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Activation of Human Peroxisome Proliferator-Activated Nuclear Receptors (PPAR γ 1) by Semi-Volatile Compounds (SVOCs) and Chemical Mixtures in Indoor Dust

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Abstract

Recently, we reported that several semi-volatile compounds (SVOCs) were competitive ligands for human peroxisome proliferator-activated nuclear receptor gamma (PPAR γ 1). We also observed significant binding from chemicals extracted from house dust at a concentration of 3 mg dust/mL in the dosing medium. To follow up on this study, a commercially available reporter gene assay (GeneBLazer PPAR γ 1 non-DA Assay, Invitrogen) was used to investigate the PPAR γ 1 activation by 30 common SVOCs (e.g., brominated flame retardants, organophosphates, and phthalates) and in house dust extracts. Twenty-eight SVOCs or their metabolites were either confirmed or for the first time were found to be weak or moderate PPAR γ 1 agonists. We also observed activation in 15 of 25 dust extracts examined. In some cases, activation was as high as 50% of the activation of the positive control (rosiglitazone). Furthermore, there was a significant and positive correlation ($r = 0.7$, $p < 0.003$) between data collected from this reporter assay and our previous ligand binding assay tested on the same dust extracts. Our results suggest that many SVOCs ubiquitous in house dust, or their metabolites, are possible PPAR γ 1 agonists. Also, chemical mixtures present in house dust at environmentally relevant levels can activate human PPAR γ 1 in a transfected cell culture system, and further research is needed to identify the primary chemical(s) driving this activity.

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Supporting Information

Additional information as noted in the text. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b01523.

Notes

The authors declare no competing financial interest.

INTRODUCTION

The peroxisome proliferator-activated nuclear receptor gamma (PPAR γ) is a master nuclear receptor that regulates lipid metabolism, cell proliferation, apoptosis, and differentiation. PPAR γ has two isoforms, i.e., PPAR γ 1 and γ 2; the former is expressed in virtually all tissues, including heart, muscle, colon, kidney, and pancreas, while the latter is primarily expressed in adipose tissue (30 amino acids longer than γ 1).¹ Activation of PPAR γ , especially γ 2, regulates adipocyte gene expression and may be a key factor for obesity.² Recently, many environmental contaminants have been shown to activate PPAR γ , leading to increased adipogenesis in cell cultures and sometimes in vivo.^{3,4} Those chemicals include several organotins (tributyltin (TBT) and triphenyltin (TPT)) and mono(2-ethylhexyl) phthalate bis(2-ethylhexyl) (MEHP) (a metabolite of the phthalate DEHP), both of which were shown to upregulate and stimulate PPAR γ .^{3,4} Several recent studies have suggested that flame retardants (FRs) and phthalates might represent important classes of compounds that could bind to and activate PPAR γ . For example, 2,2',6,6'-tetrabromo bisphenol (TBBPA), 3,3',5,5'-tetrachlorobisphenol A (TCBPA), and triphenyl phosphate (TPP) were identified as partial agonists of PPAR γ .^{5,6} In another study, benzyl butyl phthalate (BzBP) and butyl paraben showed significant activation of PPAR γ and adipogenesis using cell culture assays.⁷ Thus, there has been a lot of interest in identifying new "chemical obesogens" and investigating their potential health effects.

In our recent study, more than 20 semi-volatile organic compounds (SVOCs), primarily including flame retardants (FRs) and some of their metabolites, were tested for PPAR γ binding potential using a fluorescence polarization ligand binding assay (PolarScreen PPAR γ 1-competitor assay kit, Invitrogen).⁸ We found that several organophosphate compounds such as tributylphosphate (TBP) and tris(2-butoxyethyl) phosphate (TBOEP) showed significant binding potential at the highest dose level tested. Several of the polybrominated diphenyl ethers (PBDE) metabolites (i.e., hydroxylated PBDEs and halogenated phenols) also effectively bound to PPAR γ . As for the house dust extracts, 21 of 24 dust samples tested showed significant PPAR γ binding potency at a concentration of 3 mg dust equivalent quantity (DEQ)/mL. However, ligand binding does not necessarily indicate agonism of the receptor, leading to transcriptional events. Therefore, it is of great interest to investigate whether those identified possible PPAR γ ligands or the chemical mixtures in house dust can activate human PPAR γ .

To follow up on our previous research, PPAR γ activation using a cell-based reporter assay was used in this study. Chemicals that were previously identified as possible PPAR γ ligands were tested here for PPAR γ 1 activation. Furthermore, several other groups of chemicals such as phthalates and triaryl phosphates that are ubiquitous in house dust were also included. The aryl phosphates that contain isopropyl or *tert*-butyl substitutions are similar to TPP in chemical structure and are used as important components in FRs and plasticizers. Therefore, we included these chemicals in this study on PPAR γ 1 activation to investigate the structure-dependent activity. In addition, PPAR γ 1 activation by house dust extracts was also examined. As many SVOCs such as phthalates and FRs are not chemically bound to their commercial products, they migrate out over time and have a high accumulation in dust due to their physicochemical properties (high octanol–air partitioning coefficient Log K_{OA}). According to the U.S. EPA Exposure Factors Handbook,⁹ children ingest approximately 50 mg of dust per day, and dust ingestion has been increasingly identified as an important exposure pathway for the uptake of SVOCs (e.g., PBDEs) in the home, especially for the toddlers and infants who spend most of their time (>95%) in the indoor environment.^{10,11} Therefore, investigating PPAR γ activation using environmentally relevant house dust samples could be of more value in estimating real-world exposure and possible health effects.

MATERIALS AND METHODS

Tested Compounds

The abbreviation, structures, and supplier of all the tested compounds are shown in Table 1, Figure S1, and Text S1 in the Supporting Information (SI). In general, these chemicals included triaryl organophosphates, FM550 (and their metabolites), 2,2',4,4'-tetrabromodiphenyl ether (BDE47 and its metabolites), phthalates, halogenated phenols, and bisphenols. The tested compounds were mostly SVOCs, which were either identified as PPAR γ ligands in our previous study⁸ or have high abundances in indoor dust (e.g., phthalates). The type II diabetes drug rosiglitazone was used as a positive control. A possible endogenous PPAR γ ligand 15-Deoxy-D12,14-prostaglandin J2 (15d-PGJ2) was also run for comparison.

House Dust Extracts

House dust extracts were from our previous PPAR γ binding assay.⁸ In brief, indoor dust samples were investigator-collected from the main living areas of homes for Groups A¹¹ and D.¹² Dust samples in Group B were collected from gymnastics studios.¹³ Dust samples in Group C were investigator-collected from office environments,¹⁴ and Group E were participant-collected dust samples from the main living area using a similar method as reported in Hoffman et al.¹⁵ All dust samples were extracted with acetone:hexane (1:1, v/v) using sonication and then concentrated, filtered, cleaned by gel permeation chromatography [GPC, Envirogel GPC system (Waters, Milford, CA, U.S.A.)], and reconstituted in DMSO. A final stock with a concentration approximately 2000 mg DEQ dust/mL DMSO was prepared for PPAR γ 1 reporter assay.

PPAR γ Reporter Assay

A commercially available reporter gene assay (GeneBLazer PPAR γ 1 non-DA Assay, Invitrogen) was used to investigate the PPAR γ 1 activation of groups of possible PPAR γ ligands and house dust extracts. At least six different dosing levels were prepared for each chemical or dust extract, and triplicate analyses were conducted for each dose level. The details of the assay are fully described in the Supporting Information. An amalar blue assay, which was prepared from resazurin, was used for the cell viability test.

Data Analysis

After subtraction of fluorescence background from cell-free wells, the response ratio (RR) of fluorescence intensity at 460 versus 530 nm (designated as 460:530 nm) was calculated. All of the observed PPAR γ 1 activation by the chemicals or house dust extracts was normalized to the maximal response of rosiglitazone, and this activation percentage (Activation%) was used to describe the potency/efficacy of the samples. Activation% was calculated using the following equation:

$$\text{Activation}\% = (\text{RR}_{\text{Compound}} - \text{RR}_{\text{DMSO}}) / (\text{RR}_{\text{rosiglitazone}} - \text{RR}_{\text{DMSO}}) \times 100 \quad (1)$$

RR_{Compound}, RR_{DMSO}, and RR_{rosiglitazone} were the fluorescence response ratio of 460:530 nm in the tested compounds, DMSO control, and maximal response of rosiglitazone, respectively.

For most tested chemicals and dust extracts, the activation% was less than 30%. As suggested for weak agonists,⁷ an activation threshold (LAT) is proposed based on the limit of quantification (LOQ) used in analytical chemistry techniques to ensure the biological meaning of statistically significant effects. In this study, LAT is based on the variation of the DMSO control and calculated as the average DMSO value +10*SD of the solvent control over all experiments. The result showed that activation% less than 7% was not thought to be biologically different from the DMSO control. To compare the potency/efficacy between compounds, maximal activation% (ATV_{max}), the concentration inducing the maximal activation [ATV]_{max}, nonobservable adverse effect level (NOAEL), and the concentration inducing 15% (EC₁₅) and 20% (EC₂₀) activation was reported in this study. Specifically, EC₁₅ and EC₂₀ were used for the potency and maximal activation was used to describe the efficacy. For the mixture such as ITP, FM550, and *tert*-butyl phenyl diphenyl phosphate (BPDP), the weighted average of the molecular weight was calculated based on the composition. The statistical analyses and quality control are detailed in the Text S3.

RESULTS

PPAR γ 1 Reporter Assay Performance

Rosiglitazone was used as a positive control and showed a significant dose–response curve in the transactivation assay (Figure 1a). The calculated EC₅₀ was approximately 5.5 nM (RSD < 15%, *n* = 3), which was lower than that in the PPAR γ ligand binding assay (EC₅₀: 349 nM). It was also 1–2 orders of magnitude more sensitive than reported in previous studies, which used either a human osteosarcoma (U2OS) cell-based reporter assay (EC₅₀:

52 nM)¹⁶ or a Chinese Hamster Ovary (CHO) cell line luciferase reporter assay system (EC₅₀: 225 nM).¹⁷ 15d-PJG2, which is considered an endogenous PPAR γ 1 ligand, showed potent PPAR γ 1 activation, which began to activate PPAR γ 1 at 0.01 μ M and had a maximal activation of 85% of rosiglitazone. Generally, dose–response relationships were observed for 28 of the 35 tested compounds and are displayed in Figure 1 and Table 1. An inverted U-shaped nonmonotonic concentration response relationship was observed for several of the compounds tested. Belcher et al.¹⁷ also observed non-monotonic relationships for several weak PPAR γ 1 agonists, which he partially attributed to cytotoxicity. In this study, reduced cell viability was suggested based on results from an Alamar Blue cell viability assay. Most of the chemicals tested showed some cytotoxicity at concentrations >10 μ M, and those points were discarded in the dose–response curve fit. In some cases, there was no evident difference between the control and treated cells when evaluated for viability using the Alamar Blue assay. However, the proliferation of cell appeared to be hindered under microscopic examination (Figure S2); cells were characterized by smaller proliferation colonies compared to control samples. Morphologically, HEK293 cells exposed to high dosed chemicals or high concentrations of dust extracts displayed an enlarged round shape, losing cell–cell contact, in contrast with control cultures dominated by elongated star-shaped cell morphology. A similar observation has been reported in a previous study.¹⁸

Organophosphates

PPAR γ 1 activation varied greatly with slight changes in the chemical structure. As shown in Figure 1b, the maximal activation of those chemicals followed the order: TBPP ~ TPPi ~ Mono-ITP ~ Di-ITP < Tri-ITP < TPP < BDP (mixture). The commercial mixture BDP and TPP began to activate PPAR γ 1 at about 0.4 μ M and showed the highest PPAR γ 1 activation of approximately 60% and 42% relative to controls, respectively. Among the three isopropylated ITP isomers, mono- and di-substituted ITP isomers could slightly activate PPAR γ 1 at 3.6 μ M, and the tri-substituted ITP isomer began to activate PPAR γ 1 at 1.2 μ M and showed the highest maximal activation of 41% at the dosing concentration of 33 μ M, while all three did not show any obvious activation at low doses (<1.2 μ M). Highly reduced cell viability was observed for mono-ITP and di-ITP compared with tri-ITP at high doses (33 and 100 μ M). The phosphite analogue of TPP, TPPi was much weaker than TPP, with the maximal activation of 12%. A dose–response curve was also observed for TBOEP and TBuP, which are analogous to TBT in structure. In general, the activation was much less potent than TBT. TBuP activated PPAR γ 1 at 0.4 μ M and the maximal activation was 23%. TBOEP with a maximal activation just above the activation threshold (LAT) at 11 μ M was weaker than TBuP.

Halogenated Phenols/Bisphenols

Of the tested phenol/bisphenols, TCBPA and TBBPA were the most potent (Figure 1c and d), which was consistent with the recent finding by Riu et al.⁶ In our previous study, 3OH-BDE47, TBBA, 2,4,6-TBP, and 2,4,6-TIP were identified as potential PPAR γ ligands.⁸ In the PPAR γ 1 reporter assay, significant dose–response activation was observed for these chemicals, which began to act on PPAR γ 1 at the micromolar level. 3OH-BDE47 started to activate PPAR γ 1 at 0.8 μ M and show the highest PPAR γ 1 activation (~40%) at the highest dose (7 μ M), which was similar to the effect caused by TCBPA, although the latter had a

much lower EC₁₅. However, no activation was observed for 6-OH-BDE47, suggesting the position of the hydroxyl group affects the PPAR γ 1 activation, which was similar to the observation in our previous PPAR γ ligand binding assay.⁸ TBBA, which is a metabolite of a brominated benzoate in FM550 also showed a PPAR activation at high concentration with a maximal activation of 20% at 11 μ M. Triclosan, which showed moderate binding potency in our PPAR γ binding assay,⁸ did not initiate any activation of PPAR γ 1 in this study.

Phthalates and Their Metabolites

A dose–response relationship was observed for BzBP, DiBP, DBP, and DiNP, while no activation was observed for DEHP. The relative efficacy of PPAR γ 1 activation was: BzBP > DiBP ~ DBP > DiNP > DEHP (Figure 1e). BzBP was the most potent among all the parent phthalates with an EC₁₅ of 2.94 μ M and a maximal activation of 34%, which was consistent with the results reported in a previous study.⁷ The phthalate monoesters TBMEHP and MEHP, which are the metabolites of TBPH and DEHP, respectively, were much stronger than the parent phthalate and showed moderately potent activation of PPAR γ 1. MEHP began to activate PPAR γ 1 at 0.13 μ M, and the maximal activation was 65%. TBMEHP showed a stronger activation than MEHP at lower dosing range, but lower activation at higher dose due to the reduced viability at higher dosing levels. Another phthalate replacement bis(2-ethylhexyl) fumarate (BEHF) did not display any obvious PPAR γ 1 activation.

PPAR γ 1 Activation by Dust Samples

As shown in Figure 2, we found that 15 of the 25 tested dust extracts showed a dose–response relationship with the maximal activation at levels significantly higher than the activation threshold (LAT). In general, significant PPAR γ 1 activation was observed at ~100 μ g DEQ dust/mL. In some cases, the activation was as high as 50% of rosiglitazone. No significant activation was observed for the house dust reference material SRM2585. High variability was also observed in dust extracts from different sources. For example, the dust extracts from Groups A and D, which were collected from main living areas in homes, showed a higher activation on PPAR γ 1 than other groups (Figure 2). In contrast, Group B samples collected from gymnastic studios did not show any obvious activation.

Correlation between Ligand Binding Assay and Reporter Assay

Data from our previous paper⁸ reporting PPAR γ ligand binding potency was compared with data generated in this study on PPAR γ 1 activation to determine if the relative potencies were significantly correlated. For the individual chemicals investigated, no obvious correlation was observed between ligand binding and activation (data not shown), suggesting that binding does not necessarily induce activation. However, a significant and positive correlation ($r = 0.7$, $p < 0.003$) was observed between PPAR γ binding and PPAR γ 1 activation for the house dust extracts (Figure 3), suggesting that the binding observed in the dust mixtures are primarily producing agonistic effects.

DISCUSSION

Until now, few environmental contaminants have been shown to significantly bind and activate PPAR γ signaling. In our recent study, several SVOCs and/or their metabolites were found to competitively bind to PPAR γ .⁸ Furthermore, significant binding potency was observed for 21 of 24 dust samples. To follow up this study, PPAR γ 1 activation reporter assay was used to determine whether the binding potency observed led to activation of PPAR γ 1. In this study, 28 of 30 tested SVOC chemicals (excluding rosiglitazone and 15d-PJG2) were found to be weak to moderate agonists and showed a dose–response relationship. Most of the previously reported potential PPAR γ partial agonists (e.g., TBBPA, TCBPA, TB-MEHP, TPP, TBT, and TPT) were also confirmed in this study using a different reporter bioassay. To the best of our knowledge, several of the compounds tested here, including halogenated phenols, hydroxylated metabolites of PBDEs (i.e., 3OH-BDE47), a FM550 metabolite (i.e., TBBA), and the BPDP commercial flame retardant mixture, tri-ITP and TBuP, were all shown for the first time to have the potential to activate PPAR γ 1.

The results from this study also demonstrate that the chemical structure is very important in PPAR γ 1 activation and slight changes in the chemical structure can alter the activation significantly. For example, activation of compounds with a similar structure to TPP was examined. As shown in Figure 1b, the result showed that TPP was more potent than its organophosphite form, but both were much less potent than the organotins. The isopropylated form of TPP was also less potent in activating PPAR γ 1, suggesting the isopropylated branch might decrease the binding and activation. Alternatively, this also may be related to the relative solubility of the compounds or cytotoxicity of those chemicals, shielding potential effects at higher dose. However, it was interesting to find that the BPDP commercial mixture was more potent than TPP in PPAR γ 1 activation. In the BPDP mixture, TPP, BPDP isomers, dibutylphenyl phosphate, and tributylphenyl phosphate account for approximately 35%, 49.1%, 14.4%, and 1% of the total, respectively.¹⁹ The higher activity in this commercial mixture suggested that BPDP isomers or dibutylphenylphosphate could be more potent than TPP. Due to the lack of purified BPDP isomer standards, it is impossible to confirm this hypothesis in this study. BPDP is of interest because it is used as a FR and is also commonly used as additives in lubricants, hydraulic fluids, and plastics.²⁰ In contrast, a pure chemical standard of TBPP, the tri-*tert*-butyl substituted isomer, did not show much activation, suggesting the substitution and number of branches strongly affects activation. The comparison between TPP and its analogs also makes the contribution of TPP in FM550 and ITP clearer since the role of other ITPs could not be excluded in previous studies.^{5,17} FM550 consists of 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB, ~30%), bis (2-ethylhexyl) tetrabromophthalate (TBPH, ~8%), TPP (~17%), and ITPs (~45%).^{21,22} The ITP component is a complex flame retardant mixture containing TPP and ortho-, meta-, and para-substituted isomers of mono-, di-, tri-, and tetra-ITPs, which are believed to comprise approximately 32%, 10%, 2.4%, and 0.4% of FM550, respectively.²¹ Considering the percentage of TPP in FM550 and ITP, it is very possible that TPP is the major contributor to PPAR γ activation in both commercial mixtures.

In this study, several other FRs or their metabolites were identified as weak PPAR γ 1 agonists. BDE47 demonstrated some PPAR γ 1 activation. Several recent studies have also identified BDE47 as a possible “environmental obesogen”. For example, induction of adipocyte differentiation by BDE47 in 3T3-L1 cells has previously been observed,²³ and global gene expression analysis in 3T3-L1 cells exposed to low doses of BDE-47 induced adipocyte differentiation through various mechanisms that include PPAR γ 2 gene induction.²⁴ Suzuki et al.¹⁶ also observed a dose–response relationship between PPAR γ 2 and BDE-47 with a 5% induction concentration of 10 μ M using a U2OS reporter assay, although the difference between PPAR γ 1 and PPAR γ 2 is still unknown. In this current study, 3OH-BDE47, which is a metabolite of BDE47, was found to moderately activate PPAR γ 1 at a high dose (~40% activation at 7 μ M) and was more potent than the parent BDE-47. In the previous PPAR γ binding study, 3-OH-BDE-47 exhibited a comparable binding potency to rosiglitazone.⁸ Though the activation was not as potent, it was still more potent than its parent compound. Therefore, it might be interesting to confirm if the PPAR γ 1 activation with BDE47 was due to hydroxylated metabolites present in the incubations. 2,4,6-TBP, which is used as a fungicide or FR product, also showed a weak activation of PPAR γ 1. 2,4,6-TBP has been widely detected in environmental samples and has been identified as a potential thyroid-disrupting compound in indoor dust.²⁵ TBBA, the metabolite of a brominated benzoate present in FM550, also showed a significant PPAR γ 1 activation at higher concentrations and was recently reported as a good biomarker of exposure to FM550, being detected frequently in urine samples.^{26,27} In a rodent study, perinatal exposure to FM550 lead to obesity,²⁸ and it is possible that the observed weight gain may be linked to PPAR γ 1 activation by either TPP or TBBA and should be a focus of future research.

Several phthalates were also identified as PPAR γ 1 agonists in this study. Although phthalate metabolites such as MEHP and monobenzyl phthalate have been identified as PPAR γ 1 agonists and observed to stimulate differentiation of 3T3-L1 adipocytes,²⁹ few studies on the parent compounds have been conducted. Generally, the tested phthalates in this study were much less potent than the monoester phthalate such as MEHP. However, BzBP itself was identified as a partial agonist of PPAR γ 1 with a maximal activation of 34% and an EC₁₅ of 2.94 μ M, which was very comparable with TPP and consistent with another recent study.⁷ Concern over the link between phthalate exposure and PPAR γ 1 activation has been increasing in recent years, and several studies found significant associations between urinary metabolites of phthalates and obesity in human populations with specific age, gender, or race/ethnicity groups.^{30–32}

Here, we report for the first time that indoor dust extracts have the potential to activate PPAR γ 1. In this study, 15 out of 25 tested dust samples showed significant PPAR γ 1 activation higher than the activation threshold (LAT) (i.e., > 7% activation compared to rosiglitazone). Furthermore, the significant and positive correlation ($r = 0.7$, $p < 0.003$) between the reporter assay and ligand binding assay suggested that the bindings observed in the dust mixtures were primarily producing agonistic effects. To our knowledge, very few studies have been conducted to investigate PPAR γ 1 disruption in environmentally relevant dust samples, and this is the first time PPAR γ 1 agonistic effects from mixtures of chemicals

present in house dust extracts were found. Suzuki et al.¹⁶ observed PPAR γ 1 antagonistic effect in 9 out of 13 dust extracts, which was in contrast with findings reported here. Several reasons could be used to explain these contrasting results. First, the reporter assay in this study was more sensitive than the U2OS cell-based reporter assay used by Suzuki et al.¹⁶ For example, the IC₅₀ for rosiglitazone was 52 nM in the U2OS reporter assay, while it was 5.5 nM for the assay used in this study. Another possible difference is the different DNA domains, i.e., PPAR γ 1 and PPAR γ 2, which were used in the two studies. The U2OS cell line was transfected with PPAR γ 2, while in our study the cell line was transfected with PPAR γ 1. Furthermore, observing antagonistic effects does not necessarily imply that agonists are absent in a sample. For most antagonistic assays, a full agonist such as rosiglitazone is added, and the antagonistic effect is determined by increasing the concentration of your substrate to determine if it reduces the activation. Therefore, weak or partial agonists can be an “antagonist” when combined with the full agonist by competitively binding. It could also be possible that both agonists and antagonists were in the house dust mixtures, and further investigations should be conducted.

Great variance of PPAR γ 1 activation was observed in the dust sample from different sources. To date, no characterization of the chemical composition in the dust samples from different sources has been conducted. In our previous study, PBDEs in dust samples collected from gymnasiums¹³ were found to contain at least 1 order of magnitude higher concentrations than levels in residential dust. This suggests that the PBDEs are not the primary contributor to the PPAR γ 1 activation. However, the small sample size and heterogeneity of the house dust samples prevent any solid conclusions from being made.

In conclusion, this study was a follow-up investigation to our PPAR γ 1 ligand binding research on SVOCs and chemical mixtures in house dust. Many SVOCs or their metabolites were, for the first time, found to be weak or moderate PPAR γ 1 agonists, which may increase the size of the “environmental obesogen” family. Significant PPAR γ 1 activation was also found in 15 of 25 house dust extracts, suggesting possible PPAR γ 1 disruption by exposure to indoor dust. Further studies should be conducted to identify the causal chemicals that are the primary contributor to PPAR γ 1 activation in house dust.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Dose–response relationships observed for PPAR γ 1 activation by (a) potent PPAR γ ligands including TPT, TBT, 15d-PJG2, and rosiglitazone, (b) TPP and its related analogs, (c) and (d) phenols/bisphenols and TBT analogs, and (e) phthalates and some metabolites. “Activation%” was normalized to the maximal response of rosiglitazone (at 1 μ M). Values represent the average of triplicate measurements, and error bars represent standard deviation.

Figure 2.

Dose–response relationships for PPAR γ 1 activation by 25 house dust extracts. Dose has the unit of μg dust/mL in dosing medium, and “Activation%” was normalized to the maximal response of rosiglitazone. Groups represent dust with different sources. Control is the procedural blank. Values represent the average of triplicate measurements, and error bars represent standard deviation.

Figure 3. Correlation between PPAR γ activation% at a dose of 200 μ g dust/mL in the reporter assay and ligand competitive binding potency % of PPAR γ -LBD assay at a dose of 3 mg dust/mL. “Activation%” was normalized to the maximal response of rosiglitazone.

Table 1
Summary of PPAR γ 1 Activation by Test Compounds Using a HEK293 PPAR γ 1 Reporter Assay

chemicals	acronym	ATV _{max} ^a (%)	[ATV _{max}] ^b (μ M)	NOAEL (μ M)	EC ₁₅ (μ M)	EC ₂₀ (μ M)	efficacy ^c
triphenyl phosphate	TPP	43	11	0.4	2.12	3.27	+++
triphenyl phosphite	TPPi	12	11	1.2	NA	NA	+
tert-butyl phenyl diphenyl phosphate (mixture)	BPDP	58	11	0.4	1.09	1.59	+++++
tris (4, <i>tert</i> -butyl-phenyl) phosphate	TBPP	12	11	0.4	NA	NA	+
isopropylated triaryl phosphates (mixture)	ITP	30	11	1.2	2.28	3.32	+++
monoisopropylated triaryl phosphates	mono-ITP	15	3.6	1.2	3.6	NA	+
di-isopropylated triaryl phosphates	Di-ITP	16	3.6	1.2	3.25	NA	+
tri-isopropylated triaryl phosphates	Tri-ITP	41	33	1.2	5.7	7.76	++++
tributyl phosphate	TBuP	23	33	0.4	5.86	13.35	++
tri(2-butoxyethanol) phosphate	TBOEP	13	11	3.6	NA	NA	+
2,4,6-triodinated phenol	2,4,6-TIP	20	11	0.4	8.72	11	++
2,4,6-tribrominated phenol	2,4,6-TBP	18	7	0.8	5.89	NA	+
3,5,3',5'-tetrachlorobisphenol A	TCBPA	52	7	0.09	0.23	0.47	+++++
2,2',6,6'-tetraabromobisphenol A	TBBPA	52	11	0.13	0.32	0.41	+++++
triclosan	TCS	<8	NA	NA	NA	NA	NA
Firemaster 550 (mixture)	FM550	28	11	1.2	3.23	6	++
2,3,4,5-tetrabromobenzoic acid (metabolite)	TBBA	20	33	1.2	8.16	33	++
2,2',4,4'-tetrabromodiphenyl ether	BDE47	20	7	0.8	5.2	7	++
3-hydroxide-2,2',4,4'-tetrabromodiphenyl ether (metabolite)	3-OH-BDE47	42	7	0.8	2.01	2.993	++++
6-hydroxide-2,2',4,4'-tetrabromodiphenyl ether (metabolite)	6-OH-BDE47	<8	NA	NA	NA	NA	NA
diisobutyl phthalate	DiBP	25	33	0.4	4.47	11.1	++
diisononyl phthalate	DiNP	15	33	0.4	33	NA	+
dibutyl phthalate	DBP	23	33	0.4	6.73	13.6	++
benzylbutyl phthalate	BzBP	34	10	0.13	2.94	4.81	+++
mono-(2-ethylhexyl) tetrabromophthalate (metabolite)	TBMEHP	40	3.3	0.13	0.53	0.78	++++
mono(2-ethylhexyl)phthalate (metabolite)	MEHP	65	100	0.13	1.26	2.12	+++++
bis(2-ethylhexyl) phthalate	DEHP	NA	NA	NA	NA	NA	NA
bis(2-ethylhexyl) fumarate	BEHF	<8	NA	NA	NA	NA	NA

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chemicals	acronym	ATV _{max} ^a (%)	[ATV _{max}] ^b (μM)	NOAEL (μM)	EC ₁₅ (μM)	EC ₂₀ (μM)	efficacy ^c
triphenyltin chloride	TPT	54	0.33	0.0003	0.004	0.01	+++++
tributyltin chloride	TBT	28	0.07	0.0005	0.004	0.006	+++++
15-Deoxy-D12,14-prostaglandin I2 (endogenous ligands)	15d-PG2	85	33	0.01	0.51	0.74	+++++
rosiglitazone (positive control)	Rosi.	100	0.93	0.00001	0.00132	0.0005	+++++

^a ATV_{max}: maximal activation% of the tested compounds relative to rosiglitazone.

^b [ATV_{max}]: concentration of the tested compounds to induce the maximal activation%.

^c Efficacy: qualitatively describe the efficacy of the tested compounds based on their ATV_{max} (+ 7–20%; ++ 20–30%; +++ 30–40%; ++++ 40–50%; +++++ >50%).