ANALYSIS OF POLYCHLORINATED DIBENZO-P-DIOXINS (PCDD), DIBENZOFURANS (PCDF) AND BIPHENYLS (PCB) IN FISH USING DR-CALUX® AND GC/MS: A COMPARISON.

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Introduction

Implementation of new PCDD and PCDF limit values in food en feed products by the EU on July 2002, has increased the need for cheap, rapid, and high throughput screening techniques. A number of biological methods have been reviewed and compared by Behnisch *et al*.[1,2]. The DR-CALUXâ technology was identified as the only biotechnology that meets EU quality criteria for bioassays. In comparison to chemical analysis using HRGCMS, the DR-CALUX® bioassay provide a direct measure of the total TEQ of dioxin-like activity present in a matrix, including synergistic or antagonistic effects of all dioxin-like congeners in a complex mixture. In the present study, fish samples from various sites were analysed for PCB, PCDD, and PCDF content using GC analysis. In addition, the total TEQ of dioxin-like activity was determined using the DR-CALUX® bioassay.

Methods and materials

Fish were collected in 1999 and 2000 from various sites: the Atlantic Ocean, Celtic Sea, English Channel, North Sea, Oosterschelde, Skagerrak, and Wadden Sea. Samples of anchovy, blue whiting, cod, dab, shrimp, herring, koolfish (black), mackerel, mussel, plaice, salmon, silver smelt, sole and whiting were collected. In total 16 fish samples were analysed for PCDD/FCDF and dioxin-like PCB content by GC/MS and for total 2,3,7,8-TCDD-like content (total 2,3,7,8-TCDD TEQ) by DR-CALUX®.

GC/MS

For the analysis of PCDD/Fs and PCB-77, PCB-126, and PCB-169, fish tissue was extracted using the method developed by Bligh and Dyer. The compounds were determined using gas chromatography (GC) and high resolution mass spectroscopy (MS) after a series of clean-up and fractionation steps. For the analysis of mono-ortho substituted PCBs (PCB-105, PCB-118, and PCB-156), fish samples were dried using sodium sulfate after which the samples were Soxhlet extracted with dichloromethane/ pentane. Following extraction, the extracts were cleaned with Al_2O_3 and silica gel. The compounds were determined using GC with electron capture detection (ECD). 2,3,7,8-TCDD TEQ values were obtained from determined concentration by conversion using WHO-TEFs[3].

DR-CALUX®

For the analysis of total 2,3,7,8-TCDD TEQ in fish, a minimum of 5 gr of fish tissue was extracted by means of Accelerated Solvent Extraction (ASE) (hexane/acetone; 9:1). After extraction, the hexane/

acetone fraction was evaporated under nitrogen. A minimum of 0.5 g of extract was redissolved in hexane prior to acid-silica clean-up (33% H_2SO_4). The volume of the eluate was reduced under a gentle stream of nitrogen. 0.5 ml of the remaining eluate was mixed with 25 µl of DMSO and further dried under nitrogen. In each series of 8 samples, a procedure blank and an internal reference material were included. The cleaned samples were analysed for total 2,3,7,8-TCDD TEQ content using BDS' DR-CALUX® bioassay according to the guidelines from BDS (www.biodetectionsystems.com). In short, p-GudLuc transfected H4IIE-cells were seeded, cultured and exposed in 96-well microtiter plates. Each 96-well microtiterplate contained a complete 2,3,7,8-TCDD calibration range (0 – 300 pM 2,3,7,8-TCDD per well). Each sample was analysed in triplicate. After 24-hours of incubation, the cells were washed and lysed after which the luciferase activity was analysed using a luminometer (LUCY 2; Anthos). Total DR-CALUX® TEQ content in the samples analysed was determined by interpolation from the exponential fitted 2,3,7,8-TCDD calibration curve.

Results and Discussion

In contrast to PCDD, PCDF and PCB analysis using GC/MS and GC/ECD, DR-CALUX® bioanalysis does not discriminate between these compounds and congeners. All compounds binding to the Ah-receptor are detected and integrated by the DR-CALUX® bioassay. In table 1, the analysed DR-CALUX® TEQ as well as the sum dioxin-like PCB content, the sum PCDD/PCDF and the total sum PCB/PCDD/PCDF content as determined using GC analyses is given.

Table 1. Total dioxin-like content (TEQ) in various fish species as determined by DR-CALUX® analysis and combined GC-MS and GC-ECD analysis.

Sample	DR-CALUX® TEQ (pg 2,3,7,8 TCDD/g product)	GC TEQ (pg 2,3,7,8-TCDD/g product)		
		ΣPCB	ΣPCDD/PCDF	ΣPCB/PCDD/PCDF
Herring	3.3	2.1	2.1	4.2
Koolvis	0.3	0.2	0.1	0.3
mackerel	0.7	1.0	0.3	1.3
Mackerel	0.8	1.0	0.3	1.3
Scot salmon	1.8	2.6	1.4	4.1
Herring	1.2	1.1	1.3	2.4
Cod	0.2	0.2	0.1	0.3
Mackerel	1.7	1.6	0.6	2.2
Dab	0.9	0.8	0.5	1.4
Plaice	0.3	0.2	0.3	0.5
Blue whiting	0.2	0.5	0.1	0.6
Sardine	0.1	0.1	0.0	0.1
Silver smelt	0.5	0.5	0.4	0.9
Mussel	3.5	1.2	1.1	2.3
mussel	3.9	2.0	1.5	3.5
Dutch shrimp	1.4	0.9	1.0	2.0
Herring	3.3	2.1	2.1	4.2

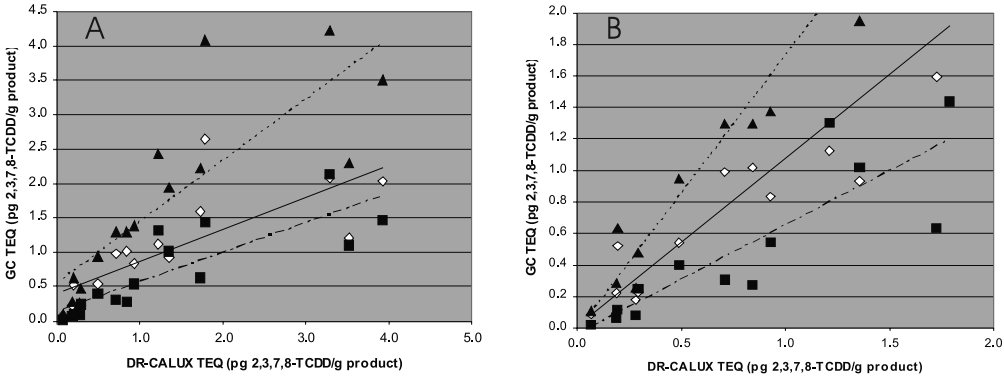

Figure 1. Correlation between analysed DR-CALUX® 2,3,7,8-TCDD TEQ content and analysed GC ΣPCB, ΣPCDD/PCDF and ΣPCB/PCDD/PCDF 2,3,7,8-TCDD TEQ content in fish samples. Correlation coefficients were calculated for all analysed fish (a) and fish with dioxin-like content in the lower range, <2 pg 2,3,7,8-TCDD TEQ/g product (b). Correlation coefficients between DR-CALUX® TEQ content and GC ΣPCB, ΣPCDD/PCDF and ΣPCB/PCDD/PCDF content are 0.70 (.....), 0.59 (– —), and 0.71 (- · - · -) respectively for (A) and 0.86 (.....), 0.81 (——), and 0.74 (- · - · -) respectively for (B).

In figure 1a, a graphical representation of these data is given. Correlation between GC and DR-CALUX® results in the lower TEQ range are included in fig. 1b. Although both analysis techniques include different extraction protocols, a good correlation between DR-CALUX® bioanalysis and GC analysis was observed. DR-CALUX® results show slightly lower total dioxin-like TEQ content in the analysed fish as compared to the ΣPCB/PCDD/PCDF GC analysed TEQ content. Antagonistic effects of certain PCB congeners present complex mixture such as in the analysed fish tissue extracts, can cause the observed discrepancy between DR-CALUX® and GC data. The present results indicate that DR-CALUX® results overestimate the PCDD and PCDF content in the analysed fish samples by a factor of 2 as compared to GCMS analysis of PCDDs and PCDFs, due to the contribution of PCBs.

References

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