

# OPTIMIZATION OF THE ANALYTICAL METHOD FOR DIOXIN-LIKE PCBs IN LOW VOLUME SAMPLES OF HUMAN SERUM OF PATIENTS FACING FERTILITY PROBLEMS

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## Introduction

Several studies indicate that human semen quality and fertility have declined over the last decades<sup>1</sup>. According to the estimation of World Health Organization about 8% of couples at the global level experience some forms of infertility problem during their reproductive lives. This percentage means that 50 to 80 million people have problems with fertility<sup>2</sup>. Several lifestyle-related (obesity, smoking) and environmental (exposure to traffic exhaust fumes, dioxins, combustion products) factors appear to negatively affect human fertility, emphasizing the importance of environmental/lifestyle impacts throughout the life course<sup>3</sup>.

Currently there is a growing scientific interest in knowing the relationship between environmental and occupational exposure to toxic substances and/or contaminants and impaired sperm quality, and the involvement of these in male sterility<sup>4</sup>. Specifically endocrine disrupting chemicals such as pesticides, herbicides, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and others.

Physico-chemical properties of these compounds result in their extreme persistence in the environment, their ubiquitous distribution from sources to remote areas via long range atmospheric transport and their ability to bioaccumulate and biomagnify in higher trophic organisms. In fact, more than 90% of human exposure to PCDD/Fs and DL-PCBs has been attributed to food intake, particularly from food of animal origins<sup>5</sup>.

The effects of the toxicity of PCDD/Fs and DL-PCBs on the male reproductive system have been studied mainly in animals which have been described: altered secretion of luteinizing hormone (LH), abnormal morphology of testis, and decrease spermatogenesis and fertility reduction<sup>6</sup>. Although studies in humans are limited and conflicting, several studies have shown that these toxins could have adverse effects on the male reproductive system.

This paper is the first step of a project that sought to study the relationship between semen quality and human exposure to DL-PCBs. The purpose of this phase is to develop an analytical method for the analysis of serum samples of small volume (1 mL). The most important aspects to consider are: minimizing both the consumption of chemical reagents and analysis time, in order to minimize the cost of the analysis, but keeping the quality standards required by EPA Method 1668C for the analysis of dioxin-like PCBs. Our main goal with this study is to provide an individualized patient study.

## Materials and methods

Blood samples were obtained by venipuncture, allowed to stand the blood was centrifuged and then the serum is separated by decantation, the serum fraction was stored in the dark at -20 ° C until analysis.

The procedure used for analytical determination was adapted from a previously published method<sup>7</sup> for the HCB, 4,4'-DDE, 4,4'-DDT and ICE 7PCB congeners analysis by gas chromatography with electron capture detection. Figure 1 shows the analytical procedure developed for the DL-PCBs in low volume samples of human serum.

The samples were analyzed by HRGC-HRMS using an Agilent HP5890 gas chromatograph equipped with programmable temperature vaporization (PTV) inlet, coupled to a Micromass Autospec Ultima-NT mass

spectrometer. An Agilent DB5-MS chromatographic column (60 m x 0.25 mm i.d. x 0.25  $\mu$ m) was used for the analysis. Isotopically labeled standards were obtained from Wellington Laboratories.

Quality assurance criteria were based on the minimum requirements describe in US EPA method 1668C for DL-PCBs. A procedural blank was associated with each batch of 4 samples and processed in the same manner. The congeners below the limit of determination (LOD) were calculated considering a concentration equal to their respective LOD.

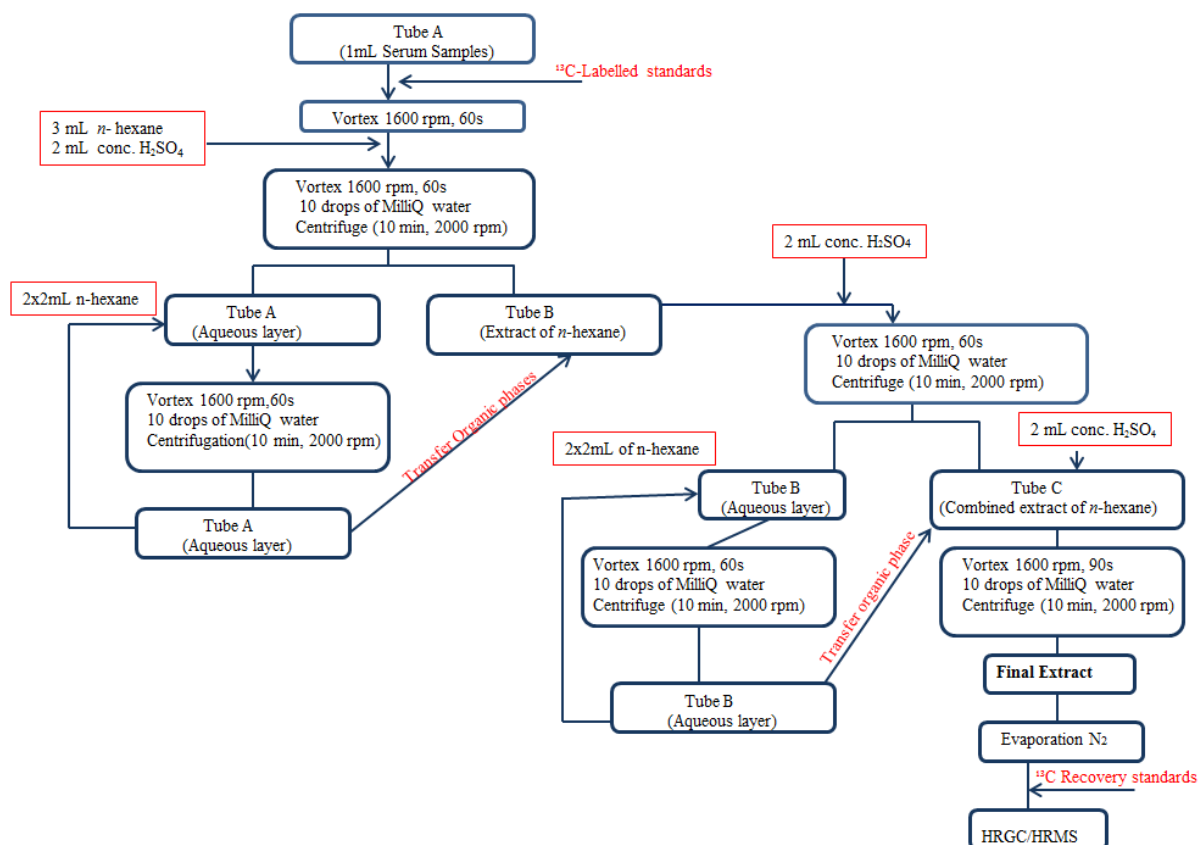


Figure 1. Scheme of analytical procedure developed.

## Results and discussion

In order to obtain biomarker values that reflect body burden of persistent organohalogen pollutants (POP), concentrations of lipophilic POP in serum or plasma are generally expressed on a lipid weight basis, and not on a fresh weight basis. In this work, the total lipid concentration was explained by the sum of the triglyceride and cholesterol concentrations, with the following regression: Total lipid = 62.3 + Triglycerides + 2.27 \* Cholesterol, developed by Phillips et al.<sup>8</sup>

In this study, 19 blood serum samples were obtained from patients enrolled for assisted reproduction at the IVF Spain. Written informed consent was obtained for each patient. Recovery rates of labelled congeners ranged from 70 to 110% and the limits of determination were generally below 1 pg/g on lipid basis.

Table 1 summarizes the concentrations of DL-PCBs in the samples analyzed. The total internal levels obtained for the patients ranged from 6.92 to 84.15 pg WHO-TEQ/g lipid for DL-PCBs with a mean value of 20.46 pg/g lipid. The dominant congeners, in order, were PCB118, 156, and 105, having mean values of 2185.7, 1102.6 and 544.9 pg/g lipid, respectively. The predominant PCBs (the sum of the three predominant congeners) contributed 67% to the mean total DL-PCB concentration in the samples analyzed.

Table 1. Levels of DL-PCB congeners in blood serum, expressed as pg/lipid base, and total DL-PCB concentrations, expressed as pg-WHO-TEQ/g lipid base.

	Mean	Min	Max
PCB-81	71.77	14.30	249.78
PCB-77	246.00	18.70	1110.13
PCB-123	156.40	22.96	399.58
PCB-118	2185.69	461.55	5216.47
PCB-114	153.47	34.21	387.25
PCB-105	544.91	136.76	1038.54
PCB-126	138.73	41.17	638.33
PCB-167	323.00	66.58	788.01
PCB-156	1102.54	196.27	2627.31
PCB-157	278.06	85.47	823.25
PCB-169	212.91	56.10	1011.36
PCB-189	344.28	102.57	1685.59
Sum DL-PCBs (pg/g lipid)	5746.32	2016.94	13227.47
pg-WHO-TEQ/g lipid	20.46	6.92	84.15

Finally, we can conclude that the main objective of this first step of the project has been achieved, an analytical method for the analysis of serum samples of small volume (1 mL) has been developed under QA/QC criteria and obtaining appropriate detection limits.

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