Environment International xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Toxicity comparison of chlorinated and brominated dibenzo-*p*-dioxins and dibenzofurans in industrial source samples by HRGC/HRMS and enzyme immunoassay

Fatin Samara [a], Barbara Wyrzykowska [a,b], Dennis Tabor [a], Dahman Touati [c], Brian K. Gullett [a,*]

[a] U.S. Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory (E305-01), 109 T.W. Alexander Drive, Research Triangle Park, NC 27711, United States
[b] Oak Ridge Institute for Science and Education Research Postdoctoral Program, P.O. Box 117, Oak Ridge, TN 37831-0117, United States
[c] ARCADIS U.S., Inc., 4915 Prospectus Drive Suite F, Durham, NC 27713, United States

ARTICLE INFO

Article history:
Received 12 August 2009
Accepted 21 December 2009
Available online xxxx

Keywords:
PBDDs/Fs
PCDDs/Fs
PBCDDs/Fs
CALUX
EIA
HRGC/HRMS

ABSTRACT

Limited information is available on the applicability of polychlorinated dibenzo-*p*-dioxin/furan (PCDD/F) toxicity assays to their brominated counterparts: polybrominated dibenzo-*p*-dioxins/furans (PBDDs/Fs). We estimated the toxicity of mixtures of chlorinated, brominated, and mixed bromochloro-dioxins and -furan (PBCDDs/Fs) laboratory standards using a chemically-activated luciferase gene expression cell bioassay (CALUX). The relative effects potency (REP) values obtained were comparable to the World Health Organization (WHO) toxic equivalency factors (TEFs) and in agreement with the concept of additive congener toxicity of mixtures of dioxins and furans. Enzyme immunoassay (EIA)-based toxic equivalents (TEQs), however, showed overestimation for PCDDs/Fs (0–4 orders of magnitudes higher) and underestimation for PBDDs/Fs (0–1 orders of magnitude lower) when compared to high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS)-based TEQ calculation (using WHO TEFs) in samples from an industrial source line. No correlation was found between the EIA and the HRGC/HRMS data, which could be attributed to differences in homologue-specific cross-reactivity responses, sample matrix type, and presence of other compounds competing for antibody binding in the immunoassay.
Published by Elsevier Ltd.

1. Introduction

Polychlorinated, polybrominated, and polybrominated/chlorinated dibenzo-*p*-dioxins/furans (PCDDs/Fs, PBDDs/Fs and PBCDDs/Fs) are present in the environment as complex mixtures. Traditionally, toxicity levels of chlorinated "dioxin-like" compounds have been determined by gas chromatography/high resolution mass spectrometry (HRGC/HRMS) analysis for congener concentrations which are weighted by congener-specific toxic equivalency factors (TEFs). The total toxic equivalency (TEQ) of a mixture is the sum of the TEF of each individual congener times its concentration and is used to estimate the risk associated with exposure. The concept of congener additivity of mixtures of chlorinated dioxins and furans using bioassays has been widely studied (Smialowicz et al., 2008; Laier et al., 2003; Scippo et al. 2004) but little or no data extend this to brominated and mixed bromo/chloro dioxins and furans. Scippo et al. (2004) have demonstrated the congener additivity of mixtures of PCDDs/Fs and dioxin-like polychlorinated biphenyls (PCBs) in the range of quantifiable concentrations of the responses. Data suggest that the biological activity of the PBDD/F and PBCDD/F compounds is similar to their chlorinated analogues (DeVito et al., 1997; Hornung et al., 1996; Kedderis et al., 1993; Mason et al., 1987; Weber and Greim, 1997). Many reports have shown that PBDDs/Fs and PBCDDs/Fs have the ability to activate the aryl hydrocarbon receptor (AhR) signal and to cross-react with dioxin antibodies individually (Behnisch et al., 2003; Samara et al., 2009; Shan et al., 2001).

In addition to the TEF concept, congener immunochemical recognition, as well as AhR recognition, can be used to estimate the TEQs for a variety of environmental samples (Brown et al., 2001; Carlson and Harrison, 1998). In the last decade, several in vitro bioassays and ligand binding assays for dioxin and dioxin-like compounds have been developed (Behnisch et al., 2001). The chemically-activated luciferase gene expression cell bioassay (CALUX) reports relative potency (REP) values dependent on receptor binding and activation, making it possible to screen for other dioxin-like compounds. Immunochemical techniques are based on the specific interaction between antibodies and antigens. The most widely used immunochemical method for pollutant detection is the enzyme immunoassay (EIA) (Estevez-Alberola and Marco, 2004). EIA measures cross-reactivities (the ability of an analyte molecule to bind to the anti-dioxin antibody in comparison to a standard molecule) and sample TEQ by responding to the toxic PCDD/F congeners in proportion to their TEFs.

* Corresponding author. Tel.: +1 919 541 1534; fax: +1 919 541 0554.
E-mail address: gullett.brian@epa.gov (B.K. Gullett).

0160-4120/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.envint.2009.12.005

Several studies of environmental samples have shown discrepancy in toxicity calculations comparing bioanalytical-derived (from CALUX and EIA) and GC/MS-derived results (Nording et al., 2007). Yang et al. (2006) compared the PCDDs/Fs toxicity levels in terms of TEQs in ambient air samples from a residential area and a waste incineration site using EIA and HRGC/HRMS. In that study the EIA-TEQs for residential district samples were higher than those by HRGC/HRMS. On the other hand, for waste incineration site samples, EIA-TEQs were lower than HRGC/HRMS. Nevertheless, these assays have been successfully used. Li et al. (1999) suggests that in the case of environmental samples, the immunoassay would demonstrate a greater analytical specificity and a relative insensitivity to interferences from other contaminants when compared to other bioanalytical techniques. This study evaluated the utility of bioanalytical techniques by comparing assay- and HRGC/HRMS-derived TEQs for PCDDs/Fs and PBDDs/Fs. The congener additivity of mixtures of chlorinated, brominated, and mixed bromo/chloro dibenzo-*p*-dioxins and dibenzofurans was studied using CALUX. We have previously estimated relative assay response factors for PBDDs/Fs using CALUX and EIA (Samara et al., 2009) and in this paper we extend the applicability of the immunoassay for calculating immunoassay TEQs versus HRGC/HRMS TEQs. Samples analyzed in this work were selected specifically because they were higher in PBDD/F concentration than PCDD/F concentration. To our knowledge this is the first paper to make this comparison in field samples from an industrial facility where samples had higher TEQs of brominated dioxin and furans than those of the chlorinated analogues.

2. Materials and methods

2.1. Reagents and solvents

Test samples comprised of individual standards in nonane and toluene of PCDDs/Fs, PBDDs/Fs, and PBCDDs/Fs were purchased from Cambridge Isotope Laboratories (Andover, MA), Wellington Laboratories–TerraChem Inc. (Shawnee Mission, KS) and Radian International LLC (Austin, TX). Individual standards and mixtures of standards for experiments were solvent-exchanged at the U.S. EPA facilities and analyzed at Xenobiotic Detection Systems Inc. (XDS, Durham, NC) by CALUX testing (Denison et al., 1998). Immunoassay experiments were performed using a High Performance Dioxin/Furan Immunoassay Kit (DF1, Cape Technologies, South Portland, ME), based on a rabbit polyclonal antibody (Carlson, 1997; Carlson and Harrison, 1998; Harrison and Carlson, 1997). HRGC/HRMS measurements were done using a Hewlett-Packard gas chromatograph 6890 Series equipped with a CTC Analytics Combi PAL autosampler (CTC Analytics, Switzerland) and coupled to a Micromass Premiere double-focusing high resolution mass spectrometer (Waters Inc., UK). Methanol, methylene chloride, hexane, dimethyl sulfoxide (DMSO) and toluene high purity solvents were purchased from Burdick and Jackson (Muskegon, MI).

2.2. CALUX analysis of polychlorinated, polybrominated and polybrominated/chlorinated dibenzo-p-dioxins/furans (PCDDs/Fs, PBDDs/Fs and PBCDDs/Fs) standard mixtures

The CALUX bioassay was used to estimate the ability of chlorinated, brominated and mixed bromo/chloro dioxins and furans to stimulate AhR-dependent gene expression individually and as mixtures. The present study utilizes a mouse hepatoma and the more stable Promega PG3-based luciferase reporter gene from XDS (Durham, NC). Individual standards and mixtures of PBDDs/Fs, PCDDs/Fs and PBCDDs/Fs were solvent-exchanged as described previously (Samara et al., 2009) by adding the standard stock (in nonane solution) to a 1.8 mL vial fitted with an insert containing DMSO. The nonane solvent was then evaporated under a stream of nitrogen and the sample was reconstituted with DMSO. The initial dilution (10 or 1 μg/mL) is then further diluted to make a six point curve. A dilution of 2,3,7,8-TCDD standards, blanks and controls were prepared in test tubes. The CALUX procedure has been reported elsewhere (Brown et al., 2001). The response for each concentration of each compound was analyzed one to three times in independent tests. All relative light unit values are corrected by subtraction of the relative light units from the blanks (standard wells containing DMSO but without 2,3,7,8-TCDD). Data for the dose–response series were fit to a sigmoidal curve described by the Hill equation using least squares best fit modeling (Brown et al., 2001). Dose–response experiments in this report were based on molar concentrations of the chemicals in order to minimize variations in results due to differences in molecular weights of the test chemicals.

2.3. Immunoassay-TEQs vs. HRGC/HRMS TEQs from industrial source samples

2.3.1. Sample collection

Gas samples were simultaneously collected at three e-waste in-duct source lines (triplicates) for both immunoassay (without recovery standards) and HRGC/HRMS (with recovery standards) analysis. These in-duct gas samples were collected from the inlet duct of the chain shredder line (A), the inlet duct of the hammer mill (B) and the inlet in-duct of the Cathode Ray Tube (CRT) assembly line (C). Previous experiments conducted in our laboratory show that this samples have high concentrations of polybrominated diphenylethers (PBDEs), (data will be reported in future publication). A decaBDE standard mixture (the most abundant BDE found in these samples) was tested with this assay for which little or no cross-reactivity was observed. The sample media used for the organic sampling was an 8 in × 10 in quartz filter and XAD trap (U.S. EPA, Emissions Measurement Center, Method 5 and Method 23). For the purpose of this paper we only tested the filter since it did not contain any surrogate or internal standard addition which can be detected by the assays.

Table 1
Estimation of CALUX REP values relative to 2,3,7,8-TeCDD for individual PCDDs/Fs, PBDDs/Fs, PBCDDs/Fs and mixtures.

Compound	CALUX REP for individual congeners molar derived [Samara et al., 2009]	CALUX REP for mixture molar derived
	EC_{50}	EC_{50}
2,3,7,8-TeCDD	1.00 ± 0.19[b]	
Mix 1		
2,3,7,8-TeBDD ^{13}C	0.99 ± 0.07[a]	0.35[a]
1,2,3,7,8-PeBDD	0.05 ± 0.01[a]	
1,2,3,7,8-PeBDF	0.11	
2,3,4,7,8-PeBDF	0.40	
2,3,7,8-TeBDF	0.41 ± 0.08[a]	
Mix 2		
1,2,3,7,8-PeCDD	0.69	0.44[a]
1,2,3,7,8-PeCDF	0.24	
2,3,4,7,8-PeCDF	0.46	
1,2,3,4,7,8-HxCDD	0.30	
1,2,3,4,7,8-HxCDF	0.30	
Mix 3		
2-Br-3,7,8-TriCDD	0.37 ± 0.04[b]	0.41[a]
3-Br-2,7,8-TriCDF	0.31 ± 0.02[b]	
2,3-DiBr-7,8-DiCDD	0.60 ± 0.02[b]	
Mix 4		
2,3,7,8-TeBDF	0.41 ± 0.08[a]	0.69[a]
1,2,3,7,8-PeCDD	0.69	
2,3,4,7,8-PeBDF	0.40	
2,3,4,7,8-PeCDF	0.46	

[a] Mean of two or three measurements.
[b] Mean of seven measurements.

Please cite this article as: Samara F, et al, Toxicity comparison of chlorinated and brominated dibenzo-p-dioxins and dibenzofurans in industrial source samples by HRGC/HRMS and enzyme immunoassay, Environ Int (2010), doi:10.1016/j.envint.2009.12.005

2.3.2. Extraction, clean-up and fractionation

Extraction of PBDDs/Fs and PCDDs/Fs from Method 23 and Method 5 filters was performed by means of Soxhlet extraction with methylene chloride (3.5 h) under restricted exposure to light (U.S. EPA, 1995; U.S. EPA, 2000). All raw extracts were concentrated using the three-ball Snyder columns, filtered, and concentrated further with nitrogen to 0.5 mL using an automated evaporator (Zymark Turbovap). For determination of PCDDs/Fs and PBDDs/PBDFs one half or one quarter of the extract was cleaned and fractionated using an automated liquid chromatography multi-column (multilayer silica, basic alumina) Power Prep Dioxin System (FMS Fluid Management Systems, Inc., USA). The volumes and concentrations of elution solvents used for PBDDs/Fs analysis are published elsewhere (Wyrzykowska et al., 2009). The PBDDs/Fs fraction was analyzed for PCDDs/Fs (carbon column clean-up step was omitted for PCDDs/Fs analysis in this study but recoveries of ^{13}C-labeled PCDDs/Fs surrogates remained within acceptance criteria of U.S. EPA Method 8290). The remainder of the extract was archived. For Method 5 filters, fractions of extracts were solvent transferred as described previously (Samara et al., 2009). Evaporations were done under a stream of nitrogen in 2 ml amber glass vials, using a specific keeper solution (100 ppm Triton X-100 in 80:20 methanol: tetraethylene glycol), with subsequent sample reconstitutions in methanol. For the EIA experiments a fraction of the extract was solvent exchanged as described in this section and tested without the clean-up and fractionation, and results showed that not much difference was observed in this assay with and without intensive clean-up of the samples (data not shown).

2.3.3. HRGC/HRMS analysis

Concentrations of PCDDs/Fs and PBDDs/Fs were determined by the HRGC/HRMS analysis. The HRMS was operated in an electron impact (35 eV and 650 μA current) selective ion recording mode at resolution $R>10{,}000$ MU (5% valley). For analysis of mono- through octa-CDDs/Fs, a 60 m DB-Dioxin (J&W Scientific, USA) GC column was used (0.15 μm film thickness × 0.25 mm i.d.). For analysis of tetra- through octa-BDD/Fs, the GC was equipped with 15 m DB-5 column (0.25 μm film thickness × 0.25 mm i.d.) (J&W Scientific, USA). The GC oven temperature for PBDDs/Fs analysis was programmed from 130 °C to 320 °C at 10 °C min^{-1} (10 min hold). The temperature program for PCDDs/Fs was from 130 °C to 260 °C at 6 °C min^{-1} with a final hold time of 50 min. The carrier gas (helium) flow rate was 1 ml min^{-1} for brominated compounds analysis and 0.9 ml min^{-1} for chlorinated compounds. A sample of 2 μl was injected under splitless mode for the analysis of PCDDs/Fs. The same was done for the analysis of PBDDs/Fs. The injection port temperature was set at 270 °C and 300 °C for chlorinated and brominated target analysis, respectively.

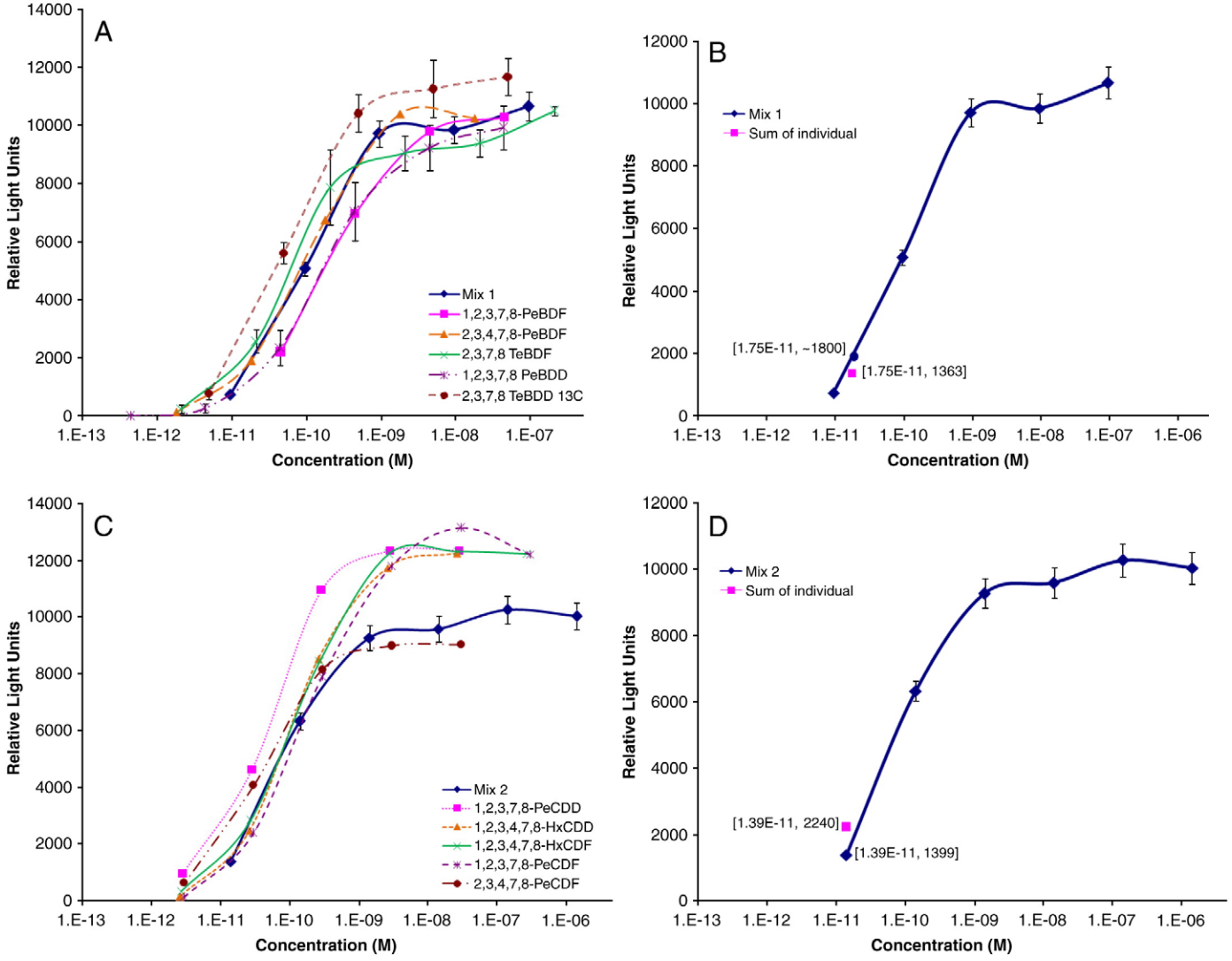

Fig. 1. Dose–response curves congeners determined by CALUX bioassay: A) individual and mixture of congeners of PeBDF, TeBDD, PeBDD and TeBDF; B) mixture and sum of individual congeners of PeBDF, TeBDD, PeBDD and TeBDF; C) individual and mixture of congeners of PeCDF, PeCDD, HxCDD and HxCDF and D) mixture and sum of individual congeners of PeCDF, PeCDD, HxCDD and HxCDF. Values with error bars represent the means ± SD of at least 3 measurements.

Please cite this article as: Samara F, et al, Toxicity comparison of chlorinated and brominated dibenzo-p-dioxins and dibenzofurans in industrial source samples by HRGC/HRMS and enzyme immunoassay, Environ Int (2010), doi:10.1016/j.envint.2009.12.005

2.3.4. EIA analysis

Samples were solvent exchanged as explained in Section 2.3.2. EIA analyses were carried out twice for each M-5 filters extract analyzed in the supplied antibody-coated tubes. The PCDD/F immunoassay which uses a rabbit polyclonal antibody has been previously described (Cape Technologies, 2006; Harrison and Carlson, 1997). Briefly, standards in the methanol/keeper solution are transferred into the antibody-coated tubes to which an aqueous sample diluent has already been added. This mixture is incubated overnight to allow capture of the analyte by the immobilized antibody. After washing the tubes with a 0.01% v/v Triton X-100 in water solution, a conjugate of a dioxin-like competitor coupled to the enzyme horseradish peroxidase is introduced to compete for the available binding sites on the antibody (those not occupied by analyte). The amount of horseradish peroxidase-competitor conjugate bound is inversely proportional to the logarithm of the dioxin concentration in the sample incubation step. After 15 min of incubation, the tubes were washed with water, enzyme substrate was added to the tubes, and color was generated by the captured horseradish peroxidase-competitor conjugate in direct proportion to the amount captured. After 30 min of incubation, stop solution was added to arrest color development. Finally optical density readings were obtained using a tube reader or spectrophotometer at 450 nm. A non-linear least squares curve fit was performed (Cape Technologies, 2004) and the median inhibition concentration (IC_{50}) for the calculated curve was determined. The IC_{50} values of test compounds were compared to the IC_{50} values of a 2,3,7,8-TCDD standard to determine the percent cross-reactivity of the test compound. The response for the concentration of each compound was analyzed at least two times. The percent negative control, which is the optical density as a percent of the negative control (keeper/methanol blank) optical density, is calculated for each 2,3,7,8-TCDD standard and sample. Concentrations were obtained using a calculation module C, accessed on the Cape Technologies website (http://cape-tech.com/). OD results were then compared to a 2,3,7,8-TCDD standard curve run in parallel.

3. Results and discussion

3.1. CALUX activity of polychlorinated, polybrominated, and polybrominated/chlorinated dibenzo-p-dioxins/furans (PCDDs/Fs, PBDDs/Fs and PBCDDs/Fs) individual congeners versus mixtures of standards

Thirteen individual congeners of PBDDs/Fs, PBCDDs/Fs, and PCDDs/Fs were selected and four mixtures of 3–5 congeners each were prepared as shown in Table 1 in order to elucidate the potential combined effects of dioxin-like compounds in mixtures. Dose–response curves were constructed for all the mixtures and compared to previously reported (Samara et al., 2009) dose–response curves of the individual congeners (Figs. 1A, C, 2A, and C). The 50% maximal response (EC_{50}) values of these curves were compared to that of 2,3,7,8-TCDD obtained during this experiments (curve not shown) to provide an estimate of the CALUX REP value (Table 1). When compared, the composite REPs (composites were

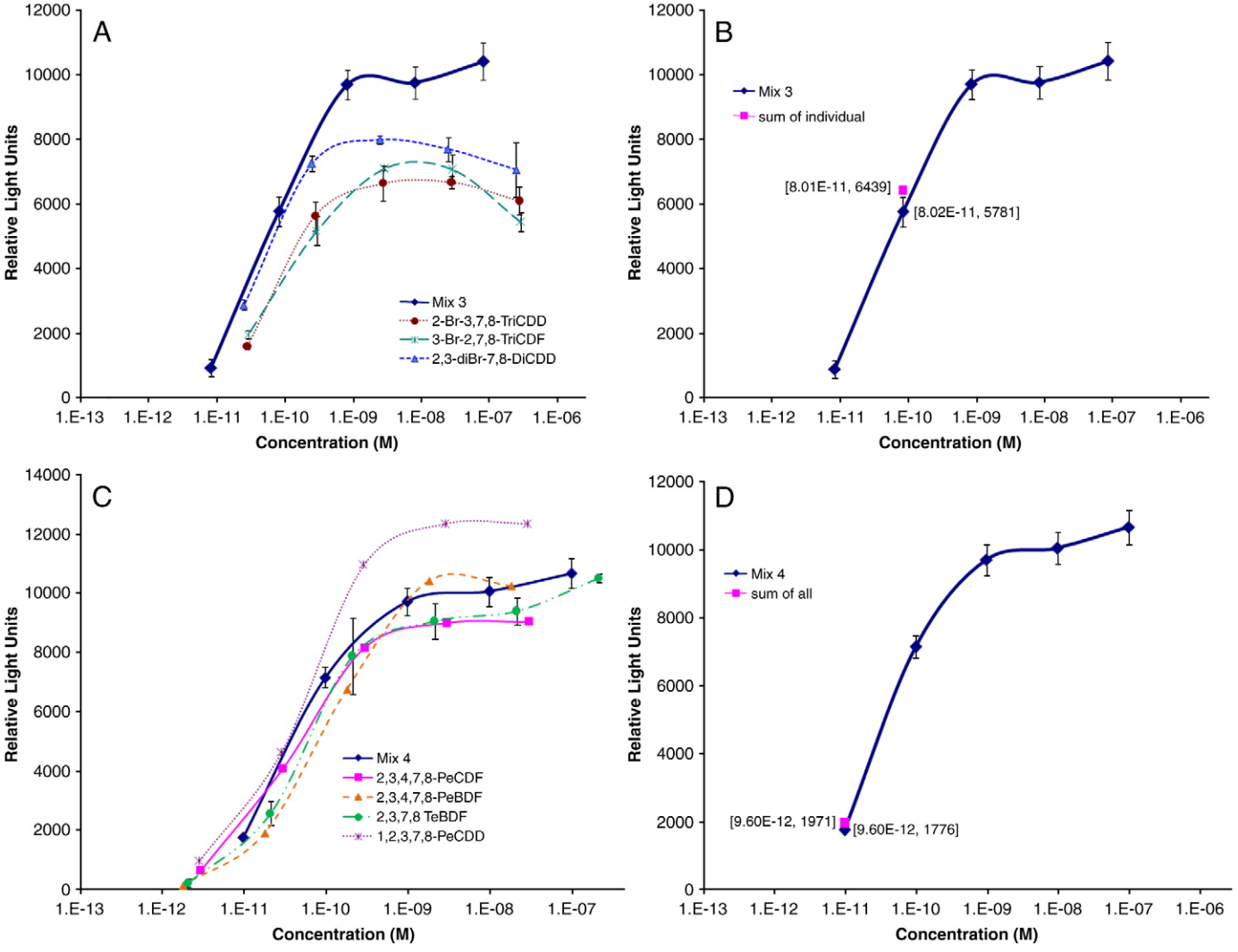

Fig. 2. Dose–response curves congeners determined by CALUX bioassay: A) individual and mixture of congeners of some PBCDD/F; B) mixture and sum of individual congeners of some PBCDD/F; C) individual and mixture of congeners of PeCDF, PeCDD, PeBDF and TeBDF and D) mixture and sum of individual congeners of PeCDF, PeCDD, PeBDF and TeBDF. Values with error bars represent the means ± SD of at least 3 measurements.

Please cite this article as: Samara F, et al, Toxicity comparison of chlorinated and brominated dibenzo-p-dioxins and dibenzofurans in industrial source samples by HRGC/HRMS and enzyme immunoassay, Environ Int (2010), doi:10.1016/j.envint.2009.12.005

Table 2
Comparative study of TEQs derived from HRGC/HRMS for PBDDs/Fs (using WHO-TEFs for PCDDs/Fs) and PCDDs/Fs vs. immunoassay-TEQs in selected industrial source line samples.

Sample ID	Total EIA-TEQ immunoassay (pg/train)	Total PBDDs/Fs WHO-TEQ HRGC/HRMS (pg/train)	Total PCDDs/Fs WHO-TEQ HRGC/HRMS (pg/train)
A-1	197 ± 42	1100	1.7
A-3	281 ± 17	5200	0.02
A-8	127 ± 42	8600	1.0
A-9	246 ± 37	8200	0.5
B-2	304 ± 60	19,000	22.8
B-4	300 ± 24	28,000	111.6
C-5	96 ± 11	310	6.5
C-6	70 ± 5	440	nd
C-7	nd	110	nd

A: inlet duct of the chain shredder line.
B: inlet duct of the hammer mill.
C: inlet in-duct of the Cathode Ray Tube (CRT) assembly line.
nd: not detected.

calculated by taking the average of the sum of the REPs for the individual congeners since they were all present at the same concentration in the mixture), for mixture 1(composed of PBDDs/Fs) (Fig. 1B) and mixture 3 (composed of PBCDDs/Fs) (Fig. 2B) were 11 % and 4%, respectively, higher than the REPs of the actual mixtures. In contrast, the composite REP for mixtures 2 (composed of PCDDs/Fs) (Fig. 1D) and 4 (composed of PCDDs/Fs and PBDFs) (Fig. 2D) were 11% and 41%, respectively, lower than the REPs of the actual mixtures. Interestingly, mixtures 1, 2, and 3 correspond to congeners of the same halogenation pattern (i.e., all chlorinated, all brominated, or all chlorinated/brominated) as opposed to mixture 4 where two congeners of PBDFs were mixed with two congeners of PCDDs/Fs.

Relative light units (RLUs) at only one data point were compared for the dose–response curves of the composite of individual congeners and the dose response curve of the mixture as shown in Figs. 1B, D, 2B, and D. Only one data point in the range of the curve was used in the case of the composite of individual congeners because the predicted curve could not be estimated. This estimation cannot be made due to the limitations of the isobole calculation method, where the predictions of the effects at higher levels or predictions cannot be applied to effect levels greater than that achieved by any single compound in the mixture. (Laier et al., 2003). The difference calculated as percent of the composite values for mixture 1 was + 32% higher than the composite REPs of individual congeners. Mixes 2, 3, and 4 had differences of −38%, −10%, and −10%, respectively, lower than the composite REPs of individual congeners (Figs. 1B, D, 2B, and D). The comparisons between the mixtures and the sums of the individual congeners obtained by REP and/or RLU suggests that PBDDs/Fs and PBCDDs/Fs have congener additive effects similar to that of PCDDs/Fs to a certain extent but a better comparison including more points should be made.

3.2. Immunoassay-TEQs vs. HRGC/HRMS TEQs from samples of an industrial source lines

We have previously shown that several brominated and mixed bromo/chloro dioxins and furans have measurable responses when using bioanalytical tools such as immunoassays and CALUX (Samara et al., 2009). In this paper we decided to use an immunoassay in order to evaluate its applicability for PBDDs/Fs TEQ measurements in samples. Table 2 shows results obtained using EIA versus HRGC/HRMS for PBDDs/Fs and PCDDs/Fs (pg/sampling train) on a total of nine extracts of samples collected from an electronic waste processing plant. These samples were selected because they had high concentrations of PBDDs/Fs and low concentrations of PCDDs/Fs. For reporting the WHO-TEQs for brominated dioxins and furans, we have used the analogous historical WHO-TEF 2005 values for PCDDs/Fs (Van den berg et al., 2006). These results show that the contribution by PCDDs/Fs in these samples to the total TEQs was more than 1000 times lower than that of PBDDs/Fs as shown in Fig. 2; hence, they will not be discussed for the rest of the paper. The sample relative percent difference (RPD) for the total EIA-TEQs vs. the total HRGC/HRMS PBDD/F WHO-TEQs in Table 2 was 186%, 194%, and 135% RPD for the sample lines A (sum of A-1, A-3, A-8, A-9), B (sum of B-2 and B-4), and C (sum of C-5, C-6 and C-7), respectively, where the highest TEQs were observed in the HRGC/HRMS PBDD/F based data. Roy et al. (2002) reported an underestimation of sample PCDDs/Fs TEQs when using this same immunoassay in soil samples, suggesting that competitive binding of interfering compounds to the antibody may have been a confounding factor. These results support those of Yang et al. (2006), suggesting that immunoassays could vary with matrix type and sample composition. Although a correlation between HRGC/HRMS and EIA values was not obtained, a very similar contamination pattern was observed in Fig. 4 where B samples had the highest concentrations followed by A and finally C.

In an immunoassay, the test response is a competitive inhibition of a polyclonal antibody specific to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and is strongest for analyte structures which are most similar to the target. For this specific assay the manufacturers have clearly specified that the anti-dioxin antibody binds to different PCDD/F congeners with different affinities and the same was observed for PBDDs/Fs in our previous work (Samara et al., 2009). The specificity of the test is predominantly for dioxins and furans that contain 3 to 6 chlorines, with a strong preference for the 2,3,7,8-Cl-substituted congeners. Fig. 3 shows the congener profile from tetra-BDDs/Fs to octa-BDDs/Fs for the sum of samples from the three sampling sites. For most of the samples the major contribution comes from hexa-BDDs/Fs and hepta-BDDs/Fs, which have very low to no cross-reactivity with the immunoassay (Samara et al., 2009; Cape Technologies, 2006).

We have previously characterized the immunoassay cross-reactivity for brominated dioxins and furans, and developed a spreadsheet program to input the GC/MS data and determine the expected EIA results (Samara et al., 2009). Fig. 4 compares the HRGC/HRMS WHO-TEQs calculated from the tetra-, penta-, and hexa-brominated dioxins/furans congeners in these industrial samples to the expected EIA-TEQs (Samara et al., 2009) and the actual EIA-TEQs obtained with the assay. For most of the samples, HRGC/HRMS WHO-TEQs were much higher than both the predicted EIA-TEQs and the actual EIA-TEQs. Interestingly, in one B sample (B-4) the expected EIA-TEQ is overestimated in respect to the HRGC/HRMS WHO-TEQs. One reason for this could

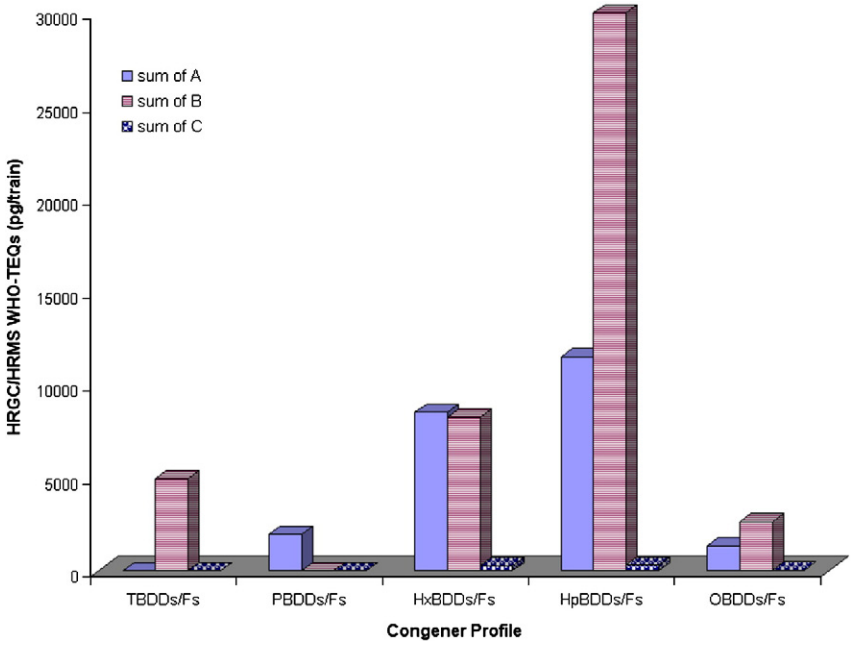

Fig. 3. Congener profile of the HRGC/HRMS WHO-TEQ reference values (pg/train) results for PBDDs/Fs (using WHO-TEFs for PCDDs/Fs) in samples collected at an e-waste recycling facility. Sample A is the sum of 4 samples at that specific line; B represents the sum of 2 samples and C, the sum of 3 samples.

Please cite this article as: Samara F, et al, Toxicity comparison of chlorinated and brominated dibenzo-p-dioxins and dibenzofurans in industrial source samples by HRGC/HRMS and enzyme immunoassay, Environ Int (2010), doi:10.1016/j.envint.2009.12.005

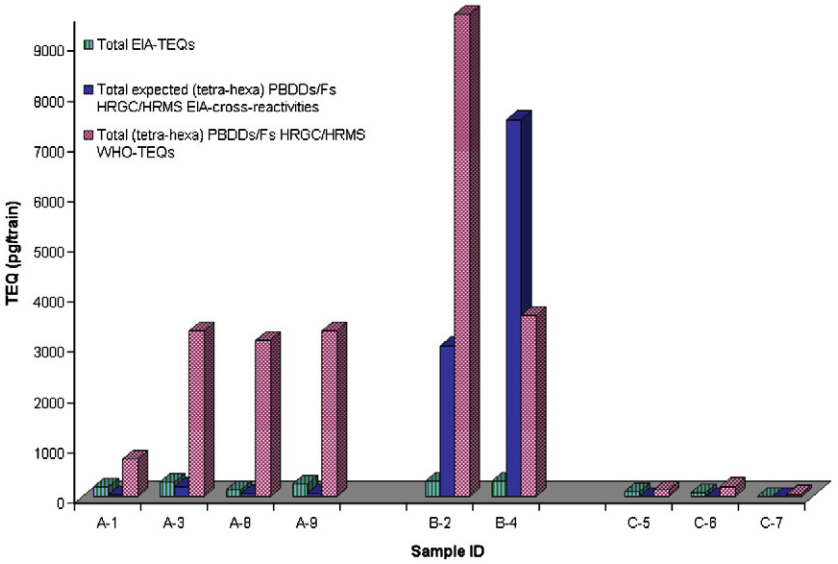

Fig. 4. Comparisons of the immunoassay-TEQs (pg/train) and HRGC/HRMS TEQs (pg/train) from (tetra-hexa) PBDDs/Fs using WHO-TEF reference values for PCDDs/Fs and the expected EIA results using our previously estimated enzyme immunoassay cross-reactivities (CR) in samples collected at an e-waste recycling facility.

be because in this sample the contribution to the total TEQ is mostly from 2,3,7,8-TBDF (data not shown) for which the immunoassay cross-reactivity is twice as much as the WHO-TEF. In the case of sample B-2 most of the contribution towards total TEQ is from 2,3,7,8-TBDF as well as 1,2,3,4,7,8-HxBDF (which has very low cross-reactivity with the assay). Although there might be more variables influencing this differences that at this moment we cannot explain. Moreover, this paper shows that the use of WHO-TEFs for PCDDs/Fs for calculating WHO-TEQs of PBDDs/Fs might overestimate the results and a better correlation between EIA and HRGC/HRMS values could be observed by taking into account only those congeners that have higher response with the assay.

4. Conclusions

Bioanalytical techniques can be used in combination with instrumental analysis to identify and characterize environmental and biological samples that contain PCDDs/Fs and PBDDs/Fs. The enzyme immunoassay has so far proven to be a valuable tool for measuring EIA-TEQs of PCDDs/Fs compounds in environmental samples and this paper shows its applicability towards measuring PBDDs/Fs as well. Our results show that the immunoassay and HRGC/HRMS observed the same concentration pattern, although EIA tends to underestimate calculated TEQs in the case of PBDDs/Fs but overestimate calculated TEQs when considering PCDDs/Fs alone. The experiments on the combined effect of mixtures of PCDD/F, PBDD/F, and PBCDD/F compounds showed congener additivity using the CALUX assay.

Acknowledgements

The authors of this paper would like to acknowledge Andrew Chu and George Clark (Xenobiotic Detection Systems, Durham, NC) for their help with the CALUX assay. Many thanks to Robert O. Harrison (Cape Technologies L.L.C, South Portland, ME) for his advice.

References

Behnisch PA, Hosoe K, Sakai S. Bioanalytical screening methods for dioxins and dioxin-like compounds — a review of bioassay/biomarker technology. Environ Int 2001;27:413–39.

Behnisch PA, Hosoe K, Sakai S. Brominated dioxin-like compounds: in vitro assessment in comparison to classical dioxin-like compounds and other polyaromatic compounds. Environ Int 2003;29:861–77.

Brown DG, van Overmeire I, Goeyens L, Denison MS, Clark GC. Analysis of brominated flame retardants and brominated dibenzodioxins and biphenyls for Ah receptor activation using the CALUX bioassay. Organohal Comp 2001;54:12–5.

Cape Technologies. L.L.C. Cape Technologies Calculation Module C. Excel workbook for iterative four parameter curve fit and analysis of sample data; 2004.

Cape Technologies. L.L.C. Cape Technologies High Performance Dioxin. Furan DF1 Kit insert (IN-DF1); 2006.

Carlson, R.E. High sensitivity immunoassay for polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans. United States Patent # 5, 674, 697. Ecochem Research, Inc. (Chaska, MN); 1997.

Carlson RE, Harrison RO. Measurement of PCDD/F TEQ by immunoassay: concept development and validation. Organohal Comp 1998;35:97–100.

Denison, M.S.; Brouwer, A.; Clark, G.C. Bioassay for detecting 2, 3, 7, 8-tetrachlorodibenzo-para-dioxin and TCDD-like compounds and novel recombinant cell line useful therefore. United States Patent # 5, 854, 010 (online at http://www.dioxins.com/images/Calux-patent%20copy.gif). Xenobiotic Detection Systems Inc. 1998. Accessed April 2008.

DeVito MJ, Diliberto JJ, Ross DG, Menache MG, Birnbaum LS. Dose–response relationships for polyhalogenated dioxins and dibenzofurans following subchronic treatment in mice I. CYP1A1 and CYP1A2 enzyme activity in liver, lung, and skin. Toxicol Appl Pharmacol 1997;147:267–80.

Estevez-Alberola M-C, Marco M-P. Immunochemical determination of xenobiotics with endocrine disrupting effects. Anal Bioanal Chem 2004;378:563–75.

Harrison RO, Carlson RE. An immunoassay for TEQ screening of dioxin/furan samples: current status of assay and applications development. Chemosphere 1997;34:915–28.

Hornung MW, Zabel EW, Peterson RE. Toxic equivalency factors of polybrominated dibenzo-*p*-dioxin, dibenzofuran, biphenyl, and polyhalogenated diphenyl ether congeners based on rainbow trout early life stage mortality. Toxicol Appl Pharmacol 1996;140:227–34.

Kedderis LB, Mills JJ, Andersen ME, Birnbaum LS. A physiologically based pharmacokinetic model for 2, 3, 7, 8-tetrabromodibenzo-p-dioxin (TBDD) in the rat — tissue distribution and CYP1A induction. Toxicol Appl Pharmacol 1993;121:87–98.

Laier P, Cederberg T, Larsen JC, Vinggaard AM. Applicability of the CALUX bioassay for screening dioxin levels in human milk samples. Food Add Contam 2003;20(6):583–95.

Li W, Wu WZ, Barbara RB, Schramm K-W, Kettrup A. A new enzyme immunoassay for PCDD/F TEQ screening in environmental samples: comparison to micro-EROD assay and to chemical analysis. Chemosphere 1999;38(14):3313–8.

Mason G, Zacharewski T, Denomme MA, Safe L, Safe S. Polybrominated dibenzo-*para*-dioxin and related-compounds — quantitative in vivo and in vitro structure–activity relationships. Toxicology 1987;44:245–55.

Nording M, Denison MS, Baston D, Persson Y, Spinnel E, Haglund P. Analysis of dioxins in contaminated soils with the CALUX and CAFLUX bioassays, an immunoassay, and gas chromatography/high-resolution mass spectrometry. Environ Toxicol Chem 2007;26(6):1122–9.

Roy S, Mysior P, Brzezinski R. Comparison of dioxin and furan TEQ determination in contaminated soil using chemical, micro-EROD, and immunoassay analysis. Chemosphere 2002;48:833–42.

Samara F, Gullett BK, Harrison RO, Chu A, Clark GC. Determination of relative assay response factors for toxic chlorinated and brominated dioxins/furans using an enzyme immunoassay (EIA) and a chemically-activated luciferase gene expression cell bioassay (CALUX). Environ Int 2009;35:588–93.

Scippo M-L, Eppe G, De Pauw E, Maghuin-Rogister G. DR-CALUX screening of food samples: evaluation of the quantitative approach to measure dioxin, furans and dioxin like PCBs. Talanta 2004;63:1193–202.

Shan G, Leeman WR, Gee SJ, Sanborn JR, Jones AD, Chang DPY, et al. Highly sensitive dioxin immunoassay and its application to soil and biota samples. Anal Chim Acta. 2001;444:169–78.

Please cite this article as: Samara F, et al, Toxicity comparison of chlorinated and brominated dibenzo-p-dioxins and dibenzofurans in industrial source samples by HRGC/HRMS and enzyme immunoassay, Environ Int (2010), doi:10.1016/j.envint.2009.12.005

Smialowicz RJ, DeVito MJ, Williams WC, Birnbaum LS. Relative Potency based on hepatic enzyme induction predicts immunosuppressive effects of a mixture of PCDDs/PCDFs and PCBs. Toxicol Appl Pharmacol 2008;227:477–84.
U.S. Environmental Protection Agency. Method 23 — Determination of Polychlorinated Dibenzo-p-dioxins and Polychlorinated Dibenzofurans from Municipal Waste Combustors. 1995.
U.S. Environmental Protection Agency. Preparation of the Components of the Modified Method 5 (Method 0010) Sampling Train for Analysis by SW-846 Method 8270. Research Triangle Park, NC. 2000.
Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, et al. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci 2006;93: 223–41.
Weber LWD, Greim H. The toxicity of brominated and mixed-halogenated dibenzo-*p*-dioxins and dibenzofurans: an overview. J Toxicol Environ Health 1997;50:195–215.
Wyrzykowska B, Tabor D, Gullett BK. Same-sample determination of ultratrace levels of polybromodiphenylethers, polybromodibenzo-*p*-dioxins/furans, and polychloro-dibenzo-p-dioxins/furans from combustion flue gas. Anal Chem 2009;81:4334–42.
Yang M, Kim Y, Oh E, Oh S, Im H, Lee E. Comparison of TEQS measured by enzyme immunoassay (EIA) and high resolution gas chromatography and mass spectrometry (HRGC/HRMS) in air samples of a residential area and a waste incineration site. Epidemiology: 2006;17(6):274.

Please cite this article as: Samara F, et al, Toxicity comparison of chlorinated and brominated dibenzo-p-dioxins and dibenzofurans in industrial source samples by HRGC/HRMS and enzyme immunoassay, Environ Int (2010), doi:10.1016/j.envint.2009.12.005