

# A modified QuEChERS approach for the screening of dioxins and furans in sediments

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**Abstract** A rapid extraction and cleanup method for the screening of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in sediments is described which combines a modified QuEChERS extraction with carbon reversed-phase solid phase extraction cleanup. This approach is compared to the classical Soxhlet extraction and multi-column cleanup method in terms of toxic equivalence quotients (TEQs), precision, instrumental chromatography, method detection limits (MDLs), recovery of <sup>13</sup>C-labelled quantitation standard, sample preparation time, workload capacity, and sustainability factors. TEQs of 32 sediment samples were found to be well correlated and differed by 16 ± 10 % between the two methods. Certified and standard reference sediments differed by 4.1 and 6.7 %, respectively. Precision and instrumental chromatography were comparable. While the modified QuEChERS method had higher MDLs and lower recoveries, in terms of preparation time and workload capacity, the modified QuEChERS approach can prepare approximately 30 samples per day as compared to 10–20 samples in 3 to 4 days for the classic method. The modified QuEChERS method was also found to be safer and greener. The appreciable improvement in capacity makes the modified QuEChERS approach a suitable alternative to the classical method for applications where turnaround time and the

number of samples that can be analyzed are more important than minimal detection limits.

**Keywords** QuEChERS · PCDD/PCDF screening · Sediment · Extraction · Ultrasonication

## Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are persistent organic pollutants that are ubiquitous in the environment [1, 2]. They are considered to be human carcinogens by the International Agency for Research on Cancer (IARC), likely carcinogens by the US EPA, and are known to cause a wide range of other toxicological effects [3–6]. The toxicity of PCDD/PCDFs is intermediated by their interactions with the aryl hydrocarbon receptor which in turn alters gene expression [7, 8]. Out of the 210 possible PCDD/PCDFs, the World Health Organization (WHO) has determined that 7 PCDDs and 10 PCDFs are of particular concern [9, 10]. All 17 are substituted at the 2,3,7,8 positions.

In order to compare the toxicity of samples containing mixtures of PCDD/PCDFs, toxic equivalence factors (TEFs) have been developed. Of all the PCDD/PCDFs, 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (TCDD) is the most toxic and is assigned a TEF of 1 while the others have been assigned a value (≤1) proportional to their relative potency. The concentrations of the 17 WHO PCDD/PCDFs in a sample are multiplied by their corresponding TEFs and summed. The resulting value is the toxic equivalence quotient (TEQ) and represents the amount of TCDD having a toxicity equivalent to the sample [11]. This approach allows for one value to be reported for each sample and simplifies risk assessment.

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Due to their lipophilic nature and resistance to metabolism, PCDD/PCDFs can accumulate in fatty tissue and biomagnification occurs up the food chain [12–14]. In fish, bioaccumulation increases with lipid content and age [15]. Biomagnification of PCDD/PCDFs in fish presents a public health risk as approximately 90 % of human exposure to PCDD/PCDFs comes from contaminated food [16], and studies have shown that contaminated fish can be an important source of exposure for those who eat fish regularly [17]. Contaminated sediments act as sources of chlorinated hydrocarbons [18], and uptake of pollutants from contaminated sediments by marine organisms has been reported [19–21]. Furthermore, because highly hydrophobic molecules such as TCDD do not exist freely in aqueous environments, it has been suggested that bioaccumulation in fish be predicted based on sediment contamination levels [22].

As a result, monitoring programs have been established to monitor and manage aquatic environments with contaminated sediments. Sediment chemistry has been shown to vary spatially [23] necessitating sample collection at different depths and locations. Many sampling locations may also be required to trace the source of a contaminant. In addition, monitoring programs must resample the same locations to account for temporal variability and to assess predictions made as well as the effectiveness of any rehabilitation action performed [24, 25]. For these reasons, a comprehensive sediment monitoring program can require a large number of samples to characterize a given area and often presents a large workload for an analytical laboratory and cost to the client.

The classical method for PCDD/PCDF analysis in sediments is briefly described here, though there is some variation amongst laboratories. After drying the sediment and homogenizing through a sieve or by grinding, the dry sediment is extracted by Soxhlet apparatus with Toluene. Chromatographic cleanup involving several packed columns is performed, and the eluted extracts are concentrated before each column and prior to instrumental analysis. This highly laborious sample preparation is followed by injection on a high resolution gas chromatograph (GC) coupled with high resolution mass spectrometry (HRMS) [26]. While considered the gold standard in terms of accuracy and precision, this technique is costly and time-consuming [27] making it unsuitable for time-sensitive situations or projects requiring a large number of samples. The classical method is also unnecessarily sensitive for contaminated sites. The Canadian quality guidelines for dioxins and furans in soil, for example, are 10 pg TEQ/g for agricultural use and 1000 pg TEQ/g for industrial use [28], while the classical method is sensitive enough for food monitoring applications with limits as low as 0.75 pg TEQ/g [29].

Many attempts have been made to reduce the cost and turnaround-time for PCDD/PCDF analysis in soils and sediments including bioassay and immunoassay methods [30, 31]. These

techniques, although faster, generally do not meet the precision and accuracy requirements for litigation [32]. Extraction techniques such as pressurized liquid extraction (PLE) and supercritical fluid extraction decrease extraction time by increasing temperature and pressure [33, 34]. Selective pressurized liquid extraction has been successfully used to accelerate the analysis of PCDD/PCDFs in sediment by combining PLE with a simultaneous cleanup [35]. These alternatives to Soxhlet extraction require drying the sample which typically takes a day. The use of microwave assisted extraction (MAE) with polar solvents eliminates the need for drying. MAE has been shown to greatly decrease extraction time for pesticides in sediments but exhibited reduced efficiency for non-polar solvents [36]. A Dean Stark trap can also eliminate the need for drying but requires complicated and costly glassware and does not reduce the extraction or clean-up time [37]. Ultrasonication with non-polar solvents has previously been used to extract organic compounds from sediments; however, this method resulted in poorer recoveries of non-polar compounds compared with Soxhlet extraction [38].

The extraction and preparation of sediment and soils using the methods listed above are labor intensive, requiring relatively large amounts of toxic solvents like toluene and dichloromethane (DCM). The trend in the past decade has been to develop and use environmentally friendly green analytical methods [39–43]. Green analytical methods: (1) reduce or eliminate the use of solvents, reagents, and preservatives, (2) minimize energy consumption, (3) minimize or simplify management of analytical waste, and (4) result in a safer environment for the analyst [40]. QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) is a rapid multi-residue green analytical method first reported in 2003 for analyzing pesticides in fruits and vegetables [43] that addresses all of these issues. Briefly, QuEChERS utilizes solid–liquid extraction in acetonitrile followed by the partitioning of the acetonitrile from the solid and aqueous layers by salting out. An aliquot of the separated acetonitrile layer is then cleaned with dispersive sorbents and analyzed. A sample prepared for pesticide analysis by QuEChERS can take as little as 30 min [43]. QuEChERS can significantly reduce the use of solvent and sample preparation steps [41] and is now considered a user-friendly green alternative to traditional liquid/solid extraction and solid phase clean-up methods [42]. Since its introduction, the use of QuEChERS has been extended to other analytes and matrices including environmental applications such as polycyclic aromatic hydrocarbons analysis in seafood [44–46] and air [47], as well as pesticides, volatile organics, and pharmaceutical products in soils [48–50].

The objective of this study was to develop a PCDD/PCDF screening method for contaminated site applications where the number of samples that can be analyzed can be increased without significant increases in detection limits. By combining a modified QuEChERS extraction with carbon reversed-

phase solid phase extraction (SPE) cleanup and adopting quality objectives appropriate for contaminated sites, analytical capacity is greatly improved and the use of toxic solvents like DCM and toluene has been reduced by 96 and 97 %, respectively. In this paper, the TEQ values obtained by the classical method are compared with the modified QuEChERS method for 32 sediment samples from contaminated sites in Ontario, Canada, as well as standard reference and certified reference sediments. The modified QuEChERS method is evaluated in terms of precision and method detection limits (MDLs). The two methods are also compared in terms of instrumental chromatography, recovery of  $^{13}\text{C}$ -labelled surrogates, sample preparation time, workload capacity, and sustainability factors. A comparison of the proposed method to the classical method and bioassay/immunoassays is summarized in Table 1.

## Materials and methods

### Consumables, sediments, and standards

#### Suppliers

Acetonitrile (ACN), toluene, DCM, hexanes (95 % *n*-hexane), nonane, concentrated sulfuric acid (reagent grade), sodium dodecyl sulfate, and magnesium sulfate were purchased from Caledon (Georgetown, ON, Canada). Ottawa sand, sodium hydroxide, silver nitrate, acetone, carbon tetrachloride ( $\text{CCl}_4$ ), water, and disposable 15 mL borosilicate test tubes were from Fisher Scientific (Ottawa, ON, Canada). Polypropylene 15 mL reservoirs, empty 3 mL cartridges, and matching glass frits were purchased from Agilent Technologies (Santa Clara, CA, USA). Mesh silica (70–230) was purchased from Rose Scientific Ltd. (Edmonton, AB, Canada), and sodium acetate was from Anachemia (Lachine, QC, Canada). Carbon was PX-21 from Amoco (Chicago, IL, USA). All solvents were purchased as distilled in glass or HPLC grade.

Sediment samples were collected and provided by the Environmental Monitoring and Reporting Branch of the Ontario Ministry of the Environment and Climate Change (Toronto, ON, Canada) from two contaminated sites in

Ontario. Reference sediment, NIST-1944 (New York / New Jersey waterway sediment) was obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA). Certified reference sediment, WMS-01 (lake sediment) and quantitation and injection standards were purchased from Wellington Laboratories (Guelph, ON, Canada). Concentrations of the standards used are provided in Table S2 of the Electronic Supplementary material (ESM) section. The reference sediments were obtained and analyzed dry.

Sulfuric acid,  $\text{CCl}_4$ , sodium hydroxide, silver nitrate, and sodium sulfate were exclusive to the classical method while magnesium sulfate, sodium acetate, and acetonitrile were used only for the modified QuEChERS method.

#### Reagent preparation

Silica and alumina were activated in an oven at 300 °C for at least 24 h prior to use. Carbon-silica packing was prepared by mixing 0.3 g of carbon with 5.7 g of activated silica and activating the mixture in an oven at 300 °C for 65 h to desorb contaminants. The carbon/silica packing was allowed to cool in a desiccator and used within 1 week of preparation. Sodium hydroxide/silica packing was prepared by dissolving 1.3 g of sodium hydroxide in 33 mL of HPLC grade water and mixing with 67 g of activated silica. Sulfuric acid/silica packing was prepared by mixing 25 ml of concentrated sulfuric acid with 56 g of activated silica. Silver nitrate/silica packing was prepared by dissolving 6 g of silver nitrate in 23 mL of HPLC grade water and mixing with 53 g of activated silica. Due to the toxicity of PCDD/PCDFs and the solvents, as well as the corrosive properties of the acid and base, all work was carried out using personal protective equipment and fume hoods when possible.

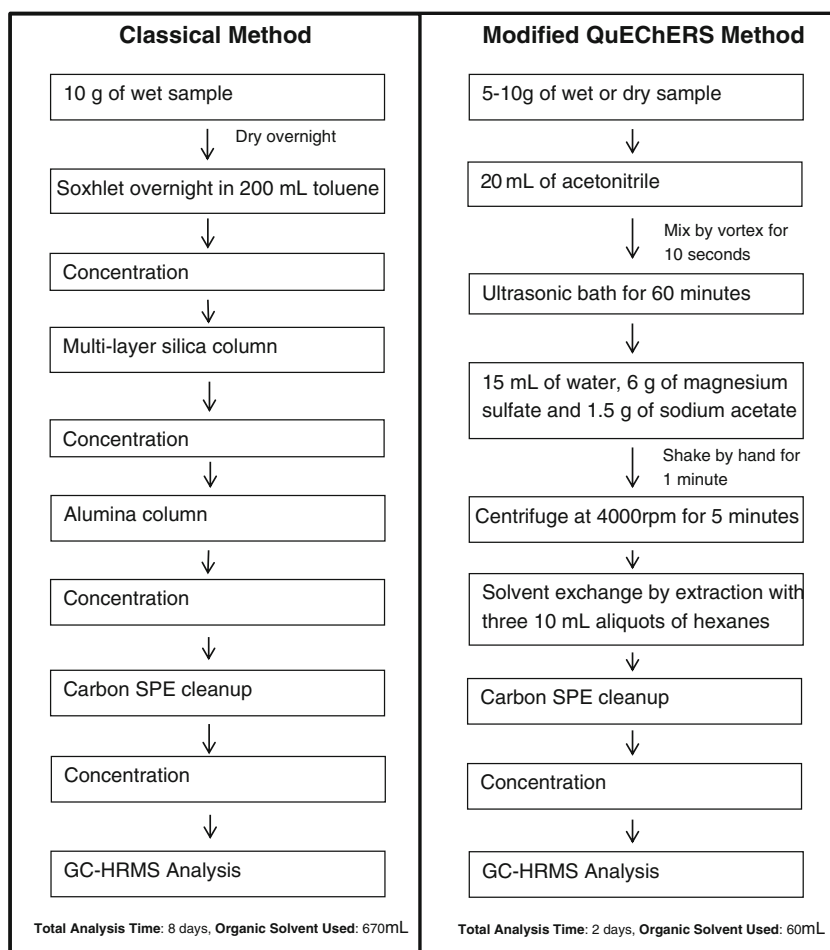
### Sample preparation

#### Classical method

As illustrated in Fig. 1, sediments were dried overnight before being homogenized by grinding. 5–10 g of each sediment sample was placed in a glass thimble and spiked with quantitation standard. After allowing the solvent to evaporate, each

**Table 1** Comparison of proposed method to existing approaches

Protocol	Advantages	Disadvantages
Classical method e.g., EPA 1613	- High sensitivity - High selectivity	- Time consuming - Costly
Bioassay/immunoassay	- Cheaper and faster detection - Reduced sample size	- Reduced precision and accuracy - Poor selectivity
Modified QuEChERS	- Fast sample preparation - Greener and safer	- Reduced precision and accuracy - Reduced sensitivity



**Fig. 1** Comparison of the classical method with the modified QuEChERS method adapted for PCDD/PCDF screening in sediment samples. Created using Microsoft Word 2010. The figure compares the flowcharts of the two methods. The classical method steps are as follows. Ten grams of wet sample is dried overnight and Soxhlet extracted overnight in 200 ml of toluene. The clean-up steps are concentration, multi-layer silica column, concentration, alumina column, concentration, carbon SPE cleanup, and concentration. The final step is GC/HRMS analysis. The total analysis time is 8 days and 670 ml of organic solvent is used. The modified QuEChERS method steps are as

follows. Five to 10 g of wet or dry sample is vortex mixed with 20 ml of acetonitrile for 10 s. The sample is placed in an ultrasonic bath for 1 h. Fifteen milliliters of water, 6 g of magnesium sulfate, and one and a half grams of sodium acetate are added. The sample is mixed by hand for 1 min and centrifuged at 4000 revolutions per minute for 5 min. The acetonitrile extract is solvent exchanged to hexanes using liquid-liquid extraction and cleaned by carbon SPE and concentrated. The final step is GC/HRMS analysis. The total analysis time is 2 days and 60 ml of organic solvent is used

thimble was placed in a Soxhlet apparatus and extracted with 200 mL of toluene overnight at six cycles per hour. The extracts were evaporated to dryness and reconstituted with 5 mL of hexanes.

Multi-layer columns were prepared by adding to a glass column in the following order 1.5 g of 10 % silver nitrate/silica, 1 g of activated silica, 2 g of sodium hydroxide/silica packing, 1 g of activated silica, 4 g of sulfuric acid/silica packing, 2 g of activated silica, and 2 g of anhydrous sodium sulfate. The extracts were added to the multi-layer columns and eluted with 100 mL of hexanes. The eluent was concentrated to 1 mL and added to an alumina column. One hundred milliliters of hexanes and 20 mL of 10 %  $\text{CCl}_4$  in hexanes were passed through the column and discarded. Fifty milliliters of DCM was passed through the column and collected.

The eluent was evaporated, reconstituted with 1 mL of hexanes, and passed through a carbon column with 40 mL of 25 % DCM in hexanes. The carbon column was inverted, and 160 mL of toluene was passed through the column. The toluene extracts were evaporated to dryness under a stream of nitrogen and transferred to GC vials for instrumental analysis.

#### Modified QuEChERS method

Figure 1 outlines the modified QuEChERS method. Sediments were not dried prior to extraction; however, an additional amount of each sample was weighed in an aluminum boat and dried overnight at 150 °C to determine moisture content. The moisture content was between 30 and 70 % in the samples analyzed and was used to report the results on a dry

basis. All sediments were homogenized by manual mixing before being weighed.

Approximately 5–10 g of each sediment sample was weighed into a centrifuge tube and spiked with quantitation standard. After allowing the solvent to evaporate, the samples were vortex-mixed with 20 mL of ACN for 10 s and placed in a Branson 5510 ultrasonic bath (Branson Ultrasonics Corp., Danbury, CT, USA) operating at 40 kHz for 60 min. A ceramic homogenizer, 15 mL of HPLC grade water, and QuEChERS salts (6 g of magnesium sulfate and 1.5 g of sodium acetate) were added to each tube. The tubes were shaken for 1 min by hand and centrifuged for 5 min at 4000 rpm. The ACN layer was pipetted into a 60-mL vial containing 15 mL of water. The analytes were solvent exchanged to hexanes by liquid-liquid extraction of the ACN-water mixture with three 10 mL aliquots of hexanes.

Carbon columns were prepared by fixing 0.25 g of the activated carbon-silica packing in polypropylene cartridges between glass frits. Each sample extract was transferred to a 15-mL reservoir attached to a carbon column and allowed to elute. A volume of 10 mL of 25 % DCM in hexanes was passed through the column. The column was inverted and attached to a new reservoir. Ten milliliters of toluene was passed through the column and collected. The toluene extracts were evaporated to dryness under a stream of nitrogen and transferred to GC vials for instrumental analysis.

### Instrumental analysis

The extracts were reconstituted with 20  $\mu$ L of injection standard containing 2000 pg each of  $^{13}\text{C}$  1,2,3,4-TCDD and  $^{13}\text{C}$  1,2,3,7,8,9-HxCDD in order to determine the recoveries of the quantitation standard compounds. The samples were analyzed using an HP7890 GC coupled to a Micromass Autospec HRMS detector (Waters Corporation, Milford, MA, USA) fitted with a DB-5 column (5 % diphenyl, 95 % dimethyl polysiloxane, 40 m  $\times$  0.18 mm, 0.18  $\mu$ m) (Agilent Technologies, Santa Clara, CA, USA). The HRMS was tuned to a resolution greater than 10,000 (10 % peak height definition) utilizing a 40-eV electron ionization (EI) in selected ion monitoring (SIM) mode. The GC-HRMS was calibrated every 3 months using a five-point calibration set. The HRMS was tuned and mass calibrated daily using perfluorokerosene as a reference compound. Seven PCDDs and ten PCDFs were quantified. Isotopic dilution was used to calculate the concentrations of all but OCDF and 1,2,3,7,8,9-HxCDD which have no labelled analogues in the quantitation standard used. Labelled OCDD was used as the quantitation standard for OCDF, and the average of 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD was used to quantify 1,2,3,7,8,9-HxCDD. The individual concentrations of each native congener were converted to TEQs using the TEFS provided in Table S5 in the ESM. Where a peak was not detected, the instrumental level

of detection was used. Information regarding the GC temperature program, calibration standards, as well as the ions monitored is provided in the ESM section.

Chlorinated diphenyl ethers (DPEs) fragment and rearrange in the ion source of the HRMS to form PCDFs. While the bulk of the DPEs in the samples is removed by the carbon cartridge, a small but important amount may still be present in the final extract. The presence of chlorinated DPEs was assessed by monitoring the appropriate masses.

### Quality control

Procedural blanks composed of Ottawa sand were used to check for inadvertent contamination. In addition, precision and recovery (PAR) samples were included composed of Ottawa sand spiked with native PCDD/PCDFs. Both blanks and PAR samples were fortified with the same amount of quantitation standard as the test samples. Due to the absence of organic carbon in the sand, this matrix represents the optimal results.

Instrumental performance was verified in terms of sensitivity, chromatography, stability, and accuracy. Sensitivity was demonstrated before each analytical run by ensuring that 0.5 pg of 2,3,7,8-TCDD injected on-column resulted in a signal to noise ratio of 3:1 or greater. To check the chromatographic resolution, a column performance mixture (1,2,3,4-, 1,2,3,7-, 1,2,3,8-, 2,3,7,8-, and 1,2,3,9-TCDD isomers) was analyzed before and after each run to confirm that the 2,3,7,8-TCDD peak is separated from its closest neighbors by a valley of no more than 30 %. To demonstrate stability and accuracy, a mid-level calibration standard was analyzed before and after each run. The standard was quantified to confirm that the observed concentrations were within 20 % of the expected values for the native PCDD/PCDFs and 30 % for the  $^{13}\text{C}$ -labelled surrogates.

### Statistical analysis

To determine the MDLs, an anticipated standard deviation was estimated from analyzed replicates of reference material WMS-01. Eight replicates of Ottawa sand were then spiked at a level not exceeding 15 times the anticipated standard deviation and analyzed. The MDLs were assumed to be independent of each other and governed by Gaussian distribution. Therefore, the congener-specific values were converted to TEQs and added in quadrature to generate a combined MDL.

## Results and discussion

### Method development

The use of ACN as the extraction solvent eliminates the need for sample drying without necessitating the Dean-Stark

apparatus and reduces the total preparation time by a full day compared with methods that incorporate sample drying. A small amount of sample must be weighed out and dried so that the results can be reported on a dry basis; however, this second aliquot can be dried and weighed by the time the sample has undergone instrumental analysis. Extracting sediment without drying or sieving also increases the uncertainty associated with subsampling due to the constant settling of heavier particles and the presence of debris such as shells, stones, and vegetation in some samples. This uncertainty also affects the aliquot used for moisture correction which in turn affects the quantitation calculations. In addition, the classical method analyzes consistent amounts of dry sample, whereas the variation in water content in the wet sediments, which ranged from 30 to 70 % moisture in the real-world samples analyzed, leads to a large variation in sample size on a dry basis.

Ultrasonication was incorporated to overcome the strong bonding of the PCDD/PCDFs to the sediment matrix. Increasing extraction time in an ultrasonic bath by increments of 15 min revealed that the extraction efficiency maximizes after approximately an hour (see ESM Fig. S1). Ultrasonic probes not only require less time but also require cleaning between samples. An ultrasonication bath allows for more samples to be extracted simultaneously and decreases the possibility of cross contamination between samples.

It was also necessary to modify the QuEChERS cleanup approach. PCDD/PCDFs are normally found in concentrations of picograms or femtograms per gram of sample together with interferences at concentrations which are orders of magnitude higher. Analyzing small aliquots of the separated ACN layer as per the original QuEChERS method resulted in a TEQ difference of 150 % to the classical method (see ESM Fig. S2) and suggested that the entire ACN extract must be carried through the cleanup stage to achieve acceptable amounts for detection. Also, the QuEChERS clean-up sorbents (400 mg of primary secondary amine (PSA), 400 mg of end-capped C18, 1200 mg MgSO<sub>4</sub>) originally intended for fruit and vegetable samples, resulted in sample extracts which contained unknown precipitates and therefore could not be injected onto the GC column and analyzed.

Carbon cleanup proved to be selective enough for qualification and quantification of PCDD/PCDFs (see ESM Table S7) but necessitated a solvent exchange to hexanes because it was found that the ACN stripped the carbon from the silica dispersant altering the elution profile of the analytes. Finding a carbon sorbent with a comparable activity to silica-dispersed PX-21 carbon that works in ACN could eliminate the need for solvent exchange and decrease preparation time further. The solvent exchange to hexanes was carried out by liquid-liquid extraction rather than evaporating the ACN and reconstituting with hexanes. Liquid-liquid extraction was found to be much quicker and provides an additional cleanup based on polarity (see ESM Fig. S4). It was found that adding

water to the ACN extract to create a roughly 2:1 ACN/water by volume mixture prior to the liquid-liquid extraction shifted the equilibrium concentration of the analytes to the hexanes layer and maximized recovery (see ESM Fig. S5).

### Comparison of TEQ results

The average percent difference between the TEQ of certified reference sediment WMS-01 obtained with QuEChERS extraction and the published TEQ was 4.1 % ( $N=10$ ). The average percent difference between the TEQ of standard reference sediment NIST-1944 obtained with QuEChERS extraction and the published TEQ was 6.7 % ( $N=10$ ). Table 2 lists the TEQs determined by the two methods for 32 real-world samples. The percent difference between the TEQ values determined by the modified QuEChERS method and those obtained by the classical method ranged from 1.0 to 38 % with an average absolute percent difference of 16 %. The small difference in TEQs obtained for the reference sediments compared to the real-world samples illustrates the added uncertainty inherent to subsampling wet sediment. Table 2 also highlights the variability in concentrations at contaminated sites. Results ranged from below the method's detection limit of 9.3 pg TEQ/g to as high as 10,000 pg TEQ/g. A compound-specific comparison of the two methods for a real-world sample is provided in ESM Fig. S3.

Figure 2 plots the TEQs of 32 real-world sediment samples determined by the modified QuEChERS method against the concentrations determined by the classical method. Linear regression analysis shows that the two sets of data are very closely correlated with an  $R^2$  value of 0.9987.

While the bias caused by the lower extraction efficiency of the modified QuEChERS method creates a concern regarding false negatives at low levels, it does not affect the ability of this method to quickly find contaminated areas and profile large contaminated sites.

### Precision

Precision was evaluated in terms of %RSD for replicates of fortified Ottawa sand. PAR samples analyzed with different sets were used to evaluate the between-run precision and are reported in Table 3. The %RSD values are comparable for the classical and the modified QuEChERS methods with average %RSD values of 8.8 and 8.6 %, respectively. The compound 1,2,3,7,8,9-HxCDD which is not quantified by isotope dilution exhibits a particularly high %RSD for the modified QuEChERS method. Table 3 also lists the within-run precision for replicates of Ottawa sand that were prepared and analyzed as one set. Compared to the classical method with an average %RSD of 2.4 %, the modified QuEChERS method is less precise with an average %RSD of 6.4 %.

Table 4 lists the results of eight replicates of a real-world sample prepared and analyzed together using the modified QuEChERS method. The real-world sample with an average %RSD of 9.3 % is the least precise in terms of %RSD due to the variability in subsampling wet sediments. The decrease in within-run precision does not affect the proposed method's fitness for purpose and is an acceptable tradeoff for the increase in analytical capacity.

### Method detection limits

Table 5 lists the congener-specific MDLs determined for the modified QuEChERS method at a confidence level

**Table 2** Comparison of the TEQ values and absolute percent deviation of sediment samples extracted by the classical method and the modified QuEChERS method

Sample	Classical method (pg/g dry)	Modified QuEChERS (pg/g dry)	Absolute deviation (%)
1	173	141	21
2	212	214	1.0
3	300	250	19
4	375	343	8.8
5	228	195	16
6	190	171	11
7	179	144	22
8	9890	7960	22
9	276	233	17
10	52.3	50.6	3.3
11	2930	2350	22
12	407	304	29
13	67.7	66.0	2.5
14	489	366	29
15	167	129	26
16	936	856	9.0
17	20.8	18.1	14
18	155	132	16
19	251	337	29
20	816	666	20
21	110	98.1	12
22	186	157	18
23	13.5	12.8	5.1
24	17.6	14.1	23
25	16.4	14.9	10
26	40.3	59.4	38
27	16.1	18.5	13
28	80.1	81.2	1.4
29	62.8	63.8	1.6
30	187	270	36
31	740	695	6.3
32	1730	1540	12

of 99 %. The individual MDLs were on average three and a half times larger than the classical method. This difference in sensitivity is likely due to the decreased extraction efficiency of the modified QuEChERS method.

A combined MDL of 9.3 pg TEQ/g was calculated for the modified QuEChERS method. Samples with TEQs below the combined MDL were excluded from the comparison of results. This MDL is sufficient for use in profiling and monitoring contaminated sites as demonstrated by the variability and levels seen in Table 2.

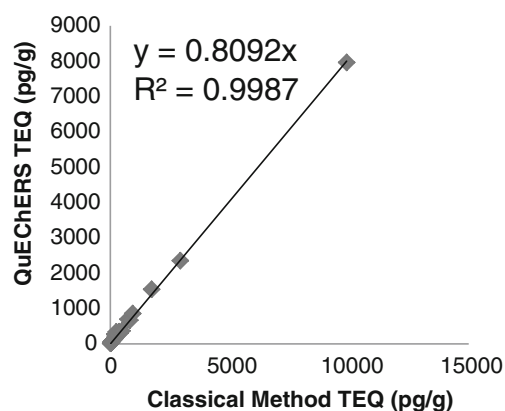
Uncertainty has been estimated for this method based on these MDLs as well as 16 duplicates of a real-world sample and can be found in ESM Table S6.

### Instrumental chromatography

Figure 3a, b shows the reconstituted chromatographs of a real-world sample prepared by both methods. Little difference can be seen between the sample extracted and cleaned by the classical method (Fig. 3a) and the modified QuEChERS method (Fig. 3b) in terms of background noise and peak shape. The instrument lock mass was monitored and examined, confirming that ion suppression was not taking place. This suggests that the carbon SPE cleanup is sufficient for this application.

### Recoveries of labelled PCDD/PCDFs

Figure 4 shows the average percent recoveries of  $^{13}\text{C}$  labelled quantitation surrogates added to eight sediment samples prior to being extracted by the two methods. The average recovery ranged from 61 to 94 % in samples prepared by the classical



**Fig. 2** Linear regression as a measure of correlation of TEQs determined by the modified QuEChERS method with TEQs determined by the classical method for paired samples. Created using Microsoft Excel 2010. A graph of the TEQs calculated for 32 real-world samples by the modified QuEChERS method plotted against the TEQs for the same samples determined by the classical method. A line of best fit is shown with a slope of 0.8092 and an  $R^2$  value of 0.9987

method. The same samples prepared by the modified QuEChERS method had an average recovery range of 26 to 65 %. These ranges are typical of the recoveries obtained by the two methods. We believe that the difference in recoveries can be explained by the lower extraction efficiency of the modified QuEChERS method. Soxhlet extraction of sediment that had been extracted by QuEChERS revealed that as much as 10 % of the analytes remained in the sediment. It was also

found that approximately 5 % also remained on the carbon column. Congeners with a higher level of chlorination exhibited poorer recoveries by the modified QuEChERS method and may fall short of the criteria set by some international regulations. The proposed method is therefore unsuitable for quantitation of the highly chlorinated PCDD/PCDF congeners. However, these congeners have low TEFs and their effect on the final results is minimal.

**Table 3** Between-run and within-run precision based on replicates of spiked Ottawa sand prepared by the classical method ( $n = 11$ ) and the modified QuEChERS method ( $n = 8$ )

Congener	Between-run				Within-run			
	Target (pg)	Mean (pg)	% RSD	% Recovery	Target (pg)	Mean (pg)	% RSD	% Recovery
Classical method								
2378-TCDF	200	200	<i>10.6</i>	101	8	7.8	2.55	100
12378-PCDF	1000	1050	7.2	105	39	40	2.03	102
23478-PCDF	1000	1020	7.5	102	39	38	2.16	97
123478-HxCDF	1000	1070	7.1	107	39	39	2.77	101
123678-HxCDF	1000	1070	7.2	107	39	39	1.90	100
234678-HxCDF	1000	1040	6.9	104	39	38	2.84	98
123789-HxCDF	1000	1050	7.5	105	39	38	2.63	99
1234678-HpCDF	1000	1080	18.6	108	39	37	2.74	95
1234789-HpCDF	1000	1080	6.9	108	39	39	2.29	101
OCDF	2000	2040	14.5	102	78	77	2.54	99
2378-TCDD	200	210	9.1	105	8	7.7	2.54	99
12378-PCDD	1000	1050	7.3	105	39	39	1.98	100
123478-HxCDD	1000	1030	8.5	103	39	38	2.62	98
123678-HxCDD	1000	1080	7.3	108	39	40	3.31	102
123789-HxCDD	1000	1110	10.9	111	39	40	2.50	104
1234678-HpCDD	1000	1050	6.5	105	39	38	1.82	98
OCDD	2000	2040	5.8	103	78	75	2.42	97
Modified QuEChERS method								
2378-TCDF	400	368	7.4	92	10	10.1	12	101
12378-PCDF	2000	2060	7.6	103	50	48.7	12	97
23478-PCDF	2000	1960	7.4	98	50	43.8	5.5	88
123478-HxCDF	2000	2070	7.8	104	50	47.1	5.5	94
123678-HxCDF	2000	2100	7.1	105	50	47.3	4.9	95
234678-HxCDF	2000	2050	6.4	102	50	45.3	4.3	91
123789-HxCDF	2000	2030	5.5	102	50	49.6	4.8	99
1234678-HpCDF	2000	2000	8.0	100	50	45.5	2.8	91
1234789-HpCDF	2000	2120	8.0	106	50	46.0	4.1	92
OCDF	4000	4110	7.9	103	100	93.5	3.4	93
2378-TCDD	400	394	10.7	98	10	10.0	11	100
12378-PCDD	2000	2080	8.4	104	50	51.0	5.3	102
123478-HxCDD	2000	2100	6.7	105	50	51.9	7.1	104
123678-HxCDD	2000	2040	7.7	102	50	46.9	5.7	94
123789-HxCDD	2000	2040	25.2	102	50	51.3	11	103
1234678-HpCDD	2000	2000	9.0	100	50	43.4	4.3	87
OCDD	4000	3980	6.1	100	100	89.6	4.3	90

The italicized %RSDs are used as measures of precision



**Table 4** Within-run precision based on eight replicates of real-world sediment prepared by the modified QuEChERS method ( $n = 8$ )

Congener	Mean (pg)	%RSD
2378-TCDF	20	<i>13</i>
12378-PCDF	14	<i>10</i>
23478-PCDF	34	<i>5.3</i>
123478-HxCDF	59	<i>4.0</i>
123678-HxCDF	58	<i>5.7</i>
234678-HxCDF	46	<i>3.7</i>
123789-HxCDF	2.1	<i>23</i>
1234678-HpCDF	73	<i>4.7</i>
1234789-HpCDF	14	<i>7.2</i>
OCDF	21	<i>11</i>
2378-TCDD	7.7	<i>17</i>
12378-PCDD	60	<i>4.0</i>
123478-HxCDD	28	<i>4.7</i>
123678-HxCDD	154	<i>5.1</i>
123789-HxCDD	95	<i>8.3</i>
1234678-HpCDD	310	<i>8.2</i>
OCDD	360	<i>23</i>

The italicized %RSDs are used as measures of precision

**Sample preparation time and capacity**

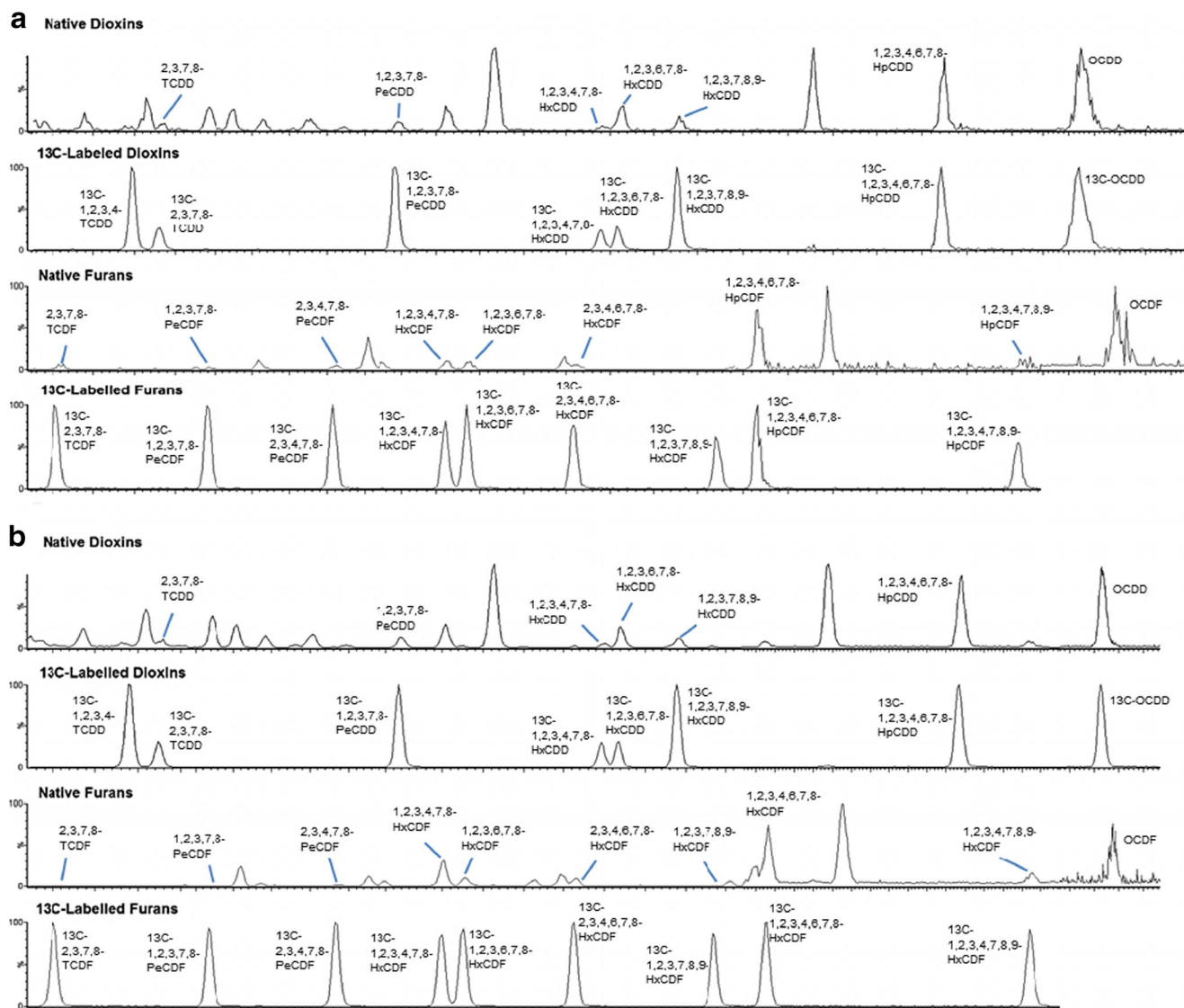
In the event of an environmental emergency, ten samples received in the morning could be prepared by one analyst using the modified QuEChERS method while another sets up and validates the GC-HRMS. In less than 8 h, the QuEChERS extracts would be ready to analyze. After approximately 12 h of instrumental analysis and 2 h of data reading, the results could be reported. Knowing the PCDD/PCDF levels within 24 h would be beneficial to decision-makers, even with an average absolute difference of  $16 \pm 10$  % compared to the best available results and further time savings may be possible through automation and faster instrumental analysis.

Assuming the analysis is performed by one sample preparation technician and one GC-HRMS analyst, the typical turn-around time for PCDD/PCDF analysis in sediments by the classical method is 10 samples in 8 to 10 days. Because no specialized glassware is required for the modified QuEChERS sample preparation, it can easily be scaled up so that the limiting factor is the HRMS capacity of about 30 analyses per day, including daily instrument validation. This increase in workload capacity more than compensates for the increased detection limits and decreased precision of the modified QuEChERS method for applications such as contaminated site profiling and monitoring.

Contamination monitoring is also an example of the complementary nature of two methods. Decision-makers can submit a greater number of samples and obtain results in a fraction of the time by the modified QuEChERS approach, thereby saving money and quickly profiling contaminated areas. If needed for

**Table 5** Comparison of congener-specific method detection limits at a 99 % confidence level for the modified QuEChERS method and classical method based on replicates of fortified Ottawa Sand

PCDD/ PCDF	Modified QuEChERS method ( $n = 8$ )				Classical method ( $n = 11$ )			
	Target value (pg/g)	Average concentration (pg/g)	Within-run standard deviation (pg/g)	Method detection limit (pg/g)	Target value (pg/g)	Average concentration (pg/g)	Within-run standard deviation (pg/g)	Method detection limit (pg/g)
2378-TCDF	10	10.1	1.18	3.54	8	7.8	0.2	0.55
12378-PeCDF	50	48.7	5.67	17.0	39	40	0.80	2.22
23478-PeCDF	50	43.8	2.40	7.20	39	38	0.81	2.25
123478-HxCDF	50	47.1	2.58	7.73	39	39	1.09	3.01
123678-HxCDF	50	47.3	2.32	6.97	39	39	0.74	2.04
234678-HxCDF	50	45.3	1.94	5.81	39	38	1.08	2.97
123789-HxCDF	50	49.6	2.36	7.07	39	38	1.01	2.78
1234678-HpCDF	50	45.5	1.26	3.77	39	37	1.01	2.80
1234789-HpCDF	50	46.0	1.90	5.69	39	39	0.89	2.47
OCDF	100	93.5	3.20	9.58	78	77	1.95	5.40
2378-TCDD	10	10.0	1.07	3.22	8	7.7	0.20	0.54
12378-PeCDD	50	51.0	2.69	8.07	39	39	0.77	2.12
123478-HxCDD	50	51.9	3.68	11.1	39	38	0.99	2.75
123678-HxCDD	50	46.9	2.68	8.04	39	40	1.32	3.64
123789-HxCDD	50	51.3	5.62	16.9	39	40	1.01	2.80
1234678-HpCDD	50	43.4	1.86	5.57	39	38	0.69	1.92
OCDD	100	89.6	3.86	11.6	78	75	1.82	5.02



**Fig. 3** Reconstructed chromatography of real-world sample extracted by classical method (a) and modified QuEChERS method (b). Created using Microsoft PowerPoint 2010. A comparison of the reconstructed

chromatographs of a real-world sample extracted by the two methods. Peaks for all 17 analyzed dioxins and furans are identified. Both chromatographs are similar in terms of background noise and peak shape

verification or litigation, a smaller number of samples could then be submitted for analysis by the classical method and serve as an ongoing validation of the modified QuEChERS method.

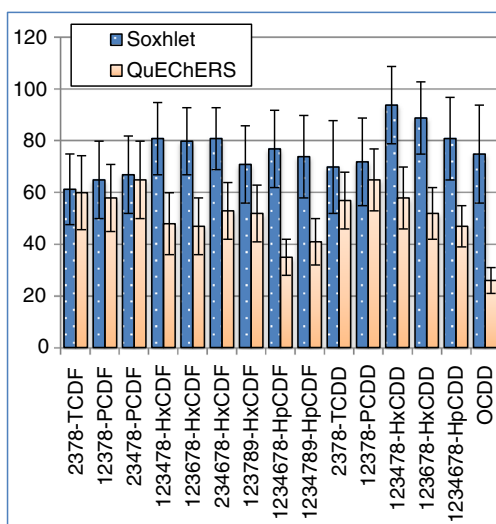
### Sustainability factors

The modified QuEChERS method has several advantages over the classical method for PCDD/PCDF analysis in sediment in terms of “green” chemistry as well as health and safety. The classical method involves extraction with a boiling solvent, whereas in the modified QuEChERS method, high temperatures are limited to the cavitation sites in the ultrasonic bath during extraction. The classical method is also typically performed with toluene while QuEChERS uses a less toxic solvent, acetonitrile, as an extraction solvent. In addition, eliminating the multi-stage silica cleanup used by the classical method eliminates the need for

concentrated sulfuric acid and sodium hydroxide used in the column packing. However, the biggest advantage of the QuEChERS approach over the classical method in terms of eco-friendliness is due to the reduction in solvent use [51] as the modified QuEChERS method uses less than 10 % of the organic solvents by volume. The overall reduction in solvent volume and the use of a less toxic extraction solvent at room temperature make the modified QuEChERS approach a greener and safer alternative to the classical method for PCDD/PCDF analysis in sediments.

### Conclusions

A sample preparation technique using ultrasonication-aided QuEChERS extraction with a carbon SPE cleanup was developed for PCDD/PCDF screening in sediments. This resulted



**Fig. 4** Average percent recoveries of <sup>13</sup>C-labelled quantitation surrogates in sediment samples extracted by the classical method and the modified QuEChERS Method ( $n = 8$ ). Created using Microsoft Excel 2010. A bar graph comparing the congener-specific recoveries of carbon 13 labelled quantitation surrogates in sediment samples extracted by the classical method and the modified QuEChERS method. For every compound, the classical method achieves a higher recovery of quantitation standard. The classical method recoveries range from approximately 60 to 95 % while the modified QuEChERS method recoveries range from approximately 25 to 65 %

in a method that had higher detection limits but was considerably faster than the classical method as well as being cheaper, safer, and “greener.” While the TEQ values determined by the modified QuEChERS method differed by 16 % on average from the classical method in real-world samples, the sample preparation time was reduced from 10 samples in 3 to 4 days to as many as 30 samples per day. This reduction in time will assist authorities in responding to environmental emergencies and the increase in capacity will ease laboratory bottlenecks and support contaminated site monitoring and remediation projects.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interests.

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