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United States National Sewage Sludge Repository at Arizona State University – A New Resource and Research Tool for Environmental Scientists, Engineers, and Epidemiologists

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Abstract

Processed municipal sewage sludges (MSS) are an abundant, unwanted by-product of wastewater treatment, increasingly applied to agriculture and forestry for inexpensive disposal and soil conditioning. Due to their high organic-carbon and lipid contents, MSS not only is rich in carbon and nutrients but also represents a ‘sink’ for recalcitrant, hydrophobic and potentially bioaccumulative compounds. Indeed, many organics sequestered and concentrated in MSS meet the U.S. Environmental Protection Agency's definition of being persistent, bioaccumulative, and toxic (PBT). In a strategic effort, our research team at the Biodesign Institute has created the National Sewage Sludge Repository (NSSR), a large repository of digested MSSs from 164 wastewater treatment plants from across the USA, as part of the Human Health Observatory (H2O) at Arizona State University (ASU). The NSSR likely represents the largest archive of digested MSS specimens in the USA. The present study summarizes key findings gleaned thus far from analysis of NSSR samples. For example, we evaluated the content of toxicants in MSS and computed estimates of nationwide inventories of mass produced chemicals that become sequestered in sludge and later are released into the environment during sludge disposal on land. Ongoing efforts document co-occurrence of a variety of PBT compounds in both MSS and human samples, while also identifying a large number of potentially harmful MSS constituents for which human exposure data are still lacking. Finally, we summarize new future opportunities and invite collaborative use the NSSR by the research community. The H2O at ASU represents a resource and research tool for environmental scientists and the larger research community. As illustrated in this work, this repository can serve to (i) identify and prioritize emerging contaminants; (ii) provide spatial and temporal trends of contaminants; (iii) inform and evaluate the effectiveness of environmental policy-making and regulations; and (iv) approximate, ongoing exposures and body burdens of mass-produced chemicals in human society.

Keywords

Environmental Specimen Banks; National Repository; Sewage Sludge; Biosolids; Human Health Observatory; Contaminants of Emerging Concern

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Introduction

Processed municipal sewage sludge (MSS) or biosolids are an abundant byproduct of wastewater treatment. About 6.5 million metric dry tonnes (7.18 million U.S. tons) of MSS is produced in the U.S. annually (NEBRA 2007). The term sewage sludge and biosolids are used interchangeably by the U.S. Environmental Protection Agency (U.S. EPA). The U.S. EPA defines biosolids as treated or processed sewage sludge that can be beneficially recycled. Thus, the term biosolids refers to the particulate-containing solid, semi-solid, or liquid byproduct that is usually applied on land as a soil amendment/fertilizer. However, part of the treated sewage sludge is also disposed of in incinerators and landfills. MSS is rich in organic-carbon and lipid content, and hence serves as a sink to hydrophobic and persistent contaminants. The U.S. EPA is required to identify and regulate toxic contaminants that may be present in MSS at levels of concern to ecological and human health, under the Clean Water Act that was passed in 1972. Hence, U.S. EPA has performed four sewage sludge surveys thus far, to identify inorganic and organic contaminants for regulation considerations (U.S. EPA 2007, U.S. EPA 2009). These surveys are important since about 50% of the MSS produced annually in the U.S. is reused as a soil amendment (NEBRA 2007, U.S. EPA 2012a). The information from the 1988-89 survey was used to establish the 'The standards for the use or disposal of sewage sludge' (Title 40 of CFR Part 503) that describes the general requirements, pollutant limits and management practices for the use or disposal of sewage sludge. Currently, the U.S. EPA has established standards for nine toxic compounds in land-applied sewage sludge, all belonging to the inorganic group of metals, including As, Cd, Cu, Hg, Mo, Ni, Pb, Se, and Zn (§503.13). The Part 503 regulation focuses on the presence of nine metals, pathogens and MSS attractiveness to vectors as a basis for determining MSS quality. MSS that meet the most stringent limits for all three of these parameters are termed Exceptional Quality (EQ) MSS. MSS that do not meet the stringent quality are termed non-EQ MSS. Facilities producing EQ MSS are not required to follow the Part 503 rule for land application. Non-EQ MSS, however, requires following the Rule with respect to pollutant limits, pathogens (Class A – "pathogen free" and Class B – some pathogens), vector attraction and public access restrictions depending upon the degree to which the quality diverges from EQ (U.S. EPA 1994).

Though organics including pharmaceuticals and personal care products (PPCPs) and select flame retardants were included in surveys conducted by the EPA, standards or limits for organic compounds have not yet been established. From the 2001 sewage sludge survey reported in 2007, U.S. EPA concluded that numeric standards or management practices are not warranted for dioxin and dioxin-like compounds in land-applied sewage sludge (U.S. EPA, 2007). Several researchers have criticized the methodology and regulation process of the U.S. EPA (Iranpour et al. 2004, McBride 2003). Additionally, recent studies have shown contamination of groundwater, surface water, and plant uptake of organic contaminants from soil amended with MSS (Clarke and Smith 2011, Lapworth et al. 2012, Sepulvado et al. 2011, Wu et al. 2010, Xia et al. 2010). The U.S. EPA has screened for only a small fraction of the more than 40,000 contaminants of emerging concern (CEC) that potentially could be present in MSS (Diamond et al. 2011). Additionally, it is estimated that about 2,000 new chemicals are introduced into commerce each year in the USA (California Department of

Toxic Substances Control 2013). Hence more frequent surveys and a wider screening approach is required in order to identify and prioritize CECs. In a strategic effort, our team at the Biodesign Institute at Arizona State University has created the National Sewage Sludge Repository (NSSR) as part of the Human Health Observatory (H2O) to address this research need.

National Sewage Sludge Repository (NSSR)

The NSSR consists of digested MSS samples collected from 164 wastewater treatment plants (WWTPs) across the United States by the U.S. EPA during their 2001 (reported in 2007) and 2006/7 (reported in 2009) National Sewage Sludge Surveys (NSSS). After completion of surveys, the samples were acquired by our laboratory and stored at -20°C for further analysis. Samples were stored initially at Johns Hopkins University, and later transferred to Arizona State University with chain of custody documentation for long-term maintenance. The NSSR likely represents the largest archive of digested MSS specimens in the U.S. The rationale for creating NSSR was to leverage the benefits of sewage sludge analysis in general and the value of samples collected as part of the U.S. EPA's surveys in particular. The primary goal of initial investigations was to determine the occurrence and identity of CECs in U.S. sludges, and to compute estimates of nationwide inventories of CECs that have not been included in prior surveys. To date we have analyzed and reported nationwide occurrence of hundreds of CECs using the NSSR. The target chemicals that we focused on included PPCPs, brominated flame retardants (BFRs), perfluorinated compounds (PFCs), alkylphenol and their ethoxylates (AP & APEOs), hormones as well as brominated dioxins and furans (Br-D/F). Work is ongoing to evaluate other toxic contents present in MSS samples. Here, we review prior work utilizing the NSSR samples, identify future research opportunities, and invite collaborative use of NSSR samples by the research community.

NSSR Sample Inventory

The NSSR consists of samples collected by the U.S. EPA during their 2001 and 2006/7 NSSS. MSS specimens were collected from 164 WWTPs from across the contiguous United States, representing the more than 16,000 WWTPs operating in the U.S (Fig. 1). The repository currently houses a total of 190 MSS specimens (Table 1; Fig. 2).

2001 NSSS Samples

MSS samples were collected by the EPA from 94 WWTPs in 32 U.S. states and the District of Columbia as part of the 2001 NSSS. Information on sampled facilities is available as supplementary material (Table S1). 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 purpose of EPA's 2001 survey was to estimate levels of dioxins, dibenzofurans, and coplanar polychlorinated biphenyls in MSS (U.S. EPA 2007). Grab samples were collected between February and March 2001, according to an established protocol, exclusively targeting facilities featuring secondary treatment (U.S. EPA 2007, U.S. EPA 1988). All samples were collected in 500 mL glass or polyethylene/polypropylene jars. Polyethylene gloves were used to prevent sample

contamination. Solid MSS samples were collected by using a polyethylene pail and polypropylene scoop (U.S. EPA 1988). Samples were directly collected from the sludge discharge chute in the pail, thoroughly mixed using the scoop and then transferred to the containers. If the facility featured two or more dewatering units, equal amounts of samples were collected from each discharge point and mixed in the polyethylene pail. While collecting liquid MSS samples from storage tanks, the pumps were ran for five minutes to clear stagnant sludge and then samples were collected in to the pail. The collected samples were thoroughly agitated in the pail using a polyethylene ladle and then transferred to the container.

Once samples were collected, identifying labels were attached to the containers and the container lids were further sealed with tape. The samples were iced, packed and shipped to laboratory for storage/analyses promptly via overnight shipping service. Samples were collected only from processed MSS intended for disposal. 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. In addition, duplicate samples were collected from 14 facilities, amounting to a total of 113 MSS samples. Three samples were lost due to breaking of containers during transportation. The NSSR currently harbors a total of 110 samples from the 2001 NSSS. The solids content of the MSS samples ranged between 1.5 and >90%.

2006/7 Targeted National Sewage Sludge Survey (TNSSS) samples

MSS samples were collected from 74 publicly owned WWTPs that participated in the U.S. EPA's TNSSS. The facilities selected in the survey are statistically representative of the target population consisting of WWTPs that: (i) were operational during 2002 and/or 2004; (ii) had a flow rate of greater than 3.8 million liters per day (MLD) [or 1 million gallons per day (MGD)]; (iii) employed a minimum of secondary treatment; and (iv) were located in the contiguous United States. From the 3,337 WWTPs that met the above criteria, the U.S. EPA statistically selected 74 facilities using a random sampling design stratified for flow [3.8 to 38 MLD (1 to 10 MGD), 38 to 380 MLD (10 to 100 MGD), and >380 MLD (>100 MGD)] from 35 U.S. states. Grab samples of MSS were collected by U.S. EPA from each facility between August 2006 and March 2007. Four of the facilities had two treatment systems for solids; hence a second sample was collected to represent both treatment systems. The solids content of the MSS samples ranged from 4% to 93%. Additionally, a duplicate grab sample was collected from six other facilities to allow for variations associated with the sampling procedure. Solid samples represented dewatered sludges collected from dewatering units like belt press, filter press, drying bed, centrifuge, compost pile or other sources. Small grab samples were collected from multiple regions in large piles (or multiple grabs from continuous processes), and then composited in a large pre-cleaned container, so that the solids represented the bulk sludge product (U.S. EPA, 2009). The composites were thoroughly mixed and transferred to final sampling containers. All samples collected were final treated product of MSS. Liquid samples were collected as free-flowing materials from storage tanks. The sampling pipe lines were purged and any mixing equipments were turned on prior to sampling, so that the collected liquids represented the bulk product. Liquid samples were collected directly into the final sample containers made of either glass or

HDPE. After sample collection, the containers were labeled with preprinted label containing EPA episode and sample number. The samples collected from each facility were packed, iced and shipped overnight to EPA sample repository in Baltimore, MD (U.S. EPA, 2009).

The objective of the TNSSS was to report the occurrence in MSS of selected contaminants of emerging concern (*e.g.*, PPCPs, BFRs) as identified by the U.S. EPA and the National Research Council (NRC). After completion of TNSSS, leftover samples were acquired by our laboratory and stored at -20°C for further analysis. From the 84 MSS samples, four were excluded from analysis because the sample containers were either missing or broken. Information on sampled facilities is available as supplementary material (Table S2).

Past Applications of the NSSR

Mass Inventories of CECs

The U.S. EPA has screened for a suite of legacy contaminants (in addition to inorganics analyses) that included polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls in its 2001 NSSS samples (U.S. EPA 2007). The toxic equivalent quantity (TEQ; expressed as toxic equivalents of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) were found to vary from 7 ng/kg to over 100 ng/kg in the MSS samples. In the 2006/7 TNSSS study (reported in 2009), the U.S. EPA screened for a suite of 72 PPCPs, six semi-volatile organics and polycyclic aromatic hydrocarbons, 25 steroids and hormones and 11 polybrominated diphenyl ethers (PBDEs), of which 98 contaminants were detected in MSS samples (U.S. EPA 2009). To date, the U.S. EPA has not evaluated these pollutants for regulatory actions. In an effort to include additional CECs in the screening process, our team at the Biodesign Institute at ASU has analyzed composite samples prepared from individual MSS samples obtained in the 2001 NSSS.

The first study from our group, conducted in 2009 screened for 72 PPCPs (the same suite of analytes screened by EPA in their 2006/7 survey) in five composites prepared from the 2001 NSSS samples (McClellan and Halden 2010). Thirty-eight analytes were detected in at least one composite sample and the concentration ranged from 0.002 to 48 mg/kg dry weight (dw). The antimicrobial compounds, triclocarban (TCC) and triclosan (TCS), were found to be the most abundant analytes at mean concentrations of 36 ± 8 and 12.6 ± 3.8 mg/kg dw, respectively. Using the percent of MSS applied on land, it was estimated that about 210 - 250 metric tonnes of total PPCPs were released to U.S. soils via MSS land application (McClellan and Halden 2010). The contaminants occurrence frequency and levels were shown to be similar to those reported by the U.S. EPA in its 2006/7 TNSSS survey, demonstrating that PPCP releases in U.S. MSS have been ongoing for several years. The contract laboratory that developed EPA Method 1694 for PPCP analysis updated their analytical methods to include 48 new analytes. Hence, a second study was conducted in 2012 by recreating the composites from the 2001 NSSS and analyzing for a total of 120 PPCPs in MSS (Chari and Halden 2012). This latter study reported on the occurrence in MSS of 26 not previously monitored PPCPs in composite samples at levels ranging from 1.65 to 673 µg/kg dw (Fig. 3a). These newly detected 26 analytes (Chari and Halden 2012) contributed an estimated additional 5 - 15 metric tonnes of PPCPs to the loading of U.S. soils previously reported (McClellan and Halden 2010). Chemical concentrations in our

2009 and 2012 PPCP analysis were statistically indistinguishable, thus validating our approach of composite sampling for economical tracking of chemical inventories in MSS samples (Chari and Halden 2012).

AP & APEOs surfactants are widely used in commercial and industrial products and are high production volume (HPV) chemicals (*i.e.*, produced and used in excess of 454,000 kg/year or one million pounds annually in the U.S.). Due to their endocrine disrupting activities, nonylphenol (NP) and derivatives have been voluntarily phased-out in several European countries (OSPAR 2006), and currently are being considered for regulations in the U.S. (U.S. EPA 2013a). In 2013, we reported the first nationwide inventories of AP & APEOs surfactants in MSS samples from the 2001 NSSS (Venkatesan and Halden 2013a). NP was the most abundant analyte (534 ± 192 mg/kg dw), followed by its mono- and di-ethoxylate derivatives (62.1 ± 28 and 59.5 ± 52 mg/kg dw, respectively) (Fig. 3b). Annual load of NP and its derivatives to U.S. soils via land application were estimated at 1,204 - 4,289 metric tonnes.

PFCs are another group CECs that has widespread applications in commercial and industrial products (Kannan 2011). Although the production of the major PFC, perfluorooctane sulfonate (PFOS) and related products was phased out in the U.S. between 2000 and 2002, continued environmental contamination of PFCs results from the use of precursor compounds such as fluorotelomer alcohols and polyfluoroalkyl phosphates (Houde et al. 2011). We reported the first nationwide inventories of 13 perfluoroalkyl substances (PFASs) in MSS samples from the 2001 NSSS (Venkatesan and Halden 2013b). PFOS was the most abundant compound detected at 403 ± 127 µg/kg dw, followed by perfluorooctanoate (PFOA) and perfluorodecanoate (PFDA) at 34 ± 22 and 26 ± 20 µg/kg dw, respectively (Fig. 3c). Total PFASs load in U.S. MSS was estimated at 2,749 – 3,450 kg/year. The study additionally showed no significant difference in concentrations of PFASs detected in MSS samples collected prior to and after the phase-out of PFOS related compounds (2002), demonstrating persistence and/or continued environmental contamination with PFCs.

Brominated flame retardants (BFRs) are ubiquitous halogenated chemicals, resulting in significant exposure and contamination of wildlife and humans (Birnbaum and Staskal 2004; De Wit 2002). Following concerns associated with these chemicals, several major commercial products of BFRs [penta- and octa-brominated diphenylethers (BDE)] were banned by the European Union in 2002 and phased-out by 2005 in the U.S. (Cox and Efthymiou 2003; U.S. EPA 2013b). We determined the national baseline levels and release inventories of 77 traditional and novel BFRs using the 2001 NSSS composite samples (Venkatesan and Halden 2014a). A total of 32 poly BDEs (PBDEs) and 9 non-BDE BFRs were detected in MSS samples, with the most abundant compound being deca-BDE (BDE-209) (Fig. 3d). The annual mean loading rate of total PBDEs and non-BDE BFRs in U.S. MSSs were estimated at 47,900–60,100 and 12,900–16,200 kg/year, respectively. A comparison of select PBDEs in MSS samples from the 2001 and 2006/7 EPA's survey featured a lower mean concentrations of PBDEs in 2006/7 samples compared to the 2001 samples. The downward trends of Σ PBDEs in U.S. MSSs indicate the desirable impact of ongoing efforts in phasing-out PBDEs in the U.S.

Currently, more than 50% of processed U.S. MSS (biosolids) is applied on land as a soil amendment (NEBRA 2007, U.S. EPA 2012a). Hence, monitoring and estimating mass inventories of these abundant CECs are important for assessing the risks associated with land application of MSS. Analyzing nationally representative samples from the NSSR can provide nationwide release inventories of CECs to U.S. soils as demonstrated in the aforementioned studies (Chari and Halden 2012, McClellan and Halden 2010, Venkatesan and Halden 2013a, Venkatesan and Halden 2013b, Venkatesan and Halden 2014a). In addition to the above CECs, our lab has analyzed for nitrosamines, hormones and Br-D/F in the NSSR samples and work is ongoing to estimate the nationwide inventories for these chemicals in MSS (Venkatesan et al., 2014; Venkatesan and Halden 2014b). The most abundant group of chemicals in MSS was composed of AP and APEOs, followed by PPCPs and BFRs (Fig. 4a). The 123 CECs detected in MSS so far were calculated to contribute about 0.04 – 0.15% to the total dry mass of MSS produced in the U.S., a mass equivalent totaling about 2,600 – 7,900 metric tonnes of hazardous chemicals annually (Venkatesan and Halden 2014b). Nationwide concentrations of CECs obtained by analyzing NSSR samples serve to inform both ecological and human health risk assessments and regulatory requirements for these recalcitrant chemicals.

Ecological and human health risks of CECs

MSS are known to represent a ‘sink’ for hydrophobic organic compounds of limited biodegradability. Chemicals accumulated in MSS are those that survived the primary and secondary treatment of WWTP unit operations, and thus are notably resistant to degradative processes (Venkatesan and Halden 2014b). Hence, screening for CECs in MSS may serve to identify and prioritize potentially problematic chemicals that are used in human society. A recent study from our group showcased the use of NSSR samples as an analytical matrix to identify hydrophobic CECs potentially posing a human health threat based on chemical abundance, environmental persistence, and bioaccumulation potential (Venkatesan and Halden 2014b). Of the 123 CECs detected in MSS so far, 91% of the top abundant CECs detected in excess of 1 ppm in MSS samples were HPV chemicals. Fifty-five chemicals were identified as being potentially bioaccumulative [*i.e.*, featuring an *n*-octanol water partitioning coefficient (K_{OW}) of greater than 10^5]; 93% of these compounds were halogenated (Fig. 4b). Eight CECs were identified as priority chemicals that require further investigation with regards to toxicity, human exposure and regulatory requirements. These included penta-brominated diphenylether (BDE-99), deca-brominated diphenylether (BDE-209), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), NP, NP-monoethoxylate, NP-diethoxylate, TCS and TCC. A comparison of chemicals detected in the NSSR samples with chemicals detected in nationally representative biological specimens from humans (CDC 2009), revealed 70% overlap. Comparison of lipid-normalized chemical concentrations in humans and MSS revealed a positive correlation ($R^2 = 0.62$), suggesting that MSS can serve as an indicator of ongoing chemical exposures and chemical body burdens in human society (Venkatesan and Halden 2014b). The approach presented in the latter study summarized how the analysis of MSS samples from the NSSR can be used to estimate (*i*) conservative rates of chemical usage; (*ii*) resistance to biotransformation, and (*iii*) bioaccumulation potential of chemicals, thus making MSS a valuable matrix for environmental monitoring of CECs. This ‘pre-screening’ approach effectively can reduce the several thousands of known

or suspected hydrophobic CECs to a manageable list of priority chemicals that merit allocation of precious resources.

Future Applications of the NSSR

Temporal and spatial trends of CECs

Our group at the Biodesign Institute has analyzed for hundreds of CECs in the 2001 NSSS samples as summarized in the previous section. Work is ongoing to characterize the CEC content in the 2006/7 TNSSS samples. This will enable us to provide time-related trends of nationwide occurrence of CECs in MSS by comparing 2001 and 2006/7 data. This information is vital in understanding the changes in chemical mass flows and usage in human society. Samples from 2001 and 2006/7 surveys by themselves cannot fully capture the temporal trends and discharges of contaminants, especially when the variation is seasonal which will require repeated sampling over shorter intervals of time. However, some of the CECs (*e.g.*, PFOS, PBDEs, NP and NPEOs) were phased-out after 2001 or are currently being phased-out in the U.S. Hence, a comparison of 2001 and 2006/7 data could aid in evaluating changes in the extent of environmental inputs nationwide and in evaluating the effectiveness of chemical regulations and policies. For example, penta- and octa-BDE formulations were voluntarily phased out in the U.S. by 2004. A comparison of concentrations of PBDEs analyzed in 2001 and 2006/7 NSSS samples showed a downward trend (a 45% decrease in mean total concentration for 11 PBDEs), indicating the desirable impact of ongoing efforts in phasing-out PBDEs in the U.S. (Venkatesan and Halden 2014a). One of the limitations from our past studies was the loss of some critical information during compositing (pooling) of MSS samples. Composite samples were prepared to obtain a reasonable mean baseline concentration for the target analytes in an economical and scientifically defensible fashion. However, compositing samples are not well suited to capture the variation in concentrations among individual WWTPs, which may show inter-plant variability in loadings and removal efficiencies. It is also possible for some trace contaminants to become diluted during mixing. Hence, future work from our center is directed towards analyzing individual samples from the NSSR in order to address inter-facility variability and provide spatial variations of CECs in MSS. With the improvement of analytical methods and introduction of new chemicals every year, the NSSR samples can be used to track the contamination levels of legacy and new compounds. Chemical regulations and policies is based on historical trends; hence, it is critical to maintain environmental specimen banks such as the NSSR to keep track of mass produced chemicals and their chemical transformation products.

Biological applications

In addition to chemicals, biological agents also are present in MSS and deserve an evaluation for resultant risks posed. Although sewage treatment greatly reduces the number of microorganisms contained in wastewater, many of these can still survive and become concentrated in MSS, creating a biological risk with respect to land application of MSS. Important organisms of concern chiefly include enteric pathogens (viruses, bacteria, and parasites) that are the leading cause of gastroenteritis and respiratory illnesses in both developing and developed countries (Godfree and Farrell 2005, Simmons and Xagorarakis

2011, Wéry et al. 2008). Bacteria such as *Salmonella* spp., *Campylobacter jejuni*, and *Escherichia coli* O157:H7 have been shown to exhibit variation in survival rates in WWTPs and have detectable concentrations in treated effluent and MSS (Wéry et al. 2008). Amongst viruses, over 140 enteric viruses are known to potentially enter into domestic sewage and MSS (Gerba et al. 2002), many of which are currently listed on the U.S. EPA's Contaminant Candidate List (CCL) as emerging contaminants (U.S. EPA 2012b). Norovirus, which is the most common cause of foodborne-disease outbreaks in the U.S., was detected in all MSS samples collected from five U.S. WWTPs (Simmons and Xagorarakis 2011). Although the detected burden of 3-7 viruses/g of dewatered MSS is low, this concentration may still pose a risk because many viruses (and some bacteria) have an infectious dose as low as 10 viral particles (Wei and Knier 2010). Protozoa and parasites are usually unable to multiply in MSS as they need a biological host; however, some parasite oocysts and helminth ova can survive for up to a year in wastewater and possibly longer in MSS, as more nutrients are available in this matrix (Sidhu and Toze 2009). One study found that 100% of processed MSS samples collected successively over a period of eight months from two facilities contained *Cryptosporidium* oocytes, 6 to 20% of which were viable (Guzman et al. 2007). These organisms pose an occupational risk to many workers as well as to the consumers of the crops once they reach market. *E. coli* and norovirus have been detected previously in air samples near sites where MSS was land applied (Brooks et al. 2005), which is a cause for concern due to the possibility of long-range atmospheric transport of these microorganisms. Thus far, only pathogens have been a focus of study with respect to MSS land application. However, non-pathogenic organisms are also of concern due to their potential of acting as allergens and of developing and spreading antibiotic resistance (Davies 1994). Any bacterium capable of genetic transfer can spread resistance genes to other bacteria. As antibiotics are a major organic contaminant in MSS (McClellan and Halden 2010), this selective pressure can induce mutations that may spread both to organisms in the biosolids and to indigenous microbial populations.

There exists a paucity of information on numbers and survival patterns of pathogens in MSS which can be remedied with better detection methods and more large-scale sampling for the presence of biological agents of concern. The NSSR samples, which are kept frozen at -20°C sealed in secure containers, represent a potentially valuable sample matrix for microbial analyses in addition to the many chemical analyses that already have been conducted or are underway. One potential study one may envision is a comparison between the two major sampling sets (2001 vs. 2006/7) for the presence of pathogens in MSS from locations nationwide. Although enumeration of microbes by culturing in previously frozen and archived samples is non-ideal and may lead to an underestimation of the true quantity and composition of microbial communities present, molecular DNA-based assays can still be conducted. One such example is the use of quantitative polymerase chain reaction (qPCR) for tracking certain viral or bacterial pathogens of concern (Wolf et al. 2010, Wong et al. 2010). . Another application could be the profiling of antibiotic resistance genes and determining potential association with the presence and levels of antibiotics known to occur in MSS (Munir et al. 2011). As land application of MSS is the most economical way of disposal, it is important that the associated biological risks are minimized. The NSSR at ASU represents one potentially viable resource for assessing these biological risks.

Limitations of the NSSR

The purpose of environmental specimen banks is to provide access to previously acquired samples, whose utility may depend in part on the duration of storage. The samples archived in the NSSR were up to 13 years old at the writing of this paper. There is a possibility that some of the labile compounds of interest may have degraded or volatilized during storage. This information would suggest that the concentrations and detection frequencies for some of the compounds reported in our past work may be lower than what would have been found if samples had been analyzed right away. On the other hand, storage of MSS also could have enabled the formation of some of the chemicals from conversion of precursor compounds present in MSS. As a condition for participation in the study, the U.S. EPA kept undisclosed the identity of the MSS samples and additional key information that would be beneficial in calculating the best estimate of mass flows of chemicals, and particularly the mass of CECs that are deposited on American soils as a result of MSS application on land. Although the facilities that were sampled during the EPA's NSSRs are known (Table S1 and S2), the MSS samples currently housed in the NSSR and the H₂O are not linked to the facilities from which they were sampled. However, the capacity (flow) and the region (Northeast, South, Midwest, and West) of the facility from which they were sampled are known. This information could be used to provide contaminant variation with respect to size and region of the facility.

Conclusions and Access to the NSSR

With the development of new technologies and the introduction of new chemicals in commerce, it is important to maintain environmental specimen banks to understand the historic and current distribution (temporal and spatial) of contaminants in the environment. The NSSR at ASU contributes in this aspect by maintaining some 190 MSS specimens collected from 164 WWTPs across the U.S., arguably the largest archive of digested MSS samples in the country. As many of the mass produced chemicals are discharged into the sewage system, digested MSS is an important matrix for environmental monitoring of persistent and bioaccumulative chemicals (Venkatesan and Halden 2014b). As illustrated in multiple case studies, the nationally representative samples housed in the NSSR can serve to provide information on the nationwide occurrence and release inventories of CECs. As shown in past studies, chemicals sequestered in MSS also may serve as an early warning system for determining ongoing chemical exposures and buildup of chemical body burden in human population (Venkatesan and Halden 2014b). There is also the potential for using the repository to track the occurrence, genetic diversity and abundance of microbial pathogens using non-culture dependent techniques, and to assess potential biological risks resultant from handling of MSS and its application on land. Efforts are underway to expand the NSSR by adding additional specimens of novel matrices and from diverse geographic locations. This work is ripe with opportunities for collaborative work to leverage the NSSR research to the largest degree possible. Researchers and parties interested in using this resource are encouraged to contact the authors of this study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

MSS sampling locations of U.S. EPA's 2001 National Sewage Sludge Survey (NSSS) and the 2006/7 Targeted NSSS (TNSSS). Samples are currently housed in the NSSR maintained as part of the larger Human Health Observatory (H2O) at the Biodesign Institute at Arizona State University.

Fig. 2.
Photographs of exemplary MSS samples archived at -20°C at Arizona State University in the NSSR as part of the larger Human Health Observatory (H2O).

Fig. 3.

Rank order plot of mean concentrations of (a) pharmaceuticals and personal care products (PPCPs) [Source: (Chari and Halden 2012)], (b) alkylphenol and their ethoxylates (AP & APEOs) – 4-nonylphenol (NP); 4-nonylphenol monoethoxylate (NP1EO); 4-nonylphenol diethoxylate (NP2EO) [Source: (Venkatesan and Halden 2013a)], (c) perfluorinated compounds (PFCs) - perfluorobutanoate (PFBA); perfluoropentanoate (PFPeA); perfluorohexanoate (PFHxA); perfluoroheptanoate (PFHpA); perfluorooctanoate (PFOA); perfluorononanoate (PFNA); perfluorodecanoate (PFDA); perfluoroundecanoate (PFUnDA); perfluorododecanoate (PFDoDA); perfluorobutane sulfonate (PFBS); perfluorohexane sulfonate (PFHxS); perfluorooctane sulfonate (PFOS); perfluorooctane sulfonamide (PFOSA) [Source: (Venkatesan and Halden 2013b)], and (d) brominated flame retardants (BFRs) – polybrominated diphenylethers (PBDEs); polybrominated biphenyls (PBBs); hexabromocyclododecane (HBCDD); decabromodiphenyl ethane (DBDPE); 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE); hexabromobenzene (HBB); pentabromoethylbenzene (PBEB) [Source: (Venkatesan and Halden 2014a)], detected in MSS composite samples from the U.S. EPA's 2001 NSSS. Error bars represent minima and maxima ($n = 5$).

Fig. 4.

(a) Relative abundance of CECs in MSS samples from the 2001 NSSS. The number above the bar represents the number of chemicals detected in each chemical group. (b) Classification of 123 chemicals detected in NSSR samples based on abundance in MSS and on *n*-octanol water partition coefficient (K_{OW}). Dashed horizontal line represent the criteria for chemical bioaccumulation potential ($\text{Log } K_{OW} = 5$). Data source: (Venkatesan and Halden 2014b).

Table 1
Summary of samples in the NSSR as part of the larger Human Health Observatory (H2O) at the Biodesign Institute at Arizona State University

Survey year	No. of facilities sampled	No. facilities with two treatment systems	No. facilities having field duplicates sampled	Total number of MSS specimens in NSSR after loss ^a	Approximate amount of sample remaining in the repository	
					% of samples	Weight (g)
2001	94	5	14	110	4	>1200
					16	800-1200
					80	400-500
2006/7	74	4	6	80	5	>1800
					15	1500-1800
					23	1200-1400
					31	900-1000
					19	600-700
					7	400-500

^aThree samples from 2001 NSSS and four samples from 2006/7 TNSSS were lost due to broken container/lost during shipment.