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Endocrine disrupting potential of total and bioaccessible extracts of dust from seven different types of indoor environment

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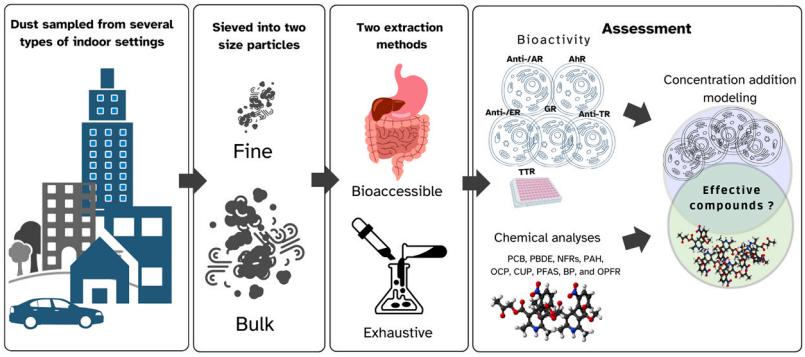
HIGHLIGHTS

- Representative indoor dust samples (2 size fractions) from 7 different indoor types.
- Total and bioaccessible extracts mimicking the gastrointestinal conditions.
- Diverse endocrine disrupting potential patterns across different microenviron ments.
- In some cases, bioaccessible extracts higher effects than organic solvent extracts.
- Low explicability of observed effects by the wide spectrum of detected chemicals.

GRAPHICAL ABSTRACT



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ABSTRACT

Information on the indoor environment as a source of exposure with potential adverse health effects is mostly limited to a few pollutant groups and indoor types. This study provides a comprehensive toxicological profile of chemical mixtures associated with dust from various types of indoor environments, namely cars, houses, pre-fabricated apartments, kindergartens, offices, public spaces, and schools. Organic extracts of two different polarities and bioaccessible extracts mimicking the gastrointestinal conditions were prepared from two different particle size fractions of dust. These extracts were tested on a battery of human cell-based bioassays to assess endocrine disrupting potentials. Furthermore, 155 chemicals from different pollutant groups were measured and their relevance for the bioactivity was determined using concentration addition modelling. The exhaustive and bioaccessible extracts of dust from the different microenvironments interfered with aryl hydrocarbon receptor, estrogen, androgen, glucocorticoid, and thyroid hormone (TH) receptor signalling, and with TH transport. Noteably, bioaccessible extracts from offices and public spaces showed higher estrogenic effects than the organic solvent extracts. 114 of the 155 targeted chemicals were detectable, but the observed bioactivity could be only marginally explained by the detected chemicals. Diverse toxicity patterns across different microenvironments that people inhabit throughout their lifetime indicate potential health and developmental risks, especially for

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children. Limited data on the endocrine disrupting potency of relevant chemical classes, especially those deployed as replacements for legacy contaminants, requires further study.

1. Introduction

Indoor pollution has come under scrutiny by the scientific community and regulatory agencies over the past decades [24]. The interest in assessing the quality of the indoor environment is mainly based on the potential presence of various xenobiotics, together with the long time that people spend in indoor environments (80 – 90% of people's life span). Indoor chemicals can have several sources, including domestic sources (appliances, building materials, furniture, etc.), indoor activities (cooking, smoking, application of insecticides, fragrances, household products, etc.), and external environments, from where chemicals can be brought-in by the occupants on clothes and shoes. Moreover, polluted outdoor air, such as in intense traffic areas might contribute to the chemical profile in nearby indoor environments. Once these contaminants are released from their sources, they are distributed throughout the indoor environment, including ad(b)sorption on dust particles [11, 19,30]. The persistence of potentially harmful chemicals, due to shelter from external degradation factors [33,41], combined with the long time spent indoors facilitates the uptake of contaminants through oral, inhalation and/or transdermal routes, and emphasizes the importance of understanding the toxicity associated with indoor dust. Human biomonitoring studies have found a positive correlation between chemical levels detected in indoor dust and in biological samples (blood and urine) of building occupants [42,43]. Once taken up, these contaminants can contribute to undesirable health outcomes [22], including endocrine disrupting effects [7,36,37,44]. In this regard, children are of a special concern, since exposure to endocrine disruptors (ED) during early childhood might result in negative effects in later stages of life [18, 28]. Moreover, the exposure of young children is enhanced by their frequent object/hand-to-mouth behaviour, thumb or finger sucking, pacifier use, crawling etc., which can result in significant dust ingestion [23,35]. To understand the toxicity of orally ingested chemicals from accidental dust exposure, studying their bioaccessibility is recommended [12,16,45].

Previous studies have addressed pollutant groups in bulk dust particles [11,17,19] and, in some cases, their distribution according to particle size [14,20,3,4]. A few studies have indicated a potential for indoor dust pollutant mixtures to interfere with the signalling of estrogen receptor (ERα), androgen receptor (AR), aryl hydrocarbon receptor (AhR), peroxisome proliferator-activated receptor (PPARγ2), glucocorticoid receptor (GR), thyroid hormone receptor (TRβ), or thyroxine (T_4) transport [7,9,27,36,37,44,45]. However, these earlier studies have limitations in the range of analysed pollutant groups and ED bioactivities, also relative to the size of the dust particles. Moreover, the variety of studied indoor types was rather limited and there is a lack of knowledge on the bioaccessibility of chemicals responsible for the endocrine disrupting potential from indoor dust.

The current study aims to address these gaps by comprehensively characterizing the pollution of dust from different types of indoor environments by endocrine-disrupting compounds. Utilizing human cell-based bioassays, the research investigates endocrine-disrupting endpoints that have been associated with diverse adverse health effects, including disturbed early (neuro)development, reproductive disorders, metabolic or immunity impairment. This is conducted in conjunction with extensive chemical analyses of a wide spectrum of contaminants. Representative samples of indoor dust were collected in seven types of microenvironments (cars, houses, kindergartens, offices, prefabricated apartments, public spaces, and schools) to provide material for testing in a battery of in vitro bioassays to characterise (i) the endocrine-disrupting effects elicited by extracts of the indoor dust in polar and nonpolar solvents, (ii) in different particle sizes (<0.25 mm and <2 mm), and (iii) the potential bioaccessibility of the bioactive compounds recovered after a physiologically based extraction test (PBET) with the inclusion of silicone elastomer (poly-dimethylsiloxane) as a lipophilic sink. Additionally, (iv) extensive chemical analyses were carried out, targeting 155 organic contaminants. The data from the chemical analyses was interlinked with data on the bioactivity obtained from the scientific literature to (v) model the explicability of the observed effects in vitro by the detected compounds.

2. Material and Methods

2.1. Materials

Simulated gastric and intestinal fluid components (pepsin, citric acid, L-sodium malate, acetic acid, hydrochloric acid, pancreatin, bile salts, and sodium bicarbonate) were purchased from Sigma-Aldrich, Germany. All used solvents, i.e., hexane, acetone, ethyl acetate, dichloromethane, and nonane, were pesticide grade or equivalent (Sigma-Aldrich, Germany). Concentrated sulfuric acid (95–97%) and high-purity grade silica gel (pore size 60 Å, 70–230 mesh) used in clean-up procedures were purchased from Sigma-Aldrich, Czech Republic. Analytical standards of target compounds and their perdeuterated (*D*-) or ^{13}C-labelled analogues were purchased from various suppliers, i.e., Dr. Ehrenstorfer GmbH (Germany), SupelCo (Germany), Wellington (Canada), CIL (USA), AccuStandard (USA), LGC Standards (UK), and NEOCHEMA GmbH (Germany). The silicone polymer material SSP-M823 0.125 mm thick was purchased from Specialty Silicone Products, Inc. Ultrapure water was purchased from Sartorius, Germany. Additional information on the used materials is included in the detailed methodology description in Supplementary Material 1 (SM1).

2.2. Methods

2.2.1. Sampling and processing of indoor dust

Pooled dust samples were collected in 2019, representing seven different types of indoor environments in the Czech Republic, i.e., cars (CA), houses (HO), kindergartens (KI), offices (OF), prefabricated apartments (PA), public spaces (PS), and schools (SC) (the description of individual sampling locations is in Table S1). The indoor dust (except cars) was collected from the household vacuum bags. The household or professional vacuum cleaners were used by inhabitants or cleaning staff for vacuuming the whole space until the bag was full. The entire bag was put into the zip-top bag, transported to the laboratory and stored at − 18 °C until processing. A household vacuum cleaner was used to collect dust from seven cars in a single vacuum bag, which was then put into the zip-top bag and transported to the laboratory and stored at − 18 °C until processing. After sampling, the content of each bag was independently sieved to a particle size < 2 mm and pooled with the others (proportionally by mass) from the same microenvironment type (except cars, which were sampled directly into one vacuum bag). The composite dust samples from the individual environments were sieved to the fractions < 2 mm (bulk dust), and < 0.25 mm (fine dust). The bulk and fine dust fraction were homogenised using a vertical rotator (Baghirra, Czech Republic). Both dust size fractions were used for the bioassays and extensive chemical analyses.

2.2.2. Dust extraction

Two extraction methods were used for the dust samples: the first, exhaustive organic extraction, is a common method to determine the content of organic pollutants in dust. The second method, PBET (Physiologically Based Extraction Test), is designed to isolate bioaccessible

chemicals by simulating the conditions of the gastrointestinal tract. The exhaustive extraction procedure for bioassays and chemical analyses followed the same protocol (as shown in Fig. 1), details in SM1.2 and Table S2. Given their relevance for oral exposure (as discussed in [32]), the fine dust samples also underwent extraction through a physiologically based extraction test (PBET). This process was conducted both with and without the incorporation of a lipophilic sink (as depicted in Fig. 1 and S1).

2.2.3. Physiologically based extraction test (PBET)

PBET test applied a sequential extraction, by a medium which emulates the composition of the gastrointestinal (GI) tract fluid, to evaluate the potential bioaccessibility of chemicals following dust ingestion [40]. In these tests, the fine dust fractions were used, since particles of this size are more likely to adhere to hands, thereby increasing the chances of dust ingestion [21,4], because of their transfer to food and/or hand-to-mouth activity. Also, the accessibility of relevant chemicals was shown to be higher from dust particles of this size [32].

In brief, 0.4 g of the fine dust fraction was extracted with 40 mL of simulated gastric fluid (details in SM1.3) and gently swirled for one hour under slow shaking at 37 ºC, yielding the gastric phase (GP) extract. To have enough material for bioassays, the extraction was performed on two 0.4 g portions of fine dust fraction and both prepared PBET extracts were combined for use in bioassays. Subsequently, 20% of the GP extract was subjected to dichloromethane extraction (as described in SM1.3), resulting in the bioaccessible extract of the gastric phase (BEGP; Fig. 1). The remaining 80% of the GP from the previous step underwent an extraction in a medium that simulated the composition of intestinal fluid and the solution was then agitated for 4 h at 37ºC (details in SM1.3). The outcome of the sequential extraction process described above resulted in the generation of the intestinal phase extract (IP). This extract then underwent dichloromethane extraction (as described in SM1.3), resulting in the production of the bioaccessible extracts of the intestinal phase (BEIP; Fig S1). For the purposes of biotesting, both BEGP and BEIP extracts were transferred to methanol by gentle evaporation under nitrogen followed by the addition of methanol.

To mimic the partitioning of bioaccessible chemicals into the lipid-rich compartments of the gastrointestinal tract, another portion of fine dust samples was extracted with the addition of a lipophilic sorptive sink incorporated into the PBET test in this study. This rendered two sets of bioaccessible samples (i) bioaccessible samples with the addition of a sorptive sink and (ii) bioaccessible samples without the addition of a sorptive sink.

2.2.4. PBET of dust with sorptive sink

Due to higher hydrophobicity, many organic chemicals sorbed in indoor dust and unintentionally ingested by humans tend to partition into lipophilic phases such as the lipid membranes of intestinal enterocytes. However, most in vitro methods used for exposure assessment of bioaccessibility do not include a lipophilic phase to mimic the chemical partitioning with the lipid membranes, which may underestimate the chemical bioaccessibility. To overcome this issue, a sorptive sink was incorporated into the PBET to maintain a concentration gradient and thus enhance the chemical desorption from dust [8,10,12]. In this study, a silicone polymer was used as a sorptive sink in PBET because of its well-known partitioning properties [34] and fast diffusion of hydrophobic organic chemicals [31].

The silicone polymer material SSP-M823 (Specialty Silicone Products, Inc), 30 × 30 cm and 0.125 mm thick, was cut into sheets of rectangular shape with a mass of approximately 0.4 g each. The mass of dust and mass of silicone were selected based on the following calculation: considering the uptake capacity of silicone ($K_{sw}\times m$ silicone) and the uptake capacity of dust ($K_{oc}\times f_{oc}$), aiming at depletive extraction, i.e., $K_{sw}\times m$ silicone $>>K_{oc}\times f_{oc}\times m$ dust. Here, K_{sw} denotes silicone/water

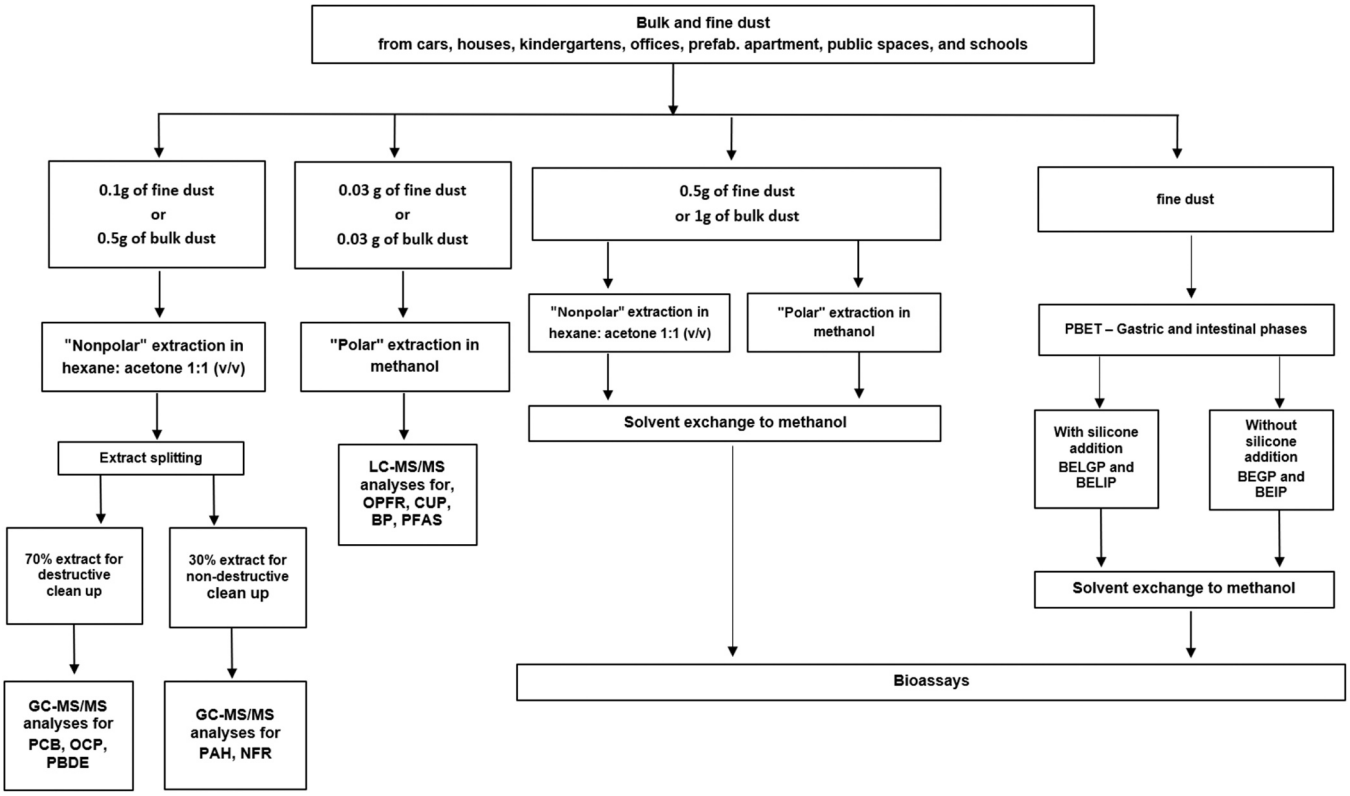

Fig. 1. General description of the dust samples processing. Abbreviations: polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), organochlorine pesticides (OCP), polybrominated diphenyl ethers (PBDE), novel flame retardants (NFR), bisphenols (BP), current-use pesticides (CUP), organophosphate flame retardants (OPFR), and per- and polyfluoroalkyl substances (PFAS).

partition coefficient of the analysed compound, K_{oc} is the dust organic carbon/water distribution coefficient and f_{oc} is the organic carbon fraction in the dust sample. For the estimation of silicone/dust phase ratio in the experiment, we assumed in the first approximation that K_{oc}=K_{sw}. Based on the calculations we selected a 1:1 silicone/dust mass ratio for PBETs with silicone addition.

Before use, the silicone sheets underwent pre-extraction in a Soxhlet extractor using ethyl acetate for 96 h to remove non-polymerized monomers. They were then washed with methanol to eliminate any remaining impurities and excess ethyl acetate. Subsequently, the silicone sheets were rinsed with high-quality water (MQ water) to remove traces of methanol. The cleaned silicone sheets were stored in MQ water in glass jars at 4 ℃ until use. 0.4 g of silicone in the form of 125 μm thick sheets was added to 0.4 g dust samples in the PBET (GP) prepared identically to conditions described in Section 2.2.3. (Fig. S1). At the end of the exposure in the gastric fluid, the silicone was retrieved, cleaned and preserved for solvent extraction and subsequent use in bioassays. A new 0.4 g silicone was added to the simulated intestinal fluids (IP) prepared, as described in Section 2.2.3. (Fig. S1) retrieved at the end of exposure, cleaned and preserved for solvent extraction and subsequent use in bioassays. The silicone sheets were taken out of GP and IP with clean stainless-steel tweezers, cleaned using MQ water, dried using lint-free paper tissue and stored in 20 mL glass vials with aluminum foil-lined screw tops at − 20 °C until further processing.

2.2.5. Extraction of silicone samples

Before extraction, the silicone sheets were taken out of the freezer and left to equilibrate to room temperature. Extraction was performed in 20 mL glass vials in 15 mL of hexane for 4 h by soft swirling on an orbital shaker (GFL 3020, Germany) at 100 rpm in the dark. Extraction in hexane was repeated and both extract portions were combined in a 100 mL point flask before the volume was reduced to 1.5–2 mL using Kuderna-Danish evaporation unit. Thereafter, the extracts were quantitatively transferred to 4 mL amber glass vials and brought to 3 mL volume. Afterwards, the PBET extracts with the silicone addition underwent solvent exchange to methanol yielding the bioaccessible extract of lipophilic gastric phase (BELGP) and bioaccessible extract of lipophilic intestinal phase (BELIP) (Fig. S1), which were employed in the bioassays.

2.2.6. Bioassays

A battery of in vitro bioassays (Table 1) was employed to assess the ED potential elicited by the prepared indoor dust extracts. A detailed description of the bioassay methodology is included in SM1.4. Calibration curves utilized to assess the bioactivity of the indoor dust in this study can be seen in SM1.6. Briefly, human cell lines stably transfected with luciferase gene under the control of the aryl hydrocarbon receptor (AhR), estrogen receptor (ERα), androgen and glucocorticoid receptor (AR/GR), thyroid hormone receptor (TR), glucocorticoid receptor (GR), and peroxisome proliferator-activated receptor gamma (PPARγ) were employed to assess AhR-mediated activity, anti-/ ERα, anti-/ AR, anti-/ TR, GR-mediated-activity, and PPARγ-mediated activity of organic and PBET dust extracts, respectively. Importantly, the assessment of specific GR activity was performed only on the samples with detectable AR/GR activity. The assessment of thyroxine (T4) displacement from its transporter transthyretin (TTR) by the organic dust extracts was done based on Ren and Guo [29]. In brief, this method focusses on the competitive displacement of a fluorescent dye (F-T4) from TTR caused by the compounds in the environmental samples. The displacement of the F-T4 after incubation with the environmental samples was assessed as a decrease in the fluorescence of the complex TTR-FT4. The neutral red uptake assay to detect cell viability was employed as described in Nováková et al. [26]. The concentrations causing cytotoxicity were excluded from the dose-response curve fit of the studied ED potentials.

The generated dose-response data were analysed using GraphPad Prism 8.4.3 (GraphPad Software, LLC ©) using the Hill function for the assessment of the effective or inhibition concentration (EC or IC, respectively) both for the samples and the reference compounds (RC). The generated EC/IC data for both RC and samples considering the molecular weight (MW) of the respective RC were used to translate the effects into Bioanalytical equivalents (BEQ_{bio}; Eq. 1) expressed as BEQ_{RC} (Table 1). The BEQbio used to express the results from the bioassays employing cell lines were calculated from EC_{10} and IC_{20} values; the displacement of thyroxine (T_4) from its transporter transthyretin (TTR) was expressed according to the observed IC_{50}.

$$\mathrm{BEQbio} = \frac{\mathrm{ECx(RC)} * MWRC}{\mathrm{ECx(sample)}} or \frac{\mathrm{ICx(RC)} * MWRC}{\mathrm{ICx(sample)}} \quad (1)$$

The BEQ values for bioaccessible samples, which did not reach the EC_{10} response, were assessed as a point estimate (P.E.) (Eq. 2) whenever the highest response of the exposed cells was statistically significant (t-Test; $P < 0.05$) compared to the respective solvent control.

Furthermore, the concentration addition model was applied to assess the contribution of the detected chemicals to the biological effects observed in the battery of bioassays, as described in Neale et al. [25]. In brief, the BEQchem (Eq. 2) was derived based on the concentration of each chemical ($c_{a,i}$) and its relative effect potencies (REP_i), derived from studies utilizing the same or closely related cell lines as employed in the present study as described in Novaková et al. (2022) and the REP_i values for the TTR assay were taken from Hamers et al. [9] utilizing an analogous assay as in the current study. The individual REPi and their source for each endpoint are listed in SM3. The obtained BEQchem is compared with the BEQbio to characterise the explicability of the observed biological effects by the detected chemicals in the sample.

$$BEQchem = \sum_{i=1}^{n} REP_i * c_{a,i} * \frac{\mathrm{MW(RC)}}{\mathrm{MW}(i)} \quad (2)$$

Table 1
Description of the battery of bioassays employed to assess the ED potentials from indoor dust extracts. (a) TCDD = 2,3,7,8 -tetrachlorodibenzo-*p*-dioxin. (b) DHT = dihydrotestosterone. (c) ND = effect not detected. For the calibration curves utilized in this study, refer to SM 1.6.

Endpoint	Endpoint abbreviation	Bioassay	Ref. compound	Ref. compound concentration range	BEQ_{RC}	EC/IC_x derived
Aryl hydrocarbon receptor-mediated activity	AhR	AZ-AhR	TCDD[a]	0.064 – 50 nM	BEQ_{TCDD}	EC_{10}
Glucocorticoid activity	GR	AzGR	Dexamethasone	0.001 – 1 μM	BEQ_{DEX}	EC_{10}
Estrogenicity	ER	HeLa9903	17-β Estradiol	0.41 – 500 pM	BEQ_{E2}	EC_{10}
Antiestrogenicity	aER	HeLa9903	Fulvestrant	0.008 – 5 nM	BEQ_{Fulv}	IC_{20}
Androgenicity / Glucocorticoid activity	AR	MDA-kb2	DHT[b]	0.01 – 100 pM	BEQ_{DHT}	EC_{10}
Antiandrogenicity	aAR	MDA-kb2	Flutamide	0.008 – 1 μM	BEQ_{Flu}	IC_{20}
Thyroid hormone receptor- agonistic activity	TR	PZ-TR	Triiodothyronine (T3)	0.009 – 150 nM	BEQ_{T3}	ND
Thyroid hormone receptor- antagonistic activity	aTR	PZ-TR	Diclazuril	4 – 100 μM	BEQ_{DCZL}	IC_{20}
Thyroxin-transthyretin displacement	TTR	TTR displ.	Thyroxine (T4)	0.9 - 2000 nM	BEQ_{T4}	IC_{50}
Peroxisome proliferator-activated receptor gamma-mediated activity	PPARγ	PPARγ-UAS-*bla* 293 H	Rosiglitazone	0.001 – 1000 nM	$BEQ_{PPARγ}$	ND[c]

Where:

$$REP_i = \frac{\mathrm{ECx(RC)}}{\mathrm{ECx}(i)} \quad or \frac{\mathrm{ICx(RC)}}{\mathrm{ICx}(i)} \tag{3}$$

The limits of quantification (LOQs) in bioassays were calculated as BEQbio (Eq. 1) of the highest non-cytotoxic concentration of the sample and EC_{10} of the reference compound. For samples which due to the low effect were assessed as point estimates, the LOQs were calculated as follows: for ECx of the reference compound, x value corresponds to 3x relative standard deviation of solvent control, which was expressed as the percentage of maximal activity of the reference compound. ECx (sample) was the highest non-cytotoxic concentration of the respective sample. LOQs for each assay and sample can be found in SM2 Table 5.

2.2.7. Chemical analyses

The dust extracts were analysed for a wide range of chemical groups including 155 target chemicals as described in SM1.7. and depicted in Fig. 1. Polychlorinated biphenyls (PCB), organochlorine pesticides (OCP), polycyclic aromatic hydrocarbons (PAH), polybrominated diphenyl ethers (PBDE), and novel flame retardants (NFR) were analysed in the nonpolar solvent extract. The polar solvent extracts were used for the quantification of currently used pesticides (CUP), perfluorinated compounds (PFAS), organophosphate flame retardants (OPFR), and bisphenols (BP). For a detailed description of the analytical methodology including the detection conditions and utilized equipment, please refer to SM1.7.

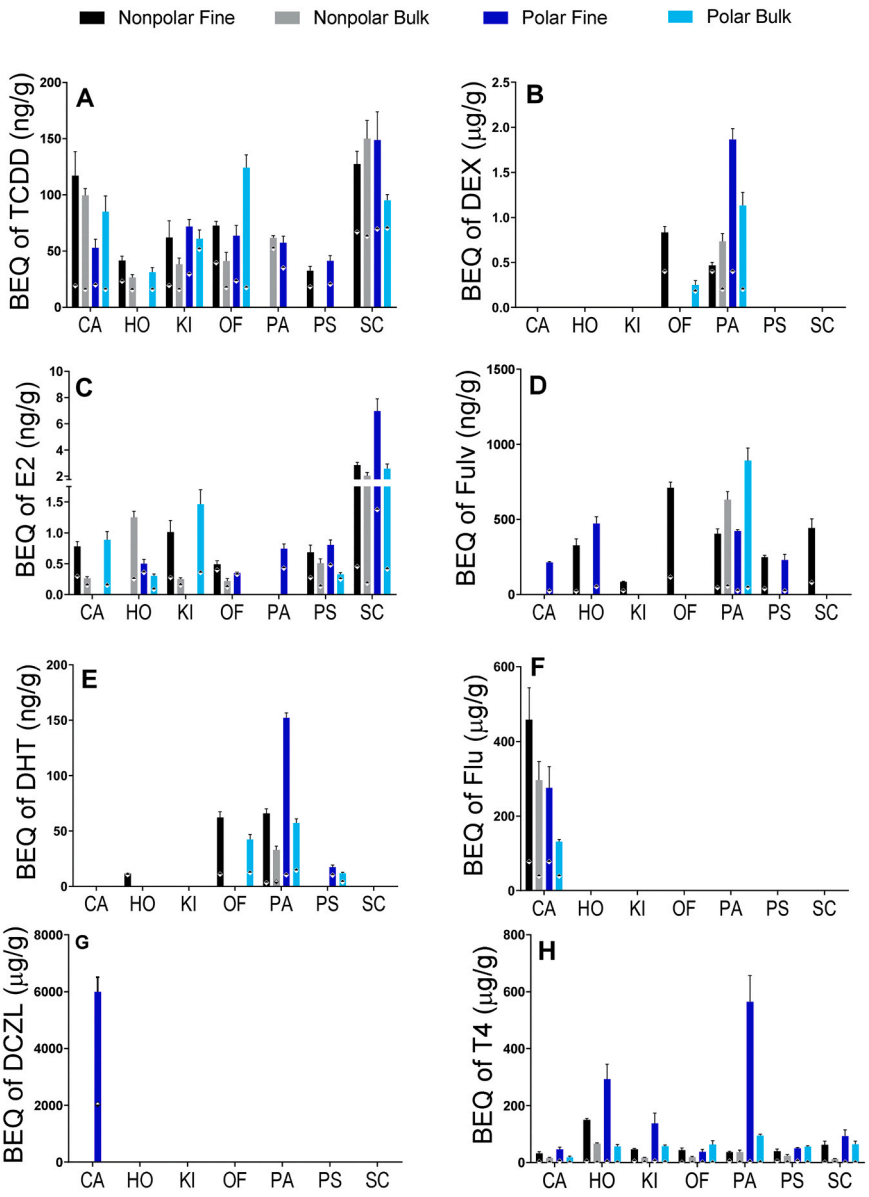

Fig. 2. Bioactivity of exhaustive extracts of different polarities assessed by in vitro reporter gene assay. Nonpolar Fine (hexane: acetone 1:1 (v/v) extract of fine dust particles (<0.25 mm)), Nonpolar Bulk (hexane: acetone 1:1 (v/v) extract of bulk dust (< 2 mm)), Polar Fine (methanolic extract of fine dust particles (< 0.25 mm), Nonpolar Bulk (methanolic extract of bulk dust (< 2 mm). A= AhR-mediated activity, B= glucocorticoid activity, C= estrogenicity, D= anti-estrogenicity, E = androgenic/glucocorticoid activity, F= anti-androgenicity, G= anti-thyroid activity, H= displacement of TTR). CA= Cars, HO= homes, KI= kindergartens, OF= Offices, PA= prefabricated apartments, PS= public spaces, SC= schools. The results are expressed as the mean ± standard error of the mean (SEM) of bioconcentration equivalent (BEQ) of the respective reference compound (Table 1). Missing columns = below limit of quantification or effect not detected. For a detailed list of BEQs refer to S1 Tables 1–2 and Fig S2.

3. Results

The extracts of dust from the seven sampled indoor microenvironments displayed different toxicity patterns, encompassing both endocrine effects and cytotoxicity. The cytotoxicity affected the detectability of specific ED effects and resulted in varying LOQs for the individual dust extracts. Detailed results on the bioactivity including BEQ values determined from individual experiments, together with the mean BEQs with standard deviation, and the LOQs for the individual dust extracts in all bioassays are listed in SM2 tables and in SM1 Figs. S2 and S3.

3.1. Bioactivity of polar and nonpolar extracts from both indoor dust size fractions

Different patterns of endocrine disrupting activity have been observed in the extracts of the two polarities from bulk and fine dust sampled from various microenvironments (Fig. 2, S2). Organic solvent extracts of dust from all sampled indoor types elicited AhR-mediated activity with the greatest levels in schools, cars and offices (Fig. 2A). In a few cases the AhR-mediated activity could be masked by greater cytotoxicity of dust extracts, such as in the case of bulk dust from public spaces and prefabricated apartments (Fig. S2A).

The results from MDA-kB2 cells indicate AR/GR agonistic responses for extracts of dust from four studied indoor types (HO, OF, PA, PS; Fig. 2E). We could not clearly differentiate the AR and GR agonism detected in the samples from OF (nonpolar fine and polar bulk) and PA (all) shown in Fig. 3E since the addition of the antagonist flutamide to suppress AR-mediated response increased the cytotoxicity. However, the agonism of HO and PS samples can be considered specific to AR, as no effect was observed in the GR-specific assay (see below). The androgenic

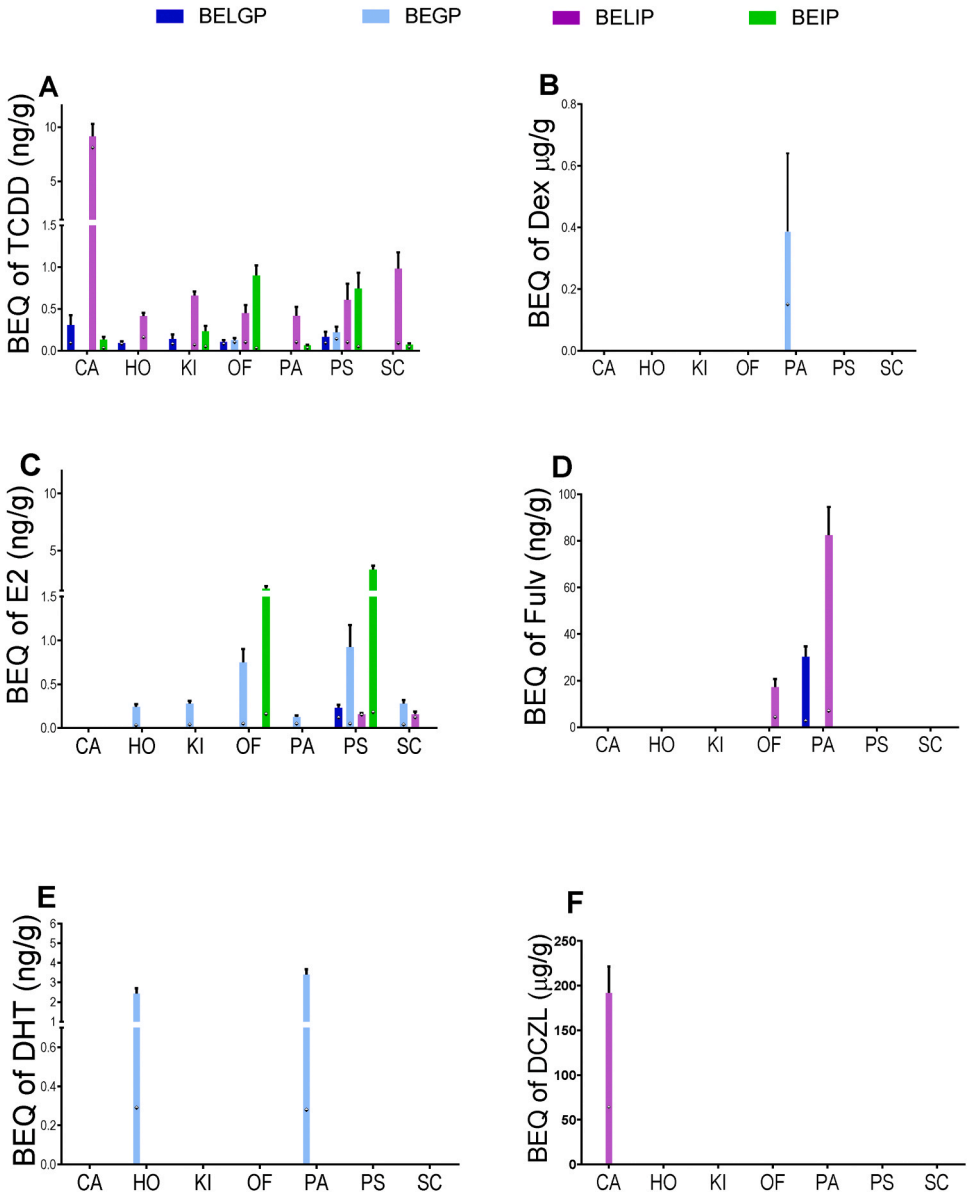

Fig. 3. Bioactivity of the bioaccessible dust extracts obtained from physiologically based extraction test (PBET) assessed through in vitro reporter gene assays. Bioaccessible extracts of intestinal (BEIP) simulation, bioaccessible extract intestinal phase simulation with addition of lipophilic sink (BELIP), bioaccessible extracts of gastric simulation (BEGP), bioaccessible extract of gastric simulation with addition of lipophilic sink (BELGP). A= AhR-mediated activity, B= glucocorticoid activity, C= estrogenicity, D= anti-estrogenicity, E = androgenic/glucocorticoid activity, F= anti-thyroid activity. CA= Cars, HO= homes, KI= kindergartens, OF= Offices, PA= prefabricated apartments, PS= public spaces, SC= schools. The results are expressed as the mean ± standard error of the mean (SEM) of bioconcentration equivalent (BEQ) of the respective reference compound (Table 1). Missing columns = below limit of quantification or effect not detected. For a detailed list of BEQs refer to S1 Tables 3–4 and Fig S3.

activity detected in the nonpolar extract of fine dust from houses (BEQ_{DHT}: 11.4 ± 1.2 ng/g) was close to its LOQ. The highest detectable specific androgenic activity was observed for the polar fine dust extract from public spaces (BEQ_{DHT}: 17.3 ± 3.4 ng/g), followed by the polar extract from bulk dust in the same environment. Nevertheless, the LOQs for school dust samples and polar bulk dust extracts from two other indoor types were higher than the androgenicity detected for HO and PS (Fig. S2E). The assessment of the specific AR/GR-mediated activities in the MDA-kB2 cell line (Fig. S2E and F) was strongly affected by the cytotoxicity of the different organic dust extracts together with antagonist flutamide, which indicates greater sensitivity of this cell line to the studied samples.

Glucocorticoid activity was detected in all organic extracts of dust from PA; with the maximal activity observed in polar extracts of both size particles. The LOQ (0.4 μg/g) was close to the detected level (BEQ_{DEX} 0.5 ± 0.1 μg/g) for PA nonpolar fine extracts. Samples from OF (nonpolar fine and polar bulk) also showed detectable GR activity (Fig. 2B). No agonism on TR and PPARγ was detected for any sample. Agonism on ER was detected in indoor dust extracts from all microenvironments, with significantly greater BEQ levels (2 – 7 ng/g of E2) across both size fractions and solvent polarities in the dust from schools compared to the other environments (Fig. 2C). Antiestrogenic activity (Fig. 2D) was also observed in samples from all assessed microenvironments. In general, antiestrogenicity was detectable primarily for fine dust extracts from all indoor types (BEQ_{FULV}: 83.7 – 710.3 ng/g). Only the samples from prefabricated apartments had higher antiestrogenicity in organic extracts of bulk dust.

The antiandrogenicity was detected just across the set of both polar and nonpolar extracts from dust of both particle sizes collected from cars (Fig. 2F), but the LOQs affected by cytotoxicity in the case of fine dust samples from schools were higher than the effects detected in extracts from car dust (Fig S2F). Antagonistic effect on the thyroid hormone receptor was detectable only for the polar extract of fine dust from cars ($BEQ_{DCZL:}$ 6000 μg/g). Nevertheless, there were LOQ values greater than this detected bioactivity in the case of bulk dust samples from cars, and fine dust samples from three indoor types (KI, PS, SC nonpolar and polar fine; Fig. S2G). The different extracts of dust from all studied microenvironments showed detectable effects on the TTR displacement assay (BEQ_{T4}: 12.4 - 564.3 μg/g). The effects were most pronounced for polar extracts of fine dust, with the greatest effects observed for samples from prefabricated apartments and homes (Fig. 2H).

3.2. Bioactivity of bioaccessible extracts obtained by PBET

The fine dust fractions were used for the PBET extraction as the fine dust organic solvent extracts generally showed greater ED activities and the fine particles are also more prone to adhere to hands, which enhances the possibility of their ingestion. Due to the limited availability of samples obtained through PBET, the bioaccessible extracts were only tested on assays where organic extracts of the respective samples exhibited bioactivity. The results of the bioactivity elicited by the bioaccessible extracts are shown in Fig. 3; detailed information on BEQs and LOQs are in SM2 Tables 3–4 and SM1 Fig. S3. The study of the bioactivity of samples subjected to the physiologically based extraction test (PBET) revealed that the samples prepared by extraction simulating the gastrointestinal tract mostly elicit lower bioactivity than the organic extracts of different polarities. However, intestinal phase extract (BEIP) of dust samples from offices and public spaces showed higher estrogenicity than their respective organic extracts (Figs. 2C, 3C). In some cases, the addition of silicone led to greater detected bioactivity, which indicates that the lipophilic phase addition improved the extraction recovery of some bioactive compounds.

As observed in the case of organic extracts, also the bioaccessible dust extracts showed widespread AhR-mediated effects across all studied indoor types (Fig. 3A), with PBET extracts mimicking the intestinal phase eliciting higher bioactivity than their gastric counterparts. In most dust samples, the addition of a lipophilic sink (BELIP) significantly increased the accessibility of the compounds with AhR-mediated activity. Estrogenicity was detected in bioaccessible dust extracts from six out of seven indoor types with the highest effect observed for intestinal phase extracts of dust from offices and public spaces (OF and PS; Fig. 3C). The gastric phase extract (BEGP) showed detectable bioaccessible estrogenicity in dust from all indoor types except of cars. Intestinal phase extracts (IP) elicited detectable estrogenicity in the case of dust from offices, public spaces and schools (Fig. 3C). Unfortunately, a higher cytotoxicity background complicated the assessment of the estrogenicity of PBET from intestinal phase extract of dust from prefabricated apartments and schools (Fig S3C and S3).

Antiestrogenic effects of the bioaccessible dust fractions were detected for both BELGP and BELIP extracts of dust from prefabricated apartments, as well as in BELIP samples from offices. The antiestrogenic effects were stronger in the addition of the lipophilic phase, which documents that the addition of silicone improved the extraction recovery of antiestrogenic compounds from these samples (Fig. 3D). AR/GR-mediated activity was only detectable in BEGP from houses and prefabricated apartments (Fig. 3E, S3E). In case of the latter (PA), it corresponded namely to the measurable GR activity of this gastric phase dust extract (Fig. 3B). No antiandrogenic activity was detectable for the PBET extracts from car dust, indicating that the responsible antiandrogenic compounds had low bioaccessibility to modelled gastrointestinal (GI) tract fluids. Anti-thyroid hormone effects were detectable only in BELIP extract from car dust ($BEQ_{DCZL:}$ 191.9 ± 50.9 μg/g) (Fig. 3F). Nevertheless, the LOQ value of BEGP from car dust was greater than the bioactivity detected for BELIP (Fig. S3F).

3.3. Chemical profile of indoor dust

We investigated the chemical profiles in polar and nonpolar organic extracts of both particle size-fractions of indoor dust (Fig. 4) to provide insight into the chemicals contributing to the detected bioactivity. These extensive chemical analyses revealed the presence of chemicals from multiple pollutant groups across all samples, including polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), organochlorine pesticides (OCP), polybrominated diphenyl ethers (PBDE), novel flame retardants (NFR), bisphenols (BP), current-use pesticides (CUP), organophosphate flame retardants (OPFR), and per- and polyfluoroalkyl substances (PFAS). The list of all analysed compounds, along with the detailed qualitative and quantitative concentration data, is available in SM3 Table 1. Generally, PAH were found to be widespread across all microenvironments. 27 out of 29 targeted PAH were detected in dust from all studied indoor types. PCB were also found to be widely distributed, with dust from kindergartens showing higher levels than the dust samples from other indoor types. 11 of 13 targeted OCP were detected in the set of samples. Dust samples from schools ($\sum_{10}$ 1 797 (fine) and ($\sum_{11}$ 1 614 (bulk) ng/g) and public spaces ($\sum_{11}$ 3 492 (fine) and $\sum_{11}$ 1 736 (bulk) ng/g) showed much higher OCP levels compared to the other sampled sites, with p,p′-DDT (school fine and bulk, and public space fine dust) and p,p′-DDE (PS bulk dust) at the highest concentrations. PBDE were found in all sampled indoor environments, with a strong dominance of BDE-209, and lesser contribution of other PBDE, with BDE-47, BDE-99, BDE-100, BDE-154, and BDE-183 detected in all tested samples. Bulk dust from houses contained the highest amount of PBDE ($\sum_{10}$ 6 141 ng/g), with BDE-209 representing 99.9% of this concentration. Also, some of the targeted NFR were found in dust across all tested microenvironments. The total amount of NFR was the highest in indoor bulk dust from schools ($\sum_{5}$ 902 ng/g), with tris (2,3-dibromopropyl) isocyanurate (TDBP-TAZTO) (871 ng/g) being the major contributor, followed by fine dust from offices ($\sum_{13}$ 703 ng/g) and public spaces ($\sum_{10}$ 190 ng/g).

Bisphenol A (BPA) was the main contributor among the BP, with the highest concentrations detected across all microenvironments. Fine dust from schools contained the highest concentration of BPA (6 779 ng/g of

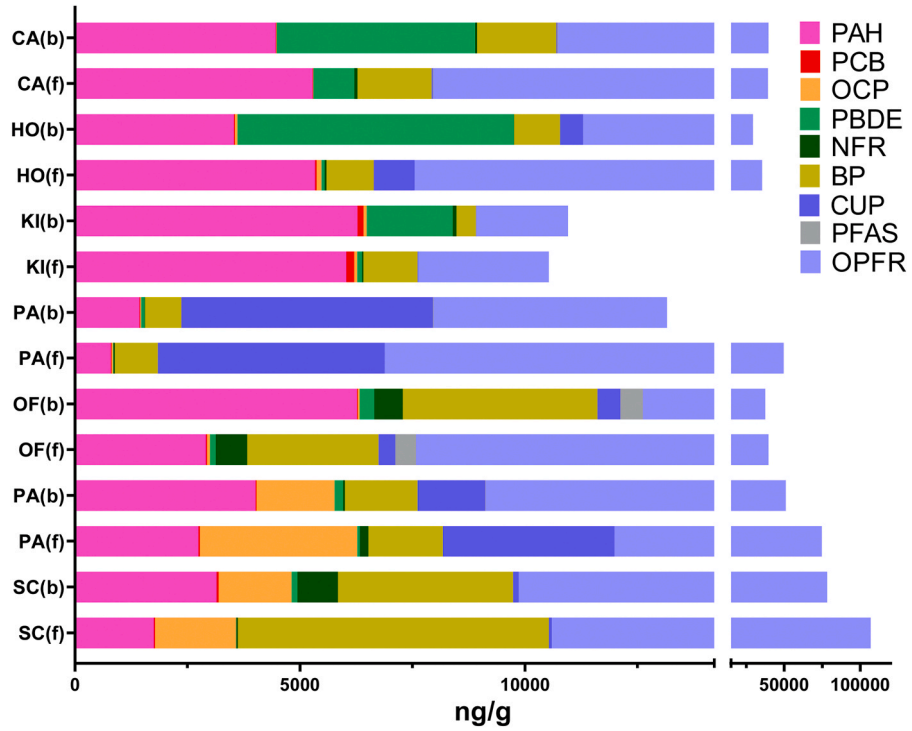

Fig. 4. Total cumulative concentrations in ng/g of target chemicals detected in indoor dust from seven microenvironments. The concentrations are given in ng of each class of target chemicals /g dust. CA= Cars, HO= homes, KI= kindergartens, OF= Offices, PA= prefabricated apartments, PS= public spaces, SC= schools. Bulk dust (b) and fine dust (f) refer to the particle size (< 2 mm and < 0.25 mm, respectively). For detailed information on each chemical please refer to S3 Table 1.

dust). Bisphenol S (BPS) and bisphenol F (BPF) were also detected in dust from all studied indoor environments. Regarding CUP, 11 out of the 36 analysed pesticides were detected in our study. Dust samples from prefabricated apartments and public spaces showed much greater levels of chlorpyrifos (up to 5546 (PA bulk) ng/g) compared to dust from other indoor types (SM3, Table1). OPFR were the pollutant group with the highest levels across the samples. The sum of 14 detected OPFR ranged from 2 038 ng/g in bulk dust from kindergartens to 96 317 ng/g in fine dust from schools. Several OPFR showed concentrations over 5000 ng/g across multiple indoor types. The greatest difference among the micro-environments was found for triphenyl phosphate, which was below LOQ in dust from two indoor types but reached 58 469 ng/g in fine dust from schools. A few PFAS were detected in 9 out of 14 tested samples. However, 14 PFAS were detected in indoor dust (fine and bulk) from offices. Their levels ($\sum_{PFAS(fine)}$ 452 and $\sum_{PFAS(bulk)}$ 496 ng/g) were much greater than in dust from any other microenvironments, where none (KI) or only a few PFAS at very low levels were detected. Among the fluorinated compounds detected in office dust, PFOA (up to 151 ng/g) and PFOS (up to 247 ng/g) had the highest concentrations, suggesting specific sources of these chemicals in offices and more limited sources in other indoor microenvironments.

3.4. Contribution of the detected chemicals to the observed bioactivity

The contribution of the detected chemicals to the observed biological effects was assessed using the concentration addition modelling. The assessment was based on REP*i* (relative potencies; SM3) obtained from the same or analogous cell models in CompTox database, peer-reviewed literature, and in-house generated data as previously shown by Novaková et al. (2022) with the addition of the REP_i for TTR binding

Table 2
Coverage of the REPs for each chemical class in the respective bioassays used in this study. Chemical class = targeted compound groups. Analysed = the total number of analysed chemicals. Detected (n) = the total number of chemicals above the limit of detections. Detected (%) = percentage of detected chemicals calculated based on the number of analysed chemicals. N/A = non-active/active chemicals according to the data available for the respective endpoint bioactivity (REPs). No data (%) = the percentage of detected chemicals with no bioactivity information (REP) available in the assessed bioassays.

		Total detected		AhR		ERα		aERα		AR/GR		aAR		GR		TTR assay	
Chemical class	Total assessed	n	%	N/A	No data (%)	N/A	No data (%)	N/A	No data (%)	N/A	No data (%)	N/A	No data (%)	N/A	No data (%)	N/A	No data (%)
PAH	29	28	97	9/9	39	9/6	50	15/2	43	15/2	43	11/8	36	16/3	36	0/0	100
PCB	9	9	100	1/0	89	6/1	22	0/3	67	7/0	22	0/7	22	1/0	89	0/2	78
OCP	13	11	85	5/0	55	5/1	45	3/3	45	6/0	45	2/5	36	8/0	27	0/0	100
PBDE	10	10	100	3/0	70	0/4	60	2/1	70	3/0	70	1/5	40	3/0	70	0/2	80
NR	23	15	65	2/0	87	2/0	87	3/0	80	0/1	93	1/2	80	4/0	73	0/0	100
BP	3	3	100	3/0	0	0/3	0	1/2	0	2/0	33	1/2	0	3/0	0	0/0	100
CUP	36	11	31	3/7	9	6/3	18	5/5	9	10/0	9	4/6	9	10/0	9	0/0	100
OPFR	16	14	88	10/1	21	6/0	57	6/1	50	5/1	57	4/2	57	12/0	14	0/0	100
PFAS	16	12	75	6/0	50	6/0	50	2/4	50	6/0	50	6/0	50	6/0	50	0/8	33

PAH = Polycyclic Aromatic Hydrocarbons, PCB = Polychlorinated biphenyls, OCP = Organochlorine pesticides, PBDE = Polybrominated diphenyl ethers, NFR = novel flame retardants, BP = Bisphenols, CUP = Current-use pesticides, OPFR = Organophosphate flame retardants, PFAS = Per- and polyfluoroalkyl substances.

inhibition from Hamers et al. [9]. The total contribution of the detected bioactive chemicals with known REP is expressed as BEQchem (Eq. 3, Chapter 2.3). Comprehensive information on the available REPs, results of chemical analyses, and BEQchem for each bioassay is included in the SM3 Tables 3–9. The availability of the specific bioactivity information for each chemical class in the respective bioassays used in this study is summarized in Table 2. The AhR-mediated activity could be marginally explained by the presence of PAH; the maximum explicability was found for bulk dust from houses (7%). The contribution of the analysed CUP to the observed AhR-mediated activity was $< 1\%$. BP significantly contributed to ER-mediated responses of extracts from office bulk dust (20.9%), and from fine and bulk dust from houses (5.2% and 8.3%, respectively), fine and bulk dust from public spaces (5.2% and 11.9%, respectively), bulk dust sampled from cars (4.4%), fine dust from prefabricated apartments (4.2%), and fine and bulk dust samples from schools (2.8% and 4.3%, respectively). Additionally, the targeted PAH contributed to the estrogenicity in bulk dust from offices (5.6%). There was very low explicability of the other effects by the detected compounds with available REPs. 0.1% of the antiestrogenic effects of extracts of fine dust collected in kindergartens was explained by the presence of the targeted PAH. The same level (0.1%) of the anti-androgenicity in extracts of bulk dust obtained from cars was explained by the targeted PAH and BP. The targeted chemicals with available REPs did not significantly contribute to the observed androgenicity, glucocorticoid activity, and T4 displacement from TTR ($< 1\%$). Nevertheless, as shown in an overview Table 2, the availability of information on the specific bioactivities and respective REPs widely differed across studied endpoints and compound classes. It demonstrates that for many of the detected compounds, even for some with widespread occurrence at very high concentrations, there were no REPs available across multiple assays. Thus, even some abundant chemicals could not be taken into account in mixture effect modelling. Across effects, there was the least knowledge on REP coverage for TTR-binding inhibition, which was a widespread effect across all studied indoor types. Among compound groups, there was the least info on flame retardants across most of the effects, despite high levels of some of these compounds. This highlight major gaps in knowledge which need to be urgently addressed.

4. Discussion

Previous research has documented potential toxicity and chemical contamination in different indoor settings highlighting a potential threat to the well-being of building occupants [11,13,2,15,39] (Tab10 in SM3). However, these studies assessed only limited types of microenvironments and endpoints. Although previous studies (SM3 Tab 10) have explored the application of in vitro models to screen ED potential in indoor dust, sparse studies have utilized these models to assess the bioaccessibility of the compounds responsible for the toxic potential. This study demonstrates the applicability of in vitro human cells/protein-based models for the assessment of bioactivity of exhaustive and bioaccessible chemical mixtures associated with various indoor environments. The objective of utilizing two different organic solvents, one “polar” and one “non-polar”, is to comprehensively extract and assess the organic chemicals from the dust matrix. Nevertheless, the acetone component in non-polar solvent mix could lead to a partial extraction of some more polar compounds. While this does not affect the target chemical analyses, it might have some impact on the bioanalytical results from bioassays.

Subsequently, the bioactivity of the exhaustive extracts, representing the full endocrine disrupting potential, is compared with that of PBET extracts, which provides insights into the effects of bioaccessible chemicals present in dust matrix. This approach enables to assess the bioaccessibility of the effective compounds, while also providing data on the overall endocrine disrupting pollutant load in the dust from different types of indoor environment. This is an important information for discussion with previous studies that employed mostly just exhaustive extractions for indoor dust pollution characterization.

Our results document widespread presence of compounds with some ED modes of action (MoA) in both exhaustive and bioaccessible extracts, while there are major differences and specific patterns among indoor types for some other MoA. The intra-study comparison of the effects of organic extracts of two different polarities and bioaccessible extracts mimicking the gastrointestinal conditions showed that organic solvent extracts mostly exhibited higher bioactivity compared to bioaccessible extracts, except for estrogenicity from intestinal phase extracts (BEIP). Additionally, the assessment of organic extracts revealed a wide range of bioactivities, including AhR-mediated and anti-estrogenic activity, and T4 displacement from TTR. The present study observed anti-androgenic and anti-thyroid activities only for extracts of dust from cars, while extracts from houses and public spaces exhibited androgenic effects. Samples from offices and prefabricated apartments showed androgenic/glucocorticoid activity.

Previous studies [27,38,5] have demonstrated widespread AhR activity in organic extracts of dust from various microenvironments like houses, offices, and lecture rooms. We found that AhR-mediated activity detected in samples from houses was comparable with the levels reported by Nováková et al. [27], while only our study found this bioactivity also in polar extracts from offices; comparison is possible due to the similarity of the models and extraction method employed in both studies. Moreover, our results show that the bioaccessible extracts from dust across the different indoor types also preserve AhR-mediated activity, even though these samples underwent different types of treatment, namely extraction by gastric and intestinal fluids including samples with the addition of silicone as a sorptive sink. We observed that the recovery of AhR-mediated effect from PBET was about 8% for dust sampled from cars (CA BELIP vs Nonpolar fine). The PBET samples from other microenvironments showed about 1% effect recovery in the AhR assay.

The employed analyses and modelling showed some common patterns across all tested organic extracts, e.g. the presence of PAH was identified as a contributing factor to the observed AhR-mediated activity. Moreover, except for PA, the detected PAH also played a role in the observed estrogenicity of organic extracts from all microenvironments. Additionally, the detected bisphenols (BP) were found to partially account for the estrogenicity observed in organic extracts from all tested microenvironments (see SM3_Tab 2). Regarding the observed ERα agonism, we showed that organic extracts of dust from schools elicited the highest effects. These samples also contained the highest detected levels of NFR, OPFR, and BP. However, the presence of bisphenol A, a known estrogenic compound, could only explain 4.3% of the observed estrogenicity. This finding indicates a substantial presence of unknown chemicals with the capacity for interfering with ERα or contribution from other detected compounds with missing info on ER agonism. It is worth noting the presence of TDBP-TAZTO, a novel flame retardant found in high levels exclusively in bulk samples from schools, for which there is currently no available REP data in the literature.

Only one previous study has employed cell lines to compare the magnitude of the effects elicited by bioaccessible extracts with their organic counterparts. This study by Zhou et al. [45] focused on compounds with GR-mediated activity in samples from Chinese households. They showed that the magnitude of antagonism on GR elicited by bioaccessible fraction (tenax-assisted bioaccessible extraction (TBE)) was consistently lower (mean 36.8% (9 – 87%)) in bioaccessible samples compared to the organic extracts. A similar trend of lower ED potentials in the bioaccessible compared to the organic extract was also observed across most effects and samples in our study. Nevertheless, the comprehensive assessment of the two types of indoor dust extracts in our study showed that in some cases bioactive compounds can be more accessible by extraction using PBET of intestinal fluids. Besides the previously studied estrogenicity of organic indoor dust extracts (SM3_Tab 10), we have also shown that the bioaccessible dust extracts from all microenvironments elicited detectable estrogenicity. Notably,

bioaccessible extracts (BEIP) from OF and PS exhibited even greater estrogenicity than their organic counterparts. This underscores the relevance of the assessment of indoor dust from various microenvironment types utilizing different organic and bioaccessible extractions.

The latter study (Zhou et al., 2022) also assessed the presence of agonism on GR from dust samples; however, none of their dust extracts elicited activation of the GR receptor using MDA-kB2 cell line. Notably, the GR agonism was only shown in a previous study on organic extracts of dust from houses in the United States [37]. Our study documents GR-mediated activity in organic extracts of indoor dust from prefabricated apartments and offices in central Europe. Moreover, this GR-mediated activity was recovered in bioaccessible extract (BEGP) by around 30.7% of the activity detected from PA polar fine and around 100% of the GR activity detected from PA nonpolar fine extract. Interestingly, this microenvironment was also more contaminated with CUP, with high predominance of chlorpyrifos. To our knowledge, our study is the first documenting GR-mediated activity in indoor dust samples collected in Europe. Moreover, it brings novel information on the differences among the studied indoor types and the significant bioaccessibility of the compounds contributing to this activity.

We also showed that organic and bioaccessible extracts exhibited antagonistic effects on ERα. Part of the anti-estrogenic effects can still be recovered in the bioaccessible extracts from OF (2.5%) and PA (9.2%) dust; BELIP vs Nonpolar Fine and Polar Bulk, respectively. The dual effects of indoor dust samples extracts, acting as both ERα agonists and antagonists, have been previously documented for samples collected from homes, offices, and lecture rooms [27]; this previous study additionally documented anti-AR effects from some of those samples. Furthermore, our study draws attention to an effect that was not previously detected: organic extracts from HO and PS also exhibited agonistic effects on the androgen receptor (AR). The AR-mediated response recovery after PBET was around 24.7% compared to the organic extract (nonpolar fine from HO). To our knowledge (SM3 Tab 10), this is the first study showing AR activity in indoor dust from both organic and bioaccessible dust extracts. This profiling underscores the multifaceted interactions of the complex mixture present in indoor dust with different endocrine receptors mediating various modes of action and the potential risk to those exposed via the oral route.

The specific aAR and aTR effects detected in dust from cars show that this neglected microenvironment can be an important source of exposure to bioactive chemicals which can be at higher levels than in other indoor settings. Interestingly, 3% of the bioactivity observed in the polar extract of fine dust could be recovered in the bioaccessible extract (BELIP), suggesting that some compounds with antagonistic activity on TR can be accessible via oral exposure. The assessment of TR and aTR effects was impaired by the relatively high cytotoxicity of all organic extracts in our study to the employed PZ-TR cell line. Importantly, the cytotoxicity triggered by the dust extracts can be an important confounding factor in the assessment of (ant)agonistic effects by in vitro assays with cell lines. This higher cytotoxicity observed in PZ-TR cell line could explain why our study was unable to detect TR-mediated effects, which were demonstrated by Nováková et al. (2002) in their analysis of organic (dichloromethane) extracts from indoor dust samples collected from lecture rooms and houses. Nevertheless, the use of a cell-free model (TTR assay) to assess the disruption on the transport of T4 enabled the detection of this thyroid hormone disrupting effects by extracts of dust from all studied indoor types.

The concentration addition model, as recommended in a component-based approach, has been applied as a suitable method for predicting mixture effects of chemicals sharing the same mode of action such as the interaction with hormone receptors ([1]; reviewed in [6]). In our study, the explanatory power of this component-based model of prediction was partially hindered by the limited information on the REPs available in literature. Although all targeted PCB and PBDE were detected, for 89% and 70%, respectively, of these chemicals REPs for AhR-mediated activity and GR activity were missing. REPs for explaining the observed effects on thyroid hormone transport (TTR assay) were not available for most detected pollutant groups, including PAH, OCP, NFR, BP, CUP, and OPFR. Half of the detected PFAS also did not have any REP available for the explicability of AhR-mediated activity, ERα, aERα, AR/GR, aAR/GR, and GR, even though this group of contaminants has drawn growing interest of the scientific and regulatory community. Notably, OPFR detected at the highest levels in the studied microenvironments lacked REPs for 21 – 57% of compounds across assays related to the observed ERα, aERα, AR/GR, aAR/GR. NFR had the highest proportion of missing REPs, with 73% of the chemicals missing REPs for explicability of the observed GR, followed by missing REPs at 80% for aERα, 87% for AhR and ERα, 93% for AR/GR, and 100% for the displacement of T4 from TTR. This limited information emphasizes a crucial gap in the study of toxicological properties of contaminants present in indoor dust that complicates the assessment of the contribution of different pollutant groups to the ED potentials and identification of effect drivers.

5. Conclusion

This study provides comprehensive information on the chemical composition and endocrine disrupting potential of extracts from two particle sizes of dust from different indoor types. Moreover, the results document the bioaccessibility of the compounds contributing to the endocrine disrupting effects since the effects were detectable even after treatment with simulated digestive conditions. The greater cytotoxicity in some cases interfered with specific bioactivity detection, which shows that ED effects can be masked by higher sensitivity of the available in vitro models to general cytotoxic effects. The extensive chemical analyses highlight the complex nature of the dust composition and the presence of banned chemicals in close proximity to building occupants, along with high levels of understudied replacement chemicals, such as OPFR and NFR. Despite the extensive chemical analysis, the limited data on the bioactivity of the target chemicals shows that the relative potencies of relevant contaminants are still understudied. Furthermore, this limited explicability also suggests the presence of unidentified or unknown compounds in indoor dust with bioactive properties. Our results show that exposure to potential endocrine-disrupting chemicals in indoor dust can vary in intensity and location, constantly affecting individuals who spend time in various indoor settings. Further investigations are necessary to identify sources and toxic drivers, aiding regulatory decisions on acceptable chemical levels.

Statement of environmental implication

This study highlights the presence of a wide spectrum of hazardous pollutants in various indoor types, their endocrine disrupting potential along with the bioaccessibility of the contributing compounds. Several endocrine disrupting effects were detected in both exhaustive and bioaccessible extracts from representative dust samples; extensive chemical analyses confirmed hazardous banned and emerging pollutants. This complex exposure scenario brings attention to potential harm to exposed people, especially children in sensitive stage of development. This comprehensive assessment of indoor pollution contributes to a better understanding of human exposure, facilitating informed risk assessment, management, and public health decisions towards safer indoor environment.

CRediT authorship contribution statement

Klára Hilscherová: Writing – review & editing, Supervision, Project administration, Conceptualization. **Vrana Branislav:** Writing – review & editing, Methodology. **Melymuk Lisa:** Writing – review & editing, Funding acquisition, Conceptualization. **Simona Rozárka Jílková:** Methodology, Investigation, Data curation. **Rusina Tatsiana:** Methodology, Investigation. **Pinto-Vidal Felipe Augusto:** Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Jiří**

Novák: Writing – review & editing, Validation, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.133778.

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