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Hormone receptor activities of complex mixtures of known and suspect chemicals in personal silicone wristband samplers worn in office buildings

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Abstract

Humans are exposed to increasingly complex mixtures of hormone-disrupting chemicals from a variety of sources, yet, traditional research methods only evaluate a small number of chemicals at a time. We aimed to advance novel methods to investigate exposures to complex chemical mixtures. Silicone wristbands were worn by 243 office workers in the USA, UK, China, and

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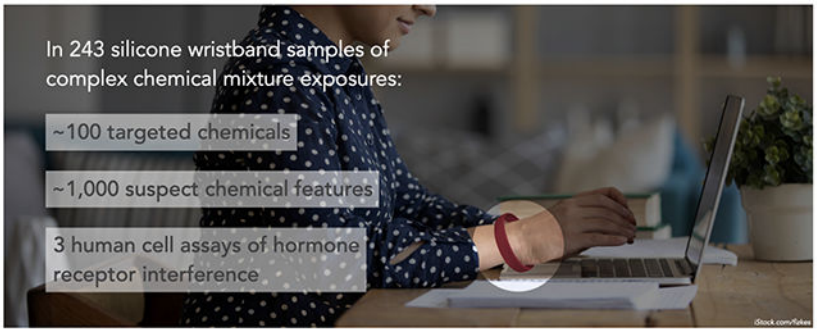
Declaration of interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Several of the co-authors (PB, AB, EF) are employed by and obtain a salary from BDS, the company that has developed the CALUX bioassays and who were responsible for bioanalysis of the wristband samples. The authors declare no other known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

India during four work shifts. We analyzed extracts of the wristbands for: 1) 99 known (targeted) chemicals; 2) 1,000+ unknown chemical features, tentatively identified through suspect screening; and 3) total hormonal activities towards estrogen (ER), androgen (AR), and thyroid hormone (TR) receptors in human cell assays. We evaluated associations of chemicals with hormonal activities using Bayesian kernel machine regression models, separately for targeted versus suspect chemicals (with detection ≥50%). Every wristband exhibited hormonal activity towards at least one receptor: 99% antagonized TR, 96% antagonized AR, and 58% agonized ER. Compared to men, women were exposed to mixtures that were more estrogenic (180% higher, adjusted for country, age, and skin oil abundance in wristband), anti-androgenic (110% higher), and complex (median 836 detected chemical features versus 780). Adjusted models showed strong associations of jointly increasing chemical concentrations with higher hormonal activities. Several targeted and suspect chemicals were important co-drivers of overall mixture effects, including chemicals used as plasticizers, fragrance, sunscreen, pesticides, and from other or unknown sources. This study highlights the role of personal care products and building microenvironments in hormone-disrupting exposures, and the substantial contribution of chemicals not often identifiable or well-understood to those exposures.

Graphical Abstract



Keywords

Chemicals; mixtures; non-targeted; assays; endocrine disruptors

1. Introduction

We are exposed to increasingly complex mixtures of chemicals in our environments: Globally, over 350,000 industrial chemicals have been registered in national or regional inventories (Wang et al., 2020). In the past decade alone, over 69,000 of those chemicals were registered, of which approximately 11,000 chemicals are not publicly identifiable due to business confidentiality. Furthermore, tens of thousands of chemicals are only registered in low- and middle-income countries (LMICs) (Wang et al., 2020), who have a disproportionate share of chemical production yet whose chemical exposures are less likely to be researched or nationally biomonitored (Goodman et al., 2020; Landrigan et al., 2018; Lucattini et al., 2018).

Given the plethora of both known and unknown chemicals in commerce, current approaches to assess and regulate chemicals one at a time do not realistically portray the way we are exposed to complex mixtures of chemicals in the real world (Drakvik et al., 2020). For one, the combination of individual chemicals in a mixture can produce compounded risk of the same health outcome through identical or different physiological mechanisms (Escher et al., 2020). In addition, certain chemicals can trigger or worsen the effect of other chemicals when present together (Kienzler et al., 2016; Orton et al., 2014). Moreover, even when a particular chemical has been successfully phased out of production, other less well-known chemicals with similar unforeseen health concerns have often been used as replacements, in a phenomenon called “regrettable substitution” (Blum et al., 2019; Maertens et al., 2021). For example, in a decades-long chain of regrettable substitution of chemical flame retardants in response to emerging health concerns about carcinogenicity, neurotoxicity, or endocrine disruption, historic polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) were replaced with polybrominated diphenyl ethers (PBDEs), followed by currently used organophosphate esters (OPEs) and now less-understood brominated flame retardants (novel BFRs) (Birnbaum and Bergman, 2010; Covaci et al., 2011; Saillenfait et al., 2018; Stapleton et al., 2012; Vuong et al., 2020). Hormone-disrupting phthalate plasticizers have also been interchanged for each other or replaced with less studied substitutes (Frederiksen et al., 2020; Zota et al., 2014). For these reasons, assessments of only a single chemical or single class of chemicals underestimate both total exposure and health risk (Drakvik et al., 2020; Kortenkamp, 2014).

It is clear now that chemical mixtures can produce effects on hormone function that are not always predicted based on single-chemical experiments (Kortenkamp et al., 2022). Endocrine-disrupting chemicals (EDCs) alter function(s) of the endocrine system through a number of different mechanisms (Bergman et al., 2013), but a key characteristic of EDCs is their direct interference with hormone receptors in the body (La Merrill et al., 2020). The ubiquitous exposure of the human population to EDCs has contributed to an increasing worldwide prevalence of endocrine-related diseases and disorders (Bergman et al., 2013), including fertility declines for both men and women (Skakkebæk et al., 2022), obesity (Heindel et al., 2022; Kassotis et al., 2022; Lustig et al., 2022), diabetes (Sargis et al., 2019), and brain-based disorders (Volk et al., 2022).

The indoor environment is a significant source of our exposure to EDCs, including many semi-volatile organic chemicals (SVOCs) that are used in building materials, building maintenance products, furnishings, and consumer products. SVOCs can migrate out of materials over time and accumulate in the dust, air, and surfaces in buildings (Lucattini et al., 2018; Mitro et al., 2016; Weschler and Nazaroff, 2008) due to their use in furniture, carpet, flooring, electronics, insulation, sealants, paint, or many other products (typically as undisclosed additives) (Cooper et al., 2020; Erickson and Kaley, 2011; Hammel et al., 2017; Wittassek et al., 2011). Research has found that certain endocrine-disrupting SVOCs investigated in our study, including plasticizers and flame retardants among other chemicals, can interfere with hormone receptors (EPA, 2019; Hamers et al., 2006; Legler et al., 2002; Seeger et al., 2016; Suzuki et al., 2013) and increase human risk of adverse effects on fertility and pregnancy outcomes (Carignan et al., 2017; Choi et al., 2019; Hauser et al., 2006; Messerlian et al., 2018; Mumford et al., 2015), development (Doherty et al., 2019a;

Linares et al., 2015; Mariana et al., 2016), and thyroid function (Allen et al., 2016; Boas et al., 2012; Preston et al., 2017; Shrestha et al., 2018).

Novel methods to assess the hormonal impacts of complex, real-world chemical mixtures and to identify the indoor building microenvironments in which we are exposed to those endocrine-disrupting chemical mixtures are essential (Escher et al., 2020). One emerging method is cell-based assays of indoor dust, which measure the amount of agonism (activation) or antagonism (suppression) of hormone receptors in human cells exposed to chemical mixtures extracted from the dust samples (Kollitz et al., 2018; Suzuki et al., 2013; Vandermarken et al., 2016). For example, our previous research found that every dust sample we collected from university buildings was hormonally active, and the degree of hormonal activity was significantly related to the summed dust concentrations of certain chemical classes commonly added to building materials, including PBDEs and OPEs (Young et al., 2021b). This dust analysis demonstrated the potential toxicity of chemical mixtures found in specific buildings. However, the chemical composition of dust can vary significantly from one room to another in the same building (Al-Omran et al., 2021; Jílková et al., 2018), and it does not always equate to the same cumulative mixture that people are ultimately exposed to across all the micro-environments they spend time in. In our current study here, we aimed to extend the cell assay methodology to silicone wristband samples of people's actual *personal* exposures to chemical mixtures inside buildings.

Silicone wristbands are novel personal passive samplers that accumulate chemicals from the air, dust, and products that individuals are exposed to through inhalation, dermal contact with those mediums, or hand-to-mouth contact (Anderson et al., 2017; Hammel et al., 2018; S. Wang et al., 2019b, 2019a). Unlike traditional internal biomarkers such as urine or blood that integrate all routes of exposure (including ingestion from diet and drinking water), these simple, non-invasive silicone wristbands allow researchers to pinpoint external, non-dietary environmental exposures and to control the sampling duration (Fuentes et al., 2022; Wacławik et al., 2022). For several types of SVOCs (such as flame retardants and plasticizers), the chemicals in personal wristbands, compared to environmental dust or air samples, better reflect actual exposures within the "personal activity cloud" surrounding an individual (Okeme et al., 2018; Rodes et al., 1991) and have been shown to correlate well with internal biomarkers (Dixon et al., 2018; Gibson et al., 2019; Hammel et al., 2020, 2018, 2016; Levasseur et al., 2021). Only one very recent study applied a human cell assay to silicone wristband extracts, which showed that the majority of chemical extracts isolated from wristband samples antagonized thyroid hormone receptor activity (Kassotis et al., 2020).

Using hormone receptor cell assays to evaluate silicone wristband extracts would be innovative in the quantification of total and proximate hormonal impacts of complex, real-world chemical mixtures that people are personally exposed to, without some of the challenges of extensive human epidemiological studies of lagged or binary health outcomes. We aimed to conduct several human hormone receptor cell assays of chemical mixture exposures collected via silicone wristbands. We also aimed to pair the results from the cell assays of silicone wristband extracts with the measurement of not only targeted chemicals but also the novel suspect chemical screening of hundreds of substances in order to enhance

our assessment of entire mixtures of chemicals, including unknown or emerging chemicals (Fuentes et al., 2022). To do this, we leveraged our previous study that collected silicone wristbands worn by office workers (Young et al., 2021a).

Using the same wristband samples, the specific objectives of this study were to: 1) quantify the estrogen, androgen, and thyroid hormone receptor activity in human cells using extracts of silicone wristbands worn only at work by 243 office workers in the USA, China, India, and the UK; 2) identify key chemical drivers of the hormonal activities based on concentrations of 99 targeted chemicals and over 1,000 features identified by suspect screening of the wristband samples; and 3) determine the associations of the overall chemical mixtures as a whole with the hormonal activities.

2. Methods

2.1. Study Population

For this study, we used silicone wristband samples from 243 office workers that had been collected as part of our previous study of the chemical exposures of 251 office workers in the USA, UK, China, and India from the Global Buildings CogFx Study. The participants were non-smoking, full-time employees working in urban office buildings. Further details on the study population and recruitment are provided in the previous manuscript (Young et al., 2021a).

We selected 243 participants who had complete chemical data for all 99 targeted chemicals. The final 243 participants were located in the USA (n=82), UK (n=37), China (n=70), and India (n=54), and they occupied 36 unique buildings (median of six participants per building). These 36 urban office buildings had a median of approximately 350 occupants during operating hours and a median 6,600 m^2 gross area.

Of the 243 participants, 209 responded to a survey about sociodemographic information from the parent study (Table S1). For gender, the participants were roughly equally split between male and female (no other gender identities were reported), with 54% female overall, 61% in the USA, 30% in the UK, 69% in China, and 37% in India. The age distributions were similar across countries (medians ranged from 27 to 33), as were the typical weekly hours spent in the office (medians ranged from 38 to 44). Racial/ethnic diversity within countries was relatively low. Only six participants were Black (3%) and three were Hispanic (1%). White participants constituted 70% and 87% of participants in the USA and UK, respectively (0% in China and India), while 19% and 3% were Asian in the USA and UK, respectively (100% and 98% in China and India). The population was well-educated, with 99% having completed education above high school and 64% at the professional, master, or doctorate level.

2.2. Study Design

The office workers participated during a study week in 2019 that was predefined for each building. The participants were instructed to wear their silicone wristband sampler continuously during four consecutive workdays, Monday–Thursday, but only during their work hours. They were told to put the wristband on as soon as they arrived at their office

building in the morning and to remove it and wrap it in foil in a sealed plastic bag on their desk before they left the office for the night. Some participants did not wear the wristband on certain day(s) when they were out of the office, but the majority (82%) wore it all four workdays, and the concentrations of chemicals in the wristband samples were scaled to a standard sampling period based on the number of cumulative hours they wore the wristband each day as reported in time log sheets. All collected wristbands were shipped back to Boston and frozen within one to two weeks. The study protocol was reviewed and approved by the Institutional Review Board at the Harvard T.H. Chan School of Public Health.

The office buildings in the UK were sampled earlier than those in the other countries (USA buildings: most in April of 2019, one in January; UK: all in February; China: all in July; India: most in May, one in June). However, in our previous manuscript, we controlled for indoor sensor-measured temperature and relative humidity in sensitivity analyses of our regression models and found that the results for country differences in chemical exposures held even after accounting for potential seasonal differences (Young et al., 2021a).

Field blanks were collected from different buildings. The field blanks consisted of wristbands prepared using the same protocol as the participant kits and shipped in the same boxes to various countries, but they were not opened or worn. We analyzed 21 field blanks in the chemical analyses and 23 in the hormone receptor assays.

2.3. Wristband Preparation and Targeted Chemical Analyses

Our protocols for the preparation and analysis of the wristband samples followed previous publications (Hammel et al., 2020, 2018, 2016; Reddam et al., 2020; Wise et al., 2020). Before sending sample kits to participants, we cleaned the red silicone wristbands using solvent extraction and wrapped the wristbands in pre-cleaned aluminum foil and Ziploc bags. The wristbands were analyzed for 99 chemicals mostly using an Exactive GC Hybrid Quadrupole-Orbitrap GC-MS/MS system (Thermo Fisher Scientific, Waltham, MA, USA) at the Michael and Annie Falk Foundation Exposomics Laboratory at Duke University. Only about one-fifth (roughly 0.75 g) of each wristband sample was used for the chemical analyses. More detail on the chemical laboratory methods and quality assurance and quality control are provided in our previous manuscript (Young et al., 2021a). The analytes consisted of 11 PCBs; 13 BFRs, including 10 PBDEs and 3 novel BFRs; 31 OPEs; 10 phthalates or phthalate alternatives; 12 pesticides; and 20 polycyclic aromatic hydrocarbons (PAHs). Abbreviations for targeted chemicals included in statistical analyses are provided in the Supplementary Material, but the primary ones we discuss here are: dichlorodiphenyldichloroethylene (*p,p*'-DDE, or DDE for short), triphenyl phosphate (TPHP), di-n-butyl phthalate (DnBP), di-isobutyl phthalate (DiBP), and tri-n-butyl phosphate (TnBP).

2.4. Suspect Screening of Chemical Features

After instrumental analysis, mass spectrometry data were processed to support suspect screening using the deconvolution plugin in the Tracefinder software. Features were filtered with a signal to noise threshold of 10, a total ion chromatography (TIC) threshold of

1000000, an Ion overlap window of 90%, and an accurate mass tolerance of 10 ppm. The RT alignment window was set to 10 seconds.

After deconvolution, samples were screened against three libraries to deduce tentative identifications of the chemical features, where available. The first library screened was the NIST17 library, which consisted of over 250,000 low resolution, electron ionization (EI) spectra. The second library used was the Thermo Hi-Res Library, which consisted of approximately 900 high resolution EI spectra generated with an orbitrap instrument by Thermo Fisher Scientific (Waltham, MA, USA). The third and final library was an in-house library, which consisted of approximately 150 high resolution EI spectra generated in-house. Library searches were first conducted using a hi-res forward search approach with a dot product threshold of 500; however, identities were further evaluated in custom R scripts. With the custom R scripts, library matches were conducted using a composite ranking system, which included all possible scoring metrics (forward and reverse), metadata information from PubChem, and cross sample scoring. Additionally, the custom R scripts allowed for blank subtraction and standard normalization for all features identified via suspect screening to remove non-relevant features. Identities of specific unknown features discussed in the text of this study were manually curated to confirm that our scripts were selecting the highest quality match.

Tentative identifications were assigned to unique features with high match scores after blank subtraction (See spreadsheet in Supplementary Material). In our workflow, identities were assigned based on an aggregate ranking system that considers forward search metrics (similarity index and high-resolution filtering [HRF] score), number of PubMed entries, number of patents, and overall abundance of response in sample list. PubChem metadata information for PubMed entries and patents was implemented with the PubChemLite database (Schymanski et al., 2021). Given the complexity of the wristband matrix and the caveats associated with library matching for unknown identifications, our workflow was best suited to identify high abundance analytes that are expected to be observed.

It is important to note that the tentative identifications presented in the Supplementary Material include repeat tentative identifications. This is an unfortunate artifact of EI spectral library matching as a means of unknown screening. With EI methods, spectral information can be limited for certain compounds, leading to difficulties in identifying correct compounds. For example, while a phthalate identification can be confident due to the presence of the signature base peak (m/z 149), we often lack fragmentation information beyond this to accurately determine which phthalate isomer the unknown feature represents. Phenolic compounds often suffer from this same phenomenon. Similarly, for compounds with many different homologs such as PCBs, we can confidently identify which homolog an unknown feature belongs to (i.e., the degree of chlorination), but cannot determine which specific isomer the feature represents without retention time matching against analytical standards.

We used the Functional Use Database (FUse) of the U.S. Environmental Protection Agency to link identified suspect features by Chemical Abstracts Service (CAS) registry number to known functional uses in consumer products and industrial processes (K. A. Phillips et al.,

2017). We supplemented this information with the EPA Chemical and Products Database and Human Metabolome Database when needed (Dionisio et al., 2018; Wishart et al., 2022).

2.5. Nuclear Hormone Receptor Assays

We cut another one-fifth piece (0.6–0.9 g) of each wristband sample to ship to the BioDetection Systems laboratory in Amsterdam, The Netherlands. The silicone wristband samples (including field blanks) were evaluated in three chemically activated luciferase gene expression (CALUX) assays: thyroid hormone receptor β (TR) antagonism, androgen receptor (AR) antagonism, and estrogen receptor α (ER) agonism.

The CALUX assays used human female osteosarcoma cell lines (U2OS) that were stably transfected with the luciferase reporter gene from the firefly. For assays of *agonism*, chemicals in the sample extract may activate the specific hormone receptor under study and thus trigger expression of luciferase and production of light. The emitted luminescence is measured with a luminometer (TriStar LB941, Berthold, Bad Wildbad, Germany) and is directly proportional to the amount of activation of the hormone receptor. For assays of *antagonism*, there is an added agonist compound (triiodothyronine [T3] for TR assay and dihydrotestosterone [DHT] for AR assay) whose agonism may be suppressed by chemicals in the sample extract and is measured as a reduction in the amount of emitted light. The measured light in the assays is then benchmarked against known reference compounds (potent agonists for agonism assays or potent antagonists for antagonism assays), which have eight serial dilutions that are measured in the assays for estimation of a full reference concentration-response curve. The bioactivities of the samples are benchmarked by interpolating a certain dilution of the sample extract onto the calibration curve of the reference compound. The chosen dilution point for agonism is the lowest sample extract concentration producing bioactivity above the LOQ, and for antagonism it is highest sample extract concentration producing bioactivity below 80% of the reference compound's maximal response (targeting the linear range of the reference curve). The final units of the bioactivities of the samples are interpreted as μg-equivalent/g-wristband (μg-eq/g) or ng-eq/g: the mass of reference compound that produces the same amount of bioactivity as a given mass of wristband sample. The reference compounds were deoxynivalenol for TR antagonism (Collet et al., 2019), flutamide for AR antagonism (Sonneveld et al., 2005), and 17β-estradiol for ER agonism (Sonneveld et al., 2005). More in-depth explanations and methods are provided in the Supplementary Material and in our previous study of these assays of indoor dust (Young et al., 2021b).

Before analysis, the wristband samples were extracted three times via a 15 min sonication in 10 mL of a 1:1 hexane:acetone mixture and then evaporated to complete dryness under nitrogen gas. Samples were then reconstituted in 25 μL of dimethyl sulfoxide (DMSO) and stored at −20°C in glass vials until analysis in 2021. Five serial dilutions (1x, 3x, 10x, 30x, and 100x) of each sample extract were then prepared in DMSO. The final prepared dilutions consisted of 0.1% DMSO. These sample extract dilutions were first evaluated for cytotoxicity of the U2OS cells by direct microscopic examination. Occasionally, dilutions which showed cytotoxicity were excluded from assessment to avoid the damage to cell

viability being misinterpreted as antagonism in the test system. Each non-cytotoxic dilution was analyzed three times in the hormone receptor assays.

The average method limits of quantification (LOQs) for our samples were 2.5 μg-eq/g-wristband in the assays of TR antagonism, 1.5 μg-eq/g-wristband for AR antagonism, and 0.013 ng-eq/g-wristband for ER agonism. For quality assurance and quality control, each plate had a reference compound series and solvent blanks. The sample extract dilutions, reference compound dilutions, and solvent blanks were all analyzed in triplicate. The LOQs for the assays of antagonism were calculated as the estimated concentration of the reference compound that produces 80% of its maximum response (in the presence of an added agonist), because only measured responses below that 80% point (in the linear range of the reference dose-response curve) were used for benchmarking the bioactivities of the samples. For the assays of agonism, the method LOQs were estimated as the average of the DMSO solvent blank plus 10 times the standard deviation of the triplicate solvent blank measurements. For samples that had no dilutions producing a response over the LOQ, the actual LOQ was corrected to represent the first dilution that was not cytotoxic, if any dilutions were cytotoxic. The 23 field blanks were mostly all below the LOQ. There were four field blanks above the LOQ for TR antagonism, one above the LOQ for AR antagonism, and one for ER agonism. The average field blank concentrations used for blank correction of sample values (conservatively assuming <LOQ as zero) were 0.65 μg-eq/g for TR antagonism, 0.083 μg-eq/g for AR antagonism, and 0.00065 ng-eq/g for ER agonism. Further information is provided in previous studies for CALUX assay procedures (Collet et al., 2019; Sonneveld et al., 2005; Young et al., 2021b).

2.6. Statistical Analyses

2.6.1. Data Preparation—Before analysis, concentrations of targeted chemicals, suspect chemicals, and hormonal activities in the wristband samples were blank corrected by subtracting average concentrations of field blanks. For descriptive statistics, visualizations, and multilevel regression models, the chemical and bioactivity concentrations were also scaled to a standard 32 h sampling time period (four 8 h workdays) based on the number of hours each participant wore their wristband, as detailed in our previous manuscript (Young et al., 2021a). However, the unscaled concentrations were used in the Bayesian kernel machine regression (BKMR) models when evaluating relationships just between the chemicals and bioactivities. Non-detect values of the chemicals and hormonal activities were substituted with half the method detection limit (Hornung and Reed, 1990) for that sample or sample batch.

Some of the suspect chemical features were tentatively identified to have the same CAS number albeit slightly different retention times and masses (51% of the frequently detected features in laboratory sample batch 1 and 48% in batch 2, whose suspect features could not be cross matched across batches). Some of these may be isomers of each other with the same formula but different arrangements (e.g., for phthalates). To avoid having multiple, highly colinear variables for potential duplicates or isomers, we condensed each set of tentative duplicates into selected principal components when the number of principal components that explained at least 70% of the cumulative variance was less than the number of duplicates,

based on principal components analysis (PCA). For example, seven frequently detected features tentatively identified as dibutyl phthalate (likely phthalate isomers) in sample batch 1 were summarized by three principal components that together explained most (76%) of the variability in concentrations of those seven features. When describing the functional uses of suspect chemical features, each set of condensed tentative duplicates only counted once.

The objectives of our main statistical analyses (n=243) were to assess the associations between chemical mixture exposures and hormonal activities and to identify important targeted and suspect chemical drivers of those bioactivities. We conducted separate models for each of the three hormonal activity outcomes. For all models, we natural log-transformed the hormonal activities because the outcome data were non-normally distributed. For preliminary evaluation of relationships between chemicals and hormonal activities, we used non-parametric Spearman correlation coefficients, for which significance was evaluated at α_{BH}=0.0192 for suspect features and α_{BH}=0.0190 for targeted chemicals following the Benjamini-Hochberg Procedure to decrease the false discovery rate when calculating hundreds of correlations (for both sample batches) (Benjamini and Hochberg, 1995). The boxplot visualizations in this paper plotted the median, first quartile hinge (25th percentile), and third quartile hinge (75th percentile), with whiskers extending from these hinges to data points that were at most 1.5 times the interquartile range away from the hinge; outliers beyond the whiskers were plotted as individual points. All analyses were conducted in R (version 4.1.2), and we used the *bkmr* package for the main models (Bobb et al., 2018).

2.6.2. Bayesian Kernel Machine Regression (BKMR)—Because of the high dimensionality and collinearity of the exposure data on many different chemicals, we used a novel statistical modeling method for complex chemical mixtures called Bayesian machine kernel regression. BKMR is a supervised variable selection approach that reduces the data while still using actual, interpretable exposure concentrations in the models (unlike e.g., principal component scores). BKMR models were advantageous over other supervised variable selection methods in our study because they: 1) evaluate cumulative mixture effects in addition to individual effects of the mixture components, 2) identify important bad actors in the chemical mixtures, 3) allow for non-linear and non-additive effects of the exposures on the outcomes (with no need to specify the exposure-response function *a priori*) and different directions of effects for different chemicals, and 4) investigate potential interactions among the chemicals.

First, we conducted BKMR models using the targeted chemical exposures. We evaluated the subset of 28 targeted chemicals that were detected in at least one half of samples in this analysis (Young et al., 2021a). We categorized these chemicals into six different groups based on their chemical classes.

Second, we conducted BKMR of the chemical features identified through suspect screening. These models had to be run separately for the two laboratory batches of samples (Batch 1: n=130; Batch 2: n=113) because the unknown features could not be cross-matched. The two sample batches were mostly random and both batches included samples from all countries (Batch 1: 61 in USA, 25 in UK, 13 in China, and 31 in India; Batch 2: 21 in USA, 12 in UK, 57 in China, and 23 in India). For inclusion in the models, we selected the features

detected in at least 50% of samples (using the slightly larger sample size that included the one or two wristbands by batch that were later excluded for lack of participant information on sampling duration). Among those, we then conducted principal components analysis to condense sets of potential duplicate or isomer features (same chemical formula) into fewer principal components, as described above in Section 2.6.1. This resulted in 587 included features for batch 1 and 600 included features for batch 2. We did not include the targeted chemical measurements in these models because those analytes can also be captured in suspect screening if abundant enough. Because the BKMR models could not include all the features at once (up to about 100 features for sample batch 1) due to sample size limitations, we randomly divided the selected features into subsets and conducted separate BKMR models on each subset. We used six subsets of up to 100 features each for sample batch 1 and seven subsets of up to 86 features each for sample batch 2 (which had a smaller sample size).

Before all the BKMR models, we first centered and scaled the exposure concentrations (which are thus interpreted in terms of standard deviations) and log-transformed the hormonal activities. Here, we used the chemical and bioactivity concentrations that were not adjusted by the amount of time the wristband was worn by each participant, to minimize any potential measurement error that might occur when only evaluating associations between measurement-based concentration variables. We adjusted for country indicator variables in all BKMR models. We also adjusted for squalene, one of the identified suspect features that is a component of natural sebum and thus could be a proxy for level of skin oil. Squalene could be a confounder given that the squalene levels (peak areas) were expected and shown to be different by gender (Figure S1; Wilcoxon $p<0.0001$), which is likely a predictor of exposure, and because more skin oil could dilute hormone-disrupting substances in the wristbands. Squalene was also significantly correlated with TR antagonism ($p<0.00001$) and ER agonism ($p=0.002$), whether directly or indirectly. Final BKMR models were implemented with at least 80,000 iterations, with the first half of iterations discarded. For the BKMR models of targeted chemicals, we used hierarchical variable selection based on the six pre-defined chemical groups, which accounted for highly correlated chemicals within certain chemical classes.

2.6.3. Multilevel Regression Models for Country and Gender—We implemented multilevel regression models ($n=209$) to evaluate associations of country and gender identity with the log-transformed, time-adjusted hormonal activities, controlled for age and amount of squalene in wristbands (skin oil indicator), and including random intercepts for shared office buildings. These models only had a sample size of $n=209$ due to missing reported demographic information. Because the model outcomes were log-transformed, we transformed the estimates into percent changes in the results. Statistical significance was evaluated at $\alpha=0.05$.

3. Results

3.1. Recap of Targeted Chemicals in Silicone Wristband Samples

As described in our earlier publication, we detected 94 of 99 measured targeted chemicals in the silicone wristband samplers worn by 243 office workers in this study (Young et al., 2021a). Most samples had detectable levels of at least one chemical within each chemical class (OPEs: 100% of samples; phthalates: 100%; PAHs: 98%; pesticides: 93%; BFRs: 91%; PCBs: 84%). There was a wide range in concentrations of chemicals included because the study population spanned four countries. Office worker exposures tended to be higher in the USA and UK for chemicals with flame retardant applications; higher in the USA for legacy banned PCBs; higher in India for pesticides; and higher in India and China for PAHs, phthalates, and a contemporary PCB (Young et al., 2021a).

3.2. Hormonal Activities in Silicone Wristband Samples

In the human cell assays, the chemical mixtures collected in every silicone wristband exposure sample were hormonally bioactive towards at least one of the three hormone receptors. Specifically, among the 243 samples, 241 (99%) antagonized thyroid hormone receptor activity, 233 (96%) antagonized androgen receptor activity, and 142 (58%) agonized estrogen receptor activity. The medians for the hormonal activities were 13.4 μg-eq/g for TR antagonism (range among bioactive samples: 1.2–220 μg-eq/g), 7.0 μg-eq/g for AR antagonism (bioactive range: 0.76–420 μg-eq/g), and 0.016 ng-eq/g for ER agonism (bioactive range: 0.0066–0.50 ng-eq/g). More summary statistics for the hormonal activities are provided in Table S2.

3.3. Differences in Hormonal Activities by Gender and Country

The female office workers tended to be exposed to chemical mixtures with higher interference with estrogen receptor and androgen receptor but lower interference with thyroid hormone receptor, independent of country. In multilevel regression models, female office workers were exposed to chemical mixtures with 110% higher androgen receptor antagonism (95% CI: 52–180%; $p<0.0001$) and 180% higher estrogen receptor agonism (95% CI: 120–260%; $p<0.0001$) compared to male office workers, adjusted for country, age, and squalene ($n=209$) (Table 1). The chemical mixture exposures of female office workers had 31% lower TR antagonism compared to male office workers (95% CI: −43–−16%; $p=0.0002$). The detection rates of ER agonism were also higher among women (83%) compared to men (30%), whereas their detection rates were similar for AR and TR (all ≥94%). The gender differences are evident in the boxplots, and the differences hold within each country (Figure 1; Figure S2). We also visualized the median hormonal activities by gender within each individual office building (Figure S3), which showed strong gender differences even among participants theoretically exposed to the same overall office building environment. We cannot rule out that unknown hormonally bioactive, endogenous substances in the wristbands (not picked up by suspect screening) may play a role in observed gender differences; however, our results were at least controlled for amount of squalene as a proxy for differences in sebum skin oil production.

Office workers in the USA tended to be exposed to chemical mixtures with higher estrogenic and higher anti-androgenic activities compared to the other three countries while those in the UK tended to experience lower estrogenic and lower anti-androgenic activities (Figure 2). The median hormonal activities in the USA, UK, China, and India were: 8.6, 4.2, 6.0, and 6.9 μg-eq/g, respectively, for AR antagonism; 0.026, 0.007, 0.014, 0.012 ng-eq/g for ER agonism; and 13, 13, 13, and 15 μg-eq/g for TR antagonism. In multilevel regression models adjusted for gender, age, and amount of squalene in wristbands (skin oil indicator), office workers in the USA were exposed to chemical mixtures with 88% higher ER agonism (p=0.002), 62% higher TR antagonism (p=0.036), and 68% higher AR antagonism (p=0.052) compared to the UK (Table 1). Office workers in India were similarly exposed to chemical mixtures with 74% higher ER agonism (p=0.010) and 71% higher TR antagonism (p=0.034) than in the UK. Office workers in China were exposed to slightly higher hormonal activities (TR: 40%, AR: 3%, ER: 5% higher) than in the UK, but these did not reach statistical significance (Table 1).

Because the multilevel models included random intercepts for the office building, we were able to find that most, but not all, of the variability in the log hormonal activities of the exposure samples were attributed to differences between individuals within buildings, not to differences between buildings. In fact, 77, 95, and 99% of the variability existed at the individual participant level for TR, AR, and ER interference, respectively, for the models presented in Table 1.

Levels of AR antagonism and ER agonism were significantly and positively correlated (Spearman r=0.63, p<0.0001), while TR antagonism was only negatively or non-significantly correlated with interference of ER (r=−0.26, p<0.0001) and AR (r=−0.07, p=0.26).

3.4. Mixture Effects of Targeted Chemicals

As visualized in Figure 3 for the BKMR models, the 243 silicone wristband samples showed strong, increasing estimated effects of the overall chemical mixtures on all three hormonal activity outcomes: thyroid hormone receptor antagonism, androgen receptor antagonism, and estrogen receptor agonism in human cells. The chemical mixtures in the model incorporated concurrent exposure to 28 different commonly detected, targeted chemicals. As described in detail in the following sections, six particular chemicals were the largest drivers of the mixture effects on hormonal activities: DiBP (a phthalate), TPHP (an OPE), PCB-11 (a PCB), DDE (a pesticide), DnBP (a phthalate), and TnBP (an OPE). Office workers in this study were commonly exposed to each of these chemicals, which had detection rates of 99, 98, 73, 61, 100%, and 54% respectively. Other chemicals may have made important contributions to the complex mixture effect even if their individual effects were not large.

3.4.1. Thyroid Hormone Receptor Antagonism—Among the 243 office worker samples, increasing levels of joint chemical exposure were strongly associated with higher antagonism of thyroid hormone receptor in CALUX human cell assays, adjusted for country and squalene (skin oil indicator) (Figure 3). For example, there was 25% higher antagonism

of TR activity when all the targeted chemical exposures in the mixture were fixed at the 75th percentile compared to the median 50th percentile (95% CI: 10–41%).

The pesticide DDE (a breakdown product of DDT) was the largest driver of the overall chemical mixture effect on TR antagonism (Figure 4). Office worker exposures to DDE were strongly associated with levels of TR antagonism by the silicone wristband sample extracts (Figure 4). An increase in office worker exposure to the pesticide DDE from its median 50th percentile to 75th percentile was associated with a 9% (95% CI: 2–16%) increase in TR antagonism by the chemical mixture, when all other chemicals were fixed at their median (50th percentile) exposure values and when adjusted for country and squalene.

PCB-11 was also a relatively important driver in the model (Figure 4; Table S3). Increasing levels of exposure to PCB-11 from its 50th to 75th percentile was associated with 6% higher antagonism of TR activity (95% CI: −5–17%). The organophosphate ester TnBP demonstrated a smaller magnitude of association with TR antagonism; an increase in TnBP exposure from the 50th to 75th percentile was associated with 4% higher TR antagonism (95% CI: −2–11%).

3.4.2. Androgen Receptor Antagonism—In the BKMR models, higher levels of joint exposure to the overall targeted chemical mixture were strongly associated with increasing levels of androgen receptor antagonism by the mixture, adjusted for country and squalene (Figure 3). There was a 52% increase in antagonism of AR (95% CI: 27–82%) associated with an increase in all the targeted chemical mixture components from their 50th percentiles (medians) to their 75th percentiles.

Phthalates, particularly DiBP and DnBP, were important drivers of the overall mixture effect on AR (Figure 4; Table S3). An increase in exposure to DiBP from its median 50th to 75th percentile was associated with 14% (95% CI: −0.1–30%) higher AR antagonism, holding all other chemicals fixed at their medians and adjusted for country and squalene (Figure 4). An increase in DnBP exposure from its 50th to 75th percentile was associated with a 7% (95% CI: −3–17%) increase in AR antagonism. There were significant correlations in wristband concentrations among the phthalates group: for example, DnBP and DiBP concentrations were highly correlated with each other (Spearman r=0.75; p<0.00001) and with bis (2-ethlyhexyl) phthalate (DEHP) (r=0.65–0.66; p<0.00001) and moderately correlated with diethyl phthalate (DEP) (r=0.41–0.56; p<0.00001).

PCB-11 and TPHP also had meaningful contributions to the mixture effect for AR antagonism (Figure 4; Table S3). An increase in exposure to PCB-11 from its 50th percentile to 75th percentile was associated with a 15% increase in antagonism of ARby the chemical mixture (95% CI: −2–36%), when all other chemicals were fixed at their median exposure values and when adjusted for country and squalene (Figure 4). An increase in exposure to TPHP from its 50th to 75th percentile was associated with 6% higher antagonism of AR (95% CI: −0.4–14%).

3.4.3. Estrogen Receptor Agonism—Increasing levels of exposure to the overall chemical mixture were strongly associated with higher levels of estrogen receptor agonism,

adjusted for country and squalene (Figure 3). The BKMR models found a 70% increase in agonism of ER by the targeted chemical mixture (95% CI: 46–99%) associated with an increase in concentrations of all the chemical mixture components from their 50th to 75th percentiles.

One phthalate in particular was an important driver of the chemical mixture effect on ER (Figure 4; Table S3). Among the office workers, increasing levels of exposure to DiBP from its 50th to 75th percentile was associated with a 38% increase in agonism of ER by the chemical mixture (95% CI: 20–59%), holding all other exposures fixed at their medians and adjusting for country and squalene (Figure 4). Exposure to an organophosphate ester, TPHP, was also strongly associated with agonism of ER (Figure 4). Increasing levels of exposure to TPHP from the 50th to 75th percentile was associated with 22% higher agonism of ER (95% CI: 11–34%).

The chemicals significantly correlated with hormonal activities in simple bivariate analyses did not always align with the chemicals with strong associations in the more robust BKMR models that account for chemical collinearities and allow for flexibility with non-linear and non-additive effects amongst the chemicals (See asterisks in Figure 4; Table S4). The exposure-response curves for meaningful chemicals are visualized in Figure S6, and the inclusion probabilities for each chemical in the models are provided in Table S3. There was no observed evidence of strong interactive effects within the chemical mixture for any of the three hormonal activity endpoints (Figure S4; Figure S5).

3.5. Mixture Effects of Suspect Chemical Features

3.5.1. Summary of Suspect Features—On average, an individual participant was exposed to 793 unknown chemicals features (or chemical “signatures”) collected in their wristband samples (median: 810; max: 1,010). In total, we observed 1,044 chemical features in laboratory sample batch 1 of the silicone wristband samples (n=130). Of the 1,044 total suspect features, 835 were frequently detected in the wristband samples (detection rate of at least 50%), and there were 587 unique features after condensing sets of duplicate/isomer features into fewer principal components. When available, tentative identifications for the 835 frequently detected, suspect features in batch 1 are presented in the Supplementary Material. We also observed 1,446 chemical features separately in laboratory batch 2 of wristband samples (n=113), but these could not be cross matched with the other random batch, so for brevity we have focused on the larger sample batch 1 in this paper and have included similar graphs for sample batch 2 in the Supplementary Material.

Fragrances were the most common primary functional use of the suspect chemical signatures (34% of those with known functional uses), followed by emollients (14%), masking agents (10%), and UV absorbers (7%), among the 168 commonly detected suspect chemicals that were assigned tentative identities with match scores of at least 95%, excluding duplicate identities (Table 2). Example tentative chemical identities for suspect features within each functional use category are presented in Table 2. A large portion (65% total) of the 168 features did not have known functional uses in the EPA database (Table 2) (K. A. Phillips et al., 2017).

The number of detected chemical features in the wristbands did not significantly differ by country (Kruskal-Wallis p=0.17) but did by gender (Wilcoxon p<0.0001). The female exposure samples had a median of 836 suspect chemical features detected while the male samples had 780 detected (using data from both sample batches). The significant difference by gender held (median 105 versus 97 detected features; p<0.0001) even among the subset of chemical features with known chemical functional uses in consumer products based on EPA's database (i.e., possibly excluding endogenous substances) and with high identity match scores (at least 95%) (K. A. Phillips et al., 2017).

Many of the common, suspect chemical features were significantly correlated with the three hormonal activity outcomes (Figure 5). For example, 137 features were significantly, positively, and at least moderately correlated with thyroid hormone receptor antagonism, 134 features with androgen receptor antagonism, and 159 features with estrogen receptor agonism (Figure 5). A much smaller number of features were significantly, *negatively*, and moderately correlated with interference with those hormone receptors (could have been agonists instead of antagonists, or vice versa). These preliminary correlations suggest that many suspect features may be important for the hormonal activities, but the following BKMR models account for non-linear relationships and the potential high collinearity between some features.

3.5.2. Cumulative Mixture Effects of Suspect Features—In BKMR models with 100 frequently detected, suspect chemical features, there were strong, positive cumulative effects of the overall chemical mixtures on all three hormonal activity outcomes in the silicone wristband samples (Figure 6). For example, there was a 30% increase in thyroid hormone receptor antagonism associated with an increase in all 100 randomly selected suspect chemical mixture components from their 50th percentiles to their 75th percentiles (95% CI: 8–56%) in sample batch 1 and random feature subset A (Figure 6). There was 45% higher antagonism of androgen receptor associated with an increase in all the chemical mixture components from their 50th to 75th percentiles (95% CI: 14–84%) in sample batch 1 and random feature subset A. Increasing exposure to all the mixture components from their 50th to 75th percentiles was associated with a 46% increase in estrogen receptor agonism in sample batch 1 and random feature subset A (95% CI: 12–91%). These overall mixture effects were very similar across most of the six rounds of BKMR models that had to be conducted separately on up to 100 randomly divided features at a time (Figure S9).

3.5.3. Suspect Chemical Features Driving Mixture Effects—There were many specific chemical features, with tentative identities, that were strongly or moderately associated with hormone receptor interference by the mixture in BKMR models, adjusted for country and squalene (Table 3). For example, increasing levels of exposure from the median 50th percentile to 75th percentile for octocrylene was associated with 8 or 4% higher antagonism of AR activity in sample batch 1 or sample batch 2, respectively, and with 1% higher agonism of ER activity in sample batch 2 (Table 3). Similarly, an increase in exposure from the 50th to 75th percentile for features representing octinoxate was associated with around 8% higher ER agonism (in both sample batch 1 and batch 2) and 9% higher AR antagonism in sample batch 2. In support of these tentative feature identities, participants

who reported any sunscreen use during the study period did have higher levels of the octocrylene and octinoxate features in their wristbands (see boxplots in Figure S10).

An increase in exposure from the 50th to 75th percentile for the feature α-isomethylionone and the feature diethyltoluamide (DEET) were associated with 11% higher and 3% higher AR antagonism, respectively (Table 3). The identification of DEET was able to be confirmed using an analytical standard. The feature octamethylcyclotetrasiloxane (also called D_4) was associated with 79% higher antagonism of AR and 48% higher agonism of ER, respectively. The positive association of the principal component representing dibutyl phthalate (or other phthalate isomer mixtures) with AR antagonism and ER agonism (Table 3) matched the associations found previously based on the targeted chemical analysis for dibutyl phthalate (Figure 4). Associations with hormonal activities for other chemical features are provided in Table 3. Any lack of strong associations between the hormonal activities and other features not shown in Table 3 does not necessarily mean they do not have effects or did not contribute to mixture effects.

4. Discussion

4.1. Effects of Chemical Mixtures on Hormone Receptors

This study advanced a first-of-its-kind method to investigate hormone receptor dysfunction by complex, real-world chemical mixtures. To do so, we sampled the exposures of a multi-national cohort of office workers by using silicone wristband samplers analyzed for known chemicals, unknown suspect chemical signatures, and *in vitro* hormonal activities. We found that the complex chemical mixtures exposing office workers were hormonally active towards estrogen, androgen, and thyroid hormone receptors. In fact, the chemical mixtures in all of the 243 wristband-based exposure samples blocked or mimicked testosterone, estrogen, or thyroid hormone receptor activation in human cells in the laboratory. Our results suggest that office workers' cumulative exposures to air, dust, and products are hormonally active, interfering with both sex hormones and thyroid hormones.

These hormone receptors regulate physiological processes in our bodies, so their interference can have critical consequences on reproductive, developmental, and metabolic health as well as certain hormone-related cancers. Estrogen receptor a regulates the female reproductive cycle; maintains breast, uterine, bone, cardiovascular, and fat tissue; contributes to breast cancer development; and for males, influences sperm morphology and concentration (Delfosse et al., 2015; Eve et al., 2020; Grimaldi et al., 2015; Hess, 2003; Liu et al., 2020). Androgen receptor regulates male embryo sexual differentiation, sperm production, and female ovarian follicle growth and ovulation (Grimaldi et al., 2015; Walters and Handelsman, 2018). Thyroid hormone receptor β is critical for normal development, growth, metabolism, and brain function (Grimaldi et al., 2015).

Many hormone-disrupting chemicals disrupt the same hormone receptor and can generate additive effects when found in complex chemical mixtures, even at low individual concentrations (Drakvik et al., 2020; Escher et al., 2020). The combined effects of cocktails of chemicals, including high proportions of unidentifiable chemicals, motivate the need to evaluate exposures to chemicals as the complex mixtures they are, instead of focusing on

one known chemical at a time. In our study, we measured nearly 100 known, targeted chemicals and over 1,000 chemical signatures via suspect screening in the wristband-based exposure samples. Our statistical models provided evidence of the strong mixture effects on the investigated hormonal signaling pathways from joint exposure to many known *and* unknown chemicals. We found that higher co-exposures of office workers to both known chemical mixtures and suspect chemical mixtures were strongly associated with considerably higher interference of all three estrogen, androgen, and thyroid hormone receptors in human cells. The strong association of suspect chemical features with the hormonal activities underpins the importance of novel laboratory methods that explore unidentified chemicals, as traditional targeted laboratory analyses may miss many bioactive chemicals that are less well-established in research.

4.2. Targeted Chemicals Driving Mixture Effects

We found that several individual chemicals were strong drivers of the effects of the exposure mixtures on the hormone receptor function in human cells. While we evaluated chemical effects on three hormone receptors in this study, it is important to note that these mixtures could also contribute to other hormone-disrupting mechanisms, such as through interference with hormone transporters, membrane-associated receptors, receptor degradation, hormone metabolism, hormone clearance, DNA methylation, and coactivator expression, among others (Balaguer et al., 2017; Tabb and Blumberg, 2006).

In the statistical models of targeted chemical mixtures, the phthalate DiBP and the organophosphate ester TPHP were strong drivers of the mixture effects on estrogen receptor agonism in the human cell tests. DiBP, TPHP, the phthalate DnBP, and PCB-11 were important drivers of mixture effects on androgen receptor antagonism, while the pesticide DDE, PCB-11, and TnBP were important for thyroid hormone receptor antagonism. There is previously published evidence of disruption by these chemicals of both hormone receptors *in vitro* and actual reproductive health outcomes *in vivo* for humans or animals, as we describe below. Even the chemicals that were not strongly associated in our BKMR models may have still played supporting roles in the overall mixture effects.

DiBP and DnBP are short-chain phthalates widely used as plasticizers in personal care products, paints, adhesives, toys, and other products (Y. Wang et al., 2019; Wittassek et al., 2011). These two phthalates were ubiquitously detected in at least 99% of the wristband samples in our study. Although DiBP was classified as inactive in the EPA CompTox Chemistry Dashboard towards ER agonism (human ovary cell line) and AR antagonism (human breast cell line) and DnBP as inactive towards AR antagonism (Williams et al., 2017), other studies did find activity in human or hamster luciferase-based assays for DiBP and DnBP (Engel et al., 2017; Takeuchi et al., 2005). The CompTox results are not necessarily contradictory with our study, as any inactivity of an individual chemical when tested in isolation may not predict the activity of the chemical in complex, interactive mixtures as a whole (Kienzler et al., 2016; Orton et al., 2014); in addition, any one particular phthalate could be acting as a proxy for total phthalate exposure. Recent review studies found moderate to robust evidence of associations of DnBP exposure in humans with semen quality, time to pregnancy, and anogenital distance (reproductive development) for

male exposure (Radke et al., 2018) and with preterm birth for female exposure (Radke et al., 2019). DiBP is structurally similar to DnBP but with less research; DiBP exposure has been associated with adverse effects in animals on male reproductive development, testosterone production, sperm histology, anogenital distance, and testicular function, and with anogenital distance in human male children (Sedha et al., 2021; Yost et al., 2019).

TPHP is an organophosphate ester commonly used as a plasticizer or flame retardant in many building materials and consumer products, including furniture, textiles, plastics, electronics, and nail polish (Mendelsohn et al., 2016; Saillenfait et al., 2018; Young et al., 2018). Several studies support our finding that TPHP is an important driver of mixture effects on AR antagonism and ER agonism. In the CompTox Dashboard and several other *in vitro* studies, TPHP was identified as an AR antagonist and ER agonist in isolation (Bajard et al., 2021; Kojima et al., 2013; Suzuki et al., 2013; Williams et al., 2017). In addition, previous research has found suggestive evidence of adverse *in vivo* effects of TPHP exposure on semen quality in rodents and humans (Bajard et al., 2021; Meeker et al., 2013; Meeker and Stapleton, 2010) and on pregnancy outcomes and behavioral development in humans (Carignan et al., 2017; Doherty et al., 2019b). TnBP is another organophosphate ester, which had a suggested association with TR antagonism, and which can serve as a plasticizer or flame retardant in building materials such as plastics, lacquers, sealants, paints, wallpaper, and concrete (Wang et al., 2017; Wei et al., 2015). The office workers in the USA, followed by UK, had much higher detection rates and concentrations of TnBP in wristbands compared to China and India, which may be indicative of the historically stringent flammability codes in the USA and UK (Young et al., 2021a). Although TnBP was classified as inactive towards TR when tested in isolation based on the CompTox data (Williams et al., 2017), a different study did observe TR antagonism by TnBP (in a hamster luciferase-based assay) (Zhang et al., 2016).

DDE is a breakdown product of the pesticide DDT, which is still used in India for malaria control but was banned by 1983 in the USA, UK, and for agricultural use in China (Qiu et al., 2004; van den Berg et al., 2017; Wong et al., 2005). Unsurprisingly, office workers in India in our study population were exposed to much higher levels and detections of DDE than in the other three countries (Young et al., 2021a). Similar to the strong association we found between DDE and thyroid hormone receptor antagonism, CompTox and another study also found TR antagonism for DDE when tested in isolation (in rat or whale cell lines) (Lühmann et al., 2020; Williams et al., 2017).

Although traditional PCBs were banned or phased out decades ago (Conolly et al., 2009; EPA, 1979; The People's Republic of China, 2007), PCB-11 is a contemporary PCB congener that is not covered under bans due to its presence as an *unintentional* byproduct in certain wall paints and other pigmented materials such as textiles and product packaging (Anezaki et al., 2014; Guo et al., 2014; Hu et al., 2008; Rodenburg et al., 2010; Shang et al., 2014; Vorkamp, 2016). PCB-11 has increasingly become a concern in buildings (Hu and Hornbuckle, 2010; Rodenburg et al., 2010; Young et al., 2021a) and has widely exposed 73% of office workers in this study. The health effects of PCB-11 have not been well-established, but two studies did find AR antagonism of PCB-11 in human and hamster luciferase cell assays (Pěnčíková et al., 2018; Takeuchi et al., 2017).

4.3. Suspect Chemicals Driving Mixture Effects

Novel suspect chemical signatures in the wristband-based exposure samples were abundant. About 587 unique signatures were frequently detected in at least half of wristband samples, and many were classified to function as fragrance ingredients based on their tentative identifications. Exposures to the complex mixtures of suspect chemical features were linked with strong, increasing effects on interference with estrogen, androgen, and thyroid hormone receptors in human cells. About 39 unique chemical features were highlighted as strong drivers of the mixture effects on at least one of the three hormonal activities in our statistical models, which could include up to about 100 suspect features at a time in the model. Many more chemical features may have still played a role in the hormonal activities even if not statistically identified in the models, given that over 130 features were significantly correlated with each bioactivity.

The important suspect chemical features included two tentatively identified as octocrylene and octinoxate, which were associated with higher estrogen receptor agonism and higher androgen receptor antagonism by the exposure samples. Based on EPA's Chemical and Products Database, octocrylene and octinoxate are both used in sunscreen, lip balm, and insect repellent; octocrylene is also used in surface sealers, while octinoxate is additionally used in air freshener, fragrance, lip color, and self-tanner.(Dionisio et al., 2018) Octocrylene was confirmed to be an AR antagonist when tested on its own in the CompTox assays, although it was inactive towards ER and octinoxate was classified as inactive towards both (Williams et al., 2017). Another study found some *in vitro* ER agonistic activity by both octocrylene and octinoxate in human cells, but no detectable AR antagonism (Lee et al., 2022). Research on the health effects of these two sunscreen-related chemicals is scarce, but rodent studies found associations of octinoxate dose with lower prostate weight, lower sperm count, and higher testosterone levels among male rats and with uterine endometrium thickening among female rats (Axelstad et al., 2011; Kwon and Choi, 2021; Seidlová-Wuttke et al., 2006). A human epidemiologic study found that higher urinary levels of octinoxate were significantly associated with lower testicular volume, reduced anogenital development, and later pubertal onset of pubic hair among students (Huang et al., 2020), while another study found that urinary levels of octocrylene were associated with polycystic ovary syndrome (PCOS) among overweight or obese women (Gu et al., 2019; Kwon and Choi, 2021). One caveat with our results is that we cannot exclude the possibility that these suspect chemical features may be highly correlated with other chemicals that are latently contributing to the effects and that we could not detect; regardless, octinoxate and octocrylene would act as proxies for the mixture formulations of their sunscreen or other products.

The suspect feature tentatively identified as DnBP (although this feature could represent other structurally similar phthalates too) was associated with higher estrogen receptor agonism and androgen receptor antagonism by the exposure samples. DnBP concentrations in the wristbands based on our targeted analysis were also slightly positively associated with those endpoints, and as confirmation, the CompTox screening of DnBP in isolation found agonistic activity towards ER (Williams et al., 2017). DnBP was classified as inactive in the CompTox screening for AR antagonism (Williams et al., 2017), but other studies found

antagonistic activity for DnBP in human or hamster cells (Engel et al., 2017; Takeuchi et al., 2005).

There were a few other suspect features of interest that showed positive associations with androgen receptor antagonism or estrogen receptor agonism by the exposure samples. DEET is a widely used insecticide that is designed to be applied directly to human skin for repelling mosquitos and ticks and preventing vector-borne illness (Dionisio et al., 2018; EPA, 2022). α-Isomethylionone is known to be used in fragrance, air freshener, deodorant, and lip color, based on the EPA Chemical and Products Database (Dionisio et al., 2018). The chemical D_4 has many different functions (K. A. Phillips et al., 2017) and can be found in personal care products (e.g., hair styling, hair coloring, deodorant, shaving cream, and eye products), caulk/sealant, construction & building materials, and auto vehicle detailing work (Dionisio et al., 2018). All three of these chemical features were classified as inactive when tested in isolation for those hormonal endpoints in CompTox screening (Williams et al., 2017), although D_4 had detectable estrogenic activity in other *in vitro* research (He et al., 2003; Quinn et al., 2007). It is possible that we may have been able to better observe effects in cumulative chemical mixtures or that the chemicals are correlated with other exposures that influence hormonal activity. Overall, our results suggest that many emerging chemicals work together in complex mixtures to contribute to interference with hormone receptors.

4.4. Differences in Hormonally Bioactive Exposures by Gender and Country

We found noticeably higher estrogenic and anti-androgenic activities of exposures for female office workers than male office workers, even adjusted for country, age, and level of skin oil excretion onto wristbands. These exposure differences by gender were evident even among male and female participants working in the same office building. Wristbands collected by female participants also had significantly more suspect chemical features detected than for male participants (difference in medians was 56 features). The different use of personal care products and cosmetics could partly contribute to the more hormonally bioactive exposures of female office workers. For example, in latent class analysis based on methods from our previous paper (Young et al., 2021a), only about 27% of male participants with reported information were classified as moderate to heavy users of personal care products (such as perfume/cologne) compared to 67% of female participants. This is not surprising given that women routinely use more than 10 different personal care products on average (Preston et al., 2021) and that hormone-disrupting phthalates and other chemicals are commonly used in perfume, makeup, shampoo, nail polish, and other products (Y. Wang et al., 2019; Wittassek et al., 2011). However, our findings cannot fully exclude the possibility that the wristbands may also be collecting other hormonal, endogenous substances on the skin that may differ by gender. There was not a clear reason for the higher TR antagonism activities observed for male office workers.

Furthermore, there were some differences by country in the levels of hormonal activities of the office worker exposures. Workers in the UK tended to be exposed to less hormonally bioactive exposures. Although many environmental, regulatory, and cultural factors could contribute to this difference, one potential reason is that the UK had tighter restrictions of certain phthalates (e.g., in cosmetics) (Sackmann et al., 2018; Wittassek et al., 2011) and

more conservative regulations of pesticides (Donley, 2019) compared to the other countries. Compared to the UK (and often USA), office workers in China and India did tend to have higher exposures to several of the chemicals that were identified in statistical models to be important drivers of the hormonal activity mixture effects, including DiBP, DnBP, and DDE, which were hypothesized in our previous paper to result at least partly from differences in chemical restrictions (Young et al., 2021a).

Interestingly, our multilevel regression models showed that there was more variability in hormonal activities of exposures between individuals working within the same office building than variability between different office buildings, even with the silicone wristband samplers only being worn during work hours. Within-building variability also dominated in the multilevel models for many specific targeted chemicals in the same wristband samples based on our previous publication (Young et al., 2021a). This result emphasizes the diversity of microenvironments *within* a building that could occur for several reasons. Furnishings, electronics, dust levels, and occupancy could all vary across different rooms within a building. An individual's level of movement and resulting "activity cloud" of contaminants surrounding them could influence exposure intensity (Rodes et al., 1991). In addition, use of personal care products and cosmetics varies from individual to individual and could influence the unique personal exposure of each participant within the building. An individual's prior exposure activity or personal care product usage from a different environment could also potentially carry over into that building. As an example, clothes can serve as reservoirs (and sources) of chemicals and thus lead to prolonged exposure, as has been shown for at least phthalates and PCBs (Licina et al., 2019; Morrison et al., 2018). This result demonstrates that we carry our cumulative exposures with us and that silicone wristbands may partly capture exposures across multiple environments (to a certain extent) even when intended to be worn only in one environment.

4.5. Comparison to Previous Studies

There has only been one previously published study that conducted cell assays of chemical mixtures collected in silicone wristband samples of exposure. Among 72 adults in North Carolina (USA) participating in a thyroid cancer cohort, about 70% of the wristband-based exposure samples showed significant antagonism towards TR (at 1% extract concentration); concentrations of several phthalates, organophosphate esters, and brominated flame retardants were positively associated with the levels of antagonism (Kassotis et al., 2020). Although the raw bioactivities could not be compared between our two studies, the frequent detection of TR antagonism with linkages to chemicals used in building materials and consumer products aligns with the results of our study. We also previously published a study conducting our same three hormone receptor cell assays on 46 samples of indoor dust from university buildings in the USA (Young et al., 2021b). We similarly found very high detection rates for antagonism of AR (87% in dust; 96% in wristbands) and TR (89% in dust; 99% in wristbands), but lower detection rates for ER agonism (96% in dust; 58% in wristbands). Dust is a major medium in the indoor built environment through which semi-volatile organic chemicals from materials accumulate and expose occupants (Mitro et al., 2016). The previous study of hormonally bioactive dust in buildings supports the current study's findings of hormonal activities in silicone wristbands,

which collected chemicals from dust, air, and products while worn by participants in office buildings.

4.6. Novelties, Strengths, and Limitations of Methods

This study has advanced an innovative method to assess the hormone-disrupting implications of our exposures to complex chemical mixtures in buildings using novel silicone wristband samplers. We evaluate interference of chemical mixture exposures with three different hormone receptors in human cells in a relatively large, four-country study, and we provide the first report of estrogenic and anti-androgenic activities in wristband-based exposure samples. These CALUX human cell-based assays of wristbands provided a relatively rapid and inexpensive indicator of the proximate 'hormonal health' implications of chemical exposures inside buildings without the challenges in some human epidemiological studies of linking one-time chemical exposures to binary health outcomes that can take years to diagnose and that depend on other highly variable risk factors or individual physiology. As another advantage in this study, the silicone wristband personal passive samplers were a useful technique to sample the external exposures of office workers. Silicone wristbands are simple and non-invasive, isolate external environmental exposures, do not have to be frozen immediately, and can easily be split into sections for multiple different laboratory analyses. We also analyzed the wristband samples for about 100 different targeted chemicals in addition to over 1,000 chemical features identified by suspect screening to understand the complexity of mixtures of both known and unknown chemicals that participants were exposed to and to tentatively identify important, emerging suspect chemicals. The targeted laboratory analyses paired with the novel, suspect screening analyses allowed us to comprehensively evaluate chemical mixture exposures and their joint effects on hormonal activities. As an additional advantage, the suspect screening analyses helped us identify endogenous substances present in the wristbands, such as squalene, that might need to be considered or controlled for in statistical models. Another strength of this study was the use of novel BKMR models to account for the large number of potentially correlated chemicals, to investigate cumulative mixture effects, and to identify 'bad actor' chemicals.

There were several limitations with this study. First, the suspect chemical screening analysis favors abundant chemicals, so it was less likely to detect low-concentration chemical classes such as PCBs and BFRs; however, we did measure many of these chemicals in our targeted analysis. Second, although we conducted a relatively large number of hormone receptor activation assays, there are many other hormone-disrupting mechanisms through which chemicals can act (Balaguer et al., 2017; Tabb and Blumberg, 2006). Third, future studies that assess cell-based assays of wristbands might consider techniques to exclude endogenous hormones and their metabolites present in skin from the wristband samplers, or at least to measure them, and to ask participants details about medications and oral contraceptive use. Even with the possible presence of potentially hormonally bioactive endogenous substances, we still observed statistically strong mixture effects of the chemical mixtures on the hormonal activity outcomes. Fourth, we statistically evaluated associations between exposures to specific chemicals and the hormonal activities but did not exactly measure an individual chemical's toxicity on its own; however, conducting assays on chemical mixtures better reflects the real-world complexity and potential synergistic

effects between chemicals. Fifth, we could not interpret the raw concentrations of hormonal activities in the wristband extracts because there were no comparative wristband studies and no translations to human internal dose or health outcomes. Finally, the cell-based hormone receptor assays of wristband-based exposure samples do not measure actual human biomarkers or health outcomes and do not necessarily portray the exact chemical mixtures that would wind up inside the human body (depending on an individual's physiology) or how much hormone disruption would occur in the body. However, the one previous study of TR antagonism by wristband extracts did find trends with elevated odds of papillary thyroid cancer (Kassotis et al., 2020). In addition, studies have shown that *in vitro* cell assays of individual hormone-disrupting chemicals do reflect known *in vivo* health outcomes (La Merrill et al., 2020; Rotroff et al., 2013; Schenk et al., 2010). Although the hormonal activities of wristband-based exposure samples cannot yet be directly translated into human health outcomes, our study provides valuable data on how buildings and products influence our exposures to complex cocktails of hundreds of both known and unknown chemicals and how those chemical mixtures have strong hormone-mimicking activities. This study used several novel methods to quantify the immediate hormone receptor-interfering effects of chemical mixtures that office workers are personally exposed to indoors.

5. Conclusions

Our study provides the first report of the *in vitro* hormonal activities of personal chemical exposures collected via silicone wristbands towards estrogen, androgen, and thyroid hormone receptors. We found that every one of the 243 office workers, who wore silicone wristband samplers in office buildings across the USA, UK, China, and India, were exposed to hormonally bioactive chemical mixtures that mimicked or blocked sex hormones or thyroid hormone in human cells in the laboratory. Our analyses of nearly 100 targeted chemicals and over 1,000 chemical signatures identified via suspect screening in the wristband samples showed that the chemical mixtures that exposed the workers were highly complex and included a large fraction of novel or usually unknown chemicals that are often overlooked. In our statistical modeling, these mixtures of both known and suspect chemicals demonstrated strong effects on the hormonal activities of the wristband-based exposure samples and highlight the fact that traditional targeted laboratory analyses likely miss many hormonally bioactive chemicals. Numerous individual chemicals were identified as being important co-drivers of the mixture effects on hormonal activities, including some chemicals with expected bioactivity, some chemicals that were not expected, and some chemicals that have very little health information. Plasticizers, fragrance chemicals, pesticides, and PCBs were among the chemicals we determined to play important roles in the mixture effects. In addition, we identified potential gender disparities in the hormonal activities of chemical exposures that may be related to cosmetic use; samples from female office workers showed higher chemical complexity, estrogenicity, and anti-androgenicity. Overall, this study highlighted that building microenvironments, and the materials and personal care products used within them, are important sources of our hormone-disrupting exposures. This study also advanced several novel methods, including the use of silicone wristbands for sampling personal external exposures, the laboratory analysis of both targeted chemicals and suspect chemical features, the *in vitro* hormone receptor cell assays of silicone wristbands,

and the novel BKMR statistical models of dozens of chemicals. These techniques allowed us to quantitatively measure the proximate 'hormonal health' implications of entire chemical co-exposures as the complex mixtures they are in real buildings for real people.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Office workers wore silicone wristbands to collect exposures to chemical mixtures
- We observed 1,044 chemical signatures in wristband samples via suspect screening
- Every wristband extract was hormonally active in human hormone receptor cell assays
- The chemical mixtures disrupted estrogen, androgen, and thyroid hormone receptors
- Exposures were influenced by personal care products, buildings, gender disparities

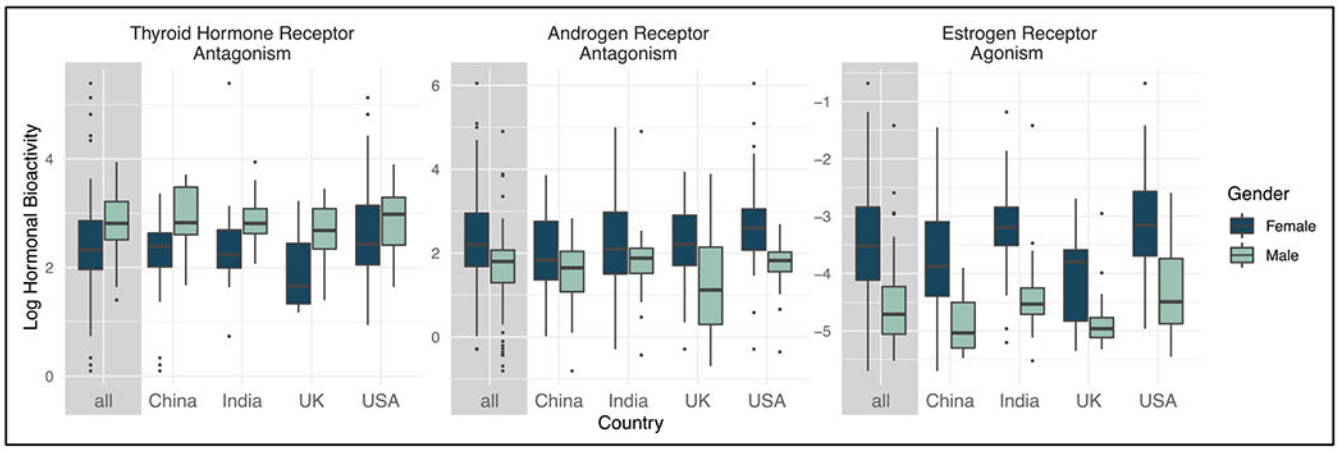

Figure 1.

Log-transformed hormonal activities of 209 silicone wristband samples towards thyroid hormone, androgen, and estrogen receptors in CALUX human cell bioassays by country and reported gender.

Note: Outcomes units are in μg-eq/g for thyroid hormone and androgen receptor interferences and in ng-eq/g for estrogen receptor interference. Concentrations were scaled to 32 hours of sampling based on the amount of time each wristband was worn. Boxplot whiskers extend to points within 1.5 times the interquartile range from the lower or upper hinge (first or third quartile, respectively).

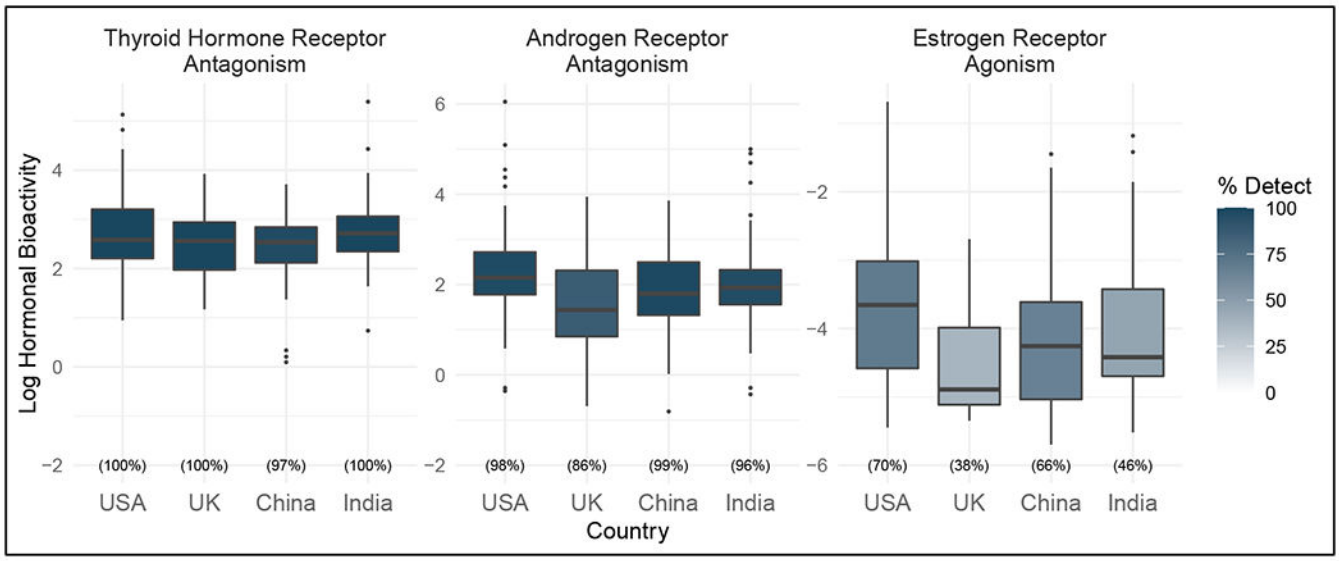

Figure 2.

Log-transformed hormonal activities of 243 silicone wristband samples towards androgen, estrogen, and thyroid hormone receptors in CALUX human cell bioassays by country and shaded by the detection rate.

Note: Outcomes units are in μg-eq/g for thyroid hormone and androgen receptor interferences and in ng-eq/g for estrogen receptor interference. Concentrations were scaled to 32 hours of sampling based on the amount of time each wristband was worn. Boxplot whiskers extend to points within 1.5 times the interquartile range from the lower or upper hinge (first or third quartile, respectively).

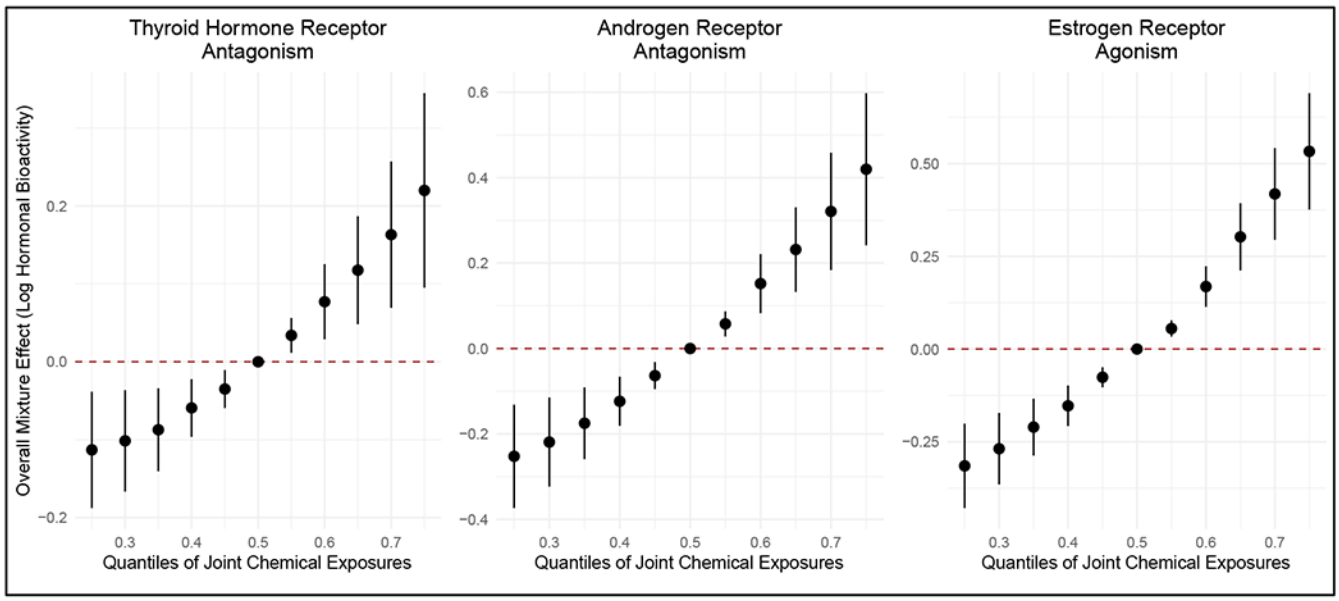

Figure 3.

Overall effect (with 95% confidence intervals) of entire targeted chemical mixture on three log hormonal activity outcomes when all exposures are fixed at various quantiles (0.25–0.75) as compared to when all exposures are fixed at their medians, in Bayesian kernel machine regression (BKMR) models based on 243 silicone wristband samples worn by office workers.

Note: Models included 28 targeted chemicals detected in at least half of samples. Models are adjusted for country and squalene (sebum indicator). Outcomes units are in μg-eq/g for thyroid hormone and androgen receptor interferences and in ng-eq/g for estrogen receptor interference.

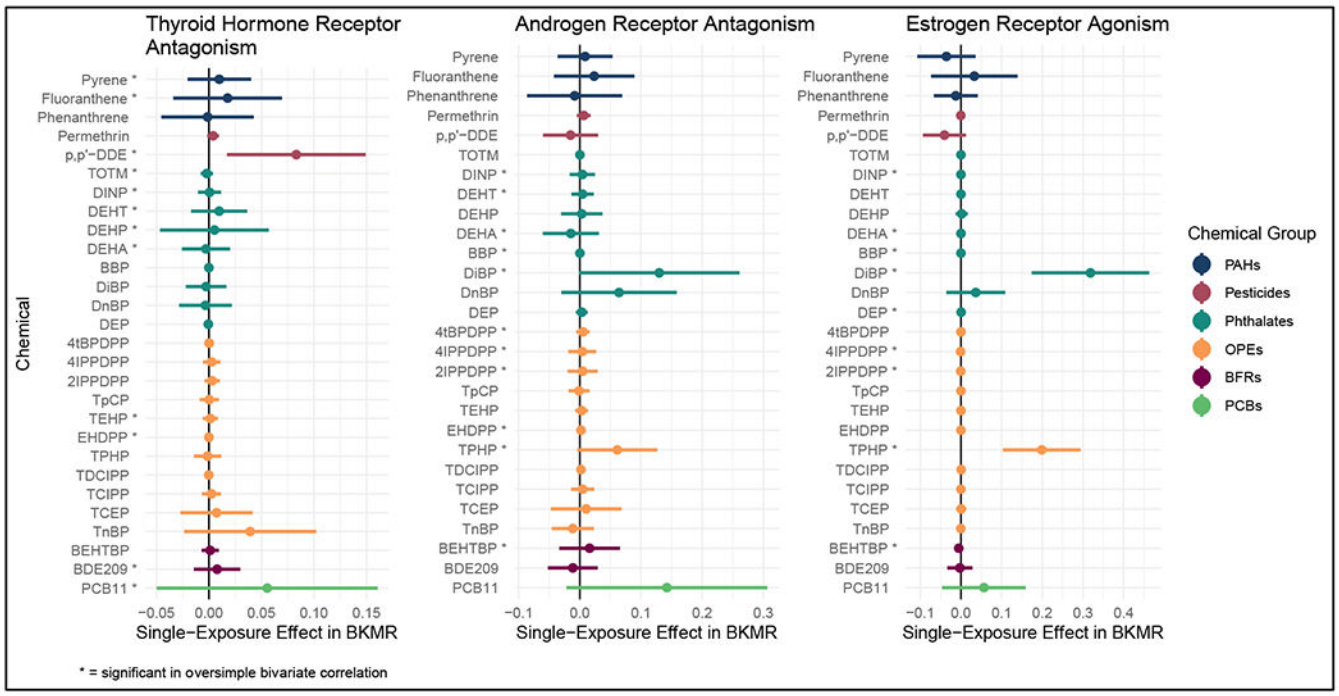

Figure 4.

Single-exposure effects (with 95% confidence intervals) of certain individual chemical mixture components on three log hormonal activity outcomes when all other chemical exposures are fixed at their median value, based on Bayesian kernel machine regression (BKMR) models of 243 silicone wristband samples worn by office workers. The effects are defined as the difference in the log hormonal activity associated with a change in the individual targeted chemical from its 50th percentile (median) to 75th percentile, holding other exposures constant at their medians.

Note: Models are adjusted for country and squalene (sebum indicator). The 28 chemicals included in the model were detected in at least half of wristband samples. As a comparison point, asterisks indicate significance in highly simple, bivariate Spearman correlation tests with outcome, where the significance level is defined as α=0.019 based on the Benjamini-Hochberg procedure. Outcomes units are in μg-eq/g for thyroid hormone and androgen receptor interferences and in ng-eq/g for estrogen receptor interference.

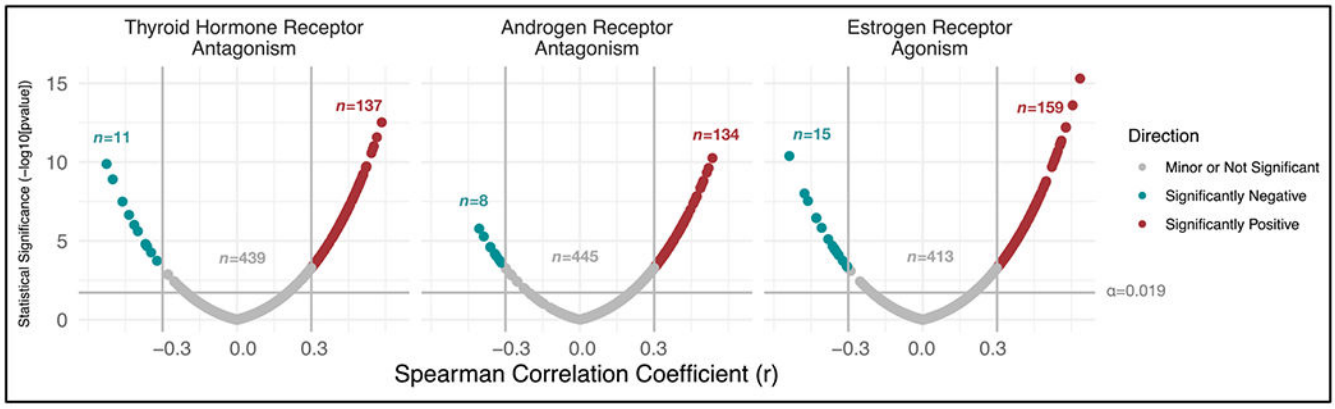

Figure 5.

Volcano plot of Spearman correlations and their statistical significances between each meaningful suspect chemical feature and each hormonal activity outcome in laboratory sample batch 1 of silicone wristband samples (n=130).

Note: meaningful suspect features were ones detected in at least 50% of samples, and any structural duplicates/isomers among the features were condensed into key principal components. Statistical significance was evaluated at α=0.019 based on the Benjamini-Hochberg procedure to reduce the false discovery rate. Volcano plots for sample batch 2 are shown in Figure S8.

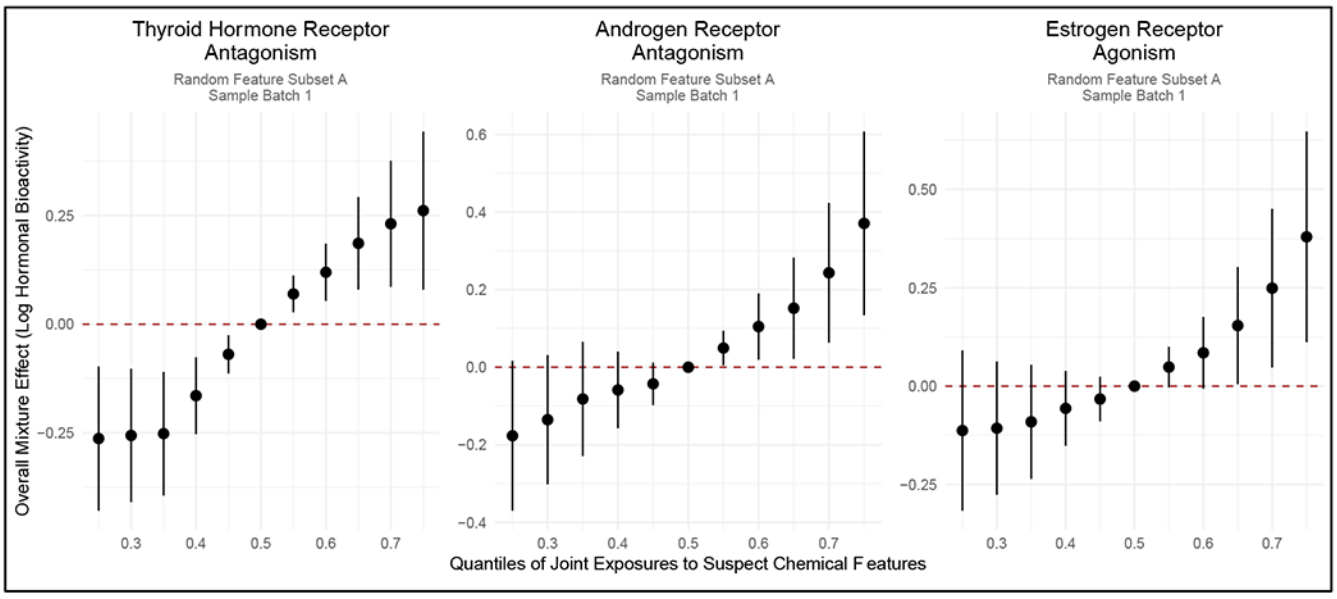

Figure 6.

Overall effect (with 95% confidence intervals) of entire mixture of 100 important suspect chemical features on three log hormonal activity outcomes when all exposures are fixed at various quantiles (0.25–0.75) as compared to when all exposures are fixed at their medians, adjusted for country and squalene, in Bayesian kernel machine regression (BKMR) models based on 130 silicone wristband samples worn by office workers.

Note: this graph shows the results for sample batch 1 (n=130) in the first of six rounds of BKMR models (models could only evaluate up to about 100 of the important features at a time, which were randomly divided); the results for every round are presented in Figure S9. Outcomes units are in μg-eq/g for thyroid hormone and androgen receptor interferences and in ng-eq/g for estrogen receptor interference.

Table 1.

Multilevel regression models of associations of country and gender with in vitro interference of three hormone receptors (in CALUX bioassays) in silicone wristband samplers worn by office workers, adjusted for amount of squalene in wristbands as a proxy for sebum.

	Thyroid Hormone Receptor Antagonism (μg-eq/g)		Androgen Receptor Antagonism (μg-eq/g)		Estrogen Receptor Agonism (ng-eq/g)	
Variable	**% Change [95% CI]**	***p***	**% Change [95% CI]**	***p***	**% Change [95% CI]**	***p***
Gender Identity (Ref: Male)						
Female	−31% [−42.9%, −16.2%] ***	0.0002	105% [51.5%, 176%] ***	<0.0001	181% [120%, 260%] ***	<0.0001
Country (Ref: UK)						
USA	62.1% [7.19%, 145%] *	0.04	68.1% [3.25%, 174%] .	0.05	88.3% [30%, 173%] **	0.002
China	39.8% [−12.1%, 122%]	0.19	3.2% [−38.3%, 72.8%]	0.91	4.67% [−28.3%, 52.9%]	0.82
India	70.8% [7.74%, 169%] *	0.03	52.2% [−10.2%, 158%]	0.14	74.4% [17.1%, 160%] *	0.01
Age (Continuous)	0.30% [−0.91%, 1.53%]	0.63	−0.22% [−2.23%, 1.65%]	0.82	−0.17% [−1.73%, 1.4%]	0.83
Squalene: Peak Area (Continuous)	0.017% [0.011%, 0.023%] ***	<0.0001	0.002% [−0.008%, 0.012%]	0.75	−0.005% [−0.013%, 0.003%]	0.22

Note: Hormonal activity outcomes were log-transformed for modeling, so model estimates were transformed to percent changes. The model used a sample size of n=209 based on the number of office workers who reported their age and gender.

Table 2.

Summary of main functional use categories reported for unique, commonly detected suspect chemical features with high identity match scores in laboratory sample batch 1 of 130 silicone wristband samples from this study, based on the Functional Use Database (FUse) of the U.S. Environmental Protection Agency. (K. A. Phillips et al., 2017)

Category of Main Functional Use	*n* (%)	Select examples of tentative identities of suspect features
Fragrance	20 (34%)	Dibutyl phthalate (DnBP); bis(2-ethylhexyl) phthalate (DEHP); α-terpineok 1-hexadecanol; oleic acid; α-hexylcinnamaldehyde
Emollient	8 (14%)	Tris(2-ethylhexyl) trimellitate (TOTM); octamethylcyclotetrasiloxane (D_4); methyl stearate; decyl palmitate; octadecyl benzoate
Masking Agent	6 (10%)	Bis(2-ethylhexyl) adipate (DEHA); α-isomethylionone; butyl benzoate; α-bisabolol; ethylene brassylate; methyl dihydrojasmonate
UV Absorber	4 (6.9%)	Octocrylene; octinoxate; benzophenone; 2-(2H-benzotriazol-2-yl)-4,6-ditertpentylphenol (UV 328)
Ubiquitous	3 (5.2%)	Diethyl phthalate (DEP); isopropyl palmitate; isopropyl myristate
Flame Retardant	3 (5.2%)	Triphenyl phosphate (TPHP); tris(1-chloro-isopropyl phosphate (TCIPP); tris(l,3-dichloro-isopropyl) phosphate (TDCIPP)
Plasticizer	3 (5.2%)	Benzyl butyl phthalate (BBzP); dicyclohexyl phthalate; isodecyl diphenyl phosphate (IDPP)
Colorant	2 (3.4%)	Pyrene; phenol
Hair Conditioner	1 (1.7%)	Squalene
Skin Protectant	1 (1.7%)	Diethyltoluamide (DEET)
Catalyst	1 (1.7%)	Methyl benzoylformate
Emulsion Stabilizer	1 (1.7%)	Cholesterol
Perfumer	1 (1.7%)	Ethanone, l-(2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl)-
Film Forming Agent	1 (1.7%)	Butyl citrate
Antimicrobial	1 (1.7%)	Chloroxylenol
Flavorant	1 (1.7%)	3,3,6-Trimethylhepta-1,5-dien-4-one
Viscosity Controlling Agent	1 (1.7%)	Hexyl dodecanoate
Unknown	110	

Note: We included 168 suspect features that were detected in at least 50% of samples in batch 1, that had tentative identities with match scores of at least 95%, and that were not duplicated identities. Suspect features in the two sample batches could not be cross-matched. The list of main and any reported functional use categories for features in both sample batches 1 and 2 are presented in Table S5.

Table 3.

Single-exposure effects of key suspect chemical features on three log hormonal activity outcomes, based on Bayesian kernel machine regression (BKMR) models conducted separately by sample batch and by random subset of up to 100 frequently detected features at a time.

Suspected Feature	Tentative Chemial Identity	Identity Match Score (%)	Reported Functional Uses in EPA Databases	Random Model Subset	% Change [95% CI]		
					Thyroid Hormone Receptor Antagonism	Androgen Receptor Antagonism	Estrogen Receptor Agonism
Sample Batch 1 (n=130)							
B1_223	*Not certain: match score < 90%*	71	*Not certain: match score < 90%*	B1-B			16% [−0.97%, 35%]
B1_261	Norbornane	95	*Unknown*	B1-A	−1.6% [−3.4%, 0.27%]		
B1_309	Methyl stearate	95	Emollient, skin conditioner, fragrance, flavorant	B1-D	−11% [−20%, −1.3%]		
B1_353	1-Tetracosanol	97	*Unknown*	B1-F			18% [1.8%, 36%]
B1_396	Pentanediamide, N,N’-di-benzoyloxy-	98	*Unknown*	B1-C		4.3% [−0.79%, 9.7%]	26% [8.9%, 45%]
B1_43	α-Isomethylionone	96	Masking agent, skin conditioner, perfumer, fragrance, flavorant	B1-C		11% [1%, 23%]	
B1_485	*Not certain: match score < 90%*	89	*Not certain: match score < 90%*	B1-F		30% [11%, 52%]	33% [11%, 59%]
B1_505	Octocrylene	97	UV absorber, UV filter, light stabilizer	B1-A		7.7% [2%, 14%]	
B1_562	*Not certain: match score < 90%*	84	*Not certain: match score < 90%*	B1-A	26% [10%, 45%]		
B1_577	*Not certain: match score < 90%*	0	*Not certain: match score < 90%*	B1-C	17% [4.9%, 32%]		−13% [−23%, −1.3%]
B1_68	Trimethylacetic anhydride	96	*Unknown*	B1-B		8.3% [−1.5%, 19%]	
B1_69	Diethyltoluamide (DEET)	98	Insecticide, insect repellent, skin protectant, active ingredient	B1-E		3.3% [−0.28%, 7.1%]	
B1_82	(−)-Epicedrol	93	*Unknown*	B1-F		13% [0.55%, 28%]	
B1_188 et al (PC1)	Tert-butyl benzoate	98	*Unknown*	B1-D			36% [10%, 69%]

Suspected Feature	Tentative Chemial Identity	Identity Match Score (%)	Reported Functional Uses in EPA Databases	Random Model Subset	% Change [95% CI]		
					Thyroid Hormone Receptor Antagonism	Androgen Receptor Antagonism	Estrogen Receptor Agonism
B1_225 et al (PC1)	Cyclohexyl palmitate	93	*Unknown*	B1-F	−8.5% [−17%, 0.38%]		
B1_362 et al (PC1)	Ethanone, 2,2-dihydroxy-1-phenyl-	96	*Unknown*	B1-E			16% [−0.86%, 35%]
B1_390 et al (PC1)	Octamethylcyclotetrasiloxane (D_4)	95	Emollient, skin conditioner, hair conditioner, fragrance, viscosity controlling agent, solvent, humectant, antistatic agent	B1-B		79% [41%, 130%]	48% [17%, 89%]
B1_393 et al (PC1)	Octinoxate	98	UV absorber, UV filter, light stabilizer, fragrance	B1-B			7.8% [−0.84%, 17%]
B1_583 et al (PC1)	(Z,E)-α-Farnesene	93	*Unknown*	B1-E	23% [7.7%, 42%]		
B1_615 et al (PC1)	Hexadecane-1,2,-diol	94	Hair conditioner, viscosity controlling agent, skin conditioner, emollient	B1-E			21% [−2.9%, 51%]
B1_991 et al (PC1)	17-Octadecynoic acid	95	*Unknown*	B1-B	22% [0.22%, 48%]		
Sample Batch 2 (n=113)							
B2_1312	*Not certain: match score < 90%*	0	*Not certain: match score < 90%*	B2-E		18% [0.95%, 39%]	
B2_1320	*Not certain: match score < 90%*	76	*Not certain: match score < 90%*	B2-G		15% [−1.2%, 34%]	
B2_1352	*Not certain: match score < 90%*	83	*Not certain: match score < 90%*	B2-B		16% [−2%, 38%]	
B2_1385	*Not certain: match score < 90%*	86	*Not certain: match score < 90%*	B2-B	16% [−1.8%, 36%]		
B2_319	Phenol, 2-(2-furyl)(2-pyrimidylamino)methyl-	94	*Unknown*	B2-B		12% [−0.78%, 28%]	
B2_331	Isobomyl formate	91	Fragrance, perfumer, flavorant	B2-D		23% [2.3%, 48%]	
B2_640	3-Hydroxybenzoyl 2-nitrobenzylidenehydrazide	94	*Unknown*	B2-F			29% [12%, 49%]
B2_677	Octinoxate	97	*Unknown*	B2-E			18% [0.16%, 40%]

Suspected Feature	Tentative Chemial Identity	Identity Match Score (%)	Reported Functional Uses in EPA Databases	Random Model Subset	% Change [95% CI]		
					Thyroid Hormone Receptor Antagonism	Androgen Receptor Antagonism	Estrogen Receptor Agonism
B2_761	Octinoxate *(different form / CAS number)*	97	UV absorber, UV filter, light stabilizer, fragrance	B2-A		9.4% [3.8%, 15%]	9.4% [0.41%, 19%]
B2_705	Hexyl benzoate	98	Fragrance, perfumer, flavorant	B2-D		5.9% [1.3%, 11%]	
B2_790	4-Fluorobenzoic acid, 2-phenylethyl ester	92	*Unknown*	B2-E		5.4% [1%, 9.9%]	
B2_917	Ethanone, 2-hydroxy-1,2-bis(4-methoxyphenyl)-	97	*Unknown*	B2-C			25% [8.3%, 44%]
B2_922	Octocrylene	97	UV absorber, UV filter, light stabilizer	B2-F		3.6% [2%, 5.3%]	1.3% [−0.014%, 2.6%]
B2_991	*Not certain: match score < 90%*	0	*Not certain: match score < 90%*	B2-E	21% [6.4%, 38%]		
B2_1384 et al (PC1)	Ambrettolide	93	Fragrance, perfumer, flavorant	B2-D	28% [4.4%, 56%]		
B2_164 et al (PC1)	*Not certain: match score < 90%*	82	*Not certain: match score < 90%*	B2-G	9.4% [−1.3%, 21%]	34% [11%, 61%]	
B2_510 et al (PC1)	Dibutyl phthalate *(could represent other phthalates)*	99	Fragrance, plasticizer	B2-D		30% [3.2%, 64%]	18% [1%, 38%]
B2_660 et al (PC1)	*Not certain: match score < 90%*	89	*Not certain: match score < 90%*	B2-B		4.9% [−0.66%, 11%]	

Note: Only chemical features that had 90% confidence intervals above or below the null for a given outcome are presented. The effects are defined as the difference in the log hormonal activity associated with a change in the individual chemical feature from its median 50th percentile to 75th percentile, holding other exposures in the model constant at their medians, and adjusted for country and squalene. Outcomes units are in μg-eq/g for thyroid hormone and androgen receptor interferences and in ng-eq/g for estrogen receptor interference. Reported functional uses are based on the EPA Functional Use database and supplemented from the EPA Chemical and Products Database.(Dionisio et al., 2018; A. L. Phillips et al., 2017)