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Analysis of PCDD/Fs and dioxin-like PCBs in atmospheric deposition samples from the Flemish measurement network: Correlation between the CALUX bioassay and GC–HRMS

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ABSTRACT

Since the CALUX (Chemically Activated LUciferase gene eXpression) bioassay is a fast, sensitive and inexpensive tool for the analysis of a high number of samples, the use of this technique in routine analysis of atmospheric deposition samples may be a valuable alternative for GC–HRMS. In this study, a validated CALUX method was used for the analysis of PCDD/Fs and dioxin-like PCBs in more than 90 atmospheric deposition samples for different locations in Flanders. The samples were taken in residential and agricultural areas, where a threshold limit of 21 pg WHO-TEQ m^{-2} d^{-1} for the sum of PCDD/Fs and dioxin-like PCBs was set, and in industrial zones and natural reserves, where no official threshold limit is available. The results from the Flemish measurement program showed correlation between CALUX and GC–HRMS for all the samples, originating from the different areas (R^2 of 0.81, 0.53 and 0.64 for dl-PCBs, PCDD/Fs and sum of both fractions, respectively). Median CALUX/GC–HRMS ratios of 2.0, 0.9 and 1.3 were reported for the PCDD/Fs, dioxin-like PCBs and the sum of both fractions, respectively. The results show that the CALUX bioassay is a valuable alternative tool for the classic GC–HRMS analysis of atmospheric deposition samples in the Flemish measurement network.

1. Introduction

Although the risks of PCDD/Fs and PCBs are well known and a lot of research has been done during the recent years, these compounds are still environmental pollutants of major concern. Even in the 21st century, high concentrations of PCDD/Fs and PCBs are still found in sediment (Sanctorum et al., 2007), soil (Kakimoto et al., 2004), fish (Baeyens et al., 2007) and many other matrices such as blood, milk, etc. (Wittsiepe et al., 2007; Nakamura et al., 2008; Croes et al., 2011a). Not all contamination is due to historical pollution (Wevers et al., 2004). In Flanders, the Flemish Environment Agency (VMM) has a monthly measurement program for the monitoring of atmospheric depositions of PCDD/Fs and PCB 126 in different locations in Flanders. Since the beginning of the monitoring campaign in Flanders in 1993, concentrations have decreased drastically, but in certain locations the warning limit, set by the VMM based on tolerable intake values and VDI guideline 2090/1, of 6 pg WHO-TEQ m^{-2} d^{-1} for mean monthly values was regularly exceeded (VMM reports PCDD/Fs and PCB 126 in deposition samples; VDI 2090/1 guidelines). In 2010, VMM adopted a new threshold value for the sum of PCDD/Fs and dioxin-like PCBs (dl-PCBs) of 21 pg WHO-TEQ m^{-2} d^{-1}, where it was assumed that PCB 126 accounts for 70% of the total dioxin-like PCB toxic equivalent. Since PCDD/Fs and dioxin-like PCBs are primarily taken up by humans through food, it was assumed that measuring points located in industrial areas have a minor impact on human health. The new warning limit was therefore only implemented for agricultural and residential areas (Desmedt and Roekens, 2011; Vandermarken et al., 2011).

Since PCDD/Fs and dioxin-like PCBs are persistent in the environment, can accumulate in the fat tissue of animals and humans and have hormone disrupting properties, it is important to have a reliable, fast and inexpensive method to monitor, on a regular basis, the concentration of these pollutants. Until now, only GC–HRMS methods are available for the quantification of PCDD/Fs and dioxin-like PCBs in atmospheric deposition samples (Moon et al., 2005; Man et al., 2006; Nizzetto et al., 2006; Oka et al.,

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2006). However, since GC–HRMS analysis is four to five times more expensive compared to the CALUX bioassay, only some priority hotspots can be measured systematically. When applying the CALUX bioassay, it would be possible to monitor more locations, spread out over whole Flanders, to get an insight in the pollution pressure and to locate possible new pollution sources. CALUX would also be interesting for monitoring the pollution spread in different wind directions and to investigate the concentration gradient at different distances from certain point sources (e.g. a waste incinerator). Very recently, for the first time, a new CALUX method was optimized and validated for the separate analysis of PCDD/Fs and dioxin-like PCBs in atmospheric deposition samples (Croes et al., 2011b). In this study, a comparison will be made between CALUX and GC–HRMS results from more than 90 atmospheric deposition samples from the Flemish measurement network. All CALUX and GC–HRMS results will be compared to the warning limits, set by the VMM, to determine false positive and false negative results. The final objective is then to define the correlation between both techniques and to investigate if the CALUX bioassay can be used as a valuable alternative of or in addition to GC–HRMS for the analysis of PCDD/Fs and dioxin-like PCBs in atmospheric deposition samples in Flanders.

2. Materials and methods

2.1. Chemicals and standards

Hexane (for PCDD/Fs and PCBs, minimum 96%), acetone (Pesti-S grade, minimum 99.9%) and toluene (for PCDD/Fs and PCBs, minimum 99.8%) were purchased from Biosolve (The Netherlands). Neutral alumina (activated, 150 mesh), silver nitrate (5 wt.% on silica gel 60), ethyl acetate pestanal, silica gel 60 for column chromatography and C18 ENVI disks were purchased from Sigma–Aldrich (Germany). Sulfuric acid (95–97%, ACS reagent), ethanol of ultra pure grade and DMSO were obtained from Merck (Germany). Anhydrous sodium sulfate was purchased from Boom (The Netherlands) and the X-CARB from XDS (USA). The glass fiber filters were purchased from Whatman (UK). The standard solution of 2,3,7,8-TCDD (50 ng mL^{-1}) was purchased from Campro Scientific (The Netherlands).

2.2. Sample collection

The samples were collected in Bergerhoff deposition gauges from 17 different geographical locations in Flanders (located in the regions Menen, Genk, Gent, Mol, Beerse, Zelzate, Merksem, Oostrozebeke, Roeselare and Stabroek) and during five different time periods (August 2009–September 2010). In total, 92 samples were analyzed by CALUX and 51 samples (from the same locations and time periods) were analyzed by GC–HRMS. The CALUX samples were analyzed at the Free University of Brussels (VUB, Brussels, Belgium) according to the validated method described by Croes et al. (2011b). Briefly, the samples were filtered using a ENVI C18 disk in-between two glass fiber filters and extracted with an accelerated solvent extractor (ASE) using a hexane/acetone (1/1, v/v) mixture. Ten milliliter extract (out of 30 mL) was cleaned up using a multi-layer silica gel column, containing acid silica (33%, w/w), silver nitrate and deactivated neutral alumina, coupled in series with a carbon column. After extraction and clean up, the dioxin-like PCB and PCDD/F fraction were evaporated and redissolved in 3 and 5 mL hexane, respectively. The PCB fractions were measured with the sensitive H1L7.5c1 mouse hepatoma cell line, while the PCDD/F fractions were measured using either the H1L6.1c3 or the more sensitive H1L7.5c1 mouse hepatoma cell lines (Denison et al., 2008; He et al., 2011; Van Langenhove et al., 2011). RLU values were expressed as% RLU induction, with the highest RLU response of the 2,3,7,8-TCDD standard calibration curve set at 100% (Fig. 1). The choice of cell line, used for measuring the sample extracts, was be made according to the response rate of the sample. If possible, a full dose-dilution curve was established to calculate the CALUX-BEQ values. The GC–HRMS samples were analyzed at SGS (Melsele, Belgium), according to the method described by Desmedt and Roekens (2011). Briefly, the solid fraction was filtered over a pre-extracted glass fiber filter and dried overnight at 55 °C, while the water fraction was extracted two times with toluene. The glass fiber filter was spiked with internal ^{13}C standards and extracted by soxhlet for 24 h with the dried toluene extract of the water fraction. The extract was then concentrated and cleaned up by column chromatography on Mixed silica and Alumina B Super I. ^{13}C-1,2,3,4-TCDD and 1,2,3,7,8,9-HxCDD were used as the syringe spike. The 17 PCDD/F congeners with 2,3,7,8-Cl-substitution and PCB126 were analyzed by HRGC–HRMS, using a DB5-MS column of 60 m × 0.25 mm × 0.1 μm and a Micromass Autospec Ultima mass spectrometer. The detection limits of all the congeners were below 1 pg m^{-2} d^{-1}, fulfilling the requirements of the VDI 2090 standard. WHO-TEF values from 1998 were used for calculating the WHO-TEQs (Van den Berg et al., 1998).

3. Results

3.1. Experimental set up for CALUX bioassay analysis

Since atmospheric deposition samples from different locations in Flanders can differ significantly in concentration of PCDD/Fs and/or dioxin-like PCBs, it is difficult to establish the optimum dilution range. Contrary to biological samples (i.e. milk and blood),

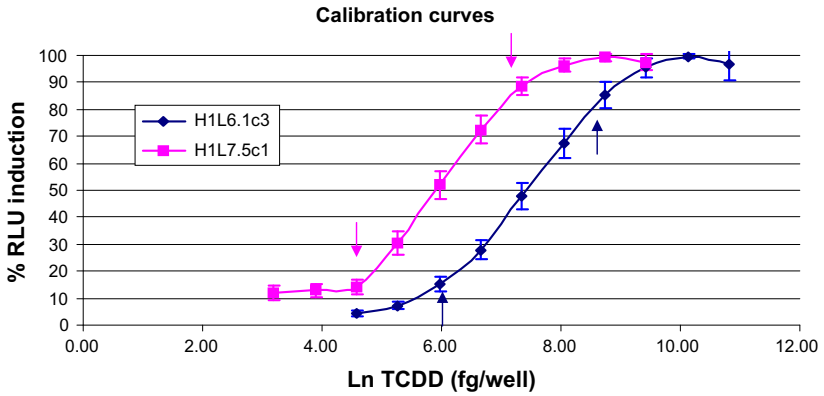

Fig. 1. 2,3,7,8-TCDD standard calibration curves for the H1L6.1c3 and the H1l7.5c1 cell lines. The working range is indicated by the arrows.

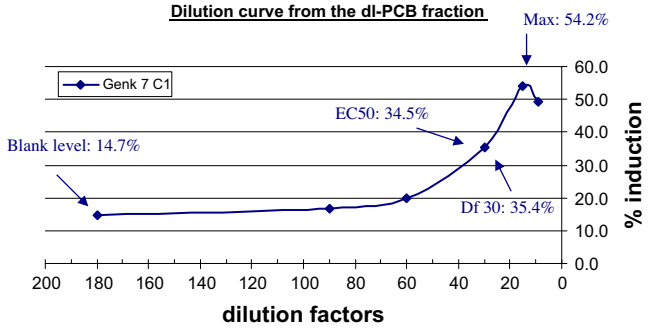

Fig. 2. An example of a full dose dilution curve for the PCB fraction of a sample extract with calculation of the EC50 value. Analysis was done with the H1L7.5c1 cell line. df is dilution factor; Max is maximum induction.

Table 1
% RLU inductions and CALUX-BEQ values, calculated with the inverse prediction method, for 4 dl-PCB sample extracts from campaign 3. df is dilution factor. Data in bold were CALUX values, close to the EC50 value.

Sample	df 7.5	df 9	df 15	df 30	df 60	df 90
	% RLU induction from the dl-PCB fraction					
Gent 9.2	14.9	**32.4**	14.6			
Menen 9.2	24.7	**25.7**	14.3			
Menen 6.1		**52.1**	**27.2**	21.3	14.1	12.6
Genk 10.2	**22.0**	16.6	15.1			
	CALUX-BEQ values in pg BEQ m^{-2} d^{-1}					
Gent 9.2	1.29	**3.68**	2.51			
Menen 9.2	2.30	**2.89**	2.45			
Menen 6.1		**5.95**	**5.03**	7.25	<LOQ	<LOQ
Genk 10.2	**1.97**	1.31	<LOQ			

it is for environmental samples not possible to establish a full dose dilution curve from a pooled sample extract that can be used as a benchmark for this type of sample matrix and that will provide the optimum dilution factor (Croes et al., 2011a). Therefore, it was decided to establish for every atmospheric deposition sample a (full dose) dilution curve, for both the dioxin-like PCB and PCDD/F fractions, to calculate the CALUX-BEQ concentration using Hill regressions ($y_i = y_0 + \frac{m \cdot x_i^h}{k^h + x_i^h} + \varepsilon_i$). First, one dilution point was measured to determine the degree of contamination. Choice of cell line and further dilutions were made depending on the% Relative Light Unit (RLU) induction of this first measurement. In the next paragraphs, a more detailed explanation about the calculation of the PCB and PCDD/F concentrations for routine analysis is given.

3.2. Calculation of the dioxin-like PCB fraction with the CALUX bioassay

Due to the lower Relative Potency (REP) values, the PCB fraction was measured with the sensitive H1L7.5c1 cell line. First, a dilution factor (df) of 15 was used for range finding (600 μL extract in one well). If the% RLU induction was lower than 25%, the sample was classified as low polluted and df of 7.5 and 9 were measured. If the% RLU induction was between 25% and 50%, the sample was medium contaminated and df of 30, 60, 90 and 9 (this to account for possible antagonistic or inhibition effects) were analyzed. When the% RLU induction was higher than 50% (highly contaminated samples), df of 30, 60, 90, 150 and/or 300 were analyzed and confirmation with the H1L6.1c3 cell line was done. Samples below the LOD/LOQ were analyzed at df 9 after spiking of the sample extract with a QC standard solution and the CALUX-BEQ value was calculated using the inverse prediction method (Elskens et al., 2011). For samples with a full dose–response curve, the effective concentration (EC) method was used, while for samples for which the Hill fit is inaccurate, the CALUX-BEQ assessment can be performed using the slope ratio method after linearization with Box–Cox transformations, i.e. $[\text{slope}]_{\text{SAMPLE}}$ over $[\text{slope}]_{\text{TCDD}}$ (Elskens et al., 2011).

In Fig. 2, an example of a full dose dilution curve for the dl-PCB fraction of a sample (location Genk 7 sample 2, campaign 1 September–August 2009) is given. At df 15 the maximum (max) induction was found (54%), while at df 180 the induction of the sample extract was approximately at DMSO blank level (14.7%). At dilution factors lower than 15, the% RLU induction was again decreasing due to matrix effects (toxic or inhibition effects). These effects were also found in the PCDD/F fraction of human serum samples (Croes et al., 2011a). In this example, the induction level corresponding to the EC50 value was 34.5% (Fig. 2). This RLU induction was then expressed as a concentration in pg BEQ m^{-2} d^{-1} by using the four-parameter Hill function and taking into account the surface of the Bergerhoff gauges (0.0231 m^2) and the sampling time (30 ± 2 d). Following this approach, a CALUX-BEQ value of 11.5 pg BEQ m^{-2} d^{-1} was found for the PCB fraction of the sample Genk 7 from campaign 1 (C1).

Table 1 gives an overview of the% RLU induction and the BEQ calculation of four dl-PCB sample extracts from campaign 3. Samples Gent 9.2, Menen 9.2 and Genk 10.2 were low contaminated, while sample Menen 6.1 showed a higher dl-PCB contamination level. Therefore, after analyzing the initial dilution factor of 15, also df 7.5 and 9 were analyzed for the samples from Gent and Menen. Samples Gent 9.2 and Menen 9.2 showed antagonism/inhibition for df 7.5, while for the sample Genk10.2 a gradual increase in% RLU induction was seen. This example shows that it is important to measure both df 7.5 and 9 for quantification of the sample extract. If only df 7.5 would be measured, a too low BEQ-value would be reported for samples Gent 9.2 and Menen 9.2. If only df 9 would be measured, the quantification would be correct for samples Gent 9.2 and Menen 9.2, but there would be an underestimation of the dl-PCB level for sample Genk 10.2, since the induction level for this sample was very low (16.6%, which is in the lower part of the curve, very close to the LOQ). The uncertainty on the result is also higher in this range of low induction. For sample Menen 6.1, the induction level at df 15 was higher compared to the other samples in Table 1 and here, more dilution points could be measured to establish the full dose–response dilution curve and to determine the EC50 value (32.4%), according to the method described above. A final concentration of 5.49 pg BEQ m^{-2} d^{-1} was reported for this sample.

This approach of establishing a full dose dilution curve where possible and analyzing the three lowest dilution factors for low contaminated samples was adopted for all dl-PCB fractions analyzed in atmospheric deposition samples.

3.3. Calculation of the PCDD/F fraction with the CALUX bioassay

For the calculation of the CALUX-BEQ of the PCDD/F fraction, a similar approach to that of the dl-PCB fraction was used. First, a range finding was done by analysis of one sample dilution (df 30; 1 mL extract in two wells) with the H1L6.1c3 cell line. If the% RLU induction was lower than 15%, the sample was classified as low polluted and further measurements were done with the more sensitive H1L7.5c1 cell line. Therefore, a df of 15 was measured with this sensitive cell line. If the% RLU induction was higher then 50%, dilution factors of 30–300 were analyzed. If the% RLU induction was lower then 50%, df of 7.5 and 30 (to account for possible antagonistic of inhibition effects) were analyzed. If the% RLU induction, measured with the H1L6.1c3 cell line was between 15% and 50%, the sample was medium contaminated and df of

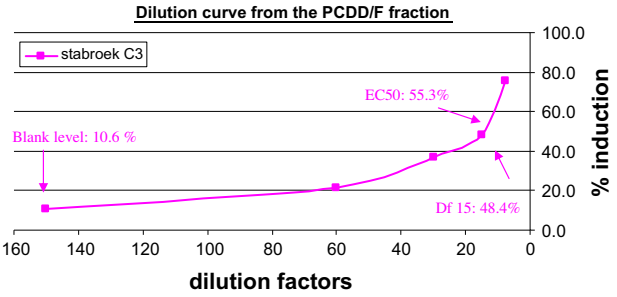

Fig. 3. An example of a full dose dilution curve for the PCDD/F fraction of a sample extract with calculation of the EC50 value. Analysis was done with the H1L6.1c3 cell line. df is dilution factor.

7.5, 15, 60 and 150 were analyzed. When the% RLU induction was higher then 50% (highly contaminated samples), df of 15, 40, 60, 150 and/or 300 were analyzed.

In Fig. 3, an example of a full dose dilution curve for the PCDD/F fraction of a sample (location Stabroek sample 2, campaign 3 December 2009–January 2010) is given. In this example, no maximum induction was reached, since there was not enough sample extract available to analyze lower dilution factors. However, for PCDD/F fractions, it can be assumed that the maximum induction will reach 100%, similar to the maximum induction of the TCDD standard calibration curve. For this sample (Stabroek from campaign C3), an EC50 value of 55.3% was determined and a CALUX-BEQ value of 38.7 pg BEQ m^{-2} d^{-1} could be reported for the PCDD/F fraction.

Table 2 gives an overview of the% RLU induction and the BEQ calculation of three PCDD/F sample extracts from campaigns C1 and C3. Sample extracts from Menen 6.2 and 9 were analyzed with the H1L6.1c3. The sample extract from Menen 9.4 was analyzed with the H1L7.5c1 cell line, since the response was below 15% RLU induction at df 30 using the H1L6.1c3 cell line. For samples Menen 6.2 and Menen 9 a full dose response curve was established with the H1L6.1c3 cell line, but the maximum could not be reached. Since the% RLU induction is still gradually increasing with lower df, it can be assumed that the maximum will be close to 100% induction. The calculated EC50 values were respectively 54.0 and 55.9% for the samples from Menen 6.2 and Menen 9, yielding concentrations of respectively 23.3 and 23.9 pg BEQ m^{-2} d^{-1}. Sample Menen 9.4 gave an induction lower than 15% at df 30 and therefore a full dose dilution curve was established with the more sensitive H1L7.5c1 cell line. Here, also the maximum of 100% was not reached, but the induction levels were also increasing with lower dilution factors. The EC50 value for this sample was 54.4%, which yielded a BEQ concentration of 14.9 pg BEQ m^{-2} d^{-1}.

This approach of establishing a full dose dilution curve where possible and analyzing the samples with lower induction levels with the more sensitive H1L7.5c1 cell line was followed for all PCDD/F fractions analyzed in atmospheric deposition samples.

3.4. Correlation between CALUX and GC–HRMS

The sample locations were divided in four regions (rural, agricultural, industrial and nature reserves). For the first two regions, the VMM adopted in 2010 a new GC–HRMS threshold value for the deposition of the sum of PCDD/Fs and dl-PCBs of 21 pg WHO-TEQ m^{-2} d^{-1}. The GC–HRMS and CALUX results for the eight sampling areas in these regions are given in Table 3. To compare the GC–HRMS and CALUX results and to evaluate the number of samples that were higher than the GC–HRMS warning limit, the sum of PCDD/Fs and dl-PCBs for GC–HRMS measurements was calculated according to the following formula: Σ(PCDD/Fs and dl-PCBs GC–HRMS) = [WHO-TEQ PCDD/Fs]$_{GC-HRMS}$ + ([WHO-TEQ PCB 126]$_{GC-HRMS}$/0.7), where it was assumed that PCB 126 accounts for 70% of the total dl-PCB toxic equivalent (Desmedt and Roekens, 2011). From Table 3, it is clear that dl-PCB values were generally lower with the CALUX bioassay than with GC–HRMS, except for some of the very low PCB concentrations (close to the limit of quantification; i.e. Beerse 1 from campaign 3). CALUX/GC–HRMS ratios ranged from 0.3 to 3.9, with a median ratio of 0.9. For the PCDD/F fraction, CALUX values were higher compared to the GC–HRMS WHO-TEQ values. CALUX/GC–HRMS ratios ranged from 0.7 to 5.5, with a median ratio of 1.9. For some sampling locations, the ratios were relatively constant in time (i.e. Stabroek), while for other locations (i.e. Beerse) more variation was found during different sampling campaigns. This can be due to interfering compounds, present in the atmospheric deposition sample extract, that can bind to the receptor of the CALUX bioassay and/or to seasonal effects. For the sum of PCDD/Fs and dl-PCBs, ratios between 0.3 and 5.1 were found, with a median value of 1.3. Also, one false-negative CALUX result (Beerse C2, with 19 pg BEQ m^{-2} d^{-1} for the sum of the CALUX results and 27 pg WHO-TEQ m^{-2} d^{-1} for the sum of the GC–HRMS results) was reported and for six measurement locations a false-positive result (Beerse C1 and C3, Menen C3, Stabroek C3, Oostrozebeke C4 and Roeselare C2) was observed.

Table 2
% RLU inductions and CALUX-BEQ values, calculated with the inverse prediction method, for 3 PCDD/F sample extracts from campaigns 1 and 3. df is dilution factor. Data in bold were CALUX values, close to the EC50 value.

Sample	df 7.5	df 15	df 30	df 60	df 150
	% RLU induction from the PCDD/F fraction				
Menen 6.2	**54.6**	31.6	26.4	14.7	8.0
Menen 9.4	66.3	**58.3**	**41.2**	20.7	9.0
Menen 9	**54.2**	40.9	29.9	19.1	11.7
	CALUX-BEQ in pg BEQ m^{-2} d^{-1}				
Menen 6.2	**23.3**	22.9	38.1	43.5	<LOQ
Menen 9.4	9.73	**14.5**	**15.9**	12.4	<LOQ
Menen 9	**23.9**	30.8	42.1	53.3	83.7

The GC–HRMS and CALUX results for the eight sampling areas in the industrial zones and the natural reserves are given in Table 4. For these areas, since 2010, no official warning limits are available. To evaluate the GC–HRMS and CALUX results, depositions were checked upon the warning limits of 6 pg WHO-TEQ m^{-2} d^{-1} (medium elevated) and 26 pg WHO-TEQ m^{-2} d^{-1} (highly elevated). From this Table, it is clear that the CALUX/GC–HRMS ratios are comparable to the results found in rural and residential areas: dl-PCBs were generally a bit lower with CALUX and PCDD/Fs were generally higher with CALUX compared to GC–HRMS. These findings were also often reported in the literature for different matrices (Tsutsumi et al., 2003; Van Overmeire et al., 2003; Van Wouwe et al., 2004) and confirm the results found during validation of the above described method (Croes et al., 2011b). The CALUX/GC–HRMS ratios ranged from 0.2 to 4.4 (median 0.9) and from 0.7 to 4.6 (median 2.2) for the dl-PCB and PCDD/Fs, respectively. For the sum of PCDD/Fs and dl-PCBs, ratios between 0.3 and 4.1 were found, with a median value of 1.5. For the PCB fraction, one false-positive CALUX result was reported (Genk 10 C1, with a CALUX value <6 pg TEQ m^{-2} d^{-1} and a GC–HRMS value >6 pg WHO-TEQ m^{-2} d^{-1}) and for three measurement locations a false-positive result (Genk 2 C3, Genk 7 C1 and Menen 6 C3) was found. For the PCDD/F fraction no false-negative results were observed, but for 12 measurement locations a false-positive result was found (Genk 10 C1 and C3, Genk 2 C1, Genk 7 C1, C2 and C3, Genk 8 C1 and C3, Genk 9 C1, C2 and C3 and Zelzate 3 C5). Duplicate or triplicate CALUX measurements were generally in good agreement, for both PCDD/Fs and dl-PCBs (e.g. Genk 10 C3, Genk 2 C5, Gent 9 C2). This

Table 3
GC–HRMS and CALUX results for the PCDD/Fs and dl-PCBs fractions, collected during five different sampling campaigns on eight different residential and agricultural locations in Flanders. The numbers in bold were higher than the new GC–HRMS threshold value of 21 pg WHO-TEQ m^{-2} d^{-1}.

Location	Campaign	GC–HRMS			CALUX			CALUX/GC–HRMS ratio		
		PCDD/Fs	PCB126	Sum PCDD/Fs and dl-PCBs	PCDD/Fs	dl-PCBs	sum PCDD/Fs and dl-PCBs	PCDD/Fs	dl-PCBs	sum PCDD/Fs and dl-PCBs
Merksem	2	4.7	2.1	7.7	4.2	2.2	6.3	0.9	1.0	0.8
Merksem	2				6.7	1.5	8.3	1.4	0.7	1.1
Merksem	5	3.9	3.1	8.3	5.5	0.9	6.4	1.4	0.3	0.8
Merksem	5				6.8	1.5	8.3	1.7	0.5	1.0
Beerse	1	14	2.1	17	78	2.8	**80**	5.5	1.3	4.8
Beerse	2	13	9.6	**27**	15	3.4	19	1.2	0.3	0.7
Beerse	3	8.7	0.8	9.8	47	3.2	**51**	5.5	3.9	5.1
Beerse	5	9.7	1.6	12	12	1.1	13	1.2	0.7	1.1
Beerse	5				7.1	0.7	7.7	0.7	0.4	0.6
Menen 10	1	4.8	29	**46**	17	16	**33**	3.5	0.6	0.7
Menen 10	2	3.3	1.8	5.9	7.6	2.1	9.8	2.3	1.2	1.7
Menen 10	3	7.2	1.8	9.8	27	2.4	**29**	3.7	1.3	3.0
Menen 10	3				23	1.5	**25**	3.2	0.8	2.5
Roeselare	2	9.3	2.9	13	15	6.7	**22**	1.6	2.3	1.6
Roeselare	2				12	5.9	17	1.2	2.0	1.3
Roeselare	4	8.0	1.3	9.9	7.3	0.8	8.0	0.9	0.6	0.8
Roeselare	4				20	0.7	21	2.5	0.5	2.1
Roeselare	5	6.9	2.6	11	9.9	3.1	13	1.4	1.2	1.2
Roeselare	5				10	3.4	14	1.5	1.3	1.3
Stabroek	1	6.7	2.3	10	13	1.8	15	2.0	0.8	1.5
Stabroek	1				13	2.0	15	2.0	0.9	1.5
Stabroek	2	4.2	0.9	5.5	15	1.9	17	3.5	2.1	3.0
Stabroek	2				8.7	1.1	10	2.1	1.2	1.8
Stabroek	3	15	1.7	17	39	2.5	**41**	2.6	1.5	2.4
Stabroek	3				22	2.5	**24**	1.5	1.5	1.4
Stabroek	5	3.6	1.0	5.0	4.6	3.0	7.6	1.3	3.0	1.5
Stabroek	5				9.2	1.7	11	2.6	1.7	2.2
Menen 8	5	5.0	8.9	18	8.3	2.4	11	1.7	0.3	0.6
Menen 8	5				11	7.1	18	2.1	0.8	1.0
Menen 8	5				13	3.1	16	2.6	0.3	0.9
Menen 8	5				7.7	3.3	11	1.5	0.4	0.6
Menen 8	5				11	3.2	14	2.1	0.4	0.8
Menen 9	1	14	140	**214**	24	48	**71**	1.7	0.3	0.3
Menen 9	2	8.4	13	**28**	29	24	**53**	3.4	1.8	1.9
Menen 9	3	7.7	4.2	14	15	3.7	18	1.9	0.9	1.3
Menen 9	3				12	2.9	14	1.5	0.7	1.1
Menen 9	3				14	1.8	15	1.8	0.4	1.1
Menen 9	3				15	2.5	17	1.9	0.6	1.3
Menen 9	3				17	1.2	18	2.2	0.3	1.3
Oostrozebeke	4	9.6	1.0	11	30	0.9	**31**	3.2	0.9	2.8
Oostrozebeke	5	31	5.5	**39**	29	2.8	**32**	0.9	0.5	0.8

means that the CALUX results were reproducible and that the differences between the CALUX and GC–HRMS results were due to interfering compounds in the extracts. Since PAHs (and other acid-labile compounds) and heavy metals were removed during extraction and clean up (Croes et al., 2011b), other unknown molecules that can bind to the Ah-Receptor probably cause the higher/lower responses found with the CALUX bioassay. More research is needed to indentify these interfering compounds. Of course, when comparing the CALUX and GC–HRMS results, it is also important to take into account the uncertainty on the GC–HRMS result. This was not done here, since the GC–HRMS measurement was performed only once on each sample.

When taking into account all 92 samples that were given in Tables 3 and 4, a median CALUX/GC–HRMS ratio of 2.0 and 0.9 was found for the PCDD/Fs and dl-PCBs, respectively. For the sum of the two fractions a ratio of 1.3 was reported. Coefficients of determination (R^2) of 0.81, 0.53 and 0.64 were found for dl-PCBs, PCDD/Fs and sum of both fractions, respectively. For the dioxin-like PCBs some more variation of the CALUX/GC–HRMS ratio was found compared to the PCDD/F fraction. This could be expected, since with GC–HRMS only PCB 126 was measured, while CALUX measures all dioxin-like PCBs.

4. Conclusions

The analysis of PCDD/Fs and dioxin-like PCBs in atmospheric deposition samples is commonly performed by GC–HRMS, but the CALUX bioassay could be used instead as a simple and fast screening and quantification method. During almost the whole year 2009–2010, atmospheric deposition samples were collected from different locations in Flanders and analyzed with both CALUX and GC–HRMS. With GC–HRMS only single sample analysis were performed, while with the CALUX bioassay duplicate or triplicate analyses were done. Also, with GC–HRMS all PCDD/F congeners and one PCB congener (PCB 126, the most toxic dl-PCB with a TEF value of 0.1) were measured, while with the CALUX bioassay quantification of the CALUX-BEQ value resulted from the analysis of a full dose–response curve and the calculation of the EC50 value using the Hill function (or Box–Cox transformation, when the Hill fit was inaccurate).

Overall, for most samples a satisfactory correlation was found between the CALUX and GC–HRMS results for the four sampling areas and for both the PCDD/Fs and dl-PCBs. Coefficients of determination (R^2) of 0.81, 0.53 and 0.64 were reported for dl-PCBs, PCDD/Fs and sum of both fractions, respectively. A median

Table 4
GC–HRMS and CALUX results for the PCDD/Fs and dl-PCBs fractions, collected during five different sampling campaigns on eight different industrial locations and 1 nature reserve (Zelzate) in Flanders. The numbers in bold were higher than the GC–HRMS warning limit of 6 pg WHO-TEQ m^{-2} d^{-1}. The numbers in italic were higher than the GC–HRMS limit of 26 pg WHO-TEQ m^{-2} d^{-1}.

Location	Campaign	GC–HRMS			CALUX			CALUX/GC–HRMS ratio		
		PCDD/Fs	PCB126	Sum PCDD/Fs and dl-PCBs	PCDD/Fs	dl-PCBs	Sum PCDD/Fs and dl-PCBs	PCDD/Fs	dl-PCBs	Sum PCDD/Fs and dl-PCBs
Genk 10	1	*2.4*	*2.2*	5.5	6.5	9.7	16	2.7	4.4	2.9
Genk 10	2	*1.4*	*0.5*	2.1	*5.7*	*2.1*	7.8	4.1	4.1	3.6
Genk 10	2				*5.8*	*0.4*	6.2	4.2	0.8	2.9
Genk 10	3	*5.0*	*1.3*	6.9	14	*2.5*	17	2.8	1.9	2.4
Genk 10	3				14	*2.0*	16	2.7	1.5	2.3
Genk 2	1	2.6	13	21	8.7	8.4	17	3.3	0.7	0.8
Genk 2	2	7.4	**27**	46	8.9	14	23	1.2	0.5	0.5
Genk 2	2				11	11	22	1.5	0.4	0.5
Genk 2	3	6.7	6.2	16	24	*4.6*	28	3.5	0.7	1.8
Genk 2	3				17	*3.8*	21	2.6	0.6	1.4
Genk 2	3				25	*2.9*	28	3.8	0.5	1.8
Genk 2	5	**47**	**188**	316	**40**	**69**	109	0.8	0.4	0.3
Genk 2	5				**41**	**67**	108	0.9	0.4	0.3
Genk 2	5				**37**	**70**	107	0.8	0.4	0.3
Genk 2	5				**38**	**43**	82	0.8	0.2	0.3
Genk 7	1	*4.2*	7.3	15	15	12	26	3.5	1.6	1.8
Genk 7	1				14	*3.8*	17	3.2	0.5	1.2
Genk 7	2	*3.3*	*4.1*	9.2	12	*3.0*	15	3.7	0.7	1.7
Genk 7	3	*4.3*	*0.7*	5.3	9.2	*2.3*	12	2.1	3.3	2.2
Genk 7	3				*5.1*	*1.1*	6.2	1.2	1.6	1.2
Genk 8	1	*2.7*	*3.8*	8.1	8.9	*2.8*	12	3.3	0.7	1.4
Genk 8	2	*2.5*	*1.0*	3.9	*2.9*	*2.9*	5.8	1.2	2.9	1.5
Genk 8	3	*4.3*	*0.5*	5.0	8.5	*1.5*	10	2.0	3.1	2.0
Genk 8	3				8.0	*0.7*	8.7	1.9	1.4	1.7
Genk 8	2	*2.5*	*1.0*	3.9	*2.7*	*3.1*	5.8	1.1	3.1	1.5
Genk 9	1	*1.8*	*1.1*	3.4	7.1	*2.0*	9.1	4.0	1.8	2.7
Genk 9	1				7.4	*1.0*	8.4	4.1	0.9	2.5
Genk 9	2	*2.3*	*1.2*	4.0	8.2	*1.7*	9.8	3.5	1.4	2.4
Genk 9	2				6.5	*1.5*	8.0	2.8	1.3	2.0
Genk 9	3	*4.0*	*1.1*	5.6	9.7	*2.0*	12	2.4	1.8	2.1
Genk 9	3				14	*1.8*	16	3.6	1.6	2.9
Gent 9	1	**27**	**112**	186	**124**	**106**	230	4.6	1.0	1.2
Gent 9	2	12	19	39	24	8.6	33	2.0	0.5	0.8
Gent 9	2				24	7.3	31	1.9	0.4	0.8
Gent 9	3	12	*4.1*	18	15	*4.4*	19	1.2	1.1	1.1
Gent 9	3				13	*3.7*	16	1.1	0.9	0.9
Menen 6	1	16	**101**	160	**53**	**77**	130	3.4	0.8	0.8
Menen 6	2	26	**61**	113	**56**	**26**	82	2.2	0.4	0.7
Menen 6	2				**52**	**43**	94	2.0	0.7	0.8
Menen 6	3	12	9.0	25	22	*5.5*	28	1.8	0.6	1.1
Menen 6	3				23	*2.7*	26	1.9	0.3	1.0
Menen 6	5	14	**41**	73	17	**27**	43	1.2	0.6	0.6
Menen 6	5				10	11	21	0.7	0.3	0.3
Mol	2	*2.7*	*0.6*	3.6	*2.5*	*1.1*	3.6	0.9	1.7	1.0
Mol	2				*2.6*	*0.9*	3.5	1.0	1.4	1.0
Mol	5	*2.4*	*0.8*	3.5	*4.7*	*0.7*	5.5	2.0	0.9	1.5
Zelzate 3	4	15	*1.2*	17	**58**	*1.0*	59	3.9	0.8	3.5
Zelzate 3	4				**68**	*1.3*	69	4.5	1.0	4.1
Zelzate 3	5	*4.3*	*0.6*	5.2	8.4	*1.1*	9.5	2.0	1.8	1.8
Zelzate 3	5				11	*2.3*	13	2.5	3.8	2.6
Zelzate 3	5				11	*1.1*	12	2.6	1.8	2.4

CALUX/GC–HRMS ratio of 2.0, 0.9 and 1.3 was found for the PCDD/Fs, dl-PCBs and sum of both fractions, respectively. The ratios sometimes varied between sampling locations and also between sampling periods on the same location. For the dl-PCBs, no difference was found for the median CALUX/GC–HRMS ratio (0.9) in the residential/agricultural areas (Table 3) compared to the median ratio found in the industrial areas/natural reserve (Table 4). For the PCDD/Fs, a slightly higher ratio (2.2) was found in the industrial areas/natural reserve compared to the residential/agricultural areas (1.9). Probably not only PCDD/F levels, but also concentrations of other pollutants (that can interfere in the CALUX assay) are influenced by sampling location and period. These results show that the CALUX bioassay can be implemented in the Flemish GC–HRMS measurement network. Especially as a screening technique, for identifying possible contaminated locations and for determining the most suitable geographical location for the quantification of PCDD/Fs and dl-PCBs in atmospheric deposition samples (taking into the account distance from a point source and the dominant wind direction), CALUX can be a valuable alternative to GC–HRMS. Also, to quantify the atmospheric depositions at new measurement locations and to follow up the concentration levels over a long time period, the CALUX bioassay can be used instead of GC–HRMS. However, total replacement of the GC–HRMS technique by CALUX for routine analysis will be rather difficult. Since previous measurement campaigns at existing locations cannot be compared without some particular assumptions to the new CALUX measurements, the long-term evaluation of atmospheric depositions in an existing location will be more difficult. Also, more research is needed to

identify, and eventually quantify, the unknown CALUX-interfering compounds. This will lead to better insights in the composition of atmospheric depositions and their impact on the environment and on the human health.

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