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Chemical mixtures isolated from house dust disrupt thyroid receptor β (TR β) signaling

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Abstract

House dust is a source of exposure to chemicals that can impact hormone regulation. This study was designed to evaluate the potential of house dust mixtures (n=137) to disrupt thyroid hormone nuclear receptor signaling in a cell-based reporter assay and examine associations with thyroid hormones (TH) measured in residents of the homes. Approximately 41% of the extracts (ranging from 10.5 –4.097 μ g of dust/mL) significantly antagonized TR β signaling by 20–67% relative to the hormone control. The concentrations of twelve flame retardants (FRs) quantified in the mixtures were significantly correlated with TR β antagonism; however, they were inactive when tested individually. We hypothesize that the observed antagonism is due to mixture effects or unidentified compounds that co-occur with FRs. Dust extract potency was significantly associated with free thyroxine (FT4, $r_s = -0.64$, $p < 0.001$), suggesting that more potent dust samples are associated with higher FT4 levels in residents. Overall, these results suggest that house dust is a significant source of exposure to TH-disrupting chemicals, and TR β may have a role in mediating effects of exposure on TH levels. Additional studies are needed to identify the chemical(s) driving the observed effects on TR β , and to determine if these changes lead to any adverse outcomes.

Introduction

It is well recognized that the indoor environment significantly impacts human health. The number of indoor chemicals suspected to adversely affect human health has increased over the past fifty years with the development of new building materials and consumer products (reviewed in¹). Many of these chemicals are not permanently bound to their products and leach or off-gas from products and re-distribute into indoor air, dust, and surfaces^{2–4}.

House dust is a highly complex mixture of hundreds (potentially thousands) of chemicals^{5–6}. Unintentional dust ingestion is a significant exposure source for many chemicals of concern, including flame retardants^{7–8}, lead⁹, and phthalates¹⁰. Human epidemiology studies

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have linked exposure to contaminants in dust to altered hormone signaling in multiple endocrine systems^{11–13}.

Changes in circulating thyroid hormone levels is a hallmark of thyroid disruption. Circulating thyroid hormone levels are tightly regulated via negative feedback the hypothalamus-pituitary-thyroid (HPT) axis¹⁴ and mediated by thyroid hormone receptors (TRs)^{15–17}. TRs are encoded by two genes (THRA and THRB) that produce three active isoforms: TR α 1, TR β 1, and TR β 2 (reviewed in¹⁸). Studies in mouse models and thyroid hormone-resistant patients have shown that the two TR β isoforms are the dominant regulators of HPT feedback, and that TR α 1 is unable to compensate for the loss of TR β function (reviewed in¹⁹). Given that normal thyroid hormone levels are vital for many physiological and developmental processes, the disruption of TR β signaling by dust contaminants could have significant effects on human health.

Previous *in vitro* and *in silico* studies that focused on individual contaminants identified in dust have yielded mixed results^{20–29}, and suggest TR β is not a major target of chemicals in house dust due to a lack of activity or predicted binding. However, individual chemical testing does not account for the potential combined effects of exposures to complex mixtures. Interactions between chemicals in a mixture can significantly influence the activity or toxicity of the mixture as a whole, and these effects may not be predictable when assessing individual compounds^{30–31}. For example, *in vitro* and *in vivo* studies have shown that combinations of estrogenic and anti-androgenic chemicals can act jointly and produce effects much greater than predicted based on individual chemical data, even when the individual chemical concentrations are below no-effect levels^{32–35}. It's possible that the thyroid-disrupting effects associated with house dust are due the combined effects of multiple substances in the mixture rather than individual chemicals.

The purpose of this study was to evaluate the potential of complex mixtures isolated from house dust to disrupt TR β signaling in a cell-based reporter assay. We employed a whole mixtures approach by testing house dust collected from private homes to more accurately represent real-world exposures. Compared to individual chemical testing, the advantages of testing whole mixtures are that all identified and unknown substances in the mixture are present and interactions between mixture components are accounted for^{30–31}. We focused on TR β over TR α because this isoform is the dominant regulator of circulating TH levels as described above. The results from the reporter assay were used to determine if the concentrations of twelve flame retardants (FRs) previously quantified in the dust³⁶ were associated with the observed activity. The TR β activities were further assessed for potential relationships with measured thyroid levels and other health outcomes in adults living in the sampled homes.

2. Materials + Methods

Chemicals.

Table S1 contains the information regarding the flame retardants and other chemicals used in this study, including the Chemical Abstracts Service (CAS) number, manufacturer, and supply number.

Dust Collection and Extraction.

Dust samples used in this study were collected as part of a study investigating flame retardant exposures and papillary thyroid cancer using a case-control design. Dust collection, extraction, and analysis have been described in detail^{36–37}. Briefly, the main living area of participants homes was vacuumed with a cellulose thimble fitted in the hose attachment of a Eureka® Mighty Might vacuum. Upon collection, thimbles were wrapped in foil and immediately frozen until extraction. Dust samples were extracted with 1:1 dichloromethane:hexane (v/v) via sonication, and concentrated to 1 mL using a nitrogen evaporation system. Extracts were split into equal volumes, and the solvent from one aliquot was evaporated to dryness and re-suspended in 100 µL DMSO for assays, while the other aliquot was used for flame retardant analysis. Brominated and organophosphate flame retardants were quantified using GC/MS as described previously^{36–37}. Brominated FRs quantified include the major PentaBDE congeners (BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154), DecaBDE (BDE-209), and the brominated components of Firemaster® 550 (FM550): 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB) and bis(2-ethylhexyl)-tetrabromophthalate (BEH-TEBP). Assessed organophosphate FRs include tris(2-chloroethyl)phosphate (TCEP), tris(1,3-dichloroisopropyl)phosphate (TDCIPP), tris(1-chloro-2-propyl)phosphate (TCIPP), and triphenyl phosphate (TPHP). FR concentrations that were below the detection limit were assigned a value equal to ½ of the minimum detection level (MDL) for statistical analysis.

Serum Analysis.

Free thyroxine (FT4), free triiodothyronine (FT3), thyroid-stimulating hormone (TSH), total cholesterol, and total triglycerides were measured by LabCorp in Burlington, NC using standard protocols³⁷. The study population has been described in detail previously^{36–37}.

GeneBLazer® Assay.

Given that multiple nuclear receptors recognize the same DNA response elements as TRβ, and complex chemical mixtures in house dust can target multiple signaling pathways, we utilized a Gal4 reporter system to specifically focus on TRβ response and circumvent potential interference from other receptors and signaling pathways. As TRβ1 and TRβ2 only differ in the N-terminus region (reviewed in¹⁸), a Gal4 system is representative of both TRβ isoforms as the Gal4-TRβ construct only includes the ligand-binding domain of the receptor. Details regarding the cell culture media and reagents can be found in Table S2. Methods for preparing the frozen GeneBLazer® TRβ-UAS-*bla* HEK 293T cell stocks can be found in the Supporting Information. Frozen cells were quickly thawed in a 37°C water bath with gentle agitation. Thawed cells were diluted in 10 mL of assay medium (phenol red-free high glucose DMEM supplemented with 2% charcoal-stripped FBS, 4 mM GlutaMAX™, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 25 mM HEPES, 100 U/mL penicillin and streptomycin). Cells were centrifuged at 200 x g for 5 minutes, and the pellet was gently re-suspended via pipetting in 2 mL of fresh assay medium. Cell were quantified, and density was adjusted to 3.6×10^5 cells/mL. Duplicate 384-well black-walled tissue culture plates with clear bottoms were plated at ~10,000 cells/well (28 µL of the cell suspension) and incubated at room temperature for 2 hours to allow for cell settling.

Originally, we planned to test for both agonist and antagonist activity; however, we chose to focus on antagonism after no agonist activity was observed with the first 50 samples. To test the ability of the dust extracts to inhibit T3-mediated TR β transcription, test wells were treated with both 0.3 nM triiodothyronine (T3) and dust extracts (4 doses per extract, 1/2 dilutions). The final volume of each well was 40 μ L, and the final DMSO concentration was 0.1%. Plates were incubated for 18 hours at 37°C with humidity and 5% CO₂. The positive control consisted of 0.3 nM T3 in the absence of a competitor chemical, and negative controls were unstimulated cells dosed with only Assay DMEM with 0.1% DMSO. The top and bottom row of each plate served as cell-free controls and contained only media. Fluorescence background was quantified from cell-free wells.

Following incubation, cells were loaded with 1 μ M CCF4-AM LiveBLAzer™ substrate that was prepared following the manufacturer's protocol³⁸. Plates were protected from light and incubated for two hours at room temperature. Blue (410/460 nm) and green (410/530 nm) fluorescence emissions were quantified on a SpectraMax M5 plate reader (Molecular Devices, San Jose, CA). Each assay was repeated on three separate occasions, with quadruplicate wells per concentration within each assay.

GeneBLAzer® Data Analysis.

Raw data was analyzed following the manufacturer's recommendations^{38–39}. Net blue and green fluorescence signals were calculated by subtracting the average fluorescence background from the cell-free wells from all control and experiment wells. The blue/green emission ratio was calculated for each well by dividing the net blue values (410/460 nm) by the corresponding net green values (410/530 nm). To quantify the receptor response over background, the response ratio (RR) was calculated by dividing the blue/green emission ratio for each test compound by the emission ratio for the DMSO control wells. The % Inhibition was calculated from the response ratio using the following formula.

$$\% \text{ Inhibition} = \left(1 - \frac{RR_{\text{TEST COMPOUND}} - RR_{\text{DMSO}}}{RR_{0.3 \text{ nM T3}} - RR_{\text{DMSO}}} \right) \times 100$$

Cell Viability Assay.

Cell viability was assessed using a resazurin reduction assay⁴⁰ and described in detail in the Supporting Information. Cytotoxicity was distinguished by a decrease in cell viability greater than 15%.

Statistical Analysis.

GeneBLAzer® results were analyzed using a one-way ANOVA followed by Dunnett's posthoc test to identify dust extract dilutions that significantly reduced TR β activity compared to the T3 control. The relationships between the final concentration of FRs in the wells and the degree of TR β antagonism (defined as % Inhibition compared to the T3 control) was assessed using Spearman correlation coefficients. For assessing relationships with the health biomarkers (FT4, FT3, TSH, total cholesterol, total triglycerides), we determined that extracted dust mass could be a potential confounder in maximal bioactivity.

A normalized metric was created for further assessment: potency ($\mu\text{g/mL}$ dust extract concentration) at which 20% TR β antagonism was observed (IC₂₀). Full dose responses of dust extracts were plotted and used to calculate the point where the curves crossed the 20% efficacy line. Values were extrapolated as necessary for samples that approached but did not reach the 20% inhibition mark; values with no apparent activity (not increasing towards the 20% mark) were not included, as potencies cannot be calculated for inactive samples. All analyses were conducted in GraphPad Prism 7. Statistical significance was set at $\alpha = 0.05$.

3. Results

Antagonist activity of the dust extracts.

The thyroid hormone triiodothyronine (T3) was used as the active hormone in the antagonist assays. The EC₅₀ for T3 was 0.206 nM (95% confidence interval = 0.195 – 0.218 nM) in our assay, which is similar to what was reported by the manufacturer (0.25 nM)³⁹ (Figure S1). Based on the T3 dose-response curve results, the antagonist assays were conducted using 0.3 nM of T3. This concentration yields a good dynamic response range and is located between the EC₅₀ and EC₈₀ (0.34 nM) concentrations as recommended by the manufacturer. Summary statistics are presented in Table 1. Of the 137 tested extracts, 57 (41.6%) inhibited TR β signaling >20% relative to the T3 control. The remaining 80 extracts (58.4%) did not affect TR β signaling and were considered inactive (see Figure 1 for representative examples of active and inactive extracts). The dust concentrations tested in this study varied based on the mass of dust collected in all the homes. While the dust concentration ranges in the active and inactive extracts were similar, the median dust concentration in active extracts was over 2-fold higher than the median concentration in inactive extracts (Table 1). TR β antagonism was significantly and positively associated with the dust mass concentration in the well (Figure 2; $r_s = 0.54$, $p < 0.001$, $n = 137$), as would be expected given that more dust would contain more chemical contaminants. Cytotoxicity was observed in one or more doses in 31 of the 137 tested extracts. Toxic doses were excluded from statistical analyses, and only doses below the cytotoxicity threshold (> 15% viability loss) were included in the analysis.

Flame retardant correlations.

To gain a better understanding of the chemicals potentially driving the observed response, we assessed the relationship between the final concentration in the wells of the twelve flame retardants in the dust mixtures and the degree of TR β antagonism. Summary statistics of the FR concentrations are presented in Table 1, and the Spearman correlation results are presented in Table 2. Antagonism was significantly and positively associated with the concentrations of all 12 flame retardants in the mixtures (Table 2, $r_s = 0.25 - 0.44$). The strongest correlations were observed with BDE-209 ($r_s = 0.44$, $p < 0.001$), TCEP, and TCIPP ($r_s = 0.39$, $p < 0.001$ for both).

TR β activities of the individual FRs.

To test if the FRs present in the dust extract mixtures may be driving the observed antagonism, each FR was tested individually in the GeneBLAzer® assay using a concentration range that included the ranges observed in the dust extracts (Figure 3). No

significant antagonism was observed with any of the 12 FRs, which suggests that the individual FRs may not be driving the observed effects.

Antagonist potency correlations with thyroid biomarkers.

Previous studies have identified associations between FR exposure and serum TH levels in individuals living in the sampled homes. For this study, we assessed the relationships between dust potency (dust concentration ($\mu\text{g/mL}$) that inhibited $\text{TR}\beta$ by 20% relative to the T3 control) and health biomarkers of residents using Spearman correlations. Potency was significantly correlated with serum FT4 ($r_s = -0.64$, $p < 0.001$, $n=51$) in adults living in sampled homes (Figure 4). The result with FT3 was suggestive but did not reach statistical significance ($r_s = -0.33$, $p < 0.10$, $n=45$). No relationship was observed between potency and TSH ($r_s = 0.14$, $p = 0.38$, $n=53$), body mass index (BMI, $r_s = 0.18$, $p = 0.17$, $n=112$), total cholesterol ($r_s = 0.13$, $p = 0.27$, $n=100$), and total triglycerides ($r_s = 0.12$, $p = 0.23$, $n=100$). A summary of the measured health metrics is presented in Table S3.

Discussion

In this study, extracts of house dust collected from 137 homes in North Carolina were screened for the ability to disrupt normal $\text{TR}\beta$ signaling in a cell-based reporter assay. Nearly half of the tested extracts antagonized $\text{TR}\beta$ signaling between 20–67% compared to the T3 control, suggesting that house dust is a significant source of $\text{TR}\beta$ -disrupting chemicals. This is significant because $\text{TR}\beta$ is essential to multiple developmental processes and normal physiology in vertebrates, including neurodevelopment and adult neurogenesis, cardiac maturation and physiology, skeletal development, and metabolism (reviewed in ^{14, 41}). The consequences of developmental thyroid disruption are often permanent. Multiple compounds identified in dust have been linked to neurological and developmental abnormalities in laboratory models, and epidemiological studies have identified links between exposure to PBDEs and neurological effects in adults and children ^{42–44}. It's hypothesized that a majority of the PBDE exposure is coming from dust in these populations. $\text{TR}\beta$ disruption is especially concerning in young children, as they are undergoing vulnerable periods of development that can be influenced by exogenous chemical signals.

This is the first study to demonstrate the antagonistic effects of dust exposure on $\text{TR}\beta$ signaling in eukaryotic cells. An earlier study by Chou et al ⁴⁵ that used a yeast-based reporter assay observed $\text{TR}\beta$ antagonism in response to nine dust samples collected from buildings and roads at a university in Taiwan, supporting the notion that chemical mixtures in house dust can disrupt $\text{TR}\beta$ signaling. Results from additional studies indicate that house dust mixtures can interfere with nuclear receptors other than $\text{TR}\beta$, such as the peroxisome proliferator-activated receptor gamma ($\text{PPAR}\gamma$). $\text{TR}\beta$ and $\text{PPAR}\gamma$ are evolutionarily-related receptors ⁴⁶ that share roles in metabolic processes such as adipogenesis and lipid homeostasis ^{47–48}. Recent studies with $\text{PPAR}\gamma$ by our laboratory ^{49–50} and others ⁵¹ found that dust mixtures had agonist effects on $\text{PPAR}\gamma$ signaling. This is significant because both the activation of $\text{PPAR}\gamma$ and inhibition of $\text{TR}\beta$ can target many of the same endpoints, such

as promoting adipogenesis⁴⁸. Together with the current results, this suggests that the impacts of contaminant mixtures in house dust on a physiological system may be complex.

Understanding and predicting the effects of mixture exposures is aided by identifying chemicals within a mixture that contribute to the observed response. Previous receptor studies have identified significant relationships between concentrations of specific chemicals in dust and receptor activity. For example, phthalate concentrations in dust collected from kindergarten classrooms in Belgium were associated with estrogen receptor (ER) activation in a cell-based bioassay⁵². A separate study found that concentrations of PBDEs and chlorinated paraffins in dust samples from five countries were significantly correlated with observed aryl hydrocarbon receptor (AhR) activities⁵³. Previous work in our lab identified a significant relationship between PPAR γ activity and fatty acids in dust, but not phthalates or organophosphates⁵⁰. In this study we found that all twelve FRs were significantly correlated with TR β antagonism, yet none of the FRs antagonized TR β signaling when tested individually. This suggests that the mechanism of action is more complex than simple mediation by the individual chemicals and may implicate co-exposures or mixture effects as a cause.

The concentrations of contaminants in house dust often correlate with each other, frequently due to both chemicals occurring in the same commercial mixtures, co-application of different chemicals to the same products, or shared sources^{11, 54–56}. However, the majority of data for contaminant correlations in house dust has focused on specific chemical classes (i.e. flame retardants or phthalates), and potential associations between diverse classes needs to be clarified. Previous studies examining relationships between contaminant concentrations in other compartments (indoor air and biological samples) have found significant correlations between chemicals from diverse classes and sources^{57–58}. A recent study from our group observed significant correlations between urinary metabolites of various contaminant classes with different use patterns (for example, the metabolite of TDCIPP and bisphenol A), which suggests a common source or exposure pathway⁵⁸. While the tested FRs did not antagonize TR β signaling, it is possible that the observed antagonism is driven by unidentified chemicals that co-occur with the flame retardants in the mixture. Contaminants such as plasticizers, colorants, and UV stabilizers are often included alongside FRs during the manufacturing process and are frequently identified in house dust^{2, 59–62}. The fact that some of these compounds have been shown to disrupt TR β signaling in laboratory studies (reviewed in⁶³) supports the notion that contaminants that co-occur with the flame retardants may be driving the observed antagonism.

A second possibility is that the observed antagonism is due to the chemicals in the dust mixtures acting together in a way that influences the overall activity of the mixture (often referred to as “mixture effects”). Previous *in vitro* and *in vivo* studies have demonstrated that the combined effects of mixtures of chemicals that share the same mechanism of action can be greater than the individually tested components, even when the concentration of each component is below the no observed effect level^{32–35}. The activity of chemical in a mixture may be enhanced (synergism) or inhibited (antagonism) by interactions with other mixtures components, and this effect may not be predictable when testing individual chemicals^{30–31}. This is illustrated by a recent study examining the effects of chemical mixtures used in

hydraulic fracturing on nuclear receptor activities⁶⁴. This study frequently observed responses that were more potent than the individual components of the mixture. Furthermore, synergistic TR β antagonism was observed with a mixture comprised of 23 chemicals, meaning the degree of TR β antagonism was greater than the predicted additive effects of the individual components. Interestingly, the synergistic effect was eliminated when bisphenol A was added to the mixture⁶⁴. The observed lack of activity of the individual FRs in this study may therefore be due to the absence of the remaining mixture components.

Many studies have identified relationships between contaminant exposure via house dust and altered thyroid levels; however, the mechanism(s) behind the relationship are unclear. In the present study, we identified a significant negative correlation between the potency of TR β antagonism in house dust and serum FT4 levels of residents living in the sampled homes. The relationship suggests more potent dust extracts are associated with higher FT4 levels, and that dust exposure may increase circulating FT4 levels by disrupting TR β -mediated negative feedback on the HPT axis. TR β regulates the synthesis of thyrotropin releasing hormone (TRH) in the hypothalamus and TSH in the pituitary, which controls TH production in the thyroid gland. In our study TR β antagonism was not significantly associated with TSH; however, it is interesting to note that the correlation was in the positive direction, which is what one would anticipate given regulatory feedback. The absence of a significant association with TSH may also suggest that TSH may not be responding to the elevated FT4 levels in these individuals, perhaps because they have not yet reached a threshold of activation. A similar situation has been observed in cases of resistance to thyroid hormone (RTH) involving a mutated TR β (reviewed in^{19, 65}). One of the classic signs of RTH is elevated thyroid hormones and unsuppressed TSH due to point mutations in the ligand binding domain of TR β . These mutations interfere with the receptor's ability to bind hormones, and TR β is unable to regulate TSH and TRH levels in response to elevated thyroid hormones. It's possible that flame retardants or other contaminants in the dust mixture are interfering TR β 's ability to regulate TSH and TRH expression, however a much larger study would be necessary to understand if dust-mediated TR β disruption is occurring via this mechanism.

In conclusion, these results demonstrate that house dust is a significant source of TR β disrupting chemicals, and TR β disruption may have a role in the altered serum TH levels observed post-exposure. The implications of these results are significant, as TR β signaling is vital to normal development, and the health and wellbeing of children and adults. Our results illustrate the importance of testing environmental mixtures, as effects may not be observed on an individual chemical basis. The significant association between TR β signaling and FT4 levels suggest that exposure to house dust may disrupt TR β feedback on the HPT axis. Future studies including effect-directed analysis to identify and characterize the active fractions of the dust mixtures and testing "mock mixtures" of chemicals would help identify the driver(s) of the observed TR β antagonism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Representative results of TR β antagonism (% Inhibition) by active (closed shapes) and inactive (open shapes) dust extracts obtained via the GeneBLAzer® β -lactamase reporter assay in HEK 293T cells as described in the Materials and Methods. The colors represent different dust extracts. Cells were treated with a range of dust extract concentrations in the presence of 0.3 nM triiodothyronine (T3). Extracts that decreased TR β activity $\geq$ 20% of the T3 control were considered active. Plotted data is the average $\pm$ SEM of three separate experiments.

Figure 2. Spearman correlation between the degree of inhibition of TRβ signaling relative to the triiodothyronine (T3) control and the tested dust extract concentrations (n=137).

Figure 3.

TR β dose-response results with the individual flame retardants. None of the (A) PBDEs, or (B) FM550 components and PFRs antagonized TR β signaling at any of the tested doses. GeneBLAzer® β -lactamase reporter assays in HEK 293T cells were conducted as described in the Materials and Methods. Each data point represents the average $\pm$ SEM of three separate experiments.

TR β Potency (20% Antagonism) and Thyroid Status in Residents

Figure 4.

Spearman correlations between the potency of TR β antagonism and serum measurements of (A) thyroid stimulating hormone (TSH), (B) free thyroxine (FT4), and (C) free triiodothyronine (FT3) of individuals living in the sampled homes. Potency is defined as the dust extract concentration that inhibited TR β signaling by 20% relative to the T3 control (0.3 nM). Potency is significantly and inversely correlated to free thyroxine (FT4), which suggests that higher FT4 levels are associated with more potent dust extracts. The relationship with free triiodothyronine (FT3) is suggestive but not significant.

Table 1.

Median and range of the TR β antagonism results (% Inhibition compared to the T3 control), the median and range concentrations of dust extracts ($\mu\text{g/mL}$) in the wells, and the median and range concentrations of each flame retardant in the wells (pg/mL) in the active and inactive samples.

	Active Extracts (n=57)		Inactive Extracts (n=80)	
	Median	Range	Median	Range
TR β Inhibition (%)	37%	20% – 67%	7%	–7% - 19%
Dust Extracts ($\mu\text{g/mL}$)	505.5	40.5 – 3,284	232.1	10.50 – 4,097
PBDEs (pg/mL)				
BDE-47	111.3	0.921 – 22,000	39.71	0.115 – 7,903
BDE-99	227.5	1.006 – 45,900	55.14	3.200 – 10,010
BDE-100	26.26	0.368 – 8,945	8.538	0.092 – 1,912
BDE-154	9.166	0.262 – 5,114	7.487	0.065 – 935.4
BDE-153	11.57	0.475 – 6,277	9.654	0.008 – 1,009
BDE-209	258.1	7.460 – 4,829	100.9	0.093 – 12,290
FM550 (pg/mL)				
EH-TBB	83.86	0.707 – 7,292	44.42	0.045 – 1,266
BEH-TEBP	99.00	0.566 – 2,615	34.18	0.566 – 564.4
PFRs (pg/mL)				
TCEP	121.6	7.113 – 4,313	86.51	0.189 – 2,104
TDCIPP	678.9	48.75 – 7,855	297.1	6.094 – 15,320
TCIPP	985.3	45.04 – 56,800	420.0	11.26 – 5,867
TPHP	560.4	8.485 – 23,980	249.4	2.253 – 26,850

Table 2.

Spearman correlations between the concentrations of individual flame retardants in the wells vs. TR β Inhibition.

	r_s
BDE-47	0.36 ***
BDE-99	0.37 ***
BDE-100	0.34 ***
BDE-153	0.27 **
BDE-154	0.25 **
BDE-209	0.44 ***
EH-TBB	0.29 ***
BEH-TEBP	0.32 ***
TCEP	0.39 ***
TDCIPP	0.33 ***
TCIPP	0.39 ***
TPhP	0.35 ***

= $p < 0.001$

**
= $p < 0.01$