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ANALYSIS OF DIOXINS IN CONTAMINATED SOILS WITH THE CALUX AND CAFLUX BIOASSAYS, AN IMMUNOASSAY, AND GAS CHROMATOGRAPHY/HIGH-RESOLUTION MASS SPECTROMETRY

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Abstract

The chemically activated luciferase expression assay, the chemically activated fluorescence expression assay, and the enzyme-linked immunosorbent assay (ELISA) are all bioanalytical methods that have been used for the detection and quantification of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs). However, no comparisons of the results obtained by these three methods have been published analyzing identical replicates of purified sample extracts. Therefore, we have evaluated the performance of each of these methods for analyzing PCDD/Fs in aliquots of extracts from aged-contaminated soil samples and compared the results with those obtained by gas chromatography/high-resolution mass spectrometry (GC/HRMS). The quantitative performance was assessed and the effects of sample purification and data interpretation on the quality of the bioassay results were investigated. Results from the bioanalytical techniques were, in principle, not significantly different from each other or from the GC/HRMS data ($p = 0.05$). Furthermore, properly used, all of the bioanalytical techniques examined were found to be sufficiently sensitive, selective, and accurate to be used in connection with soil remediation activities when aiming at the remediation goal recommended by the U.S. Environmental Protection Agency (i.e., < 1,000 pg toxic equivalency/g). However, a site-specific correction factor should be applied with the use of the ELISA to account for differences between the toxic equivalency factors and the ELISA cross-reactivities of the various PCDD/F congeners, which otherwise might significantly underestimate the PCDD/F content.

Keywords

CALUX; CAFLUX; Enzyme-linked immunosorbent assay; Soil; Dioxin

INTRODUCTION

Dioxin is a generic term used for polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) and it typically refers to the most potent or toxic PCDD/F

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congener, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The TCDD and other PCDD/F congeners with chlorine substituents in the 2,3,7,8-positions can cause a wide spectrum of biological and toxicological effects, including immunotoxicity, carcinogenicity, and adverse effects on reproduction [1–3]. The aryl hydrocarbon receptor (AhR) plays a crucial role in the mechanism mediating the toxic action of these compounds and it begins with the high-affinity binding of the ligand (PCDD/F) [4–6]. Subsequent translocation of the complex into the nucleus of the cell, dimerization of the AhR with the AhR nuclear translocator (Arnt) protein, and binding of the ligand:AhR:Arnt complex to its DNA recognition sequence leads to increased transcription and translation of certain genes, including that of cytochrome P4501A1 (*CYP1A1*). All dioxin-like compounds are assumed to act through this AhR signal transduction pathway.

Because the toxic response is mediated through a common mechanism, the toxicity of each 2,3,7,8-substituted PCDD/F congener can be compared to that of TCDD. For this purpose, toxic equivalency factors (TEFs) have been assigned to seven PCDDs, ten PCDFs, and 12 polychlorinated biphenyls (PCBs), that all meet the basic TEF criteria (i.e., they are persistent in the environment, structurally related to dioxins, exert their action through the AhR, and produce a similar spectrum of toxic and biological responses) [7,8]. The PCBs of interest might adopt a planar configuration because they lack, or only have one, chlorine in ortho-positions. A toxic equivalency (TEQ) value could be calculated for a mixture of PCDDs, PCDFs, and dioxin-like PCBs, assuming that their effects are additive [7,8]. Hence, the TEQ value is the sum of the TEF value for each congener multiplied by its concentration. Use of TEQ values facilitates risk assessments and the drafting and implementation of legislation regarding mixtures of dioxin-like compounds.

Several in vitro bioassays have been developed for assessing AhR activity and the detection and quantification of dioxins and related chemicals [9–12]. The primary advantages of AhR-based bioassays are their high throughput capacity and low cost, compared with gas chromatography/high-resolution mass spectrometry (GC/HRMS) [12]. However, deviations between the TEF values for individual congeners and their relative effective potency to induce a bioassay response, and contributions from AhR ligands for which no TEFs are available, could in principle lead to discrepancies between bioassay and GC/HRMS results. Nevertheless, these bioassays have been successfully used in several applications, including assessments of dioxin-like potency in sediments and pore water, blood plasma, sewage sludge, food control, and analyses of polycyclic aromatic hydrocarbons (PAHs) [13–17]. Intra- and interlaboratory comparison studies have confirmed their usefulness in dioxin analysis of soils and sediments [18–20]. Moreover, the performance of a *CYP1A1*-dependent ethoxy-resorufin O-deethylase (EROD) bioassay and an immunoassay for TEQ determination of environmental samples, including contaminated soil, has also been compared [21,22].

The chemically activated luciferase expression (CALUX) and chemically activated fluorescence expression (CAFLUX) cell bioassays are two state-of-the-art techniques that can be used to generate bioassay TEQ values (CA[F]LUX-TEQs) that can be compared with GC/HRMS TEQ estimates [23–26]. Both of these bioassays involve the use of genetically modified mouse hepatoma cells, and after exposure to sample extracts containing AhR ligands, they produce luciferase or enhanced green fluorescent protein, respectively. The intensity of the luminescence or fluorescence signal is directly proportional to the amount of AhR ligands in the sample and can be converted to a CA(F)LUX-TEQ by comparison to a TCDD calibration (standard) curve. The critical parameters that influence the CALUX results have been described elsewhere [27]. For environmental samples, sample preparation steps including extraction, clean-up, and fractionation have a significant effect on the composition of AhR ligands in the final extract, constituting a complex mixture of

compounds. Because many compounds produce AhR-independent toxic and biological responses and many AhR-active chemicals have not been assigned TEFs [4–6], it is important to purify extracts to eliminate such interfering substances to avoid potential bias in data interpretation.

In the study presented here, we evaluated the utility of three different bioanalytical techniques for dioxin analysis of aged, contaminated soil samples (an enzyme-linked immunosorbent assay [ELISA] [28] and two cell-based bioassays [CALUX and CAFLUX]) by comparing results obtained from these methods with PCDD/F data obtained by GC/HRMS analysis (the PCBs were only present in a limited number of samples and therefore not included in the comparison). To facilitate rigorous comparisons, identical conventional GC/HRMS preparation protocols were applied to all of the samples analyzed by all of the examined methods. This procedure includes Soxhlet extraction followed by multistep column clean-up with American and European standard methods [29,30]. Several fractions in addition to those containing PCDD/Fs were collected and tested to screen for the presence of AhR agonists and antagonists. To the best of our knowledge, this is the first time that these PCDD/F bioanalytical techniques have been tested in parallel with identical purified extracts. The overall focus of this work was on evaluating the quantitative performance of the ELISA and the CALUX and CAFLUX bioassays and to examine the possible effect of sample purification and data interpretation on the results.

MATERIALS AND METHODS

Chemicals

Solvents used for extraction and clean-up (toluene, *n*-hexane, dichloromethane, methanol) were of glass-distilled grade and supplied by Honeywell Burdick and Jackson (Muskegon, MI, USA). Tetradecane (olefin-free, p.a. grade) was from Fluka (Buchs, Switzerland), and dimethyl sulfoxide (DMSO, analytical grade) was from Scharlau (Barcelona, Spain). Silica (Kieselgel 60) and anhydrous sodium sulfate were from Merck (Darmstadt, Germany). The AX-21 carbon originated from Anderson Development (Adrian, MI, USA) but is currently not commercially available. For the cell bioassays, tissue culture media and geneticin (G418) were supplied by Invitrogen/Gibco (Carlsbad, CA, USA), fetal bovine serum (FBS) by Atlanta Biologicals (Atlanta, GA, USA), and luciferase lysis buffer and luciferin reagent by Promega Corporation (Madison, WI, USA). The TCDD that was used in the CALUX assay was a gift from S. Safe (Texas A&M University, TX, USA). The TCDD used in the CAFLUX assay and isotopically labeled standards used for GC/HRMS analyses were from Cambridge Isotope Laboratories (Andover, MA, USA). For the GC/HRMS quantification of target analytes, a standard solution containing the 17 native 2,3,7,8-substituted PCDD/Fs was used (Wellington Laboratories, Guelph, ON, Canada).

Samples

Soil samples were collected from five wood treatment sites (wood 1–10) and from a chlor-alkali site (chlor 1 and 2) in Sweden. All wood treatment sites were located in northern Sweden and were polluted with pentachlorophenol (PCP), 2,3,4,6-tetrachlorophenol (TCP), or both wood impregnation formulations containing considerable amounts of dioxins, especially hepta- and octa-CDD/Fs. A sample of an artificial soil containing 10% peat, 20% kaolin, and 70% sand with background levels of PCDD/Fs prepared by Pelagia Miljökonsult (Hörnefors, Sweden) according to guidelines published by the Organisation for Economic Co-operation and Development (OECD) was also included. All samples were air-dried at room temperature, passed through a 2-mm sieve, and thoroughly homogenized before extraction. The dry weight of the samples was determined by weighing portions of them before and after heating for at least 12 h at 105°C.

Soxhlet extraction and clean-up

Air-dried samples (2.5–5 g) were Soxhlet-extracted with toluene for 15 h, and each extract was split into aliquots for analysis by GC/HRMS, the two cell-based bioassays, and an anti-dioxin antibody-based ELISA. The sample clean-up procedure preceding all of the different detection techniques was identical, except for the addition of internal standards to the portions intended for GC/HRMS. The purified portions without internal standards were split into three separate aliquots for analysis by CALUX, CAFLUX, and ELISA. One of the original crude extract aliquots was not subjected to sample cleanup and purification and was directly examined by the CAFLUX assay for determination of total AhR agonist activity.

The clean-up procedure has previously been described in detail [31]. Briefly, the bulk of the coextracted material was removed by a multilayer column sequentially packed in-house with 35% KOH-silica (w/w), activated silica, and 40% sulfuric acid–silica (w/w) then eluted with *n*-hexane. The solvent was evaporated to about 1 ml and applied to a carbon column (AX-21/Celite, 1:12, w/w), which was eluted with dichloromethane/*n*-hexane (1:1, v/v). The column was then turned upside down and eluted with toluene. The toluene eluate was evaporated to about 1 ml and applied to an alumina oxide column, which was eluted with *n*-hexane and dichloromethane/*n*-hexane (1:1, v/v). As a final step, the dichloromethane/*n*-hexane fraction was evaporated to 0.5 ml; applied to a miniaturized multilayer column; sequentially packed with 35% KOH-silica (w/w), activated silica, and 40% sulfuric acid-silica (w/w); and eluted with *n*-hexane. The solvent of spiked aliquots of the extracts was changed to tetradecane (50 µl), and the solvent of nonspiked aliquots to DMSO (30 to 800 µl). The losses during solvent exchange were minimized by avoiding evaporation to dryness. Earlier evaluations have shown that the analyte losses are <10% with such an approach.

GC/HRMS

The instrumental analysis by GC/HRMS was carried out as previously described [31]. Selected ion monitoring at a resolution of 8,000 to 10,000 was used to quantify the 2,3,7,8-substituted PCDD/F congeners by the isotope dilution technique. The target analytes were identified by checking retention times and isotope ratios.

ELISA

The protocol of an ELISA analysis with the use of 2,3,7-trichloro-8-methyl-dibenzo-*p*-dioxin (TMDD) as surrogate standard has been described elsewhere [28]. The ELISA analysis was performed at the University of California (Davis, CA, USA). Triplicates of serial dilutions of sample extracts and standards were analyzed. The progressive inhibition of the assay caused by increasing the amounts of soil in the tested extract was related to the TMDD standard concentration–absorbance curve, and the results were converted to TMDD equivalents and interpreted as total PCDD/F contents. Calculated mean values and standard deviations of PCDD/F contents in the soil samples were all based on individual absorbance measurements of each tested dilution.

CALUX

Genetically modified mouse hepatoma cells (H1L6.1c3) were used in the CALUX cell bioanalysis [26,32]. These cells contain a stably transfected AhR-responsive firefly luciferase gene that responds to TCDD and other AhR agonists by expressing luciferase in a time-, dose-, and chemical-specific manner. The CALUX analysis was performed at University of California, Davis. Briefly, a portion (100 µl) of cell suspension containing 75,000 cells was added to each well of a Costar white, 96-well, clear-bottomed microplate (Corning, NY, USA) and incubated at 37°C for 24 h before sample addition. Serial dilutions of sample extracts and a standard (TCDD in DMSO, 0.5 to 5,000 pM) in FBS-supplemented

cell media were then added to the wells of the plate. All dilutions were tested in triplicate. The final concentration of DMSO was 1% (v/v). Cells were incubated at 37°C with treatment media for 24 h, then lysed with Promega luciferase lysis buffer, and the luciferase activity was initiated by the automatic injection of Promega stabilized luciferase reagent. The light output was then measured with an automated microplate luminometer (Anthos Lucy2, Eugendorf, Austria) and expressed as relative light units.

CAFLUX

The CAFLUX analysis was performed at the Swedish Defense Research Agency (Umeå, Sweden) with the use of genetically modified mouse hepatoma cells (H1G1.1c3) according to protocols described elsewhere [24,25]. Briefly, 70,000 cells were transferred to each well of a Nunc black, 96-well, clear-bottomed microplate (Nunc, Roskilde, Denmark) and allowed to attach for 24 h before exposure to TCDD or soil sample extracts. The cells were then treated with serial dilutions of the sample extracts or TCDD in DMSO without removing the cell culture media. A TCDD standard curve (0.3–2,000 pM) was created for each plate, and all dilutions were tested in triplicate, with a final concentration of 1% (v/v) DMSO. The background fluorescence in the intact cells was determined immediately after dosing and after incubation for 24 h at 33°C. The fluorescence in each well of the plate was measured in a microplate fluorometer (Fluostar Galaxy, BMG Labtechnologies, Offenburg, Germany) with an excitation wavelength of 485 nm and an emission wavelength of 520 nm.

CA(F)LUX-TEQ calculations

The CA(F)LUX-TEQ values for effective concentrations (ECs) were calculated at 25, 50, and 75% (EC25, EC50, and EC75) of the maximum responses on the TCDD dose–response curve. To obtain the mean CA(F)LUX-TEQ values and standard deviations for the samples (pg/g sample), the EC25, EC50, and EC75 values from the TCDD curves were divided by the amount of soil corresponding to the EC25, EC50, and EC75 response levels of the sample dose–response curves, after which the mean and standard deviations of these quotients were calculated. Both the TCDD standard curves and the sample curves were fitted with the use of SigmaPlot 2001 version 7.101 software (Systat Software, Richmond, CA, USA), to the four-parameter Hill function. For two of the CAFLUX-analyzed samples (wood 5 and wood 6), the EC75 levels were extrapolated. Furthermore, to obtain complete sample dose–response curves for four of the CAFLUX-analyzed samples (wood 2, 3, 9, and chlor 2), the response at the background level (derived from DMSO-treated cells) was used in the curve fitting (because even the most diluted extract produced a signal considerably higher than background). For samples that did not produce a complete dose–response curve, the CA(F)LUX-TEQs were calculated with the use of the individual responses of all dilutions that produced a response in the EC25 to EC75 range.

In a few cases, the inducing activity was very low, so the CA(F)LUX-TEQ values were calculated with responses below the EC25 level. However, even in these cases, the response was higher than the limit of quantification, which was defined as the mean response of triplicate determinations of the DMSO control plus 10 times the standard deviation.

RESULTS AND DISCUSSION

Comparison of the bioassay, immunoassay, and gas chromatography/mass spectrometry data

A standard (TCDD or TMDD) and a sample extract curve (wood 1) of the CALUX and CAFLUX assays and the ELISA are presented in Figure 1. The TCDD used in the CALUX analysis (in USA) and CAFLUX analysis (in Sweden) was of different origin but did give similar responses. In some cases, the concentration of PCDD/Fs in the sample extract was

not enough to generate complete assay curves. Although this could have been compensated for by the use of larger sample intakes, this was not done for practical reasons because complementary tests were difficult to perform because the tests had been done in another country (ELISA and CALUX).

The results of the ELISA, CALUX, and CAFLUX, together with GC/HRMS reference values, for aged dioxin–contaminated soil samples are compiled in Figure 2. The reported CA(F)LUX-TEQs represent the mean values calculated from those determined from several EC values (i.e., EC25, EC50, EC75) derived by fitting the standard and sample extract curves to the four-parameter Hill function, with the following exceptions: CALUX results for five of the samples (woods 6–10) and CAFLUX results for four samples (woods 7, 8, 10, and artificial soil), for which PCDD/F calculations were based on single-point estimates from the respective standard curves. The error bars correspond to the standard deviations for multiple CA(F)LUX and ELISA determinations of serial dilutions of sample extracts, except for the CALUX determination of wood 1 ($n = 2$) and the CAFLUX determinations of wood 7 ($n = 2$), for which the range of the two individual values are indicated. The luminescence for wood 5 and the artificial soil were below the detection limit. Also the inhibition of the absorbance for woods 4 to 10 and the artificial soil were below the detection limit.

Because ELISA failed to detect PCDD/Fs in eight of the 13 analyzed samples, it is clearly the least sensitive assay. However, in several cases, the ELISA response was very close to the detection limit, suggesting that an increased sample intake would have allowed ELISA detection. For two of the tested samples (wood 5 and the artificial soil), CALUX also failed to give a detectable response. Thus, CAFLUX appeared to be the most sensitive of the bioanalytical techniques.

Statistical evaluation of all data (excluding nondetects) by *t* test revealed no significant differences between the methods ($p = 0.05$), except for GC/HRMS and ELISA ($p = 0.02$). The correlation coefficients (r^2) were also calculated by linear regression for all possible combinations of detection methods (Table 1). The analyses show that CALUX was most correlated with GC/HRMS, followed by ELISA and CAFLUX. Furthermore, all the data of the bioanalytical techniques were highly correlated ($r^2 > 0.92$). It has to be stressed, however, that the ELISA underestimated all data by an order of magnitude and that the correlation analysis might be compromised because of the limited ELISA data set.

The data suggest that CALUX and CAFLUX produce sufficiently accurate data to meet criteria set by the U.S. Environmental Protection Agency for screening samples containing higher levels of dioxins than the remediation threshold of 1,000 pg PCDD/F TEQs/g for residential soils [33]. The ELISA underestimation of the dioxin content by roughly an order of magnitude (see above) does, however, call for the use of site-specific correction factors. Previous investigations of the studied soils, together with other contaminated soils, have shown that ELISA can also provide acceptable results if the data are corrected for differences between the TEF and cross-reactivity values of the PCDD/Fs present in the samples [28]. Hence, the two bioassays, and possibly the ELISA, could be used for screening PCDD/Fs in contaminated soil samples and for prioritizing samples for further analysis by GC/HRMS, thereby increasing the overall number of samples that can be screened in large-scale soil pollution surveys. For such activities, the ELISA approach would have considerable advantages because it could be most easily adapted for use in the field, which is a highly desirable feature. However, the bioassays provide more accurate determinations of relatively lightly contaminated soils, and biologically relevant sums of AhR ligands are reported. In addition, the CAFLUX bioassay is significantly cheaper and more convenient than CALUX and yields realtime induction responses. Drawbacks of the cell-based bioassays include restrictions on their application because of their use of

genetically modified cells and the limited amounts of validation data that are currently available, especially for the CAFLUX assay. Furthermore, the CAFLUX assay produces high background fluorescence, which might influence the results of lightly contaminated samples.

Bioassay data evaluation

The sample and TCDD dose–response curves yielded by the bioassays were not always parallel, and the efficacy (maximal induction response) indicated by the curves was not identical, in accordance with previous reports [34]. The discrepancies are illustrated by the differences in the slopes and maximal induction responses between the soil dose–response curve for wood 1 and the TCDD dose–response curve shown in Figure 1a and b. Consequently, the CA(F)LUX-TEQ value was dependent on the dilution chosen for quantification; thus, the relative potency at the EC25-, EC50-, and EC75-based values differed somewhat. In Figure 3a, the CAFLUX-TEQs calculated with the use of EC25, EC50, and EC75 values for all samples that gave complete or nearly complete dose–response curves are shown, and the corresponding data from the CALUX assay are shown in Figure 3b. Parallel standard and sample curves prevail when calculated values are very close. In contrast, if the values are widely dispersed, the curves are nonparallel, which suggests nondioxin compounds that influence the assay response. Thus, such samples might benefit from improved clean-up.

The effect of cleaning sample extracts on the bioassay results

The AhR can be activated by a structurally diverse range of chemicals, of which the PCDD/Fs are only one class [4–6,35]. Accordingly, accurate detection of PCDD/Fs in sample extracts requires clean-up of the extracts to remove the undesired “nonclassical” AhR ligands, which often are moderate to weak AhR inducers compared with TCDD [4–6]. These might otherwise contribute significantly to the overall bioassay induction, leading to overestimation of the potency of the extract if they are not removed. As expected, the sample cleanup procedure applied in this study removed large quantities of such interfering or undesired compounds, as illustrated by the significantly weaker (sometimes up to 100-fold weaker) CAFLUX responses induced by purified extracts than by crude extracts (Table 2). Hence, the overall activity of the crude extracts results from both classical and nonclassical AhR ligands [35] present in the extract, and accurate analysis of the target PCDD/Fs required removal of the undesired AhR agonists in the multistep column clean-up process. Although the CA(F)LUX-TEQs derived from the cleaned-up extracts should result primarily from the toxicologically relevant PCDD/Fs, and thus be comparable to the GC/HRMS-TEQ of the 2,3,7,8-PCDD/Fs, the possibility of contributions from other dioxin-like AhR agonists (i.e., polychlorinated naphthalenes) that could copurify with the PCDD/Fs could not be excluded. However, in this study, the contributions of other halogenated aromatic hydrocarbons to the overall response seemed to be limited because there was good agreement between CA(F)LUX and GC/HRMS data. Nevertheless, unwanted substances in the purified extracts could still be the reason for nonparallel curves, impairing accurate quantification. An improved cleanup on the basis of new protocols, or on a refinement of the present, would therefore be beneficial and might lead to improved CAFLUX performance for samples currently being severely overestimated by this method, especially samples containing the lowest amounts of PCDD/Fs (e.g., woods 9 and 10).

The CAFLUX responses to other fractions produced during the clean-up were also measured. The first fraction eluted from the carbon column, which would be expected to contain any multi–ortho-substituted PCBs and polychlorinated diphenylethers (PCDEs) present in the sample, did not induce any significant CAFLUX response, although GC/HRMS analysis of three of the samples revealed significant amounts of PCDEs (270–1,100

ng/g dry wt, not shown). Thus, the CAFLUX assay did not recognize PCDEs as AhR agonists and this is consistent with findings reported by other authors [36]. Furthermore, the detection of the non–ortho-PCB 3,3′,4,4′-tetrachlorobiphenyl (PCB77) in the dioxin fraction of the chlor-alkali site samples (at high picogram levels) demonstrated that AhR-active PCBs were also present in these samples. Because the multi–ortho-PCBs should elute in the first carbon column fraction, together with the PCDEs, these compounds would not contribute to the overall CAFLUX response to the cleaned-up sample extracts.

Tests for antagonism and other potential biasing factors

Because antagonists can suppress the AhR-dependent induction response [37], CAFLUX cells were exposed to DMSO or sample extracts (first fraction eluted from the carbon column, $n = 4$) spiked with known amounts of TCDD to evaluate whether the observed zero response was due to antagonists. In all cases, the magnitude of the response was correlated with the amount of TCDD added, indicating that inhibitory chemicals were not present in the extracts (data not shown).

In CAFLUX analysis, the signal might also be biased because of the presence of fluorescent substances, compounds that quench fluorescence, or both. However, no fluorescence was detected in any wells immediately after addition of the sample extracts, indicating that no fluorescent chemicals were present in the soil extracts that could affect accurate measurements of enhanced green fluorescent protein. Furthermore, no reduction in the fluorescence signal produced in TCDD-treated cells during the course of the 24-h incubations was detected after addition of the sample extracts, indicating that no fluorescence-quenching compounds were present in the crude sample extracts.

CONCLUSIONS

The ELISA and CA(F)LUX assays are bioanalytical techniques that could be used in combination with instrumental analysis to identify and characterize environmental and biological samples that contain PCDD/Fs. Given the ability of these techniques to also recognize other compounds, they will not be able to replace instrumental analysis methods. However, they can greatly facilitate monitoring studies when they are used to prescreen sample extracts to identify potentially contaminated samples and to prioritize samples for subsequent congener-specific instrumental analysis by GC/HRMS. Consequently, these bioanalytical techniques might be very useful in remediation programs for PCDD/F-polluted industrial areas when rapid screening of large numbers of samples is essential. It is worth noting that the bioassays will respond to other dioxin-like AhR agonists if present in the samples, thereby facilitating the discovery and identification (by GC/HRMS) of previously unknown compounds (in the dioxin fraction) with dioxin-like biological effects. The dioxin-like PCBs, especially the non–ortho-PCBs coeluting with the PCDD/Fs in the current clean-up, will, for instance, cause CA(F)LUX response if present in the samples. This might be the reason for the over-estimation of PCDD/F content in the chlor-alkali samples because these contain significant amounts of non–ortho-PCBs. However, these compounds are not present to any significant extent in the rest of the samples and consequently do not interfere with the CA(F)LUX-TEQ determinations of the wood treatment samples.

To maximize the benefits of the rapid bioanalytical techniques they should be combined with high-throughput extraction and clean-up techniques. We have therefore developed a simultaneous extraction and fractionation technique for PCDD/Fs in contaminated soils with the use of selective accelerated solvent extraction (ASE), and it has been successfully combined with the ELISA detection method [28]. A similar selective ASE method has been developed and used in combination with CAFLUX for determining dioxin TEQs in food and feed samples [38]. Currently, the performance of selective ASE of soil samples with

CAFLUX detection is being evaluated, and a significant reduction in costs and increase in throughput is expected.

Finally, it has to be stressed that the CA(F)LUX-TEQ levels that have been obtained in this study relate to the total amount of bioactivity present in the soils, and this does not necessarily reflect the toxicity, but merely gives information on the potential for gene induction. Although AhR-dependent gene induction is a prerequisite for AhR-dependent toxicity, additional analysis by instrumental, in vivo animal studies, or both must be carried out before any definitive conclusions regarding the toxicity of a sample or extract can be made. Accordingly, accurate exposure and risk assessment analysis would also require information on the bioavailability of the target pollutant(s), which might be obtained through the use, for instance, of mild extraction techniques.

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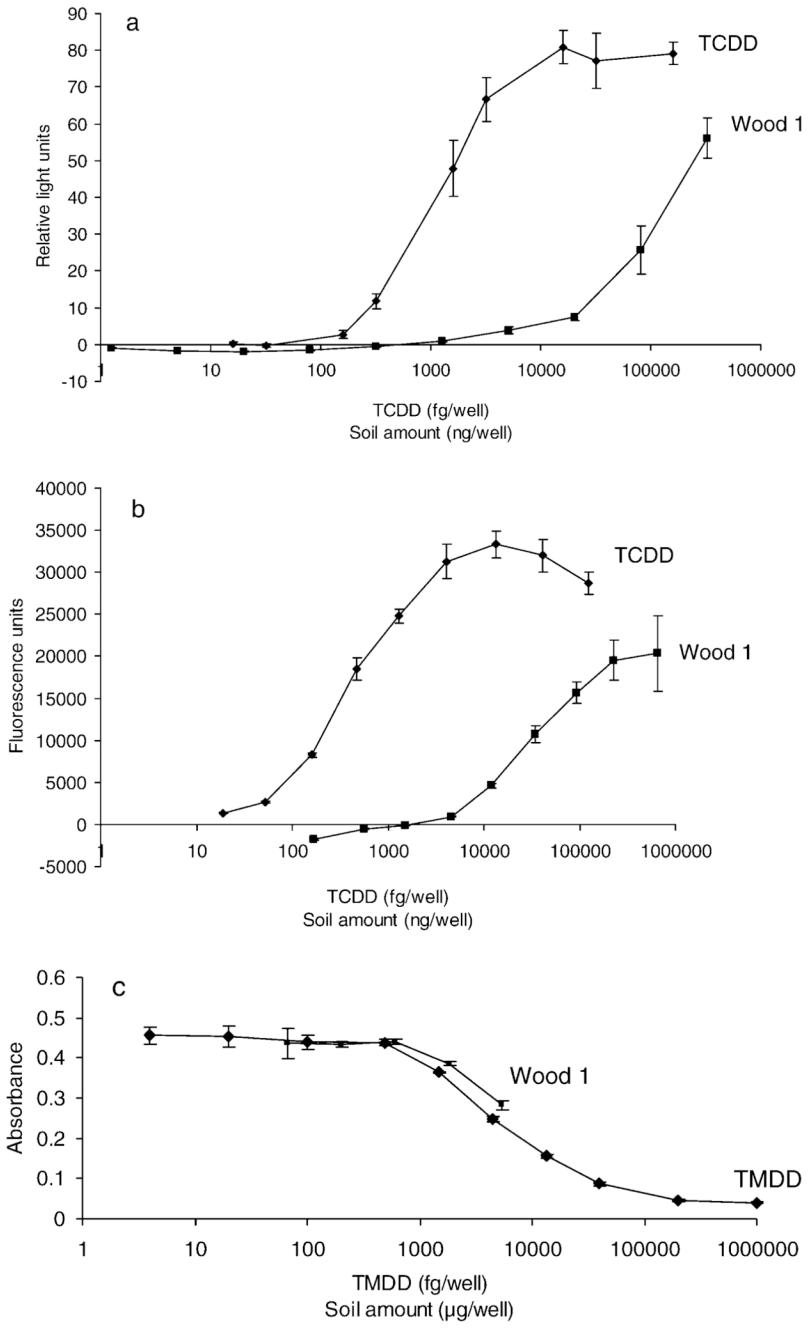

Fig. 1.

(**a**) Chemically activated luciferase expression (CALUX), (**b**) chemically activated fluorescence expression (CAFLUX), and (**c**) enzyme-linked immunosorbent assay (ELISA) dose–response curves for the standard 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or 2,3,7-trichloro-8-methyl-dibenzo-*p*-dioxin (TMDD) and the wood 1 soil extract, illustrating the slight deviations from parallelism between the CA(F)LUX standard and sample extract curves and an incomplete ELISA sample extract curve. All data have been corrected for signals from a blank sample (wells treated with dimethyl sulfoxide).

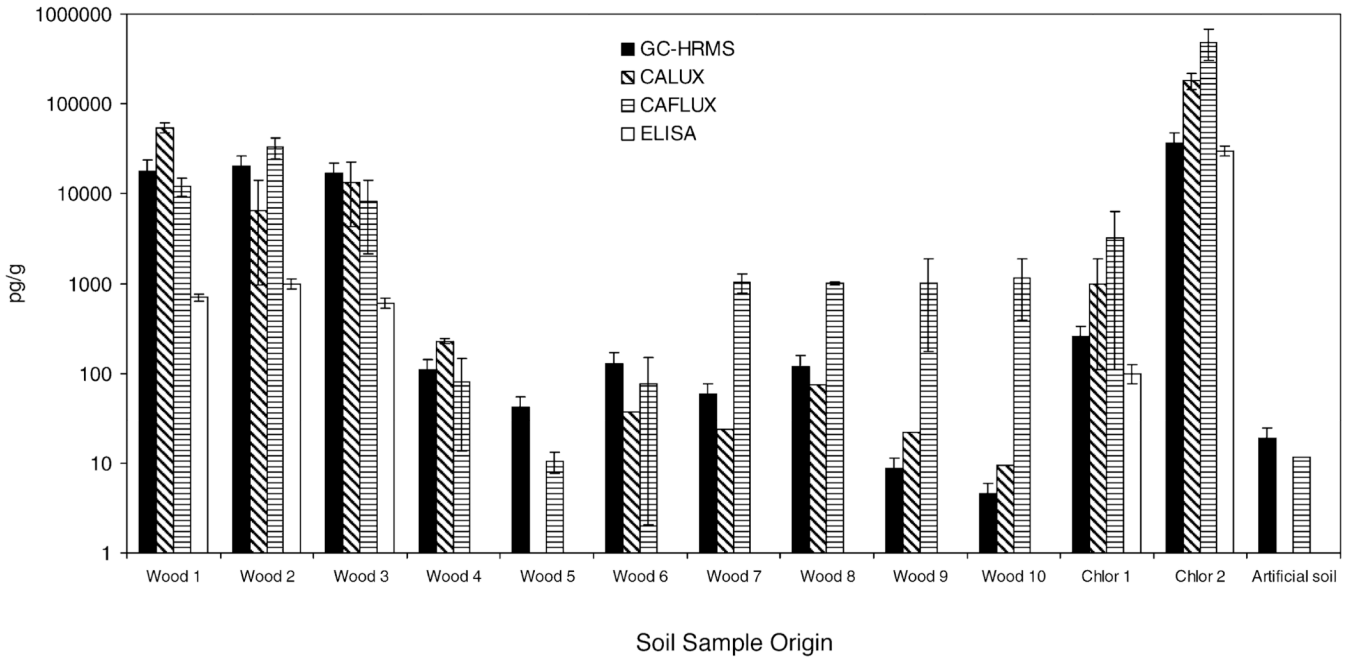

Fig. 2.

Comparisons of results of gas chromatography/high-resolution mass spectrometry (GC/HRMS) World Health Organization toxic equivalency (WHO-TEQ) reference values (pg/g dry wt) and chemically activated luciferase expression (CALUX)–TEQ (pg/g dry wt), chemically activated fluorescence expression (CAFLUX)–TEQ (pg/g dry wt), and enzyme-linked immunosorbent assay (ELISA) analysis of 2,3,7-trichloro-8-methyl-dibenzo-*p*-dioxin (TMDD) equivalents (pg/g dry wt). The GC/HRMS results were calculated with TEQ factors from Van den Berg et al. [7], including only dioxins. For comparison purposes, the ±30% uncertainty in the accredited GC/HRMS procedure used was included in the graph.

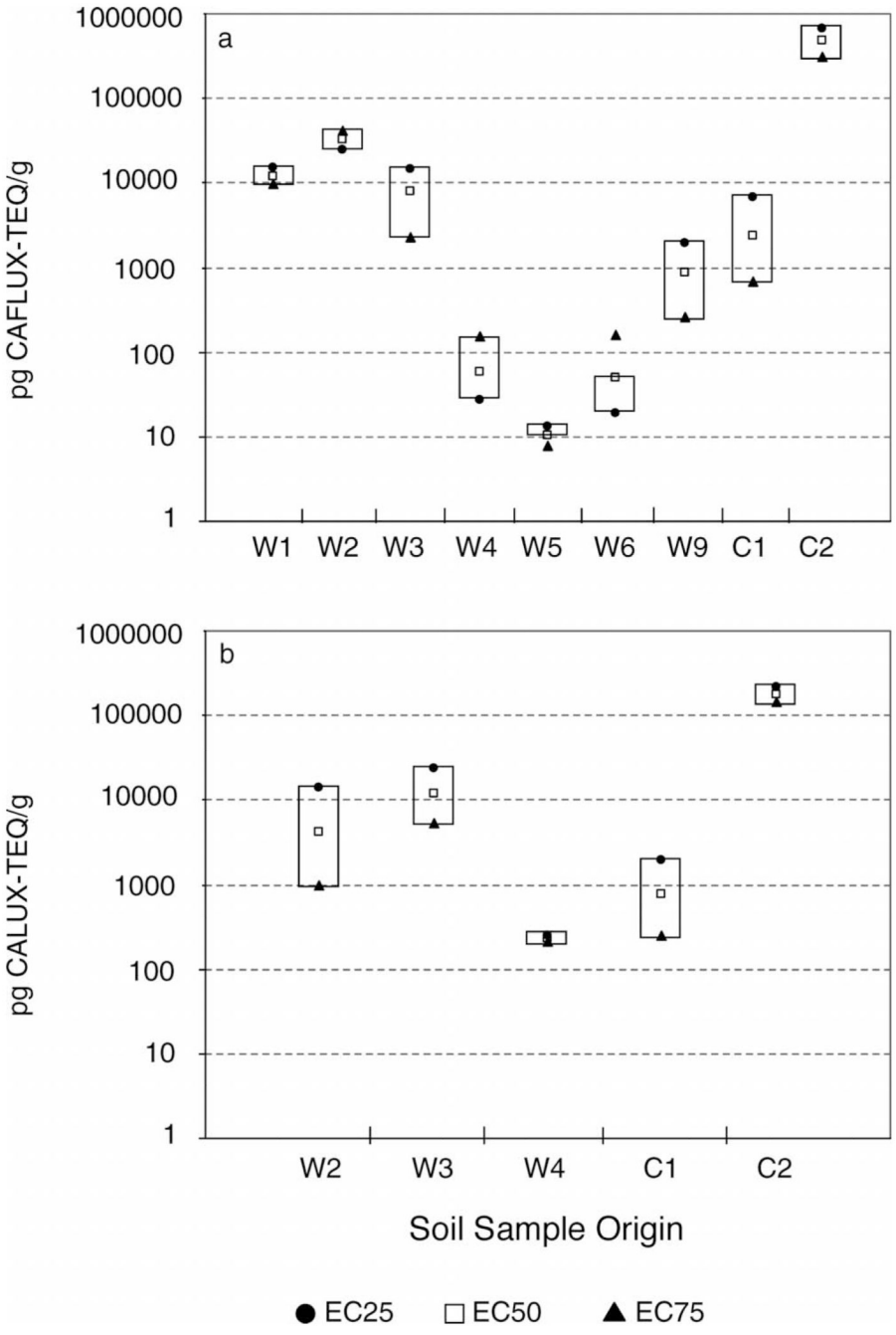

Fig. 3.

Bioassay toxic equivalency (TEQ) concentration ranges for soil samples analyzed by (**a**) chemically activated fluorescence expression (CAFLUX) and (**b**) chemically activated luciferase expression (CALUX). The uncertainties in the determinations because of deviations from parallelism between the sample and standard curves are indicated by the size of the range. Data points outside the bars were derived by extrapolation beyond the empirical data. W = wood; C = chlor.

Table 1

Correlation coefficients for the methods under study

	GC/HRMS	CALUX	CAFLUX	ELISA
GC/HRMS[a]	1	0.719	0.617	0.655
CALUX[b]		1	0.923	0.927
CAFLUX[c]			1	0.999
ELISA[d]				1

[a] Gas chromatography/high-resolution mass spectrometry.

[b] Chemically activated luciferase expression.

[c] Chemically activated fluorescence expression.

[d] Enzyme-linked immunosorbent assay.

Table 2

Results of chemically activated fluorescence expression (CAFLUX) analysis of soil samples from wood treatment sites and a chlor-alkali site in Sweden expressed as CAFLUX toxic equivalency values (TEQs)

Sampling site		CAFLUX TEQs (pg/g dry wt)	
		Crude extract	Dioxin fraction[a]
Wood	1	210,000	12,000
	2	100,000	33,000
	3	230,000	8,200
	4	1,600[b]	80
	5	710	10
	6	6,800	76
	7	12,000	1,000
	8	60,000	1,000
	9	1,600[b]	1,000
	10	12,000	1,100
Chlor	1	44,000	3,300
	2	1,000,000[b]	490,000
Artificial soil		2,400[b]	12

[a] For details regarding the quantification, see text.

[b] On the basis of individual dilutions of samples (n = 2–4).