Environmental Science & Technology

pubs.acs.org/est

Article

# Combining Nontargeted Analysis with Computer-Based Hazard Comparison Approaches to Support Prioritization of Unregulated Organic Contaminants in Biosolids

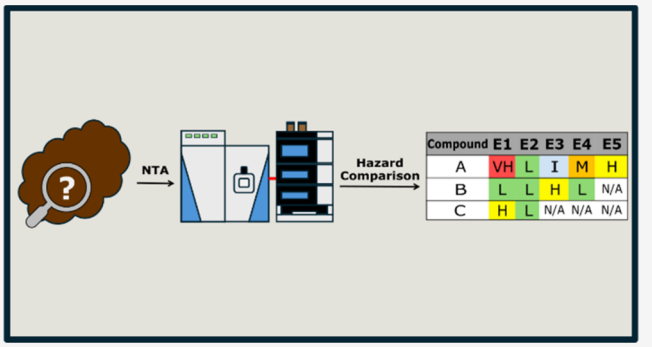
Matthew N. Newmeyer, Qinfan Lyu, Jon R. Sobus, Antony J. Williams, Keeve E. Nachman, and Carsten Prasse*

Cite This: *Environ. Sci. Technol.* 2024, 58, 12135−12146

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

**ABSTRACT:** Biosolids are a byproduct of wastewater treatment that can be beneficially applied to agricultural land as a fertilizer. While U.S. regulations limit metals and pathogens in biosolids intended for land applications, no organic contaminants are currently regulated. Novel techniques can aid in detection, evaluation, and prioritization of biosolid-associated organic contaminants (BOCs). For example, nontargeted analysis (NTA) can detect a broad range of chemicals, producing data sets representing thousands of measured analytes that can be combined with computational toxicological tools to support human and ecological hazard assessment and prioritization. We combined NTA with a computer-based tool from the U.S. EPA, the Cheminformatics Hazard Comparison Module (HCM), to identify and prioritize BOCs present in U.S. and Canadian biosolids ($n$ = 16). Four-hundred fifty-one features were detected in at least 80% of samples, with identities of 92 compounds confirmed or assigned probable structures. These compounds were primarily categorized as endogenous compounds, pharmaceuticals, industrial chemicals, and fragrances. Examples of top prioritized compounds were p-cresol and chlorophene, based on human health end points, and fludioxonil and triclocarban, based on ecological health end points. Combining NTA results with hazard comparison data allowed us to prioritize compounds to be included in future studies of the environmental fate and transport of BOCs.




**KEYWORDS:** *wastewater, sludge, environmental monitoring, chemical space, high-resolution mass spectrometry*



## INTRODUCTION

Treated sewage sludge ("biosolids") is produced as a byproduct of wastewater treatment. The U.S. Environmental Protection Agency (EPA) estimates that 3.76 million dry metric tons of biosolids were produced in 2022.[1] One of the main methods of disposal is via land application, which can improve soil qualities, provide beneficial nutrients,[2] and divert biosolids from landfilling and incineration. In 2022, 56% of all biosolids generated in the U.S. were land-applied, with over half applied on agricultural land.[1] The EPA sets quality standards for biosolids intended for land application, detailed in 40 CFR 503B,[3] which sets specific pollutant limits for select heavy metals and describes requirements for reducing pathogens and vector attraction. These quality standards, however, do not currently set limits for any organic contaminants in biosolids intended for land application.

The occurrence of organic contaminants in biosolids, referred to here as biosolid-associated organic contaminants (BOCs), has been studied for decades in the U.S. and internationally,[2,4−8] with some specific classes of chemicals receiving increased attention, including pharmaceuticals,[9−15] chemicals used in personal care products,[14−16] flame retardants,[17,18] ultraviolet (UV) filters,[17,19] and per- and polyfluorinated alkyl substances (PFASs).[16,20] As required by the Clean Water Act, the EPA conducts reviews every two years to identify additional contaminants in biosolids and sets limits if those contaminants are determined to pose a health risk to humans or the environment.[21] The EPA's Biennial Reviews and National Sewage Sludge Surveys identified chemicals present in U.S. and Canadian biosolids, and the results were recently curated,[22] with updated versions publicly available on the EPA's CompTox Chemicals Dashboard[23] (hereafter "the Dashboard").

Received: March 22, 2024
Revised: June 3, 2024
Accepted: June 4, 2024
Published: June 25, 2024

Most studies investigating BOCs have used targeted analyses to identify the presence of specific pollutants. High-resolution mass spectrometry (HRMS)-based nontargeted and suspect screening analyses are alternative approaches to studying chemical contamination in environmental media.[24−26] These methodologies are capable of detecting a broader range of analytes in a sample, including metabolites and environmental transformation products,[27] compared to targeted analyses using lower-resolution MS instruments, and are increasingly being used to study chemical contamination of biosolids, including those produced in the U.S.,[28−31] Spain,[32−34] Nigeria,[35] France,[36] Sweden,[37] and the Arctic.[38]

Data sets generated from nontargeted analysis (NTA) are typically very large, often containing measurements of thousands of chemical features. A major challenge is determining which features are in need of further risk characterization to identify chemicals of the highest concern for human or ecological health. For biosolids, different approaches have been applied to prioritize the detected contaminants. For example, Black et al. combined NTA with quantitative structure−activity relationship (QSAR) models and *in vitro* bioassays to screen and prioritize chemicals identified in California biosolids.[28,29] Here, we present an alternative approach to prioritize BOCs detected by NTA using a tool that compiles publicly available experimental hazard data with data generated using QSAR prediction algorithms from the EPA. Our objective was to demonstrate the utility of combining NTA with a computer-based hazard comparison approach to support compound prioritization. We analyzed biosolids produced by U.S. and Canadian wastewater treatment plants (WWTPs) to identify BOCs that were detected across the different samples. Then, we evaluated detected compounds based on their potential to elicit adverse human health and ecological effects (including their propensity for environmental persistence and bioaccumulation). Note that when discussing compound prioritization in this paper, we are specifically referring to the process of prioritizing compounds based on hazard comparisons and not referring to how the NTA data processing was performed. Compounds prioritized from this work can be included in future studies of the environmental transport and fate of BOCs.

## MATERIALS AND METHODS

**Biosolid Specimen Acquisition and Processing.** We received 16 unique biosolid samples from 13 different WWTPs across nine U.S. and three Canadian cities. All biosolids analyzed in this study were the final products produced from each facility, collected following the treatment and dewatering steps. Most samples were class B biosolids (11:16, 68.8%) and treated under mesophilic (10:16, 62.5%) and anaerobic conditions (13:16, 81.3%). Additional information about the analyzed biosolids can be found in Supporting Table 1.

Specimens were shipped overnight on ice and stored at −20 °C. To prepare for sample extraction, biosolids were thawed and 7.5 ± 0.3 g was weighed into 50 mL centrifuge tubes. Additionally, a pooled quality control (QC) sample was prepared by weighing 4.0 ± 0.1 g of each biosolid sample into a blender and blending until homogenized; then, 7.5 ± 0.3 g aliquots were prepared in 50 mL centrifuge tubes. All samples were stored at −20 °C until analysis.

**Chemicals and Supplies.** ACS-grade methanol and acetonitrile and Optima methanol, acetonitrile, and formic acid were obtained from Fisher Scientific. Ultrapure water was obtained from a Milli-Q Advantage A10 system (18.2 MΩ·cm, MilliporeSigma, Burlington, MA; abbreviated MQW). Liquid chromatography−mass spectrometry (LC−MS) grade ammonium fluoride was obtained from Honeywell Fluka (Muskegon, MI).

A mixture of 120 compounds was prepared ("QC mix") at 1 mg/L (0.5 mg/L for perfluorinated compounds) to evaluate the method performance and test data processing workflow parameters; details on compounds included in the mix are provided in Supporting Information and Supporting Table 2. An internal standard mixture containing 13 isotopically labeled compounds was prepared at 5 mg/L in methanol ("IS mix"). QC and IS mixtures were stored at −20 °C. Reference materials to prepare the mixtures were obtained from MilliporeSigma (St. Louis, MO), Toronto Research Chemicals (Toronto, Ontario, Canada), and Wellington Laboratories (Guelph, Ontario, Canada).

**Biosolid Extraction.** Biosolids were extracted using a Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) procedure. Extraction conditions (e.g., salts, solvents, etc.) used in this study were similar to those from a previous study investigating the effects of different QuEChERS conditions that yielded the most detectable features in raw sludge and processed biosolids using NTA.[36] One frozen aliquot of each biosolid sample and four frozen aliquots of the pooled QC were allowed to thaw at room temperature. Additionally, we randomly selected 4 biosolid samples (25% of samples) to extract in duplicate to evaluate extraction reproducibility. To prepare an extraction blank, 7.5 mL of MQW was added to an empty 50 mL centrifuge tube. The QC mix (200 μL) was added to two of the pooled QC samples (referred to as the "fortified control", containing 26.7 ng/g QC compounds [13.3 ng/g for PFAS] after fortification), and 200 μL of methanol was added to all other samples. Then, 100 μL of IS mix was added to every sample, and tubes were capped, vortex-mixed, and allowed to sit for 1 h. Subsequently, 10 mL of MQW and 10 mL of acetonitrile were added to every sample, and tubes were capped, vortex-mixed for 30 s, and sonicated for 30 min. Individually, tubes were uncapped, and the entire content of one salt pouch (6000 mg magnesium sulfate/1500 mg sodium acetate; United Chemical Technologies, Bristol, PA) was added to the sample; then, the tube was capped and vigorously shaken for ≥10 s to break up any clumping and vortex-mixed for 30 s. After salts were added to all samples, tubes were centrifuged at 3000*g* for 20 min. Next, 8 mL of the organic phase was transferred to a 15 mL tube containing dispersive solid-phase extraction (dSPE) sorbent (900 mg magnesium sulfate/300 mg PSA/150 mg CEC18, United Chemical Technologies), and the tube was capped, vigorously shaken for ≥10 s, and vortex-mixed for 30 s, and then, all tubes were centrifuged at 3000*g* for 10 min. Then, 5.5 mL of the supernatant was transferred to a separate 15 mL Falcon tube and concentrated to 1 mL, at which point 1 mL of MQW was added. Tubes were capped, vortex-mixed, and concentrated to remove the remaining acetonitrile. Samples were transferred to 1.5 mL microcentrifuge tubes and centrifuged at 20,000*g* and 4 °C for 5 min. Finally, the supernatant was transferred to a disposable polypropylene syringe with a PTFE filter attached (13 mm, 0.22 μm; Restek, Bellefonte, CA), and the sample was filtered into an amber HPLC vial.

**Instrumental Analysis.** We performed chromatographic separation using an UltiMate 3000 RSLCnano system (Thermo Scientific) with a Synergi Hydro-RP column (150


https://doi.org/10.1021/acs.est.4c02934
Environ. Sci. Technol. 2024, 58, 12135−12146

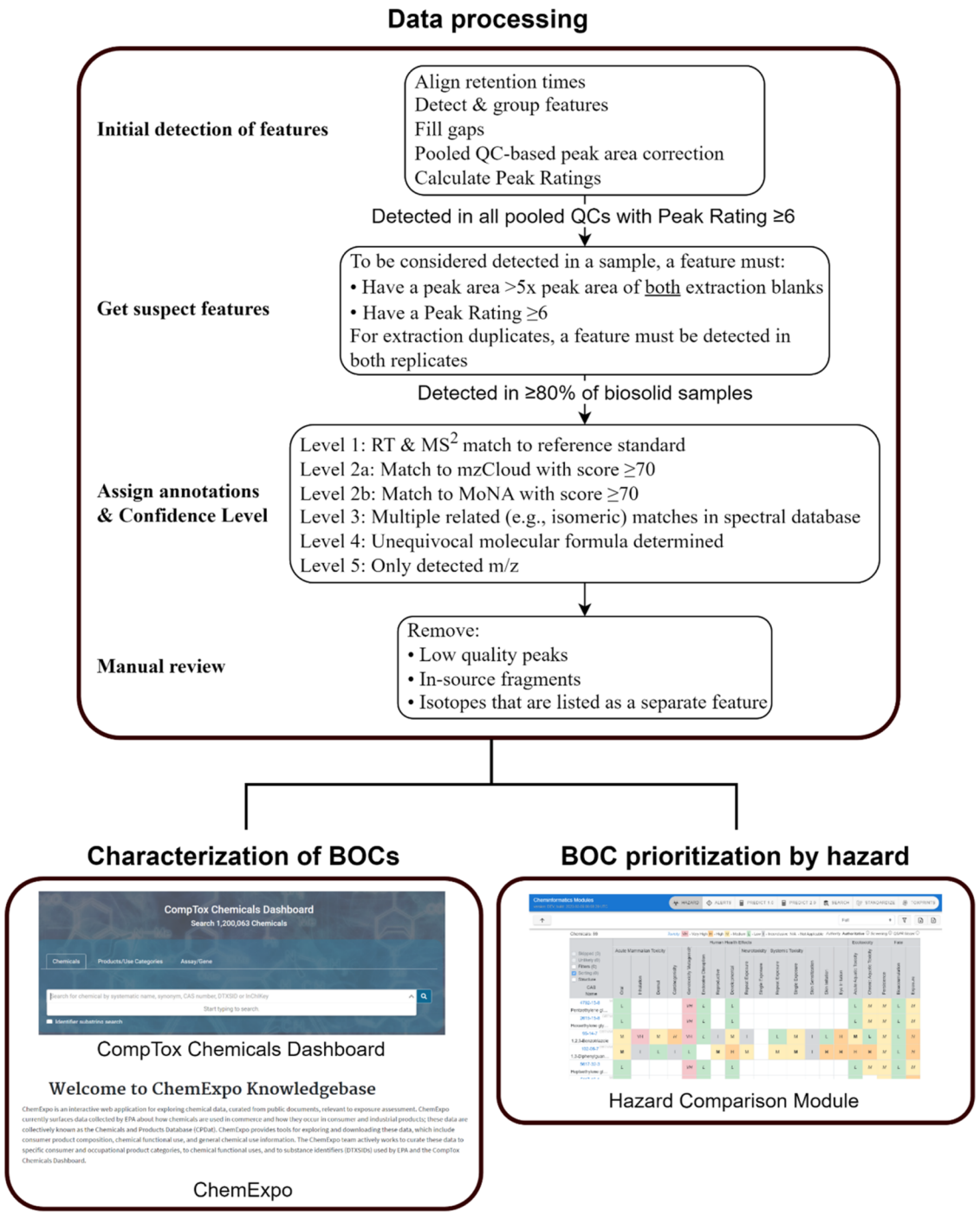



**Figure 1.** Data processing and analysis workflow.

mm × 1 mm, 4 μm; Phenomenex, Torrance, CA) and a KrudKatcher ULTRA in-line filter. We conducted experiments on maximizing the detectable chemical space[39] of our analysis by studying the effects of different mobile phase compositions and ionization modes (i.e., electrospray ionization vs atmospheric pressure chemical ionization) on features detected in biosolid samples; the design and results from that work are available in the Supporting Information. Based on our results, we analyzed all samples in two batches, both utilizing heated electrospray ionization (H-ESI): one batch in positive mode only using aqueous mobile phase (A) 1 mM ammonium fluoride in MQW and organic mobile phase (B) 0.1% formic acid in methanol and one batch in negative mode only using mobile phase (A) 1 mM ammonium fluoride and mobile phase (B) acetonitrile. Additional chromatographic conditions are available in the Supporting Information. Analytes were detected with a Q-Exactive HF Orbitrap HRMS (Thermo Scientific) with data acquired in full-scan/data-dependent $MS^2$ (top $N$ = 10) mode. H-ESI source and other mass spectrometer settings are provided in Supporting Table 3.

**Data Processing.** Data processing steps are briefly described below, and additional details are provided in the Supporting Information. Figure 1 summarizes the data processing and analysis workflow. Raw data were initially processed with Compound Discoverer (version 3.3, Thermo Scientific). Workflow settings are provided in Supporting


https://doi.org/10.1021/acs.est.4c02934
Environ. Sci. Technol. 2024, 58, 12135−12146

Table 4. The fortified controls were processed separately first to evaluate workflow parameters before all other samples. Extraction blanks ($n$ = 2), pooled QC replicates ($n$ = 6), and biosolid samples ($n$ = 20, 16 samples, and 4 duplicates) were included in the workflow together. Because positive and negative mode data were collected using different chromatography conditions, each data set was processed separately.

After generating a table of detected features, data were exported to R (v4.2) to filter features that were present in ≥80% of biosolid samples (based on $n$ = 16 as our workflow required features to be present in both replicates of the extraction duplicates). Because our objective was to identify BOCs that were widely detected across different samples to support prioritization, compounds detected only locally or regionally, or potentially hazardous compounds detected at a frequency <80%, would be expected to be excluded from this analysis. The resulting list was used to filter the original feature table in Compound Discoverer. Then, remaining features were manually reviewed to assign annotations with corresponding confidence levels according to previous recommendations,[40] with slight modifications. In this study, we differentiated between library matches from Thermo's mzCloud database (designated as level 2a) and the MassBank of North America (designated as level 2b); additional details are provided in the Supporting Information. For subsequent data analysis (see below), only compounds with confirmed (confidence level 1) or probable (confidence levels 2a and 2b) structural annotations were included because a single structural annotation was required for searching use and hazard data.

**Analytical Method Performance.** We evaluated the fortified controls to determine the proportion of fortified compounds that were detected by the method for each polarity mode. For compounds that ionized in both modes, the polarity that produced the higher intensity was used. Compounds present in the fortified control with a mean peak area <5× the peak area in the extraction blank were considered not detected. Additionally, we summarized the internal standard peak area variabilities, calculated as the coefficient of variation (CV%), for the pooled QCs and facility samples. Finally, we evaluated the extraction reproducibility for the four pairs of duplicate samples by calculating the percent differences of peak areas for all features detected in both extracts from each pair.

**Characterization of Detected BOCs.** We compared our list of level 1, 2a, and 2b compounds with the compounds present on the EPA's Chemicals in Biosolids (2022) List (hereafter "the Biosolids List"), available on the Dashboard (https://comptox.epa.gov/dashboard/chemical-lists/BIOSOLIDS2022). The 2022 List is the most current update of a curated list[22] that summarizes chemicals detected in U.S. and Canadian biosolids from EPA National Sewage Sludge Surveys and Biennial Reviews. For detected compounds that were not present on the Biosolids List, we conducted a literature search to see if there were any studies reporting their presence in biosolids; details on how the search was conducted are available in the Supporting Information.

To characterize possible sources of compounds detected in biosolids, we accessed Harmonized Functional Use[41] data from the Chemicals and Products Database (CPDat)[42] via the EPA's ChemExpo site (https://comptox.epa.gov/chemexpo/get_data/). Functional use data were downloaded and summarized for each of the level 1, 2a, and 2b compounds, where available. Most compounds had multiple reported uses, so the top use, based on the number of products listing that use, was assigned to each compound for statistical summaries; multiple functional uses were assigned in cases of ties. The CPDat database primarily contains data about how chemicals are used in consumer and industrial products and would not contain data for other compounds possibly detected in biosolids, e.g., metabolites, transformation products, or naturally occurring substances. Therefore, data gaps were manually filled in by searching additional public databases (e.g., PubChem).

**Compound Prioritization by Hazard.** To support the prioritization of detected compounds for future risk characterization, level 1, 2a, and 2b compounds were searched using the EPA's Cheminformatics Hazard Comparison Module (HCM, https://www.epa.gov/comptox-tools/cheminformatics), which compiles publicly available experimental toxicological data from tens of sources, combined with QSAR-based predictions of multiple toxicity end points, and generates a hazard profile (consisting of a categorical hazard ranking score accompanied by a confidence rating for the source of the hazard information) for each searched chemical that includes human health effects, ecotoxicity, and fate end points.[43] Hazard data are translated to letter scores of inconclusive (I), low (L), medium (M), high (H), and very high (VH) based on a modification of the Design for the Environment Alternatives Assessment Criteria for Hazard Evaluation, and a "trumping" method was utilized to select the score from the most authoritative source.[43] Application of the tool to support NTA in rapid-response scenarios was recently demonstrated.[44]

We conducted three analyses to prioritize compounds based on different groups of human and ecological end points. The human health-related end points were split into two groups based on whether the effect was anticipated to be due to chronic or short-term chemical exposure. End points in the chronic exposure group ($n$ = 7) were "Carcinogenicity", "Genotoxicity Mutagenicity", "Endocrine Disruption", "Reproductive", "Developmental", "Neurotoxicity Repeat Exposure", and "Systemic Toxicity Repeat Exposure". The remaining human health end points comprised the short-term exposure group ($n$ = 8): "Acute Mammalian Toxicity Oral", "Acute Mammalian Toxicity Dermal", "Acute Mammalian Toxicity Inhalation", "Neurotoxicity Single Exposure", "Systemic Toxicity Single Exposure", "Skin Sensitization", "Skin Irritation", and "Eye Irritation". The end points in the ecological end points group ($n$ = 4) were "Acute Aquatic Toxicity", "Chronic Aquatic Toxicity", "Persistence", and "Bioaccumulation". The associated definitions for each end point, in terms of the thresholds used to define the various grade annotations of I, L, M, H, and VH, are available on the HCM website.

For each analysis, we calculated four separate scores for each compound based on the generated Hazard Profile, as summarized below. Examples of calculating each score are available in the Supporting Information. First, individual hazard scores were converted to a numerical scale from 1 to 4 corresponding from low−very high with any inconclusive or missing hazard scores omitted; an average hazard score (eq 1) was calculated by averaging the individual hazard scores across the end points, where data were available.

$$\text{avg. hazard score} = \frac{\sum \text{individual hazard scores}}{\text{number of end points with data available}} \quad (1)$$



https://doi.org/10.1021/acs.est.4c02934
Environ. Sci. Technol. 2024, 58, 12135−12146

Next, an average quality score (eq 2) was calculated by converting the data source authority ratings to numerical scores 1, 2, and 3 for the QSAR model, screening, and authoritative, respectively, and averaging across end points, where data were available.

$$\text{avg. quality score} = \frac{\sum \text{individual quality scores}}{\text{number of end points with data available}} \quad (2)$$

A completeness score (eq 3) was calculated by averaging the number of end points for which data were available (considering inconclusive scores as unavailable) across the total number of end points queried; compounds were assigned to one of four groups based on the quartile of the completeness score (e.g., a compound was placed in the "high" group if the completeness score was ≥0.75).

$$\text{completeness score} = \frac{\text{number of end points with data available}}{\text{number of end points searched}} \quad (3)$$

Finally, a quality-adjusted hazard score (eq 4) was calculated by multiplying the average hazard and average quality score, which accounts for the authority of the sources of hazard information for each end point. This means that a chemical that has a higher percentage of lower-quality data sources would have a lower quality-adjusted hazard score due to a lower average quality score.

$$\text{quality-adjusted hazard score} = \text{avg. hazard score} \times \text{avg. quality score} \quad (4)$$

Compounds were ranked by the quality-adjusted hazard score for each of the three groups of end points analyzed.

## RESULTS AND DISCUSSION

**Analytical Method Performance.** We detected 41:73 (56.2%) and 23:47 (48.9%) QC mix compounds in the fortified controls in positive and negative modes, respectively. Compounds included in the QC mix were evaluated as neat solutions and found to be amenable to detection using this method; therefore, compounds not detected were either poorly recovered, potentially degraded in the mixture due to reactions, or susceptible to matrix effects. Table 1 summarizes the variability in peak areas for the isotopically labeled internal standards for the pooled QCs and facility samples. Peak area CV% for the pooled QCs ($n$ = 6 injections) ranged from 0.698 to 6.56% in positive mode and from 1.36 to 3.31% in negative mode. For the facility samples ($n$ = 20, including 4 extraction duplicates), the internal standard peak area CV% ranged from 10.7 to 103% in positive mode and 19.1 to 79.0% in negative mode. The larger variabilities observed among the facility samples are likely due to differences in extraction recoveries and matrix effects between the samples. In contrast, the pooled QC was extracted once and injected multiple times, representing the variability associated with the instrument performance during the analysis.

We also calculated the percent difference between peak areas for features detected in both extracts for the four pairs of extraction duplicates (Figure S4). Median percent differences across the four pairs of extraction duplicates were 12.3−15.0 and 19.9−83.8% in positive and negative modes, respectively.

**Table 1. Peak Area Variability (CV%) for Internal Standards Measured in Pooled QC and Facility Samples[a]**

| internal standard | retention time (min) | pooled QCs ($n$ = 6)[b] | facility samples ($n$ = 20)[b] |
|---|---|---|---|
| | Positive Mode | | |
| 4-hydroxybenzophenone-$d_4$ | 12.77 | 2.42 | 44.0 |
| acetaminophen-$d_4$ | 3.20 | 3.75 | 72.0 |
| acetamiprid-$d_3$ | 9.54 | 1.87 | 24.0 |
| buspirone-$d_8$ | 10.35 | 1.28 | 77.7 |
| caffeine-$^{13}C_3$ | 7.86 | 0.897 | 10.7 |
| carbamazepine-$d_{10}$ | 12.60 | 0.698 | 20.3 |
| diphenhydramine-$d_3$ | 11.34 | 3.11 | 101 |
| malathion-$d_6$ | 14.81 | 1.56 | 44.9 |
| nicotine-$d_4$ | 2.54 | 4.99 | 45.2 |
| sulfamethazine-$d_4$ | 7.45 | 6.56 | 31.0 |
| testosterone-$d_3$ | 15.20 | 5.15 | 78.5 |
| tiamulin-$^{13}C_4$ | 12.68 | 3.68 | 103 |
| | Negative Mode | | |
| 4-hydroxybenzophenone-$d_4$ | 10.33 | 1.65 | 39.1 |
| acetamiprid-$d_3$ | 7.72 | 1.36 | 19.1 |
| sulfamethazine-$d_4$ | 5.66 | 1.37 | 27.9 |
| triclosan-$^{13}C_6$ | 14.77 | 3.31 | 79.0 |

[a]Note that 16 internal standards are reported here because 3 ionized in both positive and negative modes and we are reporting both sets of results. For internal standards detected in both polarity modes, retention times are different because of different mobile phases. [b]CV % for the pooled QC represents the variability of a single sample injected multiple times while CV% for the facility samples represents the variability across 20 different extracts, each injected once; different extracts of the pooled QC were used for positive and negative modes, whereas the same facility sample extract was analyzed in both modes. Note that data after QC-based peak area corrections were used to calculate CVs.

Our results are comparable to previously reported reproducibility of compounds detected in biosolid extraction replicates using positive ionization mode.[30]

Many different preparation methods have been employed for the analysis of organic contaminants in biosolids,[45,46] and several studies have specifically investigated the performance of QuEChERS.[9,11,13,19,36,47,48] However, most of these studies utilized targeted approaches to optimize the extraction procedure for those compounds. Therefore, the precision of the targeted methods was generally very good (e.g., <20% CV) compared to the higher intersample (i.e., as measured by internal standard peak areas in facility samples in Table 1) and intrasample (i.e., as measured by percent differences of peak areas for features detected in extraction duplicates in Figure S4) variability observed for some compounds in this study.

An understudied aspect of analyzing contaminants in biosolids is the variability of matrix effects exhibited by different samples and the effect on the NTA results. This is an important consideration because NTA data processing workflows often use peak areas in lieu of concentrations for feature filtering and statistical analyses. Additionally, high variabilities across sample matrix effects could generate large uncertainties for concentration estimates generated using quantitative NTA (qNTA) approaches.[49] Dong et al.[9] evaluated matrix effects for three different biobased fertilizers following QuEChERS and observed variable matrix effects across the products; for example, at 10 ng/g, the matrix effects for chloroxuron were −42, −27, and 9% in the three different samples. Similarly, Magee et al.[50] demonstrated the high variability in matrix


https://doi.org/10.1021/acs.est.4c02934
Environ. Sci. Technol. 2024, 58, 12135−12146

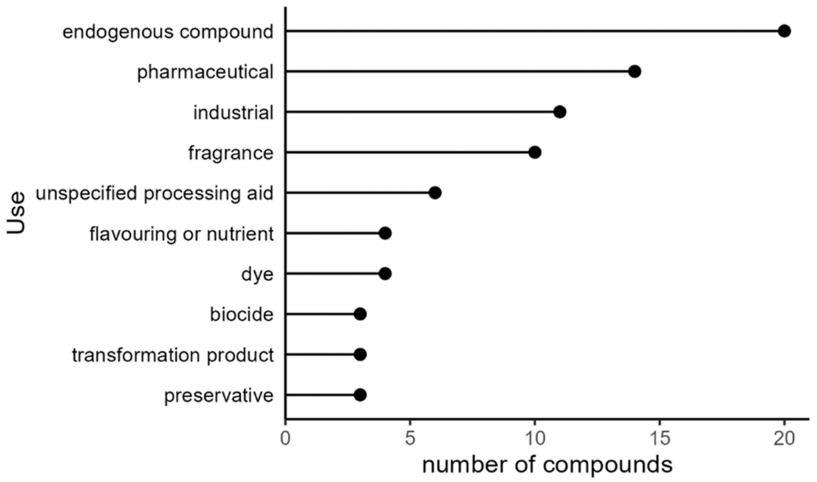



**Figure 2.** Top 10 use categories for confidence level 1, 2a, and 2b compounds; of the 89 compounds included in the analysis, 74 are represented in this Figure (83.1%). Some compounds are represented across multiple use categories; for example, 2-naphthol had the top harmonized functional uses of dye, fragrance, and intermediate. The "industrial" category included compounds with Harmonized Functional Uses of intermediate, plasticizer, catalyst, and monomers and compounds with a manually filled in use of industrial.

effects across four different pools of biosolids following a solvent extraction for the analysis of pharmaceuticals. These differences could be compensated for by a properly validated internal standard, but correcting the peak areas for hundreds to thousands of features detected by NTA, most of which have unknown identities, with only a select number of internal standards could produce erroneous results.[50] Our data processing workflow filtered compounds based on their detection (i.e., presence) in a sample, which does not require a correction for matrix effects; however, this is an important consideration for future work that either attempts to compare biosolid samples using peak areas (e.g., comparative NTA approaches) or use qNTA methods to estimate concentrations.

**Detection and Identification of BOCs.** The data processing workflow initially detected >71,000 features in positive mode and >34,000 features in negative mode among samples. Following all filtering and data cleaning steps (Figure 1), 317 and 134 features remained in positive and negative modes, respectively. Supporting Table 5 summarizes all 451 features with their assigned annotation and associated confidence level. We confirmed the identities of twenty-six compounds (confidence level 1), and there were forty-seven level 2a compounds, 19 level 2b compounds, and ten level 3 compounds; structural assignments were not possible for 77.4% of features (levels 4 and 5, 349:451). Examples of comparisons of fragmentation spectra resulting in confidence levels 1, 2a, and 2b annotations are given in Figures S4−S8. There were 66 features (14.6%) across all confidence levels that were detected in 100% of biosolid samples tested, consisting of 12 level 1, 14 level 2a, six level 2b, two level 3, twenty-two level 4, and ten level 5 features; these are designated in Supporting Table 5.

Of the 92 compounds assigned confidence levels 1, 2a, or 2b, 89 were present on the dashboard, and 71:89 (79.8%) were not present on the Biosolids List. After a search of the literature was conducted, 13:71 (18.3%) compounds that were not present on the Biosolids List were previously reported in biosolids produced in the U.S. or Canada. Several of the compounds—norharman, dextrorphan, piperine, curcumin, and celecoxib—were previously reported without concentrations,[28,29] which prevented their inclusion in the Biennial Reviews. 1,2,3-Benzotriazole, desmethylcitalopram, and galaxolidone were recently reported in Connecticut biosolids,[30,31] and lamotrigine was recently reported in 100% of tested Canadian biosolids,[51] but these studies were published after the latest Biennial Review reporting period ending in 2021. Interestingly, *O*-desmethylvenlafaxine and citalopram,[52] and bisphenol S and bisphenol F[53] were previously reported in biosolids but the compounds were not included despite the studies being cited in the respective Biennial Reviews.[54,55] To the best of our knowledge, this report is the first time that the remaining 58 compounds are reported in biosolids produced in the U.S. or Canada.

Notably, no PFAS were included among these compounds. Our QC results (Supporting Table 2) indicate that PFASs were detectable with this methodology, and during our initial data processing, we were able to confirm that PFASs were detected in some individual samples. However, no PFASs exceeded our 80% detection threshold. This threshold was chosen *a priori* in order to focus our analysis on compounds widely detected nationally and to not bias the results toward any particular chemical class. These data agree with those from Black et al.,[28] who observed detection frequencies for perfluorooctanoic acid, perfluorononanoic acid, perfluorodecanoic acid, perfluorohexanoic acid, perfluoroheptanoic acid, and perfluorooctanesulfonic acid of 71, 50, 36, 21, 14, and 7% in analyzed California biosolids, all of which fall below the 80% threshold used in the current study.

Data on Harmonized Functional Use were available for 46:92 (50.0%) of level 1, 2a, and 2b compounds; all reported Harmonized Functional Uses for each compound are provided in Supporting Table 6. Of the remaining 46 compounds without Harmonized Functional Use data, we were able to manually fill in data gaps for additional 43 compounds using other publicly available databases. All assigned use data are summarized in Supporting Table 5, and the top 10 use categories, based on the number of compounds with that assigned use, are summarized in Figure 2.

The top category was endogenous compounds ($n$ = 20 compounds; e.g., androstenedione, cholest-4-en-3-one, harman), defined here as compounds naturally produced by an organism. Pharmaceuticals ($n$ = 14) was the top use category


https://doi.org/10.1021/acs.est.4c02934
Environ. Sci. Technol. 2024, 58, 12135−12146

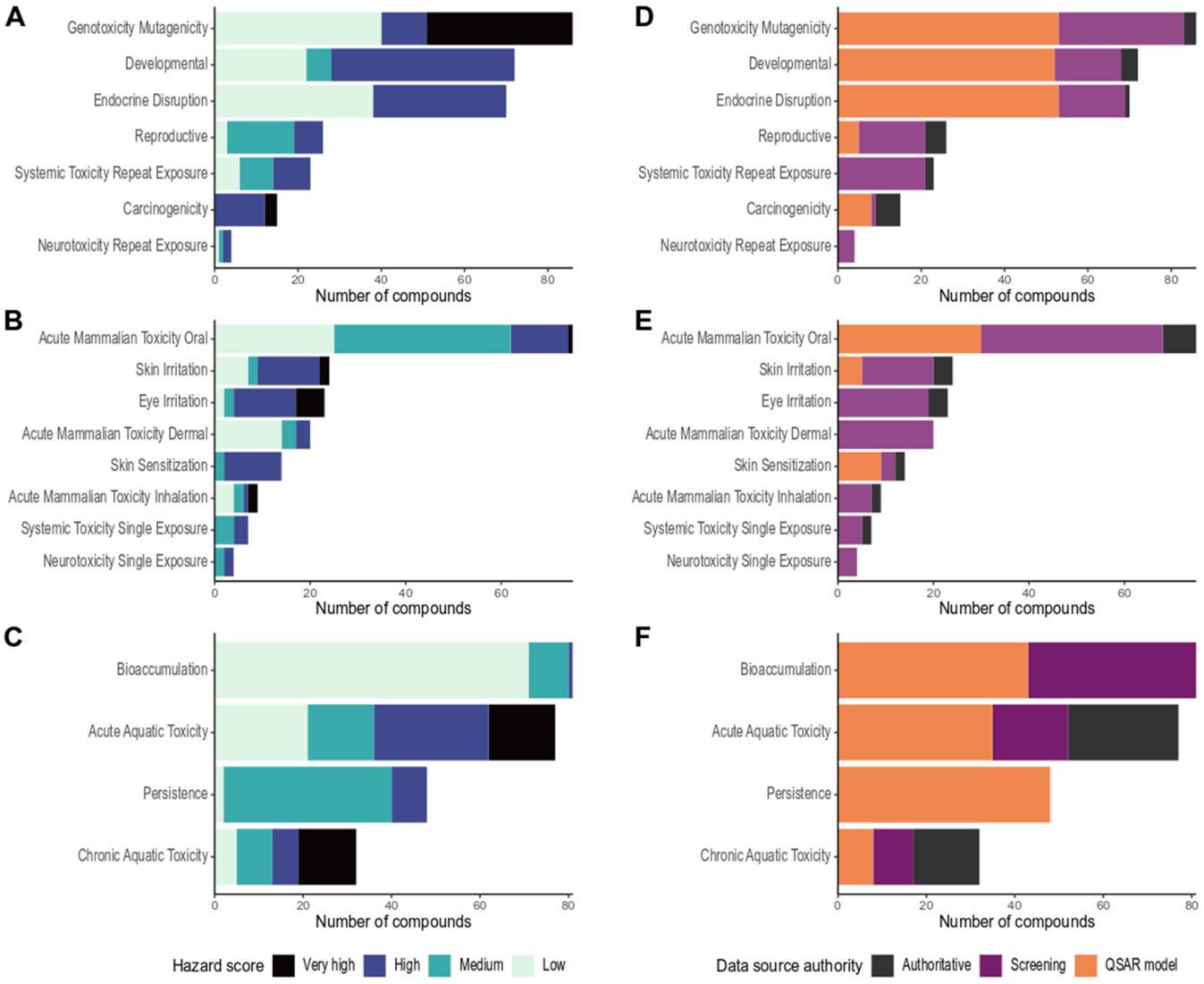



**Figure 3.** Distributions of hazard scores (A–C) and data source authorities (D–F) for end points related to chronic chemical exposure (A, D), short-term chemical exposure (B, E), and ecotoxicity and fate (C, F). The total number of compounds searched was 89.

of anthropogenic compounds and included both parent compounds (e.g., citalopram, carbamazepine, and lamotrigine) and metabolites of pharmaceuticals (e.g., desmethylcitalopram and *O*-desmethylvenlafaxine). Industrial chemicals ($n$ = 11) are defined here as any compound having a Harmonized Function Use of "intermediate", "plasticizer", "catalyst", or "monomer"; (e.g., bisphenol A, tris(2-butoxyethyl) phosphate, 1,3-diphenylguanidine), or a manually filled in use of "industrial" (e.g., dibenzylamine and 4-methylphthalic anhydride).

**Prioritization of BOCs.** We compiled hazard data for the 89 level 1, 2a, and 2b compounds that were present on the Dashboard using the HCM. The exported Hazard Profile and Hazard Records are available in Supporting Tables 7 and 8, respectively. The Hazard Records show the available data from all sources for each compound and end point; for our analyses, we utilized the integrated hazard score shown on the Hazard Profile determined by the "trumping" method.[43] Data availability and data source quality (i.e., Authoritative, Screening, or QSAR Model) varied widely across the 19 end points, as shown in Figure 3. There were seven end points for which data were available for >50% of compounds: Genotoxic Mutagenicity ($n$ = 86:89 compounds), Bioaccumulation ($n$ = 81), Acute Aquatic Toxicity ($n$ = 77), Acute Mammalian Toxicity Oral ($n$ = 75), Developmental ($n$ = 72), Endocrine Disruption ($n$ = 70), and Persistence ($n$ = 48). Due to completely missing data (i.e., no information available for any relevant end points) related to chronic chemical exposure, short-term chemical exposure, and ecological toxicity/fate, zero (0%), 14 (15.7%), and two (2.25%) compounds, respectively, were excluded from further prioritization. There is the possibility, therefore, that potentially hazardous compounds could be excluded from our analysis due to these data gaps. There were ten end points, where >50% of data was from Screening sources, and seven end points, where >50% of data was from the QSAR Model. For the remaining two end points, the plurality of data was from the QSAR Model (45.5%) for Acute Aquatic Toxicity, and from Authoritative sources (49.6%) for Chronic Aquatic Toxicity. These distributions are unique for our group of searched chemicals and would change if different compounds were searched or if data were added or updated in the HCM in the future.

The calculated Quality-adjusted Hazard Scores for compounds based on each of the three analyses are listed in Supporting Table 9, and a summary of the Top 5 prioritized


https://doi.org/10.1021/acs.est.4c02934
Environ. Sci. Technol. 2024, 58, 12135–12146

Table 2. Top 5 Prioritized Compounds for Each End Point Group Based on Quality-Adjusted Hazard Scores

| compound (confidence level) | quality-adjusted hazard score | completeness group[a] | use[b] |
|---|---|---|---|
| Human Health Effects—chronic Exposure (7 End Points) | | | |
| ketoconazole (2b) | 8.01 | medium low | pharmaceutical |
| *p*-cresol (2a) | 7.19 | high | fragrance |
| 4-androstene-3,17-dione (2a) | 6.99 | medium low | endogenous |
| clorophene (2a) | 6.72 | medium high | biocide |
| phenolphthalein (2a) | 6.68 | high | not specified |
| Human Health Effects—short-term Exposure (8 End Points) | | | |
| clorophene (2a) | 7.56 | high | biocide |
| *p*-cresol (2a) | 7.14 | high | fragrance |
| indole (2a) | 6.99 | medium low | fragrance |
| thymol (1) | 6.88 | medium high | fragrance |
| ketoconazole (2b) | 6.25 | medium low | pharmaceutical |
| Ecological End Points (4 End Points) | | | |
| fludioxonil (2a) | 12.00 | medium high | biocide |
| ketoconazole (2b) | 8.01 | high | pharmaceutical |
| 4-(1,1,3,3-tetramethylbutyl)phenol (2a) | 6.75 | high | adhesion promoter |
| triclocarban (2a) | 6.75 | high | preservative |
| carbamazepine (1) | 6.22 | high | pharmaceutical |

[a]Completeness group refers to the percentage of end points within each analysis that had available data. Compounds were assigned to one of four completeness groups based on the quartile of the completeness score. [b]Certain compounds may have multiple uses. The use listed in the table is the one with the highest number of products listing that use based on data from the CPDat. A complete list of uses for each compound in CPDat is presented in Supporting Table 6.

compounds from each analysis is presented in Table 2. Note that including the Top 5 compounds is meant to be illustrative and not reflective of any decision that these are the only compounds that should be evaluated further. Higher Quality-adjusted Hazard Scores were generally associated with higher Completeness Scores. This is not particularly surprising as hazardous compounds may be more widely studied. Missing data in the HCM can occur for multiple reasons,[43] though, so we did not want to deprioritize a potentially hazardous compound due to lower data availability; this is a decision that is protective of health. For example, a compound with a hazard score of High for Genotoxic Mutagenicity, but with no data for other end points, may still represent a concerning compound that should be studied further. While the Completeness Score did not factor into compound prioritization, it is important to summarize the amount of data available for each compound.

There are several factors to consider when evaluating these data. First, compound prioritization, as performed here, is based on hazard estimates only. While this is useful as a screening method for choosing compounds to include in subsequent studies, additional information is needed to provide quantitative characterization of compound risks, including compound-specific dose—response metrics, compound concentrations in relevant environmental media, and estimates of rates of contact with or intake of the environmental media in the population(s) of interest. For example, the top prioritized compound using the group of end points related to human health effects after chronic chemical exposure was ketoconazole, a pharmaceutical used to treat fungal infections. In 2013, the U.S. Food and Drug Administration (FDA) issued a safety announcement to limit the use of oral ketoconazole due to reported liver injury, adrenal gland problems, and harmful drug interactions, although its use in topical formulations was still considered safe.[56] The presence of ketoconazole in biosolids alone may not directly indicate a significant risk; if concentrations in relevant environmental media are low and exposures (i.e., via pathways including ingestion of biosolids, amended soils, or produce) do not result in a sufficient dose, health effects would not be expected to occur. Our evaluation, therefore, is indicative of the value of further investigation of ketoconazole.

Second, we did not attempt to apply weights to the individual end points, which could introduce additional bias.[43] Rather, we separated related end points into groups. There are opportunities to improve this process, though. For example, most of the end points related to human health effects following acute chemical exposure are based on exposure route (e.g., acute mammalian toxicity oral) or site-specific exposure (e.g., eye irritation). While we included all end points here, end point selection could be adjusted using well-characterized exposure estimates for specific scenarios, e.g., like those recently published for agricultural workers' exposure to soil,[57,58] leading to more tailored prioritizations.

## ■ ENVIRONMENTAL IMPLICATIONS

The hazards related to biosolids exposures, including those related to land application, have generally been studied with *in vitro* or *in vivo* (including both animal and plant) bioassays,[29,59−64] but these approaches do not provide compound-specific data. Most of these studies also performed a targeted analysis of organic contaminants present in biosolids,[59,61,63,64] but the chosen compounds may not necessarily be those responsible for observed effects. In contrast, the implementation of nontargeted and suspect screening approaches can detect a broader range of compounds in a sample, but not all may be of toxicological concern. Therefore, a strategy is needed to filter and prioritize features detected in NTA for further risk characterization to identify chemicals of highest concern for human or ecological health. The prioritization of NTA results using hazard or toxicity data has been implemented in several other studies, most investigating chemical contaminants in water matrices[65−69] but has also been applied to dust[70] and, recently, in biosolids,[28,29] and tools to assist analysts are increasingly


https://doi.org/10.1021/acs.est.4c02934
Environ. Sci. Technol. 2024, 58, 12135−12146

becoming available.[43,71−75] Our approach utilizing the HCM allowed us to generate a single "composite" hazard score for individual chemicals that could facilitate prioritization by combining data across several end points. It is important to note that these scores do not represent a quantitative estimate of the risk posed by each compound, nor are they definitive interpretations of a chemical's propensity to elicit a specific health outcome. Additionally, these scores apply to compounds individually and do not include hazards associated with mixtures. Instead, this approach yields information useful in comparing and prioritizing compounds for further consideration.[43] Further, while we demonstrated this approach using biosolids, it is flexible and can be applied to prioritize contaminants in other environmental media, e.g., food commodities,[76] and can readily be tailored for other exposure scenarios.

We implemented an HRMS-based nontargeted methodology to identify BOCs widely detected in U.S. and Canadian biosolids. Compounds with confirmed or probable structural annotations were primarily endogenous compounds, pharmaceuticals, industrial chemicals, and fragrances. BOCs were prioritized for further consideration based on hazard estimates from three different groups of human and ecological health end points using the EPA's Cheminformatics Hazard Comparison Module. These compounds may pose risks to human health through, for example, uptake into edible crops or leaching into groundwater following land application. Additionally, there are possible ecological risks associated with soil and groundwater leaching of BOCs. The data generated from this work will inform our larger ongoing study to understand the fate and transport of BOCs in soil, surface, and ground waters, and edible produce following land application of biosolids. Prioritized compounds will be monitored and quantified in different environmental media to support compound-specific characterizations of risk.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.4c02934.

Additional methodological details; comparison of the distributions of selected physicochemical properties for compounds present on the Biosolids 2022 List and compounds included in the QC mix; modified QuEChERS procedure for chemical space evaluation; comparison of unique molecular formulas observed under different instrumental conditions in the positive ionization mode; distribution of peak area percent differences for features detected in extraction duplicates in positive mode and negative mode; example data for three hypothetical compounds and five end points to demonstrate calculation of different scores; comparisons of fragmentation spectra resulting in confidence level 1, 2a, and 2b annotations (PDF)

Information about wastewater treatment facilities that provided biosolid samples; compounds included in the quality control mixture; mass spectrometer source and Full MS/dd-MS2 settings; compound Discoverer workflow node settings; annotations and confidence levles for features detected in biosolids; Harmonized Functional Use data available for level 1, 2a, and 2b compounds; Hazard Profile for level 1, 2a, and 2b compounds; Hazard Records for level 1, 2a, and 2b compounds; calculated composite scores for level 1, 2a, and 2b compounds from Hazard Comparison Module data (XLSX)

## ■ AUTHOR INFORMATION


### Corresponding Author

**Carsten Prasse** − *Department of Environmental Health and Engineering and Risk Sciences and Public Policy Institute, Johns Hopkins University, Baltimore, Maryland 21205, United States;* orcid.org/0000-0002-1470-141X; Email: cprasse1@jhu.edu

### Authors

**Matthew N. Newmeyer** − *Department of Environmental Health and Engineering, Johns Hopkins University, Baltimore, Maryland 21205, United States;* orcid.org/0000-0002-0653-1553

**Qinfan Lyu** − *Department of Environmental Health and Engineering, Johns Hopkins University, Baltimore, Maryland 21205, United States*

**Jon R. Sobus** − *Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27709, United States*

**Antony J. Williams** − *Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27709, United States;* orcid.org/0000-0002-2668-4821

**Keeve E. Nachman** − *Department of Environmental Health and Engineering, Risk Sciences and Public Policy Institute, and Center for a Livable Future, Johns Hopkins University, Baltimore, Maryland 21205, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.est.4c02934



### Funding

This work was supported by a U.S. EPA National Priorities grant (R840247). KEN was supported by a grant from the U.S. Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health to the Johns Hopkins Education and Research Center for Occupational Safety and Health (award number T42 OH0008428).


### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS


We would like to acknowledge and thank all of the WWTPs for their participation and for sending biosolid samples and providing details about their treatment processes. We thank C. Ring and P. Kruse for their valuable discussions related to this study. We would also like to thank D. Abrahamsson and C. Brueck for their review of our manuscript and thoughtful comments. Data presented herein did not undergo a formal quality assurance review as outlined in "US EPA Office of Research and Development's Quality Management Plan for Scientific Research". This manuscript was subjected to the US EPA internal review process. The research results presented do not necessarily reflect the views of the Agency or its policy. Mention of trade names or products does not constitute endorsement or recommendation for use.

## REFERENCES


(1) US EPA, O. W.Basic Information about Biosolids 2023 https://www.epa.gov/biosolids/basic-information-about-biosolids. (accessed December 29, 2023).

(2) Pozzebon, E. A.; Seifert, L. Emerging Environmental Health Risks Associated with the Land Application of Biosolids: A Scoping Review. *Environ. Health* **2023**, *22* (1), No. 57, DOI: 10.1186/s12940-023-01008-4.

(3) Environmental Protection Agency. 40 CFR Part 503 -- Standards for the Use or Disposal of Sewage Sludge 2023 https://www.ecfr.gov/current/title-40/part-503. (accessed December 14, 2023).

(4) Kanteraki, A. E.; Isari, E. A.; Svarnas, P.; Kalavrouziotis, I. K. Biosolids: The Trojan Horse or the Beautiful Helen for Soil Fertilization? *Sci. Total Environ.* **2022**, *839*, No. 156270.

(5) Steele, J. C.; Meng, X.-Z.; Venkatesan, A. K.; Halden, R. U. Comparative Meta-Analysis of Organic Contaminants in Sewage Sludge from the United States and China. *Sci. Total Environ.* **2022**, *821*, No. 153423.

(6) Petrie, B.; Barden, R.; Kasprzyk-Hordern, B. A Review on Emerging Contaminants in Wastewaters and the Environment: Current Knowledge, Understudied Areas and Recommendations for Future Monitoring. *Water Res.* **2015**, *72*, 3−27.

(7) Clarke, B. O.; Smith, S. R. Review of 'Emerging' Organic Contaminants in Biosolids and Assessment of International Research Priorities for the Agricultural Use of Biosolids. *Environ. Int.* **2011**, *37* (1), 226−247.

(8) Harrison, E. Z.; Oakes, S. R.; Hysell, M.; Hay, A. Organic Chemicals in Sewage Sludges. *Sci. Total Environ.* **2006**, *367* (2), 481−497.

(9) Dong, Y.; Das, S.; Parsons, J. R.; Praetorius, A.; de Rijke, E.; Helmus, R.; Slootweg, J. C.; Jansen, B. Simultaneous Detection of Pesticides and Pharmaceuticals in Three Types of Bio-Based Fertilizers by an Improved QuEChERS Method Coupled with UHPLC-q-ToF-MS/MS. *J. Hazard. Mater.* **2023**, *458*, No. 131992.

(10) Sellier, A.; Khaska, S.; Le Gal La Salle, C. Assessment of the Occurrence of 455 Pharmaceutical Compounds in Sludge According to Their Physical and Chemical Properties: A Review. *J. Hazard. Mater.* **2022**, *426*, No. 128104.

(11) Nannou, C. I.; Boti, V. I.; Albanis, T. A. A Modified QuEChERS Approach for the Analysis of Pharmaceuticals in Sediments by LC-Orbitrap HRMS. *Anal. Bioanal. Chem.* **2019**, *411* (7), 1383−1396.

(12) Chen, Y.; Yu, G.; Cao, Q.; Zhang, H.; Lin, Q.; Hong, Y. Occurrence and Environmental Implications of Pharmaceuticals in Chinese Municipal Sewage Sludge. *Chemosphere* **2013**, *93* (9), 1765−1772.

(13) Peysson, W.; Vulliet, E. Determination of 136 Pharmaceuticals and Hormones in Sewage Sludge Using Quick, Easy, Cheap, Effective, Rugged and Safe Extraction Followed by Analysis with Liquid Chromatography−Time-of-Flight-Mass Spectrometry. *J. Chromatogr. A* **2013**, *1290*, 46−61.

(14) Olofsson, U.; Bignert, A.; Haglund, P. Time-Trends of Metals and Organic Contaminants in Sewage Sludge. *Water Res.* **2012**, *46* (15), 4841−4851.

(15) McClellan, K.; Halden, R. U. Pharmaceuticals and Personal Care Products in Archived U.S. Biosolids from the 2001 EPA National Sewage Sludge Survey. *Water Res.* **2010**, *44* (2), 658−668.

(16) Abril, C.; Santos, J. L.; Martín, J.; Aparicio, I.; Alonso, E. Occurrence, Fate and Environmental Risk of Anionic Surfactants, Bisphenol A, Perfluorinated Compounds and Personal Care Products in Sludge Stabilization Treatments. *Sci. Total Environ.* **2020**, *711*, No. 135048.

(17) Ge, J.; Li, J.; Han, X.; Du, B.; Guo, Y.; Zhu, H.; Zeng, L. Triazine-Derived Ultraviolet Filters and Flame Retardants: A Nationwide Co-Occurrence and Prevalence Study in China. *Environ. Sci. Technol. Lett.* **2023**, *10*, 909−915, DOI: 10.1021/acs.estlett.3c00497.

(18) Navarro, I.; de la Torre, A.; Sanz, P.; Fernández, C.; Carbonell, G.; de los Ángeles Martínez, M. Environmental Risk Assessment of Perfluoroalkyl Substances and Halogenated Flame Retardants Released from Biosolids-Amended Soils. *Chemosphere* **2018**, *210*, 147−155.

(19) Ramos, S.; Homem, V.; Santos, L. Development and Optimization of a QuEChERS-GC−MS/MS Methodology to Analyse Ultraviolet-Filters and Synthetic Musks in Sewage Sludge. *Sci. Total Environ.* **2019**, *651*, 2606−2614.

(20) Thompson, J. T.; Robey, N. M.; Tolaymat, T. M.; Bowden, J. A.; Solo-Gabriele, H. M.; Townsend, T. G. Underestimation of Per- and Polyfluoroalkyl Substances in Biosolids: Precursor Transformation During Conventional Treatment. *Environ. Sci. Technol.* **2023**, *57* (9), 3825−3832.

(21) US EPA, O. W.Biosolids Laws and Regulations 2023 https://www.epa.gov/biosolids/biosolids-laws-and-regulations. (accessed December 29, 2023).

(22) Richman, T.; Arnold, E.; Williams, A. J. Curation of a List of Chemicals in Biosolids from EPA National Sewage Sludge Surveys & Biennial Review Reports. *Sci. Data* **2022**, *9* (1), No. 180, DOI: 10.1038/s41597-022-01267-9.

(23) 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, DOI: 10.1186/s13321-017-0247-6.

(24) Aceña, J.; Stampachiacchiere, S.; Pérez, S.; Barceló, D. Advances in Liquid Chromatography−High-Resolution Mass Spectrometry for Quantitative and Qualitative Environmental Analysis. *Anal. Bioanal. Chem.* **2015**, *407* (21), 6289−6299.

(25) Hollender, J.; Schymanski, E. L.; Ahrens, L.; Alygizakis, N.; Béen, F.; Bijlsma, L.; Brunner, A. M.; Celma, A.; Fildier, A.; Fu, Q.; Gago-Ferrero, P.; Gil-Solsona, R.; Haglund, P.; Hansen, M.; Kaserzon, S.; Kruve, A.; Lamoree, M.; Margoum, C.; Meijer, J.; Merel, S.; Rauert, C.; Rostkowski, P.; Samanipour, S.; Schulze, B.; Schulze, T.; Singh, R. R.; Slobodnik, J.; Steininger-Mairinger, T.; Thomaidis, N. S.; Togola, A.; Vorkamp, K.; Vulliet, E.; Zhu, L.; Krauss, M. NORMAN Guidance on Suspect and Non-Target Screening in Environmental Monitoring. *Environ. Sci. Eur.* **2023**, *35* (1), No. 75, DOI: 10.1186/s12302-023-00779-4.

(26) Schulze, B.; Jeon, Y.; Kaserzon, S.; Heffernan, A. L.; Dewapriya, P.; O'Brien, J.; Ramos, M. J. G.; Gorji, S. G.; Mueller, J. F.; Thomas, K. V.; Samanipour, S. An Assessment of Quality Assurance/Quality Control Efforts in High Resolution Mass Spectrometry Non-Target Workflows for Analysis of Environmental Samples. *TrAC, Trends Anal. Chem.* **2020**, *133*, No. 116063.

(27) Picó, Y.; Barceló, D. Transformation Products of Emerging Contaminants in the Environment and High-Resolution Mass Spectrometry: A New Horizon. *Anal. Bioanal. Chem.* **2015**, *407* (21), 6257−6273.

(28) Black, G. P.; Anumol, T.; Young, T. M. Analyzing a Broader Spectrum of Endocrine Active Organic Contaminants in Sewage Sludge with High Resolution LC-QTOF-MS Suspect Screening and QSAR Toxicity Prediction. *Environ. Sci.: Processes Impacts* **2019**, *21* (7), 1099−1114.

(29) 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* (10), 6729−6739.

(30) Nason, S. L.; Lin, E.; Eitzer, B.; Koelmel, J.; Peccia, J. Changes in Sewage Sludge Chemical Signatures During a COVID-19 Community Lockdown, Part 1: Traffic, Drugs, Mental Health, and Disinfectants. *Environ. Toxicol. Chem.* **2022**, *41* (5), 1179−1192.

(31) Nason, S. L.; Lin, E.; Pollitt, K. J. G.; Peccia, J. Changes in Sewage Sludge Chemical Signatures During a COVID-19 Community Lockdown, Part 2: Nontargeted Analysis of Sludge and Evaluation with COVID-19 Metrics. *Environ. Toxicol. Chem.* **2022**, *41* (5), 1193−1201.

(32) Cuñat, A.; Álvarez-Ruiz, R.; Suarez-Varela, M. M. M.; Pico, Y. Suspected-Screening Assessment of the Occurrence of Organic

Compounds in Sewage Sludge. *J. Environ. Manage.* **2022**, *308*, No. 114587.
(33) Gil-Solsona, R.; Nika, M.-C.; Bustamante, M.; Villanueva, C. M.; Foraster, M.; Cosin-Tomás, M.; Alygizakis, N.; Gómez-Roig, M. D.; Llurba-Olive, E.; Sunyer, J.; Thomaidis, N. S.; Dadvand, P.; Gago-Ferrero, P. The Potential of Sewage Sludge to Predict and Evaluate the Human Chemical Exposome. *Environ. Sci. Technol. Lett.* **2021**, *8* (12), 1077−1084.
(34) Castro, G.; Ramil, M.; Cela, R.; Rodríguez, I. Identification and Determination of Emerging Pollutants in Sewage Sludge Driven by UPLC-QTOF-MS Data Mining. *Sci. Total Environ.* **2021**, *778*, No. 146256.
(35) Nikolopoulou, V.; Ajibola, A. S.; Aalizadeh, R.; Thomaidis, N. S. Wide-Scope Target and Suspect Screening of Emerging Contaminants in Sewage Sludge from Nigerian WWTPs by UPLC-qToF-MS. *Sci. Total Environ.* **2023**, *857*, No. 159529.
(36) Bergé, A.; Buleté, A.; Fildier, A.; Vulliet, E. High-Resolution Mass Spectrometry as a Tool To Evaluate the Sample Preparation of Sludge. *Anal. Chem.* **2017**, *89* (18), 9685−9694.
(37) Veenaas, C.; Bignert, A.; Liljelind, P.; Haglund, P. Nontarget Screening and Time-Trend Analysis of Sewage Sludge Contaminants via Two-Dimensional Gas Chromatography−High Resolution Mass Spectrometry. *Environ. Sci. Technol.* **2018**, *52* (14), 7813−7822.
(38) Lee, S.; Kim, K.; Jeon, J.; Moon, H.-B. Optimization of Suspect and Non-Target Analytical Methods Using GC/TOF for Prioritization of Emerging Contaminants in the Arctic Environment. *Ecotoxicol. Environ. Saf.* **2019**, *181*, 11−17.
(39) Black, G.; Lowe, C.; Anumol, T.; Bade, J.; Favela, K.; Feng, Y.-L.; Knolhoff, A.; Mceachran, A.; Nuñez, J.; Fisher, C.; Peter, K.; Quinete, N. S.; Sobus, J.; Sussman, E.; Watson, W.; Wickramasekara, S.; Williams, A.; Young, T. Exploring Chemical Space in Non-Targeted Analysis: A Proposed ChemSpace Tool. *Anal. Bioanal. Chem.* **2023**, *415*, 35−44.
(40) 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.
(41) Phillips, K. A.; Wambaugh, J. F.; Grulke, C. M.; Dionisio, K. L.; Isaacs, K. K. High-Throughput Screening of Chemicals as Functional Substitutes Using Structure-Based Classification Models. *Green Chem.* **2017**, *19* (4), 1063−1074.
(42) Dionisio, K. L.; Phillips, K.; Price, P. S.; Grulke, C. M.; Williams, A.; Biryol, D.; Hong, T.; Isaacs, K. K. The Chemical and Products Database, a Resource for Exposure-Relevant Data on Chemicals in Consumer Products. *Sci. Data* **2018**, *5* (1), No. 180125.
(43) Vegosen, L.; Martin, T. M. An Automated Framework for Compiling and Integrating Chemical Hazard Data. *Clean Technol. Environ. Policy* **2020**, *22* (2), 441−458.
(44) Sloop, J. T.; Chao, A.; Gundersen, J.; Phillips, A. L.; Sobus, J. R.; Ulrich, E. M.; Williams, A. J.; Newton, S. R. Demonstrating the Use of Non-Targeted Analysis for Identification of Unknown Chemicals in Rapid Response Scenarios. *Environ. Sci. Technol.* **2023**, *57* (8), 3075−3084.
(45) Martín-Pozo, L.; de Alarcón-Gómez, B.; Rodríguez-Gómez, R.; García-Córcoles, M. T.; Çipa, M.; Zafra-Gómez, A. Analytical Methods for the Determination of Emerging Contaminants in Sewage Sludge Samples. A Review. *Talanta* **2019**, *192*, 508−533.
(46) Zuloaga, O.; Navarro, P.; Bizkarguenaga, E.; Iparraguirre, A.; Vallejo, A.; Olivares, M.; Prieto, A. Overview of Extraction, Clean-up and Detection Techniques for the Determination of Organic Pollutants in Sewage Sludge: A Review. *Anal. Chim. Acta* **2012**, *736*, 7−29.
(47) Benedetti, B.; Majone, M.; Cavaliere, C.; Montone, C. M.; Fatone, F.; Frison, N.; Laganà, A.; Capriotti, A. L. Determination of Multi-Class Emerging Contaminants in Sludge and Recovery Materials from Waste Water Treatment Plants: Development of a Modified QuEChERS Method Coupled to LC−MS/MS. *Microchem. J.* **2020**, *155*, No. 104732.
(48) Nannou, C. I.; Boti, V. I.; Albanis, T. A. Trace Analysis of Pesticide Residues in Sediments Using Liquid Chromatography−High-Resolution Orbitrap Mass Spectrometry. *Anal. Bioanal. Chem.* **2018**, *410* (7), 1977−1989.
(49) Groff, L. C.; Grossman, J. N.; Kruve, A.; Minucci, J. M.; Lowe, C. N.; McCord, J. P.; Kapraun, D. F.; Phillips, K. A.; Purucker, S. T.; Chao, A.; Ring, C. L.; Williams, A. J.; Sobus, J. R. Uncertainty Estimation Strategies for Quantitative Non-Targeted Analysis. *Anal. Bioanal. Chem.* **2022**, *414*, 4919−4933, DOI: 10.1007/s00216-022-04118-z.
(50) Magee, H. Y.; Maurer, M. M.; Cobos, A.; Adhikari, S.; Halden, R. U. Impact of LC-MS/MS Quantification Methods on Analytical Results by Example of Nationwide U.S. Environmental Samples. *Sci. Total Environ.* **2023**, *892*, No. 164425.
(51) Gewurtz, S. B.; Teslic, S.; Hamilton, M. C.; Smyth, S. A. Influence of Conjugation on the Fate of Pharmaceuticals and Hormones in Canadian Wastewater Treatment Plants. *ACS ES&T Water* **2022**, *2* (2), 329−338.
(52) Lajeunesse, A.; Smyth, S. A.; Barclay, K.; Sauvé, S.; Gagnon, C. Distribution of Antidepressant Residues in Wastewater and Biosolids Following Different Treatment Processes by Municipal Wastewater Treatment Plants in Canada. *Water Res.* **2012**, *46* (17), 5600−5612.
(53) Yu, X.; Xue, J.; Yao, H.; Wu, Q.; Venkatesan, A. K.; Halden, R. U.; Kannan, K. Occurrence and Estrogenic Potency of Eight Bisphenol Analogs in Sewage Sludge from the U.S. EPA Targeted National Sewage Sludge Survey. *J. Hazard. Mater.* **2015**, *299*, 733−739.
(54) U.S. Environmental Protection Agency. *Biennial Review of 40 CFR Part 503 As Required Under the Clean Water Act Section 405(d)(2)(C) Reporting Period 2013 Biennial Review*, Office of Science and Technology, Office of Water 2018. https://www.epa.gov/sites/default/files/2018-05/documents/2013_biosolids_biennial_review_final.pdf. (accessed December 20, 2023).
(55) U.S. Environmental Protection Agency. *Biennial Review of 40 CFR Part 503 As Required Under the Clean Water Act Section 405(d)(2)(C) Reporting Period 2015 Biennial Review*, Office of Science and Technology, Office of Water 2018. https://www.epa.gov/sites/default/files/2018-05/documents/2015_biosolids_biennial_review_final.pdf. (accessed December 30, 2023).
(56) US FDA. FDA Drug Safety Communication: FDA Limits Usage of Nizoral (Ketoconazole) Oral Tablets Due to Potentially Fatal Liver Injury and Risk of Drug Interactions and Adrenal Gland Problems 2013 https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-fda-limits-usage-nizoral-ketoconazole-oral-tablets-due-potentially. (accessed January 04, 2024).
(57) Lupolt, S. N.; Agnew, J.; Burke, T. A.; Kennedy, R. D.; Nachman, K. E. Key Considerations for Assessing Soil Ingestion Exposures among Agricultural Workers. *J. Exposure Sci. Environ. Epidemiol.* **2022**, *32* (3), 481−492.
(58) Lupolt, S. N.; Agnew, J.; Ramachandran, G.; Burke, T. A.; Kennedy, R. D.; Nachman, K. E. A Qualitative Characterization of Meso-Activity Factors to Estimate Soil Exposure for Agricultural Workers. *J. Exposure Sci. Environ. Epidemiol.* **2023**, *33* (1), 140−154.
(59) Ahkola, H.; Lindholm-Lehto, P.; Perkola, N.; Välitalo, P.; Meriläinen, P.; Mäenpää, K.; Stelzer, J. A. A.; Heiskanen, I.; Järvistö, J.; Nuutinen, J.; Leppänen, M. T. A Preliminary Study on the Ecotoxic Potency of Wastewater Treatment Plant Sludge Combining Passive Sampling and Bioassays. *Sci. Total Environ.* **2021**, *758*, No. 143700.
(60) Alvarenga, P.; Mourinha, C.; Farto, M.; Palma, P.; Sengo, J.; Morais, M.-C.; Cunha-Queda, C. Ecotoxicological Assessment of the Potential Impact on Soil Porewater, Surface and Groundwater from the Use of Organic Wastes as Soil Amendments. *Ecotoxicol. Environ. Saf.* **2016**, *126*, 102−110.
(61) Gupta, A.; Kumar, M.; Ghosh, P.; Swati; Thakur, I. S. Risk Assessment of a Municipal Extended Aeration Activated Sludge Treatment Plant Using Physico-Chemical and in Vitro Bioassay Analyses. *Environ. Technol. Innovation* **2022**, *26*, No. 102254.

(62) Hu, X.; Shi, W.; Wei, S.; Zhang, X.; Yu, H. Identification of (Anti-)Androgenic Activities and Risks of Sludges from Industrial and Domestic Wastewater Treatment Plants. *Environ. Pollut.* **2021**, *268*, No. 115716.
(63) Mazzeo, D. E. C.; Fernandes, T. C. C.; Levy, C. E.; Fontanetti, C. S.; Marin-Morales, M. A. Monitoring the Natural Attenuation of a Sewage Sludge Toxicity Using the Allium Cepa Test. *Ecol. Indic.* **2015**, *56*, 60−69.
(64) Renaud, M.; Chelinho, S.; Alvarenga, P.; Mourinha, C.; Palma, P.; Sousa, J. P.; Natal-da-Luz, T. Organic Wastes as Soil Amendments − Effects Assessment towards Soil Invertebrates. *J. Hazard. Mater.* **2017**, *330*, 149−156.
(65) Sjerps, R. M. A.; Vughs, D.; van Leerdam, J. A.; ter Laak, T. L.; van Wezel, A. P. Data-Driven Prioritization of Chemicals for Various Water Types Using Suspect Screening LC-HRMS. *Water Res.* **2016**, *93*, 254−264, DOI: 10.1016/j.watres.2016.02.034.
(66) Blackwell, B. R.; Ankley, G. T.; Corsi, S. R.; DeCicco, L. A.; Houck, K. A.; Judson, R. S.; Li, S.; Martin, M. T.; Murphy, E.; Schroeder, A. L.; Smith, E. R.; Swintek, J.; Villeneuve, D. L. An "EAR" on Environmental Surveillance and Monitoring: A Case Study on the Use of Exposure−Activity Ratios (EARs) to Prioritize Sites, Chemicals, and Bioactivities of Concern in Great Lakes Waters. *Environ. Sci. Technol.* **2017**, *51* (15), 8713−8724.
(67) Gros, M.; Blum, K. M.; Jernstedt, H.; Renman, G.; Rodríguez-Mozaz, S.; Haglund, P.; Andersson, P. L.; Wiberg, K.; Ahrens, L. Screening and Prioritization of Micropollutants in Wastewaters from On-Site Sewage Treatment Facilities. *J. Hazard. Mater.* **2017**, *328*, 37−45.
(68) Brunner, A. M.; Dingemans, M. M. L.; Baken, K. A.; van Wezel, A. P. Prioritizing Anthropogenic Chemicals in Drinking Water and Sources through Combined Use of Mass Spectrometry and ToxCast Toxicity Data. *J. Hazard. Mater.* **2019**, *364*, 332−338.
(69) Perin, M.; Dallegrave, A.; da Costa, J. S.; Streit, L.; de Araújo Gomes, A.; Pizzolato, T. M. Identification of the Organic Compounds in Surface Water: Suspect Screening Using Liquid Chromatography High-Resolution Mass Spectrometry and in Silico Toxicity Evaluation. *Int. J. Mass Spectrom.* **2023**, *484*, No. 116982.
(70) Rager, J. E.; Strynar, M. J.; Liang, S.; McMahen, R. L.; Richard, A. M.; Grulke, C. M.; Wambaugh, J. F.; Isaacs, K. K.; Judson, R.; Williams, A. J.; Sobus, J. R. Linking High Resolution Mass Spectrometry Data with Exposure and Toxicity Forecasts to Advance High-Throughput Environmental Monitoring. *Environ. Int.* **2016**, *88*, 269−280.
(71) Yang, J.; Zhao, F.; Zheng, J.; Wang, Y.; Fei, X.; Xiao, Y.; Fang, M. An Automated Toxicity Based Prioritization Framework for Fast Chemical Characterization in Non-Targeted Analysis. *J. Hazard. Mater.* **2023**, *448*, No. 130893.
(72) Arturi, K.; Hollender, J. Machine Learning-Based Hazard-Driven Prioritization of Features in Nontarget Screening of Environmental High-Resolution Mass Spectrometry Data. *Environ. Sci. Technol.* **2023**, *57*, 18067−18079, DOI: 10.1021/acs.est.3c00304.
(73) Reif, D. M.; Martin, M. T.; Tan, S. W.; Houck, K. A.; Judson, R. S.; Richard, A. M.; Knudsen, T. B.; Dix, D. J.; Kavlock, R. J. Endocrine Profiling and Prioritization of Environmental Chemicals Using ToxCast Data. *Environ. Health Perspect.* **2010**, *118* (12), 1714−1720.
(74) Marvel, S. W.; To, K.; Grimm, F. A.; Wright, F. A.; Rusyn, I.; Reif, D. M. ToxPi Graphical User Interface 2.0: Dynamic Exploration, Visualization, and Sharing of Integrated Data Models. *BMC Bioinf.* **2018**, *19* (1), No. 80, DOI: 10.1186/s12859-018-2089-2.
(75) Patlewicz, G.; Shah, I. Towards Systematic Read-across Using Generalised Read-Across (GenRA). *Comput. Toxicol.* **2023**, *25*, No. 100258.
(76) Brueck, C. L.; Xin, X.; Lupolt, S. N.; Kim, B. F.; Santo, R. E.; Lyu, Q.; Williams, A. J.; Nachman, K. E.; Prasse, C. (Non)Targeted Chemical Analysis and Risk Assessment of Organic Contaminants in Darkibor Kale Grown at Rural and Urban Farms. *Environ. Sci. Technol.* **2024**, *58*, 3690−3701, DOI: 10.1021/acs.est.3c09106.