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Comprehensive Nontargeted Analysis of Drinking Water Supplies to Identify Chemicals Associated with Estrogen Receptor Agonism or Present in Regions of Elevated Breast Cancer Occurrence

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Gabrielle P. Black, Berkley N. Anderson, Luann Wong, Christopher P. Alaimo, Guochun He, Michael S. Denison, Deborah H. Bennett, Daniel Tancredi, Blythe Durbin-Johnson, Bruce D. Hammock, Pujeeta Chowdhary, Rainbow Rubin, and Thomas M. Young*

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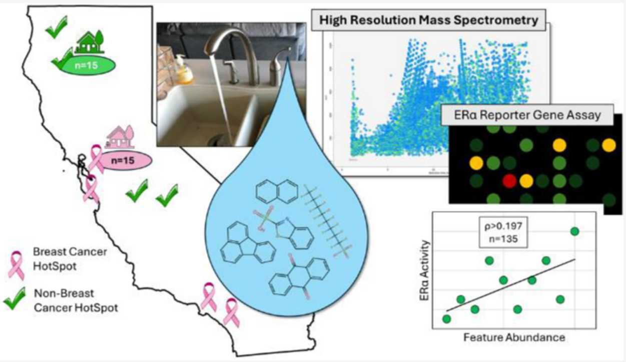
ABSTRACT: To explore the hypothesis that differential exposures to estrogen active chemicals may contribute to regional disparities in cancer incidence, a comprehensive targeted and nontargeted analysis was conducted over two seasons (2020) for drinking water samples from 120 households served by 8 public water systems (4 with historically elevated breast cancer incidence) and from 15 brands of retail water. All samples were analyzed using gas and liquid chromatography with high-resolution mass spectrometry and a bioassay for estrogen receptor agonism. Target compounds included disinfection byproducts, per- and polyfluoroalkyl substances (PFAS), trace elements, and compounds selected for their possible relation to breast cancer. Over 7500 GC and LC nontargeted molecular features passed all quality control filters in each sampling season and were prioritized for identification if they were related to measured estrogen receptor agonism or were present at higher levels in areas with high breast cancer incidence ($n = 1036$). Benzothiazole-2-sulfonic acid, acetyl tributyl citrate, and diphenyl sulfone were among the prioritized and confirmed nontarget compounds. Nine polycyclic aromatic hydrocarbons and two ketone derivatives displayed significant negative correlations with estrogen receptor agonism. Many prioritized compounds remained unidentified, as 84.4% of the LC features and 77.5% of the GC features could not be annotated with high confidence.



KEYWORDS: *high-resolution mass spectrometry, household-level water quality, estrogen receptor antagonist, CALUX bioassay, virtual effects directed analysis, bottled water contaminants*

INTRODUCTION

Breast cancer rates can vary significantly by region, and a substantial fraction of these cancers is thought to be related to environmental exposures. For example, one study of cohorts of twins found that 27% (95% confidence interval 4−41%) of breast cancer cases could be attributed to heritable factors with the balance of cases (73%) related to nonheritable factors, including lifestyle and environmental exposures.[1] Comparing environmental exposures between regions of higher and lower cancer incidence offers one opportunity to discover compounds potentially related to cancer development. In California, the greater San Francisco Bay Area, and portions of Ventura, Los Angeles, Orange, and Riverside counties have been identified as regions that had age-adjusted breast cancer incidence rates 13.7−17.6% higher than the remainder of the state during the period 2000−2008.[2] An understanding of the uneven regional distribution of breast cancer incidence led to a new effort to identify potential environmental triggers, including contaminants in drinking water.

Since most breast cancers, including 70−80% of those in California, are estrogen-dependent,[3,4] increased growth and proliferation of breast cancer can be stimulated by chemicals that can directly activate and/or enhance the activity of estrogen receptors (ERs).[5−7] Approximately 1000 endocrine disrupting chemicals (EDCs) have been identified,[8] and it has

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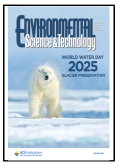

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been suggested that the increased incidence of breast cancer in industrialized countries is linked to such chemicals.[5−7,9−13] Given the well-documented and widespread presence of estrogenic chemicals in surface waters and their potential to enter drinking water supplies,[14,15] the detection, identification and characterization of estrogenic chemicals is of major importance.

Public drinking water supplies contain known carcinogens, primarily disinfection byproducts (DBPs), as well as a wide variety of compounds that may act as EDCs and/or have suspected or uncertain relationships to cancer development.[5,6,16−18] Evidence to date from both epidemiologic and animal studies has linked DBPs primarily with bladder and kidney cancers, with at least 22 of the over 600 DBPs identified to date exhibiting carcinogenic potential via these pathways. Less studied and unregulated DBPs may pose greater risks based on their increased genotoxicity compared with regulated compounds.[19−21] Known mammary gland carcinogens and EDCs encompass a broad array of chemical classes including dioxins, polychlorinated biphenyls (PCBs), phenols like bisphenol A (BPA), phthalates, per- and polyfluorinated alkyl substances (PFAS), halogenated flame retardants and solvents, pesticides, pharmaceuticals, hormones, natural products, and dyes. Most compounds within these broad chemical classes are not widely or routinely monitored in US water supplies except for those covered by the Unregulated Contaminants Monitoring Rule (UCMR), which has occurred on a 5-year cycle starting in 2000 and has included 136 constituents through Phase 5.[22] Previous efforts to identify possible links between water quality and elevated rates of breast cancer incidence in Cape Cod, MA[23] and Long Island, NY[24,25] have focused on the potential roles of wastewater inputs[26,27] and pesticide applications[24,25,28] but have not found evidence that these contaminant sources significantly impact breast cancer rates.

Nontargeted analysis (NTA) has emerged as a powerful tool for identifying chemicals potentially associated with bioactivity in environmental samples that may not be subject to monitoring or regulation. Varied workflows have been applied to connect compounds detected via NTA with specific biological effects including effects directed analysis (EDA)[29,30] and statistical approaches that have been labeled virtual EDA.[31] Both *in vitro* (e.g., estrogen receptor bioassays) or *in vivo* (e.g., whole organism toxicity tests) assays have been combined with NTA to successfully identify toxicologically significant chemicals including estrogen receptor agonists in wildfire ash,[32] sewage sludge,[33] surface water,[29,34] wastewater,[35,36] and sediments[37]

Systematically assessing whether compounds in drinking water supplies might contribute to breast cancer is confounded by numerous factors including the complex array and mixture of water sources used, combined with the diversity in type and stringency of the treatment processes employed. Superimposed on this patchwork of sources and treatment processes are variations in land uses that may degrade the quality of groundwater and surface water including: agriculture, transportation, fire-fighting, energy extraction, wastewater discharge, and industry. Inputs to water supplies from these activities can vary both seasonally and regionally. Breast cancer incidence rates are also related to urbanicity, socioeconomic status, age, race, and use of oral contraceptives. The study design applied here is not epidemiologic and does not control across regions for these factors.

The goal of the present study was to use a comprehensive characterization of public water supplies to isolate chemicals plausibly related to estrogenic activity and breast cancer. The study design included 120 households, 15 households each in eight public water systems (four systems were in areas identified as breast cancer hotspot zones and four were not) and included 15 brands of water purchased at retail outlets. The public systems were supplied by four water source types (local surface water, distant surface water, groundwater, and a mixed input of groundwater and surface water). All household (120) and bottled water (15) samples were collected twice for a total of 270 water samples. Samples were analyzed by high-resolution liquid and gas chromatography mass spectrometry techniques (targeted and nontargeted methods), estrogen receptor chemically activated luciferase gene expression bioassays, and inductively coupled plasma mass spectrometry (CALUX). Chemical identification efforts focused on compounds potentially related to breast cancer determined via statistical correlation with observed bioassay activity or comparisons between regions of above and below average breast cancer rates.

■ EXPERIMENTAL METHODS

The NTA Study Reporting Tool (SRT) was used in the preparation of this manuscript.[38]

Materials. The solvents, reagents and standards employed in this work are listed with sources, purity, and abbreviations used in the text in the Supporting Information (Tables S1 and S2).

Study Design and Sampling. Water samples were collected from 15 household kitchen sinks in each of eight regions in California. These regions included two communities representative of each of four drinking water source categories: (1) local surface water sources, (2) distant surface water sources, (3) groundwater, and (4) mixed ground and surface water. Overlaid on drinking water source types are regions, defined as hotspot zones in the subsequent discussion, that had age-adjusted breast cancer incidence rates 13.7−17.6% higher than the rest of California during the period 2000−2008 and 58.3% higher than the average rate in the four study areas that are not hotspot zones.[2] Age-adjusted breast cancer rates in the hotspot zones over this period were similar to the average levels in the US (127.7 vs 127.9 per 100 000) but significantly higher than rates in the nonhotspot zones (80.7 per 100 000). The locations selected for study (followed by their assigned acronym and public water system) include: San Mateo (SM, California Water Services), East San Francisco Bay Area (EB, East Bay Municipal Utility District), Merced (MC, City of Merced), Madera (MD, City of Madera), Los Angeles (LA, City of Los Angeles Water and Power), Irvine (IR, Irvine Ranch Water District), Yurok Tribal Land (YT, Yurok Tribal Operation), and Weaverville (WV, Weaverville Community Services District). Refer to Figure S1 for a map and Table S3 for system information. A key limitation of the sampling design is that the chosen communities and water systems in breast cancer hotspot zones (EB, IR, LA, SM) differ systematically from the nonhotspot zones (MC, MD, WV, YT) because: (i) they are larger and more urban, serving an average of 1.5 million people compared with an average of 40 000 for the nonhotspot systems, and (ii) the systems in hotspot zones all use chloramination for disinfection while the nonhotspot zones all use chlorination. In general, chloramination has been shown to produce lower concentrations of regulated DBPs like trihalomethanes and haloacetic acids, but higher concentrations of more cytotoxic and genotoxic DBPs like haloacetonitriles..[20] To our knowledge, though, none of these DBPs have been specifically linked to breast cancer. These differences are likely to impact contaminant profiles in unknown ways but were difficult to avoid because breast cancer hotspot zones are all within the two largest urban centers in California, around Los Angeles and San Francisco.[2] Because bottled water often serves as a replacement for drinking water from public systems, we also purchased and analyzed 15 brands of packaged water obtained from retail outlets in Davis, CA (BW). BW samples included samples from packaged water supplied in boxes, cans and plastic bottles and refill stations (Table S4).

Households were recruited in all regions except YT using postcards sent through the US Postal Service's Every Door Direct Mail (https://eddm.usps.com/eddm/select-routes.htm) on routes identified as areas with high pollutant loading using the CalEPA EnviroScreen tool (https://oehha.ca.gov/calenviroscreen/report/calenviroscreen-40) and with lower socioeconomic status (relative to regional averages). Households sampled in YT were recruited directly by the Yurok Tribe Environmental Department. Postcard mailings yielded sufficient volunteers in MD and WV. In the other regions (EB, SM, LA, IR, MC) additional participants were recruited via personal outreach efforts and targeted email messages to University of California campuses in Berkeley, Merced, Los Angeles and Irvine. A total of 289 volunteers signed up through an online web link, but the number responding via each recruiting pathway is not known, introducing potential biases in household characteristics. Because all households within the system received water from the same public water system, however, these effects were not expected to significantly impact tap water composition. Households were mapped using MapCustomizer.com and participants were selected to represent the broadest geographical coverage obtainable within service area boundaries of the selected utilities. Households were compensated with $25 gift cards for each sample returned. Samples were collected from each household twice, once during winter months (January through April) and again in summer months (July through September) during 2020. Over 95% of participating households provided samples in both seasons.

Sampling bottles, ice packs, instructions, and questionnaires were mailed in coolers to participants the week prior to their specified sampling date. All households within a water district were sampled on the same day during both winter and summer sampling events. Participants were asked to remove any point of use filters or devices, flush their kitchen sink for 5 min with cold water, and fill a 2.6 L amber glass bottle until it overflowed and pack it into a cooler with ice packs. A private courier service (Rapidus Inc.) picked up all 15 coolers and transported them the day of collection to Davis, CA where samples were aliquoted and placed in a cold room (5 °C) overnight. Sample preparation for trace organic and bioassay analyses were extracted the following day, while disinfection byproduct and trace element analyses were performed within 14 days of receipt. Laboratory method blanks and spiked samples were alternately processed with each batch of samples received, resulting in 9 of each type of quality control sample, collected over the entire sampling program.

Sample Preparation. Samples designated for trace element analysis (10 mL) were preserved by adding 100 μL of trace metal grade nitric acid (1% v/v). A subsample (5 mL) was transferred to a plug seal centrifuge tube and analyzed via inductively coupled plasma mass spectrometry (ICP-MS). NIST 1640A was used to evaluate system performance with recoveries ranging between 64−99%.

For organics analysis water samples (2.4 L) were adjusted to pH 6.5−7 with dilute formic acid or ammonium hydroxide. Solid phase extraction (SPE) cartridges were lab-prepared and contained Oasis HLB, Strata XAW, Strata XCW, and Isolute ENV+ to enrich neutral, cationic, and anionic species.[39,40] Cartridges were preconditioned with 5 mL methanol and 10 mL double-deionized water (DDI). Samples were loaded under vacuum at a rate of ~10 mL min^{-1} and dried under vacuum. Sample bottles were washed with 3 volumes of 4 mL 1:1 acetone:hexane and evaporated to 1 mL. SPE cartridges were sequentially eluted with (1) 5 mL of 5% MTBE in MeOH, (2) 5 mL DCM, (3) 6 mL 1:1 EtAc:MeOH with 0.5% NH_3, and last, (4) 3 mL 1:1 EtAc:MeOH with 1.7% formic acid. All eluents were combined with the bottle rinses to improve recovery of more hydrophobic compounds and were evaporated to 1 mL. 200 μL was removed for bioassay analysis and solvent exchanged into DMSO. The remaining 800 μL was evaporated to 400 μL and split into 2 × 200 μL fractions, one in MeOH:DDI water (1:5, v/v) for analysis by liquid chromatography (LC) and one in EtAc which was evaporated to near dryness and reconstituted for analysis by gas chromatography (GC). This split sample approach has been successfully employed for a wide range of compounds in our previous work[32,33,40] and allows the same underlying extract to be supplied to the GC, LC and bioassays, ensuring comparability of data across these methods but with the potential for compromising recoveries for certain subsets of compounds.

Internal standard calibration was used for both analyses. LC extracts were fortified with a suite of isotopically labeled standards at 400 ng mL^{-1} (Table S2). GC extracts were fortified with 100 ng mL^{-1} pyrene-D_{10}. Surrogate compounds were not added to samples to avoid interferences with bioassays, however spiked samples were included for each region to evaluate method performance. Recoveries averaged 57−124%, except for pentachlorophenol and 4-nitrophenol whose recoveries averaged 20 and 24%, respectively (Table S5). A 12-point, second order polynomial, internal calibration curve was used to quantify all target compounds. Target compound method detection limits (MDLs) were the higher of: (1) the lowest calibration point with a signal-to-noise ratio greater than 3 and raw height greater than 3000, or (2) the average of blank samples plus three times their standard deviation. Method quantification limits (MQLs) were the higher of (1) the lowest calibration point with a signal-to-noise ratio greater than 10 and a raw height greater than 3000, or (2) the average of blank samples plus 10 times their standard deviation. Target compounds included mammary gland carcinogens selected based on literature sources available at the time of proposal[16,27,41] and other compounds with potential linkages to cancer including disinfection byproducts, trace elements and PFAS compounds.

Data Acquisition. To avoid excessive holding times for sample extracts, the winter and summer sample sets were analyzed separately. Data processing was also done by season to address potential nontarget batch effects.[42]

Trace Elements and Disinfection Byproducts. Trace elements were measured using an Agilent 7900 ICP-MS

(Table S6). The disinfection byproduct (DBP) methods and results have been reported previously.[43] More details about both sets of methods can be found in the Supporting Information text.

Polar Organic Compounds. An Agilent 1260 Infinity HPLC was used for chromatographic separation with an Agilent Zorbax Eclipse Plus C18 column (2.1 × 100 mm, 1.8 mM). Instrument settings and chromatographic gradients are detailed in Table S7. Mass spectra were acquired using an Agilent 6530 Quadrupole Time-of-Flight mass spectrometer (QTOF-MS) using the *All-Ions* acquisition method with collision cell voltages cycling through 0, 10, 20, and 40 eV. Samples were injected in a randomized block design where a high calibration standard, a low calibration standard, and an injection blank were injected every 15 injections.

Targeted PFAS Analysis. Twenty-six PFAS targets were separated using an Agilent Poroshell HPH C18 column (2.1 mm × 100 mm, 1.8 μm). Instrument settings and chromatographic gradients are detailed in Table S8 and are based on those in a recent publication.[44] Recoveries for selected PFAS are included in Table S5. Data was acquired in *All Ions* mode as previously described, but collision cell voltages cycled through 0, 10, and 20 eV.

Nonpolar Organic Compounds. GC-QTOF-MS analysis (Agilent 8890 GC coupled to an Agilent 7250 QTOF-MS with an HP-5MS UI 30 m × 0.25 mm × 0.25 μm column, Agilent Technologies, Inc.) was performed in electron ionization (EI) mode and employed two chromatographic methods. For compounds with high ion abundance 1 μL of sample was injected into a 250 °C inlet in split mode (200:1 split ratio). For compounds with lower ion abundance 1 μL of sample was injected into a 280 °C inlet in splitless mode. Instrument settings and temperature programs for both methods are detailed in Table S8. Samples were injected in a randomized block design where a high calibration standard, a low calibration standard, and an injection blank were injected every 15 injections.

Cell Bioassays for Estrogenic Activity. Estrogen-responsive recombinant VM7Luc4E2 cells were used to analyze for estrogen active components in extracts using the ERα-CALUX method.[45] Briefly, cells were transferred into estrogen-free medium (phenol red-free α-MEM containing 10% charcoal-stripped FBS, 1.9% L-glutamine) and incubated for 3 days before plating into 96-well tissue culture plates at a density of 75 000 cells per well. Cells were allowed to attach for 24 h and then were incubated with carrier solvent DMSO (1% final concentration), 17β-estradiol (E2; 1 nM), or an aliquot (1 μL) of the indicated extract for 24 h at 37 °C. All samples and controls were analyzed in triplicate. After incubation, cells were rinsed, lysed, and shaken for 20 min to allow complete cell lysis. Luciferase activity was measured on an automated microplate luminometer with the automatic injection of 50 μL of Promega stabilized luciferase reagent as previously described.[46] Luciferase activity in relative light units (RLUs) relative to a 1 nM concentration of E2 (set at 100%) was measured. All observations were blank subtracted using RLUs observed in carrier solvent wells.

Nontargeted Alignment and Initial Annotation. LC-QTOF-MS and GC-QTOF-MS data files were each converted to an analysis base file format (Reifycs Analysis Base File Converter, ABF) and deconvoluted and aligned in MS-DIAL (version 3.66 for LC-QTOF data and version 4.90 for GC-QTOF data).[47] Alignment parameters are reported in Tables S10 (LC) and S11 (GC). Structure annotations were made by comparison to spectral libraries. LC-QTOF data was screened against two libraries: (i) a combined Agilent Pesticides, Pharmacology/Toxicology and Water Contaminants database, and (ii) libraries downloaded from the Mass Bank of North America (MoNA) for LC-MS/MS Positive Mode (99 261 spectra) and LC-MS/MS Negative Mode (47 058 spectra). GC-QTOF data was screened against the NIST17 library (306 622 spectra). To ensure the robustness of the annotations, vendor software was used to process the same data sets using Agilent Qualitative Analysis v. 10.0 (LC) and Agilent Unknowns Analysis v. 10.1 (GC). The resulting annotations were associated with aligned features using a python code that grouped features with similar retention times (± 0.04 min for GC and ±0.3 min for LC) and with its molecular ion (LC) or one of its top three abundance fragments (GC) within 0.002 amu of the m/z for the MS-DIAL feature. Up to three of the most common annotations matching a given feature are reported along with the number of samples with that annotation. Consistent annotation of a feature across samples and between the applied algorithms provided support for the validity of the annotation. Confidence levels for structural annotations were assigned using established methods following efforts to purchase standards for prioritized compounds.[48,49]

Quality assurance filtering of the full alignment data sets included 3 steps. First, features were removed if fewer than 25% of the samples had ion abundances that exceeded the upper 95% confidence interval around nine method blank samples. Second, features were removed if they had average signal-to-noise ratios below 10 across all samples. Finally, to minimize compounds found at high abundance in all GC samples, likely related to solvent impurities, features with a maximum sample abundance of less than five times the average method blank were removed. Together these filtering steps provided assurance that features passed to statistical analysis steps were meaningful and reproducible representations of compounds present in the water samples.

Feature Prioritization. Compounds within the filtered feature set were prioritized for further identification efforts based on their: (i) relationship to estrogen receptor activity, and (ii) presence at elevated levels in hotspot zones.

Estrogen Receptor Related. Spearman's (rank order) and Pearson's (linear) correlation coefficients were calculated between the abundance of each feature and the corresponding ER-CALUX activity of the sample, with the p values adjusted for multiple hypothesis testing (q values) using the Benjamini−Hochberg procedure. Chemical features with $q < 0.05$ were prioritized. Because of the large number of features present in the LC data set, an additional filter parameter required that features be present in at least 25% of the samples per season. Features related to ER-CALUX activity were also selected using an elastic net procedure, a regularized regression model that combines aspects of both LASSO and Ridge models and that has proven useful in previous nontarget studies.[32,50]

Group-Wise Contrasts. Contrasts were calculated using the limma package in R[51] and required features between two groups to be present at a log 2 fold-change of two or greater with $q < 0.05$. Contrasts were performed for the following groups: (1) all hotspot zones versus all nonhotspot zones, (2) individual hotspot zones versus all nonhotspot zones, (3) agonist samples versus nonagonist samples and (4) antagonist samples versus nonantagonist samples. For LC compounds, an abundance of 50 000 counts or above was required in at least one sample for each feature prioritized.

Targeted MS/MS and Structural Annotation of Prioritized LC NTA Features. Prioritized nontarget features from the LC workflow were reinjected in targeted MS/MS mode, where precursor ion selection was performed within a 1.2 m/z isolation window with collision cell voltages cycling through 0, 10, 20, and 40 eV at a MS_1 scan speed of 4.5 spectra s^{-1} and a MS_2 scan speed of 6 spectra s^{-1}. The MS/MS data were used to generate molecular formulas and perform *in silico* fragmentation using MSFINDER (version 3.50) and SIRIUS:CSIFingerID (SIRIUS, version 4.9.12). Molecular formula generation and structure annotation parameters are reported in the Supporting Information (Table S12). Structure annotations were merged between MSFINDER and SIRIUS based on InChIKeys. The top five ranked structures on each platform were advanced for further identification efforts, as were any of the sixth through tenth ranked structures on each platform if they were cross validated by a result in the top 100 structures on the other platform. A cumulative match score was calculated using eq 1, and structures with a score >0.33 were advanced for further identification efforts.

$$\text{Cumulative Match Score} = 0.4(\text{Rank Score}) + 0.6(\text{Match Score}) \quad (1)$$

Where Rank Scores and Match Scores are defined by eqs 2 and 3, respectively.

$$\text{Rank Score} = 0.5\left(\frac{1}{\text{MS Finder Rank}}\right) + 0.5\left(\frac{1}{\text{SIRIUS Rank}}\right) \quad (2)$$

$$\text{Match Score} = 0.5\left(\frac{\text{MS Finder Score}}{10}\right) + 0.5\left[\left(\frac{1}{|\text{CSI Score}|}\right)\times 10\right] \quad (3)$$

where the MSFINDER and SIRIUS rank scores are the ranking integer for each identification and the MSFINDER scores and CSI scores are the sum of formula and structure scores. Formula scores are calculated in MSFINDER based on mass error, isotopic ratio, formula assignment to product ions, neutral loss searching, and existence in metabolome databases. Structure scores were calculated based on accurate mass, bond dissociation energies, penalties of fragmentation linkage, and penalty of hydrogen-rearrangement rules.[52] CSI Scores (CSI:FingerID within SIRIUS) are generated based on the predicted molecular fingerprint of a compounds' fragmentation tree and spectrum.[53,54] When standards were either unavailable or unobtainable based on laboratory limitations, confirmation was attempted via inspection of online MS/MS spectra from MassBank of Europe (https://massbank.eu/), MoNA (https://mona.fiehnlab.ucdavis.edu/), and/or PubChem (https://pubchem.ncbi.nlm.nih.gov/). When reliable online MS/MS spectra were unavailable, the feature was conservatively given a level 4 annotation despite its structural prediction via *de novo* techniques.

Quantitative Structure-Activity Relationship (QSAR) Modeling. Open-source QSAR models were used to predict (i) estrogen receptor agonism, antagonism and binding for annotated structures from the LC-QTOF workflow using OPERA,[55] and (ii) carcinogenicity using six models on the VEGA platform.[56] For estrogenicity predictions in OPERA, a structure was classified as a positive result if the structure had a local applicability domain index greater than 0.4, regardless of whether it was inside or outside of the global applicability domain.[55] Features that were being investigated because of ER agonist activity and were predicted to be agonistic and bind to the estrogen receptor (ER) or those prioritized by the ER antagonist contrast and predicted to be antagonistic and bind to the ER were further investigated. Features predicted to be ER agonists, ER antagonists, or carcinogenic on three or more carcinogenic models that were being investigated because of their elevated abundance in hotspot zones, or were selected based on the Spearman's correlation, Pearson's correlation or elastic net regression were investigated further.

LC Retention Time Indexing. At the time of targeted MS/MS data acquisition, a LC retention time indexing (LC-RTI Mix) was injected, and data acquired using the *All-Ions* method. Structural annotations were evaluated for retention time plausibility using an online retention time indexing model.[57] SMILES strings, structure names, and experimental retention times were modeled against similar descriptors provided for the recommended LC-RTI Mix. Structures with "Box 1" or "Box 2" results were further investigated (Table S4).

■ RESULTS AND DISCUSSION

Estrogen Receptor Agonist Activity. Estrogen receptor agonist activity was highly variable between regions, across households within each region, and between sampling periods. No significant differences (q < 0.05) were observed in estrogenic activity between hotspot and nonhotspot groups. Activity was on average, higher in winter samples than summer samples, but seasonal variability ranged from 8 to 207% relative percent difference (Figure 1).

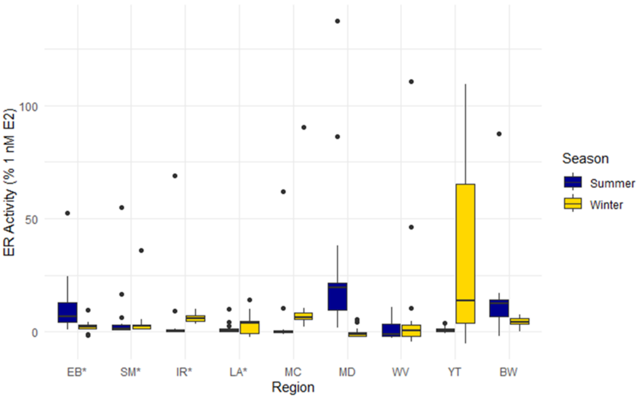

Figure 1. Estrogen receptor agonist activity measured with the ERα-CALUX assay expressed as percentage of 1 nM 17β-estradiol positive control. Asterisks denote breast cancer hotspot zones.

A 95% confidence interval was constructed around ER-CALUX method blanks, and samples that exhibited estrogenic activity above the upper limit of the confidence interval were labeled "agonists" (green shading in Tables S13 and S14; 54 winter, 46 summer) and samples with activity below the lower limit of the confidence interval were labeled "antagonists" (blue shading; 16 winter, 8 summer). Samples with observed

Table 1. Number of Nontarget Chemical Features Selected during Prioritization for Each Workflow in Each Monitoring Season

			ER agonist correlated			fold-change (>2)		
season/workflow	total prioritized	prioritized compounds annotated level 1 or 2	Pearson (±)	Spearman (±)	elastic net	hotspot	ER agonist	ER antagonist
summer/GC split	5	2	3/1	1/0	0	0	0	0
summer/GC splitless	45	18	17/4	6/21	0	5	0	1
summer/LC ESI±	512	80	52/4	46/165	6	309	17	13
winter/GC split	18	5	3/0	1/13	0	1	0	0
winter/GC splitless	110	28	7/18	5/85	0	14	0	0
winter/LC ESI±	346	53	10/5	125/86	0	235	36	3

activity between the upper and lower limits of the confidence interval were deemed “inactive”.

The concept of Bioassay Equivalent (BEQ) concentrations has been proposed to characterize the additive effect of estrogenic compounds within a mixture by reporting an equivalent concentration of 17β-estradiol that would elicit the observed bioassay response. Applying our sample concentration factor of 2400, BEQs for our bioassay results were calculated and are reported in Tables S13 and S14. Effect based trigger (EBT) values based on the BEQ concept have been developed by several research groups using different approaches to delineate levels of concern for in vitro assay results. Values estimated for the ERα-CALUX bioassay have ranged from 0.1−0.5 ng EEQ/L for surface waters[58,59] and from 0.2−3.8 ng EEQ/L for drinking water.[60−62] The maximum BEQ value observed in this study, 0.16 ng/L (MD04-S), exceeds the lowest of these thresholds (along with 6 other winter samples), but none exceeds any proposed drinking water EBTs. It should also be noted that 6 out of 7 samples above the lowest EBT value occurred in nonhotspot communities. The highest values of BEQ observed here are in the range of values observed for other US drinking waters.[63,64] Our use of a high concentration factor for bioassay samples may account for the fact that a higher fraction of ER agonist values exceeded the control samples in comparison with some previous studies that have used lower concentration factors[64]

Target Inorganic Analytes. Aluminum, chromium, copper, arsenic, nickel, zinc and barium were most prevalently detected in winter samples whereas nickel, copper, and barium were detected most frequently in summer samples. Aluminum concentrations dominated overall winter metal concentrations (detection frequency 44%) and were correlated with observed ER agonism (Pearson’s ρ = 0.36, Spearman’s ρ = 0.26). Al concentrations were highest in WV, MC, MD, and LA samples with median values of 3,567, 404, 232, and 153 μg L^{-1}. Al concentrations were far lower in summer sampling, with a maximum median of 58 μg/L and no significant correlation with ER agonism. The ability of divalent metals such as Ni and Cu or metal anions such Cr oxyanions to activate ERα through the formation of a complex within the hormone-binding domain of the receptor has been noted in previous studies[65] but these effects were not observed over the concentration ranges found in our study.

Target Organic Analytes. Of the analytes defined as potential mammary gland carcinogens measured in this study, only diethyl phthalate, pyrene, simazine, PFOS, pentachlorophenol, 4-nitrophenol, and PCB 118 were detected above method quantification levels (Tables S13−S14). Except for pyrene, none of these were detected in more than 10% of the samples. Simazine was detected in only MC (n = 2), MD (n = 2) and LA (n = 7) and only in the summer samples and ranged from 0.03−0.57 ng L^{-1}. PCB 118 was detected 4 times and only in the summer data set at concentrations of 5.48−8.76 ng L^{-1}; all detections were within MD and MC. Pentachlorophenol was detected once in the winter at 6.21 ng L^{-1} in a LA sample. Pyrene was detected in 93% of summer SM samples and 53% of summer EB samples at concentrations ranging from 2.21−9.33 ng L^{-1}. In winter samples, pyrene was detected in 100% of SM samples and only 20% of EB samples. Additionally, in winter samples, pyrene was detected in an additional 6 samples spanning other regions. A potential explanation for the elevated levels of pyrene and other PAHs detected via our nontarget workflow (see below) in SM and EB samples may be the impact of wildfires on their source waters. SM obtains water primarily from Hetch Hetchy Reservoir, which is supplied by the Tuolumne River watershed that was heavily burned in the 2013 Rim Fire (104 131 ha), while a significant fraction of EB water is obtained from Pardee Reservoir supplied from the Mokelumne River watershed that was significantly burned in the 2015 Butte fire (28 679 ha). Residual burned materials leached into the reservoirs could be a continuing source of low-level PAH contamination of the source waters.

Nine of the 30 PFAS target analytes were detected above their MQLs (0.13−7.1 ng L^{-1}; Tables S13−S14). Six and eight carbon chain sulfonates and carboxylates (perfluoro-hexanoic acid (PFHxA), -hexanesulfonate (PFHxS), -octanoic acid (PFOA) and -octanoic sulfonate (PFOS)) were the most commonly detected. Three households in WV exceeded the recently adopted US EPA maximum contaminant levels (MCL) for PFOS (4 ng L^{-1}) at concentrations between 5.84−6.18 ng L^{-1}, but based on the results for HFPO−DA (Gen-X) and PFHxA, the hazard index set by the EPA was not exceeded by any household in this study.[66] These results are consistent with the UCMR PFAS measurements taken in 2023−2024 for the systems we tested (except for YT, which was not included in UCMR data). For example, the highest PFOS concentrations observed in the present study were in WV (up to 6.2 ng/L) lower than the reported maximum UCMR value for WV (19 ng/L), which was also the highest among this group of systems. The highest PFHxS value for these systems in the UCMR results was in MC (15 ng/L) while this study also found that MC had the highest concentrations of PFHxS (7.2 ng/L). In summer samples, PFHxA, PFHpA, PFPeS, PFHxS and PFOS were negatively correlated with ER activity via Spearman’s correlations, while in winter samples, PFBS was negatively correlated via Pearson’s correlation and PFOS was positively correlated via Spearman’s correlation. The reasons that most of the correlations are negative, and the discrepancy between PFOS in winter and summer, are not clear.

Nontargeted Analysis—Prioritization. Across both GC and LC data sets and both seasons, 1036 features were prioritized out of the 16 929 aligned features that passed the filtering steps (Tables 1 and S15). Complete lists of the aligned features from each workflow and their prioritization via different pathways are provided in Tables S16−S21. Examples of prioritized compounds identified following the full workflow are discussed in the subsequent sections, but it is important to note that most prioritized compounds remain unidentified since just 15.6% of prioritized LC features and 29.8% of prioritized GC features across all samples and seasons could be annotated at confidence levels 1 or 2.

Nontargeted Analysis—LC Results. One of the compounds prioritized using the LC-HRMS workflow was identified as benzothiazole-2-sulfonic acid (BTSA). Environmental occurrence of BTSA has been connected with the oxidation of the rubber vulcanization accelerator 2-(methylthio)benzothiazole (MTBT) via metabolic or photochemical processes. Previous studies have shown, for example, that treatment of aqueous solutions of MTBT with ultraviolet light at 254 nm yields BTSA as a major product.[67] Levels of BTSA in municipal wastewaters are in the low μg/L range[68,69] and it has been found in drinking water supplies in areas where it has been detected in wastewater.[70] BTSA was widely detected in our study, with 89% of summer samples exceeding the 95% upper confidence interval around blanks (Figure 2). A search for MTBT using the GC data detected it in 11 samples, ten of them from IR, where the highest BTSA ion abundances were observed during both sampling periods. A feature corresponding to MTBT was not found in the MS-DIAL alignment; all detections occurred only via Unknowns Analysis. Although these results suggest an environmental source of BTSA, it is also possible that it may be leached from polyethylene piping within the water distribution system.[71]

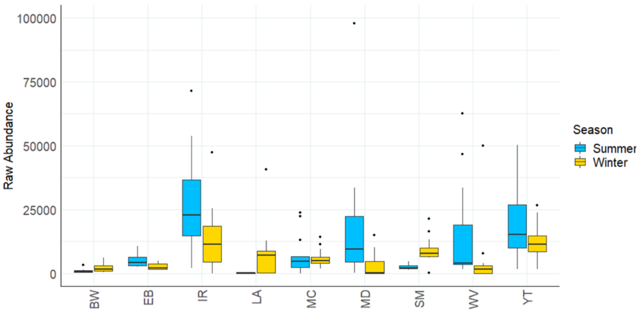

Figure 2. Benzothiazole-2-sulfonic acid ion abundances across sampling locations and seasons.

The presence of several pesticides was highlighted by the prioritization scheme. Fluridone, an herbicide used to control aquatic weeds, was elevated in the hotspots as a group and also elevated in LA (S and W) and IR (S only). Fluridone has been reported to result in endocrine disruption in the fish Delta smelt, specifically increasing E2 concentrations[72] and in a second fish model, Japanese Medaka, revealed by transcriptome data.[73] Fluridone is extensively used to control aquatic weeds and within water systems may be applied to control algae and cyanobacteria blooms.[74] Other prioritized pesticides and degradation products included diuron (W/S), atrazine (W), atrazine-desethyl (W/S), and atrazine-deisopropyl (W), each of which was less abundant in SM, EB and IR than in nonhotspot zones. These results are largely driven by the elevated concentrations of these herbicides and degradates in agriculturally impacted groundwater samples from MD and, to a lesser extent, MC. Atrazine and its desethyl and deisopropyl byproducts are listed as reproductive toxicants by the state of California.[75]

A compound that was prioritized by a negative correlation with estrogen receptor agonism, was elevated among antagonist samples, and was elevated in hotspots generally and specifically in SM samples from the summer was 4,5-dimethyl-1,2-phenylenediamine. This relative of para-phenylenediamine tire additives that have been the subject of much recent investigation[76] is most likely a dye material based on its functional classification in the EPA Comptox dashboard.[77,78] It is unclear why this compound was present at significantly higher abundances in summer samples compared with winter samples from SM (17× higher in summer).

Nontargeted Analysis—GC Results. One striking feature of the prioritization in Table 1 is the high fraction of features from the GC splitless workflow negatively correlated with ERα agonism, especially using the Spearman correlation (85/90 winter, 21/27 summer). Of these negatively correlated features, a substantial fraction are polycyclic aromatic hydrocarbons (PAHs); for example, 8/21 of the negatively correlated features in summer were PAHs (Table 2). A significant body of literature has documented the "cross-talk" between the aryl hydrocarbon receptor (AhR) and the estrogen receptor.[79−82] Underlying mechanisms contributing to the interaction between the two receptors include increased metabolism of estrogen active hormones stimulated by AhR activation or the binding of PAH metabolites to the estrogen receptor thereby blocking binding of endogenous ligands.[79] These interactions are complex and depend on specific PAH structure and molecular size. Larger (4−5 ring) PAHs have demonstrated estrogen receptor antagonism[83] and mixtures of PAHs extracted from environmental media typically exert ERα antagonism that is proportional to total PAH concentrations.[84,85] Seven of the nine PAHs listed in Table 2 have been tested for antiestrogenic activity, with three showing antiestrogenic activity while four others did not.[83] Overall,

Table 2. Polycyclic Aromatic Hydrocarbons with GC Splitless Ion Abundances That Were Negatively Correlated (q < 0.05) with ER Agonism or Prioritized via Hotspot Fold-Change

compound	prioritized alignment ID	correlation
naphthalene	W156	ER- Spearman
acenaphthylene	S358	ER- Spearman
phenanthrene[a]	S553	ER- Spearman
anthracene[a]	S559	ER- Spearman
fluoranthene[a]	S691/W689	ER- Spearman (S)/Hotspot (W)
pyrene[a]	S774	ER- Spearman
chrysene[b]	S844/S847	ER- Spearman
benzo[*k*] fluoranthene[b]	S903	ER- Spearman
indeno[1,2,3-cd] pyrene[b]	S961	ER- Spearman

[a]Tested and not found to be ER antagonists[83] [b]Identified as ER antagonists[83]

this suggests that a negative correlation between PAH concentrations and estrogen receptor agonism has some mechanistic basis, but it also highlights how compounds arising from similar sources (i.e., correlated with each other) can exhibit correlations with ER activity that are coincidental rather than causal.

A PAH-related compound widely detected in our samples (72.6 and 52.6% above the upper 95% confidence interval around method blanks in summer and winter, respectively) was 9,10-anthracenedione. It was prioritized in the summer samples via a negative Spearman's correlation with estrogen receptor agonism ($q < 0.05$) and it was confirmed with a standard. Almost 10% of the samples (24/270) contained anthraquinone at quantifiable levels ranging from 16.7 to 371 ng/L. Most detections occurred in samples from SM, followed by EB and IR. Anthraquinone is a primary byproduct when anthracene containing waters are disinfected using chlorine,[86] so its presence in the summer sample set, where numerous anthracene detections were recorded, is not surprising. This compound has been documented to induce liver, kidney and bladder cancers in rodents[87] and is already the focus of some regulatory scrutiny by its inclusion on the US Environmental Protection Agency Contaminant Candidate List (CCL5).

Elemental sulfur, in the form of cyclic octaatomic sulfur, was prioritized in both winter and summer samples by its elevated abundance in breast cancer hotspot areas, particularly IR and SM. Elemental sulfur is a key intermediate in the oxidation of sulfides and is present at high levels in both marine sediments[88] and in landfill leachates.[89] The origins of elemental sulfur in the IR and SM source waters and its potential health significance are not clear; its presence may be an indicator of redox active processes that impact other species in the water samples and produce compounds of health concern.

Acetyl tributyl citrate (ATBC) was a widely detected suspect compound (51 detections/270 samples) that was positively correlated with ER agonist activity in the summer sample set. ATBC has diverse commercial applications including use as a plasticizer in PVC piping and as an ingredient in cosmetics and nail polish. This annotation was confirmed using a standard and the concentrations of ATBC were retrospectively quantified, with a maximum concentration of 1.7 μg/L. The average concentration of ATBC observed in the 51 samples with quantifiable concentrations was 316 ng/L. The significance of the ATBC detections for human health are unclear. It is approved as a food contact additive by the US Food and Drug Administration, and it is often used in plastics like polyvinyl chloride (PVC) to replace phthalate substances with known endocrine disrupting effects. Some studies have, however, called into question the presumed safety of ATBC. For example, ATBC exhibited both estrogen receptor antagonist activity and androgen receptor agonist activity when tested using in vitro test methods under the Organization for Economic Co-operation and Development (OECD) guidelines for identifying EDCs.[90] In zebrafish, ATBC was associated with endocrine disruption of the GH/IGFs axis, a major neuroendocrine pathway. This activity was potentially related to oxidative stress, as impacts of ATBC were alleviated with treatment of an antioxidant.[91] Among alternative plasticizers to phthalates, ATBC was found to induce the most significant activation of the steroid and xenobiotic receptor (SXR) which regulates expression of cytochrome P450 3A4 (CYP3A4) in the liver and intestines.[92] CYP3A4 in turn affects the metabolism of contaminants and endogenous steroid hormones suggesting that the compound may affect estrogen receptor levels in vivo.

A fragrance compound, 4-oxoisophorone, was commonly detected in SM and LA, was prioritized by the group hotspot filter, and was confirmed with a standard. The compound has been detected in a number of European rivers including the Lippe[93] and the Ruhr.[94] Diphenyl sulfone (DPS) was another widely detected compound with 61% of samples exceeding the upper 95% confidence interval around method blanks. DPS was confirmed with a standard and could be retrospectively quantified in 5 samples (BW-09W, MC-10W, MC-15W, YT-13W, YT-15W), with concentrations ranging from 3.9−16 ng/L. DPS exhibited a significant Pearson's correlation coefficient with estrogen receptor agonism ($q < 0.05$) in the winter samples when concentrations were highest. An important commercial application of DPS is as a sensitizer in ink printing, and it is found in a variety of papers at mg/g levels.[95] During recycling DPS can be emitted to the environment in wastewater from recycling plants or it can be incorporated into recycled paper products. The occurrence of DPS in recycled papers has extended to its detection in recycled toilet paper and wastewater treatment plant effluents.[96] To our knowledge, this is the first report of DPS detections in drinking water supplies.

The foregoing analysis and discussion have focused on less studied chemicals with potential relationships to breast cancer including BTSA, ATBC, diphenyl sulfone, and oxoisophorone, and it highlighted the potential confounding role of estrogen receptor antagonists including PAHs and their derivatives. We also screened our prioritized compound list against a recently published list of 921 mammary gland carcinogens, promoters of estrogen and progesterone synthesis, or ER agonists.[97] A total of 18 of our 115 unique LC-prioritized compounds and 5 out of 53 of our unique GC prioritized compounds appeared on the mammary carcinogen list. Table S22 reproduces the information in Table S1 of Kay et al. for these priority compounds. The compounds can be grouped into four classes: hormones (natural or synthetic), phthalates, pesticides, and other as shown by shading in Table S22. Five compounds from Table S22 were also measured as part of our targeted analysis: 17β-Estradiol, 17α-Ethinylestradiol, Norgestrel, DDT, and DEHP. None of these compounds except DEHP were above their respective MDLs in any sample, which were 1.3, 2.6, 0.19, 0.5, and 9.5 ng/L, respectively. DEHP (analyzed in combination with di-n-octyl phthalate) was detected above its MDL in many samples, but was below the MQL of 1000 ng/L in all samples. The appearance of these compounds on our prioritized list suggests that they may be capable of ER activation even at ultratrace levels. The finding that 13.7% of the compounds on our priority list are included on a list of compounds potentially related to breast cancer suggests that the prioritization scheme applied here was generally effective at isolating compounds worthy of further examination.

Although none of the water samples tested exceeded published drinking water related EBTs for ER agonism, and none exceeded US EPA MCLs for any compound measured (at the time of sampling), there is still evidence that prioritized compounds, potentially related to breast cancer, were present. Among these are compounds rarely or never previously identified in drinking water, compounds with known relationship to breast cancer development, and prioritized features that could not be confidently annotated. No specific identified chemicals emerged as clear drivers of estrogen receptor agonism or variations in regional breast cancer incidence. Future studies can extend upon this work by (i) further expanding the chemical space covered by the analytical methods to encompass the full set of breast cancer relevant chemicals identified in recent work,[97] (ii) adopting methods such as chemical fractionation to better account for the fact that chemicals in mixtures exert bioactivity at levels below lowest effects levels for individual compounds,[98] and (iii) adopting broader geographic coverage to investigate regional differences in the concentrations of compounds exceeding MQLs (e.g., diethyl phthalate, pyrene, simazine, pentachlorophenol, 4-nitrophenol, PCB 118, PFOS, and 9 other PFAS), and to better match hotspot and nonhotspot zones of breast cancer incidence.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.4c12204.

Additional method information and water system map-1 Figure (PDF)

List of materials and standards, water system characteristics, instrument settings, alignment and annotation parameters, and all target and aligned nontarget data-23 Tables (XLSX)

■ AUTHOR INFORMATION

Corresponding Author

Thomas M. Young − *Department of Civil and Environmental Engineering, University of California, Davis, California 95616, United States;* orcid.org/0000-0001-7217-4753; Phone: 530-754-9399; Email: tyoung@ucdavis.edu

Authors

Gabrielle P. Black − *Department of Civil and Environmental Engineering, University of California, Davis, California 95616, United States;* orcid.org/0000-0002-1578-742X

Berkley N. Anderson − *Department of Civil and Environmental Engineering, University of California, Davis, California 95616, United States;* orcid.org/0000-0003-2554-1666

Luann Wong − *Department of Civil and Environmental Engineering, University of California, Davis, California 95616, United States*

Christopher P. Alaimo − *Department of Civil and Environmental Engineering, University of California, Davis, California 95616, United States*

Guochun He − *Department of Environmental Toxicology, University of California, Davis, California 95616, United States*

Michael S. Denison − *Department of Environmental Toxicology, University of California, Davis, California 95616, United States*

Deborah H. Bennett − *Department of Public Health Sciences, University of California, Davis, California 95616, United States;* orcid.org/0000-0001-6698-2316

Daniel Tancredi − *Department of Public Health Sciences, University of California, Davis, California 95616, United States*

Blythe Durbin-Johnson − *Department of Public Health Sciences, University of California, Davis, California 95616, United States*

Bruce D. Hammock − *Department of Entomology and Cancer Center, University of California, Davis, California 95616, United States;* orcid.org/0000-0003-1408-8317

Pujeeta Chowdhary − *Breast Cancer Prevention Partners, San Francisco, California 94109, United States*

Rainbow Rubin − *Breast Cancer Prevention Partners, San Francisco, California 94109, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.est.4c12204

Notes

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■ REFERENCES

(1) Lichtenstein, P.; Holm, N. V.; Verkasalo, P. K.; Iliadou, A.; Kaprio, J.; Koskenvuo, M.; Pukkala, E.; Skytthe, A.; Hemminki, K. Environmental and Heritable Factors in the Causation of Cancer — Analyses of Cohorts of Twins from Sweden, Denmark, and Finland. *N. Engl. J. Med.* **2000**, *343* (2), 78−85.

(2) Public Health Institute California Breast Cancer Mapping Project: Identifying Areas of Concern in California; California Breast Cancer Research Program : 2012.

(3) Parise, C. A.; Bauer, K. R.; Brown, M. M.; Caggiano, V. Breast Cancer Subtypes as Defined by the Estrogen Receptor (ER), Progesterone Receptor (PR), and the Human Epidermal Growth Factor Receptor 2 (HER2) among Women with Invasive Breast Cancer in California, 1999−2004. *Breast J.* **2009**, *15* (6), 593−602.

(4) Howlader, N.; Noone, A.-M.; Yu, M.; Cronin, K. A. Use of Imputed Population-based Cancer Registry Data as a Method of Accounting for Missing Information: Application to Estrogen Receptor Status for Breast Cancer. *Am. J. Epidemiol.* **2012**, *176* (4), 347−356.

(5) Lecomte, S.; Habauzit, D.; Charlier, T. D.; Pakdel, F. Emerging Estrogenic Pollutants in the Aquatic Environment and Breast Cancer. *Genes* **2017**, *8* (9), 229.

(6) Rutkowska, A. Z.; Szybiak, A.; Serkies, K.; Rachon, D. Endocrine disrupting chemicals as potential risk factor for estrogen-dependent cancers. *Pol. Arch. Med. Wewn.* **2016**, *126* (7−8), 562−570.

(7) Treviño, L. S.; Wang, Q.; Walker, C. L. Hypothesis: Activation of rapid signaling by environmental estrogens and epigenetic reprogramming in breast cancer. *Reprod. Toxicol.* **2015**, *54*, 136−140.

(8) *Endocrine Society Common EDCs and Where They Are Found.* https://www.endocrine.org/topics/edc/what-edcs-are/common-edcs (accessed Jan 24, 2025).

(9) Bergman, Å.; Heindel, J.; Jobling, S.; Kidd, K.; Zoeller, R. T. State-of-the-science of endocrine disrupting chemicals, 2012. *Toxicol. Lett.* **2012**, *211*, S3.

(10) Davis, D. L.; Bradlow, H. L.; Wolff, M.; Woodruff, T.; Hoel, D. G.; Antonculver, H. Medical Hypothesis - Xenoestrogens As Preventable Causes of Breast-Cancer. *Environ. Health Perspect.* **1993**, *101* (5), 372−377.

(11) Gao, H.; Yang, B. J.; Li, N.; Feng, L. M.; Shi, X. Y.; Zhao, W. H.; Liu, S. J. Bisphenol A and Hormone-Associated Cancers: Current Progress and Perspectives. *Medicine* **2015**, *94* (1), No. e211.

(12) McLachlan, J. A. Environmental signaling: from environmental estrogens to endocrine-disrupting chemicals and beyond. *Andrology* **2016**, *4* (4), 684−694.

(13) Safe, S. H. Interactions between hormones and chemicals in breast cancer. *Annu. Rev. Pharmacol. Toxicol.* **1998**, *38*, 121−158.

(14) Kuch, H. M.; Ballschmiter, K. Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per liter range. *Environ. Sci. Technol.* **2001**, *35* (15), 3201−3206.

(15) Rahman, M. F.; Yanful, E. K.; Jasim, S. Y. Endocrine disrupting compounds (EDCs) and pharmaceuticals and personal care products (PPCPs) in the aquatic environment: implications for the drinking water industry and global environmental health. *J. Water Health* **2009**, *7* (2), 224−243.

(16) Rodgers, K. M.; Udesky, J. O.; Rudel, R. A.; Brody, J. G. Environmental chemicals and breast cancer: An updated review of epidemiological literature informed by biological mechanisms. *Environ. Res.* **2018**, *160*, 152−182.

(17) Rudel, R. A.; Attfield, K. R.; Schifano, J. N.; Brody, J. G. Chemicals causing mammary gland tumors in animals signal new directions for epidemiology, chemicals testing, and risk assessment for breast cancer prevention. *Cancer* **2007**, *109* (12), 2635−2666.

(18) Newton, S. R.; McMahen, R. L.; Sobus, J. R.; Mansouri, K.; Williams, A. J.; McEachran, A. D.; Strynar, M. J. Suspect screening and non-targeted analysis of drinking water using point-of-use filters. *Environ. Pollut.* **2018**, *234*, 297−306.

(19) Richardson, S. D.; Plewa, M. J.; Wagner, E. D.; Schoeny, R.; Demarini, D. M. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. *Mutat. Res.* **2007**, *636* (1−3), 178−242.

(20) Li, X.-F.; Mitch, W. A. Drinking Water Disinfection Byproducts (DBPs) and Human Health Effects: Multidisciplinary Challenges and Opportunities. *Environ. Sci. Technol.* **2018**, *52* (4), 1681−1689.

(21) IARC *Some Drinking-Water Disinfectants and Contaminants, including Arsenic*, **2004**.

(22) US Environmental Protection Agency Occurrence Data from the Unregulated Contaminant Monitoring Rule. https://www.epa.gov/dwucmr/occurrence-data-unregulated-contaminant-monitoring-rule#:~:text=UCMR%201%20was%20published%20in,for%201%2C3%2Ddichloropropenehttps://www.epa.gov/dwucmr/occurrence-data-unregulated-contaminant-monitoring-rule#:~:text=UCMR%201%20was%20published%20in,for%201%2C3%2Ddichloropropene (accessed Jan 13, 2025).

(23) McKelvey, W.; Brody, J. G.; Aschengrau, A.; Swartz, C. H. Association between residence on Cape Cod, Massachusetts, and breast cancer. *Ann. Epidemiol.* **2004**, *14* (2), 89−94.

(24) O'Leary, E. S.; Vena, J. E.; Freudenheim, J. L.; Brasure, J. Pesticide exposure and risk of breast cancer: a nested case-control study of residentially stable women living on Long Island. *Environ. Res.* **2004**, *94* (2), 134−144.

(25) Teitelbaum, S. L.; Gammon, M. D.; Britton, J. A.; Neugut, A. I.; Levin, B.; Stellman, S. D. Reported residential pesticide use and breast cancer risk on Long Island, New York. *Am. J. Epidemiol.* **2007**, *165* (6), 643−651.

(26) Brody, J. G.; Aschengrau, A.; McKelvey, W.; Swartz, C. H.; Kennedy, T.; Rudel, R. A. Breast cancer risk and drinking water contaminated by wastewater: a case control study. *Environ. Health* **2006**, *5* (1), No. 28.

(27) Rudel, R. A.; Melly, S. J.; Geno, P. W.; Sun, G.; Brody, J. G. Identification of Alkylphenols and Other Estrogenic Phenolic Compounds in Wastewater, Septage, and Groundwater on Cape Cod, Massachusetts. *Environ. Sci. Technol.* **1998**, *32* (7), 861−869.

(28) Brody, J. G.; Aschengrau, A.; McKelvey, W.; Rudel, R. A.; Swartz, C. H.; Kennedy, T. Breast cancer risk and historical exposure to pesticides from wide-area applications assessed with GIS. *Environ. Health Perspect* **2004**, *112* (8), 889−897.

(29) Zwart, N.; Nio, S. L.; Houtman, C. J.; de Boer, J.; Kool, J.; Hamers, T.; Lamoree, M. H. High-Throughput Effect-Directed Analysis Using Downscaled in Vitro Reporter Gene Assays To Identify Endocrine Disruptors in Surface Water. *Environ. Sci. Technol.* **2018**, *52* (7), 4367−4377.

(30) Hong, S.; Lee, J.; Cha, J.; Gwak, J.; Khim, J. S. Effect-Directed Analysis Combined with Nontarget Screening to Identify Unmonitored Toxic Substances in the Environment. *Environ. Sci. Technol.* **2023**, *57* (48), 19148−19155.

(31) Eide, I.; Neverdal, G.; Thorvaldsen, B.; Arneberg, R.; Grung, B.; Kvalheim, O. M. Toxicological evaluation of complex mixtures: fingerprinting and multivariate analysis. *Environ. Toxicol. Pharmacol.* **2004**, *18* (2), 127−133.

(32) Young, T. M.; Black, G. P.; Wong, L.; Bloszies, C. S.; Fiehn, O.; He, G.; Denison, M. S.; Vogel, C. F. A.; Durbin-Johnson, B. Identifying Toxicologically Significant Compounds in Urban Wildfire Ash Using In Vitro Bioassays and High-Resolution Mass Spectrometry. *Environ. Sci. Technol.* **2021**, *55* (6), 3657−3667.

(33) Black, G. P.; He, G.; Denison, M. S.; Young, T. M. Using Estrogenic Activity and Nontargeted Chemical Analysis to Identify Contaminants in Sewage Sludge. *Environ. Sci. Technol.* **2021**, *55*, 6729−6739.

(34) Tian, Z.; Zhao, H.; Peter, K. T.; Gonzalez, M.; Wetzel, J.; Wu, C.; Hu, X.; Prat, J.; Mudrock, E.; Hettinger, R.; Cortina, A. E.; Biswas, R. G.; Kock, F. V. C.; Soong, R.; Jenne, A.; Du, B.; Hou, F.; He, H.; Lundeen, R.; Gilbreath, A.; Sutton, R.; Scholz, N. L.; Davis, J. W.; Dodd, M. C.; Simpson, A.; McIntyre, J. K.; Kolodziej, E. P. A ubiquitous tire rubber−derived chemical induces acute mortality in coho salmon. *Science* **2021**, *371* (6525), 185.

(35) Pochiraju, S. S.; Linden, K.; Gu, A. Z.; Rosenblum, J. Development of a separation framework for effects-based targeted and non-targeted toxicological screening of water and wastewater. *Water Res.* **2020**, *170*, No. 115289.

(36) König, M.; Escher, B. I.; Neale, P. A.; Krauss, M.; Hilscherová, K.; Novák, J.; Teodorović, I.; Schulze, T.; Seidensticker, S.; Kamal Hashmi, M. A.; Ahlheim, J.; Brack, W. Impact of untreated wastewater on a major European river evaluated with a combination of in vitro bioassays and chemical analysis. *Environ. Pollut.* **2017**, *220*, 1220−1230.

(37) Lee, J.; Hong, S.; Kim, T.; Lee, C.; An, S.-A.; Kwon, B.-O.; Lee, S.; Moon, H.-B.; Giesy, J. P.; Khim, J. S. Multiple Bioassays and Targeted and Nontargeted Analyses to Characterize Potential Toxicological Effects Associated with Sediments of Masan Bay: Focusing on AhR-Mediated Potency. *Environ. Sci. Technol.* **2020**, *54* (7), 4443−4454.

(38) Peter, K. T.; Phillips, A. L.; Knolhoff, A. M.; Gardinali, P. R.; Manzano, C. A.; Miller, K. E.; Pristner, M.; Sabourin, L.; Sumarah, M. W.; Warth, B.; Sobus, J. R. Nontargeted Analysis Study Reporting Tool: A Framework to Improve Research Transparency and Reproducibility. *Anal. Chem.* **2021**, *93* (41), 13870−13879.

(39) Kern, S.; Fenner, K.; Singer, H. P.; Schwarzenbach, R. P.; Hollender, J. Identification of Transformation Products of Organic Contaminants in Natural Waters by Computer-Aided Prediction and High-Resolution Mass Spectrometry. *Environ. Sci. Technol.* **2009**, *43* (18), 7039−7046.

(40) Moschet, C.; Lew, B. M.; Hasenbein, S.; Anumol, T.; Young, T. M. LC- and GC-QTOF-MS as Complementary Tools for a Comprehensive Micropollutant Analysis in Aquatic Systems. *Environ. Sci. Technol.* **2017**, *51* (3), 1553−1561.

(41) Gray, J. M.; Rasanayagam, S.; Engel, C.; Rizzo, J. State of the evidence 2017: an update on the connection between breast cancer and the environment. *Environ. Health* **2017**, *16* (1), No. 94.

(42) Hattaway, M. E.; Black, G. P.; Young, T. M. Batch correction methods for nontarget chemical analysis data: application to a municipal wastewater collection system. *Anal. Bioanal. Chem.* **2023**, *415* (7), 1321−1331.
(43) Anderson, B. N.; Black, G. P.; Young, T. M. Disinfection Byproducts in Drinking Water from the Tap: Variability in Household Calculated Additive Toxicity (CAT). *ACS ES&T Water* **2024**, *4*, 3532−3539.
(44) Black, G. P.; Wong, L.; Young, T. M. Uptake of Per- and Polyfluorinated Alkyl Substances by Dry Farmed Oats Following the Agricultural Application of Biosolids and Compost.*Environ. Sci.: Process. Impacts* 2025, in press. DOI: 10.1039/D4EM00502C.
(45) Brennan, J. C.; Bassal, A.; He, G.; Denison, M. S. Development of a recombinant human ovarian (BG1) cell line containing estrogen receptor alpha and beta for improved detection of estrogenic/antiestrogenic chemicals. *Environ. Toxicol. Chem.* **2016**, *35*, 91−100.
(46) Rogers, J. M.; Denison, M. S. Recombinant cell bioassays for endocrine disruptors: Development of a stably transfected human ovarian cell line for the detection of estrogenic and anti-estrogenic chemicals. *In Vitro Mol. Toxicol.* **2000**, *13* (1), 67−82.
(47) Tsugawa, H.; Cajka, T.; Kind, T.; Ma, Y.; Higgins, B.; Ikeda, K.; Kanazawa, M.; VanderGheynst, J.; Fiehn, O.; Arita, M. MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nat. Methods* **2015**, *12* (6), 523−526.
(48) Koelmel, J. P.; Xie, H.; Price, E. J.; Lin, E. Z.; Manz, K. E.; Stelben, P.; Paige, M. K.; Papazian, S.; Okeme, J.; Jones, D. P.; Barupal, D.; Bowden, J. A.; Rostkowski, P.; Pennell, K. D.; Nikiforov, V.; Wang, T.; Hu, X.; Lai, Y.; Miller, G. W.; Walker, D. I.; Martin, J. W.; Godri Pollitt, K. J. An actionable annotation scoring framework for gas chromatography-high-resolution mass spectrometry. *Exposome* **2022**, *2* (1), No. osac007.
(49) Schymanski, E. L.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H. P.; Hollender, J. Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. *Environ. Sci. Technol.* **2014**, *48* (4), 2097−2098.
(50) Zou, H.; Hastie, T. Regularization and variable selection via the elastic net. *J. R Stat. Soc. Ser. B* **2005**, *67*, 301−320.
(51) Ritchie, M. E.; Phipson, B.; Wu, D.; Hu, Y.; Law, C. W.; Shi, W.; Smyth, G. K. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* **2015**, *43* (7), e47.
(52) Tsugawa, H.; Kind, T.; Nakabayashi, R.; Yukihira, D.; Tanaka, W.; Cajka, T.; Saito, K.; Fiehn, O.; Arita, M. Hydrogen Rearrangement Rules: Computational MS/MS Fragmentation and Structure Elucidation Using MS-FINDER Software. *Anal. Chem.* **2016**, *88* (16), 7946−7958.
(53) Dührkop, K.; Shen, H.; Meusel, M.; Rousu, J.; Böcker, S. Searching molecular structure databases with tandem mass spectra using CSI:FingerID. *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112* (41), 12580−12585.
(54) Dührkop, K.; Fleischauer, M.; Ludwig, M.; Aksenov, A. A.; Melnik, A. V.; Meusel, M.; Dorrestein, P. C.; Rousu, J.; Bocker, S. SIRIUS 4: a rapid tool for turning tandem mass spectra into metabolite structure information. *Nat. Methods* **2019**, *16* (4), 299.
(55) Mansouri, K.; Grulke, C. M.; Judson, R. S.; Williams, A. J. OPERA models for predicting physicochemical properties and environmental fate endpoints. *J. Cheminf.* **2018**, *10* (1), No. 10.
(56) Benfenati, E.; Manganaro, A.; Gini, G.*VEGA-QSAR: AI Inside A Platform for Predictive Toxicology*, CEUR Workshop Proceedings; 2013; Vol. 1107, pp 21−28.
(57) Aalizadeh, R.; Alygizakis, N. A.; Schymanski, E. L.; Krauss, M.; Schulze, T.; Ibáñez, M.; McEachran, A. D.; Chao, A.; Williams, A. J.; Gago-Ferrero, P.; Covaci, A.; Moschet, C.; Young, T. M.; Hollender, J.; Slobodnik, J.; Thomaidis, N. S. Development and Application of Liquid Chromatographic Retention Time Indices in HRMS-Based Suspect and Nontarget Screening. *Anal. Chem.* **2021**, *93* (33), 11601−11611.
(58) van der Oost, R.; Sileno, G.; Suárez-Muñoz, M.; Nguyen, M. T.; Besselink, H.; Brouwer, A. SIMONI (Smart Integrated Monitoring) as a novel bioanalytical strategy for water quality assessment: Part I−model design and effect-based trigger values. *Environ. Toxicol. Chem.* **2017**, *36* (9), 2385−2399.
(59) Escher, B. I.; Ait-Aissa, S.; Behnisch, P. A.; Brack, W.; Brion, F.; Brouwer, A.; Buchinger, S.; Crawford, S. E.; Du Pasquier, D.; Hamers, T.; Hettwer, K.; Hilscherová, K.; Hollert, H.; Kase, R.; Kienle, C.; Tindall, A. J.; Tuerk, J.; van der Oost, R.; Vermeirssen, E.; Neale, P. A. Effect-based trigger values for in vitro and in vivo bioassays performed on surface water extracts supporting the environmental quality standards (EQS) of the European Water Framework Directive. *Sci. Total Environ.* **2018**, *628−629*, 748−765.
(60) Brand, W.; de Jongh, C. M.; van der Linden, S. C.; Mennes, W.; Puijker, L. M.; van Leeuwen, C. J.; van Wezel, A. P.; Schriks, M.; Heringa, M. B. Trigger values for investigation of hormonal activity in drinking water and its sources using CALUX bioassays. *Environ. Int.* **2013**, *55*, 109−118.
(61) Been, F.; Pronk, T.; Louisse, J.; Houtman, C.; van der Velden-Slootweg, T.; van der Oost, R.; Dingemans, M. M. L. Development of a framework to derive effect-based trigger values to interpret CALUX data for drinking water quality. *Water Res.* **2021**, *193*, No. 116859.
(62) Escher, B. I.; Neale, P. A.; Leusch, F. D. L. Effect-based trigger values for in vitro bioassays: Reading across from existing water quality guideline values. *Water Res.* **2015**, *81*, 137−148.
(63) Smalling, K. L.; Bradley, P. M.; Romanok, K. M.; Elliot, S. M.; de Lambert, J.; Focazio, M. J.; Gordon, S. E.; Gray, J. L.; Kanagy, L. K.; Hladik, M. L.; Loftin, K. A.; McCleskey, R. B.; Medlock-Kakaley, E. K.; Cardon, M. C.; Evans, N.; Weis, C. P. Exposures and potential health implications of contaminant mixtures in linked source water, finished drinking water, and tapwater from public-supply drinking water systems in Minneapolis/St. Paul area, USA. *Environ. Sci.* **2023**, *9* (7), 1813−1828.
(64) Smalling, K. L.; Romanok, K. M.; Bradley, P. M.; Hladik, M. L.; Gray, J. L.; Kanagy, L. K.; McCleskey, R. B.; Stavreva, D. A.; Alexander-Ozinskas, A. K.; Alonso, J.; Avila, W.; Breitmeyer, S. E.; Bustillo, R.; Gordon, S. E.; Hager, G. L.; Jones, R. R.; Kolpin, D. W.; Newton, S.; Reynolds, P.; Sloop, J.; Ventura, A.; Von Behren, J.; Ward, M. H.; Solomon, G. M. Mixed contaminant exposure in tapwater and the potential implications for human-health in disadvantaged communities in California. *Water Res.* **2024**, *267*, No. 122485.
(65) Martin, M. B.; Reiter, R.; Pham, T.; Avellanet, Y. R.; Camara, J.; Lahm, M.; Pentecost, E.; Pratap, K.; Gilmore, B. A.; Divekar, S.; Dagata, R. S.; Bull, J. L.; Stoica, A. Estrogen-like activity of metals in Mcf-7 breast cancer cells. *Endocrinology* **2003**, *144* (6), 2425−2436.
(66) PFAS National Primary Drinking Water Regulation. *40 CFR Parts 141 and 142*; US Environmental Protection Agency, 2024.
(67) Jiang, P.; Qiu, J.; Gao, Y.; Stefan, M. I.; Li, X.-F. Nontargeted identification and predicted toxicity of new byproducts generated from UV treatment of water containing micropollutant 2-mercaptobenzothiazole. *Water Res.* **2021**, *188*, No. 116542.
(68) Kloepfer, A.; Gnirss, R.; Jekel, M.; Reemtsma, T. Occurrence of benzothiazoles in municipal wastewater and their fate in biological treatment. *Water Sci. Technol.* **2004**, *50* (5), 203−208.
(69) Kloepfer, A.; Jekel, M.; Reemtsma, T. Occurrence, sources, and fate of benzothiazoles in municipal wastewater treatment plants. *Environ. Sci. Technol.* **2005**, *39* (10), 3792−3798.
(70) Hinnenkamp, V.; Balsaa, P.; Schmidt, T. C. Target, suspect and non-target screening analysis from wastewater treatment plant effluents to drinking water using collision cross section values as additional identification criterion. *Anal. Bioanal. Chem.* **2022**, *414* (1), 425−438.
(71) Diera, T.; Thomsen, A. H.; Tisler, S.; Karlby, L. T.; Christensen, P.; Rosshaug, P. S.; Albrechtsen, H.-J.; Christensen, J. H. A non-target screening study of high-density polyethylene pipes revealed rubber compounds as main contaminant in a drinking water distribution system. *Water Res.* **2023**, *229*, No. 119480.
(72) Jin, J.; Kurobe, T.; Ramírez-Duarte, W. F.; Bolotaolo, M. B.; Lam, C. H.; Pandey, P. K.; Hung, T.-C.; Stillway, M. E.; Zweig, L.; Caudill, J.; Lin, L.; Teh, S. J. Sub-lethal effects of herbicides

penoxsulam, imazamox, fluridone and glyphosate on Delta Smelt (Hypomesus transpacificus). *Aquat. Toxicol.* **2018**, *197*, 79−88.

(73) Jin, J.; Kurobe, T.; Hammock, B. G.; Lam, C. H.; Lin, L.; Teh, S. J. Toxic effects of fluridone on early developmental stages of Japanese Medaka (Oryzias latipes). *Sci. Total Environ.* **2020**, *700*, No. 134495.

(74) Lam, C. H.; Kurobe, T.; Lehman, P. W.; Berg, M.; Hammock, B. G.; Stillway, M. E.; Pandey, P. K.; Teh, S. J. Toxicity of herbicides to cyanobacteria and phytoplankton species of the San Francisco Estuary and Sacramento-San Joaquin River Delta, California, USA. *J. Environ. Sci. Health, Part A* **2020**, *55* (2), 107−118.

(75) California Environmental Protection Agency. *Safe Drinking Water and Toxic Enforcement Act of 1986, Notice to Interested Parties, Chemicals Listed Effective July 15, 2016 as Known to the State of California to Cause Reproductive Toxicity: Atrazine, Propazine, Simazine and their Chlorometabolites DACT, DEA and DIA*; Office of Environmental Health Hazard Assessment, 2016.

(76) Zhao, H. N.; Hu, X.; Gonzalez, M.; Rideout, C. A.; Hobby, G. C.; Fisher, M. F.; McCormick, C. J.; Dodd, M. C.; Kim, K. E.; Tian, Z.; Kolodziej, E. P. Screening p-Phenylenediamine Antioxidants, Their Transformation Products, and Industrial Chemical Additives in Crumb Rubber and Elastomeric Consumer Products. *Environ. Sci. Technol.* **2023**, *57* (7), 2779−2791.

(77) Williams, A. J.; Grulke, C. M.; Edwards, J.; McEachran, A. D.; Mansouri, K.; Baker, N. C.; Patlewicz, G.; Shah, I.; Wambaugh, J. F.; Judson, R. S.; Richard, A. M. The CompTox Chemistry Dashboard: a community data resource for environmental chemistry. *J. Cheminf.* **2017**, *9* (1), No. 61.

(78) US Environmental Protection Agency. CompTox Chemicals Dashboard. https://comptox.epa.gov/dashboard/ (accessed Sept, 20).

(79) Safe, S.; Wang, F.; Porter, W.; Duan, R.; McDougal, A. Ah receptor agonists as endocrine disruptors: antiestrogenic activity and mechanisms. *Toxicol. Lett.* **1998**, *102-103*, 343−347.

(80) Hockings, J. K.; Thorne, P. A.; Kemp, M. Q.; Sherif, S. M.; Selmin, O.; Romagnolo, D. F. The ligand status of the aromatic hydrocarbon receptor modulates transcriptional activation of BRCA-1 promoter by estrogen. *Cancer Res.* **2006**, *66* (4), 2224−2232.

(81) Kamelia, L.; Louisse, J.; De Haan, L.; Maslowska-Gornicz, A.; Ketelslegers, H. B.; Brouwer, A.; Rietjens, I.; Boogaard, P. J. The Role of Endocrine and Dioxin-Like Activity of Extracts of Petroleum Substances in Developmental Toxicity as Detected in a Panel of CALUX Reporter Gene Assays. *Toxicol. Sci.* **2018**, *164* (2), 576−591.

(82) Tarnow, P.; Tralau, T.; Luch, A. Chemical activation of estrogen and aryl hydrocarbon receptor signaling pathways and their interaction in toxicology and metabolism. *Expert Opin. Drug Metab. Toxicol.* **2019**, *15* (3), 219−229.

(83) Arcaro, K. F.; O'Keefe, P. W.; Yang, Y.; Clayton, W.; Gierthy, J. F. Antiestrogenicity of environmental polycyclic aromatic hydrocarbons in human breast cancer cells. *Toxicology* **1999**, *133* (2−3), 115−127.

(84) Arcaro, K. F.; Gierthy, J. F.; Mackerer, C. R. Antiestrogenicity of clarified slurry oil and two crude oils in a human breast-cancer cell assay. *J. Toxicol. Environ. Health, Part A* **2001**, *62* (7), 505−521.

(85) Ssempebwa, J. C.; Carpenter, D. O.; Yilmaz, B.; DeCaprio, A. P.; O'Hehir, D. J.; Arcaro, K. F. Waste crankcase oil: An environmental contaminant with potential to modulate estrogenic responses. *J. Toxicol. Environ. Health, Part A* **2004**, *67* (14), 1081−1094.

(86) Xu, X.; Xiao, R. Y.; Dionysiou, D. D.; Spinney, R.; Fu, T.; Li, Q.; Wang, Z. J.; Wang, D. H.; Wei, Z. S. Kinetics and mechanisms of the formation of chlorinated and oxygenated polycyclic aromatic hydrocarbons during chlorination. *Chem. Eng. J.* **2018**, *351*, 248−257.

(87) Doi, A. M.; Irwin, R. D.; Bucher, J. R. Influence of Functional Group Substitutions on the Carcinogenicity of Anthraquinone in Rats and Mice: Analysis of Long-Term Bioassays by the National Cancer Institute and the National Toxicology Program. *J. Toxicol. Environ. Health, Part B* **2005**, *8* (2), 109−126.

(88) Jørgensen, B. B. Sulfur Biogeochemical Cycle of Marine Sediments. *Geochem. Perspect.* **2021**, *10* (2), 145−307.

(89) Badoil, L.; Benanou, D. Characterization of volatile and semivolatile compounds in waste landfill leachates using stir bar sorptive extraction−GC/MS. *Anal. Bioanal. Chem.* **2009**, *393* (3), 1043−1054.

(90) Park, J.; Park, C.; Gye, M. C.; Lee, Y. Assessment of endocrine-disrupting activities of alternative chemicals for bis(2-ethylhexyl)-phthalate. *Environ. Res.* **2019**, *172*, 10−17.

(91) Yun, K.; Jeon, H.; Lee, J.; Kho, Y.; Ji, K. Effects of two alternative plasticizers on the growth hormone-related endocrine system, neurodevelopment, and oxidative stress of zebrafish larvae. *Environ. Pollut.* **2024**, *341*, No. 122947.

(92) Takeshita, A.; Igarashi-Migitaka, J.; Nishiyama, K.; Takahashi, H.; Takeuchi, Y.; Koibuchi, N. Acetyl Tributyl Citrate, the Most Widely Used Phthalate Substitute Plasticizer, Induces Cytochrome P450 3A through Steroid and Xenobiotic Receptor. *Toxicol. Sci.* **2011**, *123* (2), 460−470.

(93) Dsikowitzky, L.; Schwarzbauer, J.; Kronimus, A.; Littke, R. The anthropogenic contribution to the organic load of the Lippe River (Germany). Part I: qualitative characterisation of low-molecular weight organic compounds. *Chemosphere* **2004**, *57* (10), 1275−1288.

(94) Schwanen, C. A.; Schwarzbauer, J. Structural Diversity of Organic Contaminants in a meso-scaled River System. *Water, Air, Soil Pollut.* **2022**, *233* (2), No. 33.

(95) Eckardt, M.; Kubicova, M.; Tong, D.; Simat, T. J. Determination of color developers replacing bisphenol A in thermal paper receipts using diode array and Corona charged aerosol. detection-A German market analysis 2018/2019. *J. Chromatogr. A* **2020**, *1609*, No. 460437.

(96) Yoshinari, Y.; Terasaki, M. Recycled toilet paper sensitizers, a novel source of contamination in rivers. *Environ. Chem. Lett.* **2024**, *22* (2), 485−489.

(97) Kay, J. E.; Brody, J. G.; Schwarzman, M.; Rudel, R. A. Application of the Key Characteristics Framework to Identify Potential Breast Carcinogens Using Publicly Available in Vivo, in Vitro, and in Silico Data. *Environ. Health Perspect.* **2024**, *132* (1), No. 017002.

(98) Braun, G.; Herberth, G.; Krauss, M.; König, M.; Wojtysiak, N.; Zenclussen, A. C.; Escher, B. I. Neurotoxic mixture effects of chemicals extracted from blood of pregnant women. *Science* **2024**, *386* (6719), 301−309.