Dioxin and dioxin-like activity in sediments of the Belgian Coastal area (Southern North Sea)

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Introduction

Halogenated aromatic hydrocarbons (HAHs) accumulate and biomagnify in biota due to pronounced lipophilicity and resistance to metabolic degradation[1]. Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are the most important members of a class of HAHs, which produce similar toxicity patterns but differ in respective potencies. This toxicity proceeds through the action of the aryl hydrocarbon receptor (AhR), eliciting various responses including enzyme induction, immunotoxicity and tumor promotion[2]. PAHs also activate the AhR[3], but the toxicity of PAHs is mainly caused by the production of hydrophilic metabolites[4] and they cannot be considered as dioxin-like compounds. There is serious concern about these chemicals, that were frequently observed to enter the food chain.

Amongst food sources, fish is one of the major ones to survey, because they are exposed to high amounts of HAHs and PAHs in the marine environment[5]. Once entering aquatic systems dioxins will tend to escape from solution and adsorb onto non-polar suspended particles. Elucidation of the contamination routes, therefore, requires detailed assessment of the dioxins' and dioxin-like substances' levels in sediment accumulating areas. The Belgian coastal zone of the North Sea being such an area[6].

In this study we will focus on the CALUX-TEQ contamination levels of sediments at the mouth of the Scheldt, along the Belgian coast and at some offshore stations. CALUX measurements will be performed (1) without clean-up, hence including also PAHs which are not dioxin-like compounds even if they are AhR ligands and (2) with a carbon column clean-up step separating the PCDD/F fraction from interfering compounds.

Methods and Materials

Bottom samples were collected in duplicate. The sediment samples were dried in an oven, ground and subsequently extracted with toluene by Pressurized Liquid Extraction (PLE). The extract was concentrated to dryness and re-suspended in hexane. Next, samples were analyzed with and without clean-up.

For each sample clean-up, two columns were used: one filled with sodium sulfate, deactivated neutral alumina, sulfuric acid silica gel and silver nitrate on silica gel, rinsed with hexane, and one containing sodium sulfate and X-CARB (Xenobiotic Detection Systems Inc., USA), rinsed with acetone, toluene and hexane. The multilayer silica/silver nitrate/alumina column was then placed above the carbon column and the sediment extract was loaded on the column and then eluted with hexane. The upper acidic silica column was removed and the carbon column was eluted with a hexane-acetone mixture, hexane/ethyl acetate/toluene (eluting coplanar PCBs) and toluene (eluting PCDD/Fs). Extracts were concentrated to dryness in a centrifuge under vacuum and resuspended in hexane. Prior to dosing, different amounts of hexane extracts were transferred into DMSO using a centrifuge under vacuum and, finally, medium was added to each extract in DMSO.

For the CALUX bioassay, plates were seeded with cells (*pGudLuc-6.1* cell line, Xenobiotic Detection Systems Inc., USA). After 20-24h of incubation the medium of the cell suspension was removed from each well of the dosing plate and replaced with medium containing the extract in DMSO. At the same time standard solutions of TCDD in DMSO were analyzed to generate a calibration curve.

HPLC-fluorescence was used to determine the concentrations of five very toxic PAHs (benzo(a)anthracene, benzo(b) fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene and dibenzo(a,h)anthracene).

Results and discussion

The dose-response curves of the samples and the 2,3,7,8 TCDD standards have not the same shape; they are not parallel, nor they have the same efficacy. Since the curves are not parallel, the results are function of the dose analyzed (the amount of sediment analyzed per well). All results of the raw sample extracts situated in the linear part of the curve were quantified, leading to a range of concentrations instead of one single value per sample. Therefore, overall CALUX-TEQ intervals are used to indicate the degree of pollution. These intervals are based on the minimal and maximal overall CALUX-TEQ per mass of sediment.

Five PAH (benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene and dibenzo(a,h) anthracene) were analyzed by HPLC-Fluorescence for the river mouths samples. The concentrations measured were multiplied by an appropriate REP[7] to determine approximately the proportion of the overall CALUX response due to these PAH. As these 5 PAHs represent between 14 and 30% of the maximal overall CALUX-TEQ values and between 35 to 100% of the minimal overall CALUX-TEQ values respectively, it can be excepted that PAHs represent a very important fraction of contaminants contributing to the overall CALUX-TEQ value in the sediments of the Belgian coastal area.

According to the total concentrations of dioxins, PAHs and dioxin-like compounds found in sediments at the Belgian coast, the sampling stations can be grouped into two clusters. The first group gathers stations with highest concentrations: the Scheldt mouth (2800 – 7200 pg overall CALUX-TEQ/g sediment) including Zeebrugge (1600 – 4200 pg overall CALUX-TEQ/g sediment), and Nieuwpoort (600 – 2200 pg overall CALUX-TEQ/g sediment). These concentrations are at least two orders of magnitude higher than the ones found in the second group: the coastal stations Ambleteuse, Oostende, Wenduine, Knokke and the sea stations 250, ZD2, 545, 710 and 435 (1.3 – 45 pg overall CALUX-TEQ/g sediment).

The sediments from the river mouths (Zeebrugge is situated in the outflow of the Scheldt and the Yser mouths at Nieuwpoort) show the highest overall CALUX-TEQ-values (Fig. 1). Emissions by industry and municipalities, especially the port and the town of Antwerp, and the shipping in the Scheldt contribute largely to the pollution of the rivers and their tributaries. Moreover, organic pollutants tend to concentrate in the organic phase of the sediment matrix. These high amounts of organic matter are due to municipal waste, erosion material from agricultural and forest areas and detrital plant matter, transported by the rivers Scheldt and Yser. The large organic matter fraction in their sediments enhances sorption of organic pollutants, leading to high overall CALUX TEQs at the river mouths and outflows.

The coastal stations Ambleteuse, Oostende, Wenduine and Knokke and the sea stations 710, 250, 545, ZD2 and 435 all show similar overall CALUX-TEQ per mass sediment values (Fig. 1). These results suggest a general background pollution of the Belgian zone of the North Sea. The most important movement of sediment transport in the North Sea goes from the Channel in a Northeast direction towards the Dutch coast[8]. The south-westerly oriented Scheldt outflow of highly contaminated suspended matter encounters the north-easterly flowing mass of lowly contaminated suspended matter from the Channel, resulting in a gyre in front of the eastern part of the Belgian coast and an intensive homogenization of the suspended matter and associated pollutants in front of the Belgian coast[6].

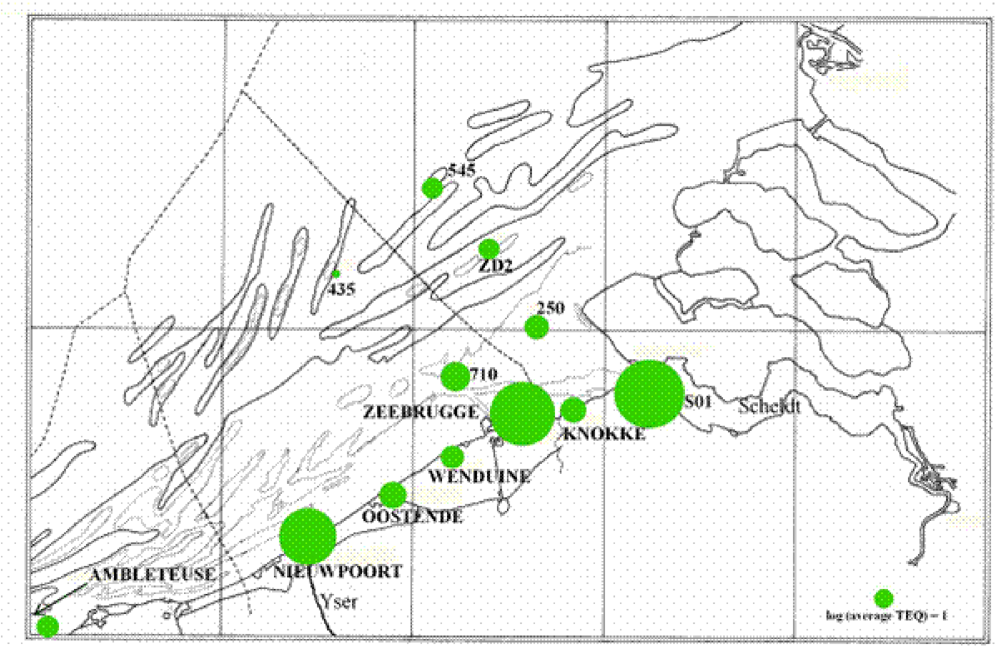

Fig. 1: Map showing overall CALUX-TEQ per mass of sediment at the sampling stations (the radius of the circle is proportional to the overall activity)

The general trend of the dioxin-CALUX-TEQ-values is similar to the one of the overall CALUX-TEQ-values, but at a different scale: the Scheldt mouth and Nieuwpoort exhibit the highest dioxin activity, whereas the other stations all show similar values. These steady levels confirm a kind of background pollution of dioxins in the Belgian coastal area.

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