Testing of 17 sample extracts and procedure blanc for their potential to interfere with TTR-T4 binding using the FITC-T4 assay

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Introduction

In response to a request by Arnika, Prague, Czech Republic, BioDetection Systems b.v. ("BDS") tested the potency of 17 sample extracts, suspected to be contaminated with PFAS, and one procedure blanc for their potency to interfere with the binding of the thyroid hormone thyroxine (T4) to the plasma transport protein Transthyretin (TTR) using the FITC-T4 binding bioassay.

Thyroid hormones are important for numerous physiological processes such as regulation of metabolism, bone remodelling, cardiac function, and mental status. Thyroid hormones are of special importance in fetal development, as development of the brain is dependent on normal levels of thyroid hormones. During the first part of pregnancy, the fetus relies entirely on transplacental transfer of maternal thyroid hormones and thus on a normal maternal thyroid function. Maternal thyroid homeostasis is also contributing to fetal development during the remaining part of pregnancy. Therefore, maintenance of normal thyroid function and levels is essential.

Most of the thyroid hormones are transported in the blood to target organs by the transport proteins TBG, TTR or albumin. Only a small portion of thyroid hormone circulating in the blood is unbound. Unbound thyroid hormone is easily secreted from the body through the urine or faeces. Although the major transport protein is TBG, TTR is of importance since it is involved in crossing the serum-placental and serum-cerebrospinal fluid barriers for the thyroid hormone thyroxine (T4).

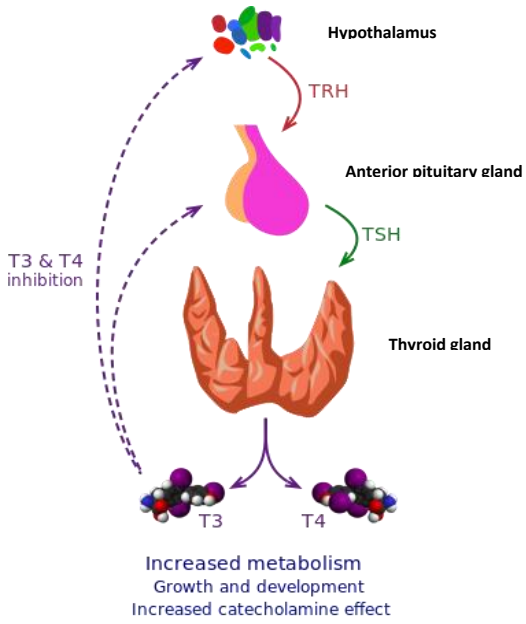

Figure 1 The thyroid system. Regulation of Thyroid hormone production

The thyroid hormone homeostasis can be disrupted by environmental chemicals at different points of interaction in the thyroid pathway, among which during transport of the hormone through the blood. Some chemicals are known to bind to the transport protein TTR thereby replacing the endogenous ligand T4. As a result, T4 is present in the circulating blood unbound and readily excreted. PFAS are such chemicals known for their capability to bind TTR thereby replacing T4.

In the TTR - FITC-T4 binding bioassay, competition between a fixed concentration of FITC-T4 and a dilution series of test items for binding TTR is determined. The measurement is based on the difference in fluorescence between bound and non-bound FITC-T4 to the TTR-binding site. Bound FITC-T4 will result in a higher fluorescence than non-bound.

The presence of increasing concentrations of PFAS, capable of competing with FITC-T4 for TTR-bindings sites, will result in a decreased amount of FITC-T4 bound TTR and thereby decreased fluorescence. Disruption of FITC-T4-TTR binding is benchmarked against the reference compound PFOA (potency factor = 1). The amount of fluorescence is proportional to the amount of FITC-T4-specific receptor binding.

In the present study, the potency of 17 migration-sample extracts and procedure blanc for disrupting thyroid hormone TTR transport was evaluated using the TTR - FITC-T4 binding bioassay.

This report summarises all analysis results obtained for the received sample extracts.

Sample processing

18 samples including 1 procedure blanc were received from Arnika, Prague, Czech Republic. Upon arrival, all samples were given a unique BDS sample code. In table 1, all sample codes and sample information are given.

Table 1 sample information

Sample Code	BDS code	UCT Code	Extract weight (g)	Extract volume (mL)
Compost-DE-12	39752	LN 24/21	1.6282	25
DE-PAP-KFC-17a	39753	LN 26/21	0.3469	25
DE-PAP-MCD-26	39754	LN 30/21	0.3539	25
Compost-DK-3	39755	LN 33/21	1.1304	25
Compost-DK-5	39756	LN 35/21	1.1966	25
DK-PAP-MCD-1	39757	LN 38/21	0.407	25
Compost-NL-3	39758	LN 41/21	0.9481	25
Compost-NL-1	39759	LN 42/21	1.8759	25
NL-MCD-01	39760	LN 45/21	0.277	25
FastF-FR-5	39761	LN 48/21	0.3152	25
Compost-FR-2	39762	LN 49/21	1.2387	25
CZ-FCM-MCD-01b	39763	LN 52/21	0.5611	25
Recycl-CZ-1	39764	LN 53/21	0.6594	25
CZ-FCM-KFC-06	39765	LN 57/21	0.2806	25
PizzaB-UK-2	39766	LN 59/21	1.0998	25
FastF-UK-2	39767	LN 62/21	0.3054	25
FastF-UK-5b	39928	LN 131/21	0.4963	25
Client procedure blanc	39929			

The solvent was evaporated under a gentle stream of N_2, until about 10 µL sample was left. This was dissolved in 1 mL of MeOH and transferred to a pre-rinsed bottle containing 100 mL HPLC water. The vial was rinsed twice with 0.5 mL of MeOH and this was also transferred into the pre-rinsed bottle.

A weak anion exchange (WAX) solid phase extraction (SPE) was performed to clean up the samples. The cartridge was conditioned using:

- 4 ml of MeOH with 0.1% NH_4OH
- 4 ml of MeOH
- 4 ml HPLC water

The sample was transferred to the cartridge at a rate of about 1 drop per second. The cartridge was washed using:

- 4 ml 25 mM NH_4Ac pH 4
- 8 ml THF:MeOH (75:25)

After washing, the cartridge was dried for 30 minutes applying vacuum. A 15 ml tube was placed under the cartridge and the sample was eluted using 4 ml of MeOH with 0.1% NH_4OH.

The extract was evaporated to dryness under a gentle stream of N_2 and re-dissolved in 50 µL of DMSO followed by preparation of serail dilutions in DMSO.

TTR - FITC-T4 binding assay

Serial dilutions of the sample extracts (2 µl) or reference material (2 µL) were incubated in Tris-buffer (pH 8.0) for 20 minutes in the presence of TTR (0.30 µM) and FITC-T4 (109 nM). The total incubation volume was 202 µl. After incubation, the fluorescence was measured using a Berthold luminometer applying an excitation wavelength of 490 nm, an emission wavelength of 535 nm and a measuring time of 0.1s.

Data analysis

Analysis results of the sample extracts expressed as induction relative to the standard reference compound PFOA, are intrapolated in the calibration curve for quantitative determination of thyroid hormone transport disruptive potential using the statistical software package Graphpad Prism V5.03.

Results

In figures 2-5, the TTR - FITC-T4 analysis results of the 17 sample extracts and procedure blanc are presented graphically. In table 2, quantified analysis results are given, expressed as the amount of PFOA equivalents per gram of sample. In addition, the lowest amount of sample showing TTR-T4 disrupting potential per litre of incubation mixture (Lowest Observed Effect Concentration; LOEC) is given.

In the client procedure blanc as well as the laboratory procedure blanc sample, a low signal was measured as reported in Table 2.

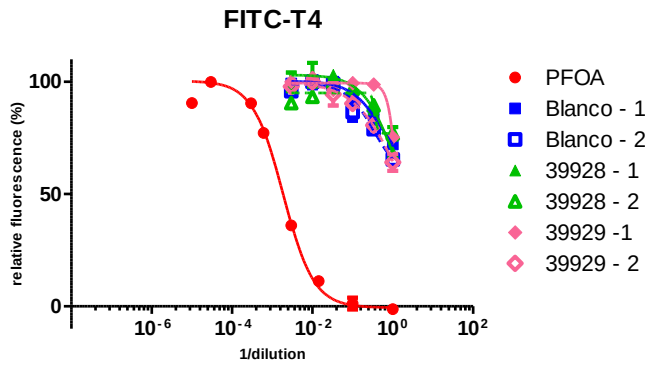

Figure 2

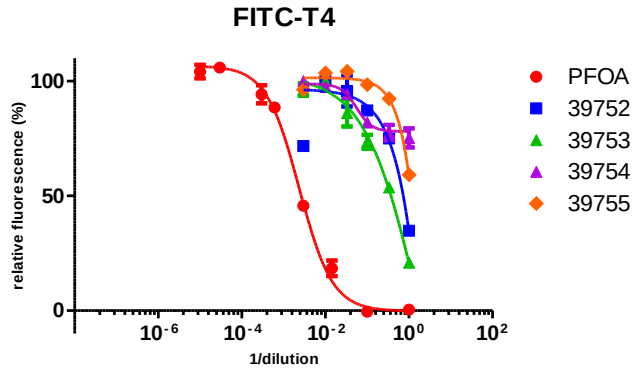

Figure 3

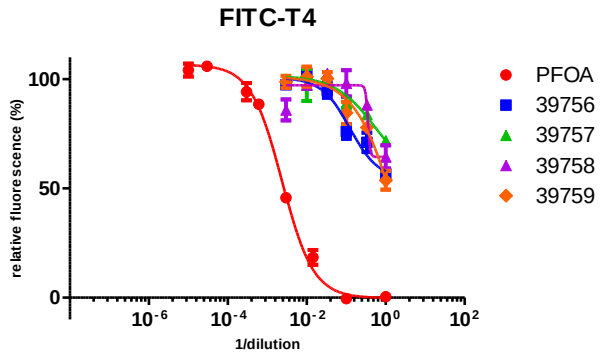

Figure 4

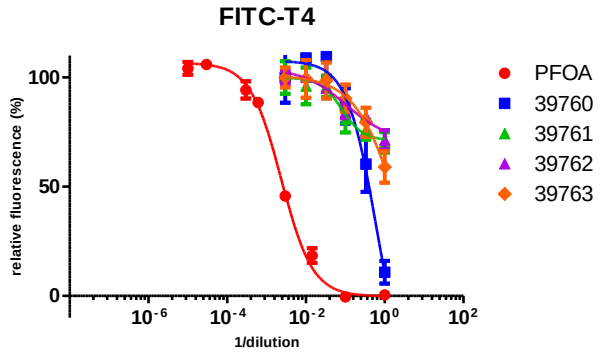

Figure 5

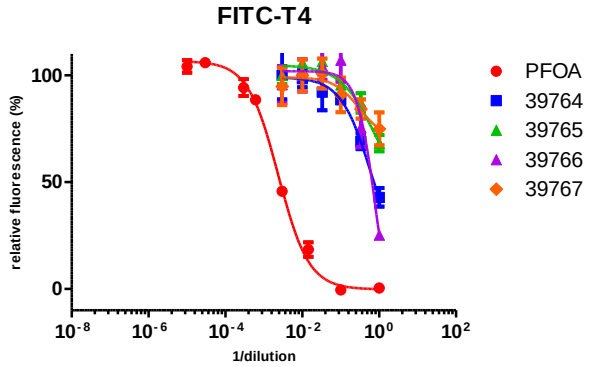

Table 2 TTR - FITC-T4 analysis results

BDS code	Client code	TTR - FITC-T4 activity (µg PFOA/g sample)	LOQ (µg PFOA/g sample)	LOEC (g sample/l incubate)
Blanc	Laboratory blanc	21	12	2.4
39752	Compost-DE-12	23	5.6	3.1
39753	DE-PAP-KFC-17a	341	26	0.2
39754	DE-PAP-MCD-26	180	26	0.4
39755	Compost-DK-3	13	8.1	5.5
39756	Compost-DK-5	74	7.7	1.0
39757	DK-PAP-MCD-1	51	23	1.4
39758	Compost-NL-3	27	10	2.7
39759	Compost-NL-1	18	4.9	4.1
39760	NL-MCD-01	200	33	0.4
39761	FastF-FR-5	220	29	0.3
39762	Compost-FR-2	21	7.4	3.5
39763	CZ-FCM-MCD-01b	52	16	1.4
39764	Recycl-CZ-1	73	14	1.0
39765	CZ-FCM-KFC-06	69	33	1.1
39766	PizzaB-UK-2	26	8.3	2.9
39767	FastF-UK-2	60	30	1.2
39928	FastF-UK-5b	39	19	1.9
39929	Client procedure blanc	21	12	3.5