

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

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4 Arjun K. Venkatesan, Hansa Y. Done and Rolf U. Halden*

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6 Center for Environmental Security, The Biodesign Institute, Security and Defense Systems

7 Initiative, Arizona State University, 781 E. Terrace Road, Tempe, AZ 85287

8 *Corresponding author phone: +1 (480) 727-0893; fax: +1 (480) 965-6603; email:

9 halden@asu.edu

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24 **Abstract**

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

46 the effectiveness of environmental policy-making and regulations, and (iv) approximate, ongoing
47 exposures and body burdens of mass-produced chemicals in human society.

48

49 **Keywords.** Environmental Specimen Banks; National Repository; Sewage Sludge; Biosolids;
50 Wastewater Treatment; Contaminants of Emerging Concern.

51

52 **Introduction**

53 Processed municipal sewage sludge (MSS) or biosolids are an abundant byproduct of wastewater
54 treatment. About 6.5 million metric dry tonnes (7.18 million US tons) of MSS is produced in the
55 USA annually (NEBRA 2007). The term sewage sludge and biosolids are used interchangeably
56 by the US Environmental Protection Agency (US EPA). The US EPA defines biosolids as
57 treated or processed sewage sludge that can be beneficially recycled. Thus, the term biosolids
58 refers to the particulate-containing solid, semi-solid, or liquid by-product that is usually applied
59 on land as a soil amendment/fertilizer. However, part of the treated sewage sludge is also
60 disposed of in incinerators and landfills. MSS is rich in organic carbon and lipid content and
61 hence serves as a sink to hydrophobic and persistent contaminants. The US EPA is required to
62 identify and regulate toxic contaminants that may be present in MSS at levels of concern to
63 ecological and human health, under the Clean Water Act that was passed in 1972.

64

65 Hence, US EPA has performed four sewage sludge surveys thus far, to identify inorganic and
66 organic contaminants for regulation considerations (US EPA 2007, 2009). These surveys are
67 important since about 50 % of the MSS produced annually in the USA is reused as a soil
68 amendment (NEBRA 2007; US EPA 2012a). The information from the 1988–1989 survey was

69 used to establish the “The standards for the use or disposal of sewage sludge” (Title 40 of CFR
70 Part 503) that describes the general requirements, pollutant limits, and management practices for
71 the use or disposal of sewage sludge. Currently, the US EPA has established standards for nine
72 toxic compounds in land-applied sewage sludge, all belonging to the inorganic group of metals,
73 including As, Cd, Cu, Hg, Mo, Ni, Pb, Se, and Zn (§503.13). The Part 503 regulation focuses on
74 the presence of nine metals, pathogens, and MSS attractiveness to vectors as a basis for
75 determining MSS quality. MSS that meet the most stringent limits for all three of these
76 parameters are termed exceptional quality (EQ) MSS. MSS that do not meet the stringent quality
77 are termed non-EQ MSS. Facilities producing EQ MSS are not required to follow the Part 503
78 rule for land application. Non-EQ MSS, however, requires following the Rule with respect to
79 pollutant limits, pathogens (class A—“pathogen free” and class B—some pathogens), vector
80 attraction, and public access restrictions depending upon the degree to which the quality diverges
81 from EQ (US EPA 1994).

82
83 Though organics including pharmaceuticals and personal care products (PPCPs) and select flame
84 retardants were included in surveys conducted by the EPA, standards or limits for organic
85 compounds have not yet been established. From the 2001 sewage sludge survey reported in
86 2007, US EPA concluded that numeric standards or management practices are not warranted for
87 dioxin and dioxin-like compounds in land-applied sewage sludge (US EPA 2007). Several
88 researchers have criticized the methodology and regulation process of the US EPA (Iranpour et
89 al. 2004; McBride 2003). Additionally, recent studies have shown contamination of
90 groundwater, surface water, and plant uptake of organic contaminants from soil amended with
91 MSS (Clarke and Smith 2011; Lapworth et al. 2012; Sepulvado et al. 2011; Wu et al. 2010; Xia

92 et al. 2010). The US EPA has screened for only a small fraction of the more than 40,000
93 contaminants of emerging concern (CEC) that potentially could be present in MSS (Diamond et
94 al. 2011). Additionally, it is estimated that about 2,000 new chemicals are introduced into
95 commerce each year in the USA (California Department of Toxic Substances Control 2013).
96 Hence, more frequent surveys and a wider screening approach are required in order to identify
97 and prioritize CECs. In a strategic effort, our team at the Biodesign Institute at Arizona State
98 University has created the National Sewage Sludge Repository (NSSR) as part of the Human
99 Health Observatory (H2O) to address this research need.

100

101 **National Sewage Sludge Repository (NSSR)**

102 The NSSR consists of digested MSS samples collected from 164 wastewater treatment plants
103 (WWTPs) across the USA by the US EPA during their 2001 (reported in 2007) and 2006/2007
104 (reported in 2009) National Sewage Sludge Surveys (NSSS). After completion of surveys, the
105 samples were acquired by our laboratory and stored at -20°C for further analysis. Samples were
106 stored initially at Johns Hopkins University and later transferred to Arizona State University with
107 chain of custody documentation for long-term maintenance. The NSSR likely represents the
108 largest archive of digested MSS specimens in the USA. The rationale for creating NSSR was to
109 leverage the benefits of sewage sludge analysis in general and the value of samples collected as
110 part of the US EPA's surveys in particular. The primary goal of initial investigations was to
111 determine the occurrence and identity of CECs in US sludges and to compute estimates of
112 nationwide inventories of CECs that have not been included in prior surveys. To date, we have
113 analyzed and reported nationwide occurrence of hundreds of CECs using the NSSR. The target
114 chemicals that we focused on included PPCPs, brominated flame retardants (BFRs),

115 perfluorinated compounds (PFCs), alkylphenol (AP) and their ethoxylates (APEOs), hormones,
116 as well as brominated dioxins and furans (Br-D/F). Work is ongoing to evaluate other toxic
117 contents present in MSS samples. Here, we review prior work utilizing the NSSR samples,
118 identify future research opportunities, and invite collaborative use of NSSR samples by the
119 research community.

120

121 **NSSR Sample Inventory**

122 The NSSR consists of samples collected by the US EPA during their 2001 and 2006/2007 NSSS.
123 MSS specimens were collected from 164 WWTPs from across the contiguous USA, representing
124 the more than 16,000 WWTPs operating in the USA (Fig. 1). The repository currently houses a
125 total of 190 MSS specimens (Table 1; Fig. 2).

126

127 **2001 NSSS Samples.** MSS samples were collected by the EPA from 94 WWTPs in 32 US states
128 and the District of Columbia as part of the 2001 NSSS. Information on sampled facilities is
129 available as supplementary material (Table S1). The facilities were selected by the US EPA to
130 obtain unbiased national estimates of chemical contaminants in US sewage sludges that are
131 disposed of primarily by land application. The purpose of EPA's 2001 survey was to estimate
132 levels of dioxins, dibenzofurans, and coplanar polychlorinated biphenyls in MSS (US EPA
133 2007). Grab samples were collected between February and March 2001, according to an
134 established proto- col, exclusively targeting facilities featuring secondary treatment (US EPA
135 1988, 2007). All samples were collected in 500 mL glass or polyethylene/polypropylene jars.
136 Polyethylene gloves were used to prevent sample contamination. Solid MSS samples were
137 collected by using a polyethylene pail and polypropylene scoop (US EPA 1988). Samples were

138 directly collected from the sludge discharge chute in the pail, thoroughly mixed using the scoop
139 and then transferred to the containers. If the facility featured two or more dewatering units, equal
140 amounts of samples were collected from each discharge point and mixed in the polyethylene
141 pail. While collecting liquid MSS samples from storage tanks, the pumps were ran for 5 min to
142 clear stagnant sludge and then samples were collected in to the pail. The collected samples were
143 thoroughly agitated in the pail using a polyethylene ladle and then transferred to the container.

144

145 Once samples were collected, identifying labels were attached to the containers, and the
146 container lids were further sealed with tape. The samples were iced, packed, and shipped to
147 laboratory for storage/analyses promptly via overnight shipping service. Samples were collected
148 only from processed MSS intended for disposal. Of the 94 WWTPs, 89 had single system (either
149 aerobic or anaerobic digestion), and five of them had two systems for sludge treatment (both
150 aerobic and anaerobic digestion). Samples were collected from each treatment systems. In
151 addition, duplicate samples were collected from 14 facilities, amounting to a total of 113 MSS
152 samples. Three samples were lost due to breaking of containers during transportation. The NSSR
153 currently harbors a total of 110 samples from the 2001 NSSS. The solid content of the MSS
154 samples ranged between 1.5 and >90 %.

155

156 **2006/7 Targeted National Sewage Sludge Survey (TNSSS) samples.** MSS samples were
157 collected from 74 publicly owned WWTPs that participated in the US EPA's Targeted National
158 Sewage Sludge Survey (TNSSS). The facilities selected in the survey are statistically
159 representative of the target population consisting of WWTPs that: (i) were operational during
160 2002 and/or 2004, (ii) had a flow rate of greater than 3.8 million liters per day (MLD) [or 1

161 million gallons per day (MGD)], (iii) employed a minimum of secondary treatment, and (iv)
162 were located in the contiguous USA. From the 3,337 WWTPs that met the above criteria, the US
163 EPA statistically selected 74 facilities using a random sampling design stratified for flow [3.8 to
164 38 MLD (1 to 10 MGD), 38 to 380 MLD (10 to 100 MGD), and >380 MLD (>100 MGD)] from
165 35 US states. Grab samples of MSS were collected by US EPA from each facility between
166 August 2006 and March 2007. Four of the facilities had two treatment systems for solids; hence,
167 a second sample was collected to represent both treatment systems. The solid content of the MSS
168 samples ranged from 4 to 93 %. Additionally, a duplicate grab sample was collected from six
169 other facilities to allow for variations associated with the sampling procedure. Solid samples
170 represented dewatered sludges collected from dewatering units like belt press, filter press, drying
171 bed, centrifuge, compost pile, or other sources. Small grab samples were collected from multiple
172 regions in large piles (or multiple grabs from continuous processes) and then composited in a
173 large pre-cleaned container, so that the solids represented the bulk sludge product (US EPA
174 2009). The composites were thoroughly mixed and transferred to final sampling containers. All
175 samples collected were final treated product of MSS. Liquid samples were collected as free-
176 flowing materials from storage tanks. The sampling pipelines were purged, and any mixing
177 equipments were turned on prior to sampling, so that the collected liquids represented the bulk
178 product. Liquid samples were collected directly into the final sample containers made of either
179 glass or HDPE. After sample collection, the containers were labeled with preprinted label
180 containing EPA episode and sample number. The samples collected from each facility were
181 packed, iced, and shipped overnight to EPA sample repository in Baltimore, MD (US EPA
182 2009).

183 The objective of the TNSSS was to report the occurrence in MSS of selected contaminants of
184 emerging concern (e.g., PPCPs, BFRs) as identified by the US EPA and the National Research
185 Council (NRC). After completion of TNSSS, left- over samples were acquired by our laboratory
186 and stored at $-20\text{ }^{\circ}\text{C}$ for further analysis. From the 84 MSS samples, four were excluded from
187 analysis because the sample containers were either missing or broken. Information on sampled
188 facilities is available as supplementary material (Table S2).

189

190 **Past Applications of the NSSR.**

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

203

204 The first study from our group conducted in 2009 screened for 72 PPCPs (the same suite of
205 analytes screened by EPA in their 2006/2007 survey) in five composites prepared from the 2001

206 NSSS samples (McClellan and Halden 2010). Thirty-eight analytes were detected in at least one
207 composite sample, and the concentration ranged from 0.002 to 48 mg/kg dry weight (dw). The
208 antimicrobial compounds, triclocarban (TCC) and triclosan (TCS), were found to be the most
209 abundant analytes at mean concentrations of 36 ± 8 and 12.6 ± 3.8 mg/kg dw, respectively.

210 Using the percent of MSS applied on land, it was estimated that about 210–250 metric tonnes of
211 total PPCPs were released to US soils via MSS land application (McClellan and Halden 2010).

212 The contaminants occurrence frequency and levels were shown to be similar to those reported by
213 the US EPA in its 2006/2007 TNSSS survey, demonstrating that PPCP releases in US MSS have
214 been ongoing for several years. The contract laboratory that developed EPA Method 1694 for
215 PPCP analysis updated their analytical methods to include 48 new analytes. Hence, a second
216 study was conducted in 2012 by recreating the composites from the 2001 NSSS and analyzing
217 for a total of 120 PPCPs in MSS (Chari and Halden 2012). This latter study reported on the
218 occurrence in MSS of 26 not previously monitored PPCPs in composite samples at levels
219 ranging from 1.65 to 673 $\mu\text{g}/\text{kg}$ dw (Fig. 3a). These newly detected 26 analytes (Chari and
220 Halden 2012) contributed an estimated additional 5–15 metric tonnes of PPCPs to the loading of
221 US soils previously reported (McClellan and Halden 2010). Chemical concentrations in our 2009
222 and 2012 PPCP analyses were statistically indistinguishable, thus validating our approach of
223 composite sampling for economical tracking of chemical inventories in MSS samples (Chari and
224 Halden 2012).

225

226 AP and APEO surfactants are widely used in commercial and industrial products and are high
227 production volume (HPV) chemicals (i.e., produced and used in excess of 454,000 kg/year or
228 one million pounds annually in the USA). Due to their endocrine disrupting activities,

229 nonylphenol (NP) and derivatives have been voluntarily phased out in several European
230 countries (OSPAR 2006) and currently are being considered for regulations in the USA (US EPA
231 2013a). In 2013, we reported the first nationwide inventories of AP and APEO surfactants in
232 MSS samples from the 2001 NSSS (Venkatesan and Halden 2013a). NP was the most abundant
233 analyte (534 ± 192 mg/kg dw), followed by its mono- and di-ethoxylate derivatives (62.1 ± 28
234 and 59.5 ± 52 mg/kg dw, respectively) (Fig. 3b). Annual load of NP and its derivatives to US
235 soils via land application were estimated at 1,204–4,289 metric tonnes.

236
237 PFCs are another group CECs that has widespread applications in commercial and industrial
238 products (Kannan 2011). Although the production of the major PFC, perfluorooctane sulfonate
239 (PFOS) and related products was phased out in the USA between 2000 and 2002, continued
240 environmental contamination of PFCs results from the use of precursor com- pounds such as
241 fluorotelomer alcohols and polyfluoroalkyl phosphates (Houde et al. 2011). We reported the first
242 nation- wide inventories of 13 perfluoroalkyl substances (PFASs) in MSS samples from the 2001
243 NSSS (Venkatesan and Halden 2013b). PFOS was the most abundant compound detected at 403
244 ± 127 $\mu\text{g}/\text{kg}$ dw, followed by perfluorooctanoate (PFOA) and perfluorodecanoate (PFDA) at 34
245 ± 22 and 26 ± 20 $\mu\text{g}/\text{kg}$ dw, respectively (Fig. 3c). Total PFASs load in US MSS was estimated
246 at 2,749–3,450 kg/year. The study additionally showed no significant difference in
247 concentrations of PFASs detected in MSS samples collected prior to and after the phase-out of
248 PFOS related compounds (2002), demonstrating persistence and/or continued environmental
249 contamination with PFCs.

250

251 BFRs are ubiquitous halogenated chemicals, resulting in significant exposure and contamination
252 of wildlife and humans (Birnbaum and Staskal 2004; De Wit 2002). Follow- ing concerns
253 associated with these chemicals, several major commercial products of BFRs [penta- and octa-
254 brominated diphenylethers (BDE)] were banned by the European Union in 2002 and phased out
255 by 2005 in the USA (Cox and Efthymiou 2003; US EPA 2013b). We determined the national
256 baseline levels and release inventories of 77 traditional and novel BFRs using the 2001 NSSS
257 composite samples (Venkatesan and Halden 2014a). A total of 32 poly-BDEs (PBDEs) and nine
258 non-BDE BFRs were detected in MSS samples, with the most abundant compound being deca-
259 BDE (BDE-209) (Fig. 3d). The annual mean loading rate of total PBDEs and non-BDE BFRs in
260 US MSSs were estimated at 47,900–60,100 and 12,900–16,200 kg/year, respectively. A
261 comparison of select PBDEs in MSS samples from the 2001 and 2006/2007 EPA’s survey
262 featured a lower mean concentrations of PBDEs in 2006/2007 samples compared to the 2001
263 samples. The downward trends of \sum PBDEs in US MSSs indicate the desirable impact of ongoing
264 efforts in phasing-out PBDEs in the USA.

265
266 Currently, more than 50 % of processed US MSS (biosolids) is applied on land as a soil
267 amendment (NEBRA 2007; US EPA 2012a). Hence, monitoring and estimating mass inventories
268 of these abundant CECs are important for assessing the risks associated with land application of
269 MSS. Analyzing nationally representative samples from the NSSR can provide nationwide
270 release inventories of CECs to US soils as demonstrated in the aforementioned studies (Chari
271 and Halden 2012; McClellan and Halden 2010; Venkatesan and Halden 2013a, b, 2014a). In
272 addition to the above CECs, our lab has analyzed for nitrosamines, hormones, and Br-D/F in the
273 NSSR samples, and work is ongoing to estimate the nationwide inventories for these chemicals

274 in MSS (Venkatesan et al. 2014; Venkatesan and Halden 2014b). The most abundant group of
275 chemicals in MSS was composed of AP and APEOs, followed by PPCPs and BFRs (Fig. 4a).
276 The 123 CECs detected in MSS so far were calculated to contribute about 0.04–0.15 % to the
277 total dry mass of MSS produced in the USA, a mass equivalent totaling about 2,600– 7,900
278 metric tonnes of hazardous chemicals annually (Venkatesan and Halden 2014b). Nationwide
279 concentrations of CECs obtained by analyzing NSSR samples serve to inform both ecological
280 and human health risk assessments and regu- latory requirements for these recalcitrant
281 chemicals.

282
283 **Ecological and human health risks of CECs.** MSS are known to represent a “sink” for
284 hydrophobic organic compounds of limited biodegradability. Chemicals accumulated in MSS are
285 those that survived the primary and secondary treatment of WWTP unit operations and thus are
286 notably resistant to degradative processes (Venkatesan and Halden 2014b). Hence, screening for
287 CECs in MSS may serve to identify and prioritize potentially problematic chemicals that are
288 used in human society. A recent study from our group showcased the use of NSSR samples as an
289 analytical matrix to identify hydrophobic CECs potentially posing a human health threat based
290 on chemical abundance, environmental persistence, and bioaccumulation potential (Venkatesan
291 and Halden 2014b). Of the 123 CECs detected in MSS so far, 91 % of the top abundant CECs
292 detected in excess of 1 ppm in MSS samples were HPV chemicals. Fifty-five chemicals were
293 identified as being potentially bioaccumulative [i.e., featuring an *n*-octanol water partitioning
294 coefficient (K_{OW}) of greater than 10^5]; 93 % of these compounds were halogenated (Fig. 4b).
295 Eight CECs were identified as priority chemicals that require further investigation with regards
296 to toxicity, human exposure, and regulatory requirements. These included penta-brominated

297 diphenylether (BDE-99), deca-brominated diphenylether (BDE-209), 1,2- bis(2,4,6-
298 tribromophenoxy)ethane (BTBPE), NP, NP- monoethoxylate, NP-diethoxylate, TCS, and TCC.
299 A compar- ison of chemicals detected in the NSSR samples with chemicals detected in nationally
300 representative biological specimens from humans (CDC 2009) revealed 70 % overlap.
301 Comparison of lipid-normalized chemical concentrations in humans and MSS revealed a positive
302 correlation ($R^2 = 0.62$), suggesting that MSS can serve as an indicator of ongoing chemical
303 exposures and chemical body burdens in human society (Venkatesan and Halden 2014b). The
304 approach presented in the latter study summarized how the analysis of MSS samples from the
305 NSSR can be used to estimate (i) conservative rates of chemical usage, (ii) resistance to
306 biotransformation, and (iii) bioaccumulation potential of chemicals, thus making MSS a valuable
307 matrix for environmental monitoring of CECs. This “pre-screening” approach effectively can
308 reduce the several thousands of known or suspected hydrophobic CECs to a manageable list of
309 priority chemicals that merit allocation of precious resources.

310

311 **Future Applications of the NSSR.**

312 **Temporal and spatial trends of CECs.** Our group at the Biodesign Institute has analyzed for
313 hundreds of CECs in the 2001 NSSS samples as summarized in the previous section. Work is
314 ongoing to characterize the CEC content in the 2006/2007 TNSSS samples. This will enable us
315 to provide time-related trends of nationwide occurrence of CECs in MSS by comparing 2001 and
316 2006/2007 data. This information is vital in understanding the changes in chemical mass flows
317 and usage in human society. Samples from 2001 and 2006/2007 surveys by themselves cannot
318 fully capture the temporal trends and discharges of contaminants, especially when the variation is
319 seasonal which will require repeated sampling over shorter intervals of time. However, some of

320 the CECs (e.g., PFOS, PBDEs, NP, and NPEOs) were phased out after 2001 or are currently
321 being phased out in the USA. Hence, a comparison of 2001 and 2006/2007 data could aid in
322 evaluating changes in the extent of environmental inputs nation- wide and in evaluating the
323 effectiveness of chemical regulations and policies. For example, penta- and octa-BDE
324 formulations were voluntarily phased out in the USA by 2004. A comparison of concentrations
325 of PBDEs analyzed in 2001 and 2006/2007 NSSS samples showed a downward trend (a 45 %
326 decrease in mean total concentration for 11 PBDEs), indicating the desirable impact of ongoing
327 efforts in phasing-out PBDEs in the USA (Venkatesan and Halden 2014a). One of the limitations
328 from our past studies was the loss of some critical information during compositing (pooling) of
329 MSS samples. Composite samples were prepared to obtain a reasonable mean baseline
330 concentration for the target analytes in an economical and scientifically defensible fashion.
331 However, compositing samples is not well suited to capture the variation in concentrations
332 among individual WWTPs, which may show inter-plant variability in loadings and removal
333 efficiencies. It is also possible for some trace contaminants to become diluted during mixing.
334 Hence, future work from our center is directed toward analyzing individual samples from the
335 NSSR in order to address inter-facility variability and provide spatial variations of CECs in
336 MSS. With the improvement of analytical methods and introduction of new chemicals every
337 year, the NSSR samples can be used to track the contamination levels of legacy and new
338 compounds. Chemical regulations and policies are based on historical trends; hence, it is critical
339 to maintain environmental specimen banks such as the NSSR to keep track of mass produced
340 chemicals and their chemical transformation products.

341

342 **Biological applications.** In addition to chemicals, biological agents also are present in MSS and
343 deserve an evaluation for resultant risks posed. Although sewage treatment greatly reduces the
344 number of microorganisms contained in wastewater, many of these can still survive and become
345 concentrated in MSS, creating a biological risk with respect to land application of MSS.
346 Important organisms of concern chiefly include enteric pathogens (viruses, bacteria, and
347 parasites) that are the leading cause of gastroenteritis and respiratory illnesses in both developing
348 and developed countries (Godfree and Farrell 2005; Simmons and Xagorarakis 2011; Wéry et al.
349 2008). Bacteria such as *Salmonella* spp., *Campylobacter jejuni*, and *Escherichia coli* O157:H7
350 have been shown to exhibit variation in survival rates in WWTPs and have detect- able
351 concentrations in treated effluent and MSS (Wéry et al. 2008). Among viruses, over 140 enteric
352 viruses are known to potentially enter into domestic sewage and MSS (Gerba et al. 2002), many
353 of which are currently listed on the US EPA's Contaminant Candidate List (CCL) as emerging
354 contaminants (US EPA 2012b). Norovirus, which is the most common cause of foodborne
355 disease outbreaks in the USA, was detected in all MSS samples collected from five US WWTPs
356 (Simmons and Xagorarakis 2011). Although the detected burden of three to seven viruses per
357 gram of dewatered MSS is low, this con- centration may still pose a risk because many viruses
358 (and some bacteria) have an infectious dose as low as 10 viral particles (Wei and Kniel 2010).
359 Protozoa and parasites are usually unable to multiply in MSS as they need a biological host;
360 however, some parasite oocysts and helminth ova can survive for up to a year in wastewater and
361 possibly longer in MSS, as more nutrients are available in this matrix (Sidhu and Toze 2009).
362 One study found that 100 % of processed MSS samples collected successively over a period of 8
363 months from two facilities contained *Cryptosporidium* oocysts, 6 to 20 % of which were viable
364 (Guzman et al. 2007). These organisms pose an occupational risk to many workers as well as to

365 the consumers of the crops once they reach market. *E. coli* and norovirus have been detected
366 previously in air samples near sites where MSS was land applied (Brooks et al. 2005), which is a
367 cause for concern due to the possibility of long-range atmospheric transport of these
368 microorganisms. Thus far, only pathogens have been a focus of study with respect to MSS land
369 application. However, non-pathogenic organisms are also of concern due to their potential of
370 acting as allergens and of developing and spreading antibiotic resistance (Davies 1994). Any
371 bacterium capable of genetic transfer can spread resistance genes to other bacteria. As antibiotics
372 are a major organic contaminant in MSS (McClellan and Halden 2010), this selective pressure
373 can induce mutations that may spread both to organisms in the biosolids and to indigenous
374 microbial populations.

375

376 There exists a paucity of information on numbers and survival patterns of pathogens in MSS
377 which can be remedied with better detection methods and more large-scale sampling for the
378 presence of biological agents of concern. The NSSR samples, which are kept frozen at $-20\text{ }^{\circ}\text{C}$
379 sealed in secure containers, represent a potentially valuable sample matrix for microbial analyses
380 in addition to the many chemical analyses that already have been conducted or are underway.
381 One potential study one may envision is a comparison between the two major sampling sets
382 (2001 vs. 2006/2007) for the presence of pathogens in MSS from locations nationwide. Al-
383 though enumeration of microbes by culturing in previously frozen and archived samples is non-
384 ideal and may lead to an underestimation of the true quantity and composition of microbial
385 communities present, molecular DNA-based assays can still be conducted. One such example is
386 the use of quantitative polymerase chain reaction (qPCR) for tracking certain viral or bacterial
387 pathogens of concern (Wolf et al. 2010; Wong et al. 2010). Another application could be the

388 profiling of antibiotic resistance genes and determining potential association with the presence
389 and levels of antibiotics known to occur in MSS (Munir et al. 2011). As land application of MSS
390 is the most economical way of disposal, it is important that the associated biological risks are
391 minimized. The NSSR at ASU represents one potentially viable resource for assessing these
392 biological risks.

393

394 **Limitations of the NSSR.**

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

410 were sampled are known. This information could be used to provide contaminant variation with
411 respect to size and region of the facility.

412

413 **Conclusions and Access to the NSSR.**

414 With the development of new technologies and the introduction of new chemicals in commerce,
415 it is important to maintain environmental specimen banks to understand the historic and current
416 distribution (temporal and spatial) of contaminants in the environment. The NSSR at ASU
417 contributes in this aspect by maintaining some 190 MSS specimens collected from 164 WWTPs
418 across the USA, arguably the largest archive of digested MSS samples in the country. As many
419 of the mass produced chemicals are discharged into the sewage system, digested MSS is an
420 important matrix for environmental monitoring of persistent and bioaccumulative chemicals
421 (Venkatesan and Halden 2014b). As illustrated in multiple case studies, the nationally
422 representative samples housed in the NSSR can serve to provide information on the nationwide
423 occurrence and release inventories of CECs. As shown in past studies, chemicals sequestered in
424 MSS also may serve as an early warning system for determining ongoing chemical exposures
425 and buildup of chemical body burden in human population (Venkatesan and Halden 2014b).
426 There is also the potential for using the repository to track the occurrence, genetic diversity and
427 abundance of microbial pathogens using non-culture dependent techniques and to assess
428 potential bio- logical risks resultant from handling of MSS and its application on land. Efforts are
429 underway to expand the NSSR and the H20 by adding additional specimens of novel matrices
430 and from diverse geographic locations. This work is ripe with opportunities for collaborative
431 work to leverage the NSSR research to the largest degree possible. Researchers and parties
432 interested in using this resource are encouraged to contact the authors of this study.

433

434 **Acknowledgement.**

435 We thank Rick Stevens, Harry B. McCarty, and the US EPA for providing the biosolids samples
436 from the 2001 and 2006/2007 National Sewage Sludge Surveys. This study was supported in part
437 by the Johns Hopkins Center for a Livable Future and by National Institute of Environmental
438 Health Sciences grants 1R01ES015445, 1R01ES020889, and their respective supplements. The
439 content of this work is solely the responsibility of the authors and does not necessarily represent
440 the official views of the NIEHS or the National Institutes of Health (NIH).

441

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556

557 **Table 1.** Summary of samples in the NSSR 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					

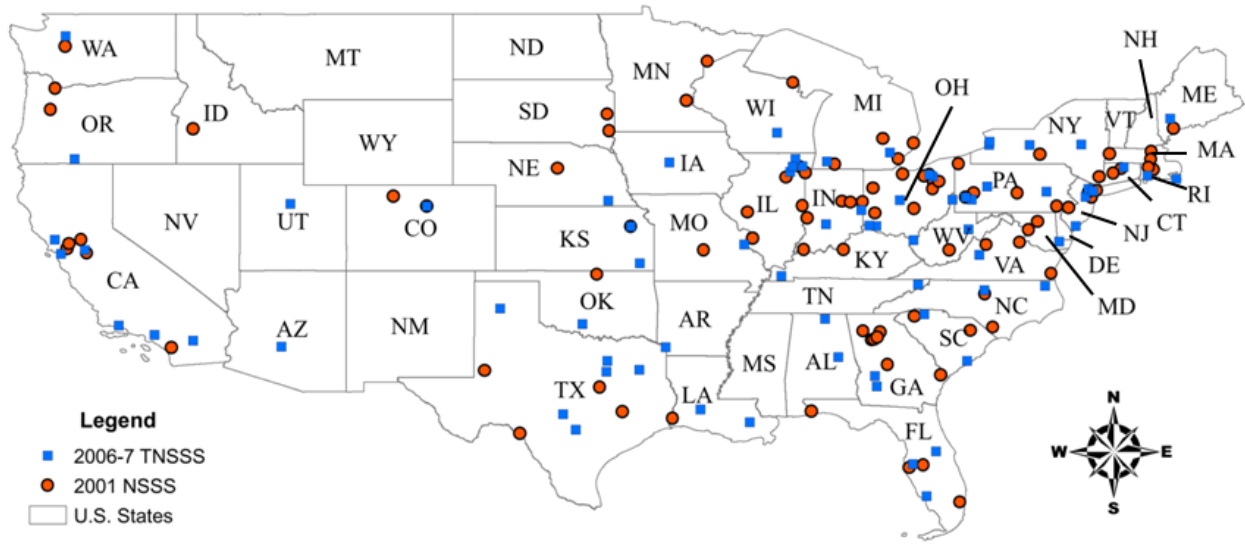
^aThree samples from 2001 NSSS and four samples from 2006/7 TNSSS were lost due to broken container/lost during shipment.

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Figures



561

562 **Fig. 1.** MSS sampling locations of U.S. EPA's 2001 National Sewage Sludge Survey (NSSS)
563 and the 2006/7 Targeted NSSS (TNSSS). Samples are currently housed in the NSSR maintained
564 at the Biodesign Institute at Arizona State University.

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578 **Fig. 2.** Photographs of exemplary MSS samples archived at -20°C at Arizona State University as
579 part of the NSSR.

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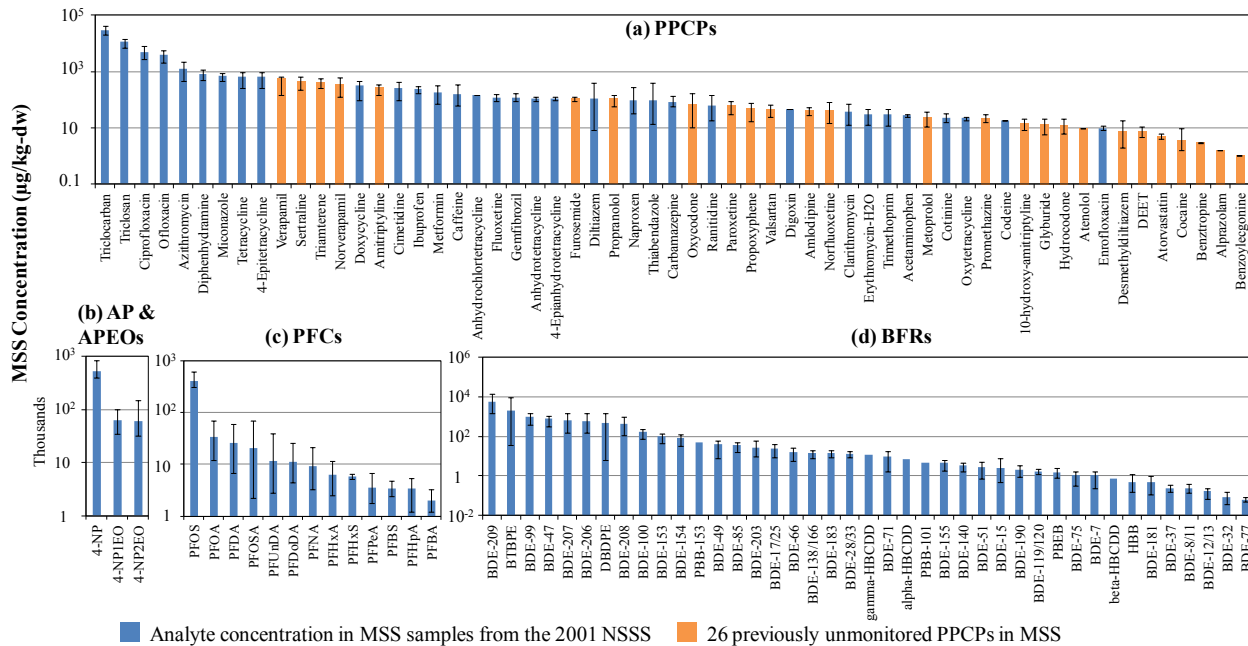
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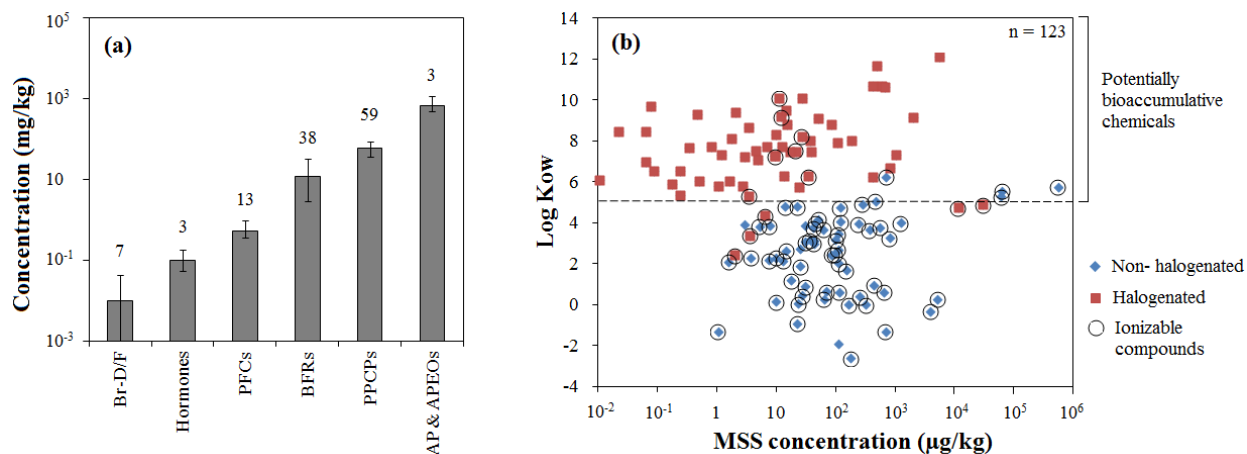
594



595 Analyte concentration in MSS samples from the 2001 NSSS 26 previously unmonitored PPCPs in MSS

596 **Fig. 3.** Rank order plot of mean concentrations of (a) pharmaceuticals and personal care products
597 (PPCPs) [Source:(Chari and Halden 2012)], (b) alkylphenol and their ethoxylates (AP &
598 APEOs) – 4-nonylphenol (NP); 4-nonylphenol monoethoxylate (NP1EO); 4-nonylphenol
599 diethoxylate (NP2EO) [Source: (Venkatesan and Halden 2013a)], (c) perfluorinated compounds
600 (PFCs) - perfluorobutanoate (PFBA); perfluoropentanoate (PFPeA); perfluorohexanoate
601 (PFHxA); perfluoroheptanoate (PFHpA); perfluorooctanoate (PFOA); perfluorononanoate
602 (PFNA); perfluorodecanoate (PFDA); perfluoroundecanoate (PFUnDA); perfluorododecanoate
603 (PFDoDA); perfluorobutane sulfonate (PFBS); perfluorohexane sulfonate (PFHxS);
604 perfluorooctane sulfonate (PFOS); perfluorooctane sulfonamide (PFOSA) [Source: (Venkatesan
605 and Halden 2013b)], and (d) brominated flame retardants (BFRs) – polybrominated
606 diphenylethers (PBDEs); polybrominated biphenyls (PBBs); hexabromocyclododecane
607 (HBCDD); decabromodiphenyl ethane (DBDPE); 1,2-bis(2,4,6-tribromophenoxy)ethane
608 (BTBPE); hexabromobenzene (HBB); pentabromoethylbenzene (PBEB) [Source: (Venkatesan

609 and Halden 2014a)], detected in MSS composite samples from the U.S. EPA's 2001 NSSS. Error
610 bars represent minima and maxima ($n = 5$).



611

612 **Fig. 4.** (a) Relative abundance of CECs in MSS samples from the 2001 NSSS. The number

613 above the bar represents the number of chemicals detected in each chemical group. (b)

614 Classification of 123 chemicals detected in NSSR samples based on abundance in MSS and on

615 *n*-octanol water partition coefficient (K_{OW}). Dashed horizontal line represent the criteria for

616 chemical bioaccumulation potential ($\text{Log } K_{OW} = 5$). Data source: (Venkatesan and Halden

617 2014b).

618