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Wastewater Treatment Plants as Chemical Observatories to Forecast Ecological and Human Health Risks of Manmade Chemicals

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Thousands of chemicals have been identified as contaminants of emerging concern (CECs), but prioritizing them concerning ecological and human health risks is challenging. We explored the use of sewage treatment plants as chemical observatories to conveniently identify persistent and bioaccumulative CECs, including toxic organohalides. Nationally representative samples of sewage sludge (biosolids) were analyzed for 231 CECs, of which 123 were detected. Ten of the top 11 most abundant CECs in biosolids were found to be high-production volume chemicals, eight of which representing priority chemicals, including three flame retardants, three surfactants and two antimicrobials. A comparison of chemicals detected in nationally representative biological specimens from humans and municipal biosolids revealed 70% overlap. This observed co-occurrence of contaminants in both matrices suggests that the analysis of sewage sludge can inform human health risk assessments by providing current information on toxic exposures in human populations and associated body burdens of harmful environmental pollutants.

Thousands of organic chemicals have been identified as contaminants of emerging concern (CECs)[1]. Sampling and identification of CECs in various environmental matrices for prioritization of CECs is often time consuming, tedious, and costly. Hence, several methods of screening for potential CECs have been proposed[1–7]. Screening methods typically consider the persistence, bioaccumulation potential and toxicity of chemicals (PBT approach). However, this approach does not consider two critical aspects influencing the risks posed by chemicals to humans and ecosystems: current chemical production rates and the individual behavior of chemicals in real-world biological systems. Tools informing on chemical usage rates and real-world biodegradability of chemicals thus would be a welcome addition to the toolbox of risk assessors tasked with prioritizing and managing CECs. Most chemicals used in consumer products are ultimately washed down the drain and are collected in municipal sewers (Fig. 1). Efficient chemical monitoring at wastewater treatment plants (WWTPs) thus may provide up-to-date information on chemical usage rates for epidemiological assessments. This so-called "sewage epidemiology" approach has been employed by other researchers to evaluate illicit-drug use in communities via measurement of drug levels in influent wastewater[8–10]. Here, we demonstrate the use of sample repositories from U.S. WWTPs nationwide to conveniently derive information on the occurrence and identity of CECs as well as their bioaccumulation potential and propensity to withstand degradation processes. The underlying hypothesis of this work is that WWTPs can serve as chemical observatories to study the prevalence and likely fate of chemicals and their bioaccumulation potential in human society and the environment.

Secondary treatment of municipal sewage consists of a biological treatment operation employing a highly complex and concentrated microbial community. Chemicals managing to withstand unscathed the passage through primary and secondary WWTP unit operations have to be considered notably resistant to aerobic degradation processes (which typically are employed in secondary treatment) and thus may have the potential to also persist in the environment upon release. Biosolids, *i.e.*, treated municipal sludge fit for application on land are known to represent a 'sink' for hydrophobic organic compounds of limited biodegradability. Hundreds of organic chemicals including polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) and specific pharmaceuticals and personal care products (PPCPs), such as polychlorinated aromatic antimicrobials have been shown to accumulate to notable amounts in biosolids[11–17]. Empirical and deterministic models have been

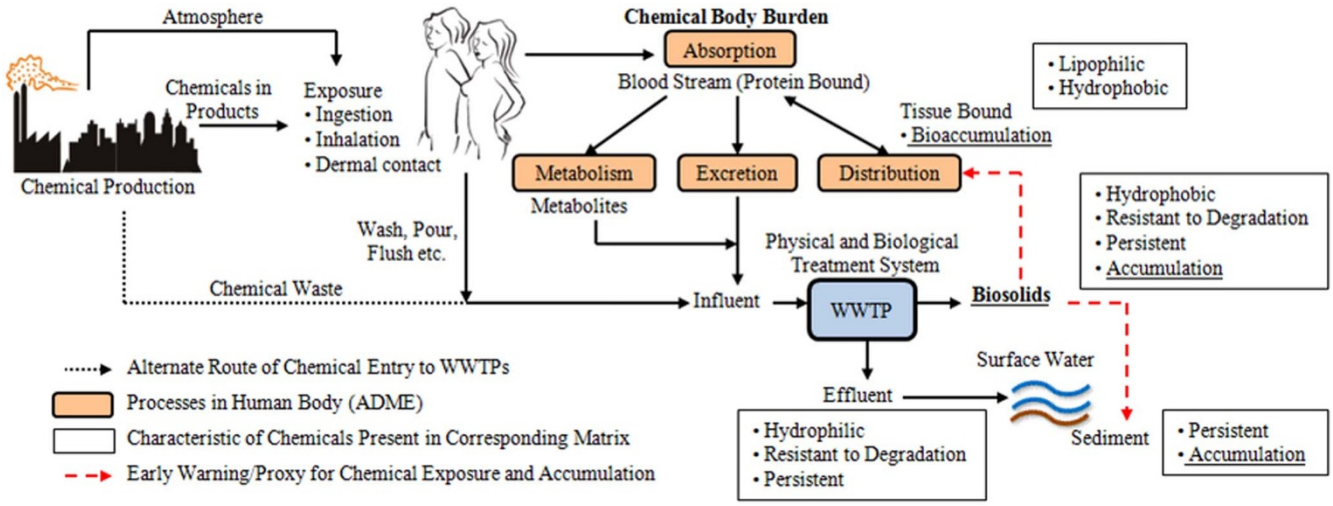

Figure 1 | Fate and transport of anthropogenic chemicals through human society and the built wastewater environment (Courtesy: Arizona State University).

proposed for predicting chemical loading to WWTPs and for identifying potentially problematic high-production volume (HPV) chemicals based on the fraction sorbed to biosolids[18–20]. In the present study, we explore the use of municipal biosolids as an analytical matrix to identify hydrophobic CECs potentially posing a human health threat based on chemical abundance, environmental persistence, and bioaccumulation potential, as indicated by a lack of transformation during aerobic and anaerobic digestion in modern WWTPs, and subsequent accumulation in the carbon- and lipid-rich biosolids which may serve as a proxy to the human body (Fig. 1).

Results

WWTPs as chemical observatories. A total of 123 chemicals were detected in biosolids, of which the nationwide occurrence of 17 brominated chemicals in U.S. biosolids is reported here for the first time (see Supplementary Table S9 online). The most abundant group of chemicals in biosolids were alkylphenol surfactants (AP and APEOs), followed by PPCPs and BFRs (Fig. 2a). Chemicals monitored in biosolids in this study were calculated to contribute about 0.04–0.15% of the total dry mass of biosolids produced in the U.S. annually, a mass equivalent to 0.4–1.5 g/kg of dry sludge or a total of 2,600–7,900 metric tonnes of chemicals annually. However, this estimate likely is lower than the true value, since other organics known to occur in biosolids (*e.g.*, linear alkylbenzene sulfonates, PCBs, etc.) were not included in this work. The study design further excluded hundreds of hydrophilic compounds that, while potentially being recalcitrant to the degradation processes, lack the potential for sequestration in biosolids and accumulation in humans.

Most abundant CECs in biosolids. The top abundant individual compounds detected in excess of 1000 μg/kg-dw composite biosolids samples emerging from the pool of 231 chemicals assayed for include: BFRs [deca BDE-209, 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), and penta-brominated BDE-99], surfactants [nonylphenol (NP) and their ethoxylates (NPEOs)]; antimicrobials (triclosan and triclocarban); and antibiotics (azithromycin, ciprofloxacin, and ofloxacin) (Fig. 2b). BFRs are widely used in plastics, textiles, electronics, and several household products to prevent fires. The penta-BDE market was dominated by North America in the 1990s, and declined after these compounds were banned in Europe in 2004[21–23]. Since then the demand for deca-BDE increased and only recently has been considered for regulation in the U.S.[21]. Although BTBPE is considered to be a low-volume BFR and was excluded from EPA's targeted national sewage sludge survey (TNSSS)[12], it falls under the category of highly abundant persistent chemicals list using the present study approach. BFRs are known to persist and bioaccumulate in the environment and also can be transformed to other toxic chemicals, including brominated dioxins and furans (Br-D/F)[24]. Br-D/F chemicals were also detected in the present study at low levels (see Supplementary Table S9 online). The pathway by which these compounds enter WWTPs is unclear, since BFR containing products are not meant to be flushed or discharged directly to the sewer. Their abundance in biosolids indicates an alternative pathway or route of entry to the environment that must be further studied. The surfactants and antimicrobials are known to be of high production volume [HPV - compounds featuring an annual usage volume in excess of 450,000 kg (1 million pounds)], representing persistent CECs that have been widely detected in various environmental matrices[14,25–27]. The three antibiotics (azithromycin, ciprofloxacin, and ofloxacin) fall into the category of highly abundant compounds, due to their extensive consumption in the United States. Sales of ciprofloxacin in the U.S. were already high at $1.97 billion in 2001 due to the anthrax scare[28]. Azithromycin is also one of the world's best selling antibiotics according to online sources and its sales volume in the U.S. is about $1.3 billion[29]. Although ciprofloxacin and ofloxacin have low partition coefficients (Fig. 3), sorption to biosolids is driven by electrostatic interactions with microorganisms in the sludge[30,31]. These chemicals have also been widely detected in U.S. surface water[26,32] and their fate depends on environmental pH levels. Human health concerns of antibiotics revolve primarily around their ability to induce resistance to multiple drugs in microbial pathogens. Buildup of antibiotics in the human body is a lesser concern, since the antibiotics' various polar moieties facilitate rapid urinary excretion; however, recent studies show bioaccumulation potential of antibiotics in macroorganisms[33,34]. Ciprofloxacin was shown to be one of the most frequently detected antibiotics in fish (muscle tissue) from the Haihe River in China[33].

In general, the fate of chemicals in the environment is determined by their partition coefficients and biodegradability. As noted above, the environmental behavior of certain chemicals cannot be predicted reliably from deterministic physical-chemical models. Idiosyncratic structural features and biological interactions may result in chemical fates which deviate substantially from those predicted from deterministic models alone. The here presented approach for estimating chemical fate from empirical observations made in actual biological systems (secondary wastewater treatment and sludge digestion units) thus may complement accepted contemporary approaches used in

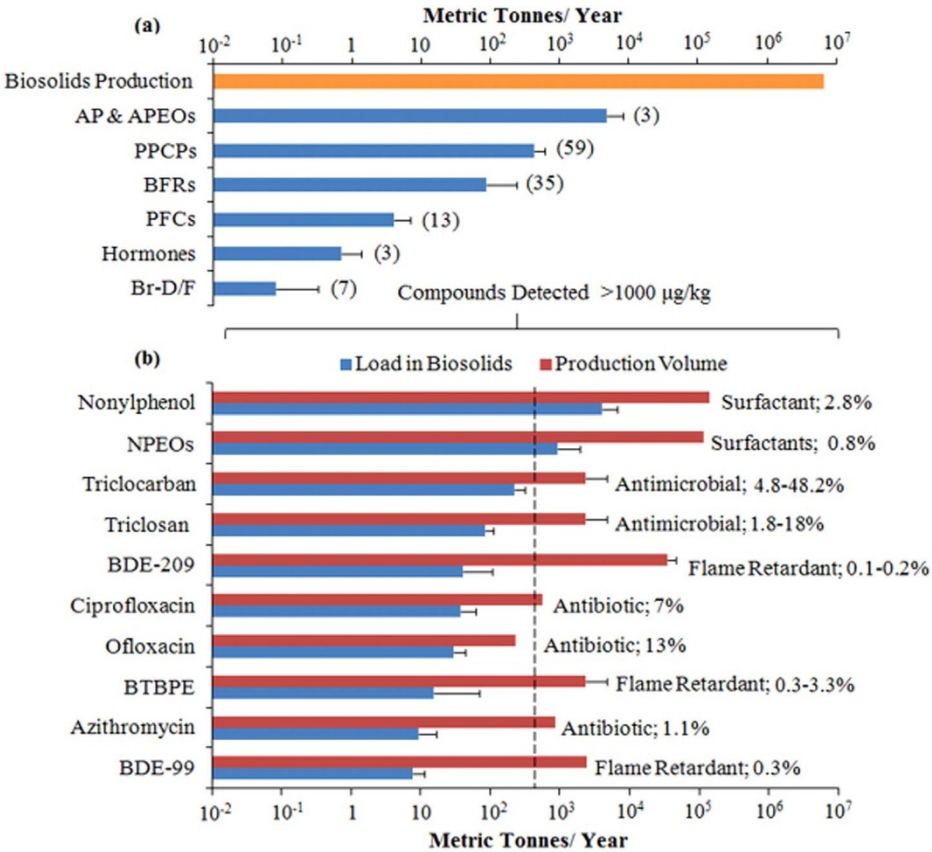

Figure 2 | (a) Annual mass of U.S. biosolids and of emerging contaminants sequestered in biosolids following municipal wastewater treatment, including sludge digestion. Numbers in parenthesis represent the number of chemicals detected in each chemical group. Refer to the text for a definition of acronyms used. (b) Occurrence in biosolids of chemicals detected in excess of 1000 µg/kg-dw and their corresponding annual U.S. production volumes. Labels to the right identify primary chemical uses and the percentage of production mass that becomes sequestered in biosolids. Dotted vertical line represents the annual production volume required for HPV classification (450,000 kg or 1 million U.S. pounds/year). Error bars represent the upper limit of average biosolids load and production volume, respectively.

risk assessment. When utilizing this proposed diagnostic approach, the secondary treatment step of municipal WWTPs essentially serves as a large-scale, aerobic preliminary biodegradability test for these manmade chemicals, whereas treatment steps for excess sewage sludge can serve to assess a chemical's propensity to partition into biota and resist anaerobic biodegradation processes.

Predicting consumption rates and environmental occurrence of CECs. The estimated annual biosolids loads of the top most-abundant chemicals detected in excess of 1000 µg/kg-dw (ppm) in composite biosolids were compared with their respective production volume in the U.S. (Fig. 2b). Between 0.1% and 48% of the individual compounds' production volume was found to be sequestered in biosolids. The highest percentage was determined for the antimicrobial triclocarban, and the lowest was observed for one particular congener (BDE-99) of penta-brominated diphenylether flame retardants. In addition, ten of the 11 most abundant compounds in biosolids (detected > 1000 µg/kg-dw) are HPV chemicals; for the single exception, the antibiotic ofloxacin (Fig. 2b), reliable production volume estimates were unavailable. These high levels of sequestration in sludge indicate the compound's stability and persistence from its production and use until disposal. The fact that 91% of the top 11 abundant compounds are HPV chemicals, indicate the integrity of biosolids matrix in capturing hydrophobic chemicals relative to their production volume. For example, the production of NP and NPEOs exceeds the volume of other abundant chemicals by more than an order of magnitude (see Supplementary Table S10 online), and these

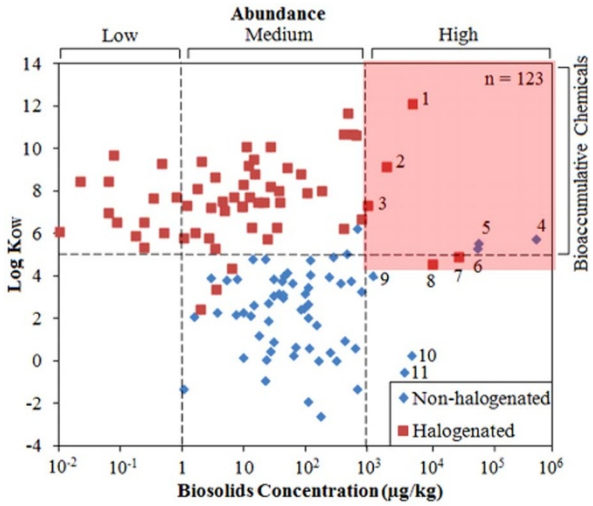

Figure 3 | **Classification of 123 chemicals detected in biosolids based on abundance in biosolids and on *n*-octanol water partition coefficient (K_{OW}).** Numbers represent highly abundant chemicals: (1) BDE-209, (2) BTBPE, (3) BDE-99, (4) NP, (5) NP1EO, (6) NP2EO, (7) triclocarban, (8) triclosan, (9) azithromycin, (10) ciprofloxacin, (11) ofloxacin. The shaded region in the top right is populated by priority CECs of high abundance and high bioaccumulation potential.

two surfactants were the most abundant chemicals detected in biosolids (Fig. 2a).

High chemical usage rates imply substantial chemical releases to the environment and thus an increased risk of exposure for susceptible receptors. Biosolids are known to be highly enriched in hydrophobic chemicals, which render them an ideal matrix for environmental monitoring. Hydrophobic compounds detected in biosolids in the parts-per-trillion (<1 μg/kg-dw) range are expected to feature very low environmental occurrences, good biodegradability, or a combination of the two. In contrast, chemicals occurring in biosolids in the parts-per-million (ppm) range (>1000 μg/kg-dw) pose a potentially significant concern, due to high usage, resistance to biodegradation and pronounced partitioning into biosolids destined for application on land. Based on these established facts, chemicals in biosolids may be prioritized in terms of potential environmental occurrence and risk by separating them into three groups indicating low (<1) medium (1 – 1000) and high (>1000) abundance in biosolids, expressed in units of μg/kg-dw (Fig. 3). Among the 123 compounds detected in this survey of 231 analytes, 12 showed low abundance, 100 showed medium abundance, and 11 featured high abundance. The selection of the ceiling threshold of 1,000 μg/kg-dw is supported by European and U.S. regulations that focus on compounds occurring in excess of 1 mg/kg (ppm) in biosolids[35]. Since tracking of the present-day production volume of chemicals used in commerce is difficult, ranking chemical abundance (as high, medium, and low) from concentrations detectable in process flows in WWTPs nationwide is a useful tool and particularly promising for compounds that are disposed of customarily into wastewater (e.g., triclocarban, an active ingredient in antimicrobial soaps).

Predicting chemical body burden and bioaccumulation potential of CECs. The national report on human exposure potential to environmental chemicals by the Center of Disease Control and Prevention (CDC) is one of the most comprehensive exposure assessments of environmental chemicals in the U.S. population. About 139 organic chemicals have been detected in human blood, serum, urine, and tissue samples that represent the U.S. population[36]. Chemical levels detected in humans reflect the amount of chemical that actually gets into the body by all routes of exposure (ingestion, inhalation, and dermal absorption)[36]. When combining the number of chemicals detected in the present study with those detected in EPA's national sewage sludge surveys, a total of 187 organic chemicals have been detected in nationwide representative biosolids samples. Out of these, 52 chemicals have already been analyzed by CDC, with 36 detects and 16 non-detects in human samples (Fig. 4a; see Supplementary Table S11 online). That is, about 70% of chemicals detected in biosolids were also detected in humans. The 16 non-detect chemicals were all detected in biosolids at levels < 25 μg/kg-dw and fall into the categories of medium and low abundance compounds, indicating lower consumption rates. Out of the 36 detects, 34 chemicals were detected in human serum samples, and two (triclosan and 1-hydroxypyrene) were detected in urine samples. It is well established that the partitioning of hydrophobic chemicals to sludge is due to their association with the lipid fraction of microbial biomass[37,38]. This mechanism of partitioning is similar to the way these chemicals bioaccumulate in macroorganisms, wildlife and humans. Hence for comparison purposes, the lipid-normalized concentrations of the 34 analytes commonly detected in human serum and biosolids samples were plotted against each other (Fig. 4b; see Supplementary Table S12 online). The plot revealed a linear trend ($R^2 = 0.62$) suggesting a similarity in the relative exposure to these chemicals by humans and WWTPs. This relationship strengthens our hypothesis of using the biosolids matrix as an indicator chemical exposure and body burden in human populations, and suggests a need to investigate the occurrence in humans of not previously monitored chemicals detectable as persistent contaminants in biosolids.

The approach of biosolids analysis illustrated here additionally can serve as an early warning system for potentially bioaccumulative chemicals. Chemicals detected in biosolids were categorized as bioaccumulative based on the criterion set by the Stockholm Convention, i.e., an *n*-octanol/water partitioning coefficient (K_{OW}) of 10^5 or greater. Using this approach, 55 chemicals were identified as being potentially bioaccumulative; 93% of which were halogenated, remarkably (Fig. 3). Also, 14 out of the 55 chemicals have already been detected in human samples reported elsewhere[39–42], thereby emphasizing the need to assess the body burden, toxicity and risk posed to human health by the remaining 31 chemicals. Of the 55 bioaccumulative chemicals, six were identified as highly abundant, 37 as having medium abundance and 12 as low-abundance chemicals with respect to their detection levels in biosolids. The six highly abundant chemicals included three BFRs and three surfactants (BDE-99, BDE-209, BTBPE, NP, NP1EO, and NP2EO), which have been shown to bioaccumulate in aquatic animals[23,43–45]. Although the abundant antimicrobials triclosan and triclocarban do not quite satisfy the criterion for bioaccumulation per se (log K_{OW} of 4.9 for triclocarban and 4.6 for triclosan), they are known to bioaccumulate in aquatic organisms[46,47]. Hence, a total of eight abundant chemicals (BDE-99, BDE-209, BTBPE, NP, NP1EO, NP2EO, triclosan and triclocarban) are highlighted in Figure 3 as priority chemicals with respect to both abundance and bioaccumulation potential.

Some of these chemicals have already been identified as priority CECs in past studies that focused on similar prioritization effort[6,7]. Triclosan was identified as one of the priority CECs in recycled water systems using a similar science-based framework developed to prioritize contaminants' inclusion in reclaimed water monitoring programs[7]. The authors used environmental concentrations and toxicological relevance of CECs to help in the prioritization effort. Another study identified BTBPE and triclocarban among the top 10 (brominated and chlorinated, respectively) persistent and bioaccumulative (P&B) priority chemicals within a pool of 22,263 chemicals from the Canadian Domestic Substance List (DSL) and U.S. EPA's Toxic Substances Control Act (TSCA) Inventory Update Rule (IUR) database by using quantitative structure activity relationship (QSAR)[6]. The top priority chemicals were selected based on the production volume, bioconcentration factor and persistence of CECs. Though these criteria for selection are similar to the present study, the biosolids analysis approach additionally incorporates environmentally relevant concentrations and fate of CECs in real-world biological systems to identify priority chemicals. In addition, it can convey important information on the actual persistence of chemicals in environmental systems, as some chemicals may biodegrade rapidly in the laboratory but fail to do so in field settings, where sorption and a lack of bioavailability can impede attenuation.

Figure 3 additionally emphasizes the established fact that halogenated compounds are more hydrophobic and lipophilic when compared to non-halogenated compounds. This explains their dominance in the bioaccumulation region (Fig. 3; top right). It must also be noted that out of the 36 chemicals detected in both humans and biosolids samples (Fig. 4a), 35 chemicals (97%) were halogenated. An analysis of the compounds included in the U.S. Safe Drinking Water Act of the EPA shows that 74 percent of regulated organic compounds carry one or more halogen substituent[48]. It is well known that the replacement of hydrogen with halogen atoms is positively correlated with a chemical's size, hydrophobicity, persistence and toxicity[49–51].

Discussion

Chemical abundance in biosolids should be interpreted as a multifaceted, cumulative proxy to the (i) current rate of chemical usage, (ii) resistance to biotransformation, and (iii) partitioning behavior

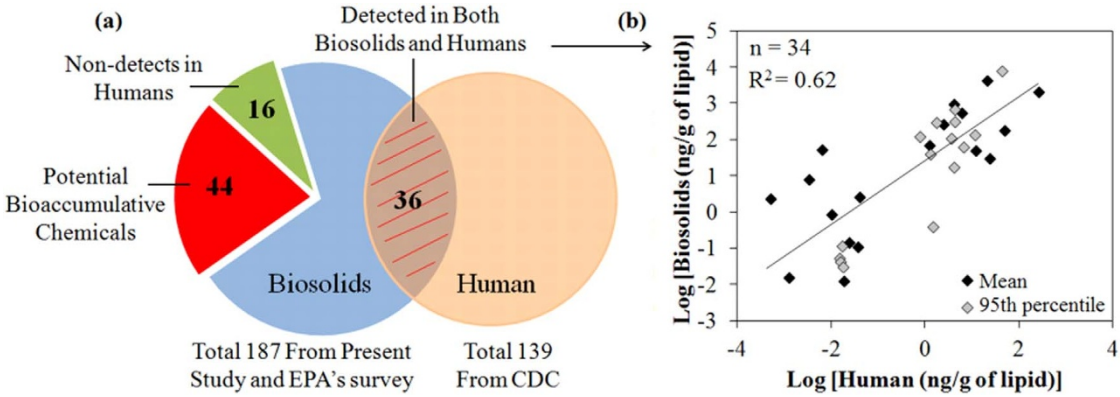

Figure 4 | (a) Comparison of organic chemicals detected in biosolids (from present study and EPA's national sludge survey together) and in human samples from the national report on human exposure to environmental chemicals study by CDC, representing the U.S. population. The hashed portion represents the common chemicals detected in both biosolids and humans. (b) Comparison of lipid-normalized concentration of the 34 chemicals detected in human serum and biosolids samples. Analytes with available geometric mean concentrations in human serum were compared with mean concentrations detected in biosolids. For analytes with unavailable mean concentration in human serum, 95th percentile values were compared.

(and thus bioaccumulation potential) as shown in this study. The present work explored the new approach of using biosolids as an analytical matrix to identify potentially harmful, mass-produced chemicals of human health concern. Admittedly, the 231 CECs investigated here represent only a modest sample of the tens of thousands of potential CECs present in modern society. By necessity, the current approach was limited to compounds for which analytical methods were readily available. Future work will be required to extend this effort to include other CECs, and to identify a much wider range of mass-produced priority chemicals, their byproducts and transformation products. Use of gas chromatography-mass spectrometry in scan mode followed by spectral analysis using database searches may aid in this chemical discovery process.

Additionally, chemicals sequestered in biosolids may serve as an early warning system for determining potential bioaccumulative chemicals and chemical body burdens in population. This 'pre-screening' step effectively can reduce the several thousands of known or suspected hydrophobic CECs to a manageable list of priority chemicals. Furthermore, it also can serve to conveniently screen experimentally for transformation products of man-made and natural compounds which likely are persistent and bioaccumulative but for which production and environmental loading data are unavailable. From the present study, we infer that the eight compounds falling into the "most abundant" category have to be considered priority contaminant candidates from a public health perspective, deserving scrutiny with respect to their potential for exposure, bioaccumulation and potential adverse health effects in biota, including humans. While the screening approach presented here provides important insights into the abundance and bioaccumulation potential of chemicals, it does not inform on chemical toxicity, which is critical for risk assessment of chemicals. Hence, for a comprehensive risk screening, chemicals emerging as a priority contaminant candidate from the present screening approach should further be evaluated with expert knowledge of their absorption, distribution, metabolism, and excretion (ADME) and their potential toxicity to humans and ecosystems.

Overall, the approach presented here was found to be in concordance with results of prior risk prioritization approaches[6,7] while also offering insights into current chemical consumption rates. An interesting correlation was found between chemicals that bioaccumulate in humans and those that persist during wastewater treatment and accumulate in sludge (Fig. 4b). Such a correlation intrinsically is vulnerable to sample bias, however. Therefore, future work is needed to further validate and carefully define the limits of the chemical prioritization approach presented here.

Methods

Sample description. Biosolids samples analyzed in the present study were originally collected by U.S. EPA as part of the 2001 National Sewage Sludge Survey (NSSS). After completion of 2001 NSSS, the samples were acquired by our laboratory and stored in amber glass jars (500 mL) at −20°C for further analysis. The facilities were selected by the U.S. EPA to obtain unbiased national estimates of chemical contaminants in U.S. sewage sludges that are disposed of primarily by land application. The samples were collected between February and March 2001 according to an established protocol, only from facilities that included secondary treatment. Representative samples were collected in 500 mL glass or polyethylene jars from 94 WWTPs in 32 U.S. States and the District of Columbia. Information on specific sampling locations is available in the peer-reviewed literature[11]. Samples were collected only from processed sewage sludges intended for disposal. The biosolids composites analyzed in this study constitute a representative sample (94 facilities) of the more than 16,000 U.S. WWTPs. Of the 94 WWTPs, 89 had single system (either aerobic or anaerobic digestion) and five of them had two systems for sludge treatment (both aerobic and anaerobic digestion). Samples were collected from each treatment systems amounting to a total of 113 biosolids samples. Three of these samples were excluded from analysis due to broken containers. The rest of the 110 biosolids samples were randomly grouped into five composite samples, each containing solids from between 21 and 24 individual samples. Sampling procedure and preparation of composites are described in detail elsewhere[11]. A duplicate of composite sample #1 was prepared to serve as a blind duplicate. Composite samples were prepared to establish national baseline levels for these compounds; the validity of the present approach has been demonstrated previously[11,15–17].

Sample analysis. The samples were analyzed by a commercial lab (AXYS Analytical Services Ltd., Sydney, British Columbia, Canada) that developed EPA Method 1694 for pharmaceuticals and personal care products, and that specializes in the analysis of traditional and emerging contaminants. AXYS is a nationally accredited commercial lab in Canada and also is accredited by the National Environmental Laboratory Accreditation Program (NELAC) in Florida and New Jersey. The biosolids composites were analyzed for pharmaceuticals and personal care products (PPCPs), alkylphenols and their ethoxylates (AP and APEOs), brominated flame retardants (BFRs), perfluorinated compounds (PFCs), brominated dioxins and furans (Br-D/F), hormones and sterols. The concentrations were determined by isotope dilution for compounds with available labeled analog. Concentrations of compounds without a labeled analog were determined by external calibration. All concentrations are reported on a dry weight (dw) basis. Analytes were extracted and analyzed for each group of compounds as follows:

PPCPs. Samples were analyzed according to AXYS Method MLA – 075, a modification of U.S. EPA Method 1694. For the purpose of compound detection, the 120 analytes were divided into five groups. All analytes were separated by liquid chromatography and detected by tandem mass spectrometry (LC-MS/MS) (see Supplementary Table S1 online). List 1–3 and 5 were extracted at a pH 2.0 (acid extraction), while fourteen analytes in list 4 required basic extraction at pH 10. Prior to extraction, 1 g of dried samples were adjusted to the required pH and spiked with surrogates/labeled analogs. Solid samples were extracted by sonication with acetonitrile, concentrated by rotary evaporation, and diluted with ultra pure water to 200 mL. The extracts were then filtered, cleaned up by solid phase extraction (SPE), and analyzed by LC-MS/MS in positive and negative modes. Lists 1, 2, 4 and 5 were analyzed in positive electro-spray ionization (ESI) mode, whereas List 3 was analyzed in negative ESI mode (EPA Method 1694; EPA-821-R-08-002). Detailed analysis procedures and method performance are described elsewhere[15].

AP and APEOs. The samples were base digested and liquid-liquid extracted with hexane followed by non-aqueous acetylation. Derivitized extracts were then cleaned

up using a 5% silica chromatography column. A recovery standard was added prior to analysis. Instrumental analysis was performed on a RTX-5 capillary gas chromatography column coupled to a low-resolution mass spectrometer (LRMS) (see Supplementary Table S2 online). The LRMS was operated in electron ionization (EI) mode using multiple ion detection (MID) acquiring at least 2 characteristic ions for each target analyte and surrogate standard. Detailed analysis procedures and method performance are described elsewhere[16].

BFR. A selected suite of brominated diphenylethers (BDEs) were analyzed according to the protocol described in EPA Method 1614. Samples were spiked with isotopically labeled BDE surrogate standards, solvent extracted, spiked with a cleanup surrogate standard and cleaned up on a series of chromatographic columns that included layered acid/base silica, Florisil and alumina columns. The final extract was spiked with isotopically labeled internal standards prior to instrumental analysis. Analysis of BDE was performed using a capillary gas chromatograph coupled to a high resolution mass spectrometer (HRMS). A DB-5HT capillary column (30 m, 0.25 mm i.d. × 0.1 μm film thickness) was coupled to the MS source. Two masses from the molecular ion cluster were used to monitor each of the target analytes (EPA Method 1614; EPA-821-R-07-005). Non-BDE BFRs were analyzed by the same method by introducing additional native, labeled surrogates, and calibration standard solutions (see Supplementary Table S3 online).

For polybrominated biphenyl analysis (PBB), about 1 g (dry) of sample was spiked with 13C-labeled polychlorinated biphenyl (PCB) quantification standards and Soxhlet extracted with dichloromethane. The extracts were cleaned up using Florisil columns. The final extracts were concentrated and spiked with recovery standards prior to analysis by high performance liquidchromatography/tandem mass spectrometry (HRGC/HRMS) (see Supplementary Table S4 online).

For hexabromocyclododecane (HBCDD) analysis, about 1.5 g (dry) sample was spiked with 13C-labelled surrogate standards and Soxhlet extracted with dichloromethane. The extracts were cleaned up using Florisil column. The final extracts were concentrated and spiked with recovery standards prior to analysis by high performance liquid chromatography/tandem mass spectrometry (HPLC/MS-MS) (see Supplementary Table S5 online).

PFCs. About 5 g of dried samples were spiked with isotope-labeled surrogates and analytes were extracted by shaking initially with dilute acetic acid solution and then twice with methanolic ammonium hydroxide solution, each time collecting the supernatants. The supernatants were combined and treated with ultra pure carbon powder. The resulting solution was diluted with water and cleaned up by SPE. The eluate was then spiked with recovery standards prior to analysis. The sample extract was then separated by high performance liquid chromatography reversed phase C18 column using a solvent gradient. The column was coupled to a triple quadrupole mass spectrometer in Multiple Reaction Monitoring (MRM) mode (see Supplementary Table S6 online). Detailed analysis procedures and method performance are described elsewhere[17].

Br-D/F. Samples were spiked with isotope labeled surrogate standards, dried with sodium sulphate followed by Soxhlet extraction in toluene/acetone. The crude extracts were then cleaned by column chromatography on a layered acid/base silica column. The extract was then spiked with isotope-labeled internal standards and analyzed by capillary gas chromatography/high resolution mass spectrometry (GC/HRMS). A DB-5HT capillary column was used for separation. Two masses from the molecular ion cluster were used to monitor each of the target analytes (see Supplementary Table S7 online).

Sterols and hormones. About 1 g of dried samples was acidified to pH 2 and spiked with surrogate standards. The sample was then extracted by sonication with aqueous buffered acetonitrile and with pure acetonitrile, concentrated by rotary evaporation, and diluted with ultra pure water to 200 mL. The extracts were then cleaned up by SPE. After addition of recovery standards, the cleaned extracts were analyzed by high performance liquid chromatography coupled to a triple quadrupole mass spectrometer. The analysis was run in MRM mode in both positive and negative ESI mode (see Supplementary Table S8 online).

Quality assurance. Several tests were performed before sample analysis to ensure system and laboratory performance. A calibration standard solution with labeled and native analytes was used to verify calibration accuracy. Retention times of the analytes had to be within ±15 seconds of the respective retention time established during the previous calibration. Lab blanks were analyzed before each sample. Duplicate analysis was performed for each batch with more than six samples. To evaluate analysis precision, a blind duplicate was included in the sample set.

Modeling annual load of chemicals in biosolids. Annual load was determined for all detected analytes based on the annual biosolids production of 5.1–6.4 million metric dry tonnes (5.6–7 million dry U.S. tons) estimated for the year 2001 in the U.S. (Supplementary Table S9)[52–54].

[Annual load = (chemical concentration in biosolids) μg/kg * (10^{-9} kg/μg) * (5.1–6.4 × 10^{9} kg of biosolids/year)]

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Author contributions

A.K.V. prepared biosolids composite samples, performed data analysis and wrote the first draft of the paper. R.U.H. and A.K.V. designed the study. R.U.H. conceived the project and supervised the data interpretation and manuscript preparation.

Additional information

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