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Screening of dioxin-like compounds by complementary evaluation strategy utilising ELISA, micro-EROD, and HRGC-HRMS in soil and sediments from Montevideo, Uruguay

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ABSTRACT

Polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF) are persistent, toxic, and bioaccumulate in the environment. Due to their high analytical costs, these compounds are hardly regulated and mostly not monitored in the Third World. To overcome this, bioassays have been proposed as low-cost alternative methods. Two of the most established bioanalytical tools, the dioxin antibody-based enzyme-linked immunosorbent assay ELISA and the micro-EROD bioassay are evaluated and compared to high resolution gas chromatography and high resolution mass spectrometry (HRGC/HRMS) analytical methodology. The methods were tested using thirteen soils and sediment samples selected from diverse sites in Montevideo, Uruguay. The WHO_{2005} total toxic equivalent (WHO_{2005}-TEQ) of soils ranged from 2.4 to 2212 (ng WHO_{2005}-TEQ/kg dry sample) and from 0.14 to 9.4 (ng WHO_{2005}-TEQ/kg dry sample) in sediments. This study shows significant contamination related to dioxin-like compounds, particularly in sites where uncontrolled burnings were carried out. ELISA and micro-EROD bioassay correlated well with HRGC/HRMS, R Spearman 0.773 and 0.913, respectively and were highly correlated to each other, R Spearman 0.879. Preliminary threshold values of bioassay toxic equivalents of 330 (ng/kg dry sample) for the micro-EROD bioassay and 220 (ng/kg dry sample) for ELISA are proposed.

1. Introduction

The occurrence of some persistent organic pollutants in the environment, such as polychlorinated biphenyls (PCB), is expected to decrease with time due to prohibition in its production and stringent control in use. Still, other compounds such as polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF) are generated unintentionally by combustions and as by-products in the chemical industry. Polycyclic aromatic hydrocarbons (PAH) are mainly associated with incomplete combustion processes generated by different means of transport, industrial processes, agricultural burning practices, and domestic fires (Essumang et al., 2011). However, technological advances in industrial processes and combustion methodology together with emission abatement policies has led to a decrease in the formation of these pollutants in the developed countries but not necessarily in economic stressed countries.

Dioxin-like chemicals are characterised by properties, such as toxicity, environmental persistency, and potential to accumulate through the food chain hence the importance to determine their concentrations in different environmental matrices and their final

Abbreviations: PCDD, polychlorinated dibenzo-*p*-dioxins; PCDF, polychlorinated dibenzofurans; PCB, polychlorinated biphenyls; PAH, polycyclic aromatic hydrocarbons; HRGC/HRMS, high resolution gas chromatography and high resolution mass spectrometry; DMEM, Dulbecco's Minimum Essential Medium; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TE values, TCDD toxicity equivalent values; TMDD, 2,3,7-trichloro-8-methyl-dibenzo-*p*-dioxin; BSA, bovine serum albumin; PBS, phosphate buffer solution; PBST, phosphate buffered saline Tween 20; TMB, 3,3′,5,5′-tetramethylbenzidine; REP, relative potency; CR, cross-reactivity; ASE, accelerated solvent extraction; $EROD_{PAH}$-EQ, PAH EROD equivalents; EROD-EQ, TCDD EROD equivalents; $EROD_{Total}$-EQ, TCDD + PAH EROD equivalents.

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fate in the environment. In particular, some of them are able to penetrate through the cell membrane and act as enzyme inducers through specific binding to intracellular receptors such as the aryl hydrocarbon receptor (AhR) (Estabrook, 1996). Its activation in presence of xenobiotics can cause serious health effects such as teratogenesis and tumour promotion (Hankinson, 1995). Dioxin-like compounds able to activate this mechanism can be determined by instrumental analysis for each single compound and, by immunochemical methods and bioassays eliciting a global response based on the interaction of all these compounds together.

Data related to occurrence concentrations and spatial distributions of these chemicals is generally scarce and particularly in the Third World countries where activities like unregulated burnings on fields and uncontrolled combustions related to recycling garbage are common practices. The final fate of these pollutants in different environmental matrices is influenced by subsequent dry or wet deposition of atmospheric emissions after undergoing aerial transport (Hites, 1991; Smith and Jones, 2000). As a consequence, soil and sediments are sinks where these compounds tend to be accumulated. In cases where uncontrolled combustions are frequent practices, dioxin-like compounds are generated *in situ*. Unfortunately, in the regions where these activities are widespread, there is very little or no information reported in literature on areas suspected as potentially contaminated sites.

The employment of screening techniques to determine levels of contamination as well as spatial and temporal tendencies is a strategy to carry out low cost monitoring. The bioanalytical screening methods, specifically ELISA and micro-EROD, were chosen in consideration of costs, laboratory facility requirements, and assay reliability. The micro-EROD assay mimics the biological processes that are suspected to participate in the mechanisms of toxicity of dioxin-like compounds. Additionally, agonistic or antagonistic effects of dioxin-like compounds can be determined by this method by measuring all AhR-binding compounds from the sample able to elicit a response. The results are calculated as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalents (Behnisch et al., 2003; Schwirzer et al., 1998). On the other hand, ELISA gives a complete response determined by the specificity of the antibody which has been reported to correlate with the toxicity of the sample (Nichkova et al., 2004; Shan et al., 2001; Sugawara et al., 2002; Trindade et al., 2008).

In this work, HRGC/HRMS and the two selected bioassays are used for the study of persistent organic compounds in soil and sediment samples taken from urban and sub-urban areas from Montevideo, Uruguay. 17 PCDD/F, 16 PAH, and 18 PCB were instrumentally analysed. Homologue patterns and compound tendencies are discussed based on these analytical results in order to get more information about the sample loading compositions and possible identification of sources of contamination. The WHO_{2005} toxic equivalency factor (WHO_{2005}-TEF) for humans for each PCDD/F and PCB compound (Van den Berg et al., 2006) was used to calculate the WHO_{2005} total toxic equivalent (WHO_{2005}-TEQ) of each sample. This is calculated as the sum of the product of the concentration of each congener multiplied by its respective WHO_{2005}-TEF. These analytical results were compared with those obtained by the micro-EROD and the ELISA bioassay after standardisation from the analytical values into their correspondent bioassay values. This is done using response factors for micro-EROD and cross reactivity factors for ELISA. Based on this assessment, preliminary cut-off values for the two bioanalytical methods can be proposed to identify the environmental samples that are suspected of being non-compliant and require further analysis by HRGC/HRMS.

2. Materials and methods

2.1. Sampling

Eight soil samples (0–5 cm depth) were taken from peripheral neighborhoods of Montevideo where garbage recycling was suspected as described in Trindade et al. (2008). Samples were collected close to the burning sites (1–2 m) in case of detecting combustion points. Stones and vegetation were removed from the samples before drying overnight at 100 °C. The samples were grounded, sieved through 2 mm screen, and homogenised. Additionally, five superficial sediment samples (0–5 cm depth) were taken at the Rio de la Plata estuary using a van Veen grab. Three of the five sediments were taken from the Bay of Montevideo, a place with high potential contamination (Sediments 2, 3, and 4). Sediment 1 was from the diffusion area of the final discharge point of the municipal wastewater treatment plant and Sediment 5 from a zone of low population density upstream the city. Stones and shells were removed. Samples were subsequently transferred and transported in refrigerated containers to the laboratory. Sediments were dried overnight at 100 °C. After drying, they were sieved through a 315 μ screen and sieved further through a 60 μ screen. In total, 13 samples were collected from diverse city sites enabling us to have soils and sediments from a variety of sources for this study.

2.2. Bioanalytical determinations

2.2.1. Extraction and clean-up

Soil and sediment samples between 2 and 5 g were each Soxhlet extracted with toluene for 24 h to obtain the organic bio-accumulative compounds. The concentrated extract was then purified in a single clean-up column step where the non-persistent compounds and organic interferences were removed (Schwirzer et al., 1998). The column consisting of 10 g active silica gel, 20 g silica gel (44% H_2SO_4), 40 g silica gel (4% H_2O), 10 g Na_2SO_4 from bottom to top was eluted with n-hexane. The extract was split in two parts to be analysed by micro-EROD and ELISA.

2.2.2. Micro-EROD bioassay

Micro-EROD assay using rat hepatoma cell line H4IIEC3/T (HII4E) expressing cytochrome P4501A1 upon exposure to AhR agonists was performed according to Schwirzer et al. (1998). The H4IIE cells were grown in Dulbeccós Minimum Essential Medium (DMEM) supplemented with fetal calf serum (10%), penicillin (100 U/mL), and streptomycin (100 μg/mL) and incubated at 37 °C under atmospheric conditions of 7% CO_2 and 95% humidity. The EROD bioassay was carried out for 24 h (all compounds able to elicit a response) and for 72 h of incubation (only persistent compounds). For the quantification, dose–response curves obtained with standards of TCDD were performed for each bioassay. Blanks, samples, and standards were performed in triplicates in at least two independent experiments. Results were given as ng TCDD toxicity equivalent values (TE values) kg^{-1}dry sample.

2.2.3. ELISA bioassay

Extracts were prepared such as indicated in Section 2.2.1. This analysis was performed essentially as described previously (Nichkova et al., 2004; Shan et al., 2001). Microtitter plates were coated overnight at 4 °C with 100 μL III-BSA antigen (0.2 μg/mL in coating buffer). Minimisation of the unoccupied binding sites was performed by treatment with BSA 1% as blocking agent in PBS (200 μL/well) at room temperature for 1 h. After washing four times with PBS-Tween 0.05% and once with water, plates were

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