

# Ecotoxicology and Human Environmental Health



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~~house dust disrupt~~  
Environmental



thyroid receptor

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House  
Page  
Dust



Extraction

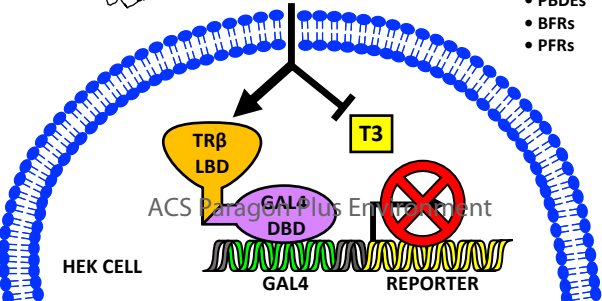


Analysis

Environmental Science & Technology

Flame Retardants:

- PBDEs
- BFRs
- PFRs



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1 RESEARCH ARTICLE

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3

4 **Chemical mixtures isolated from house dust disrupt**  
5 **thyroid receptor  $\beta$  (TR $\beta$ ) signaling**

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34

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37 CDK, KH, PLF, and HMS have nothing to disclose.

38

**39 Abstract:**

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

54

55

## 56 **Introduction**

57 It is well recognized that the indoor environment significantly impacts human health. The number  
58 of indoor chemicals suspected to adversely affect human health has increased over the past fifty years  
59 with the development of new building materials and consumer products (reviewed in<sup>1</sup>). Many of these  
60 chemicals are not permanently bound to their products and leach or off-gas from products and re-  
61 distribute into indoor air, dust, and surfaces<sup>2-4</sup>.

62 House dust is a highly complex mixture of hundreds (potentially thousands) of chemicals<sup>5-6</sup>.  
63 Unintentional dust ingestion is a significant exposure source for many chemicals of concern, including  
64 flame retardants<sup>7-8</sup>, lead<sup>9</sup>, and phthalates<sup>10</sup>. Human epidemiology studies have linked exposure to  
65 contaminants in dust to altered hormone signaling in multiple endocrine systems<sup>11-13</sup>.

66 Changes in circulating thyroid hormone levels is a hallmark of thyroid disruption. Circulating  
67 thyroid hormone levels are tightly regulated via negative feedback the hypothalamus-pituitary-thyroid  
68 (HPT) axis<sup>14</sup> and mediated by thyroid hormone receptors (TRs)<sup>15-17</sup>. TRs are encoded by two genes  
69 (THRA and THRB) that produce three active isoforms: TR $\alpha$ 1, TR $\beta$ 1, and TR $\beta$ 2 (reviewed in<sup>18</sup>). Studies  
70 in mouse models and thyroid hormone-resistant patients have shown that the two TR $\beta$  isoforms are the  
71 dominant regulators of HPT feedback, and that TR $\alpha$ 1 is unable to compensate for the loss of TR $\beta$   
72 function (reviewed in<sup>19</sup>). Given that normal thyroid hormone levels are vital for many physiological and  
73 developmental processes, the disruption of TR $\beta$  signaling by dust contaminants could have significant  
74 effects on human health.

75 Previous *in vitro* and *in silico* studies that focused on individual contaminants identified in dust  
76 have yielded mixed results<sup>20-29</sup>, and suggest TR $\beta$  is not a major target of chemicals in house dust due to a  
77 lack of activity or predicted binding. However, individual chemical testing does not account for the  
78 potential combined effects of exposures to complex mixtures. Interactions between chemicals in a mixture  
79 can significantly influence the activity or toxicity of the mixture as a whole, and these effects may not be  
80 predictable when assessing individual compounds<sup>30-31</sup>. For example, *in vitro* and *in vivo* studies have

81 shown that combinations of estrogenic and anti-androgenic chemicals can act jointly and produce effects  
82 much greater than predicted based on individual chemical data, even when the individual chemical  
83 concentrations are below no-effect levels<sup>32-35</sup>. It's possible that the thyroid-disrupting effects associated  
84 with house dust are due the combined effects of multiple substances in the mixture rather than individual  
85 chemicals.

86 The purpose of this study was to evaluate the potential of complex mixtures isolated from house  
87 dust to disrupt TR $\beta$  signaling in a cell-based reporter assay. We employed a whole mixtures approach by  
88 testing house dust collected from private homes to more accurately represent real-world exposures.  
89 Compared to individual chemical testing, the advantages of testing whole mixtures are that all identified  
90 and unknown substances in the mixture are present and interactions between mixture components are  
91 accounted for<sup>30-31</sup>. We focused on TR $\beta$  over TR $\alpha$  because this isoform is the dominant regulator of  
92 circulating TH levels as described above. The results from the reporter assay were used to determine if the  
93 concentrations of twelve flame retardants (FRs) previously quantified in the dust<sup>36</sup> were associated with  
94 the observed activity. The TR $\beta$  activities were further assessed for potential relationships with measured  
95 thyroid levels and other health outcomes in adults living in the sampled homes.

96

## 97 **2. Materials + Methods**

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

100

101 **Dust Collection and Extraction.** Dust samples used in this study were collected as part of a study  
102 investigating flame retardant exposures and papillary thyroid cancer using a case-control design. Dust  
103 collection, extraction, and analysis have been described in detail<sup>36-37</sup>. Briefly, the main living area of  
104 participants homes was vacuumed with a cellulose thimble fitted in the hose attachment of a Eureka®  
105 Mighty Might vacuum. Upon collection, thimbles were wrapped in foil and immediately frozen until

106 extraction. Dust examples were extracted with 1:1 dichloromethane:hexane (v/v) via sonication, and  
107 concentrated to 1 mL using a nitrogen evaporation system. Extracts were split into equal volumes, and the  
108 solvent from one aliquot was evaporated to dryness and re-suspended in 100  $\mu$ L DMSO for assays, while  
109 the other aliquot was used for flame retardant analysis. Brominated and organophosphate flame retardants  
110 were quantified using GC/MS as described previously<sup>36-37</sup>. Brominated FRs quantified include the major  
111 PentaBDE congeners (BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154), DecaBDE (BDE-209),  
112 and the brominated components of Firemaster® 550 (FM550): 2-ethylhexyl-2,3,4,5-tetrabromobenzoate  
113 (EH-TBB) and bis(2-ethylhexyl)-tetrabromophthalate (BEH-TEBP). Assessed organophosphate FRs  
114 include tris(2-chloroethyl)phosphate (TCEP), tris(1,3-dichloroisopropyl)phosphate (TDCIPP), tris(1-  
115 chloro-2-propyl)phosphate (TCIPP), and triphenyl phosphate (TPHP). FR concentrations that were below  
116 the detection limit were assigned a value equal to  $\frac{1}{2}$  of the minimum detection level (MDL) for statistical  
117 analysis.

118  
119 **Serum Analysis.** Free thyroxine (FT4), free triiodothyronine (FT3), thyroid-stimulating hormone (TSH),  
120 total cholesterol, and total triglycerides were measured by LabCorp in Burlington, NC using standard  
121 protocols<sup>37</sup>. The study population has been described in detail previously<sup>36-37</sup>.

122  
123 **GeneBLAzer® Assay.** Given that multiple nuclear receptors recognize the same DNA response elements  
124 as TR $\beta$ , and complex chemical mixtures in house dust can target multiple signaling pathways, we utilized  
125 a Gal4 reporter system to specifically focus on TR $\beta$  response and circumvent potential interference from  
126 other receptors and signaling pathways. As TR $\beta$ 1 and TR $\beta$ 2 only differ in the N-terminus region  
127 (reviewed in<sup>18</sup>), a Gal4 system is representative of both TR $\beta$  isoforms as the Gal4-TR $\beta$  construct only  
128 includes the ligand-binding domain of the receptor. Details regarding the cell culture media and reagents  
129 can be found in Table S2. Methods for preparing the frozen GeneBLAzer® TR $\beta$ -UAS-*bla* HEK 293T cell  
130 stocks can be found in the Supporting Information. Frozen cells were quickly thawed in a 37°C water bath  
131 with gentle agitation. Thawed cells were diluted in 10 mL of assay medium (phenol red-free high glucose

132 DMEM supplemented with 2% charcoal-stripped FBS, 4 mM GlutaMAX™, 1 mM sodium pyruvate, 0.1  
133 mM non-essential amino acids, 25 mM HEPES, 100 U/mL penicillin and streptomycin). Cells were  
134 centrifuged at 200 x g for 5 minutes, and the pellet was gently re-suspended via pipetting in 2 mL of fresh  
135 assay medium. Cell were quantified, and density was adjusted to  $3.6 \times 10^5$  cells/mL. Duplicate 384-well  
136 black-walled tissue culture plates with clear bottoms were plated at ~10,000 cells/well (28  $\mu$ L of the cell  
137 suspension) and incubated at room temperature for 2 hours to allow for cell settling.

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

147 Following incubation, cells were loaded with 1  $\mu$ M CCF4-AM LiveBLAzer™ substrate that was  
148 prepared following the manufacturer's protocol<sup>38</sup>. Plates were protected from light and incubated for two  
149 hours at room temperature. Blue (410/460 nm) and green (410/530 nm) fluorescence emissions were  
150 quantified on a SpectraMax M5 plate reader (Molecular Devices, San Jose, CA). Each assay was repeated  
151 on three separate occasions, with quadruplicate wells per concentration within each assay.

152  
153 **GeneBLAzer® Data Analysis.** Raw data was analyzed following the manufacturer's recommendations  
154 <sup>38-39</sup>. Net blue and green fluorescence signals were calculated by subtracting the average fluorescence  
155 background from the cell-free wells from all control and experiment wells. The blue/green emission ratio  
156 was calculated for each well by dividing the net blue values (410/460 nm) by the corresponding net green  
157 values (410/530 nm). To quantify the receptor response over background, the response ratio (RR) was

158 calculated by dividing the blue/green emission ratio for each test compound by the emission ratio for the  
159 DMSO control wells. The % Inhibition was calculated from the response ratio using the following  
160 formula.

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

161  
162 **Cell Viability Assay.** Cell viability was assessed using a resazurin reduction assay<sup>40</sup> and described in  
163 detail in the Supporting Information. Cytotoxicity was distinguished by a decrease in cell viability greater  
164 than 15%.

165  
166 **Statistical Analysis.** GeneBLAzer® results were analyzed using a one-way ANOVA followed by  
167 Dunnett's posthoc test to identify dust extract dilutions that significantly reduced TR $\beta$  activity compared  
168 to the T3 control. The relationships between the final concentration of FRs in the wells and the degree of  
169 TR $\beta$  antagonism (defined as % Inhibition compared to the T3 control) was assessed using Spearman  
170 correlation coefficients. For assessing relationships with the health biomarkers (FT4, FT3, TSH, total  
171 cholesterol, total triglycerides), we determined that extracted dust mass could be a potential confounder in  
172 maximal bioactivity. A normalized metric was created for further assessment: potency ( $\mu\text{g/mL}$  dust  
173 extract concentration) at which 20% TR $\beta$  antagonism was observed (IC<sub>20</sub>). Full dose responses of dust  
174 extracts were plotted and used to calculate the point where the curves crossed the 20% efficacy line.  
175 Values were extrapolated as necessary for samples that approached but did not reach the 20% inhibition  
176 mark; values with no apparent activity (not increasing towards the 20% mark) were not included,  
177 as potencies cannot be calculated for inactive samples. All analyses were conducted in GraphPad Prism 7.  
178 Statistically significance was set at  $\alpha = 0.05$ .

179  
180 **3. Results**

181

182 **Antagonist activity of the dust extracts.** The thyroid hormone triiodothyronine (T3) was used as the  
183 active hormone in the antagonist assays. The EC<sub>50</sub> for T3 was 0.206 nM (95% confidence interval = 0.195  
184 - 0.218 nM) in our assay, which is similar to what was reported by the manufacturer (0.25 nM)<sup>39</sup> (Figure  
185 S1). Based on the T3 dose-response curve results, the antagonist assays were conducted using 0.3 nM of  
186 T3. This concentration yields a good dynamic response range and is located between the EC<sub>50</sub> and EC<sub>80</sub>  
187 (0.34 nM) concentrations as recommended by the manufacturer. Summary statistics are presented in  
188 Table 1. Of the 137 tested extracts, 57 (41.6%) inhibited TRβ signaling >20% relative to the T3 control.  
189 The remaining 80 extracts (58.4%) did not affect TRβ signaling and were considered inactive (see Figure  
190 1 for representative examples of active and inactive extracts). The dust concentrations tested in this study  
191 varied based on the mass of dust collected in all the homes. While the dust concentration ranges in the  
192 active and inactive extracts were similar, the median dust concentration in active extracts was over 2-fold  
193 higher than the median concentration in inactive extracts (Table 1). TRβ antagonism was significantly and  
194 positively associated with the dust mass concentration in the well (Figure 2;  $r_s = 0.54$ ,  $p < 0.001$ ,  $n = 137$ ), as  
195 would be expected given that more dust would contain more chemical contaminants. Cytotoxicity was  
196 observed in one or more doses in 31 of the 137 tested extracts. Toxic doses were excluded from statistical  
197 analyses, and only doses below the cytotoxicity threshold (> 15% viability loss) were included in the  
198 analysis.

199  
200 **Flame retardant correlations.** To gain a better understanding of the chemicals potentially driving the  
201 observed response, we assessed the relationship between the final concentration in the wells of the twelve  
202 flame retardants in the dust mixtures and the degree of TRβ antagonism. Summary statistics of the FR  
203 concentrations are presented in Table 1, and the Spearman correlation results are presented in Table 2.  
204 Antagonism was significantly and positively associated with the concentrations of all 12 flame retardants  
205 in the mixtures (Table 2,  $r_s = 0.25 - 0.44$ ). The strongest correlations were observed with BDE-209 ( $r_s =$   
206  $0.44$ ,  $p < 0.001$ ), TCEP, and TCIPP ( $r_s = 0.39$ ,  $p < 0.001$  for both).

207

208 **TR $\beta$  activities of the individual FRs.** To test if the FRs present in the dust extract mixtures may be  
209 driving the observed antagonism, each FR was tested individually in the GeneBLAzer® assay using a  
210 concentration range that included the ranges observed in the dust extracts (Figure 3). No significant  
211 antagonism was observed with any of the 12 FRs, which suggests that the individual FRs may not be  
212 driving the observed effects.

213  
214 **Antagonist potency correlations with thyroid biomarkers.** Previous studies have identified  
215 associations between FR exposure and serum TH levels in individuals living in the sampled homes. For  
216 this study, we assessed the relationships between dust potency (dust concentration ( $\mu\text{g}/\text{mL}$ ) that inhibited  
217 TR $\beta$  by 20% relative to the T3 control) and health biomarkers of residents using Spearman correlations.  
218 Potency was significantly correlated with serum FT4 ( $r_s = -0.64$ ,  $p < 0.001$ ,  $n=51$ ) in adults living in  
219 sampled homes (Figure 4). The result with FT3 was suggestive but did not reach statistical significance ( $r_s$   
220  $= -0.33$ ,  $p < 0.10$ ,  $n=45$ ). No relationship was observed between potency and TSH ( $r_s = 0.14$ ,  $p = 0.38$ ,  
221  $n=53$ ), body mass index (BMI,  $r_s = 0.18$ ,  $p = 0.17$ ,  $n=112$ ), total cholesterol ( $r_s = 0.13$ ,  $p = 0.27$ ,  $n=100$ ),  
222 and total triglycerides ( $r_s = 0.12$ ,  $p = 0.23$ ,  $n=100$ ). A summary of the measured health metrics is  
223 presented in Table S3.

224

## 225 **Discussion**

226 In this study, extracts of house dust collected from 137 homes in North Carolina were screened  
227 for the ability to disrupt normal TR $\beta$  signaling in a cell-based reporter assay. Nearly half of the tested  
228 extracts antagonized TR $\beta$  signaling between 20-67% compared to the T3 control, suggesting that house  
229 dust is a significant source of TR $\beta$ -disrupting chemicals. This is significant because TR $\beta$  is essential to  
230 multiple developmental processes and normal physiology in vertebrates, including neurodevelopment and  
231 adult neurogenesis, cardiac maturation and physiology, skeletal development, and metabolism (reviewed  
232 in <sup>14, 41</sup>). The consequences of developmental thyroid disruption are often permanent. Multiple compounds

233 identified in dust have been linked to neurological and developmental abnormalities in laboratory models,  
234 and epidemiological studies have identified links between exposure to PBDEs and neurological effects in  
235 adults and children<sup>42-44</sup>. It's hypothesized that a majority of the PBDE exposure is coming from dust in  
236 these populations. TR $\beta$  disruption is especially concerning in young children, as they are undergoing  
237 vulnerable periods of development that can be influenced by exogenous chemical signals.

238 This is the first study to demonstrate the antagonistic effects of dust exposure on TR $\beta$  signaling in  
239 eukaryotic cells. An earlier study by Chou et al<sup>45</sup> that used a yeast-based reporter assay observed TR $\beta$   
240 antagonism in response to nine dust samples collected from buildings and roads at a university in Taiwan,  
241 supporting the notion that chemical mixtures in house dust can disrupt TR $\beta$  signaling. Results from  
242 additional studies indicate that house dust mixtures can interfere with nuclear receptors other than TR $\beta$ ,  
243 such as the peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ). TR $\beta$  and PPAR $\gamma$  are  
244 evolutionarily-related receptors<sup>46</sup> that share roles in metabolic processes such as adipogenesis and lipid  
245 homeostasis<sup>47-48</sup>. Recent studies with PPAR $\gamma$  by our laboratory<sup>49-50</sup> and others<sup>51</sup> found that dust mixtures  
246 had agonist effects on PPAR $\gamma$  signaling. This is significant because both the activation of PPAR $\gamma$  and  
247 inhibition of TR $\beta$  can target many of the same endpoints, such as promoting adipogenesis<sup>48</sup>. Together  
248 with the current results, this suggests that the impacts of contaminant mixtures in house dust on a  
249 physiological system may be complex.

250 Understanding and predicting the effects of mixture exposures is aided by identifying chemicals  
251 within a mixture that contribute to the observed response. Previous receptor studies have identified  
252 significant relationships between concentrations of specific chemicals in dust and receptor activity. For  
253 example, phthalate concentrations in dust collected from kindergarten classrooms in Belgium were  
254 associated with estrogen receptor (ER) activation in a cell-based bioassay<sup>52</sup>. A separate study found that  
255 concentrations of PBDEs and chlorinated paraffins in dust samples from five countries were significantly  
256 correlated with observed aryl hydrocarbon receptor (AhR) activities<sup>53</sup>. Previous work in our lab identified  
257 a significant relationship between PPAR $\gamma$  activity and fatty acids in dust, but not phthalates or  
258 organophosphates<sup>50</sup>. In this study we found that all twelve FRs were significantly correlated with TR $\beta$

259 antagonism, yet none of the FRs antagonized TR $\beta$  signaling when tested individually. This suggests that  
260 the mechanism of action is more complex than simple mediation by the individual chemicals and may  
261 implicate co-exposures or mixture effects as a cause.

262         The concentrations of contaminants in house dust often correlate with each other, frequently due  
263 to both chemicals occurring in the same commercial mixtures, co-application of different chemicals to the  
264 same products, or shared sources<sup>11, 54-56</sup>. However, the majority of data for contaminant correlations in  
265 house dust has focused on specific chemical classes (i.e. flame retardants or phthalates), and potential  
266 associations between diverse classes needs to be clarified. Previous studies examining relationships  
267 between contaminant concentrations in other compartments (indoor air and biological samples) have  
268 found significant correlations between chemicals from diverse classes and sources<sup>57-58</sup>. A recent study  
269 from our group observed significant correlations between urinary metabolites of various contaminant  
270 classes with different use patterns (for example, the metabolite of TDCIPP and bisphenol A), which  
271 suggests a common source or exposure pathway<sup>58</sup>. While the tested FRs did not antagonize TR $\beta$   
272 signaling, it is possible that the observed antagonism is driven by unidentified chemicals that co-occur  
273 with the flame retardants in the mixture. Contaminants such as plasticizers, colorants, and UV stabilizers  
274 are often included alongside FRs during the manufacturing process and are frequently identified in house  
275 dust<sup>2, 59-62</sup>. The fact that some of these compounds have been shown to disrupt TR $\beta$  signaling in  
276 laboratory studies (reviewed in<sup>63</sup>) supports the notion that contaminants that co-occur with the flame  
277 retardants may be driving the observed antagonism.

278         A second possibility is that the observed antagonism is due to the chemicals in the dust mixtures  
279 acting together in a way that influences the overall activity of the mixture (often referred to as “mixture  
280 effects”). Previous *in vitro* and *in vivo* studies have demonstrated that the combined effects of mixtures of  
281 chemicals that share the same mechanism of action can be greater than the individually tested  
282 components, even when the concentration of each component is below the no observed effect level<sup>32-35</sup>.  
283 The activity of chemical in a mixture may be enhanced (synergism) or inhibited (antagonism) by  
284 interactions with other mixtures components, and this effect may not be predictable when testing

285 individual chemicals<sup>30-31</sup>. This is illustrated by a recent study examining the effects of chemical mixtures  
286 used in hydraulic fracturing on nuclear receptor activities<sup>64</sup>. This study frequently observed responses  
287 that were more potent than the individual components of the mixture. Furthermore, synergistic TR $\beta$   
288 antagonism was observed with a mixture comprised of 23 chemicals, meaning the degree of TR $\beta$   
289 antagonism was greater than the predicted additive effects of the individual components. Interestingly, the  
290 synergistic effect was eliminated when bisphenol A was added to the mixture<sup>64</sup>. The observed lack of  
291 activity of the individual FRs in this study may therefore be due to the absence of the remaining mixture  
292 components.

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

310 TRH expression, however a much larger study would be necessary to understand if dust-mediated TR $\beta$   
311 disruption is occurring via this mechanism.

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

321

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326

327

328

329 **Tables**

330 **Table 1.** Median and range of the TR $\beta$  antagonism results (% Inhibition compared to the T3 control), the  
 331 median and range concentrations of dust extracts ( $\mu\text{g/mL}$ ) in the wells, and the median and range  
 332 concentrations of each flame retardant in the wells ( $\text{pg/mL}$ ) in the active and inactive samples.

	Active Extracts (n=57)		Inactive Extracts (n=80)	
	Median	Range	Median	Range
TR $\beta$ 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 ( $\text{pg/mL}$ )				
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 ( $\text{pg/mL}$ )				
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 ( $\text{pg/mL}$ )				
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

333

334

335

336 **Table 2.** Spearman correlations between the concentrations of individual flame retardants in the wells vs.  
337 TR $\beta$  Inhibition.

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

338 \*\*\* =  $p < 0.001$

339 \*\* =  $p < 0.01$

340

## 341 **Figure Legends**

342

343 **Figure 1.** Representative results of TR $\beta$  antagonism (% Inhibition) by active (closed shapes) and inactive  
344 (open shapes) dust extracts obtained via the GeneBLAzer $\text{\textcircled{R}}$   $\beta$ -lactamase reporter assay in HEK 293T cells  
345 as described in the Materials and Methods. The colors represent different dust extracts. Cells were treated  
346 with a range of dust extract concentrations in the presence of 0.3 nM triiodothyronine (T3). Extracts that  
347 decreased TR $\beta$  activity  $\geq$  20% of the T3 control were considered active. Plotted data is the average  $\pm$   
348 SEM of three separate experiments.

349

350 **Figure 2.** Spearman correlation between the degree of inhibition of TR $\beta$  signaling relative to the  
351 triiodothyronine (T3) control and the tested dust extract concentrations (n=137).

352

353 **Figure 3.** TR $\beta$  dose-response results with the individual flame retardants. None of the (A) PBDEs, or (B)  
354 FM550 components and PFRs antagonized TR $\beta$  signaling at any of the tested doses. GeneBLAzer $\text{\textcircled{R}}$   $\beta$ -  
355 lactamase reporter assays in HEK 293T cells were conducted as described in the Materials and Methods.  
356 Each data point represents the average  $\pm$  SEM of three separate experiments.

357

358 **Figure 4.** Spearman correlations between the potency of TR $\beta$  antagonism and serum measurements of (A)  
359 thyroid stimulating hormone (TSH), (B) free thyroxine (FT4), and (C) free triiodothyronine (FT3) of  
360 individuals living in the sampled homes. Potency is defined as the dust extract concentration that  
361 inhibited TR $\beta$  signaling by 20% relative to the T3 control (0.3 nM). Potency is significantly and inversely  
362 correlated to free thyroxine (FT4), which suggests that higher FT4 levels are associated with more potent  
363 dust extracts. The relationship with free triiodothyronine (FT3) is suggestive but not significant.

364

365 **Supporting Information Descriptions**

366 **Table S1.** Chemicals used in the GeneBLAzer® assay.

367

368 **Table S2.** Cell culture reagents used in the GeneBLAzer® assay.

369

370 **Table S3.** Health metrics summary.

371

372 **SI Methods: Frozen Cell Stocks.** Methods for preparing the frozen TR $\beta$ -UAS-*bla* HEK 293T cell stocks

373 needed for the GeneBLAzer® assay.

374

375 **SI Methods: Cell Viability Assay.** Methods for the resazurin reduction cell viability assay.

376

377 **Figure S1.** T3-TR $\beta$  dose-response curve.

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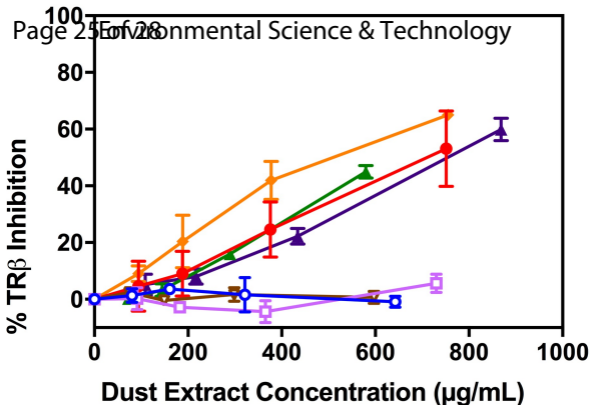
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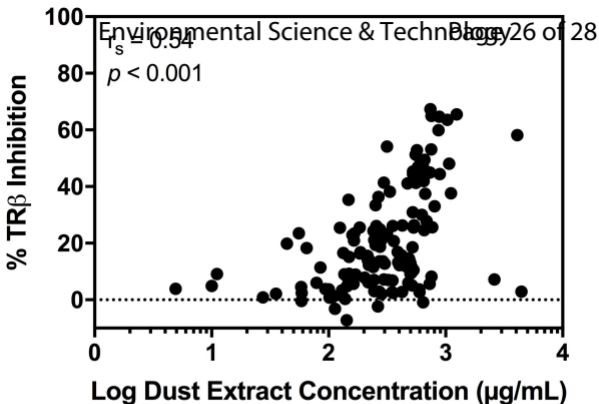
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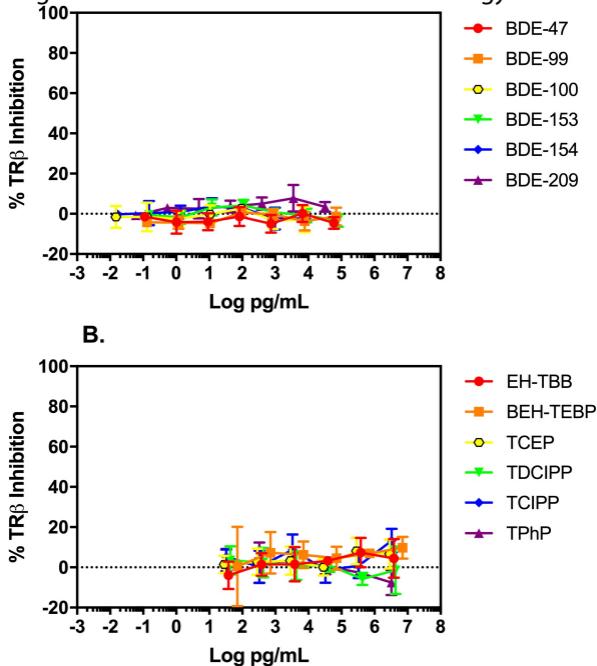
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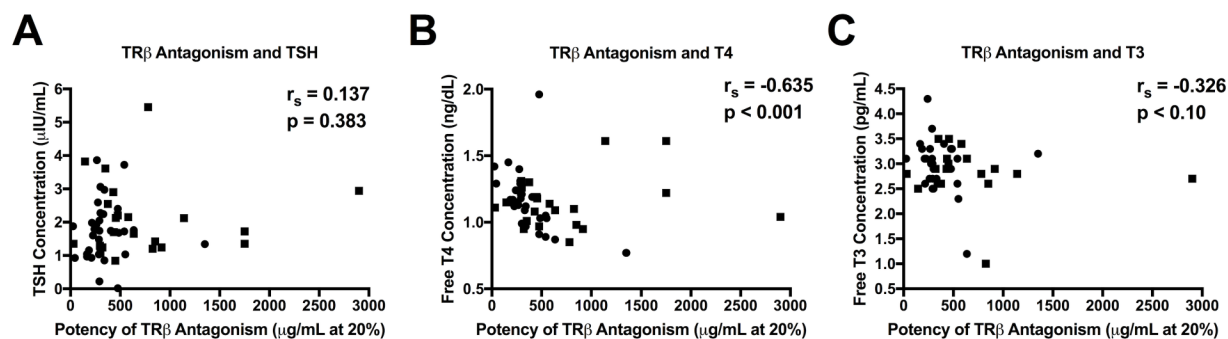
**Figure 1.** Representative results of TR $\beta$  antagonism (% Inhibition) by active (closed shapes) and inactive (open shapes) dust extracts obtained via the GeneBLAzer $\text{\textcircled{R}}$   $\beta$ -lactamase reporter assay in HEK 293T cells as described in the Materials and Methods. The colors 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 $\beta$  activity  $\geq 20\%$  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 TR $\beta$  signaling relative to the triiodothyronine (T3) control and the tested dust extract concentrations (n=137).



**Figure 3.** TR $\beta$  dose-response results with the individual flame retardants. None of the (A) PBDEs, or (B) FM550 components and PFRs antagonized TR $\beta$  signaling at any of the tested doses. GeneBLAzer $\beta$   $\beta$ -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.

**TR $\beta$  Potency (20% Antagonism) and Thyroid Status in Residents**

**Figure 4.** Spearman correlations between the potency of TR $\beta$  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 $\beta$  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.