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Rapid screening of dioxin-contaminated soil by accelerated solvent extraction/purification followed by immunochemical detection

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Abstract Since soils at industrial sites might be heavily contaminated with polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), there is a need for large-scale soil pollution surveys and, thus, for cost-efficient, high-throughput dioxin analyses. However, trace analysis of dioxins in complex matrices requires exhaustive extraction, extensive cleanup, and very sensitive detection methods. Traditionally, this has involved the use of Soxhlet extraction and multistep column cleanup, followed by gas chromatography—high-resolution mass spectrometry (GC/HRMS), but bioanalytical techniques may allow much more rapid, cost-effective screening. The study presented here explores the possibility of replacing the conventional method with a novel approach based on simultaneous accelerated solvent extraction (ASE) and purification, followed by an enzyme-linked immunosorbent assay (ELISA). Both the traditional and the novel cleanup and detection approaches were applied to contaminated soil samples, and the results were compared. ELISA and GC/HRMS results for Soxhlet-extracted samples were linearly correlated, although the ELISA method slightly underestimated the dioxin levels. To avoid an unacceptable rate of false-negative results, the use of a safety factor is recommended. It was also noted that the relative abundance of the PCDDs/PCDFs, evaluated by principal component analysis, had an impact on the ELISA performance. To minimize this effect, the results may be corrected for differences between the ELISA cross-reactivities and the corresponding toxic equivalency factor values. Finally, the GC/HRMS and ELISA results obtained following the two sample preparation methods agreed well; and the ELISA and GC/HRMS results for ASE extracts were strongly correlated (correlation coefficient, 0.90). Hence, the ASE procedure combined with ELISA analysis appears to be an efficient approach for high-throughput screening of PCDD-/PCDF-contaminated soil samples.

Keywords Immunoassay · ELISA · Soil · Dioxin · Accelerated solvent extraction

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Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) comprise a group of ubiquitous environmental contaminants (also known as dioxins) which pose a threat to the well-being of humans and other organisms owing to their resistance to biological and chemical degradation, their potential to bioaccumulate, and toxic properties (causing, for instance, reproductive disruption and cancer in wildlife as well as adverse human health effects [1, 2]). Dioxins are unintentionally formed during combustion processes, chlorine bleaching of pulp, and the synthesis of chlorine gas and various organochlorine chemicals, such as pentachlorophenol [3–7]. Consequently, soil at industrial sites is often heavily contaminated with dioxins and there is a concern that these compounds will eventually appear in terrestrial organisms such as frogs and earthworms, and thus be introduced into the food web [8, 9].

Trace analysis of dioxins in complex matrices requires exhaustive extraction, extensive cleanup, and very sensitive detection methods. European and American standard methods for dioxin analysis involve conventional Soxhlet extraction, multistep column cleanup, and gas chromatography coupled with high-resolution mass spectrometry (GC/HRMS) [10, 11]. This analytical approach is time, labor, and resource intensive, requiring substantial invest-

ments in sample preparation, equipment, data evaluation, and quality control. Given the need for large-scale soil pollution surveys, there is an urgent requirement for alternative cost-efficient, high-throughput dioxin analysis methods. Possible options include several bioanalytical screening methods for both dioxins and related compounds [12]. A common feature of these methods is the use of biological derived components to obtain analyte-specific responses. However, using such methods, we can only determine total dioxin concentrations rather than the complete congener profiles provided by GC/HRMS. Despite this limitation, bioanalytical methods may still be appropriate as screening tools to (1) eliminate negligibly contaminated samples, (2) identify more heavily contaminated samples for further analysis by confirmatory techniques such as GC/HRMS, and (3) rank samples for priority of analysis. Furthermore, bioanalytical methods often allow large numbers of samples to be processed in parallel, using simple protocols, providing rapid, cost-effective screening systems. However, further improvements and validation are necessary to gain acceptance of bioanalytical methods as reliable complements to chemical analysis. The assay protocols also have to be clearly defined and meet widely accepted performance criteria.

Bioanalytical methods can be divided into immunoassays and bioassays. Research on immunochemical methods for dioxin detection has been in progress for many years and there have been several successful attempts to develop antibody-based techniques, as reviewed by Harrison and Eduljee [13]. For instance, a highly sensitive polyclonal antibody-based enzyme-linked immunosorbent assay (ELISA) has been developed using 2,3,7-trichloro-8-methyl-dibenzo-*p*-dioxin (TMDD) as a surrogate standard [14, 15]. This ELISA has been further optimized, validated, and applied to human milk, soil, biota, sediment, and serum samples [16–18]; hence, it may efficiently detect dioxins in diverse soils, differing in the degree of pollution, soil type, organic content, etc. However, sample preparation (extraction and cleanup) is of paramount importance for the end results, irrespective of the detection technique. Unfortunately, this currently involves intensive laboratory work, reducing the potential benefits of the ELISA. Consequently, alternative sample preparation methods are under development, which are considerably faster and consume less solvent [19–23]. For instance, accelerated solvent extraction (ASE) [24], a procedure based on extraction at elevated temperature and pressure (also known as pressurized liquid extraction), has been used for PCDD/PCDF extraction from contaminated soil, yielding similar results to Soxhlet extraction [25]. Furthermore, ASE may be used with a fat retainer in the ASE cell to selectively extract polychlorinated biphenyls (PCBs) from food, feed, and biota samples [26–29]. In order to further streamline the sample preparation, new assemblies were developed that are compatible with commercially available ASE equipment [29]. The assemblies were packed with an activated carbon/Celite mixture together with the sample, facilitating simultaneous extraction and in-cell fractionation. Nording et al. [30] used a similar setup for successful determinations of the dioxin contents of fish oil and fish meal with a cell-based bioassay.

To our knowledge, simultaneous ASE-based extraction and purification combined with immunodetection has not yet been used for soil samples, but this approach is likely to significantly simplify the analysis of dioxins in soil. In the study reported here, we explored the possibility of using ASE and ELISA as a replacement for the more tedious, conventional method involving Soxhlet and multicolumn cleanup followed by GC/HRMS. This was done by comparing estimates of the dioxin contents of various soil samples, obtained using the immunoassay and GC/HRMS, that had been extracted and purified using both the novel and the conventional approaches. We also elucidated variations in PCDD/PCDF congener profiles by principal component analysis (PCA), thereby assessing the effects of PCDD/PCDF profile in the various soils on the immunoassay performance. The results indicate that the ASE (with in-cell carbon fractionation) and immunoassay approach is a promising method for rapid dioxin screening of diverse soil samples.

Experimental

Chemicals

The solvents used for extraction and cleanup (acetone, toluene, *n*-hexane, dichloromethane, and methanol) were of glass-distilled grade from Honeywell Burdick & Jackson (Muskegon, MI, USA) with the exception of *n*-heptane (*proanalysi*), which was supplied by Merck (Darmstadt, Germany). Dimethyl sulfoxide (DMSO, analytical grade) was from Scharlau (Barcelona, Spain) and tetradecane (olefin-free, *proanalysi*) from Fluka (Buchs, Switzerland). Silica (Kieselgel 60) and anhydrous sodium sulfate were from Merck and Celite 545 from Fluka. The AX-21 carbon originated from Anderson Development Co. (Adrian, MI, USA), but is not currently commercially available. Bovine serum albumin (BSA), goat antirabbit immunoglobulin G (IgG) conjugated to horseradish peroxidase, 3,3′,5,5′-tetramethylbenzidine (TMB), and Tween 20 used in the ELISA experiments were from Sigma (St. Louis, MO, USA). Phosphate buffered saline (PBS) solution (pH 7.5) was prepared by dissolving 8 g NaCl, 0.2 g KH_2PO_4, 2 g $Na_2HPO_4 \cdot 7\ H_2O$, and 0.2 g KCl/l deionized water, and PBST was prepared by adding 0.05% Tween 20 to the PBS. The coating buffer (pH 9.6) was 1.6 g Na_2CO_3 and 2.9 g $NaHCO_3$/1 deionized water. The substrate solution was 400 μl of 0.6% TMB in DMSO and 100 μl of 1% H_2O_2 in 25 ml of 100 mM citrate/acetate buffer (pH 5.5). High-binding 96-well microtiter plates were from Nunc (Roskilde, Denmark). Development of the coating antigen (III-BSA) and the antibody (7598) have been described elsewhere [15, 17]. Synthesis of the surrogate standard, TMDD, used in the ELISA has been previously reported [14]. The isotopically labeled standards ([$^{13}C_{12}$]-labeled 1,2,3,4-TCDD, and 1,2,3,4,7,8,9-HpCDF, to assess recovery, and the [$^{13}C_{12}$]-labeled 2,3,7,8-substituted PCDDs/PCDFs, except for

1,2,3,4,7,8,9-HpCDF, as internal standards) used for GC/HRMS analysis were obtained from Cambridge Isotope Laboratories (Andover, MA, USA). A standard solution containing the 17 native 2,3,7,8-substituted PCDDs/PCDFs supplied by Wellington Laboratories, Ontario, Canada, was used to quantify target analytes.

Samples

Ten soil samples were included in the study: four from small-scale and industrial waste combustion sites in Uruguay (combustion I, II, III, and IV), four from wood treatment sites in Sweden (wood I, II, III, and IV), and two from a Swedish chloralkali site (chlor I and II). An artificial soil (containing 10% peat, 20% kaolin, and 70% sand) with background levels of PCDDs/PCDFs was also included, prepared by Pelagia Miljökonsult (Hörnefors, Sweden) according to guidelines adopted by the Organization for Economic Cooperation and Development. All samples were air-dried at room temperature, passed through a 2-mm sieve, and thoroughly homogenized before extraction. To estimate total organic content, the loss on ignition was determined by heating portions of the samples for 5 h at 550 °C, after heating for at least 12 h at 105 °C to remove water.

Soxhlet extraction

The samples were Soxhlet-extracted with toluene for 15 h and each extract was split into several parts. One was left untreated for ELISA analysis of the crude extract, one was spiked with internal standard (a mixture of [$^{13}C_{12}$]-labeled PCDDs/PCDFs), cleaned up, and analyzed by GC/HRMS, while the other was cleaned up and used for ELISA analysis without adding any isotopically labeled compounds.

Cleanup

Spiked and nonspiked aliquots were cleaned up in accordance with protocols described elsewhere [31], following the EU standard methods [11]. Briefly, we used four consecutive columns (Fig. 1). First, a multilayer silica column packed with 35% KOH–silica (w/w), activated silica, 40% sulfuric acid–silica (w/w), and Na_2SO_4, which was eluted with *n*-hexane. Second, a carbon column containing AX-21/Celite (1/12, w/w), which was eluted with dichloromethane–*n*-hexane (1/1, v/v), after which the column was inverted and eluted with toluene. Third, an alumina column, for the toluene fraction, which was eluted with *n*-hexane and dichloromethane–*n*-hexane (1/1, v/v). Finally, a miniaturized multilayer silica column, for the dichloromethane–*n*-hexane fraction, which was eluted with *n*-hexane. The silica retains polar compounds such as aldehydes and ketones, the sulfuric acid–silica effectively removes oxidizable compounds and polycyclic aromatic hydrocarbons, *inter alia*, and the KOH–silica eliminates base-sensitive compounds [32]. Molecules with a planar configuration bind strongly to active carbon owing to its graphitic structure, so PCDDs/PCDFs have to be eluted after inverting the column. The alumina column has even more powerful retention properties than silica, facilitating PCDD-/PCDF-specific cleanup by retaining these analytes [33, 34]. Fractions were collected after each successive cleanup step (fractions A, B, and C, respectively). Finally, the solvent of spiked extracts was changed to tetradecane, and the solvent of nonspiked extracts to DMSO. Prior to GC/HRMS analysis, [$^{13}C_{12}$]-1,2,3,4-TCDD and [$^{13}C_{12}$]-1,2,3,4,7,8,9-HpCDF were added to the extracts to assess recovery.

Accelerated solvent extraction

Soil samples were also extracted by simultaneous ASE and purification, combining extraction and in-cell carbon fractionation of PCDDs/PCDFs. The ASE was performed

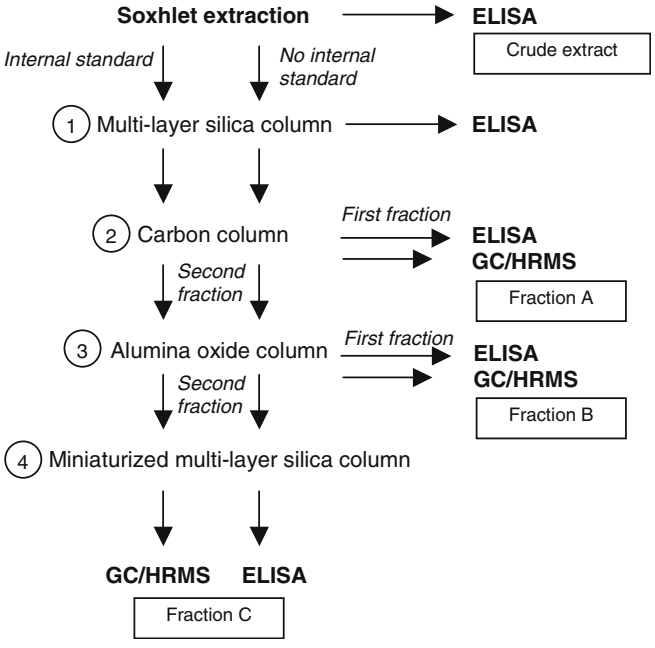

Fig. 1 Cleanup procedure used prior to enzyme-linked immunosorbent assay (*ELISA*) and Gas chromatography–high-resolution mass spectrometry (*GC/HRMS*) analysis. After Soxhlet extraction, the extracts were divided and cleaned up in parallel with or without internal standards. An aliquot of the sample extract was analyzed by ELISA without purification (crude extract). In the first purification step, a multilayer silica column was used. After this step, an aliquot was analyzed by ELISA. Polychlorinated dibenzo-*p*-dioxin (*PCDD*)/polychlorinated dibenzofuran (*PCDF*) fractionation was obtained by the use of activated carbon and the first eluate was analyzed by both ELISA and GC/HRMS (fraction A). The second eluate was applied to an alumina column. After elution of this column, the first fraction was analyzed by both ELISA and GC/HRMS (fraction B), and the second was applied to a final miniaturized multilayer silica column to remove interfering residues. The PCDD/PCDF content of the samples was determined by ELISA and GC/HRMS analyses of fraction C

using an ASE 200 accelerated solvent extractor (Dionex, Sunnyvale, CA, USA), equipped with 33-ml stainless steel extraction cells. Each cell was packed with approximately 2 g AX-21/Celite (1/3, w/w), cellulose filters, Na_2SO_4, and about 1 g of the sample mixed with about 3 g Na_2SO_4 (Fig. 2). The extraction procedure was started by continuously pumping solvent through the cell and simultaneously heating it to a preset temperature. This was followed by a static step at constant temperature and pressure. The cell was then flushed with fresh solvent under low pressure, completing the first extraction cycle. Additional static cycles followed and the sequence was ended by flushing out the residual solvent using nitrogen. In the present method, two cycles with *n*-heptane and one with *n*-heptane–acetone (2.5:1) were performed in sequence before the cells were inverted and back-flushed with four toluene cycles. The temperature, pressure, duration of the static step for each cycle, and flush volume are given in Fig. 2. The toluene fractions, which contained the PCDDs/PCDFs, were pooled and evaporated to a volume of approximately 1 ml. The extracts were split into two parts. Internal standards were added to one aliquot, while the other was left unspiked for ELISA analysis. Thereafter, the extracts were applied to miniaturized multilayer silica columns, packed with 0.3 g KOH–silica, 0.3 g activated silica, 0.6 g 40% sulfuric acid–silica, and Na_2SO_4, and were eluted with 8 ml *n*-hexane in order to remove interfering residues. Finally, the solvent of the spiked extracts was changed to tetradecane, and that of nonspiked extracts to DMSO.

ELISA procedure

The ELISA analysis of extracts from the Soxhlet extraction or ASE procedure was carried out in all essentials following previously described protocols [17, 18]. Briefly, the microtiter plates were coated with 100 μl/well III-BSA coating antigen at a concentration of 0.2 μg/ml overnight. The plates were washed with PBST and 200 μl blocking solution (0.5% BSA in PBS) was added to each well. After 30-min incubation at room temperature, the plates were washed with PBST and 50 μl/well of sample extract or TMDD standard, diluted in PBS (1/1, v/v), was added. Without delay, 50 μl of antibody 7598 (diluted 1:5000 in PBS with 0.2% BSA) was added to each well and the analyte and coating antigen were allowed to bind competitively to the antibodies for 90 min at room temperature. After washing the plates with PBST, 100 μl goat antirabbit IgG conjugated to horseradish peroxidase (diluted 1:3000 in PBST) was added to each well. The plates were incubated for 60 min at room temperature and, after washing again with PBST, 100 μl substrate solution was added to each well. After 20-min incubation at room temperature in darkness, the reaction was stopped by adding 50 μl/well of 2 M sulfuric acid. The absorbance of the reaction mixtures was then read at 450 and 650 nm using a Spectramax microplate reader (Molecular Devices, Sunnyvale, CA, USA) in dual-wavelength mode. Each dilution of sample extract or standard was analyzed in triplicate and the results were converted to TMDD equivalents, interpreted as total PCDD/PCDF contents. Method blanks and an artificial soil sample were analyzed for quality control purposes. None of the tested fractions (A, B, and C) or crude extracts from the method blank or artificial soil caused ELISA inhibition, except the crude extract from the artificial soil.

Standard curves were generated by plotting absorbance vs. the logarithm of the TMDD concentration (0.08–

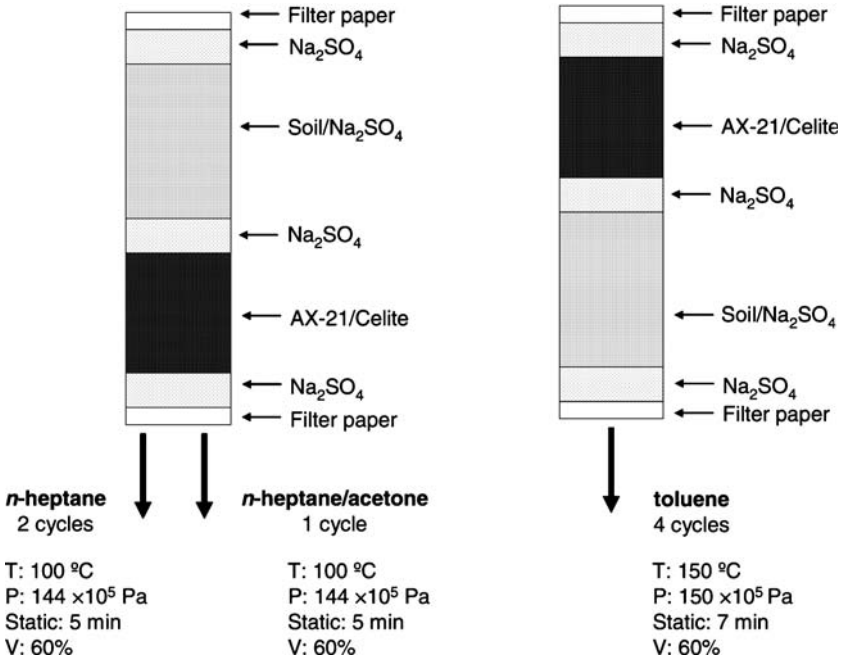

Fig. 2 Packing and elution sequence of the accelerated solvent extraction (*ASE*) cell used for simultaneous extraction and purification. The elution was as follows: two cycles of *n*-heptane, one cycle of *n*-heptane–acetone (*left*), after which the cell was inverted and back-flushed with four cycles of toluene (*right*). The temperature (*T*), pressure (*P*), static extraction time, and flush volume (*V*), expressed as percentage of cell volume, for each cycle of the different solvents, are presented *below* each graph

20,000 pg TMDD/ml was used). The curves were fitted to a four-parameter logistic equation:

$$y = \left\{ (A - D) \Big/ \left[1 + (x/C)^B \right] \right\} + D,$$

where A is the maximum absorbance at zero analyte concentration, B is the slope of the curve at the inflection point, C is the concentration of analyte giving 50% inhibition (IC_{50}), and D is the minimum absorbance at infinite concentration. The cross-reactivity (CR) for TMDD/2,3,7,8-TCDD is 1.3 [17], and it has been shown that the curves for the two compounds are statistically similar [15]. However, TMDD is less toxic than 2,3,7,8-TCDD and is therefore preferred as a standard.

Gas chromatography–high-resolution mass spectrometry

The instrumental analysis by GC/HRMS was done in accordance with previously described protocols [31], following EU standard methods [11]. The selected ion-monitoring mode and a resolution of 8,000 or greater were used in quantification of the 2,3,7,8-substituted PCDD/PCDF congeners with the isotope dilution technique.

Principal component analysis

Relationships among the Soxhlet-extracted samples with regard to congener profiles were evaluated by PCA using SIMCA-P 9.0 software (Umetrics, Umeå, Sweden) [35]. Prior to PCA, data describing the relative abundance of PCDD/PCDF congeners in each sample were mean-centered, scaled to unit variance, and log-transformed. Two principal components were calculated.

Results and discussion

ELISA characteristics

Seven ELISA calibration curves, run on three different days, were statistically analyzed. The averaged curve had maximum (A) and minimum (D) absorbance values of 0.673±0.359 and 0.047±0.029, respectively, a slope of 0.940±0.124, and a regression coefficient of 0.995±0.002. The results indicate that the method has a detection limit of 28±6 pg TMDD/ml DMSO (defined as the concentration giving 80% of the maximum response), and an IC_{50} of 123±15 pg TMDD/ml DMSO.

Impact of the cleanup procedure on the ELISA performance

To obtain reliable ELISA results, matrix effects have to be eliminated by removing compounds that might interfere with the immunochemical reaction [36]. Matrix effects on ELISA performance can be attributed to both nonspecific (unknown) and specific (cross-reactants) interferences that disrupt the antibody–antigen reaction [18]. Such compounds may include hydrocarbons and polar compounds like phthalates, phenols, and carboxylic acids [13]. The samples included in the current study originated from different sources, had differing organic matter contents (0.49–27%; Table 1), and were expected to contain varying amounts and types of interfering compounds. To remove these interferences, the conventional extraction and cleanup method used prior to GC/HRMS was used as a starting

Table 1 Gas chromatography–high-resolution mass spectrometry (*GC/HRMS*) WHO toxic equivalent values expressed as picograms per gram dry weight and enzyme-linked immunosorbent assay (*ELISA*) results expressed as picograms of 2,3,7-trichloro-8-methyl-dibenzo-*p*-dioxin equivalents per gram dry weight

Sampling site		LOI (%)	GC/HRMS	ELISA			
				Crude extract		Fraction C	
				Mean (% RSD)	*n*	Mean (% RSD)[a]	*n*
Wood	I	23	18,000	20,000 (53)	9	700 (10)	6
	II	1.3	20,000	5,800 (73)	9	1,000 (13)	6
	III	2.2	17,000	1,800 (67)	6	610 (13)	6
	IV	0.49	110	NA		ND	
Chloralkali	I	4.0	260	2,800 (44)	6	100 (24)	3
	II	1.9	37,000	170,000 (58)	6	30,000 (12)	6
Combustion	I	27	1,100	NA		280 (13)	6
	II	7.4	20	NA		110 (11)	6
	III	6.0	2,200	NA		830 (20)	6
	IV	11	110	NA		36 (15)	6
Artificial soil		11		820 (10)	6	ND	

[a]The relative standard deviations (*RSDs*) were calculated using values from triplicate analyses of a single sample extract dilution (n=3) or several dilutions of the sample extract (n>3)

LOI loss on ignition, *NA* not analyzed, *ND* not detected

point for ELISA analysis. There was a clear need to clean up crude extracts (including crude extracts of the artificial soil) because they severely inhibited the immunoassay, leading to severe overestimation of the samples' dioxin contents (Table 1, crude extract and fraction C).

In accordance with previous experience [18], the use of a multilayer silica column significantly reduced the effects of the interferences, and acceptable ELISA data were obtained for four out of six samples (data not shown). However, for the remaining two samples, matrix effects made quantification impossible; hence, further cleanup of Soxhlet extracts was necessary and was applied to all the extracts analyzed. Carbon fractionation was employed for this purpose, followed by alumina fractionation and miniaturized multilayer column cleanup (Fig. 1). Fraction A, containing *inter alia* multi-*ortho*-PCBs and polychlorinated diphenylethers (PCDEs), if present in the sample, did not cause any ELISA inhibition in the three samples tested (wood II, chlor I and II). Notably, GC/HRMS analysis of one of the samples, from the wood treatment site, showed significant amounts of PCDEs (1,100 ng/g dry weight). Thus, the ELISA was not affected by PCDEs.

The ELISA quantification of PCDDs/PCDFs in fraction C gave relative standard deviations (RSDs) within acceptable limits (less than 30%; Table 1). Thus, the extensive cleanup successfully eliminated matrix effects, resulting in lower mean dioxin estimates and RSDs than those obtained using the crude extracts. The levels found ranged between 36 and 30,000 pg/g dry weight, illustrating that the amounts of PCDDs/PCDFs at the different sites were highly variable. The dioxin contents of one of the contaminated samples were found to exceed the USEPA preliminary remediation goal of 1,000 pg toxic equivalents (TEQ)/g, and six exceeded the guideline value of 250 pg TEQ/g for industrial areas recommended by the Swedish Environmental Protection Agency (SEPA) [37, 38]. These soils would therefore be classified as highly contaminated and in need of remediation treatment.

Comparison of dioxin results from ELISA and chemical analysis

Measured ELISA and GC/HRMS results for fraction C (Table 1) were linearly correlated, with a correlation coefficient of 0.64 and a slope of 0.57 (Fig. 3); hence, ELISA underestimates the PCDD/PCDF content of the samples, implying that a safety factor of at least the inverse value of the slope has to be introduced to avoid an unacceptable rate of false-negative results by ELISA.

On the basis of the ELISA limit of detection (LOD) of 28±6 pg TMDD/ml DMSO, the theoretical LOD for the overall method (sample preparation and ELISA) was 28 280 pg TMDD equivalents/g dry weight using a sample size of 0.1–1 g and a final volume of 1 ml DMSO. Hence, the ELISA may have overestimated the dioxin content of the least contaminated sample (combustion II) because it had PCDD/PCFF levels close to the estimated LOD for the overall method. Furthermore, residual matrix interferences (including cross-reactants) have the greatest impact on lightly contaminated samples. The presence of compounds like planar PCBs, for instance, PCB 77 (0.1% CR) [17], 2,3,7,8-tetrabromodibenzo-*p*-dioxin (98% CR), and 2,3,7,8-tetrabromodibenzofuran (67% CR) could lead to overestimation of dioxin contents. Although identification of a broad spectrum of potential cross-reactants was beyond the scope of the current study, the planar PCBs 77, 126, and 169 were analyzed by GC/HRMS. They were found to be virtually nonexistent in samples from Sweden, but their combined WHO TEQ values amounted to 5–10% of the PCDD/PCDF WHO TEQs in samples from Uruguay; hence, the possibility that these compounds contributed to the ELISA inhibition could not be excluded. However, their contribution was only found to be of quantitative importance for combustion II (for which planar PCBs constituted 65% of the predicted ELISA TMDD equivalents from PCDDs/PCDFs, calculated as described later, while corresponding values for the other samples were less than 30%).

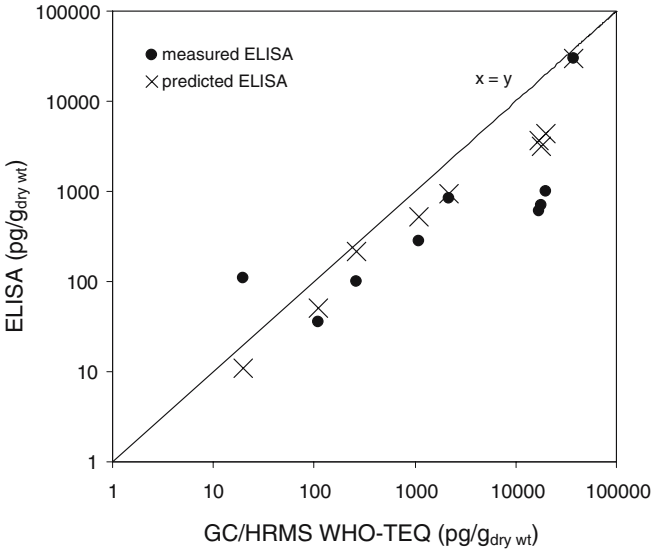

Fig. 3 Correlation between ELISA 2,3,7-trichloro-8-methyl-dibenzo-*p*-dioxin (*TMDD*) equivalents (measured and predicted) and GC/HRMS WHO toxic equivalent (*TEQ*) values (Fig. 1, fraction C)

As already indicated, any detailed discussion regarding comparisons between ELISA and chemical analysis has to consider predicted ELISA values, since differences between the ELISA CR and WHO toxic equivalency factor (TEF) among the individual 2,3,7,8,-PCDDs/PCDFs in each sample complicate comparisons of ELISA and GC/HRMS data [17, 39]. ELISA gives only a single value, which integrates the effects of various 2,3,7,8-TCDD-like compounds with varying CR. In contrast, GC/HRMS gives specific values for known analytes, but may not detect some 2,3,7,8-TCDD-like compounds, for which standards are not available. In the present study, the GC/HRMS TEQs were measured with a congener LOD of 1 pg/g dry weight and an RSD of 5–16% [40]. Nondetected congeners were ignored (set to 0) when calculating total WHO TEQ values. The predicted ELISA value was calculated by multiplying the

appropriate CR for every PCDD/PCDF [17] by its concentration, determined by GC/HRMS, and adding the contributions. Based on previously published CR values [17], the following CR estimates for nontested congeners were applied: 1,2,3,6,7,8-HxCDD (0.01), OCDD (0.0001), 1,2,3,4,7,8-HxCDF (0.0001), 2,3,4,6,7,8-HxCDF (0.054), 1,2,3,4,7,8,9-HpCDF (0.0006), and OCDF (0.0001). The CR for 1,2,3,7,8,9-HxCDD was measured and was found to be 0.028. The predicted ELISA (CR-corrected) results were somewhat lower than the GC/HRMS results (Fig. 3); however, the predicted ELISA values were always closer than the measured ELISA values to the GC/HRMS values, and the difference between predicted and measured ELISA data could be explained by losses of analytes during sample preparation (except for combustion II, as discussed earlier). The recoveries for two isotopically labeled internal standards, one TCDD and one HpCDF, added prior to cleanup of extracts were 76–115% ([$^{13}C_{12}$]-2,3,7,8-TCDD) and 21–119 % ([$^{13}C_{12}$]-1,2,3,4,6,7,8-HpCDF) as determined by GC/HRMS, respectively. This was within the acceptance criteria laid down in Ref. [11]. The poorer recovery of the more highly chlorinated congener was likely due to losses in the alumina column, which is known to be sensitive to minor variations in the elution strength of the solvents used [33, 34]. GC/HRMS analyses of fraction B confirmed the presence of PCDDs/PCDFs. ELISA analysis of fraction B also indicated the presence of dioxin-like compounds. The losses did not affect instrumental quantification since internal standards were used, but may have caused underestimations when the multistep column cleanup method was used in combination with ELISA. This indicates that suitable internal standards should also be used in immunochemical analysis. However, the development of such a standard is very difficult as the internal standard has to behave in the same way as the other analytes throughout the extraction and cleanup and, at the same time, have a low CR. For future studies it is suggested that the alumina column should be omitted prior to ELISA analysis.

A further notable feature of the predicted ELISA values is the indication that ELISA especially underestimated the dioxin contents of three of the soil samples from sites where wood treatment had occurred (wood I, II, and III) owing to their relatively high contents of congeners with substantially lower CR values than the corresponding TEFs, for example, 1,2,3,4,6,7,8-HpCDD (CR 0.003, TEF 0.01) and 1,2,3,4,6,7,8-HpCDF (CR 0.0006, TEF 0.01) [17, 39]. PCDD/PCDF contamination at these sites originated from the use of tetra- and pentachlorophenol formulations, which are known to be contaminated with PCDDs/PCDFs, predominately 1,2,3,4,6,7,8-HpCDF and OCDD [7, 41]. Furthermore, the other samples were expected to have other congener profiles. The production of chlorine gas at the chloralkali site (chlor I and II) causes a typical "chloralkali pattern" of PCDDs/PCDFs, characterized by furans [5], and uncontrolled combustion (combustion I, II, III, and IV) can give rise to a variety of PCDDs/PCDFs [3]. Thus, it is not surprising that the predicted ELISA values differ between the sites. The use of site-specific correction factors is therefore recommended, especially for soils with inputs from pollution sources known to generate dioxin profiles with a high abundance of

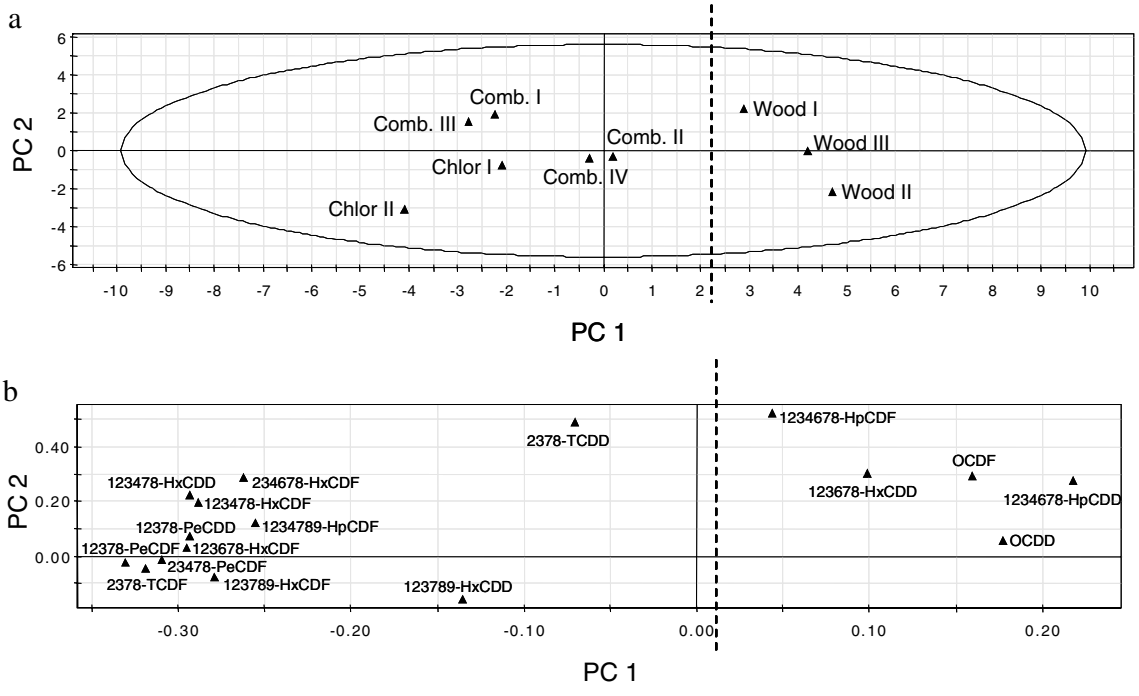

Fig. 4 **a** Score plot showing relations between the soil samples and **b** loading plot showing the corresponding relationships among the 2,3,7,8-PCDD/PCDF congeners. *PC* principal component

congeners with CR-to-TEF ratios that deviate significantly from unity.

PCA of the relative abundance of congeners

PCA was used to analyze the PCDD/PCDF congener profiles (the relative abundance of each congener; obtained by dividing its concentration by the total concentration of the 17 quantified congeners). The first two components of the model explained 75% of the variance. About two thirds of the overall variance was captured by the first principal component (PC 1), which was related to the relative abundance of the PCDD/PCDF congeners. The score plot illustrates how the different objects (samples) are related to each other (Fig. 4a). The positions of the variables (congeners) in the loading plot (Fig. 4b) are related to the positions of the objects and can be used to interpret groupings in the score plot. The three samples (wood I, II, and III) with predicted ELISA underestimates are clearly separated from the other samples. According to the loading plot, this is due to them having significantly larger proportions of the following congeners: 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8-HpCDF, OCDF, and OCDD, all of which are located far to the right. Furthermore, a small proportion of the rest of the congeners located more to the left also contributed to the predicted underestimation. Hence, with the aid of PCA, it was possible to identify types of congener profile that are likely to lead to underestimates. A high abundance of congeners that do not cross-react is unfavorable for ELISA analysis, and a correction factor is needed to calibrate the results.

The congener profiles of two samples (wood II and chlor II) that are located far apart in the score plot, and thus have dissimilar characteristics, are shown in Fig. 5. For one of the samples (wood II), 1,2,3,4,6,7,8-HpCDD and OCDD dominated the profile, while the other (chlor II) was dominated by low-chlorinated furans. The predicted ELISA performance for chlor II was in good agreement with GC/HRMS data, while it was poor for wood II. Thus, PCA of GC/HRMS data for selected representative samples is a convenient way to identify samples with unfavorable congener profiles, which may require calibration using site-specific (or area-specific) ELISA correction factors.

Simultaneous ASE and purification

Preliminary tests indicated that a final cleanup step should be applied to extracts derived by ASE with in-cell carbon fractionation for accurate ELISA results. To assess possible ELISA interferences due to remaining matrix components, aliquots of a purified ASE extract from an artificial soil were spiked with TMDD at ten different levels and analyzed with ELISA (data not shown). The curves obtained after tenfold, or higher, dilutions of the extracts

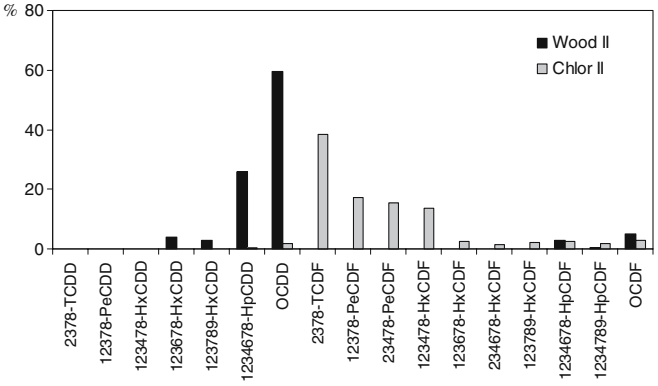

Fig. 5 PCDD/PCDF congener profile for two representative samples situated far apart in the score plot of the principal component analysis model (Fig. 4a)

were parallel to curves obtained using standards dissolved in DMSO, and the matrix effects could therefore be regarded as minor.

The repeatability of the ASE and ELISA procedure was tested by replicate analysis of several dilutions of a sample extract and the RSD was found to be in general less than 15%. Thus, the procedure seemed to fulfill the basic quality requirements for the official control of dioxins by screening methods laid down in European legislation [42] and the ELISA quantification could be regarded as reliable (with negligible matrix effects).

Figure 6 shows ELISA and GC/HRMS results for two sets of samples; one prepared using the conventional approach (i.e., Soxhlet extraction and multistep column cleanup), and one prepared using the simultaneous ASE and purification approach. The results for the two sample preparation techniques correlated well. The correlation coefficients were 0.78 for the GC/HRMS and 0.99 for the ELISA results, confirming that the ASE procedure exhaustively extracts PCDDs/PCDFs and efficiently removes potentially interfering substances from contaminated soil samples. The correlation between ELISA and GC/HRMS results for the ASE extracts was also good (correlation coefficient, 0.90). Furthermore, the PCDD/PCDF profiles obtained with the two sample preparation approaches were similar (data not shown).

An efficient way to apply the new screening procedure would be to split ASE extracts before the final evaporation into DMSO, so that ELISA could be used to prescreen for samples with levels exceeding the guideline values, and the same extract could be subjected to GC/HRMS for confirmatory analysis, if necessary. Hence, GC/HRMS time and resources could be reserved for analyzing significantly contaminated samples, rather than wasting them on screening lightly contaminated samples. This would allow much higher sample throughputs within a given budget. Furthermore, an ELISA prescreen would allow lightly contaminated samples to be run first, before the mass spectrometer is contaminated by samples containing high levels of analytes.

Thus, simultaneous ASE and purification was found to be an attractive, fast, and cost-efficient substitute for

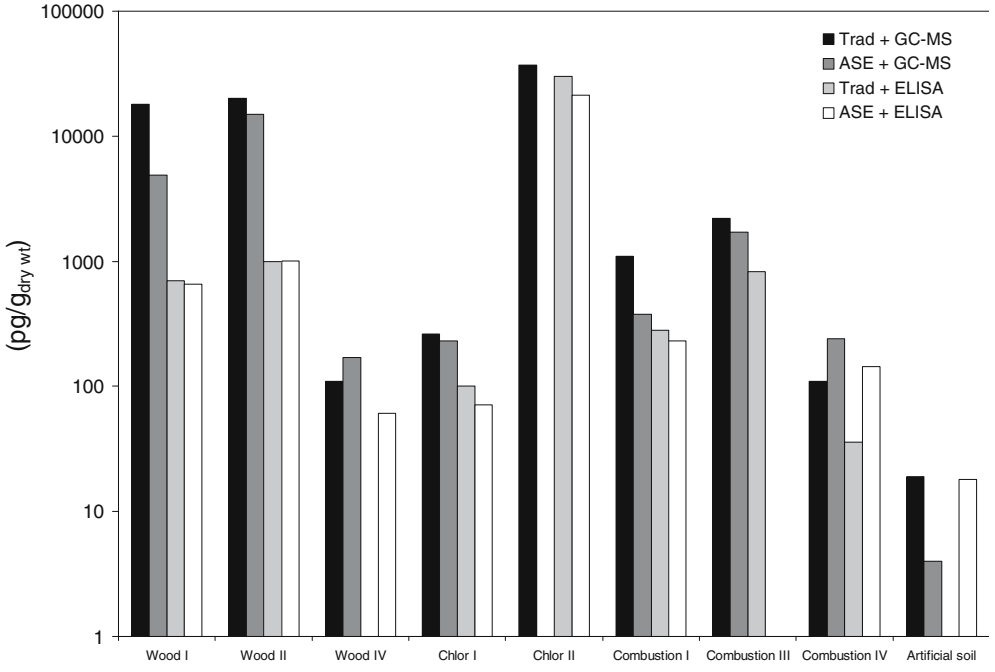

Fig. 6 GC/HRMS and ELISA results for samples extracted using the traditional approach, i.e., Soxhlet extraction and multistep column cleanup, and samples extracted using the novel simultaneous ASE and purification protocol. GC/HRMS results are expressed as WHO TEQ values, while ELISA results are expressed as TMDD equivalents

Soxhlet extraction with multistep purification, and to be suitable for both ELISA and GC/HRMS analysis. Another important benefit of this sample preparation technique prior to ELISA is that it reduces the risk for analyte losses, and thus should improve the data quality. Further validation of the simultaneous ASE and purification protocol is under way, to produce more precise measures of accuracy and precision, and to test its robustness. In this process, attempts will be made to further reduce the solvent consumption and exchange the AX-21 carbon for an equivalent material from a commercial source, partly to facilitate the wider acceptance of ASE with ELISA detection for high-throughput and cost-efficient screening of PCDD-/PCDF-contaminated soil samples.

Conclusions

Our findings imply that ELISA can, with acceptable results, be used for screening of PCDD-/PCDF-contaminated soil samples after Soxhlet extraction and multistep column cleanup. To avoid false-negative results, a safety factor, based on the relationship between GC/HRMS and ELISA results, can be used. However, a site-specific correction factor would be even better, since the PCDD/PCDF profile (evaluated by PCA) can affect the ELISA results. Furthermore, simultaneous ASE and purification in combination with ELISA analysis is a promising approach for high-throughput screening of PCDD-/PCDF-contaminated soil, as shown by the satisfactory correlation between the ELISA and GC/HRMS results. The principal advantages of the novel ASE are the time, labor, and cost savings it offers, which complement the efficiency of the ELISA screening. Furthermore, analyte losses that may bias the results are suppressed. Hence, Soxhlet extraction with tedious multistep sample cleanup might in the future be complemented or even replaced by applying the novel ASE approach prior to ELISA analysis.

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References

1. Bernes C (1998) Persistent organic pollutants: a Swedish view of an international problem. Swedish Environmental Protection Agency, Stockholm
2. Rodriguez-Pichardo A, Camacho F, Rappe C, Hansson M, Smith AG, Greig JB (1991) Hum Exp Toxicol 10:311–322
3. Bumb RR, Crummett WB, Cutie SS, Gledhill JR, Hummel RH, Kagel RO, Lamparski LL, Luoma EV, Miller DL, Nestrick TJ, Shadoff LA, Stehl RH, Woods JS (1980) Science 210:385–390
4. Olie K, Vermeulen PL, Hutzinger O (1977) Chemosphere 8:455–459
5. Rappe C, Kjeller LO, Kulp SE (1991) Chemosphere 23:1629–1636
6. Lexén K, de Wit C, Jansson B, Kjeller LO, Kulp SE, Ljung K, Söderström G, Rappe C (1993) Chemosphere 27:163–170
7. Hagenmaier H, Brunner H (1987) Chemosphere 16:1759–1764
8. Matscheko N, Tysklind M, de Wit C, Bergek S, Andersson R, Sellström U (2002) Environ Toxicol Chem 21:2515–2525
9. Kadokami K, Takeishi M, Kuramoto M, Ono Y (2002) Environ Toxicol Chem 21:129 137
10. US Environmental Protection Agency (1994) Tetra- through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS, EPA 821-B-94-005. EPA method 1613. US Environmental Protection Agency, Washington, DC
11. European Committee for Standardization (1997) Stationary source emissions—determination of the mass concentration of PCDDs/PCDFs. EN 1948:2–3. European Committee for Standardization, Brussels

12. Behnisch PA, Hosoe K, Sakai S (2001) Environ Int 27:413–439
13. Harrison RO, Eduljee GH (1999) Sci Total Environ 239:1–18
14. Sanborn JR, Gee SJ, Gilman SD, Sugawara Y, Jones AD, Rogers J, Szurdoki F, Stanker LH, Stoutamire DW, Hammock BD (1998) J Agric Food Chem 46:2407–2416
15. Sugawara Y, Gee SJ, Sanborn JR, Gilman SD, Hammock BD (1998) Anal Chem 70:1092–1099
16. Sugawara Y, Saito K, Ogawa M, Kobayashi S, Shan G, Sanborn JR, Hammock BD, Nakazawa H, Matsuki Y (2002) Chemosphere 46:1471–1476
17. Shan G, Leeman WR, Gee SJ, Sanborn JR, Jones AD, Chang DPY, Hammock BD (2001) Anal Chim Acta 444:169–178
18. Nichkova M, Park E-K, Koivunen ME, Kamita SG, Gee SJ, Chuang J, Van Emon JM, Hammock BD (2004) Talanta 63:1213–1223
19. Lopez-Avila V (1999) Crit Rev Anal Chem 29:195–230
20. Björklund E, von Holst C, Anklam E (2002) Trends Anal Chem 21:39–52
21. Sparr Eskilsson C, Björklund E (2000) J Chromatogr A 902:227–250
22. Turner C, Sparr Eskilsson C, Björklund E (2002) J Chromatogr A 947:1–22
23. Björklund E, Nilsson T, Bøwadt S (2000) Trends Anal Chem 19:434–445
24. Richter BE, Jones BA, Ezzell JL, Porter NL, Avdalovic N, Pohl C (1996) Anal Chem 68:1033–1039
25. Richter BE, Ezzell JL, Knowles DE, Hoefler F (1997) Chemosphere 34:975–987
26. Björklund E, Müller A, von Holst C (2001) Anal Chem 73:4050–4053
27. Müller A, Björklund E, von Holst C (2001) J Chromatogr A 925:197–205
28. Sporring S, Björklund E (2004) J Chromatogr A 1040:155–161
29. Sporring S, Wiberg K, Björklund E, Haglund P (2003) Organohalogen Compd 60:1–4
30. Nording M, Sporring S, Wiberg K, Björklund E, Haglund P (2005) Anal Bioanal Chem 381:1472–1475
31. Liljelind P, Söderström G, Hedman B, Karlsson S, Lundin L, Marklund S (2003) Environ Sci Technol 37:3680–3686
32. Zebühr Y (1992) Trace analysis of polychlorinated dibenzo-p-dioxins, dibenzofurans and related compounds in environmental matrices. Stockholm University, Stockholm
33. Swerev M, Ballschmiter K (1987) Anal Chem 59:2536–2538
34. Thielen DR, Olsen G (1988) Anal Chem 60:1332–1336
35. Wold S, Esbensen K, Geladi P (1987) Chemom Intell Lab Syst 2:37–52
36. Watkins BE, Stanker LH, Vanderlaan M (1989) Chemosphere 19:267–270
37. Fields TJ (1998) Directive from the EPA office of solid waste and emergency response 9200.4–26. US Environmental Protection Agency, Washington, DC
38. Swedish Environmental Protection Agency (1997) Development of generic guideline values. Model and data used for generic guideline values for contaminated soils in Sweden. Report 4639. Swedish Environmental Protection Agency, Stockholm
39. Van den Berg M, Birnbaum L, Bosveld ATC, Brunström B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FXR, Liem AKD, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Wærn F, Zacharewski T (1998) Environ Health Perspect 106:775–792
40. Maier EA, Kurz R. Darskus R (1999) The certification of the mass fractions of five polychlorodibenzo-1,4-dioxins (D48, D54, D66, D67, D70), seven polychlorodibenzofurans (F83, F94, F114, F118, F121, F124, F130), three chlorobenzenes (1,2,3-TriCB, 1,2,3,4-TeCB, PeCB), and five chlorophenols (3-CP, 3,4-DiCP, 2,4,5-TriCP, PeCP) in two contaminated soils. Report EUR 18863 EN. European Commission, Brussels
41. Koistinen J, Paasivirta J, Suonperä M (1995) Environ Sci Technol 29:2541–2547
42. European Commission (2002) Commission Directive 2002/69/EC of 26 July 2002 laying down the sampling methods and the methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in foodstuffs. Official Journal of the European Communities, European Commission, Brussels