IMPLEMENTATION OF THE CALUX BIOASSAY FOR THE ANALYSIS OF PCDD/F'S AND DIOXIN-LIKE PCB'S IN ATMOSPHERIC DEPOSITION SAMPLES FROM THE FLEMISH MONITORING NETWORK

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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. 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 measurement campaign in Flanders in 1995, concentrations have decreased drastically. However, in certain locations the warning limit for agricultural and residential areas, set by the VMM based on tolerable intake values, VDI guideline 2090/1 and a model study by VITO, of 21 pg TEQ m^{-2} day^{-1} for mean monthly values[1] (sum of PCDD/Fs and dioxin-like PCBs) is regularly exceeded. Since PCDD/Fs and dioxin-like PCBs (dl-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 dl-PCBs in atmospheric deposition samples[2, 3]. In this study, a new method for the analysis of PCDD/Fs and dl-PCBs in atmospheric deposition samples with the CALUX bioassay was developed, optimized and validated. More than 100 samples from the Flemish monitoring network were analyzed with both CALUX and GC-HRMS to define the correlation between both techniques and to investigate whether the CALUX bioassay can be used instead of or in addition to GC-HRMS for the analysis of PCDD/Fs and dl-PCBs in atmospheric deposition samples in Flanders.

Materials and methods

The sample preparation and bioassay measurement protocols were described previously by Croes et al., 2011[4] Briefly, the method consists of 4 steps: filtration, extraction, clean up and CALUX bioassay analysis. To avoid the use of large amounts of toxic solvents, new techniques were used for filtration and extraction: a C18 filter was used instead of a liquid/liquid extraction and an Accelerated Solvent Extractor (ASE) was used instead of the traditional soxhlet extraction. After pre-oxidation of the sample extract with concentrated sulphuric acid, clean up was done using a multi-layer silica gel column coupled to a carbon column. The PCDD/F and PCB fractions were finally analyzed with the H1L7.5c1 and/or the H1L6.1c3 mouse hepatoma cell lines[5,6].

Results and discussion

In this study, a new CALUX method was developed and validated. The limit of quantification was 1.4 pg CALUX-BEQ m^{-2} day^{-1} for the PCBs and 5.6 pg CALUX-BEQ m^{-2} day^{-1} for the PCDD/Fs, when using the new sensitive H1L7.5c1 cell line. The GC-HRMS recovery of spiked atmospheric deposition samples was for all ^{13}C PCDD/F congeners between 55 and 112%, with a mean recovery of 90%. CALUX recoveries of spiked procedural blanks were between the accepted ranges of 80-120%. Repeatability and reproducibility were satisfactory and no interferences from metals were detected.

For more than 100 atmospheric deposition samples from industrial, agricultural, residential and wildlife areas, collected between September 2009 and May 2010, PCDD/Fs and dl-PCBs were analyzed by the CALUX bioassay. BEQ determination was done in the optimum dilution range, around the EC50 value of the sample dose-response curve. In contrast to biological samples (i.e. blood[7] and milk[8]), for environmental samples it is 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 for the determination of an optimum dilution factor. Therefore, it was decided that for every atmospheric deposition sample a (full dose) dilution curve would be generated for both the dioxin-like PCB and PCDD/F fractions and these would be used to calculate the CALUX-BEQ concentration.

In Table 1, GC-HRMS and CALUX results are given for subset of 19 samples, for both the PCDD/Fs and the dl-PCBs, from 5 different agricultural or residential areas in Flanders. The samples were taken during 4 sampling campaigns. To compare the GC-HRMS and CALUX results and to evaluate the number of samples that were above the GC-HRMS warning limit, the sum of PCDD/Fs and dl-PCBs for GC-HRMS measurements was calculated according to the following formula (equation 1), where it was assumed that PCB 126 accounts for 70 % of the total dl-PCB toxic equivalent[9].

$$\Sigma \text{ PCDD/Fs and dl-PCBs GC-HRMS} = [\text{WHO-TEQ PCDD/Fs}]_{\text{GC-HRMS}} + ([\text{WHO-TEQ PCB 126}]_{\text{GC-HRMS}} / 0.7)$$

Equation 1: calculation of the TEQ for the sum of PCDD/Fs and dl-PCBs with GC-HRMS

From Table 1, it is clear that dl-PCB values were generally lower with the CALUX bioassay than with GC-HRMS, except for very low PCB concentrations (close to the limit of quantification; i.e. Beerse 1 from campaign 3). For the PCDD/F fraction, CALUX values were higher compared to the GC-HRMS WHO-TEQ values. CALUX/GC-HRMS ratios ranged from 1.2 to 5.5, with a median ratio of 2.0. For some sampling locations, the ratios were relatively constant (i.e. Roeselare 6), while for other locations (i.e. Beerse 1) more variation was found during different sampling campaigns. This can be due to other interfering compounds that are present in the atmospheric deposition samples and can vary in concentration level over time (seasonal effects). For the sum of PCDD/Fs and dl-PCBs, ratios between 0.7 and 5.1 were found, with a median value of 1.5.

Overall, for most samples a satisfactory correlation was found between the CALUX and GC-HRMS results, for both the PCDD/Fs and dl-PCBs. There was also a close agreement between the CALUX-BEQ values from atmospheric deposition samples taken in duplicate from the same sampling location. This shows that the CALUX bioassay can be implemented in the Flemish GC-HRMS measurement network. Especially as a screening technique, for identifying possible contaminated new measurement locations and for determining the most suitable geographical location for the quantification of PCDD/Fs and dl-PCBs in atmospheric deposition samples (taking into account distance and wind direction from a point source), CALUX can be a valuable tool.

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<table>
<tr><th>Location</th><th>Campaign</th><th colspan="3">GC-HRMS</th><th colspan="3">CALUX</th><th colspan="3">CALUX/ GC-HRMS ratio</th></tr>
<tr><th></th><th></th><th>PCDD/Fs</th><th>PCB126</th><th>sum PCDD/Fs & dl-PCBs</th><th>PCDD/Fs</th><th>dl-PCBs</th><th>sum PCDD/Fs & dl-PCBs</th><th>PCDD/Fs</th><th>dl-PCBs</th><th>sum PCDD/Fs & dl-PCBs</th></tr>
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<tr><td>Menen 10</td><td>1</td><td>4,8</td><td>29</td><td>46</td><td>17</td><td>16</td><td>33</td><td>3,5</td><td>0,6</td><td>0,7</td></tr>
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Table 1: GC-HRMS and CALUX results for the PCDD/Fs and dl-PCBs from a subset of 19 samples, collected during 4 different sampling campaigns on 5 different locations in Flanders. The numbers in red were higher than the GC-HRMS warning limit.