Contribution of Polybrominated Dibenzo-*p*-dioxins and Dibenzofurans (PBDD/Fs) to the Toxic Equivalency of Dioxin-like Compounds in Archived Biosolids from the U.S. EPA's 2001 National Sewage Sludge Survey

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ABSTRACT

The World Health Organization recently proposed the inclusion of brominated congeners in addition to chlorinated congeners, when computing the toxic equivalency (TEQ) of dioxin-like compounds (DLCs) in assessments of human health risks. In the present study, 12 polybrominated dibenzo-*p*-dioxins and furans (PBDD/Fs) were analyzed by gas chromatography/high resolution mass spectrometry (GC-HRMS) in composited, archived biosolids collected in 32 U.S. States and the District of Columbia from 94 wastewater treatment plants by the U.S. Environmental Protection Agency in its 2001 National Sewage Sludge Survey. Two PBDDs and five PBDFs were detected in biosolids composites at varying frequency (40-100%), with a total mean concentration of 10,000 (range: 630–42,800) ng/kg dry weight, of which 1,2,3,4,6,7,8-hepta-BDF constituted about 95% by mass. Relative to commercial polybrominated diphenyl ether (PBDE) formulations, the ratio of PBDD/Fs to PBDEs in biosolids was 55-times higher (~0.002% vs. ~0.11%), indicating potential PBDE transformation or possibly additional sources of PBDD/Fs in the environment. The TEQ contribution of PBDD/Fs was estimated at 162 (range: 15–672) ng/kg 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), equivalent to 75% (range: 12–96%) of the total TEQ in biosolids. The TEQ of DLCs released annually to U.S. soils as a result of land application of biosolids was estimated at 720 g (range: 530–1600 g). Among all known DLCs determined in biosolids, brominated analogs contributed 370% more TEQ than did chlorinated congeners, indicating the necessity of including brominated DLCs in the exposure and risk assessment of land-applied biosolids.

INTRODUCTION

Studies on polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDDs and PBDFs) are relatively scarce compared to their toxic chlorinated analogs, *i.e.,* polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs). However, over the past decade evidence has accumulated on the widespread occurrence of PBDD/Fs in biological and environmental matrices [1-5]. Mass produced and widely used commercial mixtures of brominated flame retardants serve as an important, continuing source of exposure to PBDD/Fs for humans and wildlife [6]. PBDFs and polybrominated biphenyls (PBBs) are present as impurities in commercial polybrominated diphenylether (PBDE) mixtures, and the global annual emission of PBDFs resulting from the production/usage of PBDEs in 2001 was estimated at 2300 kg [7]. In addition to representing unwanted impurities in flame retardants, PBDD/Fs also are formed during combustion of products containing PBDEs in municipal and industrial waste incinerators [2, 8, 9]. Waste incinerators have been shown as a major source for global atmospheric emissions of dioxins [10]. Total PBDD/F concentrations between 113 and 800,000 pg/g dry weight (dw) have been detected in plant leaves, electronic shredder residues and soil samples collected from an e-waste recycling facility in China [11]. Additionally, tetra-BDD/Fs have been shown to occur in marine biota and sediments from natural processes [12]. Widespread distribution of PBDD/Fs in marine environments also helps to explain their presence in seafood, providing a direct route for human exposure [13-15]. PBDD/Fs have also been detected in house and office dust, presumably as a result of wear and tear processes of products containing brominated flame retardants [3, 4, 16].

Risk assessment of dioxins and dioxin-like chemicals (DLCs) relies on the concept of toxic equivalency (TEQ) developed by the World Health Organization (WHO). This methodology

58 expresses the composite chemical risk resultant from complex mixtures of DLCs in a single
59 value, using as a benchmark the most toxic form of dioxin, *i.e.,* 2,3,7,8-tetrachlorodibezo-*p*-
60 dioxin or TCDD. TEQ values are calculated by summing up the products obtained when
61 multiplying concentrations of individual DLC congeners with their respective Toxic Equivalency
62 Factors (TEFs). The TEF methodology in current use considers PCDD/Fs and dioxin-like
63 polychlorinated biphenyls (dl-PCBs) only, and the inclusion of PBDD/Fs and polybrominated
64 biphenyls (PBBs) has been proposed only recently [4, 17]. A comparison of the relative potencies
65 (REPs) compiled from *in vitro* and *in vivo* studies of brominated and chlorinated analogues
66 revealed only minor differences, showing overlap in most cases and for one brominated analogue
67 even higher potency when compared to its chlorinated analogue ($TEF_{PBB\ 77} > TEF_{PCB\ 77}$) [4]. An
68 expert panel of the WHO and the United Nations Environment Programme (UNEP) discussed
69 the possible inclusion of brominated analogues of DLCs in the TEF methodology in 2011 [4]. The
70 panel concluded that PBDD/Fs and dl-polybrominated biphenyls (dl-PBBs) may contribute
71 significantly to human background exposure to TEQs, resulting in a recommendation of using
72 similar interim TEF values for brominated and chlorinated congeners for human health risk
73 assessment.
74
75 Studies reporting the occurrence and fate of PBDD/Fs in wastewater treatment plants (WWTPs)
76 are scarce. Provided that significant amounts of PBDEs are detected in wastewaters and sewage
77 sludge [18, 19], it is highly likely for PBDD/Fs to co-occur in this compartment of the built water
78 environment. In 2001, the U.S. Environmental Protection Agency (U.S. EPA) conducted a
79 national sewage sludge survey (NSSS) to estimate dioxin and DLCs in biosolids (processed
80 sewage sludge considered fit for disposal on land) to enable multi-pathway exposure and risk

assessment of the practice of land application of biosolids. Samples from 94 WWTPs were analyzed by U.S. EPA for a suite of PCDDs, PCDFs and PCBs to estimate the TEQ of U.S. biosolids. The U.S. EPA reported TEQ values ranging from 3 to 718 ng/kg of TCDD and concluded that numeric standards or management practices are not warranted for dioxin and DLCs in land-applied biosolids [20]. In response to the recommendation of the WHO-UNEP panel, we analyzed archived samples collected by the U.S. EPA during its 2001 National Sewage Sludge Survey (NSSS), for a suite of PBDDs and PBDFs to provide the first nationwide occurrence inventories of brominated DCLs in U.S. biosolids. Study objectives were: (i) to determine concentrations of PBDD/Fs in U.S. biosolids using gas chromatography - high resolution mass spectrometry (GC-HRMS); (ii) to compare the concentration ratio of PBDD/Fs to PBDEs in biosolids relative to that found in commercial flame retardant formulations; (iii) to compute the TEQ of brominated DLCs in U.S. biosolids; (iv) to assess the relative contributions of brominated and chlorinated DLCs to the total TEQ of U.S. biosolids; and (v) to assess the need for analyzing brominated analogs when assessing health risks from DLCs in biosolids.

MATERIALS AND METHODS

Sample Description. Biosolids samples were collected by the U.S. EPA during their 2001 NSSS from 94 WWTPs in 32 U.S. States and the District of Columbia. The facilities were selected by the U.S. EPA to obtain unbiased national estimates of dioxins and DLCs in U.S. biosolids that are disposed of primarily by land application [20]. After completion of 2001 NSSS, the samples were acquired by our laboratory and, stored in amber glass jars at –20 ^{o}C, were integrated into the Human Health Observatory at Arizona State University as a shared resource, termed the U.S. National Sewage Sludge Repository [21]. Archived samples were randomly grouped into five

104 composite samples, each containing solids from between 21 and 24 individual samples. The
105 approach of compositing biosolids has been validated for pharmaceuticals and personal care
106 products (PPCPs), brominated flame retardants (BFRs), alkylphenol surfactants and
107 perfluorinated compounds (PFCs), known to provide defensible national baseline levels of
108 contaminants in U.S. biosolids [18, 22-25]. The composites analyzed in this study constitute a
109 representative sample (94 facilities) of the more than 16,000 WWTPs in the U.S. Additional
110 information on sampling locations, biosolids sampling and composite preparation is provided as
111 supplementary material (Table S1).
112
113 **PBDD/Fs Analysis.** The biosolids composites were analyzed in collaboration with the
114 commercial laboratory (AXYS Analytical Services Ltd., Sydney, British Columbia, Canada) that
115 had developed EPA Method 1694 for PPCPs, and that specializes in the analysis of traditional
116 and emerging contaminants. A suite of tetra-through octa-brominated dioxins and furans
117 congeners (six PBDDs and six PBDFs) were analyzed in the present study (Table 1;
118 Supplemental Table S2). About 10 g of the samples were spiked with a suite of ^{13}C-labeled
119 analogs of PBDDs and PBDFs, and dried using sodium sulfate. The dried samples were Soxhlet
120 extracted in toluene: acetone (80:20 by volume) mixture. The resulting extracts were cleaned up
121 by column chromatography on a layers acid/base silica column. Cleaned extracts were reduced in
122 volume (100 µL) and spiked with isotopically-labeled internal standard ($^{13}C_{12}$-1,2,3,7,8,9-
123 HexaCDD) prior to analysis by GC-HRMS. GC-HRMS analyses were performed using a
124 Micromass Autospec Ultima magnetic sector HRMS (Water, Millford, MA) equipped with a
125 Hewlett-Packard 6890 GC (Agilent, Santa Clara, CA). Analysis of samples and standards were
126 conducted using low light levels to minimize photodegradation of PBDD/Fs. Analyte separation

was performed on a DB-5HT capillary column (30 m, 0.25 mm i.d. x 0.1 μm; Agilent, Santa Clara, CA). The HRMS was operated at a static mass resolution in the voltage selected ion-recording mode using selected perfluorokerosene (PFK) ions as a reference mass lock (Supplemental Table S2). Two masses from the molecular ion cluster were used to monitor each of the target analytes and ^{13}C-labeled surrogate standards. Target concentrations were determined by isotope dilution or internal standard quantification procedures using Micromass OPUSQUAN software. All concentrations are reported on a dry weight (dw) basis. QA/QC protocol is described in supplementary material available online.

RESULTS AND DISCUSSION

Method Performance. The method detection limits (MDLs) for the analytes ranged between 2 and 10 pg/g dw of biosolids (Supplemental Table S3). Recoveries from matrix spike experiments for the various analytes ranged between 50.1 and 207%. The only analyte that exceeded the method control limits of 50-150% recovery was 2,3,7,8-TBDF at 207%; since 2,3,7,8-TBDF contributed only 0.08% to the total mass of PBDD/Fs levels in biosolids, this non-ideal recovery was considered inconsequential to the overall objectives of the study. Analysis precision expressed as relative percentage difference (RPD) ranged between 4 to 55% for the analytes, with an average of 20% for the 12 analytes. Only two analytes, 2,3,4,7,8-PeBDF (43%) and 1,2,3,4,6,7,8-HpBDF (55%), exceeded the method control limit of 40% RPD; non-homogeneity of biosolids samples is known to impact analysis precision, and high RPDs (average of 42%) have been observed previously for organics in biosolids samples from the U.S EPA's NSSS [26]. Lab blanks showed no laboratory contamination for the 12 analytes of interest.

PBDD/Fs in U.S. Biosolids. Two PBDDs and five PBDFs were detected in biosolids composites from the 2001 U.S. EPA's NSSS (Table 1). The most abundant compound was 1,2,3,4,6,7,8-HpBDF, detected at an average concentration of 9,540 (range: 580 to 40,900) ng/kg dw, followed by 1,2,3,4,7,8-hxBDF and 1,2,3,4,7,8/1,2,3,6,7,8-hxBDD at 330 (range: 40 – 1290) and 76 (range: 16 – 195) ng/kg dw, respectively. The total mean concentration of PBDDs and PBDFs detected in biosolids composites was 10,000 (range: 630 to 42,800) ng/kg dw, of which 1,2,3,4,6,7,8-HpBDF constituted about 95%. Since octa-BDD was quantified against $^{13}C_{12}$-1,2,3,4,7,8-HxBDD, the recovery for OBDD may be lower than that of the labeled surrogate compound. Furthermore, octa-BDF was identified and quantified using a retention time and response factor predicted from the octa-BDD compound, the certainty of which is unknown for the present method. As a result, the concentrations of octa-BDD/F detected in biosolids are reported as 'information values' only and were not included in the data analysis (Supplemental Table S4). By analyzing composites prepared from nationally representative samples of biosolids (from 94 WWTPs), the present study provides the first baseline levels for these toxic chemicals in biosolids. To the best of our knowledge, there exists only one study in the literature that reports the occurrence of PBDDs and PBDFs in sewage sludge from a U.S. WWTP. Raw sewage sludge (not dewatered) collected from a WWTP in Manhattan, New York, on September 19 and 25, 2001, was analyzed for select PBDD/Fs as part of the study that measured the environmental impacts of the September 11 terrorist attack on the World Trade Center in 2001 [27]. The total concentration of tetra-through hexa-BDD/Fs reported were 696 and 246 ng/kg dw for sample collected on September 19 and 25, 2001, respectively [27]. These levels are similar to the total tetra-through hexa-BDD/F levels reported in the present study (average concentration of 430 ng/kg dw). In analogy to the present study, PBDFs were more abundant than PBDDs in raw

173 sewage sludge samples from New York [27]. Another study that analyzed 13 biosolids samples
174 collected from German WWTPs revealed the detection of mono- through penta-BDFs at a mean
175 total concentration of 1,170 (range: 210 to 3,050) ng/kg dw [28]. The average concentration of
176 tetra-BDF was 137 (range: 30 and 230) ng/kg dw in German biosolids, which was an order of
177 magnitude higher than those in U.S. biosolids reported in the present study. Conversely, penta-
178 BDF was rarely detected in German sewage sludge (10 ng/kg at 33% detection frequency)
179 compared to the present study (average concentration of 87 ng/kg at 80% detection frequency in
180 U.S. biosolids), although the composite sampling approach of the current work may have skewed
181 the results of detection frequency.
182
183 The origin, occurrence level, and fate of PBDD/Fs in WWTPs are still mostly unclear today.
184 PBDFs have been shown to be the predominant dioxin-like impurities in technical PBDE
185 mixtures, and the congener profile of PBDF impurities seemed to correspond to the degree of
186 bromination of commercial PBDE mixtures [6]. Our group had reported significant levels of
187 PBDEs in the past from analyzing the same set of biosolids composites studied here [18]. A total
188 PBDE concentration in biosolids of 9400 ± 960 ng/g dw (32 congeners) was detected, of which
189 deca-BDE was the most abundant congener, constituting 57% of the total PBDE load in
190 biosolids. It has been shown that octa-BDF and 1,2,3,4,6,7,8-HpBDF were the most abundant
191 and major impurities detected in commercial deca-BDE formulations [6]. The abundance of deca-
192 BDE relative to other commercial mixture of PBDEs in commerce and in biosolids could have
193 contributed to the observed high concentration of 1,2,3,4,6,7,8-HpBDF among all PBDD/Fs
194 detected in biosolids from the present study. However, octa-BDE, although detected at high
195 concentration (see Table S4), is excluded from the present analysis due to uncertain method

196 validity for this congener. The average contributions of total PBDDs and total PBDFs detected in
197 the present study relative to the total mass of PBDEs present were 0.001% and 0.11%,
198 respectively (Figure 1). In contrast, it was reported previously that PBDDs were not detectable
199 (<100 – 200 ng/g or <0.00002%) in commercial PBDE mixtures, and PBDFs accounted for only
200 0.002% by weight [7]. The higher fraction of PBDD/Fs in biosolids relative to commercial PBDE
201 formulations may be the result of (i) preferential attenuation of PBDEs compared to PBDD/Fs
202 contained in flame retardants, (ii) transformation of PBDEs to PBDD/Fs post consumption, (iii)
203 contributions from additional, not yet identified sources of PBDD/Fs, or (iv) a combination of
204 the above. A wide variety of possible sources of PBDD/Fs in the environment has been
205 summarized elsewhere [6], of which thermolysis and photolysis of BFRs in the environment,
206 industrial discharges and residues from residential fires that could potentially enter the sewer
207 system may constitute important sources for the occurrence of PBDD/Fs in WWTPs and
208 biosolids.
209
210 The annual load of total PBDD/Fs in U.S. biosolids was estimated at 140 ± 134 kg for the year
211 2001, from the present study (Table 1). This translates to an estimated release of 83 ± 81 kg/year
212 of PBDD/Fs to U.S. land as a result of land application of biosolids. However, the voluntary
213 phase-out of major formulations of PBDEs suggests a likely drop of PBDD/Fs releases in the
214 U.S. over the course of the past decade. For example, a comparison of mean levels of select
215 congeners of PBDEs in biosolids sampled in 2001 and 2006/7 revealed a drop of 45% over 5
216 years, indicating the desirable reduction in mass due to ongoing efforts to phase-out PBDEs in
217 the U.S. [18]. If a similar drop in concentrations also applies to PBDD/Fs, which is plausible but
218 speculative at this time given the lack of confirmatory data, the load of PBDD/Fs in U.S.

219 biosolids calculated for the year 2006/7 would be approximately 77 ± 74 kg. This estimate is
220 based solely on the relative abundance in U.S. biosolids of PBDD/Fs relative to PBDEs and does
221 not consider other potential contributions.
222
223 **Toxic Equivalency of DLCs in U.S. Biosolids.** In response to the WHO-UNEP panel
224 conclusion of including brominated analogs of DLCs in human health risk assessments, the
225 present study estimated the TEQ of PBDDs and PBDFs in a representative sample of U.S.
226 biosolids from across the nation. The relative potency (REP) ranges of PBDDs and PBDFs are
227 summarized in Table 1; these data were compiled previously by others using data from *in vitro*
228 and *in vivo* studies [4]. We used this information to calculate the relative contribution to the TEQ
229 of DLCs in biosolids for each analyte detected in the present study (Figure 2). For compounds
230 not detected in biosolids, the TEQ was calculated using one-half of the applicable method
231 detection limits. The total TEQ from brominated analogs of DLCs was estimated at an average of
232 72 (range: 2 to 482, calculated using the low and high levels of REP) ng/kg dw, with
233 1,2,3,4,6,7,8-HpBDF contributing 36% to the total TEQ from PBDD/Fs alone, followed by
234 1,2,3,4,7,8-HxBDF and 2,3,4,7,8-PeBDF at 22% and 17% contributions, respectively. Though
235 the REP of PBDFs is lower than that of PBDDs, the estimated TEQ of PBDFs was more than six
236 times higher than that of PBDDs; thus, among brominated DLCs, PBDFs have to be considered
237 the major contributors to the overall TEQ of biosolids. Treating non-detects as zero in the TEQ
238 estimation resulted in only a 3% reduction of the TEQ computed [TEQ of 70 (range: 2 to 482)
239 ng/kg]; hence, the treatment of MDL values had a negligible impact on the outcome of the TEQ
240 estimation.
241

242 The U.S. EPA's 2001 survey reported TEQs of chlorinated DLCs (PCDD/Fs and PCBs) ranging
243 from 3 to 718 ng/kg dw in biosolids samples [20]. The majority of the samples analyzed (n = 113)
244 featured a TEQ range of 7 to 55 ng/kg. For comparison purpose, weighted averages of TEQ
245 values for PCDD/Fs and PCBs were calculated here by grouping TEQ levels reported by U.S.
246 EPA for the 113 individual samples used in the present study. This way, the TEQs plotted in
247 Figure 3 for the chlorinated analogues of dioxins and DLCs represent TEQ values that would
248 have been detected if the samples were composited, as performed in the present study. TEQ
249 values for PBDD/Fs were recalculated here using interim TEFs according to the WHO-UNEP
250 TEQ scheme [4] (Supplemental Table S5). Average TEQs of chlorinated and brominated dioxins
251 and DLCs in U.S. biosolids were 44 (range: 22 – 100) and 162 (range: 15 – 672) ng/kg of
252 TCDD, respectively. Thus, inclusion of PBDD/Fs in the risk assessment increased the toxicity
253 associated with dioxins and DLCs by 370% (range: 15 – 2,659%). This implies that the TEQ
254 originally reported by U.S. EPA for the 2001 NSSS may represent only 21% (range: 4 – 87%) of
255 the actual total DLC risk. Whereas PBDEs also possess dioxin-like potency, their REP values are
256 several orders of magnitude lower than TCDD [29-31]. Six such PBDEs (BDE- 47, 77, 99, 100, 119,
257 153) were also detected in the biosolids analyzed here and accounted for an additional mean
258 TEQ of 12 (range: 5 – 16) ng/kg of TCDD (Figure 3; Supplemental Table S6). The grand total
259 TEQ resulting from chlorinated and brominated DD/Fs, DLCs and PBDEs was estimated to
260 average at 217 (range: 57 – 703) ng/kg TCDD in U.S. biosolids, of which PBDD/Fs contributed
261 the largest fraction (75% on average), followed by the chlorinated dioxins and DLCs (20%
262 combined) and PBDEs (5%). This translates to a total TEQ of 1300 (range: 960 – 2900) g/year
263 sequestered in U.S. biosolids annually, of which about 720 (range: 530 – 1600) g is released to
264 U.S. soils as a result of land application of biosolids. However, PBDEs currently are not included

265 in the WHO-TEQ scheme. The significant contribution from brominated DLCs increases the
266 risks associated with biosolids application on land, and highlights the need for updating old
267 estimates and expanding the number of analytes in future risk assessments to better capture the
268 total toxicity of DLC risk in biosolids. Currently, the U.S. EPA has concluded that numeric
269 standards or management practices are not warranted for dioxin and DLCs in land-applied
270 biosolids, since only 6% of the samples were observed to have TEQ values of >100 ng/kg in the
271 survey [20]. In light of the data presented here, the results of the 2001 NSSS have to be interpreted
272 as representing underestimates of the total risk, since brominated dioxins and DLCs were not
273 analyzed in the samples but figure prominently into the risk equation.
274
275 **Study Limitations.** This first national assessment of baseline levels of PBDD/Fs in U.S.
276 biosolids leveraged archived samples from the 2001 EPA NSSS and involved the analysis of
277 mega composite samples. This study approach minimizes the number of samples to be analyzed
278 and is known to provide robust, defensible mean concentrations of contaminants in biosolids
279 albeit rendering impossible a determination of true minimum and maximum concentrations in
280 individual samples[23]. We assessed the potential impact of using the composite approach for the
281 determination of mean concentrations reported in this study. Accordingly, the average TEQ of
282 PCDD/Fs and PCBs calculated from the U.S. EPA's NSSS report for individual samples was 46
283 (range: 3 – 718) ng/kg (n = 113), whereas the mean of weighted average concentrations
284 computed for the five composites was 44 (range: 22 – 100) ng/kg. The relatively small
285 discrepancy between the two estimates of only 4% suggests that the data quality of the reported
286 mean concentrations for PBDD/Fs is good. In contrast, the concentration spread among
287 individual samples that served to create the composites remains unknown. Similarly, the

detection frequencies for analytes reported here very likely was skewed by the approach of compositing samples. Unfortunately, as a condition for participation in the study, the U.S. EPA kept undisclosed the sampling location and additional key information on the biosolids samples analyzed, including sludge treatment systems employed, population served by the plant, etc. Although the WWTPs sampled were known (see Supplemental Table S1), the biosolids samples provided to us were not linked to a specific WWTP to maintain anonymity of the samples. This information would have been beneficial in identifying potential sources of the PBDD/Fs. Since the EPA redacted this essential information, compositing of samples did little to diminish the informational value of the samples used in this study.

The TEQ levels reported in the present study may not be applicable to present-day samples, since many of the PBDE mixtures have been phased-out and other brominated replacement chemicals are being used in commerce. Hence, the dioxin and DLC profiles in biosolids produced today are expected to differ from those determined here for samples collected in 2001. The mechanism or pathway by which PBDD/Fs reach or are formed in WWTPs is still not well understood and should be explored in the future. This would aid in controlling PBDD/Fs source terms and serve to reduce the overall TEQ burden of DLCs in biosolids. Finally, the present study did not include PBBs and octa-BDD/Fs in the TEQ analysis, and additional, yet unidentified, DLCs may be present in biosolids and the environment. Similar conclusions have been drawn in the past, where PBDD/Fs and other unidentified DLCs contributed significantly to the total TEQ and to *in vitro* dioxin-like activity of indoor dust [16].

310 Overall, the above limitations were of negligible consequence for meeting the objectives of this
311 study. Indeed, the present work constitutes the first national assessment of TEQ in biosolids for
312 brominated DLCs in the U.S. and worldwide. As such, it represents a valuable contribution to the
313 current understanding of the occurrence of and human health risks posed by DLCs and
314 specifically PBDD/Fs in biosolids. A major conclusion of this work is that a safety assessment of
315 DLCs in biosolids is incomplete and potentially misleading if brominated DLCs are left out of
316 the risk equation.
317

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325 represent the official views of the NIEHS or the National Institutes of Health (NIH).
326

327 **Supporting Information Available.**

328 Included in the Supplementary Material available online is information on biosolids samples, a
329 list of facilities sampled by the U.S. EPA, relevant GC-HRMS parameters, quality assurance
330 data, method performance data, and estimated TEQ values of PBDD/Fs and PBDEs in U.S.
331 biosolids. This information is available free of charge via the Internet at http://pubs.acs.org.
332

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445 **Table 1.** Concentrations, loads, and detection frequencies of PBDD/Fs in U.S. biosolids from the 2001 EPA NSSS.

Compound	Relative Potency Ranges (REP)[a]	Concentration in Biosolids (ng/kg dw) Avg (Min, Max)[b]	Detection Frequency[c] (%)	Estimated Annual Load (kg/year) (Min-Max)	
				In Biosolids[d]	Land Applied[e]
1,2,3,4,7,8/1,2,3,6,7,8-Hexabromodibenzo-*p*-dioxin (HxBDD)	0.01-0.3	76 (16, 195)	60	0.1-1	0.05-0.8
1,2,3,7,8,9-HxBDD	0.017-0.15	60 (7, 116)	40	0.05-1	0.02-0.5
2,3,7,8-Tetrabromodibenzofuran (TeBDF)	0.1-0.97	9 (5, 17)	100	0.04-0.1	0.01-0.07
1,2,3,7,8-Pentabromodibenzofuran (PeBDF)	0.004-0.69	27 (10, 45)	40	0.1-0.3	0.03-0.2
2,3,4,7,8-PeBDF	0.08-0.4	60 (6, 200)	100	0.1-1	0.02-0.8
1,2,3,4,7,8- Hexabromodibenzofuran (HxBDF)	0.008-0.09	330 (40, 1290)	100	0.3-8	0.1-5
1,2,3,4,6,7,8-Heptabromodibenzofuran (HpBDF)	0.0014-0.004	9540 (580, 40900)	100	5-260	2-157

[a]REPs were compiled and reported in [4]
[b]Avg, average; Min, minimum; Max, maximum; dw, dry weight
[c]Even numbers in increments of 20 result from the use of 5 mega composite samples
[d]Values calculated based on estimated biosolids production in 2001 (see Supplementary Material)
[e]Calculated based on the estimated percentage of biosolids applied on land in the year 2001 (50-60%) [32-34]

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Figures

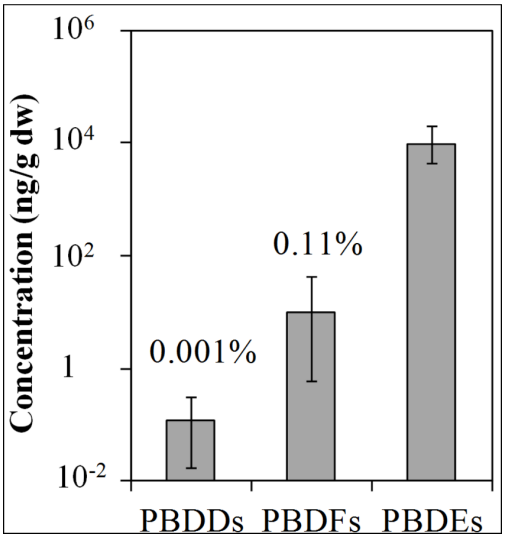
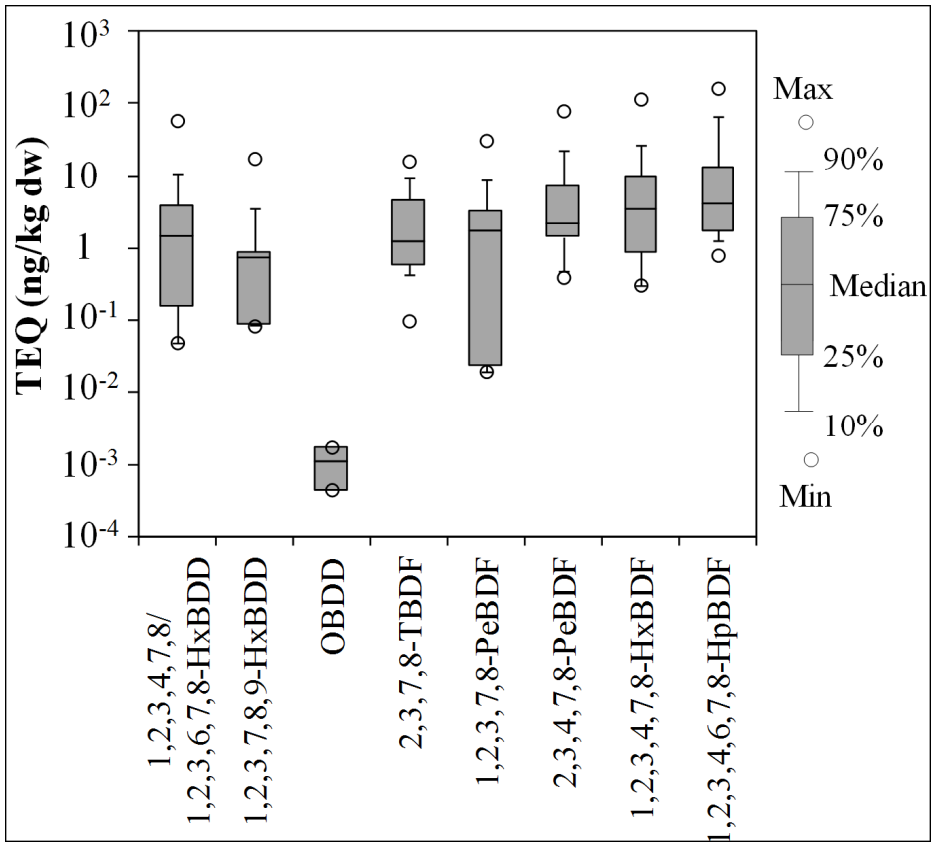


Figure 1. Concentration of PBDDs and PBDFs relative to total PBDEs levels detected in biosolids composites (n = 5) prepared from 113 samples collected by the U.S. EPA during its 2001 national sewage sludge survey from 94 facilities in 32 U.S. States and the District of Columbia. Percentages above the bar represent the respective levels relative to total PBDE levels in biosolids. Error bars represent minima and maxima.



464

465 **Figure 2.** Toxic equivalency (TEQ) ranges in U.S. biosolids of detected PBDD/Fs estimated
466 from minimum and maximum of relative potency (REP) values reported in the literature [4] (Table
467 1). TEQ values for non-detects were estimated by using one-half of the method detection limit
468 of each analyte.

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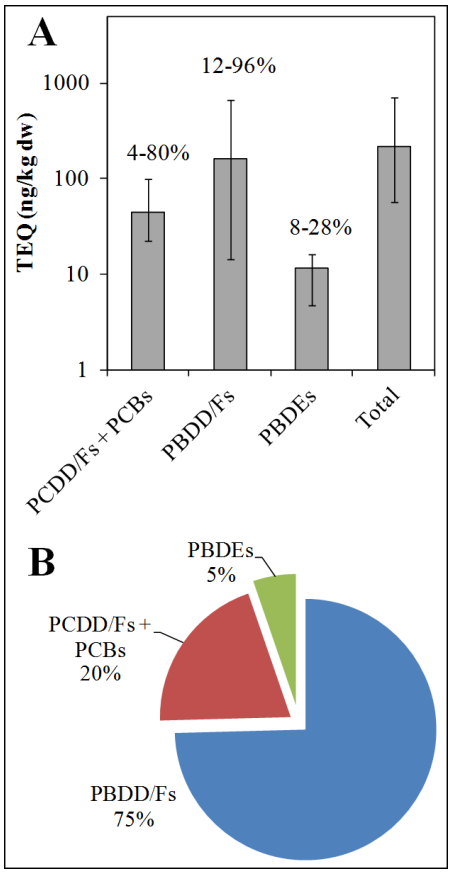
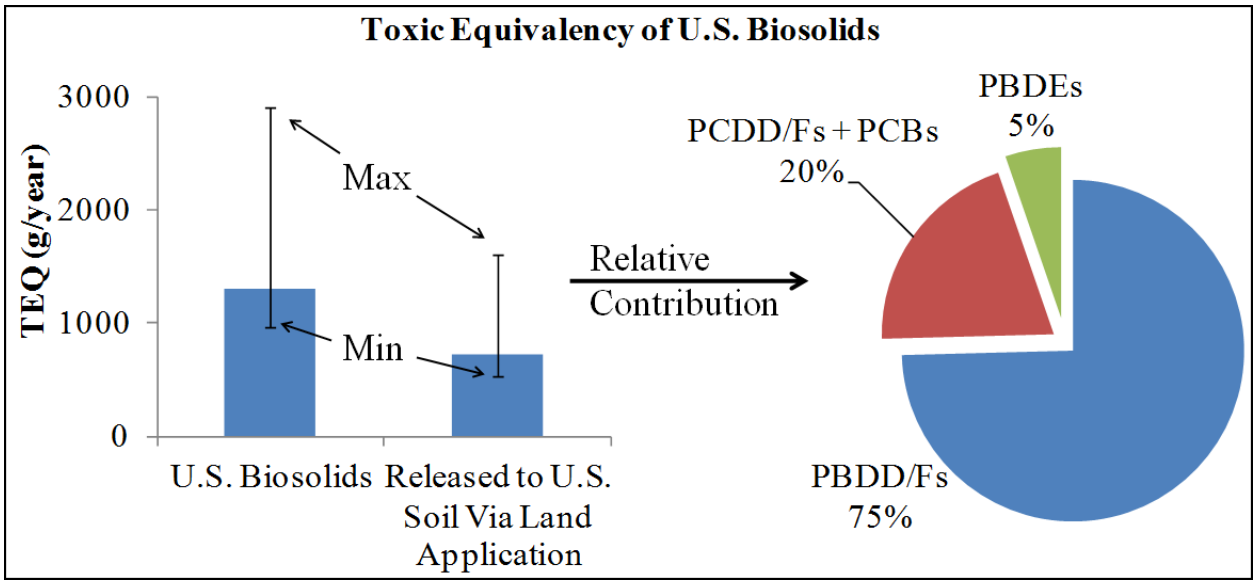


477

478 **Figure 3.** (A) Comparison of TEQs from chlorinated (PCDD/Fs, PCBs) and brominated
479 (PBDD/Fs) analogues of dioxin-like compounds (DLCs) and from PBDEs in U.S. biosolids.
480 TEQ values for PBDD/Fs were calculated using interim TEFs similar to chlorinated analogs as
481 suggested by the WHO-UNEP TEQ scheme [4]. For comparison purpose, weighted average of
482 TEQ of chlorinated analogs of DLCs were calculated by grouping TEQ levels reported by U.S.
483 EPA for the individual samples that were pooled to prepare the five composites analyzed in the
484 present study. TEQs for PBDEs were estimated using relative potency values (REP) and
485 concentrations in U.S. biosolids reported elsewhere [18, 35]. The percentage above the bar
486 represents the corresponding contribution to the total TEQ of DLC in U.S. biosolids. (B)
487 Average contribution of chlorinated dioxins and DLCs, PBDD/Fs and PBDEs to the total TEQ of
488 U.S. biosolids.



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490 **Table of Contents Art**