Isomer specific analysis of mono-ortho and non-ortho PCBs, PCDDs and PCDFs in human blood plasma

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Introduction

Recent toxicological studies have shown that some of the mono- and non-ortho polychlorinated biphenyls (PCBs) act by the same mechanism of toxicity, through interaction with the Ah-receptor, as the chlorinated dioxins. It is suggested that the toxic potential of these congeners can be expressed in 2,3,7,8-TCDD equivalents. In the Nordic risk assessment of PCBs[1] they have been assigned toxic equivalents factors (TEFs). These congeners, together with their TEF values, are listed in Table 1.

The analytical method used at our laboratory for analysing polychlorinated dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in human blood plasma has been described earlier[2] and has produced good results in a WHO inter-calibration study[3]. The aim of the present work was to develop further this analytical method and to include the mono- and non-ortho PCBs listed in Table 1.

Experimental

The idea was to make as small changes as possible in the present method and also to use disposable lab ware in order to reduce the risk of contamination. Minimising contamination is particularly important since there is a natural restriction in the amount of sample available, usually about 100-150 ml blood plasma resulting in 0.5 - 1g of fat after extraction. Storr-Hansen has shown that alumina separates the PCBs according to the chlorine substitution pattern. The congeners with a planar conformation are retained longer than other PCBs[4]. Therefore a column with basic alumina was included in the analytical method with a view to separating the non- and mono-ortho PCBs from less toxic PCBs.

The less toxic PCBs were eluted from the alumina column with n-hexane (fraction I), mono-ortho PCBs with methylenchlorid/n-hexane [1:99] (fraction II) and the non-ortho PCBs, PCDDs and PCDFs with methylenchlorid/n-hexane [50:50] (fraction III). However, approximately 10 % of the mono-ortho substituted PCBs were eluted in fraction III. By using a small Carbopack C column for further clean-up of fraction III residues of mono-ortho PCBs can be recovered and subsequently transferred to fraction III. A flow chart for the clean-up procedure is shown in Figure 1.

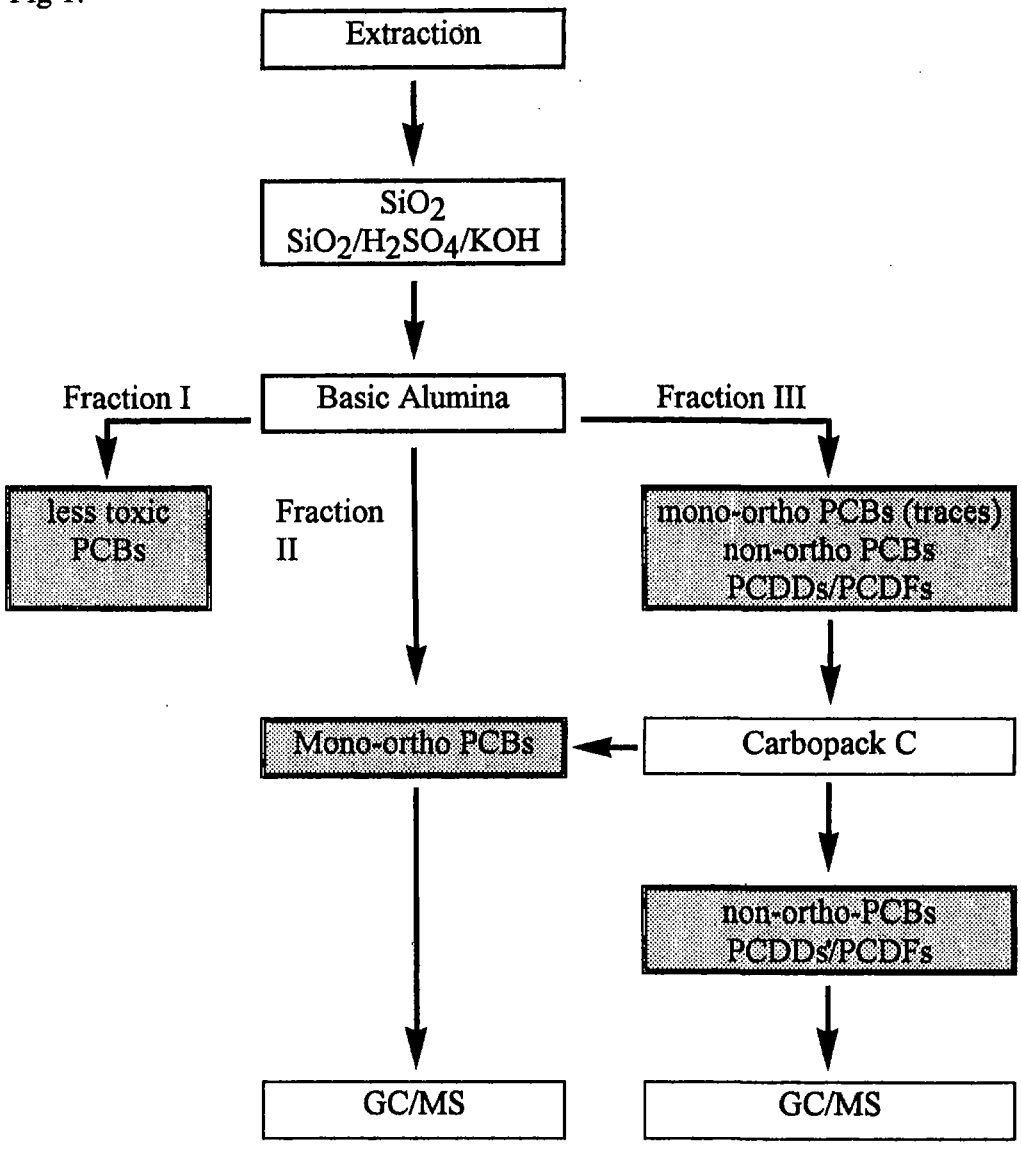
To validate the method blood samples were analysed. Before clean-up the samples were spiked with ^{13}C-labelled 2378-dioxins, 2378-dibenzofurans and non-ortho PCB isomers (IUPAC # 77, 126, 169) within the range of 100-250 pg. Labelled mono-ortho PCB isomers # 118 and 105 were also included but in the range of 10-20 000 pg. The results show that recovery of all these labelled substances is between 50 and 100 %. Isomer specific quantification was performed by means of HRGC/HRMS, using a 60 m DB-5MS column and a VG70-250S double focusing high-resolution mass spectrometer.

This method will be applied in a study funded by the European Community and the Swedish Environmental Protection Board entitled: Assessment of early signs of biological action following human exposure to polyhalogenated dibenzo-p-dioxins and related substances.

Table 1.

Category	Congener	IUPAC	TEF
non-ortho	3,3′,4,4′	77	0.0005
	3,3′,4,4′,5	126	0.1
	3,3′,4,4′,5,5′	169	0.01
mono-ortho	2,3,3′,4,4′	105	0.0001
	2,3,4,4′,5	114	0.0005
	2,3′,4,4′,5	118	0.0001
	2′,3,4,4′,5	123	0.0001
	2,3,3′,4,4′,5	156	0.001
	2,3,3′,4,4′,5′	157	0.001

Fig 1.


Extraction
SiO_2
$SiO_2/H_2SO_4/KOH$
Basic Alumina
Fraction I
less toxic PCBs
Fraction II
Fraction III
mono-ortho PCBs (traces)
non-ortho PCBs
PCDDs/PCDFs
Mono-ortho PCBs
Carbopack C
non-ortho-PCBs
PCDDs/PCDFs
GC/MS
GC/MS

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