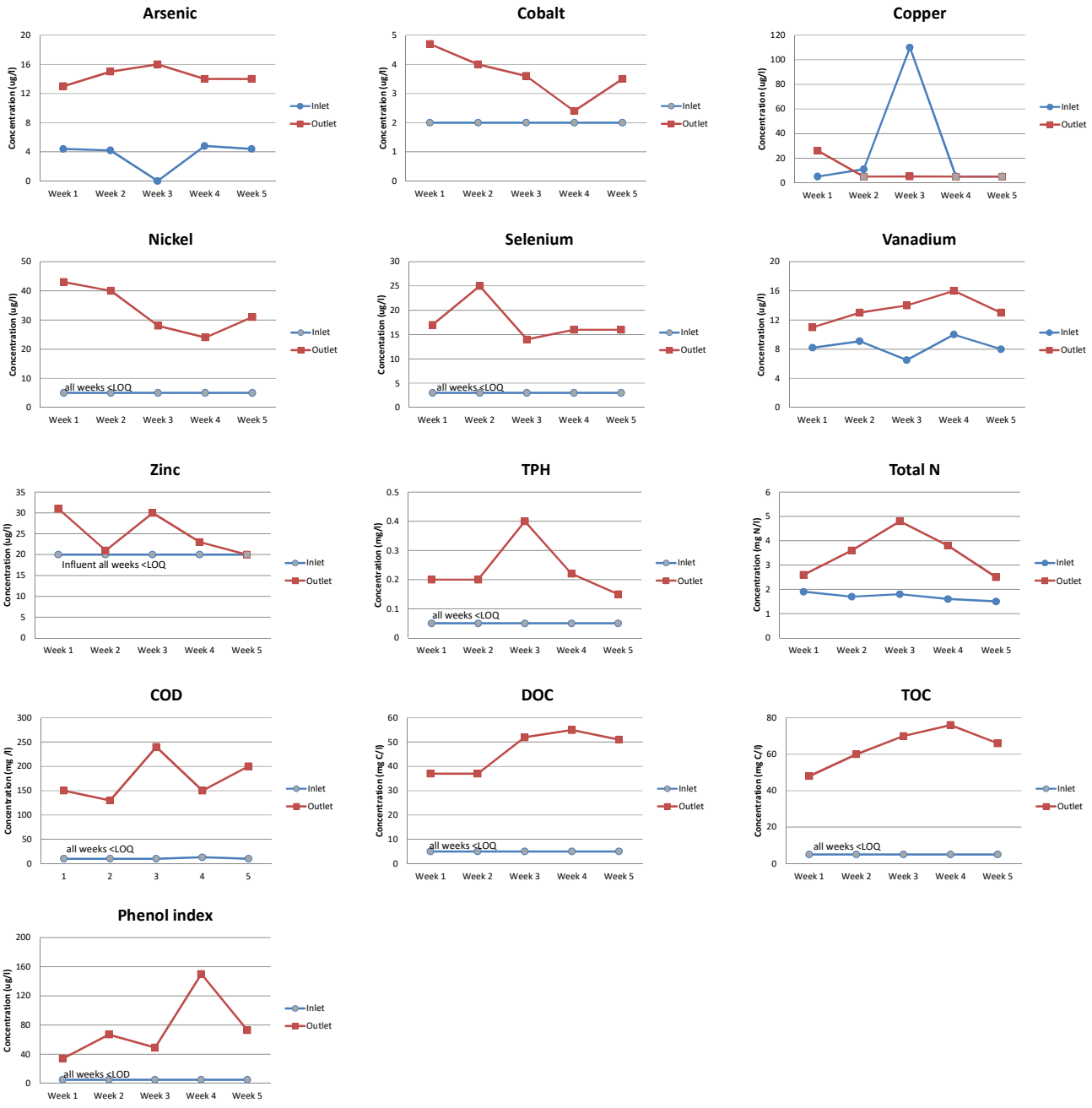


Figure 8: Concentrations of metals, TPH, total N, DOC, COD, TOC, and phenol index in spot samples of refinery A taken in the inlet (blue circles) and outlet (red squares) zones. Symbols in grey are below the LOQ.



3.1.2. *V. fischeri* (Microtox) in spot samples

Before the start of the toxicity tests, pH, ammonia concentration and conductivity were measured to check for possible confounding factors. These were all within range (Postma *et al.*, 2002) and did not cause any toxic effects. Furthermore, concentrations of several metals and BE-SPME were analysed in all spot samples and might be used to explain observed toxicity.

For each refinery, toxicity to the bacteria *Vibrio fischeri* (Microtox) was detected in at least one of the five spot samples (**Table 5**), although both frequency and strength of the toxic effects differed between the refineries. EC_{50} values below 20 volume% (percentage of effluent in final exposure media) in 4 out of 5 samples were detected in the outlet of refinery A thus showing toxic effects. EC_{50} values could not be calculated for any of the spot samples for refinery B, thus showing no toxicity except for a slight effect in one sample based on EC_{20} values. Toxicity in the outlet spot samples of refinery C was intermediate between refinery A and B as EC_{50} values could be calculated for two out of the five spot samples. It is important to note that the outlet waters are diluted in the receiving waters and a dilution factor of at least 10 is often used to correct for these dilutions.

Table 5: *V. fischeri* (Microtox) results in outlet spot samples. EC_{20} and EC_{50}-values (volume % of effluent in final exposure media) are shown together with their 95% confidence limits. TU-values are based on EC_{50} values ($TU=100/EC_{50}$).

Refinery	Weeknr	EC_{20} (vol%)	95% conf. int	EC_{50} (vol%)	95% conf. int	TU-value
A	1	8,1	7,3-8,9	18,2	16,8-19,6	5,5
	2	< 5,6	-	13,6	12,8-14,5	7,4
	3	< 6,3	-	< 6,3	-	> 15,9
	4	< 6,3	-	< 6,3	-	> 15,9
	5	< 6,3	-	27,6	26,0-29,4	3,6
B	1	> 45	-	> 45	-	<2,2
	2	11,4	10,3 - 12,6	> 45	-	<2,2
	3	> 45	-	> 45	-	<2,2
	4	> 45	-	> 45	-	<2,2
	5	> 45	-	> 45	-	<2,2
C	1	< 5,6	-	17,3	15,2-19,6	5,8
	2	7,3	5,9-9,0	32,0	26,3-39,0	3,1
	3	15,9	14,0-18,0	> 45	-	<2,2
	4	16,6	13,7-20,1	> 45	-	<2,2
	5	37,4	29,0-48,2	> 45	-	<2,2

The presence of HCs forms an essential element in comparing and understanding the toxicity to *V. fischeri* (Microtox) in spot samples and PSS extracts. This is discussed for *V. fischeri* (Microtox) into detail in §3.4. However, toxicity in spot samples can also be caused by other contaminants, e.g. metals. For instance, the highest TPH concentration was found in the outlet of refinery B and the lowest in refinery A and C, while the *V. fischeri* toxicity was the highest in the outlet of refinery A, and the lowest in B. On the other hand, the concentrations of COD and phenol were the highest in the outlet of refinery A.

To determine whether metals contributed the *V. fischeri* toxicity, metal concentrations in the different spot samples are presented in Section 4.1.1 and appendix B-D. Maximum concentrations are summarised in **Table 6** and compared with lowest EC_{50}-values found in literature. From this, it can be concluded that metal concentrations were in general much lower (factor 30-2460; for zinc factor 4) than the EC_{50}-values found in literature. Metal toxicity is therefore unlikely to occur. Refinery A is an exception as the copper concentration in the first outlet spot sample (week 1) was 26 µg/l, more or less comparable to the lowest EC_{50}-value

found in literature[5]. At the same time this increased copper concentration was only found in the first week, while toxic effects to *V. fischeri* were also observed in the other four spot samples (Copper range: <5 – 5.1 µg/l). It is therefore concluded that toxic effects by increased copper concentrations might have had some effect in the *V. fischeri* (Microtox)-assay for refinery A, but it is unlikely that copper was the only or main causative factor.

Table 6: Maximum metal concentrations (µg/l) in the five spot samples for each refinery (inlet and outlet). Concentrations below the detection limit are denoted in italic using a value of 0,5*det limit. Concentrations can be compared with lowest EC_{50}-values found in a short literature search.

	Refinery A		Refinery B		Refinery C			Literature	Ref.
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet		Lowest EC_{50} (µg/l)	
Arsenic	4.8	16	*2*	4	*2*	*2*	<<	33.400	1)
Cadmium	*0.5*	*0.5*	*0.5*	*0.5*	*0.5*	*0.5*	<<	1.230	2)
Chromium	5.2	*2.5*	*2.5*	*2.5*	*2.5*	*2.5*	<<	6.700	3)
Cobalt	*1.0*	4.7	*1.0*	3.2	*1.0*	*1.0*		?	
Copper	110	26	*2.5*	*2.5*	*2.5*	*2.5*		30	2)
Mercury	*0.03*	0.08	*0.03*	*0.01*	*0.01*	*0.01*	<<	29	3)
Lead	*2.5*	*2.5*	*2.5*	*2.5*	*2.5*	*2.5*	<<	80	1)
Nickel	*2.5*	43	2.5	41	5.0	7.1	<<	9.520	2)
Selenium	*1.5*	25	*1.5*	20	3.0	39		?	
Vanadium	10	16	*2.5*	21	*2.5*	*2.5*		?	
Zinc	*10*	31	*10*	76	*10*	*10*	<	270	2)

[1] Kaiser & DeVillers (1994); [2] Terratox (http://www.terrabase-inc.com); [3] Elnabarawy et al (1988)

3.1.3. BE-SPME in spot samples

BE-SPME can be used as possible proxy for narcotic toxicity of HC mixtures (Parkerton et al. 2000). The SPME fiber simulates the bioconcentration process, provides information on the bioavailable fraction of the HCs and can be used to assess narcosis-type or baseline toxicity. The relationships between BE-SPME and toxicity tests were therefore studied as well.

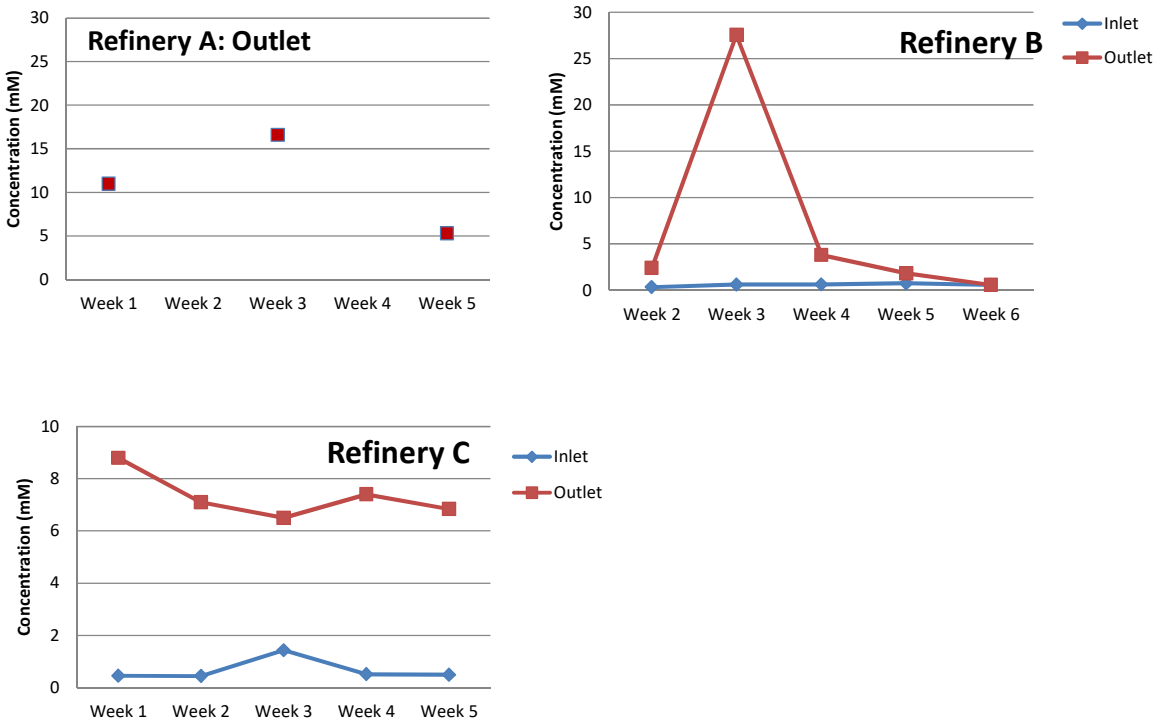
The BE-SPME was determined in all spot samples (**Figure 9** and Appendix B-D). The determination of BE-SPME concentrations of both inlet and outlet water of refinery A was compromised, since the glue that attached the SPME fiber to the holder dissolved during exposure and therefore the SPME fiber could not be analysed. This was found for all triplicate analysis as well as in some repeated experiments, which suggests that a chemical is present in the water that dissolves the glue (SigmaAldrich, 2020). Therefore, no BE-SPME data was available for the inlet and only three time points for the outlet waters of refinery A (**Figure 7**). BE-SPME concentrations varied between 5.3 and 17 mM in refinery A outlet water.

For refinery B and C the BE-SPME concentrations were at all time intervals significantly lower in the inlet than in the outlet waters; median concentrations were 4 to 14 times lower in inlet than outlet waters.

[5] Geometric mean of literature data is EC_{50} = 352 µg/l

Median concentrations in the outlet waters of refinery A was 11 mM, for refinery B 7.2 mM, and for refinery C 7.3 mM. A BE-SPME peak was found in week 3 (27.6 mM) for the outlet of refinery B. BE-SPME can be compared to CTLBB values (µmol/g octanol), with the assumption that octanol is a good surrogate for lipid in organism (McGrarth and Di Torro, 2009). This makes is possible to directly compare the BE-SPME with the CTLBB considering that critical body burdens are equivalent to BE-SPME concentrations (Redman et al., 2018). The CTLBB values for algal (49 µmol/g octanol, McGrarth and Di Torro, 2009), *Vibrio fischeri* (252 µmol/g octanol, Redman et al., 2007), and *Daphnia magna* (115 µmol/g octanol, McGrarth and Di Torro, 2009) are in a comparable range to the BE-SPME concentrations and chronic effects could therefore be expected to occur in undiluted outlet samples.

Figure 9: BE-SPME concentrations (mM) in spot samples of Refinery A, B, and C (see also Appendix B-D). For Refinery A only a limited number of samples could be measured due to the fact that the fibers for both the inlet and outlet waters were detached from the SPME fiber holders. Y axis: BE-SPME concentration; X axis: weekly sampled spot samples.



The BE-SPME concentrations in spot samples (**Figure 10**) can be compared to the results of a previous Concawe study on effluents and XAD-extracted[6] effluents (Whale et al., 2022). **Figure 10** shows the BE-SPME concentrations versus the *V. fischeri* inhibition of all spot samples and for dilutions of the original inlet and outlet waters (45, 22,5, 11.25 and 5.6 vol%). The BE-SPME concentrations are corrected for the dilutions. Based on the data from Whale et al. (2022) and Redman et al. (2018a,b) it can be concluded that moderate effects in the *V. fischeri* assay ($20<EC_{50}<45$ vol% effluent) often occur at BE-SPME concentrations about 10 mM, while strong toxic effects ($EC_{50}\leq 20$ vol%) were observed at BE-SPME concentrations above 100 mM (Redman et al., 2018a,b, Whale et al., 2022). In Redman et al., 2018b

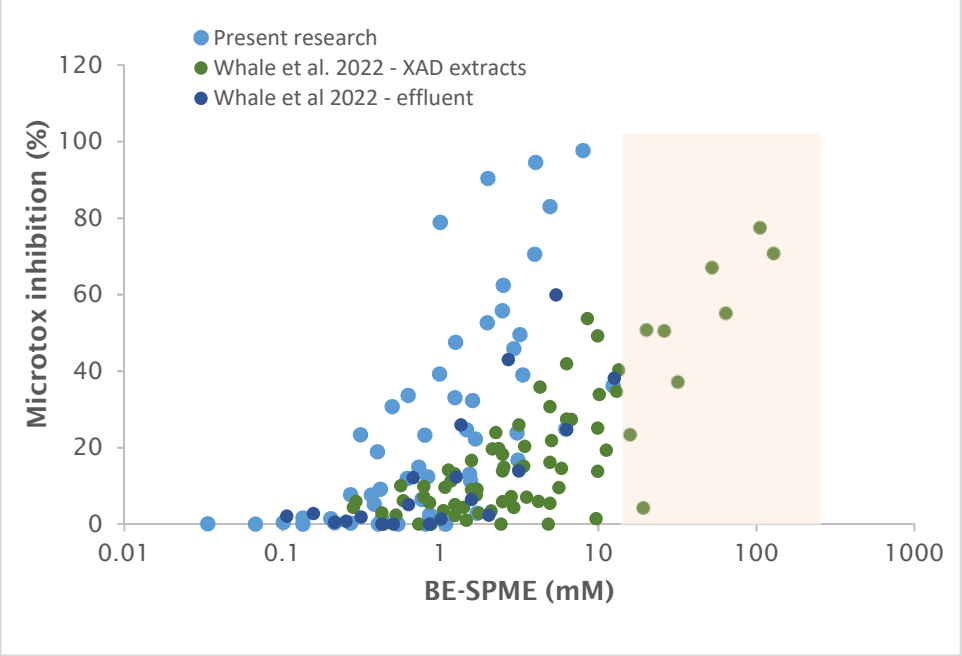
[6] XAD is a resin used to concentrate organic compounds from water (e.g. Daignault et al; Wat Res. 22/7: 803-813; 1988)

an acute critical BE-SPME concentrations is defined based on a species sensitivity distribution ranging from 13.6 to 240 mM with a median value of 37 mM. The present data are roughly in accordance with this expectation. For example, BE-SPME concentrations between 7 and 28 mM were observed in six different spot samples and in all of them toxic effects in the *V. fischeri* (Microtox) assay were observed. In the four spot samples of refinery B, in which no toxic effect to *V. fischeri* was observed, BE-SPME concentrations were all below 4 mM.

At the same time, in the present study, toxic effects seem to be slightly stronger compared to the XAD-extracts studied by Whale et al. (2022). Especially for samples containing around 10 mM BE-SPME, TUs in the present study are in general higher compared to Whale et al. (2022; blue symbols in **Figure 10** are higher than the green symbols). The present samples and the dark blue symbol samples are both effluents, and the green samples are XAD extracts of effluents.

It is therefore concluded that for the present spot samples non-specific toxicity of HCs (which is closely correlated to BE-SPME measurements; Letinski et al., 2014), is a likely contributor to the observed toxic effect in the *V. fischeri* (Microtox) assay, while toxic effects of other compounds is also expected.

Figure 10: BE-SPME vs *V. fischeri* (Microtox inhibition %) in the current study and the results of other studies (Concawe, 2010, Whale 2022). Data of the current study includes *V. fischeri* inhibition data of four dilutions (45, 22,5, 11.25 and 5.6 vol%). Shaded area is the acute critical BE-SPME concentration range (13.6 to 240 mM) based on species sensitivity distribution calculations (Redman et al., 2018b).



3.2. PASSIVE SAMPLERS (PSS)

3.2.1. Chemical analysis in PSS

Concentrations of HCs in the PSS ranged between 4 and 24 µg/g silicone in the inlet waters, and between 172 and 758 µg/g silicone in the outlet waters (**Table 7**). The PAH concentrations in the samplers are much lower and varied between 0.003 to 0.66 µg/g silicone in the inlet waters, and between 0.07 and 21.7 µg/g silicone in the outlet waters. For all refineries, HC and PAH concentrations in the outlet water were higher than the inlet water. The HC PSS concentrations are a factor of 7, 190, 47 higher in outlet than the inlet waters for refinery A, B, and C, respectively, showing that inlet had limit contribution of HCs to the outlet. The PAH concentration of PAHs in the outlet are 2, 434, and 23 times higher than the inlet for refineries A, B, and C, respectively, and show to be different from the factors of HCs. The PSS HC results follow a similar trend between refineries as the TPH concentrations found in the spot samples (**Table 7**).

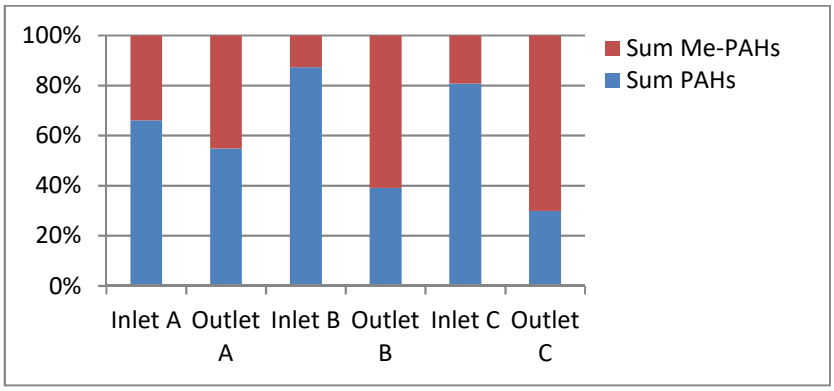
Table 7: HCs and PAH concentrations in PSS (on µg/g silicone sheet), and as dissolved water concentration (C_W) in the inlet and outlet waters. For reference, mean and median (in brackets) TPH concentrations in spot samples are shown as well.

	TPH spot	HC PSS	HCs C_W	PAH PSS	PAH C_W
	µg/L	µg/g silicone	µg/L	µg/g silicone	ng/L
Inlet A	<50	24	1.4	0.66	24
Outlet A	234 (200)	172	5.6	1.00	181
Inlet B	<50	4	0.6	0.05	11
Outlet B	2078 (580)	758	26.0	21.7	2440
Inlet C	50 (50)	10	0.7	0.003	0.5
Outlet C	526 (530)	472	4.2	0.07	0.3

By using the water sampling rates (R_{SR}) and equations [1], [3], [4] the dissolved water concentrations were calculated. A summary of HCs and PAHs concentrations found in the PSS and calculated dissolved concentrations (C_W) is given in **Table 7**, and details of each block are given in Appendix N. This section only discuss the HC and PAH concentrations, and the relationships with toxicity is discussed in §3.2.3 and §3.3. As expected, the dissolved water concentrations (Cw) are much lower than the TPH concentrations of the spot samples which was determined by extracting both HCs bound to suspended matter and the dissolved fraction in the water. Moreover, apolar HCs are mainly bound to suspended matter. Calculated dissolved HC water concentrations (C_W) in the outlet are higher than in the inlet (factors of 4, 43, 6 for refinery A, B, and C, respectively). HC concentration in the outlet water of refinery B was the highest. This patterns is in agreement with TPH concentrations pattern found in the spot samples (higher concentrations for outlet and highest concentration in outlet of refinery B).

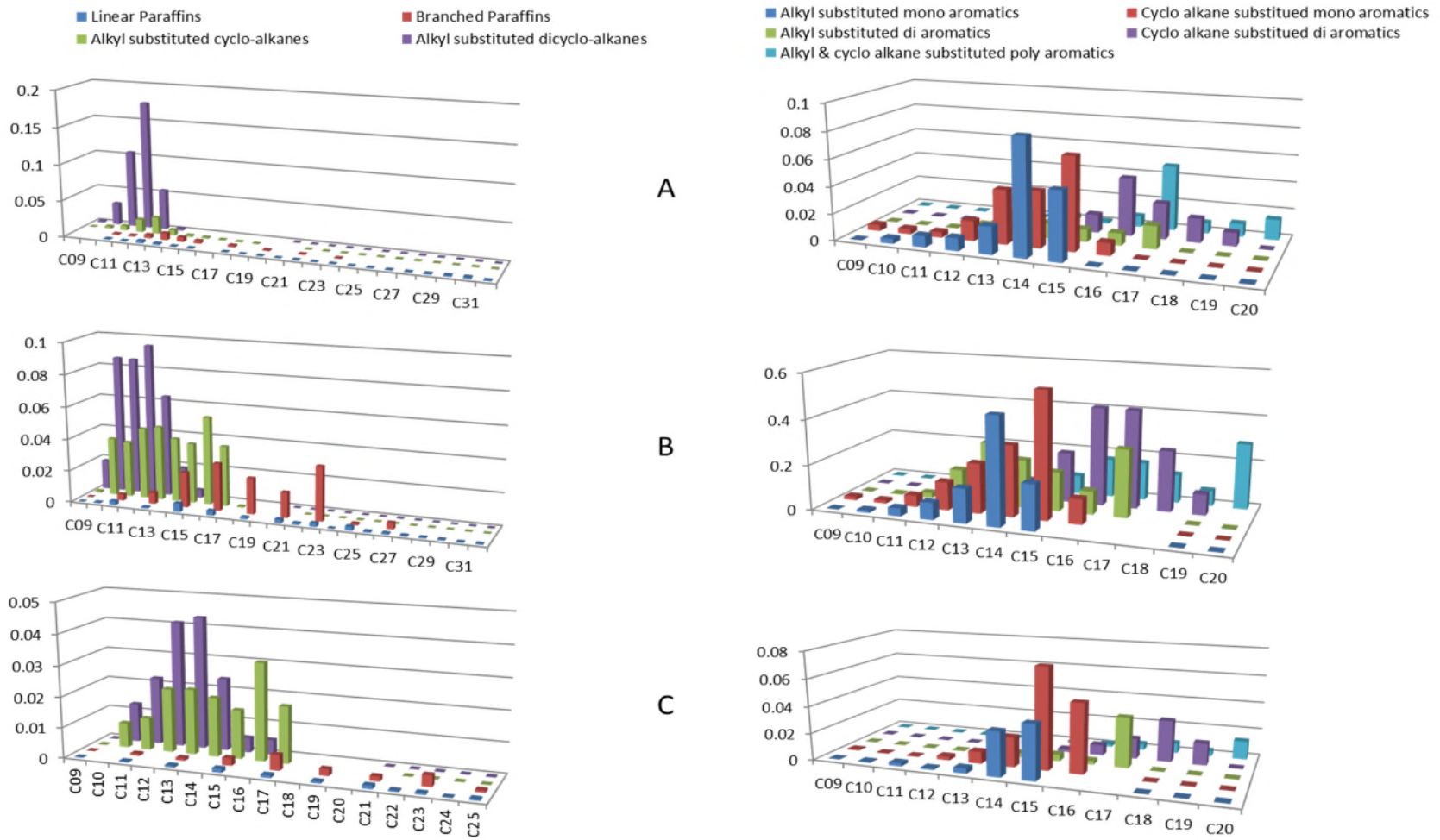
The predicted dissolved concentrations of PAHs in the outlet waters vary between 0.3 to 2440 ng/L. Relative higher amounts of methylated-PAHs (Me-PAHs) are found in outlet waters compared to inlet (**Figure 11**). The higher ratio of Me-PAH was expected as refinery products contain elevated levels of Me-PAHs.

Figure 11: Percentage of the sum of parent PAHs (PAH) and sum of methylated-PAHs (me-PAHs) in PSS extracts of the inlet and outlet waters.



Detailed information on the HC speciation (GCxGC) in the PSS can be found in Appendix F-K. As an example the HC speciation for all outlet samples are given in **Figure 12**. C_{11}-C_{17} alkyl substituted cyclo and di-cyclo alkanes dominant the patterns for the aliphatics, and the aromatics are dominated by C_{12}-C_{17} carbons in the PSS extracts. In all outlets the sum concentration of aromatics (55-87%) are higher than the sum aliphatic concentrations (13-45%). Also in the inlets the aromatics are higher (59-100%) than the aliphatics (0-41%).

Figure 12: HC speciation (GCxGC) in PSS extracts of the outlet waters of the refineries A, B, and C. Left aliphatics and right aromatics. On the x-axis the concentration in ug/l outlet water, and the y-axis shows the carbon number.



3.2.2. Toxicity in the PSS extracts

In vivo tests

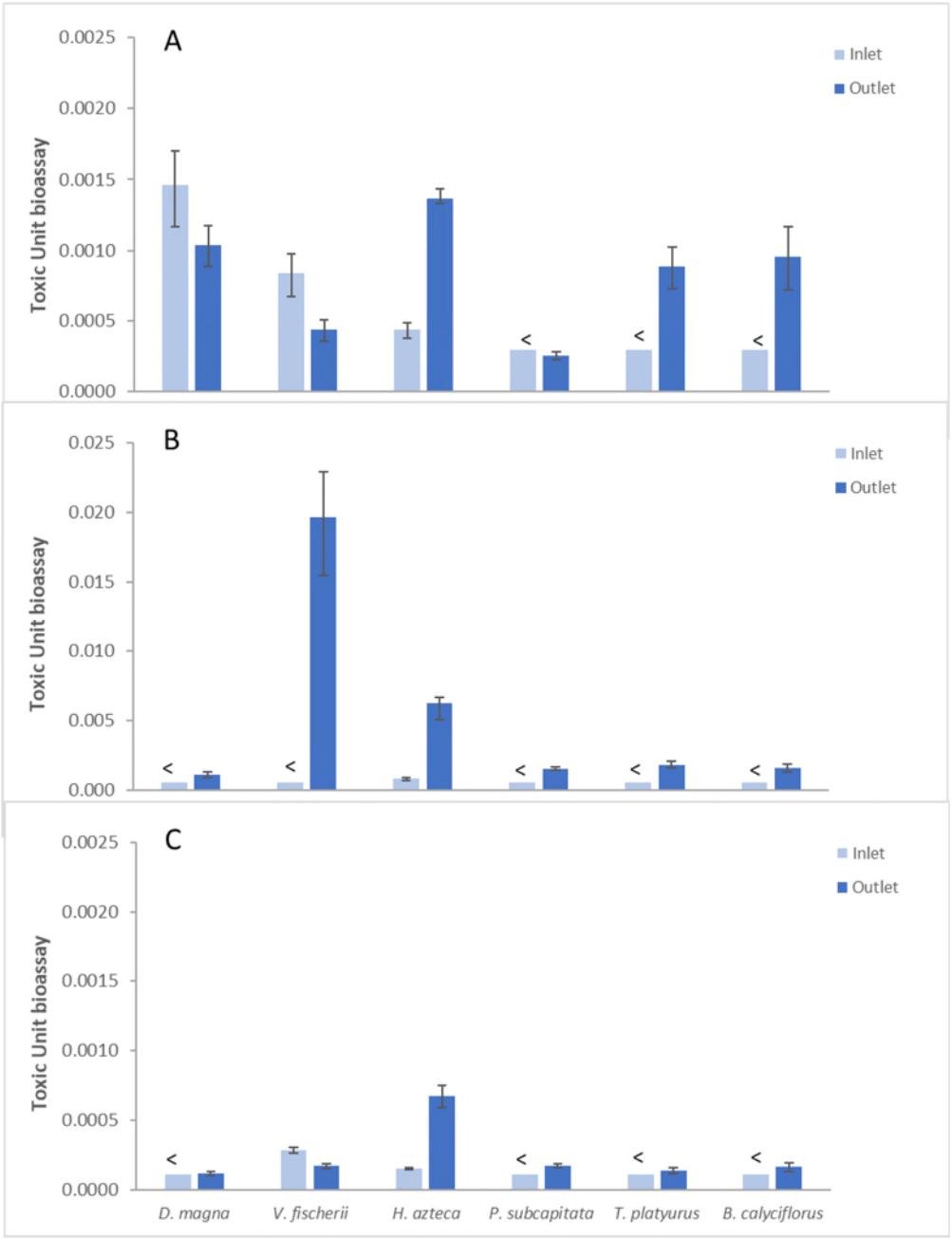
The dose-response data of the *in vivo* assays on the PSS extracts and concentration factors water-DMSO are given in Appendix L and O.

EC_{50} values from *in vivo* tests were calculated for each sample and bioassay based on the concentration series tested (the unit of these EC_{50} is the concentration of sample extract in final exposure media in volume/volume percentages; §3.4.1). However, a direct comparison of these EC_{50} values between samples and refineries is not possible as the sampling rate of PSS depends on the amount of water passing by during the five to six week exposure. Performance reference compounds (PRCs; §3.2) were therefore added to each PSS and the rate with which the amounts of these reference compounds decreases during the exposure period is a measure for the water sampling rate (Rs-value; appendix E). This Rs-value can further be recalculated into an average concentration factor (Appendix L). To aid comparisons, EC_{50} values were therefore converted into TUs ($TU_{bioassay}$) and corrected for this obtained concentration factor in the PSS (see §3.3).

An example on the $TU_{bioassay}$ calculation: A EC_{50} value of 0.0156 vol% DMSO-extract was found for the bioassay with *D. magna* in the outlet sample for refinery B. This means that a 50% effect level was reached in a 100 / 0.0156 = 6410 times diluted DMSO-extract of the PSS. At the same time this PSS extract concentrated the effluent by a factor of $5.73*10^6$, see Appendix L for water-DMSO concentrations factors. This means that the 50% effect level was reached in $5.73*10^6/6410$ = 894 times concentrated effluent by which the TU is 1/894 = 0.0011.

The $TU_{bioassay}$ values for the different inlet and outlet samples from refineries A, B and C are summarized in **Figure 13** for the bioassays with the waterflea *D. magna*, the bacterium *V. fischeri*, the amphipod *H. azteca*, the algae *P. subcapitata*, the shrimp *T. platyurus* (Thamnotoxkit), and the rotifer *B. calyciflorus* (Rotoxkit). A $TU_{bioassay}$ value below 1 indicates that no acute toxicity is expected in the undiluted inlet or outlet water. This apply to all bioassays in refinery A, B, and C. Most inlet waters showed lower $TU_{bioassay}$ values than outlet waters, except for refinery A were the $TU_{bioassay}$ values for *D. magna* and *V. fischeri* were higher in the inlet than the outlet waters. The highest $TU_{bioassay}$ values were found for the outlet water of refinery B with a maximum $TU_{bioassay}$ (0.02) for *V. fischeri*. This observation with strongest bioassay activity in the outlet of refinery B is in accordance with the chemical analysis as the outlet of refinery B was characterized by high HC and PAH concentrations (**Table 7**).

Figure 13: TU values of the various in vivo bioassays ($TU_{bioassay}$) on PSS extracts, recalculated to resemble values for the original inlet and outlet samples of refineries A, B and C. Confidence limits are calculated based on the 95% confidence limits of each EC_{50} value. A TU >1 means that acute toxic effects might be expected in the undiluted inlet or outlet sample. Note: Y-axis of refinery B is on a different scale.



In contrast, bioassays with the bacterium *V. fischeri* in spot samples showed strongest effects in the outlet of refinery A while no acute toxicity (only a slight effect in the 2nd week) was found for the outlet of refinery B. This difference might be due to the inherent complicity of using bioassays in PSS extracts as a quantitative measure for toxicity in the original in- and outlet sample. The reason for this is the

need for an accurate concentration factor. For individual chemicals these concentration factors can accurately be calculated using the silicone water partitioning coefficient as the target compounds are known (e.g. Vrana et al., 2007). However, the chemicals causing toxicity are not known and often consist of a complex mixture of polar and apolar compounds each with its own concentration factor. Nevertheless, the aforementioned calculation of expected toxicity is still the most common way of interpreting bioassay responses in PSS extracts. In addition, BTEX compounds present in spot samples are less concentrated with PSS than higher K_{ow} HCs, and BTEX compounds in the PSS extracts are mainly evaporated during the PSS sample preparation process.

In vitro toxicity tests

In vitro toxic effects in the different PSS extracts are summarised in **Table 8** for the bioassays related to AhR-agonists (DR-Luc and PAH-Calux), estrogenic (ER-Luc), or androgenic (agonistic and antagonistic) (AR-EcoScreen) activities. Concentrations were recalculated on concentration basis (per liter of water), which makes it possible to compare with other studies. An example of the calculation of the *in vitro* assay concentration on water basis is given in a box below.

Table 8: In vitro assay results expressed on water basis. DR-Luc, PAH-Calux, ER-Luc and AR-ECOscreen equivalent concentrations of the various in vitro bioassays in the inlet and outlet waters of refineries A, B and C. A higher value shows a higher activity.

Refinery	DR-Luc (pg TCDD eq./L water)	PAH-Calux (ng/l B[a]P eq.)	ER-LUC (pg E2 eq./L water)	AR-ECOscreen (µg FLU eq./L water) antagonistic
A inlet	39	4.4	5.0	0.12
A Outlet	20	14	9.2	0.66
B Inlet	1.2	0.7	6.2	0.02
B Outlet	369	223	9.2	0.83
C Inlet	2.8	0.3	0.2	0.05
C Outlet	26	7.4	2.6	0.21

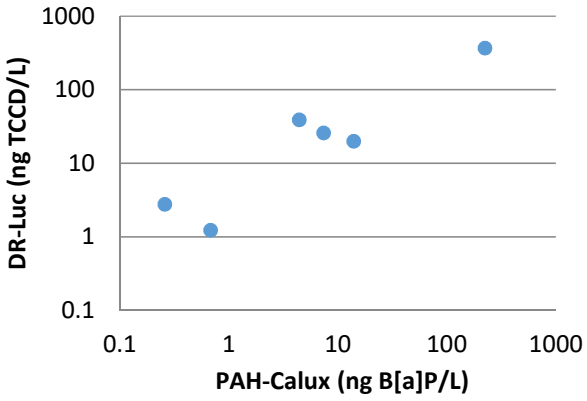
An example of the *in vitro* assay calculation:
The measured DR-LUC concentration of the DMSO-extract of inlet refinery A was 418 nM TCDD eq. DMSO extract (Appendix P), which means 418 nmol TCDD eq./ L DMSO extract. This is equal to 418 fmol TCDD eq./ µL DMSO extract. 1 µL DMSO extract is related to 3.44 L of sampled water (Appendix P). This means in one litre of water 418 / 3.44 = 122 fmol TCDD eq./L water. With a molecular mass for TCDD of 322 this gives 122 * 322 = 39127 fg TCDD eq./L, which is 39 pg TCDD eq./L water.

AhR-agonistic (DR-Luc, PAH-Calux), and androgenic-antagonistic activity (AR-ECoScreen) potencies in the outlet water were always higher when compared to respective inlet samples (**Table 8**), except for the DR-LUC for the inlet and outlet water for refinery A which were in a similar range. The largest difference in AhR-agonistic activity between inlet and outlet waters was found for refinery B (about a factor of 2000).

The strongest AhR-agonistic toxicity was observed for the outlet of refinery B and is in accordance with the highest PAH concentrations found in the PSS (**Table 6**).

A strong correlation between both AhR activity assays (DR-Luc and PAH-Calux) was observed (**Figure 14**). Both assays react on PAHs, which are present in all samples, and therefore will respond to the samples. It is suggested that for future sampling only one of these assays is needed to provide information on the AhR-activity of refinery waters if the interest is on PAHs only.

Figure 14: AhR activation of the DR-Luc (pg TCDD eq. /L) and PAH-Calux (ng B[a}P eq. / L) of the inlet and outlet samples.



Only AR-antagonist effects were found in the inlet and outlet waters. Most industrial chemicals have shown to be AR-antagonistic. A screening study of 253 industrial chemicals using the AR-EcoScreen showed that only two compounds were AR-agonistic: 2-tert-butylanthraquinone and benzoanthrone (Araki et al.,2005).

Higher ER-agonistic activity was found in the outlet compared to the inlet waters (**Table 8**). Many different types of compounds can cause ER-agonistic activity, such as steroids (e.g. estrogens), nonylphenols, bisphenols, phthalates etc. Hydroxy-PAHs also have shown estrogenic activity based on *in vitro* test systems (e.g. Charles et al., 2000; Fertuck et al., 2001; van Lipzig et al., 2005; Hayakawa et al., 2007). As ER-agonistic compounds were not chemically determined in the refinery waters it is unknown which compounds caused the increased activity.

The DR-LUC, ER-LUC and AR-ECOscreen in combination with silicone PSS were used in the TIPTOP project to assess Dutch rivers and WWTPs effluents (Hamers et al., 2018). The concentrations of DR-LUC, ER-LUC and AR-ECOscreen were 9-17 pg TCCD/ EQ/L, 0.7-8 pg E2 eq./L, and 0.04-0.54 µg FLU eq./L, respectively. The DR-LUC, ER-LUC and AR-ECOscreen concentrations of inlet and outlet of refinery A, B and C are in the same order as found in these rivers and WWTP effluents, except for the DR-LUC in the outlet of refinery B which is much higher (369 pg TCDD eq./L).

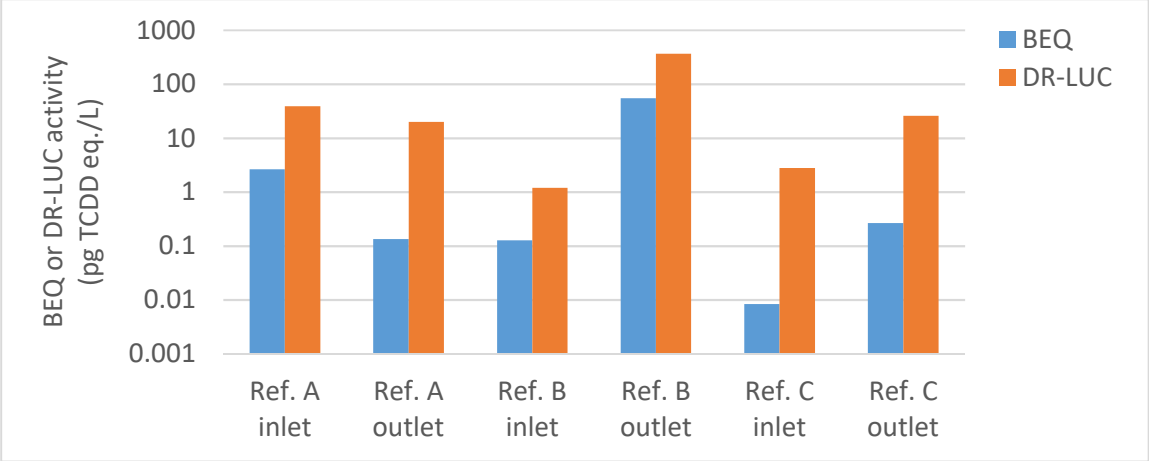
The in vitro assay results expressed on water basis for the DR-Luc, PAH-Calux, ER-Luc and AR-ECOscreen equivalent concentrations can be compared to proposed effect-based trigger values. Escher et al. (2018) and Van der Oost et al. (2017) provide trigger values for these assays. The values of Escher et al. (2018) were based on EU-EQS values and mixture effects, and the values of van der Oost et al. (2017) were developed using an approach to derive “low-ecological risk” values including field investigations. For AhR activity Van der Oost reports a trigger value of 150 ng

B[a]P-EQ/L and Escher of 6.21 ng B[a]P eq./L for the PAH-Calux. The measured PAH-Calux (**Table 9**) of all inlet waters are below these trigger values, but the outlet waters are above the trigger value of Escher, and the outlet of refinery B is above both trigger values. None of the refinery inlet and outlet waters exceeded the value for the DR-Calux of 50 pg TCDD eq./L (Van der Oost et al., 2017), except the outlet water from refinery B (369 pg TCDD eq./L) which was above the DR-Calux trigger value. However, the compounds causing the DR-Luc activity are probably PAHs and not chlorinated dioxins/furans. The trigger value is based on TCDD and not based on PAHs.

All outlet and inlet waters are below the trigger values of the ER-Luc, and antiandrogenic activity (AR-ECOscreen). Trigger values for the ER-Luc are 0.5 and 0.1 ng EEQ/L by Van der Oosten and Escher, respectively. The trigger values for the antiandrogenic activity are 25 and 14.4 (µg FluEQ/L) by Van der Oosten and Escher, respectively.

BEQ - The bioanalytical equivalent concentration (BEQ) for AhR agonist can be calculated by multiplying the REP value (Appendix P) of a PAH with C_w of each PAH (equation [8]). The BEQ can be used to provide information on the expected *in vitro* activity of the sample based on the chemically measured PAH concentrations. BEQs are given in **Figure 15**, and the details are in Appendix Q. By comparing the BEQ with the measured DR-LUC activity (**Table 8** and **9**) information is obtained on how much of the DR-LUC activity is explained by the measured PAHs. The measured PAHs explain a small part of the DR-LUC activity (0.3% to 15%) (**Figure 15**). This probably is due to the fact than many more PAHs are present in the sample, which were not determined, with AhR activity.

Figure 15: BEQ or DR-LUC concentrations (pg TCDD eq./L) in refineries A, B and C. For the BEQ calculations the REPs of Machala et al. (2001) were used.



On a BEQ basis the outlet of refinery B has the highest AhR-activity (**Table 8**). Of all PAHs, chrysene contributes the most to the BEQ, both for the DR-Luc (**Figure 16**). Interestingly, the BEQ concentrations in the inlet of refinery A is higher than outlet, which is due to a different PAH pattern found in the inlet than outlet waters and the contribution of each individual PAHs to the BEQ.

Figure 16: BEQ concentrations for the DR-Luc (pg TCDD eq./l) in the inlet and outlet samples of refineries A, B, and C based on the predicted dissolved PAH concentrations (C_W) from the PSS extracts using equation [9]. Note: Y-axis between the refineries are on a different scale.

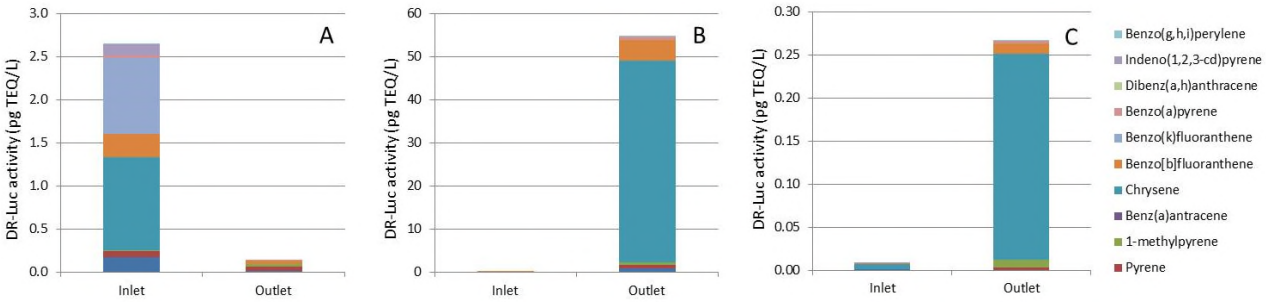


Table 9: BEQ concentrations based on REPs for the DR-Luc (TCCD equivalents) in the refinery samples and C_W (dissolved water concentrations) and measured PAHs by BIU or VU. As reference also the total dissolved PAH concentration is provided (C_W).

Refinery	PAH C_W ng/L	BEQ BIU pg TCDD-EQ/L	BEQ VU pg TCDD-EQ/L	BEQ BIU ng B[a]P-EQ/L	BEQ VU ng B[a]P-EQ/L
A inlet	24	1.6	2.6	0.083	0.12
A outlet	181	0.21	0.13	0.010	0.004
B inlet	11	0.026	0.13	0.002	0.007
B outlet	2440	44.8	54.7	2.9	4.0
C inlet	0.5	0.002	0.008	0.000	0.000
C outlet	0.3	0.035	0.27	0.001	0.015

3.2.3. Relationships between PSS concentrations, estimated dissolved water concentrations, toxic units

The GCxGC HC block concentrations in the PSS were used to predict dissolved water concentrations (C_W), and to predict toxic units (TU_{chem}), see appendix N.

An example of predicted HC blocks in dissolved water concentrations (C_W), HC blocks in PSS, and predicted acute TU_{chem} based on C_W for *D. magna* for refinery A outlet water is given in **Figure 15**. In general, for all refinery outlets the HCs in the dissolved fraction are dominated by aromatic compounds, which is expected as these are more water soluble than aliphatic compounds.

Secondly, as expected, the HC pattern in water strongly deviates from the pattern found in the PSS as the PSS mimics the bioaccumulation process and measures the bioavailable HC fraction (dissolved HCs). The measured HC block data in the samplers shows that higher K_{ow} are more accumulated in the PSS than lower K_{ow} HCs, as expected (**Figure 17**). Note, that C6-C9 (e.g. BTEX) compounds are have high losses during the sample preparation of the PSS extracts due to the evaporation step (KD/mini KD evaporation). The dominating HC in water based on PSS are the C_9 naphthenic monoaromatics, while in the PSS longer carbon chain mono-aromatics and dinaphthenics dominates. Interestingly, the TU pattern of the HCs (TU_{chem}) in

the PSS shows a similar pattern as the HCs found in the PSS, indicating that the samplers mimic the bioconcentration process following the TLM. This was expected as the TU_{chem} is based on the GCxGC HC blocks and on the TLM which depends on K_{ow} and therefore is linked to the bioconcentration process.

Quantitative relationships, based on all PSS samples, were calculated between the dissolved water concentration and TU_{chem}, and between PSS total HC concentrations and TU_{chem} for all 5 species, see **Table 10**. The relationships show that the logarithm of the predicted dissolved water concentrations (C_W) is linearly related to the logarithm predicted acute TUs (**Figure 18A**). In addition, the logarithm PSS concentrations is linearly related to the logarithm predicted acute TU (TU_{chem}) (**Figure 18B**). Based on these relationships C_W and PSS concentrations can be calculated that are equal to a TU=1. These critical values can be used to compare with BE-SPME and can be used in future studies. The critical PSS concentration range from 54 mM for *H. azteca* to 1643 mM for *V. fischeri* (**Table 9**). The PSS concentrations are on average 7 times higher than the CTLBB. The critical PSS concentrations are in accordance with the results of Bera et al. (2018) which studied the relationship between passive dosing of petroleum products, using silicone tubing and rings, and predicted acute toxicity. They showed that a BE-SPME concentration of about 20 mM equals a TU of ca. 0.9 using a CTLBB of 146 mM for a typical species. Our data showed also a strong correlation between the critical PSS concentration and the CTLBB for the five species (**Figure 19**).

Figure 17: HC blocks (blue aliphatics, red aromatics) in PSS (PSS, mM), predicted dissolved water concentrations (C_W, µM), and predicted acute TU_{chem} based on C_W for D. magna for refinery A outlet water.

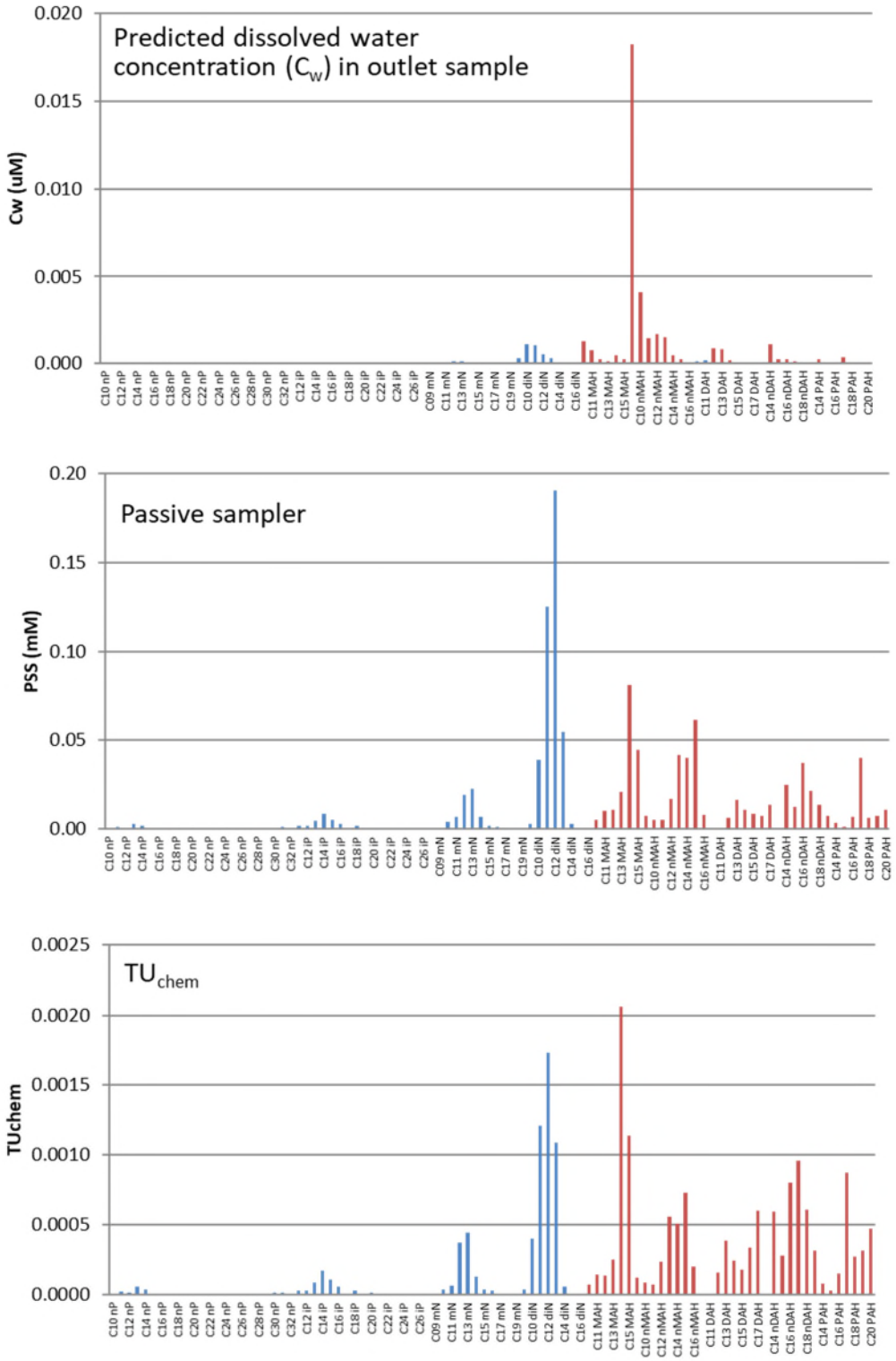


Figure 18: A: Predicted HC water concentrations (Cw) (µg/l) in inlet and outlet waters from refineries A, B, C based on GCxGC HC speciation data of PSS samplers vs. predicted acute TUs (TU_{chem} for *D. magna*, *V. fischeri*, *H. azteca*, *P. subcapitata*, and *D. rerio*. B: GCxGC HC speciation data in PSS (µmol/mL silicone; PSS concentration) vs. predicted acute TUs (TU_{chem}, EC_{50}) for *D. magna*, *V. fischeri*, *H. azteca*, *P. subcapitata*, and *D. rerio*.

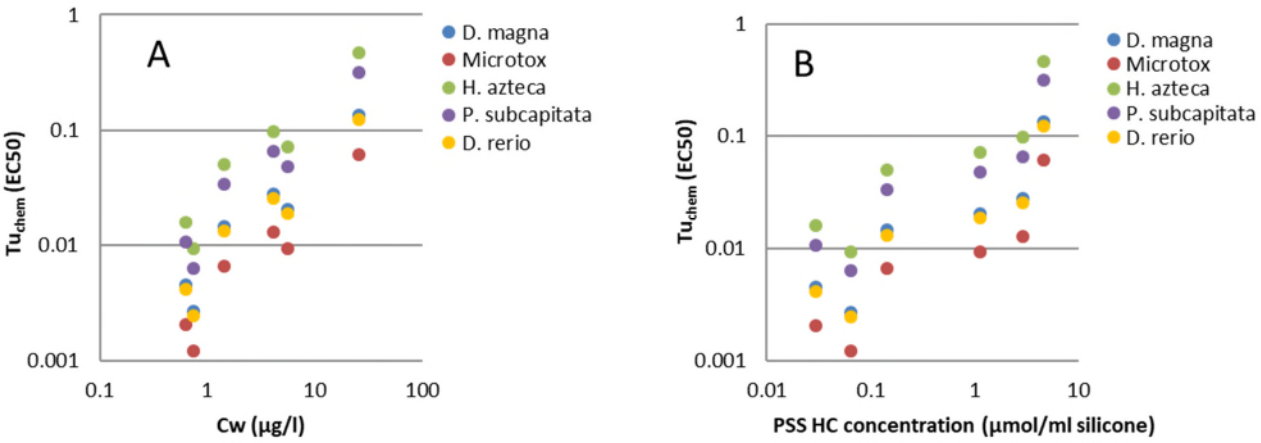
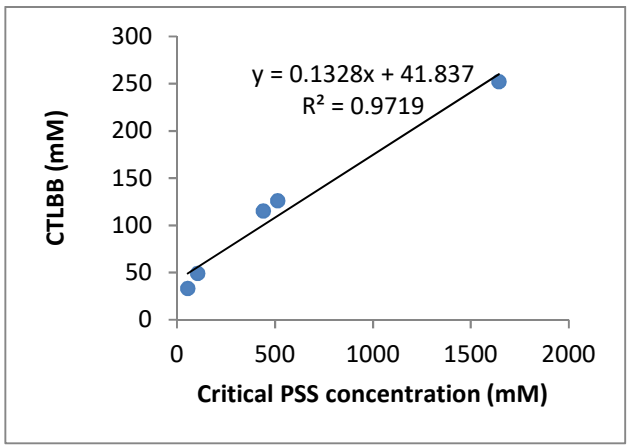


Table 10: Quantitative relationships between $logC_W$ (µg/L) and $logTU_{chem}$, and logPSS (mM) and $logTU_{chem}$ Also the CTLBB from Di Torro et al. (2000) are given as reference. Critical C_w or critical PSS concentration is the concentration at TU=1.

	Relationship C_W – TU_{chem}	Critical C_W (µg/L) at TU=1	Relationship PSS - TU_{chem}	Critical PSS (mM) at TU=1	CTLBB (mM) Di Toro et al. (2000)
D. magna	$logTU_{chem}$ = 0.946 * $logC_W$ - 2.223	224	$logTU_{chem}$ = 0.5957 * C_W - 1.5749	440	115
V. fischeri	log TU_{chem} = 0.946 *$logC_W$ - 2.563	512	$logTU_{chem}$ = 0.5957 * C_W -1.9156	1643	252
H. azteca	log TU_{chem} = 0.946 * $logC_W$ -1.681	60	$logTU_{chem}$ = 0.5957 * C_W - 1.0327	54	33
P. subcapitata	log TU_{chem} = 0.946 * log C_W - 1.852	91	$logTU_{chem}$ = 0.5957 * C_W -1.2044	105	49
D. rerio	log TU_{chem} = 0.946T *$logC_W$ - 2.262	246	$logTU_{chem}$ = 0.5957 * C_W -1.6146	513	126

Figure 19: Critical PSS concentration (TU=1) vs. CTLBB for *D. magna*, *V. fischeri*, *H. Azteca*, *P. subcapitata*, and *D. rerio*.



3.3. TOXICITY IN PSS AS AN PROXY FOR TOXICITY IN THE INLET AND OUTLET WATER

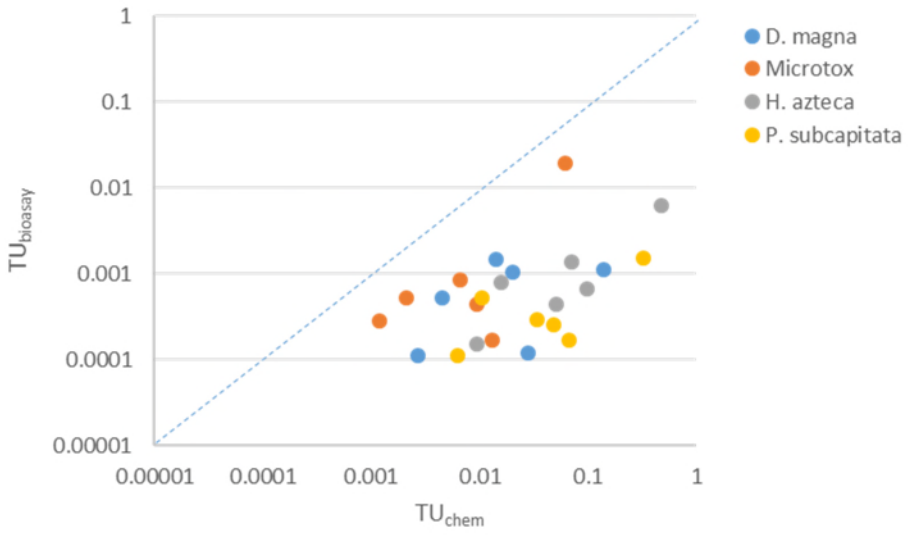
In the present case of refinery effluents (apolar) hydrocarbons are expected to be (one of) the main factors causing toxicity (see also relation BE-SPME and *V. fischeri* (Microtox) in spot samples; §3.1.3). For these substances toxic effects of mixtures can accurately be estimated based on the calculated water concentrations from PSS and the species specific critical target lipid body burden (CTLBB) for narcosis (**Table 10**). Among the seven organisms used in the present research, CTLBB values are known for the waterflea *D. magna*, the bacterium *V. fischeri*, the amphipod *H. azteca*, the algae *P. subcapitata*, and the zebrafish *D. rerio*. Calculated TU values were all below 1 (**Table 10**), indicating that the HCs typically studied in refinery effluents did not cause acute effects in neither inlet nor outlet samples of refineries A, B and C. This might even apply to chronic toxicity as most TU values are below 0.1 (a factor of 10 between acute and chronic toxicity is often assumed). The exception is the outlet of refinery B were TU values ranged from 0.06 up to 0.47. Especially for the amphipod *H. azteca* (TU=0.47) it might be argued that, due to the uncertainties around the TU calculations, some acute toxicity and especially chronic toxicity is to be expected in the outlet sample, which is in accordance to the expectation based on the BE-SPME-values in spot samples (§3.1.3). But also chronic toxicity is expected for *D. magna*, *P. subcapitata*, and *D. rerio* all with a TU >0.1. HCs in refineries B outlet contributed to this toxicity. Note however, that after discharge the outlet water is diluted within the initial mixing zone at least with a factor of 10, which finally will result in TU<0.1 in the receiving environment beyond this initial mixing zone.

Table 10: Predicted acute TUs (TU_{chem}, EC_{50}) for *D. magna*, *V. fischeri*, *H. azteca*, *P. subcapitata*, and *D. rerio* based on HC blocks measured with GCxGC in the PSS extracts and predicted dissolved water concentrations PSS dissolved water concentrations (C_W).

	TU_{chem}.				
Refinery	***D. magna***	***V. fischeri***	***H. azteca***	***P. subcapitata***	***D. rerio***
A inlet	0.014	0.0066	0.0502	0.034	0.013
A outlet	0.020	0.0093	0.071	0.048	0.019
B inlet	0.0045	0.0021	0.016	0.0106	0.004
B outlet	0.14	0.062	0.47	0.32	0.12
C inlet	0.0027	0.0012	0.0094	0.0063	0.002
C outlet	0.028	0.013	0.098	0.066	0.026

Comparing TU_{chem} (**Table 10**) with $TU_{bioassay}$ (Appendix S) shows that TU_{chem}, based on GCxGC HC data and TLM, is always higher than the measured TU with the *in vivo* bioassays ($TU_{bioassay}$) (**Figure 20**). For *V. fischeri* the difference between TU_{chem} and $TU_{bioassay}$ was the smallest (factor 3 to 77, median 6). For *P. subcapitata* the difference was the largest (factor 20-388, median 155). No correlation can be observed between the $TU_{chem}/TU_{bioassay}$ factor and the TPH or PSS HC concentrations. The difference between TU_{chem} and $TU_{bioassay}$ can be due to the uncertainty in the calculated concentration factor. It can also be due that the bioassays detect maybe more active chemicals (e.g. naphtanic acids, polar compounds) than only the measured HCs by GCxGC.

Figure 20: TU_{chem} vs. $TU_{bioassay}$ for D. magna, *V. fischeri*, H. Azteca, and P. subcapitata.



3.4. COMPARISON SPOT AND PASSIVE SAMPLING

A direct comparison can be made between *V. fischeri*-toxicity in the PSS extracts and the spot samples. As discussed above, $TU_{bioassay}$ values calculated using PSS extracts are all below 1, while spot samples (**Table 4**) showed acute toxicity in the outlet of refinery A and C. This is confirmed with the chemical analysis of HCs that also showed low TU values (TU_{chem}) based on the measured HCs (**Table 11**).

Table 11: TUs in the *V. fischeri* (Microtox) assay (based on EC_{20} and EC_{50} values) for both spot samples (TU) and extracts of the PSS ($TU_{bioassay}$) and TUs predicted on GCxGC data (TU_{chem}) for all three refineries. TU-values are based on EC values via $TU=100/EC_X$.

Spot samples					PSS	
Refinery		**Week**	**TU (EC_{20})**	**TU (EC_{50})**	**$TU_{bioassay}$**	**TU_{chem}**
A	Outlet	1	12.3	5.5		
	"	2	>18	7.4		
	"	3	>18	>16		
	"	4	>18	>16		
	"	5	>18	3.6		
	Outlet	1-5			0.00044	0.0093
	Inlet	1-5			0.00084	0.0066
B	Outlet	2	<2.2	<2.2		
	"	3	8.8	<2.2		
	"	4	<2.2	<2.2		
	"	5	<2.2	<2.2		
	"	6	<2.2	<2.2		
	Outlet	1-6			0.020	0.062
	Inlet	1-6			0.00052	0.0021
C	Outlet	1	>18	5.8		
	"	2	13.7	3.1		
	"	3	6.3	<2.2		
	"	4	6.0	<2.2		
	"	5	2.7	<2.2		
	Outlet	1-6			0.00017	0.013
	Inlet	1-6			0.00028	0.0012

This indicates that HCs were not the main causative factors for the observed toxicity to the bacterium *V. fischeri* (Microtox) in spot samples for refinery A and C. On the other hand, *V. fischeri* (Microtox) toxicity data correlated to the BE-SPME results of the spot samples.

V. fischeri TUs in the spot samples were much higher compared to the extracts of the PSS (**Table 7**). The difference for refineries A and C varied between a factor of 445 up to 1700 (EC_{50}-values), while the difference for refinery B was smaller; a factor of 35. This difference between the toxicity of spot samples and (apolar) PSS indicates that other compounds might be of importance for refinery effluents, as was also indicated by Leonards *et al.* (2017). This might be attributed to polar compounds not extracted by silicone rubbers at all (in general compounds with a $LogK_{OW}$ value <3) as well as to the more polar HCs ($3<LogK_{OW}<4$).

As such, differences in the polarity of the compounds causing toxicity in the spot samples might result in different toxicity in the PSS extracts. It was for example most remarkable that the outlet of refinery B showed the lowest toxicity in the spot samples, while toxicity in the PSS extracts was the highest compared to refineries A and C.

For example, it was noted that average BE-SPME concentrations in the spot samples of refinery B were in the same range compared to refinery A and C (7 – 11mM; Section 4.3), while TPH in the spot samples of refinery B was on average a factor of 4-8 higher compared to refinery A and C. This shows that BE-SPME provides another type of assessment of the HC emission, the dissolved fraction, than the total HC concentration (dissolved plus particle bound HCs), such as measured with TPH. For the PSS the difference in HC varied between 2 – 4 (highest value still in refinery B), while the difference in the PAH PSS concentration increased to a factor of 200-300 between refinery B and A or C (Section 4.5), showing that differences exist in the dissolved fraction of aliphatics and aromatics between refinery outlets and therefore different risks.

4. APPLYING PSS WITHIN REFINERY EFFLUENT ASSESSMENTS: TECHNICAL CONSIDERATIONS FOR PSS VS. SPOT SAMPLING

4.1. ADVANTAGES AND DISADVANTAGES OF PASSIVE SAMPLING

One of the central questions in this study, related to project objective 5, was to understand the advantages and disadvantages of PSS for the determination of HCs and toxicity in refinery waters. The study showed that PSS can be used for determination of apolar chemicals and related toxicity in refineries effluents, but there are various challenges using PSS for EBT assessments, see below. The placement and deployment of the PSS was successfully tested at three refineries (project objective 1).

One of the advantages of passive sampling is that a large volume of water is sampled and, therefore, HCs can be detected even at low water concentrations. This study showed that PSS deployed for 5-6 weeks produce enough material, based on 12 silicon sheets, to perform GCxGC, TPH, and PAH analysis, and also to test seven *in vivo* assays and five mechanistic based *in vitro* assays (project objectives 2 and 3).

Another advantage is that the collected HCs are the dissolved fraction of HCs present in the water; the fraction that is responsible for the bioaccumulation and toxicity. As expected the total HC concentration patterns in back calculated water samples deviate from the HC concentration pattern in the PSS as the samplers mimic the bioconcentration process. Due to the large amount of dissolved HCs collected by PSS the dissolved concentrations of PAHs could be determined, which is normally difficult with spot samples due to the low concentrations.

Passive sampling has also the advantage that the sampling is time-integrated and therefore determines time-weighted average concentrations; in this study average concentrations of a period of 5-6 weeks were obtained. Such time-weighted average concentrations of HCs provide information on the long-term exposure which is more related to chronic toxicity.

Possible confounding factors in the determination of effluent toxicity, such as ammonia, nitrite, pH, can be excluded with the PSS, while these are sometimes important confounding factors in toxicity tests with spot samples.

The PSS have also a number of disadvantages. Short peak concentrations are for example poorly represented in the time-weighted average concentrations, and therefore acute toxicity can be less well studied with PSS extracts.

More importantly, bioassay results from the extracts can hardly be used as a quantitative prediction of toxicity in the original samples. This is caused by uncertainties around the concentration factors. While concentration factors for individual substances are sufficiently accurate to calculate dissolved concentrations in water, this is not the case for bioassay results as the components causing the toxicity are unknown. For the present study bioassay results were converted using an average concentration factor.

Furthermore, the interpretation of the data is complicated due to the fact that some of the HCs are in equilibrium ($\log K_{OW}<6$) and some are in the linear uptake phase ($\log K_{OW}>6$). As each HC has its own sampled water rate the conversion of a bioassay response to an activity per liter water is difficult for silicon sheet samplers, as the average sampled water rate is much influenced by the HCs present.

It was found that bioassay results based on an average sampled water volume based on PSS is not a good predictor of the real toxicity in the receiving water due to the uncertainty of the concentration factor. This conclusion is supported by a recent paper of the TIPTOP project (De Weert et al., 2020). This paper concluded that the conversion is possible for adsorption-based PSS (Speedisks) but difficult for partitioning-based silicone rubber samplers, and that the conversion of bioactivities in these samplers to bioactivities in the water body should be done with great precaution (De Weert et al., 2020).

Another important point to consider is that the PSS mimic the bioaccumulation process, without biotransformation, and that the bioassays are therefore exposed to a "bioaccumulated" HC profile (C_M) (**Figure 21**). As the organisms are again accumulating the HCs from the PSS extract the exposure HC pattern in the bioassay medium deviates much from the HC pattern in the refinery streams (**Figure 21**). This 2-step bioconcentration step, one from water to PSS and secondly from PSS extract to the bioassay organism, could bias the toxicity.

To get a full picture of all chemicals present in effluents different types of PSS materials are needed to extract these from the water, and the extracts of all of these PSS types should be tested with bioassays to get a complete picture of the toxicity. This in contrast to spot sample testing, which covers the testing of all chemicals present.

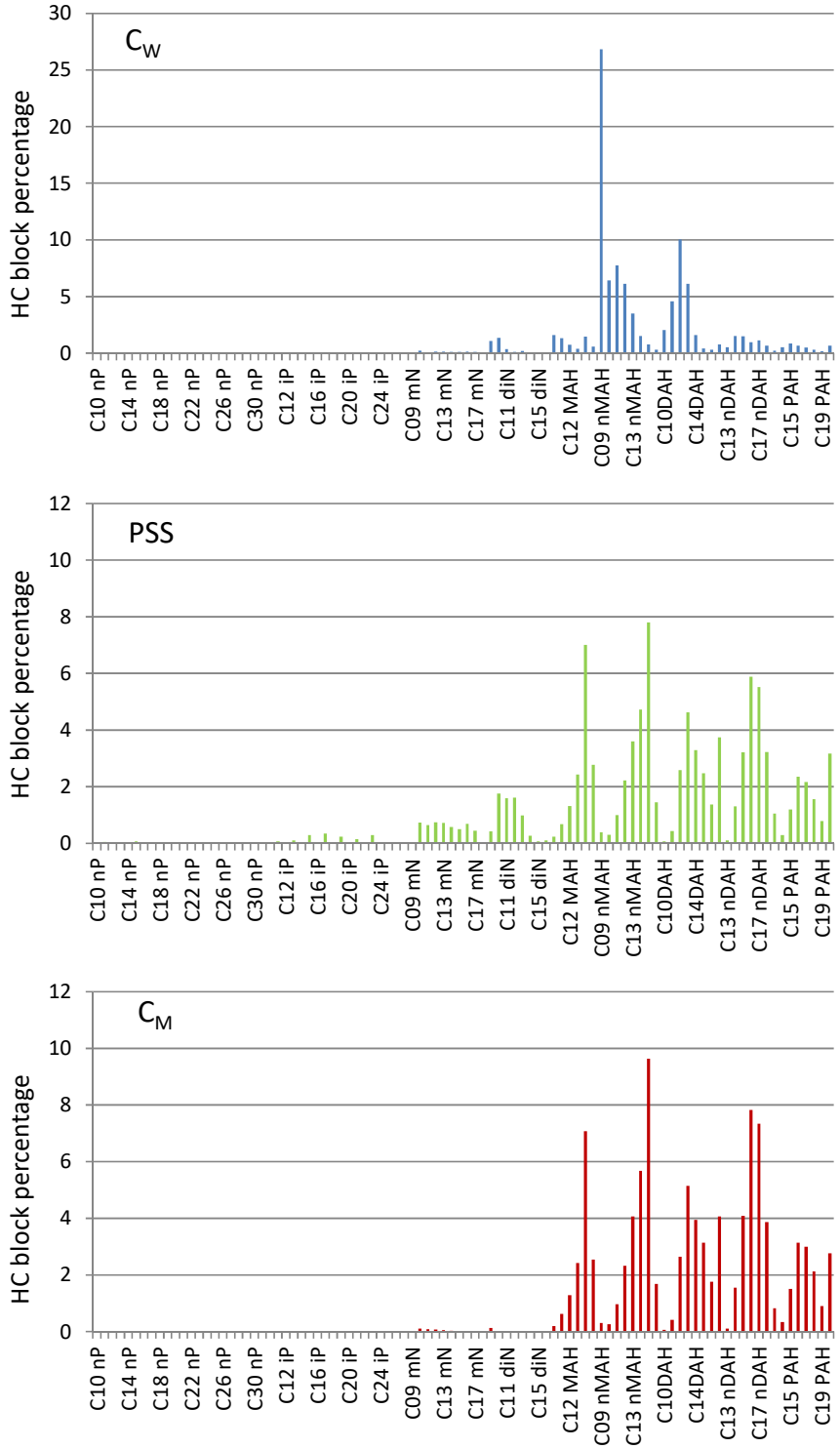
Practical considerations

The project has shown a number of practical considerations performing PSS combined with EBM at refineries. Firstly, this was the first study using PSS at refinery outlet waters, and it showed that the used PSS materials, silicon based, were able to concentrate enough HCs to perform both chemical as toxicity tests. Secondly, the selection of appropriate sample locations with enough water flow and space for the deployment of the PSS rackets is an important factor to consider when performing PSS at industrial sites. This was only possible as we followed a sequential approach, starting at one site followed by the next, and a pre-visit was performed before the sampling started at the first refinery. At one refinery a special sampling area was created for the deployment of the samplers. At two refineries the PSS samplers were placed and retrieved by personnel of VU as the site personnel did not have experience with PSS sampling. At one refinery PSS samplers were shipped by courier and placed by the refinery, which worked very efficiently. This shows that experts are needed to deploy the PSS, perform the chemical and bioassay analysis, and perform the calculations for the chemical and toxicity assessment. PSS for EBM assessment can therefore not yet be used on a routine basis.

The biggest challenge in this project was to convert the toxicity data to real water samples, as was discussed above. This will be a challenge for most PSS EBM projects when partitioning-based PSS are used. Spot sampling at all three refineries worked smoothly as the sampling protocol (Appendix A) have been used in previous sampling campaigns (e.g. Hjort et al., 2021, Whale et al., 2022).

In the TIPTOP project a cost calculation was performed between routine chemical monitoring of 45 priority substances (12 months spot samples of 40 k€ per location), and a PSS EBM campaign using two PSS samplers and a set of *in vitro* and *in vivo* assays and data analysis, which was estimated to be 34 k€ per location (Hamers et al., 2018). These costs are comparable to the current project, except additional costs for the pre-visit and visits to deploy and retrieve the PSS samplers at two refineries. Costs will be higher if PSS samplers are deployed several times per year.

Figure 21: Percentage of HC block predicted dissolved water concentration (C_W), PSS extracts, and dissolved water concentrations in bioassay medium (C_M).



4.2. CONSIDERATIONS HOW CAN PSS COMBINED WITH EBM HELP TOWARDS MAKING BETTER DECISIONS/ASSESSMENTS

The main aim of the project was to evaluate the deployment of passive sampling combined with EBM assessment to assess the impact of refining operation on the final effluent toxicity and determine the added value of PSS combined with EBM. In the next section conclusions on the 6 project objectives will be given.

1) *Perform time-integrated sampling using PSS devices deployed in refinery main inlet waters entering the refinery and final effluent waters discharged to the surrounding water (outlet waters)*

   In the current study PSS were successfully deployed and retrieved after 5-6 weeks from the outlet and inlet waters of three refineries. Because expert labs and detailed knowledge is needed to properly interpret results from studies that combine PSS with EBM testing, this approach is not yet ready for routine studies but more suitable for targeted, field specific surveillance studies.

2) *Determine concentrations of HC blocks, total petroleum hydrocarbons (TPH), and BE-SPME in PSS and compare to spot sampling*

   TPH, HC speciation and BE-SPME were determined in extracts of the PSS sheets. Many HC blocks could be determined in the PSS extract, which shows that PSS can be used in a refinery setting to determine chemical parameters.

3) *Determine toxicity profiles in the PSS extracts using a battery of bioassays consisting of seven small-volume in vivo organism-level bioassays representing four trophic levels and five mechanistic in vitro bioassays*

   Extracts of the PSS were used to assess the *in vivo* and *in vitro* toxicity of the inlet and outlet waters, and were recalculated to original waters. The results show that no acute or chronic toxicity was expected for the original water samples as the back calculated were all much lower than 0.1. The exception is the outlet of refinery B were TU values ranged up to 0.47 for the amphipod *H. azteca*. Acute toxicity and especially chronic toxicity are to be expected in this outlet sample as well as the TU for *D. magna*, *P. subcapitata*, and *D. rerio* are between 0.1 and 0.3. Note however, that the outlet waters will be diluted within the initial mixing zone with at least a factor 10 reducing the concentrations in the receiving environment beyond the zone of initial mixing. The PSS EBM results are based on average concentrations factors and therefore contains more uncertainty than the chemical dissolved water concentrations.

   In vitro bioassay results show that compounds with a mechanism-specific toxicity (AhR-activity, estrogenicity, anti-androgenicity) are present in elevated concentrations in refinery outlets. The *in vitro* assays should therefore mainly be used as an early warning system to detect chemicals with a specific mode-of-action. For one outlet (refinery B) the AhR-activity exceeded a trigger value (van der Oost et al., 2017) before dilution in the initial mixing zone.; in case a dilution factor of 10 is included to address the mixing zone dilution, the AhR-activity will be below this trigger value. The *in vitro* assays showed the added value of incorporating mode of action specific bioassays in the testing battery to detect effects of the mixture of unknown chemicals but with known toxicity mechanism. These results could trigger further chemical analysis on specific chemicals. Effect-directed analysis (EDA) is a technique that can used to shed more light on which chemicals are responsible for these specific type of toxicity. EDA with high-throughput assays, as used in this study, in combination with high-resolution fractionation are the

ideal combination to elucidate the compounds that are responsible for the toxicity (e.g. Zwart et al., 2018).

4) *Determine correlation between passive sampling response and BE-SPME measurements*

The PSS provided information on the bioavailable fraction of hydrophobic compounds that could directly linked to acute toxicity. Quantitative relationships were determined between the dissolved water concentration and PSS total HC concentrations. Critical PSS total HC concentrations were determined for acute toxicity and these are linked to BE-SPME.

5) *Assess the added value of using passive sampling compared to spot sampling*

PSS has the advantage of estimating the bioavailable concentration of hydrophobic compounds, and it provides time-weighted concentrations. PSS concentrated HCs from water in combination with HC speciation (GCxGC) provides information on the dissolved fraction which is not provided by spot sampling and TPH analysis as these provide total HC concentrations (sum of HC bounds to particles and dissolved fraction). Dissolved HC concentrations can provide a better estimate of expected ecological effects in the receiving environment than the total concentrations, and PSS also provide a time-integrated assessment. The comparison of toxicity from the spot samples with the PSS provided first indications that other compounds than HCs might be present in refinery waters and contribute to a large extent to the toxicity, based on the *V. fischeri* (Microtox) assay results. The used PSS extracts hydrophobic chemicals only and the current study suggests that also other types of chemicals are probably contributing to the toxicity, which requires a broader type of chemical screening or target analysis in refinery outlet waters.

A comparison between TUs based on HCs measured (GCxGC) (TU_{chem}) and determined by *in vivo* assays ($TU_{bioassay}$) in the PSS extract showed that TU_{chem} was always lower than $TU_{bioassay}$, in general 1 to 2 orders of magnitude. This could be due to the uncertainty in $TU_{bioassay}$ calculation or by other compounds than HCs that contributed to the toxicity.

6) *Determine the effect of chemical analysis for the observed toxicity*

The present results indicated that apolar HCs collected with the PSS might be the most dominant apolar toxicants, especially the aromatic HCs, for refinery outlet waters. For these cases (ecological) effects in the receiving surface waters can be assessed using HCs analysis and PETRORISK. The PSS provided information on the contribution of HCs to the acute toxicity and the results showed that aromatic HCs were the main hydrophobic compounds that contributed to the toxicity for *D. magna, V. fisheri, H. azteca, P. subcapitata,* and *D. rerio* in the refinery inlet and outlet waters. This conclusion was based on the predicted dissolved HC water concentrations in the bioassay medium using Petrotox.

However, the results would also indicate that other compounds not extracted with silicone rubber samplers seem to be causative factors for the observed toxic effects for all test species. This is also illustrated with the *V. fischeri* results that showed less than 5% of the observed toxicity in the refinery outlet water was due to HCs. In those cases, risk assessments based on GCxGC analyses might underestimate the 'true' risks for the receiving surface waters. More research is therefore needed to track down these other compounds, using for instance other types of PSS materials and/or non-target LC-MS screening or suspect analysis of specific chemicals used in the refinery process. This can be performed on extracts of especially polar PSS but also the apolar samplers. In

addition, the extracts of the polar PSS can be tested with a battery of *in vivo* and *in vitro* assays, which was also done in the TIPTOP project (Hamers et al., 2018). The TIPTOP project, using both silicone sheets and Speedisk, showed that most of the toxicity from Dutch rivers and WWTP effluents were found with the Speedisk, and therefore are more related to more polar compounds.

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7. GLOSSARY

BE-SPME:	Biomimetic solid phase microextraction
GCxGC:	Two-dimensional gas chromatography
HC:	Hydrocarbon
Inlet water:	Main water entering the refinery
Original water samples:	The passive samplers concentrated the compounds from the water. Extracts of the passive sampler material were tested with toxicity tests and the outcomes were calculated back to the original water concentration
Outlet water:	Final effluent water discharged to the receiving water
PSS:	Passive sampler(s)
TLM:	Target Lipid Model
TPH:	Total petroleum hydrocarbons
TU:	Toxic unit
$TU_{bioassay}$:	Effects (EC_{50}) found with the *in vivo* tests that were exposed to the passive sampler extracts. Toxic units based on the effects in each concentration series were converted to the original inlet and outlet water based on the concentration factor calculated using the PRCs.
TU_{chem}:	Predicted acute toxicity units for *D. magna*, *V. fischeri*, *H. azteca*, *P. subcapitata*, and *D. rerio*[1] based on GCxGC HC blocks data measured in the passive sampler extracts. Dissolved water concentrations (C_W) of the GCxGC data were predicted and compared to EC_{50} of the target lipid model (TLM). This toxic unit is recalculated to the original water samplers.
TU_{medium}:	Toxic unit of the HCs in the DMSO extract that was added to the bioassay medium at a concentration equal to the EC_{50} value. To calculate the TU_{medium} GCxGC data of the passive sampler extracts were used to predict the dissolved water concentrations of each HC block in the bioassay medium using Petrotox. The calculated dissolved water concentration was divided by the TLM LC_{50}. TU_{medium} shows if the HCs added in DMSO to the bioassay medium could explain the observed toxicity of the assays at EC_{50} level.

[1] Calculations could not be performed for the other organisms used as no CTLBB were available

APPENDIX A SAMPLING PROTOCOL CONCAWE PASSIVE SAMPLING/ EFFECT-BASED METHODS PROJECT

Please note that this sampling protocol comprises three sections:

1. Preliminary assessment of sampling locations (passive samplers and spot sampling)
2. Protocol for deployment and recovering of passive sampler devices
3. Spot sampling collection protocol (including spot sampling and shipping of sample procedure)

Requested Actions:

- **Please complete and return section I: Preliminary assessment of sampling locations** to mike.spence@concawe.eu and markus.hjort@concawe.eu
- **Please review section II: Protocol for deployment and recovering of passive sampler devices** when the samplers arrive on site to ensure that all the required materials are available, correctly labelled, and in good condition prior to deployment and/or recovery of samplers.
- **Please review section III: Sample collection protocol** when the sampling vessels arrive on site to ensure that all the required materials and vessels are available, correctly labelled, and in good condition prior to sampling.
- **At the time of spot sampling please complete Tables 6, 7 & 8 in the sample collection protocol**. Note that these capture a description of the sample and also the treatment process parameters both **before** and **after** the sampling event.
- Please contact mike.spence@concawe.eu and markus.hjort@concawe.eu if you have any questions, or to return the completed protocol tables

I. Preliminary assessment of sampling locations

Please complete the tables on the following page.

This data is requested to ensure that any problems or questions regarding the proposed sampling locations are addressed before sampling begins. Note that we have entered the number of samples we propose to collect at your site. Extra lines are available in the tables in case it is decided that additional samples are required.

Background Information

In the selection of sampling points please be aware of factors that could complicate the interpretation of the data. These include:

- Sampling locations that are at the end of pipeline side branches, where the water quality may be different from that of the main flow stream
    - ➢ Please select effluent sampling points to avoid these features. If this is not possible please make a note of this in the preliminary assessment table below.

Important!

1. Please return this protocol as soon as the effluent sampling locations have been decided
2. Please ensure that the staff who will collect the samples in the field are involved in the completion of this preliminary assessment
3. We ask that for this project composite sampling devices are NOT used, as these will complicate the interpretation of the data
4. If you have any questions regarding this questionnaire please contact: mike.spence@concawe.eu and markus.hjort@concawe.eu

Refinery name:	
Contact email address:	
Number of sampling locations:	

Table 1: Description of passive sampling location

Concawe Sample ID:	Site ID for sampling point (e.g. from engineering plan, or lab ID)	Description of sampling point (e.g. overflow tank, channel, basin, sewer, pump outlet, connect pipe)	Analysis performed weekly
PSD RR-1			
SS/PSD RR-2			

Table 2: Description of spot sampling location

Concawe Sample ID:	Site ID for sampling point (e.g. from engineering plan, or lab ID)	Description of sampling point (e.g. overflow tank, channel, basin, sewer, pump outlet, connect pipe)	Analysis performed weekly
SS/PSD RR-2			

Table 3: What equipment will be used to collect the spot sample at each location?

Concawe Sample ID:	Grab sampler equipped with a bottle	Weighted Beaker Sampler	Bucket	Electrical pump	Other (please specify)
SS/PSD RR-2					

NOTE: Spot sampling to be done on same location as effluent passive sampler is deployed. Spot sample to be taken weekly for 5 weeks.

II. Protocol for deployment and recovering of passive sampler devices

Please refer to the information returned in the **Preliminary assessment of sampling locations at the start of this document** and update these tables in the event of any changes to the planned sampling locations, or the devices used to collect samples.

Table 4: Record of treatment unit operating parameters BEFORE sampler is being deployed

Note: Concawe identifiers have been entered for the samples we propose to collect at your site. Extra lines are available in the tables in case it is decided that additional samples are required.

Concawe Sample ID:	Sampling date, time		Flowrate	NH4	NO3	COD	pH	Dissolved oxygen (DO)	Temperature
	Date	Time	UNITS:	UNITS:	UNITS:	UNITS:	UNITS:	UNITS:	UNITS:
PSD RR-1									
PSD RR-2									

Table 5: Record of operating parameters AFTER sampling

Concawe Sample ID:	Sampling date, time		Flowrate	NH4	NO3	COD	pH	Dissolved oxygen (DO)	Temperature
	Date	Time	UNITS:	UNITS:	UNITS:	UNITS:	UNITS:	UNITS:	UNITS:
PSD RR-1									
PSD RR-2									

Procedure for deployment and recovering of passive sampler devices

Deployment of passive sampler

The sampling frame is illustrated in Figure 1 (figure taken from Smedes and Booij, 2012). Sampler silicon sheets will be provided in closed glass bottles which are labelled for each sampling site (PSD RR-1 and PSD RR-2). **DO NOT CHANGE SILICONE SHEETS BETWEEN SAMPLING SITES** as some samplers contain a calibrant. The sheets should be mounted on the mounting stems of the sampler holder and are kept in place by a fixing rod (see Figure 1). **Please, use supplied gloves when mounting** the silicon sheets.

In **total 18 silicon sheets** and **6 Speeddisks** will be mounted on the frame for each sampling location (example see Figure 2).

Mount 18 silicon sheets on the holder as follows (see also Figure 1). At deployment, pull out the rod. Take a sheets from a jar and transfer it onto the holder, repeat till all 18 sheets are placed on the holders. Each rod can fix 6 sheets. Guide the fixing rod through the holes on the stem and fasten it with a cable strap.

Then fix **6 Speedisk samplers** with cable straps to the frame and directly deploy the sampling device at the sampling location.

The frame is designed to protect the sampler holder against damage caused by bumping. A rope or chain is used to connect the sampler to an object in the field. Sampler frames are made of corrosion-resistant metal (stainless steel).

VERY IMPORTANT is to keep the sampler **underwater** during sampling. **Exposure** of the **sampler sheets to air should be minimized**.

Figure 1: Sampler frame. Picture from Smedes and Booij, 2012. ICES protocol.

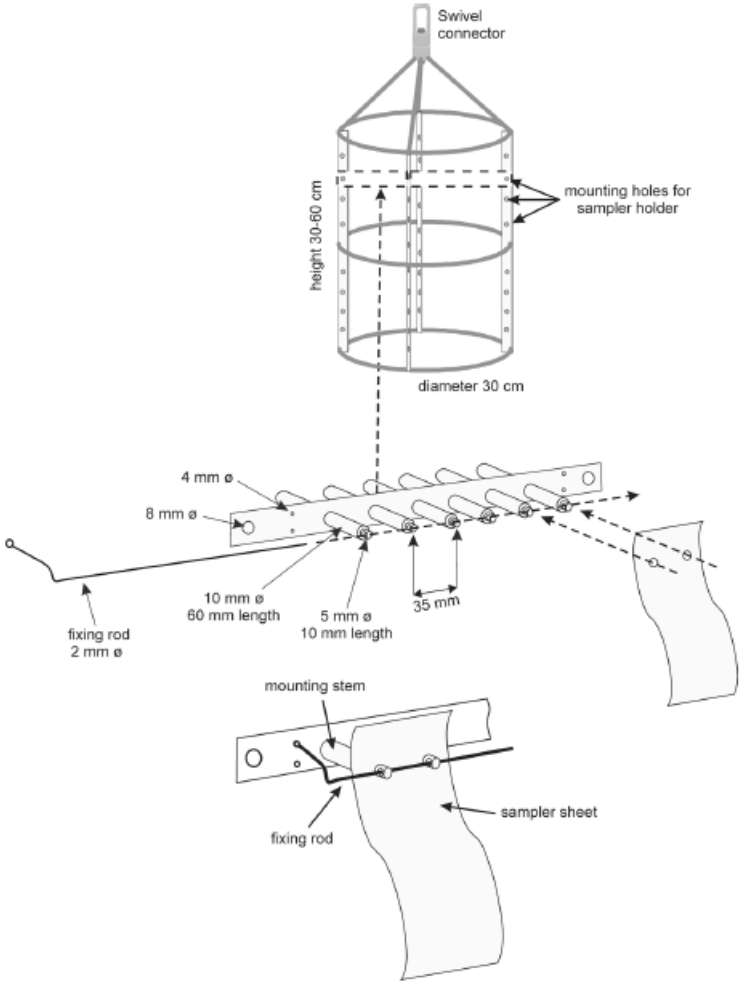


Figure 2: Placement of silicone and Speeddisk samplers on frame.

Recovery and shipment passive sampler

Depending on the site it is possible that the sampler sheets are covered by a thin biofilm, or with organisms. This is not a problem for the analysis. A clean working surface is required for handling the samplers, and local exposure water is used for rinsing and cleaning. Clean the sheets with some water from the exposure location. **Use gloves, as dispatched when handling the samplers. Keep the cleaning as short as possible.**

Each recovered sampler is placed back in a storage bottle which will be supplied. Transfer all bottles to a freezer (– 20°C). The samplers should be shipped by courier at (– 20°C). Reload the bottles carefully packed into the plastic shipping boxes. Return the samples by **express courier** (e.g. DHL, Fedex).

References

Smedes, F. and K. Booij. 2012. Guidelines for passive sampling of hydrophobic contaminants in water using silicone rubber samplers. ICES TECHNIQUES IN MARINE ENVIRONMENTAL SCIENCES, NO. 52.

Spot sample collection protocol

Please refer to the information returned in the **Preliminary assessment of sampling locations at the start of this document** and update these tables in the event of any changes to the planned sampling locations, or the devices used to collect samples.

Record of Effluent treatment plant operating parameters

IMPORTANT: Please complete the tables on the following page to record the effluent treatment unit operating parameters BEFORE and also AFTER sampling.

Data on treatment system parameters before and after the sampling event is requested to:

1. Avoid sample collection at times when the system is working outside its normal operating limits
2. Provide insight into the stability of the system during the sampling event

WWTP stability checks

IMPORTANT: If, prior to sampling, the effluent treatment unit operating parameters are outside the normal operating window, please delay sampling if possible until conditions return to normal

Table 6: Record of treatment unit operating parameters BEFORE sampling

Note: Concawe identifiers have been entered for the samples we propose to collect at your site. Extra lines are available in the tables in case it is decided that additional samples are required.

Concawe Sample ID:	Sampling date, time		Flowrate	NH4	NO3	COD	pH	Dissolved oxygen (DO)	Temperature
	Date	Time	UNITS:	UNITS:	UNITS:	UNITS:	UNITS:	UNITS:	UNITS:
SS RR-2									

Table 7: Record of operating parameters AFTER sampling

Concawe Sample ID:	Sampling date, time		Flowrate	NH4	NO3	COD	pH	Dissolved oxygen (DO)	Temperature
	Date	Time	UNITS:	UNITS:	UNITS:	UNITS:	UNITS:	UNITS:	UNITS:
SS RR-2									

Table 8: Record of collected sample appearance

Please complete the following form to indicate the appearance of the effluent during sampling

Concawe Sample ID:	Visual appearance (e.g. clear/cloudy, colour)	Temperature (hot/cold/ambient)	Visual signs of hydrocarbons (sheen, oil droplets/ emulsion)?	Other comments (e.g. odour,
SS RR-2				

Procedure for Spot sample collection

IMPORTANT NOTES:

- Please ensure that the sample line or collection device is flushed so that the effluent quality (visual, olfactory) is stable prior to sample collection
- Please do NOT rinse the sampling vessels with sample prior to collection, as this will wash out the preservative chemicals!
- Please specify any **treatment chemicals** added in WWTP units in **comments** field of sample data table.

Sampling materials checklist

You will receive the following documentation and materials inside shipping boxes (samples must be repacked and returned inside these boxes, which are approx. 30x 40x 60cm (W,D,H)):

Documentation:

1. Safety data sheets for preservative chemicals (also sent by email in advance)
    - Material safety data sheet (MSDS), 4% nitric acid
    - Material safety data sheet (MSDS), dilute sulphuric acid pH<2
    - Material safety data sheet (MSDS), dilute phosphoric acid pH<2
2. Sample collection protocol (duplicate copy of this document)

Safety equipment (inside one of boxes – indicated on label):

- Disposable gloves
- Safety glasses
- Emergency eye wash bottles (500ml saline and 200ml neutraliser)

Sampling materials and vessels for EACH spot sample:

For **each sampling point** the following bottles and equipment are provided. The sample bottles should be filled according to the instructions given below. **Please note that each bottle is wrapped in protective bubble wrap- please retain this for the safe return shipment of the bottles!**

Bottles:

1. 1x Glass bottle for TPH in water analysis (1000 ml, pre-loaded with dilute sulphuric acid pH<2).
2. 1x Plastic bottle for COD analysis (100 ml, no preservative).
3. 1x Glass bottle for DOC analysis (250 ml, no preservative).
4. 1x Glass bottle for TOC analysis (250 ml, no preservative).
5. 1x Glass bottle for 16 EPA PAH analysis (1000 ml, pre-loaded with diluted sulphuric acid pH<2).
6. 1x Glass bottle for Total suspended solid (TSS) analysis (1000ml, no preservative)
7. 1x Plastic bottle for metal analysis: (250 ml, pre-loaded with dilute nitric acid pH<2)
8. 1x Glass bottle for Microtox analysis (1000 ml).
9. 1x Glass bottle for BE-SPME analysis (1000 ml).

INSTRUCTIONS SAMPLING FOR ALL LOCATIONS

- Collect samples as agreed in the Preliminary assessment of sampling locations- please refer to the sample location information you sent to us prior to sampling
- Fill the bottles, which have been pre-labelled, with the corresponding sample. TIP: Separate out the bottles for each sampling location before starting sampling to avoid missing any.

Use the enclosed gloves and safety glasses for your safety, as some sample bottles contain dilute sulphuric, phophoric or nitric acids. Secure all caps firmly. The sample must be a freshly collected sample.

Perform the following instructions:

1. Bottles for TPH
   Fill the glass **bottles** and **TPH** completely with the sample, leaving **NO HEADSPACE**. **DO NOT RINSE** the bottle before use as the bottle contains preservative chemicals. Firmly fit screw cap provided with bottle.

2. **Plastic bottle for metals:**
   The plastic bottle for elemental analysis: total contains 4% nitric acid. Therefore **DO NOT RINSE** these bottles. Fill these bottles completely with sample and firmly fit the screw cap provided.

3. **Bottles for COD:**
   Fill the **COD bottle** completely with sample and firmly fit screw cap provided with bottle

4. **Bottles for DOC, TOC:**
   Fill each of the **DOC,** and **TOC to approx. 90%** with sample and firmly fit screw cap provided with bottle.

5. **Bottles for TSS, Microtox and BE-SPME:**
   Fill the **TSS, Microtox and BE-SPME bottles** completely with sample and firmly fit screw cap provided with bottle

6. **Bottles for PAH:**
   Fill the **PAH bottles** completely with the sample, leaving **NO HEADSPACE**.
   DO NOT RINSE the bottle before use as the bottle contains preservative chemicals. Firmly fit screw cap provided with bottle.

SHIPMENT OF FILLED SAMPLE BOTTLES
Re-package the filled sample bottles in the bubble wrap provided, and **carefully** reload them into the plastic shipping boxes. Return the samples by **express courier**

APPENDIX B CONCENTRATIONS OF PARAMETERS IN SPOT SAMPLES OF REFINERY A

Parameter	Unit	Water inlet zone	Water inlet zone	Water inlet zone	Water inlet zone	Water inlet zone	Outlet zone	Outlet zone	Outlet zone	Outlet zone	Outlet zone	T-test
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 1	Week 2	Week 3	Week 4	Week 5	*p-value*
Arsenic	µg/l	4.4	4.2	<4	4.8	4.4	13	15	16	14	14	<0.05
Cadmium	µg/l	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	n.d.
Chromium	µg/l	5.2	<5	<5	<5	<5	<5	<5	<5	<5	<5	n.d.
Cobalt	µg/l	<2	<2	<2	<2	<2	4.7	4	3.6	2.4	3.5	<0.05
Copper	µg/l	<5	11	110	<5	<5	26	<5	5.1	<5	<5	>0.05
Mercury	µg/l	<0.06	<0.06	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.08	<0.05	n.d.
Lead	µg/l	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	n.d.
Nickel	µg/l	<5	<5	<5	<5	<5	43	40	28	24	31	<0.05
Selenium	µg/l	<3	<3	<3	<3	<3	17	25	14	16	16	<0.05
Vanadium	µg/l	8.2	9.1	6.5	10	8	11	13	14	16	13	<0.05
Zinc	µg/l	<20	<20	<20	<20	<20	31	21	30	23	<20	<0.05
Total N	mg N/l	1.9	1.7	1.8	1.6	1.5	2.6	3.6	4.8	3.8	2.5	<0.05
DOC	mg C/l	<5	<5	<5	<5	<5	37	37	52	55	51	<0.05
TOC	mg C/l	<5	<5	<5	<5	<5	48	60	70	76	66	<0.05
TPH	mg/l	<0.05	<0.05	<0.05	<0.05	<0.05	0.2	0.2	0.4	0.22	0.15	<0.05
Phenol index	µg/l	<5	<5	<5	<5	<5	34	67	49	150	73	<0.05
COD	mg/l	<10	<10	<10	13	<10	150	130	240	150	200	<0.05
TSS	mg/l	6.2	14.2	7.3	8.0	6.2	91	96	160	110	79	<0.05
BE-SPME	mM	*	*	*	*	*	11	*	17	*	5.3	n.d.

* Sample analysed but the BE-SPME could not be determined. T-test, If concentrations were measured in outlet but the concentrations were below the LOQ in the inlet the LOQ values were taken to calculate if concentrations were significantly different. n.d. not possible to determine as both outlet as inlet concentrations were below the LOQ.

APPENDIX C CONCENTRATIONS OF PARAMETERS IN SPOT SAMPLES OF REFINERY B

Parameter	Unit	Inlet	Inlet	Inlet	Inlet	Inlet	Outlet	Outlet	Outlet	Outlet	Outlet	T-test
		Week 2	Week 3	Week 4	Week 5	Week 6	Week 2	Week 3	Week 4	Week 5	Week 6	P value
Arsenic	µg/l	<4	<4	<4	<4	<4	4.1	<4	<4	<4	<4	n.d.
Arsenic	µg/l	<4	<4	<4	<4	<4	4.1	<4	<4	<4	<4	n.d.
Cadmium	µg/l	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	n.d.
Chromium	µg/l	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	n.d.
Cobalt	µg/l	<2	<2	<2	<2	<2	3.2	<2	<2	<2	<2	n.d.
Copper	µg/l	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	n.d.
Mercury	µg/l	<0.02	<0.02	<0.02	<0.06	<0.06	<0.02	<0.02	<0.02	<0.02	<0.02	n.d.
Lead	µg/l	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	n.d.
Nickel	µg/l	<5	<5	<5	<5	<5	41	11	11	5.4	6.1	<0.05
Selenium	µg/l	<3	<3	<3	<3	<3	15	8.6	20	17	15	<0.05
Vanadium	µg/l	<5	<5	<5	<5	<5	21	18	9.7	7.6	6.8	<0.05
Zinc	µg/l	<20	<20	<20	<20	<20	59	76	47	34	28	<0.05
Total N	mg N/l	<1	<1	<1	<1	<1	2	1.3	1.5	1.1	2.4	<0.05
DOC	mg C/l	<5	<5	<5	<5	<5	20	16	18	13	na	<0.05
TOC	mg C/l	5.0	<5	<5	5.1	<5	26	28	23	15	na	<0.05
TPH	mg/l	<0.05	<0.05	<0.05	<0.05	<0.05	0.83	8.6	0.58	0.07	0.31	<0.05
Phenol index	µg/l	<5	<5	9.0	10.0	<5	<5	25	<5	11.0	9.0	>0.05
COD	mg/l	<10	<10	<10	10	<10	50	40	42	27	na	<0.05
BE-SPME	mM	0.3	0.6	0.6	0.7	0.6	2.4	27.6	3.8	1.8	0.6	<0.05

T-test, If concentrations were measured in outlet but the concentrations were below the LOQ in the inlet the LOQ values were taken to calculate if concentrations were significantly different. n.d. not possible to determine as both outlet as inlet concentrations were below the LOQ.

APPENDIX D CONCENTRATIONS OF PARAMETERS IN SPOT SAMPLES OF REFINERY C

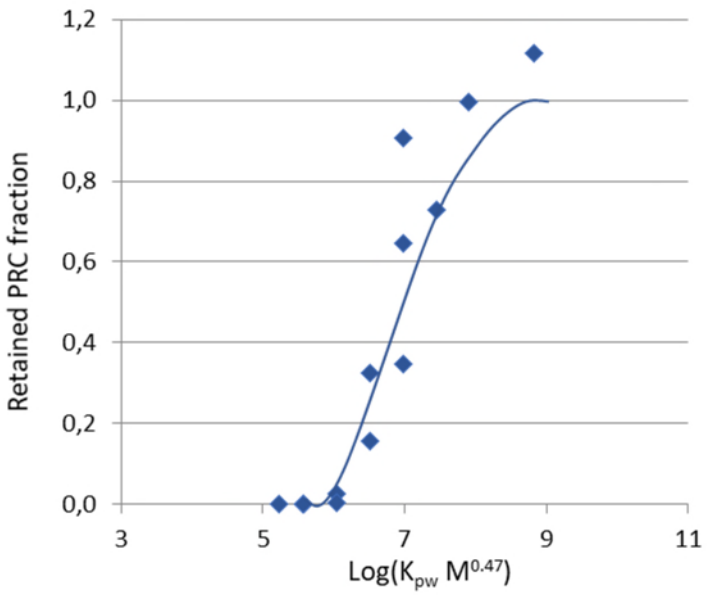
Parameter	Unit	Water inlet zone	Water inlet zone	Water inlet zone	Water inlet zone	Water inlet zone	Outlet zone	Outlet zone	Outlet zone	Outlet zone	Outlet zone	T-test
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 1	Week 2	Week 3	Week 4	Week 5	*P value*
Arsenic	µg/l	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	n.d.
Cadmium	µg/l	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	n.d.
Chromium	µg/l	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	n.d.
Cobalt	µg/l	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	n.d.
Copper	µg/l	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	n.d.
Mercury	µg/l	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	n.d.
Lead	µg/l	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	n.d.
Nickel	µg/l	5	5	5	5	5	5.4	6.8	6.5	6.7	7.1	<0.05
Selenium	µg/l	3	3	3	3	3	26	31	30	39	34	<0.05
Vanadium	µg/l	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	n.d.
Zinc	µg/l	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	n.d.
Total N	mg N/l	1	1	1	1	1	14	13	14	15	13	<0.05
DOC	mg C/l	6.6	6.6	6.6	6.6	6.8	27	28	26	24	24	<0.05
TOC	mg C/l	7.8	10	8.2	8.3	7.9	30	33	31	26	30	<0.05
TPH	mg/l	0.05	0.05	0.05	0.05	0.05	0.53	0.66	0.44	0.65	0.35	<0.05
Phenol index	µg/l	5	5	5	5	5	14	9.0	12	7	9	<0.05
COD	mg/l	11	12	12	12	12	57	56	59	54	59	<0.05
TSS	mg/l	0.85	0.64	0.48	0.61	0.76	14.7	27.3	29.5	20.2	22.8	<0.05
BE-SPME	mM	0.5	0.5	1.4	0.5	0.5	8.8	7.1	6.5	7.4	6.8	<0.05

T-test, If concentrations were measured in outlet but the concentrations were below the LOQ in the inlet the LOQ values were taken to calculate if concentrations were significantly different. n.d. not possible to determine as both outlet as inlet concentrations were below the LOQ.

APPENDIX E WATER SAMPLING RATES (RS) SILICONE PASSIVE SAMPLER SHEETS

	Rs (L/day)
Refinery A inlet	14
Refinery A outlet	84
Refinery B inlet	7
Refinery B outlet	48
Refinery C inlet	39
Refinery C outlet	345

Example of PRCs in PSS of outlet refinery C. Retained PRC fraction vs. Log(K_{pw} $M^{0.47}$).



APPENDIX F HC SPECIATION DATA (GCXGC) OF THE INLET AND OUTLET SAMPLES OF REFINERY A INLET

	Aliphatic Hydrocarbons					Aromatic Hydrocarbons				
	Linear Paraffins %	*Branched Paraffins* %	*Alkyl substituted cyclo-alkanes* %	*Alkyl substituted dicyclo-alkanes* %	*Alkyl substituted poly cyclo-alkanes* %	*Alkyl substituted mono aromatics* %	*Cyclo alkane substituted mono aromatics* %	*Alkyl substituted di aromatics* %	*Cyclo alkane substitued di aromatics* %	*Alkyl & cyclo alkane substituted poly aromatics* %
C07										
C08										
C09	<LOD	<LOD	<LOD	<LOD		<LOD	0.36			
C10	<LOD	<LOD	<LOD	<LOD		1.85	0.52	0.04		
C11	0.63	0.46	1.32	<LOD		3.57	0.74	0.11		
C12	<LOD	0.34		<LOD		2.44	0.91	0.58		
C13	1.68	1.25	1.71	<LOD		2.27	1.37	1.63	0.03	
C14	0.54	1.84		<LOD		6.95	2.67	2.38	0.32	0.15
C15	<LOD	0.26	1.16	<LOD		4.85	5.97	1.97	2.43	1.86
C16	0.34	1.31	0.92	<LOD		<LOD	2.23	1.67	3.60	2.02
C17	<LOD	<LOD	0.53	<LOD		<LOD	<LOD	2.99	4.32	2.81
C18	0.29	0.35	0.31	<LOD					3.12	1.88
C19	<LOD	<LOD	<LOD	<LOD					0.90	1.18
C20	<LOD	<LOD	<LOD							3.72
C21	<LOD	<LOD	<LOD							
C22	<LOD	<LOD								
C23	<LOD	<LOD								
C24	<LOD	<LOD								
C25	<LOD	<LOD								
C26	0.49	<LOD								
C27	0.57	<LOD								
C28	0.72	<LOD								

	Aliphatic Hydrocarbons					Aromatic Hydrocarbons				
	Linear Paraffins %	*Branched Paraffins* %	*Alkyl substituted cyclo-alkanes* %	*Alkyl substituted dicyclo-alkanes* %	*Alkyl substituted poly cyclo-alkanes* %	*Alkyl substituted mono aromatics* %	*Cyclo alkane substitued mono aromatics* %	*Alkyl substituted di aromatics* %	*Cyclo alkane substitued di aromatics* %	*Alkyl & cyclo alkane substituted poly aromatics* %
C29	1.21	<LOD								
C30	1.75	<LOD								
C31	2.47	<LOD								
C32	1.16	<LOD								
C33										
C34										
C35										
C36										
C37										
C38										
C39										
C40										

APPENDIX G HC SPECIATION DATA (GCXGC) OF THE INLET AND OUTLET SAMPLES OF REFINERY A OUTLET

	Aliphatic Hydrocarbons					Aromatic Hydrocarbons				
	Linear Paraffins %	*Branched Paraffins* %	*Alkyl substituted cyclo-alkanes* %	*Alkyl substituted dicyclo-alkanes* %	*Alkyl substituted poly cyclo-alkanes* %	*Alkyl substituted mono aromatics* %	*Cyclo alkane substituted mono aromatics* %	*Alkyl substituted di aromatics* %	*Cyclo alkane substitued di aromatics* %	*Alkyl & cyclo alkane substituted poly aromatics* %
C07										
C08										
C09	<LOD	<LOD	<LOD	0.19		<LOD	0.49			
C10	<LOD	<LOD	0.25	2.67		0.33	0.34	0.64		
C11	0.82	0.12	0.52	9.22		0.73	0.36	0.31		
C12	0.58	0.13	1.54	15.33		0.83	1.31	0.47		
C13	0.27	0.42	1.98	4.79		1.78	3.59	1.33	0.26	
C14	0.17	0.82	0.63	0.27		7.46	3.63	0.97	2.22	0.29
C15	0.58	0.55	0.19	<LOD		4.46	6.25	0.85	1.18	0.12
C16	0.56	0.34	0.15	<LOD		<LOD	0.81	0.78	3.79	0.72
C17	<LOD	<LOD	0.80	<LOD		<LOD	<LOD	1.48	2.32	4.27
C18	0.38	0.28	0.35						1.55	0.69
C19	0.69	<LOD	0.24						0.88	0.85
C20	0.20	0.12	<LOD							1.33
C21	0.73	<LOD	<LOD							
C22	0.16	0.72	<LOD							
C23	0.13	<LOD								
C24	0.18	0.47								
C25	0.18	<LOD								
C26	0.23	<LOD								
C27	0.33	<LOD								
C28	0.53	<LOD								

	Aliphatic Hydrocarbons					Aromatic Hydrocarbons				
	Linear Paraffins %	*Branched Paraffins* %	*Alkyl substituted cyclo-alkanes* %	*Alkyl substituted dicyclo-alkanes* %	*Alkyl substituted poly cyclo-alkanes* %	*Alkyl substituted mono aromatics* %	*Cyclo alkane substitued mono aromatics* %	*Alkyl substituted di aromatics* %	*Cyclo alkane substitued di aromatics* %	*Alkyl & cyclo alkane substituted poly aromatics* %
C29	0.12	<LOD								
C30	0.14	<LOD								
C31	0.22	<LOD								
C32	0.64	<LOD								
C33										
C34										
C35										
C36										
C37										
C38										
C39										
C40										

APPENDIX H HC SPECIATION DATA (GCXGC) OF THE INLET AND OUTLET SAMPLES OF REFINERY B INLET

	Aliphatic Hydrocarbons					Aromatic Hydrocarbons				
	Linear Paraffins %	*Branched Paraffins* %	*Alkyl substituted cyclo-alkanes* %	*Alkyl substituted dicyclo-alkanes* %	*Alkyl substituted poly cyclo-alkanes* %	*Alkyl substituted mono aromatics* %	*Cyclo alkane substituted mono aromatics* %	*Alkyl substituted di aromatics* %	*Cyclo alkane substitued di aromatics* %	*Alkyl & cyclo alkane substituted poly aromatics* %
C07										
C08										
C09							1.58			
C10	<LOD	<LOD	<LOD	<LOD		5.62	1.75	0.19		
C11	<LOD	<LOD	<LOD	<LOD		9.64	1.97	0.68		
C12	<LOD	<LOD	<LOD	<LOD		5.64	1.47	1.95		
C13	<LOD	<LOD	<LOD	<LOD		6.47	0.69	1.03	0.12	
C14	<LOD	<LOD	<LOD	<LOD		15.61	0.59	0.19	0.07	0.34
C15	<LOD	<LOD	<LOD	<LOD		10.30	7.53	1.08	0.34	0.38
C16	<LOD	<LOD	<LOD	<LOD		<LOD	4.41	0.42	1.38	0.30
C17	<LOD	<LOD	<LOD	<LOD		<LOD	<LOD	1.78	1.40	0.51
C18	<LOD	<LOD	<LOD	<LOD					0.49	5.57
C19	<LOD	<LOD	<LOD	<LOD					0.16	8.36
C20	<LOD	<LOD	<LOD	<LOD						<LOD
C21	<LOD	<LOD	<LOD							
C22	<LOD	<LOD								
C23	<LOD	<LOD								
C24	<LOD	<LOD								
C25	<LOD	<LOD								
C26	<LOD	<LOD								
C27	<LOD	<LOD								
C28	<LOD	<LOD								

	Aliphatic Hydrocarbons					Aromatic Hydrocarbons				
	Linear Paraffins %	*Branched Paraffins* %	*Alkyl substituted cyclo-alkanes* %	*Alkyl substituted dicyclo-alkanes* %	*Alkyl substituted poly cyclo-alkanes* %	*Alkyl substituted mono aromatics* %	*Cyclo alkane substitued mono aromatics* %	*Alkyl substituted di aromatics* %	*Cyclo alkane substitued di aromatics* %	*Alkyl & cyclo alkane substituted poly aromatics* %
C29	<LOD	<LOD								
C30	<LOD	<LOD								
C31	<LOD	<LOD								
C32	<LOD	<LOD								
C33										
C34										
C35										
C36										
C37										
C38										
C39										
C40										

APPENDIX I HC SPECIATION DATA (GCXGC) OF THE INLET AND OUTLET SAMPLES OF REFINERY B OUTLET

	Aliphatic Hydrocarbons					Aromatic Hydrocarbons				
	Linear Paraffins %	*Branched Paraffins* %	*Alkyl substituted cyclo-alkanes* %	*Alkyl substituted dicyclo-alkanes* %	*Alkyl substituted poly cyclo-alkanes* %	*Alkyl substituted mono aromatics* %	*Cyclo alkane substituted mono aromatics* %	*Alkyl substituted di aromatics* %	*Cyclo alkane substitued di aromatics* %	*Alkyl & cyclo alkane substituted poly aromatics* %
C07										
C08										
C09			0.02	0.26			0.23			
C10	0.01	<LOD	0.52	1.24		0.16	0.20	0.05		
C11	0.04	0.06	0.50	1.23		0.52	0.75	0.32		
C12	<LOD	<LOD	0.64	1.37		1.09	1.81	2.06		
C13	0.01	0.10	0.67	0.91		2.18	3.20	4.01	0.09	
C14	<LOD	<LOD	0.57	0.26		6.79	4.53	3.08	1.21	0.27
C15	0.08	0.32	0.54	0.07		2.89	8.03	2.49	3.20	1.17
C16	<LOD	<LOD	0.79	0.12		<LOD	1.60	1.48	6.27	2.47
C17	0.04	0.43	0.54	<LOD		<LOD	<LOD	4.31	6.27	2.42
C18	<LOD	<LOD	0.01	<LOD		<LOD	<LOD	<LOD	3.88	1.85
C19	0.02	0.32	<LOD	<LOD					1.34	0.98
C20	<LOD	<LOD	<LOD	<LOD					<LOD	4.17
C21	0.03	0.23	<LOD							
C22	0.01	<LOD								
C23	0.03	0.49								
C24	0.00	<LOD								
C25	0.04	0.02								
C26	0.01	<LOD								
C27	0.01	0.06								
C28	0.01	<LOD								

	Aliphatic Hydrocarbons					Aromatic Hydrocarbons				
	Linear Paraffins %	*Branched Paraffins* %	*Alkyl substituted cyclo-alkanes* %	*Alkyl substituted dicyclo-alkanes* %	*Alkyl substituted poly cyclo-alkanes* %	*Alkyl substituted mono aromatics* %	*Cyclo alkane substitued mono aromatics* %	*Alkyl substituted di aromatics* %	*Cyclo alkane substitued di aromatics* %	*Alkyl & cyclo alkane substituted poly aromatics* %
C29	0.01	<LOD								
C30	0.01	<LOD								
C31	0.00	<LOD								
C32	0.01	<LOD								
C33										
C34										
C35										
C36										
C37										
C38										
C39										
C40										

APPENDIX J HC SPECIATION DATA (GCXGC) OF THE INLET AND OUTLET SAMPLES OF REFINERY C INLET

	Aliphatic Hydrocarbons					Aromatic Hydrocarbons				
	Linear Paraffins %	*Branched Paraffins* %	*Alkyl substituted cyclo-alkanes* %	*Alkyl substituted dicyclo-alkanes* %	*Alkyl substituted poly cyclo-alkanes* %	*Alkyl substituted mono aromatics* %	*Cyclo alkane substituted mono aromatics* %	*Alkyl substituted di aromatics* %	*Cyclo alkane substitued di aromatics* %	*Alkyl & cyclo alkane substituted poly aromatics* %
C07										
C08										
C09							0.83			
C10	<LOD	<LOD	<LOD	<LOD		4.07	1.01	0.08		
C11	2.55	1.89	<LOD	<LOD		5.29	1.05	0.25		
C12	<LOD	1.97	<LOD	<LOD		4.77	0.93	0.85		
C13	4.14	3.81	<LOD	<LOD		4.07	0.46	0.34	<LOD	
C14	2.34	5.47	<LOD	<LOD		6.94	0.80	0.70	<LOD	0.07
C15	0.58	0.74	<LOD	<LOD		5.27	4.56	1.71	0.45	0.35
C16	1.53	2.79	<LOD	<LOD		<LOD	1.68	1.26	1.30	0.23
C17	<LOD	<LOD	<LOD	<LOD		<LOD	<LOD	3.22	1.42	0.50
C18	0.86	1.72	<LOD	<LOD					0.55	0.96
C19	<LOD	<LOD	<LOD	<LOD					<LOD	3.25
C20	<LOD	<LOD	<LOD	<LOD						<LOD
C21	<LOD	<LOD	<LOD							
C22	<LOD	<LOD								
C23	<LOD	<LOD								
C24	<LOD	<LOD								
C25	<LOD	<LOD								
C26	<LOD	<LOD								
C27	0.65	<LOD								
C28	1.03	<LOD								

	Aliphatic Hydrocarbons					Aromatic Hydrocarbons				
	Linear Paraffins %	*Branched Paraffins* %	*Alkyl substituted cyclo-alkanes* %	*Alkyl substituted dicyclo-alkanes* %	*Alkyl substituted poly cyclo-alkanes* %	*Alkyl substituted mono aromatics* %	*Cyclo alkane substitued mono aromatics* %	*Alkyl substituted di aromatics* %	*Cyclo alkane substitued di aromatics* %	*Alkyl & cyclo alkane substituted poly aromatics* %
C29	1.99	<LOD								
C30	2.87	<LOD								
C31	2.06	<LOD								
C32	1.81	<LOD								
C33										
C34										
C35										
C36										
C37										
C38										
C39										
C40										

APPENDIX K HC SPECIATION DATA (GCXGC) OF THE INLET AND OUTLET SAMPLES OF REFINERY C OUTLET

	Aliphatic Hydrocarbons					Aromatic Hydrocarbons				
	Linear Paraffins %	*Branched Paraffins* %	*Alkyl substituted cyclo-alkanes* %	*Alkyl substituted dicyclo-alkanes* %	*Alkyl substituted poly cyclo-alkanes* %	*Alkyl substituted mono aromatics* %	*Cyclo alkane substituted mono aromatics* %	*Alkyl substituted di aromatics* %	*Cyclo alkane substitued di aromatics* %	*Alkyl & cyclo alkane substituted poly aromatics* %
C07										
C08										
C09							0.09			
C10	<LOD	<LOD	1.13	1.78		0.08	0.03	0.00		
C11	0.05	0.10	1.46	3.08		0.32	0.04	0.01		
C12	<LOD	<LOD	2.92	5.74		0.14	0.38	0.05		
C13	0.09	0.12	2.98	6.03		0.53	1.23	0.25	0.01	
C14	<LOD	<LOD	2.70	3.31		4.55	3.07	0.40	0.02	0.02
C15	0.18	0.34	2.23	0.67		5.59	10.36	0.66	0.26	0.21
C16	<LOD	<LOD	4.45	0.68		<LOD	7.11	0.37	1.06	0.26
C17	0.13	0.72	2.60	<LOD		<LOD	<LOD	5.15	2.08	0.61
C18	<LOD	<LOD	<LOD	<LOD					4.27	1.02
C19	0.09	0.31	<LOD	<LOD					2.24	0.53
C20	<LOD	<LOD	<LOD	<LOD						1.88
C21	0.16	0.22	<LOD							
C22	0.06	<LOD								
C23	0.08	0.51								
C24	0.02	<LOD								
C25	0.07	0.11								
C26	<LOD	<LOD								
C27	<LOD	<LOD								
C28	<LOD	<LOD								

	Aliphatic Hydrocarbons					Aromatic Hydrocarbons				
	Linear Paraffins %	*Branched Paraffins* %	*Alkyl substituted cyclo-alkanes* %	*Alkyl substituted dicyclo-alkanes* %	*Alkyl substituted poly cyclo-alkanes* %	*Alkyl substituted mono aromatics* %	*Cyclo alkane substitued mono aromatics* %	*Alkyl substituted di aromatics* %	*Cyclo alkane substitued di aromatics* %	*Alkyl & cyclo alkane substituted poly aromatics* %
C29	<LOD	<LOD								
C30	<LOD	<LOD								
C31	<LOD	<LOD								
C32	<LOD	<LOD								
C33										
C34										
C35										
C36										
C37										
C38										
C39										
C40										

APPENDIX L CONCENTRATION FACTORS (CF) FROM SAMPLED VOLUME OF WATER IN THE DMSO EXTRACT USED FOR *IN VIVO* TESTING AND THE VOLUME OF DMSO EXTRACT. THESE FACTORS WERE USED TO CALCULATE THE TUs

Refinery	Inlet	Outlet
A	3.42×10^6	8.247×10^6
B	1.92×10^6	5.73×10^6
C	9.45×10^6	32.7×10^6

APPENDIX M *IN VITRO* ASSAY RESULTS EXPRESSED AS PMOL/G SILICONE SHEET

Refinery	DR-Luc (pmol TCDD EQ/ g silcone)	PAH-Calux (nmol B[a]P EQ / g silicone)	ER-Luc (pmol E2 EQ / g silicone)	AR-ECOscreen (nmol Flutamide EQ / g silicone)
A inlet	5.7	0.83	0.87	20
A Outlet	16	14	8.7	619
B Inlet	0.06	0.042	0.36	1.39
B Outlet	113	87	3.3	298
C Inlet	0.65	0.08	0.06	13
C Outlet	46	17	5.5	434

APPENDIX N HC BLOCK IN PASSIVE SAMPLER (PSS), PREDICTED DISSOLVED WATER CONCENTRATION (C_W), AND TU_{CHEM} FOR *D. MAGNA*, *V. FISCHERI*, *HYALELLA AZTECA*, *PSEUDOKIRCHNERIELLA SUBCAPITATA* FOR REFINERY A, B, AND C INLET AND OUTLET WATERS

Ref. A inlet

HC block	PSS GCxGC µg/g silicone	LogKow	Cw µg/l	TU_{chem} *D. magna*	TU_{chem} Microtox	TU_{chem} *Hyalella azteca*	TU_{chem} *Pseudokirchneriella subcapitata*
C11 nP	0.15	6.42	0.00635	0.0001	0.0001	0.0005	0.0003
C12 nP	0.00	6.98	0.00000	0.0000	0.0000	0.0000	0.0000
C13 nP	0.41	7.55	0.01689	0.0003	0.0002	0.0011	0.0008
C14 nP	0.13	8.11	0.00543	0.0001	0.0000	0.0003	0.0002
C15 nP	0.00	8.68	0.00000	0.0000	0.0000	0.0000	0.0000
C16 nP	0.08	9.24	0.00345	0.0001	0.0000	0.0002	0.0001
C17 nP	0.00	9.80	0.00000	0.0000	0.0000	0.0000	0.0000
C18 nP	0.07	10.38	0.00293	0.0000	0.0000	0.0001	0.0001
C19 nP	0.00	10.90	0.00000	0.0000	0.0000	0.0000	0.0000
C20 nP	0.00	11.46	0.00000	0.0000	0.0000	0.0000	0.0000
C21 nP	0.00	12.02	0.00000	0.0000	0.0000	0.0000	0.0000
C22 nP	0.00	12.58	0.00000	0.0000	0.0000	0.0000	0.0000
C23 nP	0.00	13.14	0.00000	0.0000	0.0000	0.0000	0.0000
C24 nP	0.00	13.70	0.00000	0.0000	0.0000	0.0000	0.0000
C25 nP	0.00	14.35	0.00000	0.0000	0.0000	0.0000	0.0000
C26 nP	0.12	14.92	0.00490	0.0000	0.0000	0.0002	0.0001
C27 nP	0.14	15.49	0.00573	0.0001	0.0000	0.0002	0.0001
C28 nP	0.17	15.94	0.00720	0.0001	0.0000	0.0002	0.0002
C29 nP	0.29	16.64	0.01215	0.0001	0.0000	0.0004	0.0003
C30 nP	0.43	17.06	0.01757	0.0001	0.0001	0.0005	0.0004
C31 nP	0.60	17.78	0.02486	0.0002	0.0001	0.0007	0.0005
C32 nP	0.28	18.18	0.01168	0.0001	0.0000	0.0003	0.0002
C11 iP	0.11	6.35	0.00459	0.000	0.0000	0.0004	0.0002
C12 iP	0.08	6.90	0.00343	0.000	0.0000	0.0003	0.0002
C13 iP	0.31	7.45	0.01262	0.000	0.0001	0.0009	0.0006
C14 iP	0.45	7.99	0.01849	0.000	0.0002	0.0012	0.0008
C15 iP	0.06	8.58	0.00261	0.000	0.0000	0.0002	0.0001
C16 iP	0.32	9.10	0.01317	0.000	0.0001	0.0007	0.0005
C17 iP	0.00	9.76	0.00000	0.000	0.0000	0.0000	0.0000
C18 iP	0.09	10.04	0.00351	0.000	0.0000	0.0002	0.0001
C19 iP	0.00	10.33	0.00000	0.000	0.0000	0.0000	0.0000
C20 iP	0.00	11.43	0.00000	0.000	0.0000	0.0000	0.0000
C21 iP	0.00	11.99	0.00000	0.000	0.0000	0.0000	0.0000
C22 iP	0.00	12.55	0.00000	0.000	0.0000	0.0000	0.0000

HC block	PSS GCxGC µg/g silicone	LogKow	Cw µg/l	TU_{chem} *D. magna*	TU_{chem} Microtox	TU_{chem} *Hyalella azteca*	TU_{chem} *Pseudokirchneriella subcapitata*
C23 iP	0.00	13.12	0.00000	0.000	0.0000	0.0000	0.0000
C24 iP	0.00	13.69	0.00000	0.000	0.0000	0.0000	0.0000
C25 iP	0.00	14.11	0.00000	0.000	0.0000	0.0000	0.0000
C10mN	0.00	5.37	0.00000	0.000	0.0000000	0.0000	0.0000
C11mN	0.32	5.95	0.00109	0.000	0.0000104	0.0001	0.0001
C12mN	0.00	6.47	0.00000	0.000	0.0000000	0.0000	0.0000
C13mN	0.42	7.02	0.01717	0.000	0.0001543	0.0012	0.0008
C14mN	0.00	7.59	0.00000	0.000	0.0000000	0.0000	0.0000
C15mN	0.28	8.13	0.01170	0.000	0.0000912	0.0007	0.0005
C16mN	0.22	8.70	0.00928	0.000	0.0001	0.0005	0.0003
C17mN	0.13	9.28	0.00533	0.000	0.0000	0.0003	0.0002
C18mN	0.08	9.83	0.00315	0.000	0.0000	0.0002	0.0001
C10	0.00	4.94	0.00000	0.000	0.0000	0.0000	0.0000
C11	0.00	5.48	0.00000	0.000	0.0000	0.0000	0.0000
C12	0.00	5.99	0.00000	0.000	0.0000	0.0000	0.0000
C13	0.00	6.63	0.00000	0.000	0.0000	0.0000	0.0000
C14	0.00	7.23	0.00000	0.000	0.0000	0.0000	0.0000
C15	0.00	7.81	0.00000	0.000	0.0000	0.0000	0.0000
C16	0.00	8.39	0.00000	0.000	0.0000	0.0000	0.0000
C10 MAH	0.45	3.98	0.14	0.000	0.0000	0.0002	0.0001
C11 MAH	0.87	4.53	0.07	0.000	0.0000	0.0003	0.0002
C12 MAH	0.60	5.06	0.02	0.000	0.0000	0.0002	0.0001
C13 MAH	0.55	5.62	0.00	0.000	0.0000	0.0002	0.0001
C14 MAH	1.69	6.08	0.07	0.002	0.0008	0.0059	0.0040
C15 MAH	1.18	6.54	0.05	0.001	0.0005	0.0038	0.0026
C09 nMAH	0.09	2.96	0.27	0.00002	0.0000	0.0001	0.0000
C10 nMAH	0.13	3.48	0.1197	0.00002	0.0000	0.0001	0.0000
C11 nMAH	0.18	3.93	0.0614	0.00002	0.0000	0.0001	0.0001
C12 nMAH	0.22	4.38	0.0271	0.00002	0.0000	0.0001	0.0001
C13 nMAH	0.33	4.84	0.0144	0.00003	0.0000	0.0001	0.0001
C14 nMAH	0.65	5.32	0.0092	0.00005	0.0000	0.0002	0.0001
C15 nMAH	1.46	5.84	0.0063	0.00010	0.0000	0.0004	0.0002
C16 nMAH	0.54	6.34	0.02246	0.00048	0.0002	0.0017	0.0011
C10DAH	0.01	3.35	0.0111	0.000	0.0000	0.0000	0.0000
C11DAH	0.03	3.80	0.0122	0.000	0.0000	0.0000	0.0000
C12DAH	0.14	4.23	0.0242	0.000	0.0000	0.0001	0.0001
C13DAH	0.40	4.70	0.0231	0.000	0.0000	0.0002	0.0002
C14DAH	0.58	5.14	0.0125	0.000	0.0000	0.0003	0.0002
C15DAH	0.48	5.61	0.0035	0.000	0.0000	0.0002	0.0001
C16DAH	0.41	6.06	0.01683	0.001	0.0003	0.0022	0.0015

HC block	PSS GCxGC µg/g silicone	LogKow	Cw µg/l	TU_{chem} *D. magna*	TU_{chem} Microtox	TU_{chem} *Hyalella azteca*	TU_{chem} *Pseudokirchneriella subcapitata*
C17DAH	0.73	6.62	0.03012	0.001	0.0005	0.0038	0.0025
C13 nDAH	0.01	4.09	0.002	0.000	0.0000	0.0000	0.0000
C14 nDAH	0.08	4.76	0.004	0.000	0.0000	0.0000	0.0000
C15 nDAH	0.59	5.16	0.012	0.000	0.0000	0.0003	0.0002
C16 nDAH	0.88	5.61	0.006	0.000	0.0000	0.0004	0.0003
C17 nDAH	1.05	6.04	0.04345	0.002	0.0007	0.0055	0.0037
C18 nDAH	0.76	6.43	0.03139	0.001	0.0005	0.0037	0.0025
C19 nDAH	0.22	6.99	0.00910	0.000	0.0001	0.0010	0.0007
C14 PAH	0.04	4.57	0.0029	0.000	0.0000	0.0000	0.0000
C15 PAH	0.45	4.97	0.0142	0.000	0.0000	0.0002	0.0002
C16 PAH	0.49	5.38	0.0062	0.000	0.0000	0.0002	0.0001
C17 PAH	0.69	5.48	0.0068	0.000	0.0000	0.0003	0.0002
C18 PAH	0.46	6.12	0.0283	0.001	0.0004	0.0034	0.0023
C19 PAH	0.29	6.60	0.0189	0.001	0.0003	0.0022	0.0015
C20 PAH	0.91	6.93	0.0119	0.000	0.0002	0.0013	0.0009

Ref. A outlet

HC block	PSS GCxGC µg/g silicone	Cw µg/l	TU_{chem} *D. magna*	TU_{chem} Microtox	TU_{chem} *Hyalella azteca*	TU_{chem} *Pseudokirchneriella subcapitata*
C10 nP	0.00	0.00000	0.00000	0.00000	0.00000	0.00000
C11 nP	0.14	0.00091	0.00002	0.00001	0.00007	0.00005
C12 nP	0.10	0.00065	0.00001	0.00001	0.00005	0.00003
C13 nP	0.46	0.00306	0.00006	0.00003	0.00021	0.00014
C14 nP	0.29	0.00192	0.00003	0.00002	0.00012	0.00008
C15 nP	0.10	0.00066	0.00001	0.00001	0.00004	0.00003
C16 nP	0.10	0.00064	0.00001	0.00000	0.00004	0.00002
C17 nP	0.00		0.00000	0.00000	0.00000	0.00000
C18 nP	0.07	0.00044	0.00001	0.00000	0.00002	0.00001
C19 nP	0.01	0.00008	0.00000	0.00000	0.00000	0.00000
C20 nP	0.03	0.00023	0.00000	0.00000	0.00001	0.00001
C21 nP	0.01	0.00008	0.00000	0.00000	0.00000	0.00000
C22 nP	0.03	0.00018	0.00000	0.00000	0.00001	0.00000
C23 nP	0.02	0.00014	0.00000	0.00000	0.00001	0.00000
C24 nP	0.03	0.00020	0.00000	0.00000	0.00001	0.00001
C25 nP	0.03	0.00021	0.00000	0.00000	0.00001	0.00000
C26 nP	0.04	0.00026	0.00000	0.00000	0.00001	0.00001
C27 nP	0.06	0.00037	0.00000	0.00000	0.00001	0.00001
C28 nP	0.09	0.00060	0.00001	0.00000	0.00002	0.00001
C29 nP	0.18	0.00116	0.00001	0.00000	0.00004	0.00002
C30 nP	0.24	0.00162	0.00001	0.00001	0.00005	0.00003
C31 nP	0.35	0.00230	0.00002	0.00001	0.00007	0.00004
C32 nP	0.11	0.00073	0.00001	0.00000	0.00002	0.00001
C11 iP	0.20	0.00132	0.00003	0.00001	0.00011	0.00007
C12 iP	0.22	0.00143	0.00003	0.00001	0.00011	0.00007
C13 iP	0.69	0.00458	0.00009	0.00004	0.00031	0.00021
C14 iP	1.42	0.00938	0.00017	0.00008	0.00059	0.00040
C15 iP	0.95	0.00630	0.00011	0.00005	0.00037	0.00025
C16 iP	0.58	0.00382	0.00006	0.00003	0.00021	0.00014
C17 iP	0.00		0.00000	0.00000	0.00000	0.00000
C18 iP	0.36	0.00237	0.00003	0.00002	0.00012	0.00008
C19 iP	0.00		0.00000	0.00000	0.00000	0.00000
C20 iP	0.20	0.00135	0.00002	0.00001	0.00006	0.00004
C21 iP	0.00		0.00000	0.00000	0.00000	0.00000
C22 iP	0.12	0.00081	0.00001	0.00000	0.00003	0.00002
C23 iP	0.00		0.00000	0.00000	0.00000	0.00000
C24 iP	0.08	0.00054	0.00001	0.00000	0.00002	0.00001
C25 iP	0.00		0.00000	0.00000	0.00000	0.00000
C26 iP	0.00		0.00000	0.00000	0.00000	0.00000
C27 iP	0.00		0.00000	0.00000	0.00000	0.00000
C09 mN	0.00	0.00000	0.00000	0.00000	0.00000	0.00000
C10 mN	0.44	0.00558	0.00004	0.00002	0.00013	0.00009
C11 mN	0.89	0.00300	0.00006	0.00003	0.00022	0.00015
C12 mN	2.66	0.01758	0.00038	0.00017	0.00131	0.00088
C13 mN	3.41	0.02257	0.00044	0.00020	0.00155	0.00104
C14 mN	1.09	0.00721	0.00013	0.00006	0.00046	0.00031
C15 mN	0.33	0.00216	0.00004	0.00002	0.00013	0.00009
C16 mN	0.25	0.00168	0.00003	0.00001	0.00009	0.00006
C17 mN	0.12	0.00081	0.00001	0.00001	0.00004	0.00003

HC block	PSS GCxGC µg/g silicone	Cw µg/l	TU_{chem} *D. magna*	TU_{chem} Microtox	TU_{chem} *Hyalella azteca*	TU_{chem} *Pseudokirchneriella subcapitata*
C18 mN	0.06	0.00040	0.00001	0.00000	0.00002	0.00001
C19 mN	0.04	0.00028	0.00000	0.00000	0.00001	0.00001
C09 diN	0.32	0.03646	0.00003	0.00002	0.00012	0.00008
C10 diN	4.49	0.15207	0.00040	0.00018	0.00140	0.00094
C11 diN	15.87	0.15622	0.00121	0.00055	0.00420	0.00283
C12 diN	26.40	0.08240	0.00172	0.00079	0.00601	0.00405
C13 diN	8.23	0.05443	0.00108	0.00049	0.00378	0.00254
C14 diN	0.47	0.00308	0.00006	0.00003	0.00020	0.00013
C15 diN	0.00		0.00000	0.00000	0.00000	0.00000
C16 diN	0.00		0.00000	0.00000	0.00000	0.00000
C10 MAH	0.56	0.16957	0.00007	0.00003	0.00026	0.00018
C11 MAH	1.25	0.10780	0.00014	0.00006	0.00049	0.00033
C12 MAH	1.42	0.03623	0.00014	0.00006	0.00048	0.00032
C13 MAH	3.07	0.02190	0.00025	0.00012	0.00089	0.00060
C14 MAH	12.84	0.08490	0.00206	0.00094	0.00718	0.00483
C15 MAH	7.59	0.05016	0.00113	0.00052	0.00395	0.00266
C09 nMAH	0.705	2.15705	0.00012	0.00006	0.00042	0.00028
C10 nMAH	0.58	0.54660	0.00008	0.00004	0.00029	0.00020
C11 nMAH	0.62	0.20960	0.00008	0.00003	0.00026	0.00018
C12 nMAH	2.26	0.27529	0.00024	0.00011	0.00084	0.00056
C13 nMAH	6.04	0.25911	0.00056	0.00025	0.00195	0.00131
C14 nMAH	6.25	0.08887	0.00050	0.00023	0.00176	0.00118
C15 nMAH	10.34	0.04471	0.00073	0.00033	0.00253	0.00171
C16 nMAH	1.40	0.00927	0.00020	0.00009	0.00069	0.00046
C10DAH	0.01	0.01416	0.00000	0.00000	0.00001	0.00001
C11DAH	0.05	0.02477	0.00001	0.00001	0.00004	0.00003
C12DAH	0.81	0.13794	0.00016	0.00007	0.00055	0.00037
C13DAH	2.29	0.13310	0.00039	0.00018	0.00134	0.00091
C14DAH	1.67	0.03610	0.00025	0.00011	0.00086	0.00058
C15DAH	1.39	0.01012	0.00018	0.00008	0.00063	0.00042
C16DAH	1.35	0.00890	0.00034	0.00015	0.00118	0.00080
C17DAH	2.55	0.01687	0.00060	0.00028	0.00210	0.00142
C13 nDAH	0.04	0.00831	0.00001	0.00000	0.00002	0.00002
C14 nDAH	3.79	0.19453	0.00059	0.00027	0.00207	0.00139
C15 nDAH	2.03	0.04161	0.00028	0.00013	0.00098	0.00066
C16 nDAH	6.52	0.04743	0.00080	0.00036	0.00278	0.00187
C17 nDAH	4.00	0.02646	0.00096	0.00044	0.00334	0.00225
C18 nDAH	2.68	0.01770	0.00060	0.00028	0.00211	0.00142
C19 nDAH	1.50	0.00991	0.00032	0.00015	0.00111	0.00075
C14 PAH	0.50	0.03979	0.00008	0.00004	0.00029	0.00019
C15 PAH	0.21	0.00664	0.00003	0.00001	0.00011	0.00007
C16 PAH	1.21	0.01510	0.00015	0.00007	0.00054	0.00036
C17 PAH	7.35	0.07267	0.00087	0.00040	0.00304	0.00205
C18 PAH	1.18	0.00781	0.00027	0.00012	0.00095	0.00064
C19 PAH	1.46	0.00964	0.00032	0.00014	0.00110	0.00074
C20 PAH	2.30	0.01519	0.00048	0.00022	0.00166	0.00112

Ref. B inlet

HC block	PSS GCxGC µg/g silicone	Cw µg/l	TU_{chem} *D. magna*	TU_{chem} Microtox	TU_{chem} *Hyalella azteca*	TU_{chem} *Pseudokirchneriella subcapitata*
C10 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C11 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C12 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C13 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C14 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C15 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C16 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C17 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C18 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C19 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C20 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C21 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C22 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C23 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C24 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C25 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C26 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C27 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C28 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C29 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C30 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C31 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C11 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C12 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C13 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C14 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C15 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C16 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C17 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C18 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C19 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C20 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C21 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C22 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C23 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C24 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C25 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C10 mN	0.00	0.00000	0.000	0.000	0.000	0.000
C11 mN	0.00	0.00000	0.000	0.000	0.000	0.000
C12 mN	0.00	0.00000	0.000	0.000	0.000	0.000
C13 mN	0.00	0.00000	0.000	0.000	0.000	0.000
C14 mN	0.00	0.00000	0.000	0.000	0.000	0.000
C15 mN	0.00	0.00000	0.000	0.000	0.000	0.000
C16 mN	0.00	0.00000	0.000	0.000	0.000	0.000
C17 mN	0.00	0.00000	0.000	0.000	0.000	0.000

HC block	PSS GCxGC µg/g silicone	Cw µg/l	TU_{chem} *D. magna*	TU_{chem} Microtox	TU_{chem} *Hyalella azteca*	TU_{chem} *Pseudokirchneriella subcapitata*
C10 diN	0.00	0.00000	0.000	0.000	0.000	0.000
C11 diN	0.00	0.00000	0.000	0.000	0.000	0.000
C12 diN	0.00	0.00000	0.000	0.000	0.000	0.000
C13 diN	0.00	0.00000	0.000	0.000	0.000	0.000
C14 diN	0.00	0.00000	0.000	0.000	0.000	0.000
C15 diN	0.00	0.00000	0.000	0.000	0.000	0.000
C16 diN	0.00	0.00000	0.000	0.000	0.000	0.000
C10 MAH	0.25	0.0756	0.000	0.000	0.000	0.000
C11 MAH	0.43	0.0369	0.000	0.000	0.000	0.000
C12 MAH	0.25	0.0064	0.000	0.000	0.000	0.000
C13 MAH	0.29	0.0021	0.000	0.000	0.000	0.000
C14 MAH	0.70	0.0446	0.001	0.000	0.004	0.003
C15 MAH	0.46	0.0294	0.001	0.000	0.002	0.002
C09 nMAH	0.07	0.2151	0.000	0.000	0.000	0.000
C10 nMAH	0.08	0.0731	0.000	0.000	0.000	0.000
C11 nMAH	0.09	0.0298	0.000	0.000	0.000	0.000
C12 nMAH	0.07	0.0080	0.000	0.000	0.000	0.000
C13 nMAH	0.03	0.0013	0.000	0.000	0.000	0.000
C14 nMAH	0.03	0.0004	0.000	0.000	0.000	0.000
C15 nMAH	0.34	0.0015	0.000	0.000	0.000	0.000
C16 nMAH	0.20	0.0126	0.000	0.000	0.001	0.001
C10DAH	0.01	0.0108	0.000	0.000	0.000	0.000
C11DAH	0.03	0.0139	0.000	0.000	0.000	0.000
C12DAH	0.09	0.0149	0.000	0.000	0.000	0.000
C13DAH	0.05	0.0027	0.000	0.000	0.000	0.000
C14DAH	0.01	0.0002	0.000	0.000	0.000	0.000
C15DAH	0.05	0.0003	0.000	0.000	0.000	0.000
C16DAH	0.02	0.0012	0.000	0.000	0.000	0.000
C17DAH	0.08	0.0051	0.001	0.000	0.002	0.002
C13 nDAH	0.01	0.0013	0.000	0.000	0.000	0.000
C14 nDAH	0.00	0.0002	0.000	0.000	0.000	0.000
C15 nDAH	0.01	0.0003	0.000	0.000	0.000	0.000
C16 nDAH	0.06	0.0004	0.000	0.000	0.000	0.000
C17 nDAH	0.06	0.0040	0.000	0.000	0.001	0.000
C18 nDAH	0.02	0.0014	0.000	0.000	0.000	0.000
C19 nDAH	0.01	0.0005	0.000	0.000	0.000	0.000
C14 PAH	0.02	0.0012	0.000	0.000	0.000	0.000
C15 PAH	0.02	0.0005	0.000	0.000	0.000	0.000
C16 PAH	0.01	0.0002	0.000	0.000	0.000	0.000
C17 PAH	0.02	0.0002	0.000	0.000	0.000	0.000
C18 PAH	0.25	0.0015	0.000	0.000	0.000	0.000
C19 PAH	0.37	0.0159	0.001	0.000	0.002	0.001
C20 PAH	0.00	0.0239	0.001	0.000	0.003	0.002

Ref. B outlet

HC block	PSS GCxGC µg/g silicone	Cw µg/l	TU_{chem} *D. magna*	TU_{chem} Microtox	TU_{chem} *Hyalella azteca*	TU_{chem} *Pseudokirchneriella subcapitata*
C10 nP	0.07	0.00028	0.000	0.000	0.000	0.000
C11 nP	0.27	0.0024	0.000	0.000	0.000	0.000
C12 nP	0.00	0.0000	0.000	0.000	0.000	0.000
C13 nP	0.09	0.0008	0.000	0.000	0.000	0.000
C14 nP	0.00	0.0000	0.000	0.000	0.000	0.000
C15 nP	0.59	0.0054	0.000	0.000	0.000	0.000
C16 nP	0.00	0.0000	0.000	0.000	0.000	0.000
C17 nP	0.33	0.0030	0.000	0.000	0.000	0.000
C18 nP	0.00	0.0000	0.000	0.000	0.000	0.000
C19 nP	0.13	0.0011	0.000	0.000	0.000	0.000
C20 nP	0.00	0.0000	0.000	0.000	0.000	0.000
C21 nP	0.23	0.0021	0.000	0.000	0.000	0.000
C22 nP	0.11	0.0010	0.000	0.000	0.000	0.000
C23 nP	0.24	0.0022	0.000	0.000	0.000	0.000
C24 nP	0.01	0.0001	0.000	0.000	0.000	0.000
C25 nP	0.27	0.0025	0.000	0.000	0.000	0.000
C26 nP	0.09	0.0008	0.000	0.000	0.000	0.000
C27 nP	0.10	0.0009	0.000	0.000	0.000	0.000
C28 nP	0.07	0.0007	0.000	0.000	0.000	0.000
C29 nP	0.07	0.0007	0.000	0.000	0.000	0.000
C30 nP	0.05	0.0005	0.000	0.000	0.000	0.000
C31 nP	0.03	0.0003	0.000	0.000	0.000	0.000
C32 nP	0.05	0.0005	0.000	0.000	0.000	0.000
C11 iP	0.43	0.0039	0.000	0.000	0.000	0.000
C12 iP	0.00	0.0000	0.000	0.000	0.000	0.000
C13 iP	0.78	0.0071	0.000	0.000	0.000	0.000
C14 iP	0.00	0.0000	0.000	0.000	0.000	0.000
C15 iP	2.39	0.0219	0.000	0.000	0.001	0.001
C16 iP	0.00	0.0000	0.000	0.000	0.000	0.000
C17 iP	3.24	0.0295	0.000	0.000	0.002	0.001
C18 iP	0.00	0.0000	0.000	0.000	0.000	0.000
C19 iP	2.45	0.0224	0.000	0.000	0.001	0.001
C20 iP	0.00	0.0000	0.000	0.000	0.000	0.000
C21 iP	1.73	0.0158	0.000	0.000	0.001	0.000
C22 iP	0.00	0.0000	0.000	0.000	0.000	0.000
C23 iP	3.69	0.0337	0.000	0.000	0.001	0.001
C24 iP	0.00	0.0000	0.000	0.000	0.000	0.000
C25 iP	0.12	0.0011	0.000	0.000	0.000	0.000
C26 iP	0.00	0.0000	0.000	0.000	0.000	0.000
C27 iP	0.43	0.0039	0.000	0.000	0.000	0.000
C09 mN	0.11	0.0051	0.000	0.000	0.000	0.000
C10 mN	3.95	0.0505	0.000	0.000	0.001	0.001
C11 mN	3.80	0.0128	0.000	0.000	0.001	0.001
C12 mN	4.84	0.0441	0.001	0.000	0.003	0.002
C13 mN	5.05	0.0461	0.001	0.000	0.003	0.002
C14 mN	4.33	0.0396	0.001	0.000	0.003	0.002
C15 mN	4.09	0.0374	0.001	0.000	0.002	0.001

HC block	PSS GCxGC µg/g silicone	Cw µg/l	TU_{chem} *D. magna*	TU_{chem} Microtox	TU_{chem} *Hyalella azteca*	TU_{chem} *Pseudokirchneriella subcapitata*
C16 mN	5.99	0.0547	0.001	0.000	0.003	0.002
C17 mN	4.10	0.0374	0.001	0.000	0.002	0.001
C18 mN	0.07	0.0006	0.000	0.000	0.000	0.000
C09 diN	2.00	0.2279	0.000	0.000	0.001	0.001
C10 diN	9.39	0.3180	0.001	0.000	0.003	0.002
C11 diN	9.34	0.0919	0.001	0.000	0.002	0.002
C12 diN	10.36	0.0323	0.001	0.000	0.002	0.002
C13 diN	6.92	0.0632	0.001	0.001	0.004	0.003
C14 diN	2.00	0.0183	0.000	0.000	0.001	0.001
C15 diN	0.57	0.0052	0.000	0.000	0.000	0.000
C16 diN	0.88	0.0080	0.000	0.000	0.000	0.000
C10 MAH	1.21	0.3659	0.000	0.000	0.001	0.000
C11 MAH	3.91	0.3364	0.000	0.000	0.002	0.001
C12 MAH	8.27	0.2109	0.001	0.000	0.003	0.002
C13 MAH	16.55	0.1182	0.001	0.001	0.005	0.003
C14 MAH	51.49	0.4700	0.011	0.005	0.040	0.027
C15 MAH	21.95	0.2003	0.005	0.002	0.016	0.011
C09 nMAH	1.77	5.4063	0.000	0.000	0.001	0.001
C10 nMAH	1.54	1.4456	0.000	0.000	0.001	0.001
C11 nMAH	5.67	1.9303	0.001	0.000	0.002	0.002
C12 nMAH	13.75	1.6762	0.001	0.001	0.005	0.003
C13 nMAH	24.26	1.0400	0.002	0.001	0.008	0.005
C14 nMAH	34.32	0.4878	0.003	0.001	0.010	0.006
C15 nMAH	60.91	0.2634	0.004	0.002	0.015	0.010
C16 nMAH	12.10	0.1105	0.002	0.001	0.008	0.006
C10DAH	0.35	0.4444	0.000	0.000	0.000	0.000
C11DAH	2.42	1.1107	0.001	0.000	0.002	0.001
C12DAH	15.61	2.6681	0.003	0.001	0.011	0.007
C13DAH	30.43	1.7685	0.005	0.002	0.018	0.012
C14DAH	23.36	0.5041	0.003	0.002	0.012	0.008
C15DAH	18.87	0.1376	0.002	0.001	0.009	0.006
C16DAH	11.21	0.1023	0.004	0.002	0.014	0.009
C17DAH	32.66	0.2981	0.011	0.005	0.037	0.025
C13 nDAH	0.66	0.1537	0.000	0.000	0.000	0.000
C14 nDAH	9.18	0.4708	0.001	0.001	0.005	0.003
C15 nDAH	24.28	0.4971	0.003	0.002	0.012	0.008
C16 nDAH	47.59	0.3460	0.006	0.003	0.020	0.014
C17 nDAH	47.58	0.4343	0.016	0.007	0.055	0.037
C18 nDAH	29.42	0.2685	0.009	0.004	0.032	0.022
C19 nDAH	10.16	0.0927	0.003	0.001	0.010	0.007
C14 PAH	2.02	0.1598	0.000	0.000	0.001	0.001
C15 PAH	8.90	0.2784	0.001	0.001	0.004	0.003
C16 PAH	18.70	0.2335	0.002	0.001	0.008	0.006
C17 PAH	18.39	0.1817	0.002	0.001	0.008	0.005
C18 PAH	14.02	0.1279	0.004	0.002	0.016	0.010
C19 PAH	7.47	0.0682	0.002	0.001	0.008	0.005
C20 PAH	31.65	0.2889	0.009	0.004	0.032	0.021

Ref. C inlet

HC block	PSS GCxGC µg/g silicone	Cw µg/l	TU_{chem} *D. magna*	TU_{chem} Microtox	TU_{chem} *Hyalella azteca*	TU_{chem} *Pseudokirchneriella subcapitata*
C11 nP	0.27	0.00354	0.000	0.000	0.000	0.000
C12 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C13 nP	0.43	0.00575	0.000	0.000	0.000	0.000
C14 nP	0.24	0.00325	0.000	0.000	0.000	0.000
C15 nP	0.06	0.00081	0.000	0.000	0.000	0.000
C16 nP	0.16	0.00212	0.000	0.000	0.000	0.000
C17 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C18 nP	0.09	0.00120	0.000	0.000	0.000	0.000
C19 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C20 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C21 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C22 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C23 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C24 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C25 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C26 nP	0.00	0.00000	0.000	0.000	0.000	0.000
C27 nP	0.07	0.00090	0.000	0.000	0.000	0.000
C28 nP	0.11	0.00144	0.000	0.000	0.000	0.000
C29 nP	0.21	0.00277	0.000	0.000	0.000	0.000
C30 nP	0.30	0.00399	0.000	0.000	0.000	0.000
C31 nP	0.21	0.00286	0.000	0.000	0.000	0.000
C32 nP	0.19	0.00251	0.000	0.000	0.000	0.000
C11 iP	0.20	0.00263	0.000	0.000	0.000	0.000
C12 iP	0.21	0.00273	0.000	0.000	0.000	0.000
C13 iP	0.40	0.00529	0.000	0.000	0.000	0.000
C14 iP	0.57	0.00759	0.000	0.000	0.000	0.000
C15 iP	0.08	0.00103	0.000	0.000	0.000	0.000
C16 iP	0.29	0.00387	0.000	0.000	0.000	0.000
C17 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C18 iP	0.18	0.00239	0.000	0.000	0.000	0.000
C19 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C20 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C21 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C22 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C23 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C24 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C25 iP	0.00	0.00000	0.000	0.000	0.000	0.000
C10 mN	0.00	0.00000	0.000	0.000	0.000	0.000
C11 mN	0.00	0.00000	0.000	0.000	0.000	0.000
C12 mN	0.00	0.00000	0.000	0.000	0.000	0.000
C13 mN	0.00	0.00000	0.000	0.000	0.000	0.000
C14 mN	0.00	0.00000	0.000	0.000	0.000	0.000
C15 mN	0.00	0.00000	0.000	0.000	0.000	0.000
C16 mN	0.00	0.00000	0.000	0.000	0.000	0.000
C17 mN	0.00	0.00000	0.000	0.000	0.000	0.000

HC block	PSS GCxGC µg/g silicone	Cw µg/l	TU_{chem} *D. magna*	TU_{chem} Microtox	TU_{chem} *Hyalella azteca*	TU_{chem} *Pseudokirchneriella subcapitata*
C10 diN	0.00	0.00000	0.000	0.000	0.000	0.000
C11 diN	0.00	0.00000	0.000	0.000	0.000	0.000
C12 diN	0.00	0.00000	0.000	0.000	0.000	0.000
C13 diN	0.00	0.00000	0.000	0.000	0.000	0.000
C14 diN	0.00	0.00000	0.000	0.000	0.000	0.000
C15 diN	0.00	0.00000	0.000	0.000	0.000	0.000
C16 diN	0.00	0.00000	0.000	0.000	0.000	0.000
C10 MAH	0.43	0.12850	0.000	0.000	0.000	0.000
C11 MAH	0.55	0.04746	0.000	0.000	0.000	0.000
C12 MAH	0.50	0.01269	0.000	0.000	0.000	0.000
C13 MAH	0.43	0.00304	0.000	0.000	0.000	0.000
C14 MAH	0.72	0.00963	0.000	0.000	0.001	0.001
C15 MAH	0.55	0.00732	0.000	0.000	0.001	0.000
C09 nMAH	0.09	0.26506	0.000	0.000	0.000	0.000
C10 nMAH	0.10	0.09833	0.000	0.000	0.000	0.000
C11 nMAH	0.11	0.03733	0.000	0.000	0.000	0.000
C12 nMAH	0.10	0.01189	0.000	0.000	0.000	0.000
C13 nMAH	0.05	0.00207	0.000	0.000	0.000	0.000
C14 nMAH	0.08	0.00119	0.000	0.000	0.000	0.000
C15 nMAH	0.48	0.00206	0.000	0.000	0.000	0.000
C16 nMAH	0.00	0.00000	0.000	0.000	0.000	0.000
C10DAH	0.01	0.01003	0.000	0.000	0.000	0.000
C11DAH	0.03	0.01216	0.000	0.000	0.000	0.000
C12DAH	0.09	0.01515	0.000	0.000	0.000	0.000
C13DAH	0.04	0.00207	0.000	0.000	0.000	0.000
C14DAH	0.07	0.00157	0.000	0.000	0.000	0.000
C15DAH	0.18	0.00130	0.000	0.000	0.000	0.000
C16DAH	0.13	0.00175	0.000	0.000	0.000	0.000
C17DAH	0.34	0.00447	0.001	0.000	0.002	0.001
C13 nDAH	0.00	0.00000	0.000	0.000	0.000	0.000
C14 nDAH	0.00	0.00000	0.000	0.000	0.000	0.000
C15 nDAH	0.05	0.00097	0.000	0.000	0.000	0.000
C16 nDAH	0.14	0.00098	0.000	0.000	0.000	0.000
C17 nDAH	0.15	0.00198	0.000	0.000	0.000	0.000
C18 nDAH	0.06	0.00076	0.000	0.000	0.000	0.000
C19 nDAH	0.00	0.00000	0.000	0.000	0.000	0.000
C14 PAH	0.01	0.00058	0.000	0.000	0.000	0.000
C15 PAH	0.04	0.00114	0.000	0.000	0.000	0.000
C16 PAH	0.02	0.00030	0.000	0.000	0.000	0.000
C17 PAH	0.05	0.00051	0.000	0.000	0.000	0.000
C18 PAH	0.10	0.00069	0.000	0.000	0.000	0.000
C19 PAH	0.34	0.00133	0.000	0.000	0.000	0.000
C20 PAH	0.00	0.00452	0.000	0.000	0.000	0.000

Ref. C outlet

HC block	PSS GCxGC µg/g silicone	Cw µg/l	TU_{chem} *D. magna*	TU_{chem} Microtox	TU_{chem} *Hyalella azteca*	TU_{chem} *Pseudokirchneriella subcapitata*
C11 nP	0.24	0.0004	0.000	0.000	0.000	0.000
C12 nP	0.00	0.0000	0.000	0.000	0.000	0.000
C13 nP	0.42	0.0006	0.000	0.000	0.000	0.000
C14 nP	0.00	0.0000	0.000	0.000	0.000	0.000
C15 nP	0.86	0.0013	0.000	0.000	0.000	0.000
C16 nP	0.00	0.0000	0.000	0.000	0.000	0.000
C17 nP	0.62	0.0009	0.000	0.000	0.000	0.000
C18 nP	0.00	0.0000	0.000	0.000	0.000	0.000
C19 nP	0.40	0.0006	0.000	0.000	0.000	0.000
C20 nP	0.00	0.0000	0.000	0.000	0.000	0.000
C21 nP	0.74	0.0011	0.000	0.000	0.000	0.000
C22 nP	0.26	0.0004	0.000	0.000	0.000	0.000
C23 nP	0.37	0.0006	0.000	0.000	0.000	0.000
C24 nP	0.10	0.0001	0.000	0.000	0.000	0.000
C25 nP	0.35	0.0005	0.000	0.000	0.000	0.000
C11 iP	0.46	0.0007	0.000	0.000	0.000	0.000
C12 iP	0.00	0.0000	0.000	0.000	0.000	0.000
C13 iP	0.55	0.0008	0.000	0.000	0.000	0.000
C14 iP	0.00	0.0000	0.000	0.000	0.000	0.000
C15 iP	1.59	0.0024	0.000	0.000	0.000	0.000
C16 iP	0.00	0.0000	0.000	0.000	0.000	0.000
C17 iP	3.37	0.0051	0.000	0.000	0.000	0.000
C18 iP	0.00	0.0000	0.000	0.000	0.000	0.000
C19 iP	1.45	0.0022	0.000	0.000	0.000	0.000
C20 iP	0.00	0.0000	0.000	0.000	0.000	0.000
C21 iP	1.04	0.0016	0.000	0.000	0.000	0.000
C22 iP	0.00	0.0000	0.000	0.000	0.000	0.000
C23 iP	2.42	0.0037	0.000	0.000	0.000	0.000
C24 iP	0.00	0.0000	0.000	0.000	0.000	0.000
C25 iP	0.50	0.0007	0.000	0.000	0.000	0.000
C10 mN	5.33	0.0681	0.000	0.000	0.002	0.001
C11 mN	6.89	0.0233	0.000	0.000	0.002	0.001
C12 mN	13.75	0.0208	0.000	0.000	0.002	0.001
C13 mN	14.08	0.0213	0.000	0.000	0.001	0.001
C14 mN	12.72	0.0192	0.000	0.000	0.001	0.001
C15 mN	10.52	0.0159	0.000	0.000	0.001	0.001
C16 mN	20.98	0.0317	0.001	0.000	0.002	0.001
C17 mN	12.28	0.0186	0.000	0.000	0.001	0.001
C10 diN	8.38	0.2839	0.001	0.000	0.003	0.002
C11 diN	14.55	0.1432	0.001	0.001	0.004	0.003

HC block	PSS GCxGC µg/g silicone	Cw µg/l	TU_{chem} *D. magna*	TU_{chem} Microtox	TU_{chem} *Hyalella azteca*	TU_{chem} *Pseudokirchneriella subcapitata*
C12 diN	27.08	0.0845	0.002	0.001	0.007	0.005
C13 diN	28.44	0.0430	0.001	0.000	0.003	0.002
C14 diN	15.63	0.0236	0.000	0.000	0.002	0.001
C15 diN	3.17	0.0048	0.000	0.000	0.000	0.000
C16 diN	3.20	0.0048	0.000	0.000	0.000	0.000
C10 MAH	0.38	0.1158	0.000	0.000	0.000	0.000
C11 MAH	1.52	0.1307	0.000	0.000	0.001	0.000
C12 MAH	0.66	0.0167	0.000	0.000	0.000	0.000
C13 MAH	2.50	0.0179	0.000	0.000	0.001	0.000
C14 MAH	21.48	0.0325	0.001	0.000	0.003	0.002
C15 MAH	26.36	0.0398	0.001	0.000	0.003	0.002
C09 nMAH	0.44	1.3532	0.000	0.000	0.000	0.000
C10 nMAH	0.14	0.1285	0.000	0.000	0.000	0.000
C11 nMAH	0.19	0.0643	0.000	0.000	0.000	0.000
C12 nMAH	1.78	0.2169	0.000	0.000	0.001	0.000
C13 nMAH	5.82	0.2497	0.001	0.000	0.002	0.001
C14 nMAH	14.48	0.2058	0.001	0.001	0.004	0.003
C15 nMAH	48.88	0.2114	0.003	0.002	0.012	0.008
C16 nMAH	33.56	0.0507	0.001	0.000	0.004	0.003
C10DAH	0.01	0.0121	0.000	0.000	0.000	0.000
C11DAH	0.04	0.0205	0.000	0.000	0.000	0.000
C12DAH	0.24	0.0416	0.000	0.000	0.000	0.000
C13DAH	1.17	0.0679	0.000	0.000	0.001	0.000
C14DAH	1.91	0.0412	0.000	0.000	0.001	0.001
C15DAH	3.09	0.0226	0.000	0.000	0.001	0.001
C16DAH	1.76	0.0027	0.000	0.000	0.000	0.000
C17DAH	24.30	0.0367	0.005	0.002	0.017	0.012
C13 nDAH	0.07	0.0156	0.000	0.000	0.000	0.000
C14 nDAH	0.11	0.0055	0.000	0.000	0.000	0.000
C15 nDAH	1.24	0.0254	0.000	0.000	0.001	0.000
C16 nDAH	5.02	0.0365	0.001	0.000	0.002	0.001
C17 nDAH	9.81	0.0148	0.001	0.000	0.002	0.001
C18 nDAH	20.12	0.0304	0.001	0.000	0.004	0.002
C19 nDAH	10.59	0.0160	0.001	0.000	0.002	0.001
C14 PAH	0.12	0.0092	0.000	0.000	0.000	0.000
C15 PAH	0.99	0.0309	0.000	0.000	0.000	0.000
C16 PAH	1.23	0.0153	0.000	0.000	0.001	0.000
C17 PAH	2.86	0.0283	0.000	0.000	0.001	0.001
C18 PAH	4.83	0.0073	0.000	0.000	0.001	0.001
C19 PAH	2.51	0.0038	0.000	0.000	0.000	0.000
C20 PAH	8.86	0.0134	0.000	0.000	0.001	0.001

APPENDIX O DOSE-RESPONSE TABLES FOR IN VIVO ASSAYS ON PASSIVE SAMPLER EXTRACT AND CONCENTRATIONS FACTORS WATER - DMSO USED TO CALCULATE TUS

Concentration	*Thamnocephalus platyurus* Survival (%)					
	Sample A inlet	Sample A outlet	Sample B inlet	Sample B outlet	Sample C inlet	Sample C outlet
Control	100	100	100	100	100	100
0.2	100	97	100	100	100	97
0.39	100	100	100	100	97	100
0.78	100	100	100	100	100	97
1.56	100	97	100	100	100	100
3.13	100	90	100	100	100	97
6.25	100	90	100	97	100	93
12.5	100	80	100	13	100	97
25	100	3	100	0	97	37
50*	100	0	97	0	100	7
100**	100	0	93	0	97	0

* 50 vol% = 2,5µl of passive sampler extract and 2,5 µl pure DMSO in 5 ml test medium, and so forth for all lower concentrations
** 100 vol% = 5µl of passive sampler extract in 5 ml test medium

Concentration	*Daphnia magna* Survival (%)					
	Sample A inlet	Sample A outlet	Sample B inlet	Sample B outlet	Sample C inlet	Sample C outlet
Control	100	100	100	100	100	100
0.2	100	100	100	100	100	100
0.39	100	100	100	100	100	100
0.78	100	100	100	100	100	100
1.56	100	100	100	100	100	100
3.13	100	100	100	100	100	100
6.25	100	100	100	93	100	100
12.5	83	33	100	83	100	100
25	17	3	100	3	100	47
50*	10	0	100	3	100	7
100**	3	0	100	0	100	0

* 50 vol% = 2,5µl of passive sampler extract and 2,5 µl pure DMSO in 5 ml test medium, and so forth for all lower concentrations
** 100 vol% = 5µl of passive sampler extract in 5 ml test medium

Concentration	*Pseudokirchneriella subcapitata* Growth inhibition (%)					
	Sample A inlet	Sample A outlet	Sample B inlet	Sample B outlet	Sample C inlet	Sample C outlet
Control	0.0	0.0	0.0	0.0	0.0	0.0
0.2	-2.3	-2.1	1.3	5.7	1.2	10.2
0.39	-2.0	-2.0	-1.9	4.8	8.4	7.8
0.78	-3.9	-1.6	-0.9	2.5	5.8	9.3
1.56	-6.8	-4.4	-1.7	3.7	3.6	9.9
3.13	-4.2	-6.4	-1.6	9.9	2.7	9.2
6.25	-6.5	-2.6	7.4	22.1	6.6	11.3
12.5	-8.3	9.1	-3.1	55.8	7.2	40.3
25	-3.7	31.3	-2.6	99.9	5.1	62.5
50*	-3.9	50.4	-2.6	100.0	7.1	89.5
100**	15.3	67.9	2.0	100.0	14.6	99.8

* 50 vol% = 2,5µl of passive sampler extract and 2,5 µl pure DMSO in 5 ml test medium, and so forth for all lower concentrations
** 100 vol% = 5µl of passive sampler extract in 5 ml test medium

Concentration	*Vibrio fischerii* Inhibition of light emission (%)					
	Sample A inlet	Sample A outlet	Sample B inlet	Sample B outlet	Sample C inlet	Sample C outlet
Control	0	0	0	0	0	0
0.39	1.4	2.9	0.9	35.9	1.3	3.6
0.78	-2.5	4.0	0.7	44.3	2.7	13.7
1.56	-4.9	9.2	1.1	62.1	2.1	17.0
3.13	1.0	18.0	-1.0	71.3	2.6	23.9
6.25	12.8	27.5	1.7	76.8	5.1	31.9
12.5	26.5	33.7	3.9	81.3	21.5	44.1
25	47.8	46.6	6.0	86.9	36.0	57.0
50*	59.0	55.4	18.2	89.4	57.3	66.3
100**	69.9	74.0	32.6	92.6	75.4	70.5

* 50 vol% = 2,5µl of passive sampler extract and 2,5 µl pure DMSO in 5 ml test medium, and so forth for all lower concentrations
** 100 vol% = 5µl of passive sampler extract in 5 ml test medium

Concentration	*Hyalella azteca* Survival (%) Sample A inlet	Sample A outlet	Sample B inlet	Sample B outlet	Sample C inlet	Sample C outlet
Control	100	100	100	100	100	100
0.2	100	100	100	100	100	100
0.39	100	100	100	100	100	100
0.78	100	100	100	100	100	100
1.56	100	100	100	100	100	100
3.13	100	100	100	37	100	93
6.25	100	87	100	0	100	10
12.5	100	0	100	0	100	0
25	100	0	100	0	100	0
50*	87	0	73	0	83	0
100**	7	0	17	0	0	0

* 50 vol% = 2,5µl of passive sampler extract and 2.5 µl pure DMSO in 5 ml test medium, and so forth for all lower concentrations
** 100 vol% = 5µl of passive sampler extract in 5 ml test medium

APPENDIX P IN VITRO ASSAY RESULTS ON PASSIVE SAMPLER EXTRACT

Concentrations in DMSO extract of in vitro assays and the liter of water per µL of DMSO.

	DR-LUC in DMSO extract (nM TCDD EQ)	ER-Luc in DMSO extract (nM E2 EQ)	AR-Ecoscreen antagonism in DMSO extract (uM Flutamide EQ)	Liter water sampled per µL DMSO extract
Refinery A Inlet	418	64	1489	0.29
Refinery A Outlet	534	292	20783	0.12
Refinery B Inlet	7.2	42	166	0.54
Refinery B Outlet	6581	194	17301	0.17
Refinery C Inlet	80	8	1637	0.11
Refinery C Outlet	2636	311	24764	0.03

APPENDIX Q RELATIVE POTENCIES (REP) OF PAHS

REPs for the DR-Luc (2,3,7,8-TCDD as reference compound) from Machala et al. (2001), and for the PAH-Calux (Benzo[a]pyrene as reference compound) form (Pieterse et al., 2013).

PAH	REP PAH-CALUX from Pieterse et al. 2013
Benzo[a]anthracene	0.3
Chrysene	0.8
Benzo[b]fluoranthene	5
Benzo[k]fluoranthene	3.7
Benzo[j]fluoranthene	1.3
Benzo[a]pyrene	1
Indeno[1,2,3-cd]pyrene	1.3
Dibenzo[a,h]anthracene	1.3
Σ228 mass-PAHs	1.4
Dibenzo[a,e]pyrene	0.3
Dibenzo[a,i]pyrene	0.2
Dibenzo[a,h]pyrene	0.2

PAH	REP DR-CALUX (6h) from Machala et al. (2001)
Fluoranthene	0.0000984
Pyrene	0.0000295
Benzo[a]anthracene	0.000000764
Cyclopenta[cd]pyrene	6.20E-06
Chrysene	1.41E-02
Unidentified me-Chrysenes*	0.0405
Benzo[b]fluoranthene	4.90E-02
Benzo[k]fluoranthene	0.28
Benzo[a]pyrene	0.01
Indeno[1,2,3-cd]pyrene	0.86
Dibenzo[a,h]anthracene	0.06
Benzo[ghi]perylene	2.27E-05
Dibenzo[a,l]pyrene	2.52E-05
Dibenzo[a,e]pyrene	1.08E-03
Dibenzo[a,i]pyrene	4.29E-02
Dibenzo[a,h]pyrene	2.65E-02

* REP based on 5-methylchrysene

APPENDIX R CALCULATED BEQ CONCENTRATIONS (PG TCDD EQ./L) IN INLET AND OUTLET WATERS USING DR-LUC REPS.

	Ref. A	Ref. A	Ref. B	Ref. B	Ref. C	Ref. C
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
Naphthalene						
Acenaphthylene						
Acenaphthene						
Fluorene						
Phenanthrene						
2-Methylphenanthrene						
2-Methylanthracene						
1-Methylanthracene						
1-Methylphenanthrene						
Anthracene						
Fluoranthene	0.17	0.014	0.0077	0.87	0.0009	
Pyrene	0.075	0.052	0.0017	0.83	0.0001	0.0035
1-methylpyrene	0.0048	0.027		0.47	0.000011	0.0083
Benz(a)antracene	0.0001			0.0020		
Triphenylene						
Chrysene	1.08		0.1090	47	0.0065	0.24
Benzo[b]fluoranthene	0.26	0.0391	0.0092	4.7	0.0006	0.012
Benzo(k)fluoranthene	0.89					
Benzo(e)pyrene						
Benzo(a)pyrene	0.024	0.0022		0.80	0.0000	0.0030
Perylene						
Dibenz(a,h)anthracene						
Indeno(1,2,3-cd)pyrene	0.13				0.0003	
Benzo(g,h,i)perylene	0.0000074	0.0000005		0.000078		0.0000051
BEQ (pg TCCD eq./L)	**2.6**	**0.1**	**0.1**	**54.7**	**0.01**	**0.3**

APPENDIX S $TU_{BIOASSAY}$ FOR THE IN VIVO ASSAYS FOR REFINERIES A, B, AND C

Sample	$TU_{bioassay}$	*D. magna*	*V. fischeri*	*H. azteca*	*P. subcapitata*	*T. platyurus*	*B. calyciflorus*
Sample A	Inlet	0.00146	0.00084	0.00044	0.00029	0.00029	0.00029
Sample A	Outlet	0.00104	0.00044	0.00136	0.00025	0.00089	0.00096
Sample B	Inlet	0.00052	0.00052	0.00079	0.00052	0.00052	0.00052
Sample B	Outlet	0.00112	0.01962	0.00624	0.00153	0.00182	0.00158
Sample C	Inlet	0.00011	0.00028	0.00015	0.00011	0.00011	0.00011
Sample C	Outlet	0.00012	0.00017	0.00067	0.00017	0.00014	0.00017

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