Title
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Authors
Kollitz, Erin M
Kassotis, Christopher D
Hoffman, Kate
et al.

Peer reviewed

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

Erin M. Kollitz[†], **Christopher D. Kassotis[†]**, **Kate Hoffman[†]**, **P. Lee Ferguson[†]**, **Julie Ann Sosa[‡]**, and **Heather M. Stapleton[†,*]**

[†]Nicholas School of the Environment, Duke University, Box 90328, Durham, North Carolina 27708, United States

[‡]Department of Surgery, University of California at San Francisco, 513 Parnassus Avenue, San Francisco, California 94117, United States

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 μg of dust/mL) significantly antagonized TRβ signaling by 20–67% relative to the hormone control. The concentrations of twelve flame retardants (FRs) quantified in the mixtures were significantly correlated with TRβ 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 TRβ 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 TRβ, 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 in[1]). 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], lead[9], and phthalates[10]. Human epidemiology studies

[*]Corresponding Author Information: Phone: (919) 613-8717, heather.stapleton@duke.edu.

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) axis [14] and mediated by thyroid hormone receptors (TRs) [15–17]. TRs are encoded by two genes (THRA and THRB) that produce three active isoforms: TRα1, TRβ1, and TRβ2 (reviewed in [18]). Studies in mouse models and thyroid hormone-resistant patients have shown that the two TRβ isoforms are the dominant regulators of HPT feedback, and that TRα1 is unable to compensate for the loss of TRβ function (reviewed in [19]). Given that normal thyroid hormone levels are vital for many physiological and developmental processes, the disruption of TRβ 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 TRβ 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 TRβ 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 TRβ over TRα 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 dust [36] were associated with the observed activity. The TRβ 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 examples 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 protocols [37]. 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 in [18]), 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 TRβ transcription, test wells were treated with both 0.3 nM triiodothyronine (T3) and dust extracts (4 doses per extract, ½ dilutions). The final volume of each well was 40 μL, and the final DMSO concentration was 0.1%. Plates were incubated for 18 hours at 37˚C with humidity and 5% CO_2. 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 μM CCF4-AM LiveBLAzer™ substrate that was prepared following the manufacturer's protocol [38]. 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 assay [40] 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 TRβ activity compared to the T3 control. The relationships between the final concentration of FRs in the wells and the degree of TRβ 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 (μg/mL dust extract concentration) at which 20% TRβ antagonism was observed (IC_{20}). 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. Statistically 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 EC_{50} 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 EC_{50} and EC_{80} (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 TRβ signaling >20% relative to the T3 control. The remaining 80 extracts (58.4%) did not affect TRβ 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). TRβ 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 TRβ 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).

TRβ 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 (μg/mL) that inhibited TRβ 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 TRβ signaling in a cell-based reporter assay. Nearly half of the tested extracts antagonized TRβ signaling between 20–67% compared to the T3 control, suggesting that house dust is a significant source of TRβ-disrupting chemicals. This is significant because TRβ 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. TRβ 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 TRβ signaling in eukaryotic cells. An earlier study by Chou et al [45] that used a yeast-based reporter assay observed TRβ 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 TRβ signaling. Results from additional studies indicate that house dust mixtures can interfere with nuclear receptors other than TRβ, such as the peroxisome proliferator-activated receptor gamma (PPARγ). TRβ and PPARγ are evolutionarily-related receptors [46] that share roles in metabolic processes such as adipogenesis and lipid homeostasis [47–48]. Recent studies with PPARγ by our laboratory [49–50] and others [51] found that dust mixtures had agonist effects on PPARγ signaling. This is significant because both the activation of PPARγ and inhibition of TRβ can target many of the same endpoints, such

as promoting adipogenesis [48]. 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 bioassay [52]. 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) activities[53]. Previous work in our lab identified a significant relationship between PPARγ activity and fatty acids in dust, but not phthalates or organophosphates [50]. In this study we found that all twelve FRs were significantly correlated with TRβ antagonism, yet none of the FRs antagonized TRβ 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 pathway [58]. While the tested FRs did not antagonize TRβ 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 TRβ signaling in laboratory studies (reviewed in [63]) 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 activities [64]. This study frequently observed responses that were more potent than the individual components of the mixture. Furthermore, synergistic TRβ antagonism was observed with a mixture comprised of 23 chemicals, meaning the degree of TRβ 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 mixture [64]. 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 TRβ 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 TRβ-mediated negative feedback on the HPT axis. TRβ 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 TRβ 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 suggests 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 TRβ (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 TRβ. These mutations interfere with the receptor's ability to bind hormones, and TRβ 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 TRβ's ability to regulate TSH and TRH expression, however a much larger study would be necessary to understand if dust-mediated TRβ disruption is occurring via this mechanism.

In conclusion, these results demonstrate that house dust is a significant source of TRβ disrupting chemicals, and TRβ disruption may have a role in the altered serum TH levels observed post-exposure. The implications of these results are significant, as TRβ 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 TRβ signaling and FT4 levels suggest that exposure to house dust may disrupt TRβ 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 TRβ antagonism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

References

1. Weschler CJ Changes in indoor pollutants since the 1950s. Atmos. Environ 2009, 43 (1), 153–169.

2. Rudel RA; Camann DE; Spengler JD; Korn LR; Brody JG Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. Environ. Sci. Technol 2003, 37 (20), 4543–4553. [PubMed: 14594359]

3. Ma WL; Subedi B; Kannan K The occurrence of bisphenol A, phthalates, parabens and other environmental phenolic compounds in house dust: A review. Curr. Org. Chem 2014, 18 (17), 2182–2199.

4. Mitro SD; Dodson RE; Singla V; Adarnkiewicz G; Elmi AF; Tilly MK; Zota AR Consumer product chemicals in indoor dust: A quantitative meta-analysis of US studies. Environ. Sci. Technol 2016, 50 (19), 10661–10672. [PubMed: 27623734]

5. Zhang J; Kamstra JH; Ghorbanzadeh M; Weiss JM; Hamers T; Andersson PL In silico approach to identify potential thyroid hormone disruptors among currently known dust contaminants and their metabolites. Environ. Sci. Technol 2015, 49 (16), 10099–10107. [PubMed: 26207645]

6. Hilton DC; Jones RS; Sjödin A A method for rapid, non-target screening for environmental contaminants in household dust. J. Chromatogr. A 2010, 1217, 6851–6856. [PubMed: 20864112]

7. Lorber M Exposure of Americans to polybrominated diphenyl ethers. J. Expo. Sci. Environ. Epidemiol 2008, 18 (1), 2–19. [PubMed: 17426733]

8. Roosens L; Abdallah MA; Harrad S; Neels H; Covaci A Exposure to hexabromocyclododecanes (HBCDs) via dust ingestion, but not diet, correlates with concentrations in human serum: preliminary results. Environ. Health. Persp 2009, 117 (11), 1707–1712.

9. Lanphear BP; Matte TD; Rogers J; Clickner RP; Dietz B; Bornschein RL; Succop P; Mahaffey KR; Dixon S; Galke W; Rabinowitz M; Farfel M; Rohde C; Schwartz J; Ashley P; Jacobs DE The contribution of lead-contaminated house dust and residential soil to children's blood lead levels. Environ. Res 1998, 79, 51–68. [PubMed: 9756680]

10. Langer S; Bekö G; Weschler CJ; Brive LM; Toftum J; Callesen M; Clausen G Phthalate metabolites in urine samples from Danish children and correlations with phthalates in dust samples from their homes and daycare centers. Int. J. Hyg. Environ. Health 2014, 217, 78–87. [PubMed: 23623597]

11. Johnson PI; Stapleton HM; Mukherjee B; Hauser R; Meeker JD Associations between brominated flame retardants in house dust and hormone levels in men. Sci. Total. Environ 2013, 445, 177–184. [PubMed: 23333513]

12. Meeker JD; Johnson PI; Camann D; Hauser R Polybrominated diphenyl ether (PBDE) concentrations in house dust are related to hormone levels in men. Sci. Total. Environ 2009, 407 (10), 3425–3429. [PubMed: 19211133]

13. Meeker JD; Stapleton HM House dust concentrations of organophosphate flame retardants in relation to hormone levels and semen quality parameters. Environ. Health. Persp 2010, 118 (3), 318–323.

14. Ortiga-Carvalho TM; Chiamolera MI; Pazos-Moura CC; Wondisford FE Hypothalamus-Pituitary-Thyroid Axis. Compr. Physiol 2016, 6 (3), 1387–1428. [PubMed: 27347897]

15. Dupré SM; Guissouma H; Flamant F; Seugnet I; Scanlan TS; Baxter JD; Samarut J; Demeneix BA; Becker N Both thyroid hormone receptor (TR)β1 and TRβ2 isoforms contribute to the regulation of hypothalamic thyrotropin-releasing hormone. Endocrinology 2004, 145 (5), 2337–2345. [PubMed: 14726446]

16. Abel ED; Ahima RS; Boers ME; Elmquist JK; Wondisford FE Critical role for thyroid hormone receptor β2 in the regulation of paraventricular thyrotropin-releasing hormone neurons. J. Clin. Invest 2001, 107 (8), 1017–1023. [PubMed: 11306605]

17. Gauthier K; Chassande O; Plateroti M; Roux JP; Legrand C; Pain B; Rousset B; Weiss R; Trouillas J; Samarut J Different functions for the thyroid hormone receptors TRα and TRβ in the control of thyroid hormone production and post-natal development. The EMBO Journal 1999, 18 (3), 623–631. [PubMed: 9927422]

18. Vennström B; Liu H; Forrest D Chapter 7: Thyroid Hormone Receptors In Nuclear Receptors: Current Concepts and Future Challenges, Bunce CM; Campbell MJ, Eds. Springer Science & Business Media: New York, 2010; pp 183–201.

19. Ortiga-Carvalho TM; Sidhaye AR; Wondisford FE Thyroid hormone receptors and resistance to thyroid hormone disorders. Nat. Rev. Endocrinol 2014, 10 (10), 582–591. [PubMed: 25135573]

20. Kojima H; Takeuchi S; Itoh T; Iida M; Kobayashi S; Yoshida T In vitro endocrine disruption potential of organophosphate flame retardants via human nuclear receptors. Toxicology 2013, 314 (1), 76–83. [PubMed: 24051214]

21. Kojima H; Takeuchi S; Uramaru N; Sugihara K; Yoshida T; Kitamura S Nuclear hormone receptor activity of polybrominated diphenyl ethers and their hydroxylated and methoxylated metabolites in transactivation assays using chinese hamster ovary cells. Environ. Health. Persp 2009, 117 (8), 1210–1218.

22. Kojima H; Takeuchi S; Van den Eede N; Covaci A Effects of primary metabolites of organophosphate flame retardants on transcriptional activity via human nuclear receptors. Toxicol. Lett 2016, 245, 31–39. [PubMed: 26778350]

23. Ibhazehiebo K; Iwasaki T; Kimura-Kuroda J; Miyazaki W; Shimokawa N; Koibuchi N Disruption of thyroid hormone receptor-mediated transcription and thyroid hormone-induced purkinje cell dendrite arborization by polybrominated diphenyl ethers. Environ. Health. Persp 2011, 119 (2), 168–175.

24. Suvorov A; Bissonnette C; Takser L; Langlois MF Does 2,2 ',4,4 '-tetrabromodiphenyl ether interact directly with thyroid receptor? J. Appl. Toxicol 2011, 31 (2), 179–184. [PubMed: 20737425]

25. Klopčič I; Skledar G; Maši LP; Dolenc MS Comparison of in vitro hormone activities of novel flame retardants TBB, TBPH and their metabolites TBBA and TBMEPH using reporter gene assays. Chemosphere 2016, 160, 244–251. [PubMed: 27380226]

26. Zhang Q; Ji CY; Yin XH; Yan L; Lu MY; Zhao MR Thyroid hormone-disrupting activity and ecological risk assessment of phosphorus-containing flame retardants by in vitro, in vivo and in silico approaches. Environ. Pollut 2016, 210, 27–33. [PubMed: 26701863]

27. Ren XM; Cao LY; Yang Y; Wan B; Wang SF; Guo LH In vitro assessment of thyroid hormone receptor activity of four organophosphate esters. J. Environ. Sci-China 2016, 45, 185–190. [PubMed: 27372132]

28. Hamers T; Kamstra JH; Sonneveld E; Murk AJ; Kester MHA; Andersson PL; Legler J; Brouwer A In vitro profiling of the endocrine-disrupting potency of brominated flame retardants. Toxicol. Sci 2006, 92 (1), 157–173. [PubMed: 16601080]

29. Nakamura N; Matsubara K; Sanoh S; Ohta S; Uramaru N; Kitamura S; Yamaguchi M; Sugihara K; Fujimoto N Cell type-dependent agonist/antagonist activities of polybrominated diphenyl ethers. Toxicol. Lett 2013, 223 (2), 192–197. [PubMed: 24076165]

30. Kienzler A; Berggren E; Bessems J; Bopp S; van der Linden S; Worth A Assessment of Mixtures - Review of Regulatory Requirements and Guidance JRC Science and Policy Report.; European Commission: Joint Research Center, Ispra, Italy, 2014.

31. Kortenkamp A; Backhaus T; Faust M State of the Art Report on Mixture Toxicity - Final Report, Executive Summary; Study Contract No. 070307/2007/485103/ETU/D.1.; European Commission: December 22, 2009.

32. Orton F; Ermler S; Kugathas S; Rosivatz E; Scholze M; Kortenkamp A Mixture effects at very low doses with combinations of anti-androgenic pesticides, antioxidants, industrial pollutant and chemicals used in personal care products. Toxicol. Appl. Pharmacol 2014, 278, 201–208. [PubMed: 24055644]

33. Rajapakse N; Silva E; Kortenkamp A Combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhances steroid hormone action. Environ. Health. Persp 2002, 110 (9), 917–921.

34. Silva E; Rajapakse N; Kortenkamp A Something from "nothing" - eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. Environ. Sci. Technol. 2002, 36, 1751–1756. [PubMed: 11993873]

35. Thrupp TJ; Runnalls TJ; Scholze M; Kugathas S; Kortenkamp A; Sumpter JP The consequences of exposure to mixtures of chemicals: Something from 'nothing' and 'a lot from a little' when fish are exposed to steroid hormones. Sci. Total. Environ 2018, 619–620, 1482–1492.

36. Hammel SC; Hoffman K; Lorenzo AM; Chen A; Phillips AL; Butt CM; Sosa JA; Webster TF; Stapleton HM Associations between flame retardant applications in furniture foam, house dust levels, and residents' serum levels. Environ. Int 2017, 107, 181–189. [PubMed: 28750223]

37. Hoffman K; Lorenzo A; Butt CM; Hammel SC; Henderson BB; Roman SA; Scheri RP; Stapleton HM; Sosa JA Exposure to flame retardant chemicals and occurrence and severity of papillary thyroid cancer: A case-control study. Environ. Int 2017, 107, 235–242. [PubMed: 28772138]

38. Invitrogen LiveBLAzer™ FRET - B/G Loading Kit with CCF2-AM and CCF4-AM Protocol. https://www.thermofisher.com/order/catalog/product/K1095 (accessed February 8).

39. Invitrogen GeneBLAzer® TR beta UAS-bla HEK 293T cell based assay protocol. https://www.thermofisher.com/order/catalog/product/K1686 (accessed February 27).

40. Riss TL; Moravec BS; Niles AL; Duellman S; Benink HA; Worzella TJ; Minor L, Cell Viability Assays In Assay Guidance Manual [Internet], Sittampalam GS; Coussens NP; Brimacombe K; al., e., Eds. Lilly Eli & Company and the National Center for Advancing Translational Sciences: Bethesda, MD, 2013 [Updated 2016 Jul 1]; pp 355–385.

41. Pascual A; Aranda A Thyroid hormone receptors, cell growth and differentiation. BBA-Gen. Subjects 2013, 1830 (7), 3908–3916.

42. Chen AM; Yolton K; Rauch SA; Webster GM; Hornung R; Sjödin A; Dietrich KN; Lanphear BP Prenatal polybrominated diphenyl ether exposures and neurodevelopment in U.S. children through 5 years of age: The HOME study. Environ. Health. Persp 2014, 122 (8), 856–862.

43. Dingemans MM; van den Berg M; Westerink RH Neurotoxicity of brominated flame retardants: (in)direct effects of parent and hydroxylated polybrominated diphenyl ethers on the (developing) nervous system. Environ. Health. Persp 2011, 119 (7), 900–907.

44. Costa LG; de Laat R; Tagliaferri S; Pellacani C A mechanistic view of polybrominated diphenyl ether (PBDE) developmental neurotoxicity. Toxicol. Lett 2014, 230 (2), 282–294. [PubMed: 24270005]

45. Chou PH; Lee CH; Ko FC; Lin YJ; Kawanishi M; Yagi T; Li IC Detection of hormone-like and genotoxic activities in indoor dust from Taiwan using a battery of *in vitro* bioassays. Aerosol Air Qual. Res 2015, 15 (4), 1412–1421.

46. Bertrand W; Brunet FG; Escriva H; Parmentier G; Laudet V; Robinson-Rechavi M Evolutionary genomics of nuclear receptors: From twenty-five ancestral genes to derived endocrine systems. Mol. Biol. Evol 2004, 21 (10), 1923–1937. [PubMed: 15229292]

47. Liu YY; Brent GA Thyroid hormone crosstalk with nuclear receptor signaling in metabolic regulation. Trends Endocrin. Met 2010, 21 (3), 166–173.

48. Lu CX; Cheng SY Thyroid hormone receptors regulate adipogenesis and carcinogenesis via crosstalk signaling with peroxisome proliferator-activated receptors. J. Mol. Endocrinol 2010, 44 (3), 143–154. [PubMed: 19741045]

49. Fang ML; Webster TF; Stapleton HM Activation of human peroxisome proliferator-activated nuclear receptors (PPARγ1) by semi-volatile compounds (SVOCs) and chemical mixtures in indoor dust. Environ. Sci. Technol 2015, 49 (16), 10057–10064. [PubMed: 26172262]

50. Fang ML; Webster TF; Stapleton HM Effect-directed analysis of human peroxisome proliferator-activated nuclear receptors (PPARγ1) ligands in indoor dust. Environ. Sci. Technol 2015, 49 (16), 10065–10073. [PubMed: 26172369]

51. Suzuki G; Tue NM; Malarvannan G; Sudaryanto A; Takahashi S; Tanabe S; Sakai S; Brouwer A; Uramaru N; Kitamura S; Taldgami H Similarities in the endocrine-disrupting potencies of indoor dust and flame retardants by using human osteosarcoma (U2OS) cell-based reporter gene assays. Environ. Sci. Technol 2013, 47 (6), 2898–2908. [PubMed: 23398518]

52. Vandermarken T; De Galan S; Croes K; Van Langenhove K; Vercammen J; Sanctorum H; Denison MS; Goeyens L; Elskens M; Baeyens W Characterisation and implementation of the ERE-

CALUX bioassay on indoor dust samples of kindergartens to assess estrogenic potencies. J. Steroid Biochem 2016, 155, 182–189.

53. Wong F; Suzuki G; Michinaka C; Yuan B; Takigami H; de Wit CA Dioxin-like activities, halogenated flame retardants, organophosphate esters and chlorinated paraffins in dust from Australia, the United Kingdom, Canada, Sweden and China. Chemosphere 2017, 168, 1248–1256. [PubMed: 27814953]

54. Stapleton HM; Misenheimer J; Hoffman K; Webster TF Flame retardant associations between children's handwipes and house dust. Chemosphere 2014, 116, 54–60. [PubMed: 24485814]

55. Dodson RE; Perovich LJ; Covaci A; Van den Eede N; Ionas AC; Dirtu AC; Brody JG; Rudel RA After the PBDE phase-out: A broad suite of flame retardants in repeat house dust samples from California. Environ. Sci. Technol 2012, 46 (24), 13056–13066. [PubMed: 23185960]

56. Kademoglou K; Xu FC; Padilla-Sanchez JA; Haug LS; Covaci A; Collins CD Legacy and alternative flame retardants in Norwegian and UK indoor environment: Implications of human exposure via dust ingestion. Environ. Int 2017, 102, 48–56. [PubMed: 28190611]

57. Rudel RA; Dodson RE; Perovich LJ; Morello-Frosch R; Camann DE; Zuniga MM; Yau AY; Just AC; Brody JG Semivolatile endocrine-disrupting compounds in paired indoor and outdoor air in two northern California communities. Environ. Sci. Technol 2010, 44 (17), 6583–90. [PubMed: 20681565]

58. Hoffman K; Hammel SC; Phillips AL; Lorenzo AM; Chen A; Calafat AM; Ye X; Webster TF; Stapleton HM Biomarkers of exposure to SVOCs in children and their demographic associations: The TESIE study. Environ. Int 2018, 119, 26–36. [PubMed: 29929048]

59. Peng H; Saunders DMV; Sun JX; Jones PD; Wong CKC; Liu HL; Giesy JP Mutagenic azo dyes, rather than flame retardants, are the predominant brominated compounds in house dust. Environ. Sci. Technol 2016, 50 (23), 12669–12677. [PubMed: 27934287]

60. Peng H; Saunders DMV; Sun JX; Jones PD; Wong CKC; Liu HL; Giesy JP Correction to Mutagenic azo dyes, rather than flame retardants, are the predominant brominated compounds in house dust. Environ. Sci. Technol 2017, 51 (6), 3593–3593. [PubMed: 28281751]

61. Wang L; Asimakopoulos AG; Moon HB; Nakata H; Kannan K Benzotriazole, benzothiazole, and benzophenone compounds in indoor dust from the United States and east Asian countries. Environ. Sci. Technol 2013, 47 (9), 4752–4759. [PubMed: 23544437]

62. Hwang HM; Park EK; Young TM; Hammock BD Occurrence of endocrine-disrupting chemicals in indoor dust. Sci. Total. Environ 2008, 404 (1), 26–35. [PubMed: 18632138]

63. Mughal BB; Fini JB; Demeneix BA Thyroid-disrupting chemicals and brain development: an update. Endocr. Connect 2018, 7 (4), R160–R186. [PubMed: 29572405]

64. Kassotis CD; Klemp KC; Vu DC; Lin CH; Meng CX; Besch-Williford CL; Pinatti L; Zoeller RT; Drobnis EZ; Balise VD; Isiguzo CJ; Williams MA; Tillitt DE; Nagel SC Endocrine-disrupting activity of hydraulic fracturing chemicals and adverse health outcomes after prenatal exposure in male mice. Endocrinology 2015, 156 (12), 4458–4473. [PubMed: 26465197]

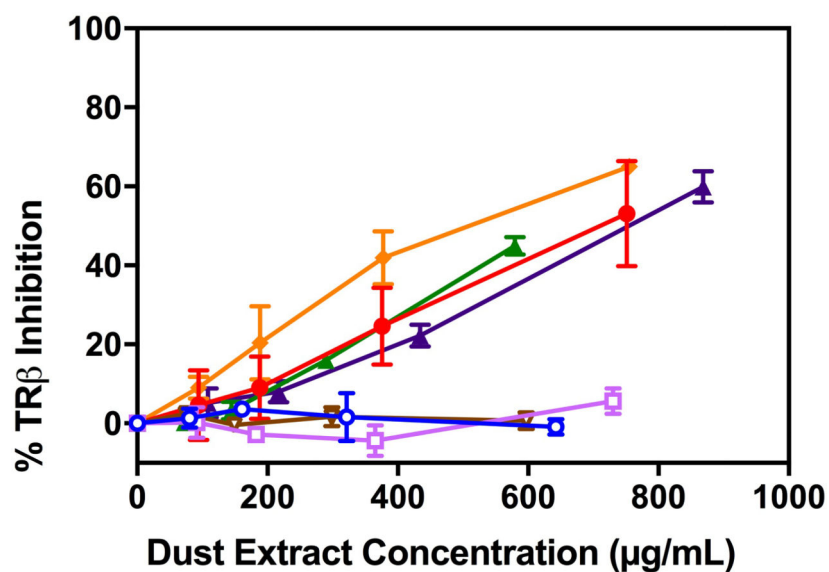65. Brent GA Mechanisms of thyroid hormone action. J. Clin. Invest 2012, 122 (9), 3035–3043. [PubMed: 22945636]

Figure 1.
Representative results of TRβ 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 TRβ activity ≥ 20% of the T3 control were considered active. Plotted data is the average ± SEM of three separate experiments.
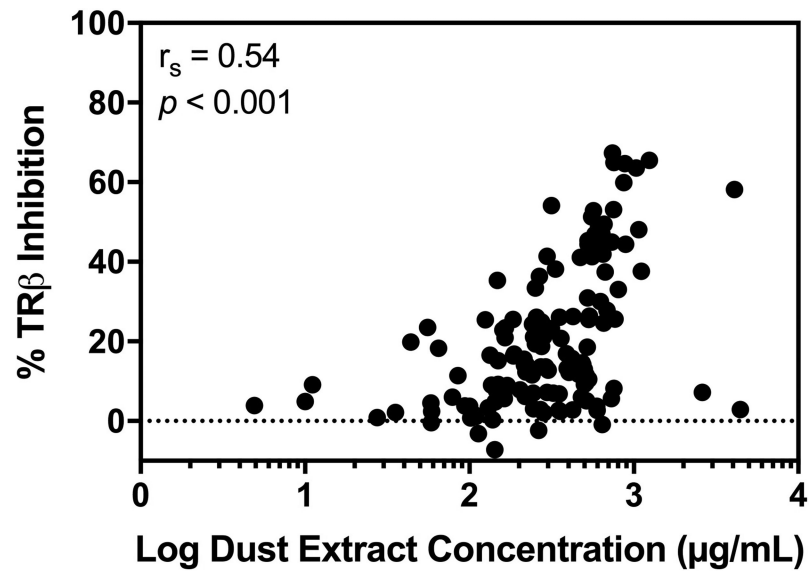
Figure 2.
Spearman correlation between the degree of inhibition of TRβ signaling relative to the triiodothyronine (T3) control and the tested dust extract concentrations (n=137).
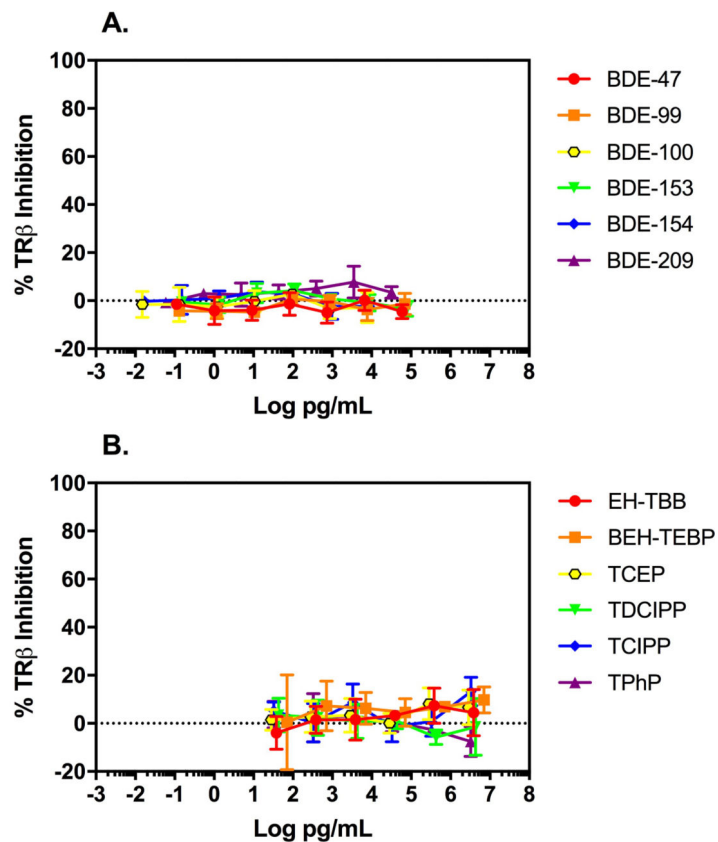
Figure 3.
TRβ dose-response results with the individual flame retardants. None of the *(A)* PBDEs, or *(B)* FM550 components and PFRs antagonized TRβ signaling at any of the tested doses. GeneBLAzer® β-lactamase reporter assays in HEK 293T cells were conducted as described in the Materials and Methods. Each data point represents the average ± SEM of three separate experiments.

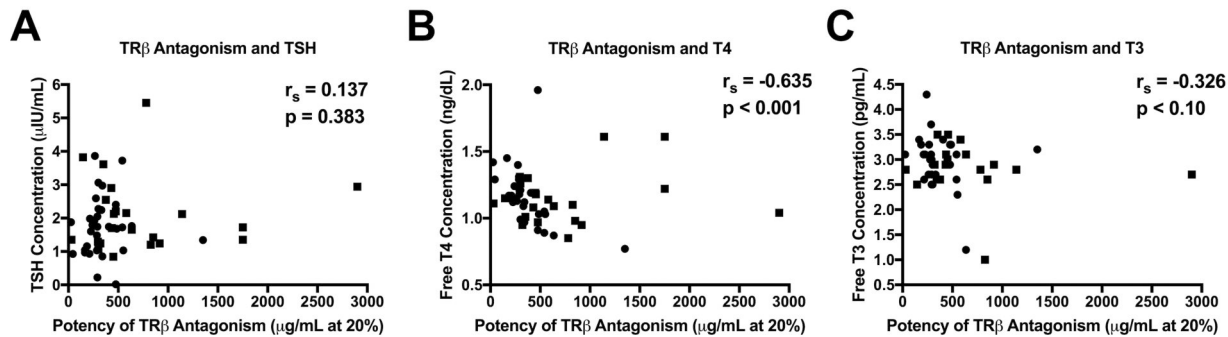## TRβ Potency (20% Antagonism) and Thyroid Status in Residents

Figure 4.
Spearman correlations between the potency of TRβ 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 TRβ 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 TRβ antagonism results (% Inhibition compared to the T3 control), the median and range concentrations of dust extracts (μg/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
TRβ Inhibition (%)	37%	20% – 67%	7%	−7% - 19%
Dust Extracts (μg/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. TRβ 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 ***

*** $= p < 0.001$

** $= p < 0.01$

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