Evaluation of Thyroid Hormone Disruption by PFAS in WWTP Influent/Effluent and Surface Waters in the Netherlands

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1 Introduction

PFAS form a mixture of thousands of synthetic compounds widely distributed in the environment (e.g. surface water). An alternative approach to chemical analysis of 20 target PFAS substances is to monitor the total contribution of PFAS in the environment by applying the effect-based PFAS reporter gene bioassay. In the present study, the feasibility to use the PFAS reporter gene bioassay for quantitative monitoring of the sum of in vitro toxicity of total PFAS in water management resulted in a good correlation between PFAS reporter gene bioanalytical results and converted chemical analytical (LCMS) results (both in µg PFOA-equivalents/l water). The results indicate that a combination of effect-based analysis by e.g. PFAS reporter gene bioassay and targeted congener specific chemical analysis would be a suitable strategy to better cover the whole complex mixture of known plus unknown PFAS and to assess them in surface/waste water for a sustainable water management.

2 Materials and Methods

2.1 Materials

Twenty surface waters and nine WWTP influent/effluent samples were previously collected for targeted chemical analysis of 23 PFAS congeners. Permission was granted to use remaining materials for PFAS reporter gene analyses. Furthermore, the chemical data on PFAS content of the collected samples was shared for comparison with PFAS reporter gene bioanalytical results.

2.2 Methods

Extraction of PFAS: Prior to PFAS reporter gene analysis, PFAS were extracted using a weak anion exchange (WAX) SPE cartridge. Approximately 500 ml surface water or 1 liter of WWTP influent/effluent was filtered on glass-fiber filters. WAX-SPE (Oasis WAX, Waters 186002493) columns were conditioned (4 ml MeOH/0.1% NH4OH; 4 mL MeOH; 4 mL super-demi water) after which the indicated volumes of sample were loaded on the columns. After washing the columns (4 mL 25 mM NH4AC pH 4; 8 ml THF/MeOH (75:25)), PFAS were eluted from the WAX-SPE using 4 ml MeOH/0.1% NH4OH. Eluates were evaporated (N2; 45 °C) and reconstituted in 15 µl of DMSO.

2.3 PFAS reporter gene bioassay

The bioassay was carried out under conditions described in detail previously (Collet et al., 2020; Behnisch et al. 2021). Serial dilution of sample extracts in DMSO were prepared and incubated in Tris-buffer (pH 8.0) overnight at 4°C in the presence of a fixed concentration of TTR and T4. As standard reference substance, serial dilutions of PFOA in DMSO were incubated (1.0*10− 6 – 3.0*10− 3 mol/L). Next, TTR-bound and free T4 were separated on a pre-cooled Bio-Gel P-6DG column. To avoid any unspecific proteins interference, serum-free exposure medium was added to collected eluates containing TTR-bound T4 and added to seeded and pre-incubated TRβ reporter gene cells. After 24 hrs., the luciferase activity was measured on a luminometer and reported as total PFOA equivalent per liter processed water.

3 Results and Discussion

PFAS are widely used in industrial applications and consumer products because of their physical/chemical characteristics such as low grade of degradation, surfactant properties, thermic and flame resistance. Numerous studies have shown that PFAS are widely distributed in the environment and are a potential risk for human and animal health. To study the sources of PFAS contamination and to assess the levels of PFAS in our aquatic environment, monitoring this group of compounds is of major importance. Currently, monitoring according to EU water framework directives, is based on chemical-analysis of only 20 target PFAS substances. An alternative approach is to monitor the total contribution of PFAS in the environment by applying effect-based biomonitoring. Instead of measuring individual compounds, effect-based monitoring uses the common biological effects elicited by compounds sharing the same biological mode-of-action. We have developed the effect-based PFAS reporter gene bioassay to measure and monitor PFAS, based on their common property to bind to specific thyroid hormone transport proteins and thereby interfering with the thyroid-hormone system with possible adverse health consequences.

In figure 1, typical dose-response curves of PFAS reporter gene analysis results of WWTP influent/effluent samples are presented. In figure 4, WWTP influent/effluent samples, total PFAS concentrations ranged from 5.2 up to 190 µg PFOA eq./l water whereas in figure 3, fresh surface waters, results ranged from 0.5 up to 7.8 µg PFOA eq./l water. To compare the total PFAS CALUX analysis data with targeted LCMS data of single PFAS congeners, individual PFAS congener concentrations (upperbound values) were multiplied with 13 in vitro derived relative potency (REP) values (Behnisch et al., 2021) or 23 in vivo derived lower/ higher relative potency factor (RPFs) (Bil et al., 2021) and added together to obtain the sum µ PFOA-equivalents per liter water. In figure 2, sum PFOA equivalents in surface waters following conversion of LCMS data using either in vitro derived REPs or in vivo derived RPFs are presented. Results show a similar trend of concentrations using either in vitro REP or in vivo RPFs for conversion of chemical analysis data.

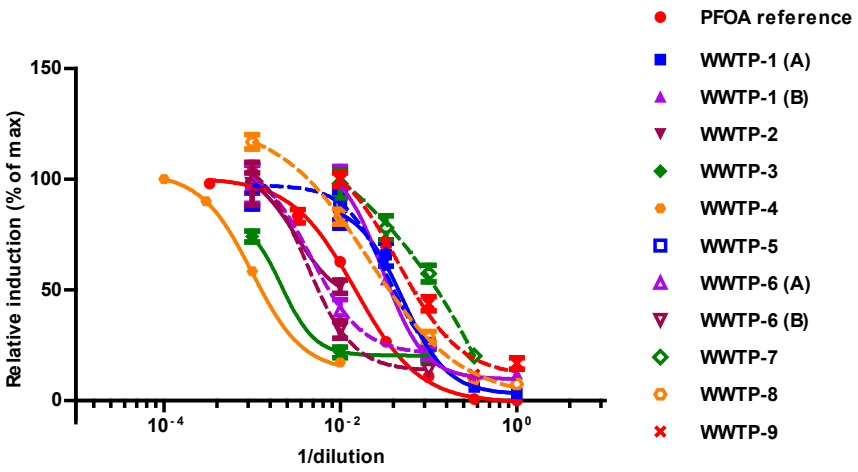

Figure 1: Concentration response curves of dilution series of WWTP influent/effluent sample extracts by PFAS reporter gene bioanalysis. PFAS reporter gene activity is expressed as induction relative to maximum induction of the PFOA reference series (relative induction RI%).

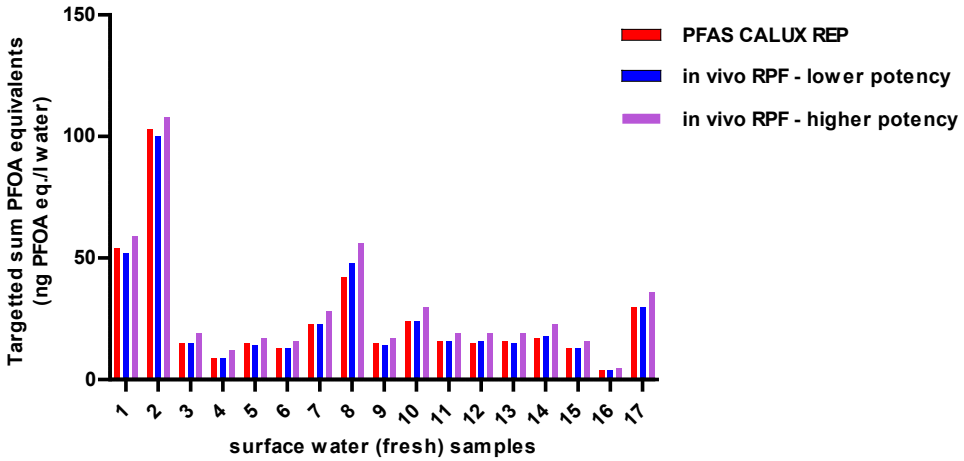

Figure 2: Comparison of converted LC/MS-data (PFAS-13) using in vitro derived REP-values (Behnisch et al., 2021) or in vivo derived lower and higher relative potency factor (RPFs) (Bil et al., 2021).

In figure 3 and table 1, the correlation between total PFAS reporter gene analysis results and sum PFOA equivalent concentrations following conversion of LC/MS-data (REPs multiplied with upperbound concentrations of the chemical analysis) for **surface water**, is given.

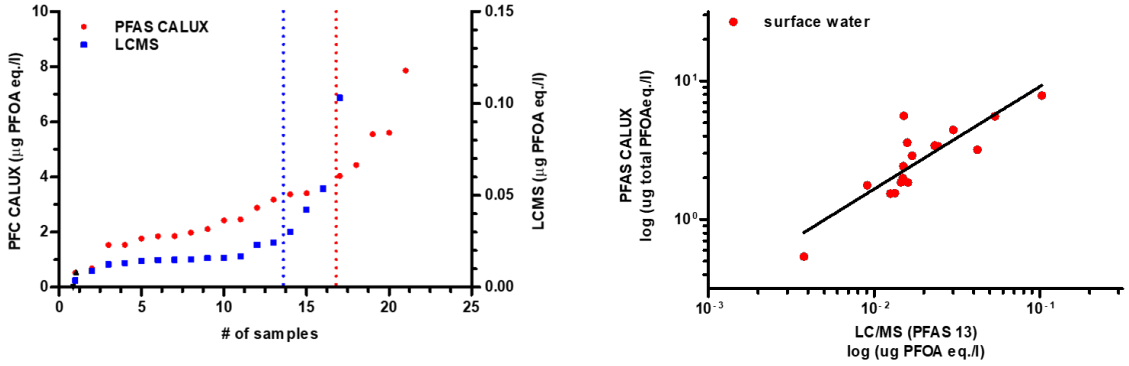

Figure 3: Correlation between the sum PFOA content using converted LCMS-data (in vitro REPs; Behnisch et al., 2021; with upperbound concentrations of the chemical analysis) and PFAS reporter gene assay (Total PFAS) analysis results of **surface** waters (Concentrations in ng/kg)

Table 1: Comparison of 5% and 80% percentile values measured by PFAS reporter gene assay analysis (Total PFAS) with converted LCMS-data (PFAS-13; upperbound concentrations) using in vitro derived REP-values (Behnisch et al., 2021) for **surface water**.

	PFAS CALUX (ug total PFOA eq./l)	UPPERBOUND LC/MS (PFAS 13) (ug PFOA eq./l)
5% Percentile	0.56	0.0039
80% Percentile	4.3	0.035

In figure 4 and table 2, the correlation between total PFAS reporter gene analysis results and sum PFOA equivalent concentrations following conversion of LC/MS-data (REPs) for **surface and waste water**, is given.

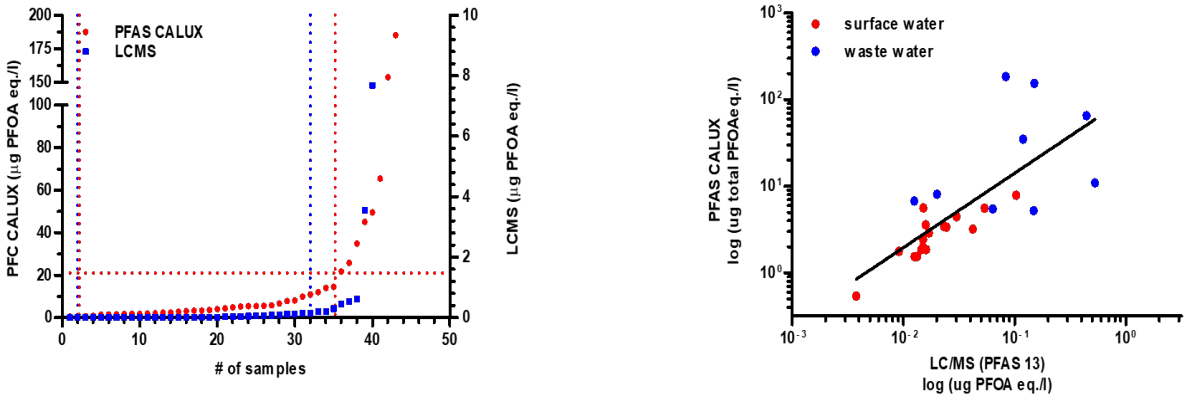

Figure 4: Correlation between the sum PFOA content using converted LCMS-data (in vitro REPs; Behnisch et al., 2021) and PFAS reporter gene assay (Total PFAS) analysis results of **surface waters** (red) and **waste water** samples (blue). (Concentrations in ng/kg)

Comparing figure 3 and 4 shows, that the PFAS reporter gene assay shows a good correlation to the chemical analysis data, albeit that the concentrations found using the PFAS reporter are significantly higher than the chemical results of the waste water samples. This indicates that in such waste water more unknown PFOA-like in vitro toxicity activities and compounds additional to the only 13 PFAS compounds here measured play a significant role for their thyroid transport hormone disruption.

Table 2: Comparison of 5% and 80% percentile values measured by PFAS reporter gene assay analysis (Total PFAS) with converted LC/MS-data (PFAS-13) using in vitro derived REP-values (Behnisch et al., 2021) for combined surface waters and waste water TP influent/effluent samples.

	PFAS CALUX (ug total PFOA eq./l)	UPPERBOUND LC/MS (PFAS 13) (ug PFOA eq./l)
5% Percentile	0.71	0.0094
80% Percentile	22	0.19

Regarding PFOA-equivalents, it is currently in Europe in the discussion to add to the water guideline EU/2013/39 a total toxicity equivalents sum value of 4,4 ng PFOA-equivalents/l (based on at the moment 20 PFAS). For the converted chemical analysis results is this comparable to the 5% percentile value of our study ranging here between 3.9 ng PFOA-eq./l (surface water) and 9.4 ng PFOA-eq./l (wastewater, see Table 1). In case of the bioanalysis results by PFAS reporter gene assays the 5%-percentile value is much higher with values of 560 ng PFOA-eq./l (surface water) and 710 ng PFOA-eq./l (wastewater) (Table 1), indicating that additional unknown PFAS may play a significant role in this in vitro thyroid toxicity pathway.
Regarding the Total PFAS analysis request in the European guideline EU 2184 (2020) of 0.5 µg total PFAS/l (based on 20 PFAS), we can conclude on our first data here that the total PFAS content can be tested by using the sensitivity of our PFAS CALUX with a LOQ of 0.1 µg total PFOA-eq./l (based on all TTR TR active PFAS).
In general, bio-based analysis results by our PFAS reporter gene assay show higher PFOA in vitro toxic equivalent concentrations than targeted chemical analysis data of summed PFAS. PFAS reporter gene bioassay results are based on total PFAS content in the samples studied whereas for chemical analysis, only 23 PFAS congeners are studied and summed, most probably explaining the higher PFAS reporter gene analysis results.
Our studies shows that sensitive effect- and in vitro toxicity based bioanalysis tools can play an important role to assess the total toxicity of the whole group of PFAS- chemicals and their mixtures.

4 Conclusions

Effect-based PFAS bioassay data correlate well with LC/MS-derived converted data showing that in vitro toxicity analysis of total PFAS content in water samples using the PFAS reporter gene bioassay is a promising and suitable strategy to cover complex mixtures of PFAS and to assess PFAS in water and the environment in general.

5 Acknowledgments

The Project called “Monitoring effects of PFAS” has been funded by Rijkswaterstaat; Ministry of Infrastructure and Water Management, the Netherlands.

6 References

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