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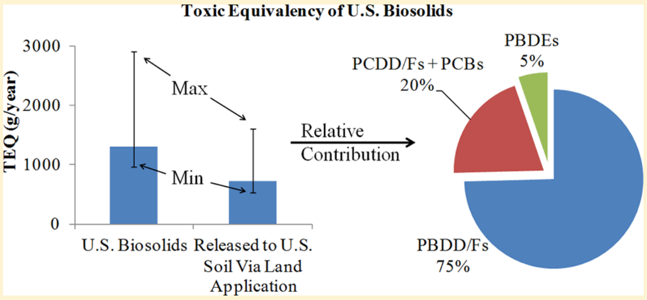
Contribution of Polybrominated Dibenzo-*p*-dioxins and Dibenzofurans (PBDD/Fs) to the Toxic Equivalency of Dioxin-like Compounds in Archived Biosolids from the U.S. EPA's 2001 National Sewage Sludge Survey

Arjun K. Venkatesan and Rolf U. Halden*

Center for Environmental Security, The Biodesign Institute, Security and Defense Systems Initiative, Arizona State University, 781 East Terrace Mall, Tempe, Arizona 85287-5904, United States

Ⓢ *Supporting Information*



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 in the composited, archived biosolids that were collected in 32 U.S. states and the District of Columbia from 94 wastewater treatment plants by the United States Environmental Protection Agency in its 2001 national sewage sludge survey. Two PBDDs and five PBDFs were detected in the biosolids composites at varying frequencies (40−100%) with a total mean concentration of 10,000 ng/kg dry weight (range: 630−42,800), 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%), which indicates potential PBDE transformation or possibly additional sources of PBDD/Fs in the environment. The TEQ contribution of PBDD/Fs was estimated at 162 ng/kg 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (range: 15−672), which is 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 the 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, which indicates the need to include 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 those on their toxic chlorinated analogs, that is, polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs); however, in 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 (BFRs) serve as important, continuing sources 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; the global annual emission of PBDFs resulting from the production and usage of PBDEs in 2001 previously was estimated to be 2300 kg.[7] In addition to representing unwanted impurities in flame retardants, PBDD/Fs also are formed during the combustion of products that contain PBDEs in municipal and industrial waste incinerators.[2,8,9] Waste incinerators were shown as a major source of 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 electronic waste recycling facility in China.[11] Additionally, tetra-BDD/Fs were shown to occur in marine biota and sediments from natural processes.[12] The widespread distribution of PBDD/Fs in marine environments also helps to explain their presence in seafood, which provides a direct route for human exposure.[13−15] PBDD/Fs have also been detected in house and office dust, presumably as a result of the wear and tear processes of products that contain BFRs.[3,4,16]

A 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 expresses the composite chemical risk that results

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Table 1. Concentrations, Loads, and Detection Frequencies of PBDD/Fs in U.S. Biosolids from the 2001 U.S. EPA NSSS

compound	REP ranges[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 ref 4. [b] Avg, average; min, minimum; max, maximum. [c] Even numbers in increments of 20 result from the use of 5 mega composite samples. [d] Values were calculated based on estimated biosolids production in 2001 (see the Supporting Information). [e] Calculated based on the estimated percentage of biosolids applied on land in the year 2001 (50−60%).[32−34]

from complex mixtures of DLCs in a single value and uses the most toxic form of dioxin as a benchmark, that is, 2,3,7,8-tetrachlorodibezo-*p*-dioxin or TCDD. TEQ values are calculated as the sum of the products obtained when the concentrations of individual DLC congeners are multiplied with their respective toxic equivalency factors (TEFs). The TEF methodology in current use considers PCDD/Fs and dioxin-like polychlorinated biphenyls (dl-PCBs) only, and the inclusion of PBDD/Fs and PBBs has been proposed only recently.[4,17] A comparison of the relative potencies (REPs) compiled from *in vitro* and *in vivo* studies of brominated and chlorinated analogs revealed only minor differences and showed overlap in most cases; for one brominated analog, even higher potency was observed compared to that of its chlorinated analog ($\text{TEF}_{\text{PBB 77}} > \text{TEF}_{\text{PCB 77}}$).[4] An expert panel of the WHO and the United Nations Environment Programme (UNEP) discussed the possible inclusion of brominated analogs of DLCs in the TEF methodology in 2011.[4] The panel concluded that PBDD/Fs and dioxin-like polybrominated biphenyls (dl-PBBs) may contribute significantly to human background exposure to TEQs, which resulted in a recommendation to use similar interim TEF values for brominated and chlorinated congeners for human health risk assessments.

Studies that report the occurrence and fate of PBDD/Fs in wastewater treatment plants (WWTPs) are scarce. Provided that significant amounts of PBDEs are detected in wastewater and sewage sludge,[18,19] it is highly likely for PBDD/Fs to co-occur in this compartment of the built water environment. In 2001, the U.S. Environmental Protection Agency (U.S. EPA) conducted a national sewage sludge survey (NSSS) to estimate dioxin and DLC levels in biosolids (processed sewage sludge considered fit for disposal on land) to enable a multipathway exposure and risk assessment of the practice of the land application of biosolids. Samples from 94 WWTPs were analyzed by the U.S. EPA for a suite of PCDDs, PCDFs, and PCBs to estimate the TEQ values of U.S. biosolids. The U.S. EPA reported TEQ values in the range of 3−718 ng/kg of TCDD and concluded that numeric standards or management practices are not warranted for dioxins and DLCs in land-applied biosolids.[20] In response to the recommendation of the WHO−UNEP panel, we analyzed archived samples that were collected by the U.S. EPA during its 2001 NSSS for a suite of PBDDs and PBDFs to provide the first nationwide occurrence inventories of brominated DCLs in U.S. biosolids. The study objectives were: (i) to determine the concentrations of PBDD/Fs in U.S. biosolids using gas chromatography/high resolution mass spectrometry (GC-HRMS); (ii) to compare the concentration ratios of PBDD/Fs to PBDEs in biosolids relative to those found in commercial flame retardant formulations; (iii) to compute the TEQ value of brominated DLCs in U.S. biosolids; (iv) to assess the relative contributions of brominated and chlorinated DLCs to the total TEQ value of U.S. biosolids; and (v) to assess the need for the analysis of brominated analogs in health risk assessments of 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 the completion of the 2001 NSSS, the samples were acquired by our laboratory, stored in amber glass jars at −20 °C, integrated into the Human Health Observatory at Arizona State University as a shared resource, and termed the U.S. National Sewage Sludge Repository.[21] The archived samples were randomly grouped into five composites, each contained solids from between 21 and 24 individual samples. The approach of compositing biosolids was validated for pharmaceuticals and personal care products (PPCPs), BFRs, alkylphenol surfactants, and perfluorinated compounds (PFCs), which demonstrates this methodology to provide defensible national baseline levels of contaminants in U.S. biosolids.[18,22−25] The composites analyzed in this study constitute a representative sample (94 facilities) of the more than 16 000 WWTPs in the United States. Additional information on the sampling locations, biosolids sampling, and composite preparation is provided in the Supporting Information (Table S1).

PBDD/Fs Analysis. The biosolids composites were analyzed in collaboration with the commercial laboratory (AXYS Analytical Services Ltd., Sydney, British Columbia, Canada) that had developed EPA Method 1694 for PPCPs and that specializes in the analysis of traditional and emerging contaminants. A suite of tetra-through octa-brominated dioxins and furans congeners (six PBDDs and six PBDFs) were analyzed in the present study (Table 1, Table S2). About 10 g of the samples were spiked with a suite of ^{13}C-labeled analogs of PBDDs and PBDFs and dried using sodium sulfate. The dried samples were Soxhlet extracted in a toluene/acetone (80:20 by volume) mixture. The resulting extracts were cleaned up by column chromatography on a layered acid/base silica column. The cleaned extracts were reduced in volume (100 μL) and spiked with an isotopically labeled internal standard ($^{13}C_{12}$-1,2,3,7,8,9-HexaCDD) prior to analysis by GC-HRMS. GC-HRMS analyses were performed using a Micromass Autospec Ultima magnetic sector HRMS (Water, Millford, MA) equipped with a Hewlett-Packard 6890 gas chromatograph (Agilent, Santa Clara, CA). The analyses of samples and standards were conducted using low light levels to minimize the photodegradation of the PBDD/Fs. Analyte separation was performed on a DB-5HT capillary column (30 m, 0.25 mm i.d. × 0.1 μm; Agilent, Santa Clara, CA). The HRMS was operated at a static mass resolution in the voltage-selected ion recording mode with the selected perfluorokerosene (PFK) ions as reference mass locks (Table S2). Two masses from the molecular ion cluster were used to monitor each of the target analytes and ^{13}C-labeled surrogate standards. The target concentrations were determined by isotope dilution or internal standard quantification procedures using Micromass OPUS-QUAN software. All of the concentrations were reported on a dry weight (dw) basis. The quality assurance and quality control (QA/QC) protocol is described in the Supporting Information.

■ RESULTS AND DISCUSSION

Method Performance. The method detection limits (MDLs) for the analytes ranged between 2 and 10 pg/g dw of biosolids (Table S3, Supporting Information). The recoveries from the 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% of the total mass of PBDD/Fs levels in the biosolids, this nonideal recovery was considered inconsequential to the overall objectives of the study. The analysis precision, expressed as the relative percentage difference (RPD), ranged between 4 and 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; the nonhomogeneity of biosolids samples is known to impact analysis precision, and high RPDs (average of 42%) were 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 the 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, which was detected at an average concentration of 9,540 ng/kg dw (range: 580−40,900 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 ng/kg dw (range: 40−1290 ng/kg dw) and 76 ng/kg dw (range: 16−195 ng/kg dw), respectively. The total mean concentration of PBDDs and PBDFs detected in the biosolids composites was 10,000 ng/kg dw (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 octa-BDD may be lower than that of the labeled surrogate compound. Furthermore, octa-BDF was identified and quantified using retention time and response factor that were 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 the biosolids are reported as "information values" only and were not included in the data analysis (Table S4, Supporting Information). 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, 2001 terrorist attack on the World Trade Center.[27] The total concentration of tetra- through hexa-BDD/Fs reported were 696 ng/kg dw and 246 ng/kg dw for the samples collected on September 19 and 25, 2001, respectively.[27] These levels are similar to the total tetra- through hexa-BDD/F levels that are reported in the present study (average concentration of 430 ng/kg dw). Analogously to the present study, PBDFs were more abundant than were PBDDs in raw sewage sludge samples from New York.[27] Another study that analyzed 13 biosolids samples collected from German WWTPs revealed the detection of mono- through penta-BDFs at a mean total concentration of 1,170 ng/kg dw (range: 210 to 3,050 ng/kg dw).[28] The average concentration of tetra-BDF was 137 ng/kg dw (range: 30 and 230 ng/kg dw) in German biosolids, which was an order of magnitude higher than those in U.S. biosolids reported in the present study. Conversely, penta-BDF was rarely detected in German sewage sludge (10 ng/kg at 33% detection frequency) compared to the present study (average concentration of 87 ng/kg at 80% detection frequency in U.S. biosolids), although the composite sampling approach of the current work may have skewed the results of the detection frequency.

The origin, occurrence level, and fate of PBDD/Fs in WWTPs are still mostly unclear today. PBDFs were shown to be the predominant dioxin-like impurities in technical PBDE mixtures, and the congener profile of PBDF impurities seems to correspond to the degree of bromination of commercial PBDE mixtures.[6] Our group reported significant levels of PBDEs in the past from the analysis of the same set of biosolids composites studied here.[18] A total PBDE concentration in biosolids of 9400 ± 960 ng/g dw (32 congeners) was detected, of which deca-BDE was the most abundant congener, constituting 57% of the total PBDE load in the biosolids. It has been shown that octa-BDF and 1,2,3,4,6,7,8-HpBDF were the most abundant and major impurities detected in commercial deca-BDE formulations.[6] The abundance of deca-BDE relative to other commercial mixtures of PBDEs in commerce and in biosolids could have contributed to the observed high concentration of 1,2,3,4,6,7,8-HpBDF among all of the PBDD/Fs detected in the biosolids from the present study. However, octa-BDE, although detected at a high concentration (see Table S4, Supporting Information), was excluded from the present analysis because of the uncertain method validity for this congener. The average contributions of total PBDDs and total PBDFs detected in the present study relative to the total mass of PBDEs present were 0.001% and 0.11%, respectively (Figure 1). In contrast, it was reported previously that PBDDs were not detectable (<100−200 ng/g or <0.00002%) in commercial PBDE mixtures, and PBDFs accounted for only 0.002% by weight.[7] The higher fraction of PBDD/Fs in the biosolids relative to the commercial PBDE formulations may be the result of (i) the preferential

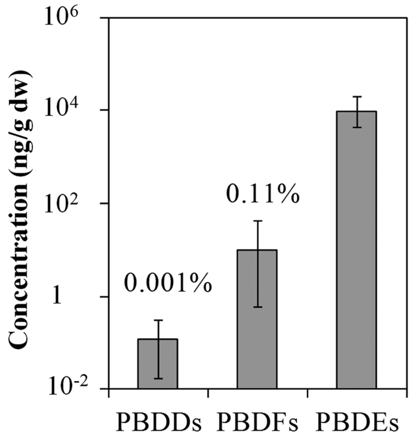

Figure 1. Concentration of PBDDs and PBDFs relative to the total PBDE levels detected in biosolids composites (n = 5) prepared from 113 samples collected by the U.S. EPA during its 2001 national sewage sludge survey from 94 facilities in 32 U.S. states and the District of Columbia. Percentages above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

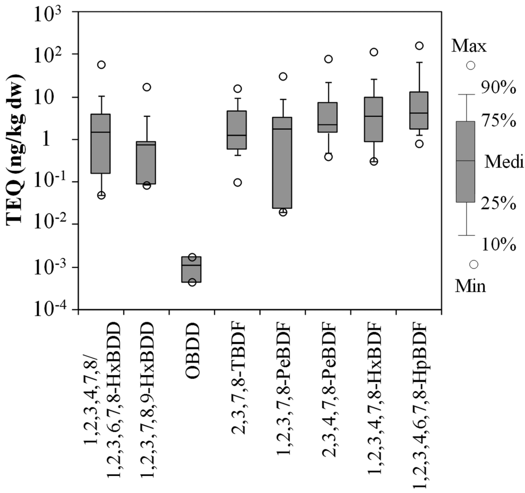

Figure 2. TEQ ranges in U.S. biosolids of the detected PBDD/Fs estimated from the minimum and maximum of REP values reported in the literature[4] (Table 1). TEQ values for nondetects were estimated by using one-half of the MDL of each analyte.

attenuation of PBDEs compared to PBDD/Fs contained in flame retardants; (ii) the transformation of PBDEs to PBDD/Fs post consumption; (iii) contributions from additional, not yet identified sources of PBDD/Fs; or (iv) a combination of the above. A wide variety of possible sources of PBDD/Fs in the environment were summarized elsewhere,[6] for example, thermolysis and photolysis of BFRs in the environment; industrial discharges and residues from residential fires subsequently enter the sewer system and constitute potentially important sources of PBDD/Fs in WWTPs and biosolids.

In the present study, the annual load of total PBDD/Fs in U.S. biosolids was estimated to be 140 ± 134 kg for the year 2001 (Table 1). This translates to an estimated release of 83 ± 81 kg/year of PBDD/Fs to U.S. land as a result of the land application of biosolids; however, the voluntary phase-out of major formulations of PBDEs suggests a likely drop of PBDD/Fs releases in the United States over the course of the past decade. For example, a comparison of mean levels of select congeners of PBDEs in the biosolids sampled in 2001 and 2006/7 revealed a drop of 45% over 5 years, which indicates the desirable reduction in mass due to the ongoing efforts to phase-out PBDEs in the United States.[18] If a similar drop in concentrations also applies to PBDD/Fs, which is plausible but speculative at this time given the lack of confirmatory data, the load of PBDD/Fs in U.S. biosolids calculated for the year 2006/7 would be approximately 77 ± 74 kg. This estimate is based on solely the relative abundance of PBDD/Fs in U.S. biosolids relative to PBDEs and does not consider other potential contributions.

Toxic Equivalency of DLCs in U.S. Biosolids. In response to the WHO−UNEP panel conclusion to include brominated analogs of DLCs in human health risk assessments, we estimated the TEQ values of PBDDs and PBDFs in a representative sample of U.S. biosolids from across the nation. The REP ranges of the PBDDs and PBDFs are summarized in Table 1; these data were compiled previously by others using data from *in vitro* and *in vivo* studies.[4] We used this information to calculate the relative contribution to the TEQ values of DLCs in biosolids for each analyte detected in the present study (Figure 2). For compounds not detected in biosolids, the TEQ value was calculated using one-half of the applicable MDLs. The total TEQ value from brominated analogs of DLCs was estimated at an average of 72 ng/kg dw (range: 2 to 482 ng/kg dw, calculated using the low and high levels of REP), with a 36% contribution of 1,2,3,4,6,7,8-HpBDF to the total TEQ value from PBDD/Fs alone, followed by 1,2,3,4,7,8-HxBDF and 2,3,4,7,8-PeBDF at 22% and 17% contributions, respectively. Though the REP of PBDFs is lower than that of PBDDs, the estimated TEQ value of PBDFs was more than six times higher than that of PBDDs; thus, among brominated DLCs, PBDFs have to be considered the major contributors to the overall TEQ values of biosolids. Nondetects were treated as zero in the TEQ value estimation and resulted in only a 3% reduction of the TEQ value computed [TEQ value of 70 ng/kg (range: 2 to 482 ng/kg)]; hence, the treatment of MDL values had a negligible impact on the outcome of the TEQ value estimation.

The U.S. EPA's 2001 survey reported TEQ values of chlorinated DLCs (PCDD/Fs and PCBs) ranging from 3−718 ng/kg dw in biosolids samples.[20] The majority of the samples analyzed (n = 113) featured a TEQ range of 7−55 ng/kg. For comparison purposes, the weighted averages of TEQ values for PCDD/Fs and PCBs were calculated here by grouping the TEQ levels reported by the U.S. EPA for the 113 individual samples used in the present study. This way, the TEQ values plotted in Figure 3 for the chlorinated analogs of dioxins and DLCs represent the TEQ values that would have been detected if the samples were composited, as performed in the present study. The TEQ values for PBDD/Fs were recalculated here using interim TEFs according to the WHO−UNEP TEQ scheme[4] (Table S5, Supporting Information). The average TEQ values of chlorinated and brominated dioxins and DLCs in U.S. biosolids were 44 ng/kg (range: 22−100 ng/kg) and 162 ng/kg (range: 15−672 ng/kg) of TCDD, respectively. Thus, the inclusion of PBDD/Fs in the risk assessment increased the toxicity associated with dioxins and DLCs by 370% (range: 15−2660%). This implies that the TEQ value originally reported by the U.S. EPA for the 2001 NSSS may represent only 21% (range: 4−87%) of the actual total DLC risk. Although PBDEs also possess dioxin-like potency, their REPs were several orders of magnitude lower than that of

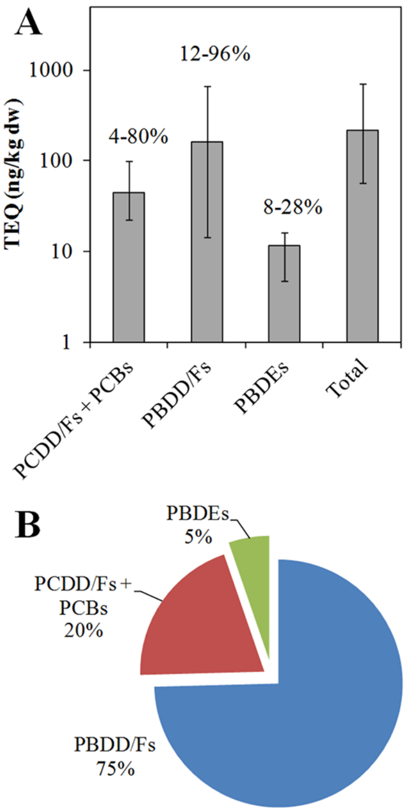

Figure 3. (A) Comparison of TEQ values from chlorinated (PCDD/Fs, PCBs) and brominated (PBDD/Fs) analogs of DLCs and from PBDEs in U.S. biosolids. TEQ values for PBDD/Fs were calculated using interim TEFs similar to the chlorinated analogs, as suggested by the WHO−UNEP TEQ scheme.[4] For comparison purposes, the weighted average of the TEQ value of the chlorinated analogs of DLCs were calculated by the grouping of TEQ levels reported by U.S. EPA for the individual samples that were pooled to prepare the five composites analyzed in the present study. TEQ values for PBDEs were estimated using REP values and concentrations in U.S. biosolids reported elsewhere.[18,35] The percentage above the bar represents the corresponding contribution to the total TEQ value of DLCs in U.S. biosolids. (B) Average contribution of chlorinated dioxins and DLCs, PBDD/Fs, and PBDEs to the total TEQ value of U.S. biosolids.

TCDD.[29−31] Six such PBDEs (BDE- 47, 77, 99, 100, 119, 153) were also detected in the biosolids analyzed here and accounted for an additional mean TEQ value of 12 ng/kg (range: 5−16 ng/kg) of TCDD (Figure 3; Table S6, Supporting Information). The grand total TEQ value that resulted from the chlorinated and brominated DD/Fs, DLCs, and PBDEs was estimated to average at 217 ng/kg (range: 57−703) TCDD in U.S. biosolids, of which PBDD/Fs contributed the largest fraction (75% on average), followed by the chlorinated dioxins and DLCs (20% combined), and PBDEs (5%). This translates to a total TEQ value of 1300 g/year (range: 960−2900 g/year) TCDD that are sequestered in U.S. biosolids annually, of which about 720 g (range: 530−1600 g) are released to U.S. soils as a result of the land application of biosolids. However, PBDEs currently are not included in the WHO−UNEP TEQ scheme. The significant contribution from brominated DLCs increases the risks associated with biosolids application on land and highlights the need to update old estimates and expand the number of analytes in future risk assessments to better capture the total toxicity of DLC risk in biosolids. Currently, the U.S. EPA has concluded that numeric standards or management practices are not warranted for dioxins and DLCs in land-applied biosolids since only 6% of the samples were observed to have TEQ values of > 100 ng/kg in the survey.[20] In light of the data presented here, the results of the 2001 NSSS must be interpreted as a representation of underestimates of the total risk since brominated dioxins and DLCs were not analyzed in the samples but figure prominently into the risk equation.

Study Limitations. This first national assessment of baseline levels of PBDD/Fs in U.S. biosolids leveraged archived samples from the 2001 U.S. EPA NSSS and involved the analysis of mega composite samples. This study approach minimizes the number of samples to be analyzed and is known to provide robust, defensible mean concentrations of contaminants in biosolids, although it renders impossible the determination of the true minimum and maximum concentrations in individual samples.[23] We assessed the potential impact of using the composite approach for the determination of mean concentrations reported in this study. Accordingly, the average TEQ value of PCDD/Fs and PCBs calculated from the U.S. EPA's NSSS report for individual samples was 46 ng/kg (range: 3−718 ng/kg) (n = 113), whereas the mean of the weighted average concentrations computed for the five composites was 44 ng/kg (range: 22−100 ng/kg). The relatively small discrepancy between the two estimates of only 4% suggests that the data quality of the reported mean concentrations for PBDD/Fs is good. In contrast, the concentration spread among individual samples that served to create the composites remains unknown. Similarly, the detection frequencies for analytes reported here very likely were skewed by the approach of composited samples. Unfortunately, as a condition for participation in the study, the U.S. EPA kept undisclosed the sampling locations and additional key information on the biosolids samples analyzed, including the sludge treatment systems employed, population served by the plant, etc. Although the WWTPs sampled were known (see Table S1, Supporting Information), the biosolids samples provided to us were not linked to a specific WWTP to maintain the anonymity of the samples. This information would have been beneficial to identify potential sources of the PBDD/Fs. Since the U.S. EPA redacted this essential information, the 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. Therefore, the dioxin and DLC profiles in biosolids produced today are expected to differ from those determined here for the 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 the control of PBDD/F source terms and serve to reduce the overall TEQ burden of DLCs in biosolids. Finally, the present study did not include PBBs or octa-BDD/Fs in the TEQ analysis, and additional yet unidentified DLCs may be present in biosolids and the environment. Similar conclusions were drawn in the past where PBDD/Fs and other unidentified DLCs contributed significantly to the total TEQ value and to the *in vitro* dioxin-like activity of indoor dust.[16]

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

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Information on biosolids samples, a list of facilities sampled by the U.S. EPA, relevant GC-HRMS parameters, quality assurance data, method performance data, and estimated TEQ values of PBDD/Fs and PBDEs in U.S. biosolids. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +1 (480) 727-0893; fax: +1 (480) 965-6603; e-mail: halden@asu.edu.

Notes

The authors declare no competing financial interest.

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