

## CHEMICAL AND TOXICOLOGICAL RISK ASSESSMENT OF NORTH SEA SURFACE SEDIMENTS. BROMINATED FLAME RETARDANTS AND DIOXIN-TYPE TOXICITY

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### Introduction

Recently, the OSPAR Commission published the Quality Status Report 2000 (QSR2000), which includes the assessment of the environmental quality of Region II, the Greater North Sea<sup>1</sup>. The QSR2000 executive summary lists several subject areas with limitations in knowledge, and recommends, a.o., that steps should be taken to close these gaps, (quote) “in particular the occurrence and effects of hazardous substances” (end quote<sup>1</sup>). As a response to this, the Dutch North Sea Directorate commissioned the National Institute for Coastal and Marine Management / RIKZ to carry out both a chemical and a biological risk assessment by, respectively, the identification and determination of the concentrations of selected substances (organic contaminants) in sediment extracts, and (2) by the determination of the responses of a suite of bacterial bioassays to these extracts.

Substances and assays were selected after evaluation of different priority pollutant lists and selection procedures, i.e. the OSPAR List of Chemicals for Priority Action and the OSPAR 1998 List of Candidate Substances<sup>2</sup>, the European Union COMMPS procedure<sup>3</sup>, the European Commission list of Priority substances under the Water Framework Directive<sup>4</sup>, and consulting the primary managing authority of the Dutch sector of the North Sea, the North Sea Directorate.

At *Dioxin2002*, we report the results of Brominated Flame Retardants (BFRs): brominated diphenylethers (PBDE), biphenyls (PBB) and hexabromocyclododecane (HBCD) analysis, and of the DR-CALUX bioassay for dioxin-type toxicity.

### Materials and Methods

#### *Sampling*

Sampling sites were chosen so as to cover different parts of the Dutch sector of the North Sea continental shelf, i.e. both coastal areas and remote, ‘reference’ areas. Surface sediments were sampled using a box-core; at each location, the top 5 cm of six individual cores were pooled and further treated as one sample. Each sample was sieved through a 63µm nylon mesh, freeze-dried and homogenized.

#### *Brominated flame-retardants*

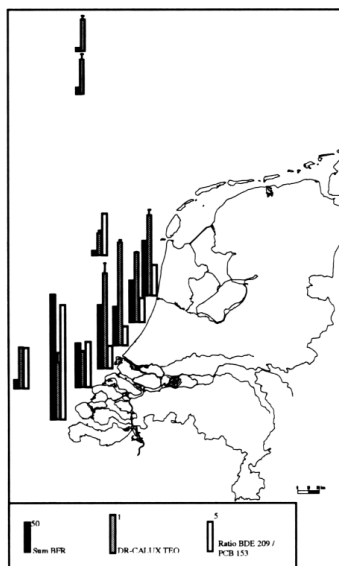
Were determined using methods adapted from de Boer *et al.* (2000)<sup>6</sup>. Briefly, after Soxhlet hexane:acetone (3:1, v/v) extraction of the freeze-dried sediment, the extract was concentrated by rotary evaporation and dissolved in 2 ml dichloromethane. Clean up of the extract was carried out by gel permeation chromatography (GPC). The GPC eluate was concentrated to ca. 1 ml under a nitrogen flow, transferred to iso-octane and further purified by silica gel chromatography and sulphuric acid treatment. The obtained iso-octane extract was analysed with GC-MS using EC/NCI mode with methane as reagent gas (BDE-209 on a 15 m DB-5 column (0.25 mm ID, 0.25 µm film thickness),

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other BFRs on a CP-Sil-8 column; 30 m x 0.21 x 0.20  $\mu\text{m}$ ). Peak identification was based on retention time and the recognition of the Br-ion ( $m/z$  79/81), and quantification based on the  $m/z$  79 ion. The following compounds were determined: PBB congeners 15, 49, 52, 101, 153 and 209; PBDE congeners 28, 47, 66, 71, 75, 77, 85, 99, 100, 119, 138, 153, 154, 190 and 209, and hexabromocyclododecane (HBCD; PBB and PBDE nomenclature being similar to that of the PCBs<sup>6</sup>).

## *Dioxin-type toxicity*

Freeze-dried sediments were extracted as above. The extract was concentrated to 5 ml (rotary evaporation). A 2 ml aliquot of the extract was purified using a multilayer silica column (30x1cm, filled with, from top to bottom, 1 cm anhydrous sodium sulfate, 7 g silica impregnated with 44 % sulphuric acid, 1 g silica, 2 g silica impregnated with 33% sodium hydroxide, 1 g silica, 1.5 g silica impregnated with 10 % silver nitrate). After pre-eluting with 25 mL hexane, 1 mL extract was added and eluted with 130 mL hexane. The other 2 ml of the Soxhlet extract was purified using GPC (as above). The eluates of the multilayer silica column and the GPC fraction were each concentrated to 5  $\mu\text{l}$  and dissolved in 50  $\mu\text{l}$  DMSO. The presence of Ah receptor active compounds was determined using the DR-CALUX assay<sup>7</sup>.



**Figure 1.** Total Brominated Flame Retardant concentrations (BFR;  $\mu\text{g}\cdot\text{kg}^{-1}$  OC, black bars), Dioxin-type toxicity (DR-CALUX;  $\text{pg TEQ/g}$  dry weight, grey bars) and concentration ratio of BDE-209 and PCB 153 (white bars) in North Sea surface sediments.

## Results and Discussion

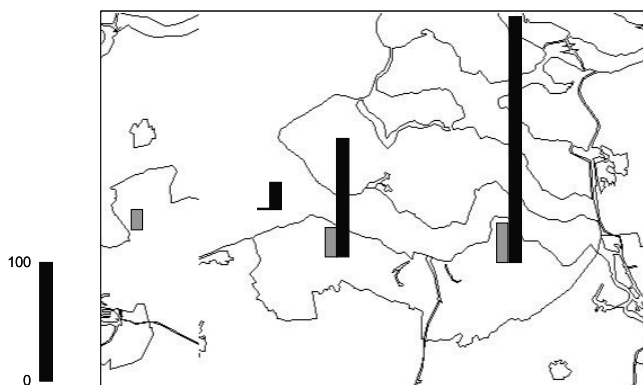
### *Brominated flame-retardants*

All PBB levels were below detection level (0.05-0.70  $\mu\text{g kg}^{-1}$  d.w.). BDE 209 levels at the outflow of the Scheldt estuary (Appelzak), was highest with 1839  $\mu\text{g kg}^{-1}$  OC (or 32  $\mu\text{g/kg}$  dw; Figure 1). On a dry-weight basis, this value is much higher than that of de Boer *et al.* (2000)<sup>5</sup>. These authors reported a decreasing PBDE trend from 510  $\mu\text{g kg}^{-1}$  at the upper estuary to less than 4  $\mu\text{g kg}^{-1}$  near Vlissingen at

the mouth. As the PBDE levels in suspended matter in the Western Scheldt were variable and depended on sampling period, the results of the present study are probably within the normal concentration range of these compounds. BDE 209 levels quickly decreased with increasing distance from likely sources (river mouths), to about  $40 \mu\text{g kg}^{-1}$  OC. These levels are comparable with those found by Zegers *et al.* (2000)<sup>8</sup> in a dated sediment core from the Waddensea.

This pattern of off-shore to near-shore increasing concentrations was found for hexabromocyclododecane (HBCD) as well, with maxima at the outflows of the rivers Scheldt, Rhine-Meuse and the North Sea Canal (IJmuiden).

As a tracer for the possible source of BDE 209, the concentration ratio BDE 209 / PCB 153 was used. While PCBs in the North Sea may be considered as a conservative contaminant with respect to organic carbon (OSPAR Commission, 2000), PBDE levels may still be increasing. A higher BFR/PCB ratio therefore indicates the proximity of a BFR source. From Figure 2, where data on PCB and PBDE levels in suspended solids in the Scheldt estuary have been added, it is clear that a BDE 209 source may likely be found upstream of the Scheldt river, as was earlier indicated<sup>5</sup>.



**Figure 2.** Ratios of decabromodiphenylether (BDE 209) and 2,2',4,4',5,5'- hexachlorobiphenyl (PCB 153) in sediment (grey) and suspended solids (black) of the Scheldt estuary.

#### *Dioxin-type toxicity*

Was detected in all sediment extracts (Figure 1), with highest responses at near-shore locations. Maximum responses were found at the outflow of the Rhine estuary and the North Sea Canal. A striking result of this study is the vastly increased DR-CALUX response in samples treated with GPC compared to the multilayer silica procedure. On average, GPC-treated samples contain 75 times more dioxin-type toxicity than silica treated samples.

Table 1 gives the between-groups Pearson correlation coefficients of both types of response and of several groups of organic micropollutants that were determined in this study.

From Table 1 it is clear that PCBs correlate well with Si-TEQ, which is due to the fact that the silica method mainly collected stable compounds such as chlorinated dioxins, furans and PCBs, in contrast to the GPC method where macromolecules and cytotoxic sulfur are removed from the extract. From this and from the apparent correlation between GPC-TEQ and  $\Sigma$  PAH (Table 1), it is hypothesized that the sediment samples contain as yet unidentified compounds with dioxin-type toxicity, e.g. PAH-type or

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**Table 1.** Pearson correlation matrix of DR-CALUX responses using silica and GPC clean-up (Si-TEQ resp. GPC-TEQ), total organic micropollutant concentration ( $\Sigma$  POP), total BFR, total PAH and total PCB. Contaminant concentrations were standardized to OC before statistical evaluation.

	Si-TEQ	GPC-TEQ	S POP	Total BFR	Total PAH	Total PCB
Si-TEQ	1.000					
GPC-TEQ	0.458	1.000				
$\Sigma$ POP	0.660	0.660	1.000			
Total BFR	0.466	0.121	0.676	1.000		
Total PAH	0.620	0.694	0.995	0.620	1.000	
Total PCB	0.900	0.478	0.730	0.555	0.699	1.000

natural compounds. Further studies are underway to elucidate the identity and activity of these compounds, using information from the literature as guidance<sup>9</sup>.

## References

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