ANALYTICAL METHODOLOGIES FOR DETERMINATION OF PCB IN BIOLOGICAL MATERIAL - INTERCALIBRATION STUDIES UNVEIL TODAY'S STATE OF THE ART

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Introduction

Production of technical PCB, characterised by several of the 209 possible chlorination products of biphenyl, occurred mainly from 1930 to the late 1970's. An overall world production until 1980 of 2 x 10^9 kg has been estimated[1]. As early as 1936 workplace threshold limits were set due to observed toxic effects in connection with occupational exposure. But, the widespread environmental contamination and its effects were not fully accounted for until the late 60's, when the global occurrence of PCB in all biological material was a fact.

In analysing chlorinated pesticides, such as DDT found in the 50's to be present in the lipid fraction of biological materials, chemists in the 60's - by the newly introduced GC techniques - found interfering peaks in the chromatograms which soon were identified as biomagnifying PCB residues[2,3]. These findings eventually led to restrictions in the 70's, in both the use and production of PCB in most countries. Also measures to minimise the generation and spread of PCBs from secondary sources were taken. Today, some thirty years and tens of thousands of PCB analyses later, our analytical techniques are tuned to detect each and every individual PCB congener down to ppt level in biota. And due to extensive efforts in combating the PCB problem since the 80's declining PCB trends are reported in several Nordic and European countries.

Most analyses of PCB in biological material in the 60's and 70's were carried out by gas chromatography using packed columns and low sensitivity detectors. Quantification was done by comparing the low-resolution pattern of the PCB peaks with technical mixtures of PCBs. The introduction of glass or fused silica columns and use of EC detectors in the 80's improved both the selectivity and sensitivity of the analyses. The growing applications in the 90's including mass specific detectors, LRMS as well as HRMS, has further sharpened the analytical procedures. The availability and use today of numerous standard compounds, native congeners as well as isotope labelled PCBs, has added a great deal of reliability and comparability to the analytical methodologies.

Materials and Methods

To illustrate the current analytical methodologies used in leading laboratories in the Nordic countries (including some European, US and Japanese laboratories) in analysing PCBs in biological material the reported methodologies in two recent inter-laboratory studies are summarised here. The summary is based on 14 laboratories within the IUPAC fish oil study[4] and

19 laboratories in the WHO/EURO milk study[5]. There is some overlap between the 33 participating laboratories, i.e. some of the laboratories took part in both studies.

Results and Discussion

Method summary. The main steps in the PCB analyses are: a) the *extraction* of the fat including the analytes, b) quantitative *determination* of the amount *of fat*, c) *clean up*, i.e. isolation of the analytes from the fat and from other interfering components, d) *detection* and e) *quantification* of the analytes.

*a) extraction**	*number of labs used*
not reported	2
none, dilution	7
Soxhlet	4
liquid extraction	16
column extraction	2
SFE	2

** solvents used: iso-octane (dilution), toluene (Soxhlet), hexane, diethyl ether, ethanol, acetone, cyclohexane, pentane (liquid or column) and CO_2 (SFE)*

*b) fat determination***	*number of labs used*
total; gravimetrical	17
aliquot; gravimetrical	2
pre-extracted (IUPAC)	14

***in the IUPAC study the sample was pre-extracted and the weight as such was used as fat weight*

c) clean up		*number of labs used*
fat reduction		
	SPE	1
	SFE	2
	GPC	6
	acid/base***	24
fractionation		
	HPLC	1
	open column****	28
	SFE, SPE and other	4

****H_2SO_4 treatment most common*
*****column materials used: modified silica, Alumina, Florisil and carbon*

d) detection		*number of labs used*
	GC-ECD*****	16
	single column	*(5)*
	double columns	*(11)*
	GC-MS******	17
	HR (R>1000)	*(8)*
	LR (R<1000)	*(9)*

******GC columns used: (all capillary 30-60 m) Ultra1, Ultra 2, DB5, SPB5, RTX5, DBDIOX, DB17, SPB17, DB210, SIL19CB, CP-SIL5, CP-SIL8CB, SPB20 and SPB-Octyl*
*******MS technique: SIR, EI*

*e) quantification********	*number of labs used*
external standards	3
internal standards	30
single	*(11)*
several	*(19)*

some native standards used: ^{12}C-PCB # 22, 29, 72, 66, 104, 112, 155, 185, 196, 199 and 207
isotope labelled standards: ^{13}C- PCB # 28, 52, 101, 105, 118, 138, 153, 180, 194, 202

********in both studies referred here individual concentrations of PCBs were to be reported.*

Comparability of results. In the IUPAC study the 14 laboratories were found to have a standard deviation (S_r) of the within-laboratory repeatability of 4-17%, for individual congeners, for 8 of the labs, the rest were within 45%. The standard deviation of between laboratory reproducibility (S_L) was between 15 and 48% for 9 of the labs, 4 of the labs were off by up to 80% and one was >100% off. The following PCB congeners were the ones that were most accurately determined in the fish oils: 28, 52, 66, 74, 101, 105, 114, 118, 128, 153, 156, 167, 170, 180 and 183. Other congeners were not included in calculating the standard deviations.

In reporting total PCB levels (also denoted sum of PCBs) in a biological sample several approaches can be used, and will eventually result in greater deviations in the results generated in different laboratories than when reporting concentrations of single congeners. The comparison, for instance in trend studies, of total levels of PCB reported in biological material today compared to the reports from the 60's through the 80's, based on quantification with Arochlore and other technical mixtures as standards, is a problem. Also the use of different quantification techniques and lack of documentation of more precise calculations make comparisons very difficult.

State of the art 1999. From the above interlaboratory studies one can conclude that the *state of the art methodology* today for PCB determination, as far as this is defined by *most used methodology*, is one that uses a 'very traditional' liquid extraction, followed by 'traditional' open column chromatography clean up, and finally a 'less traditional' detection and quantification step. By 'traditional' is meant the progression in methodology since the early 50's. Further, in comparing results between different laboratories the reported PCB levels in the same sample can be assumed to have a standard deviation of less than 50%.

As concluded above, little has changed regarding the sample pre-treatment since the first PCB analyses in the 50's and 60's. Dramatically though, has the detection and quantification developed since then by improved GC materials, mass selective detectors and accurate labelled and native standards. Recent developments in sample handling by use of different new extraction techniques,

such as SFE[6,7] [8]ASE[9] and MASE[10] i.e. microwave assisted extraction, shows that the extraction and clean up parts of the procedure are now likely to change.

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References

[1] Ballschmiter K and Zell M. Analysis of polychlorinated biphenyls (PCB) by glass capillary gas chromatography. *Fresenius Z. Anal. Chem*. **1980**, 302, 20
[2] Jensen S. Report of a new chemical hazard. *New. Sci*., **1966**, 32, 612
[3] Westöö G, Norén K and Andersson M. Levels of organochlorine pesticides and polychlorinated biphenyls in margarine, vegetable oils, and some foods of animal origin on the Swedish market in 1967-1969. *Vår Föda*, **1970**, 22, 9
[4] Lindström G, van Bavel B, Järemo M, Karlsson L, Rappe C and Hardell L. The use of supercritical fluid extraction (SFE) as a sample preparation method in the analyses of PCDD, PCDF and PCB in human tissue. *Organohalogen Compounds*, **1995**, 23, 27
[5] Liem AKD, Lindström GUM, Schlabach M, Gort SM and Hoogerbrugge R. Second Round of IUPAC/CFC/WG-HHEC Project 650/80/94. **1998**, Document nr 143/98 LOC, RIVM, Bilthoven
[6] WHO/EURO, Fourth round of interlaboratory quality assessment study on levels on PCBs, PCDDs and PCDFs in human milk and plasma. Meeting report, Prague 1998
[7] van Bavel B, Järemo M, Karlsson L and Lindström G. Development of a solid phase carbon trap for simultaneous determination of PCDDs, PCDFs, PCBs and pesticides in environmental samples using SFE-LC. *Anal. Chem*. **1996**, 68, 7, 1279
[8] van der Velde, Hijman WC, Linders SHMA and Liem AKD. SFE as clean-up technique for ppt-levels of PCBs in fatty samples. *Organohalogen Compounds*, **1996**, 27, 247
[9] Application Note 316, Dionex. Extraction of PCBs from environmental samples using accelerated solvent extraction (ASE)
[10] Onuska FI and Terry KA. Microwave extraction in analytical chemistry of pollutants: polychlorinated biphenyls. *J. High Resolut. Chromatogr*., **1995**, 18, 417