SOURCES OF ERRORS IN CLINICAL CHEMICAL ANALYSIS OF PCBS AND RECOMMENDED PRACTICES TO AVOID THEM

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Introduction

During the mid- to late-1980's, the use of packed column gas chromatography (PGC) with Aroclor[tm] standards and Webb-McCall "peak matching" quantitation[1] were the norm for analyzing polychlorinated biphenyls (PCBs) in blood in human studies, including those by the Centers for Disease Control (CDC) in the United States (US)[2]. However, significant advances have been made since that time in the analysis of PCBs in biological samples utilizing high-resolution gas chromatography (HRGC) with electron capture detection (ECD) or mass spectrometry (MS) detection to produce homolog, or preferably, congener-specific data for PCBs. Analysis with HRGC-ECD or MS with homolog or congener based quantitation is now the generally accepted standard. Surprisingly, many reports continue to be encountered of commercial clinical laboratories analyzing human blood samples using PGC-ECD or HRGC-ECD with Aroclor[tm] based quantitation. Aroclor[tm] analysis of human blood samples is not reliable and is not a recommended practice. Many of the more common potential sources of errors which need to be considered in any study involving the analysis of PCBs in blood are outlined below, and recommendations are provided for clinical laboratory analysis of PCBs with respect to 1) matrix, 2) sample containers, 3) extraction and cleanup methodology, 4) lipid versus whole weight based quantitation, 5) PCB quantitation methods, and 6) quality assurance/quality control (QA/QC) considerations. These recommendations are based on the authors' many years of combined experience with this type of analysis.

Materials and Methods

This paper is based on the review of numerous blood (whole blood, serum, plasma) samples analyzed by a variety of different analytical techniques.

Results and Discussion

Human blood samples can be analyzed for PCBs in different compartments, the major ones being whole blood, plasma, or serum[3]. While partitioning of polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/F) between blood components has been studied[3-4], similar studies are limited for PCBs[5]. Since PCB distributions between the components have not been extensively studied, methods for conversion between these components are unreliable. Therefore, an important factor regarding the specific blood component that will be analyzed by a clinical laboratory is what comparison population(s) will be used. In the United States (US), data are available for selected PCB congeners in serum for the general population for the 1999-2004 time period through the National Health and Nutrition Evaluation Survey (NHANES)[6-7] and have been published in the scientific literature[8-9]. For a particular study, a specifically and appropriate selected background population may also be used, provided the same matrix and analytical method is used.

The matrix being sampled (whole blood, plasma, serum) will be a major determinant for the sampling container to be used. The type of sampling tube used and storage conditions have been found to impact the PCB results[10]. For PCBs in whole blood or plasma, lavender or blue serological top (ethylene diamine tetraacetic acid (EDTA) additive) tubes are generally used, while for PCB analysis, serum samples obtained after clotting in red-top plain glass tubes are generally preferred due to less potential interferences. The NHANES serum sampling procedures for PCBs are publicly available and provide specific, easy to follow guidance.

Another issue which must be considered in clinical chemical analysis of PCBs is the concentration of circulating lipoproteins (lipids) in the blood. Previous studies at CDC (and others) have established that postprandial increases in circulating lipids can result in wet weight concentration changes of approximately 25 percent within a day for the same person[11]. However, these fluctuations disappear when the results are adjusted for the lipids concentration. The CDC study referenced above and NHANES utilize total cholesterol (TC) and triglyceride (TG) concentrations measured enyzmatically and input to a specific algorithm to produce a calculated Total Lipids (TL) concentration[12]. This is now the generally accepted approach, particularly when unfasted subjects are measured or when comparisons are made between laboratories or cohorts. The alternative of gravimetric lipid analysis requires a considerable amount of technical skill and large sample sizes to produce accurate and reproducible results and has been found to result in much larger uncertainties. However, an experienced laboratory can produce valid results using this approach with fasted subjects and consistent and appropriate lipid extraction analytical methods.

The PCBs in a blood sample are determined by extraction, cleanup and quantitation. A variety of extraction methods using single or multi-component solvent systems have been reported to provide reasonably reliable results. Extracted blood samples will almost always require cleanup to remove lipids and other potential interferences and to concentrate the samples further, generally followed by liquid or solid-phase extraction (SPE) cleanup. The utilization of recovery surrogates as well as cleanup standards allows for the evaluation of the efficacy and reliability of the extraction and cleanup step. Scrupulous cleaning of glassware (e.g., by baking at 400°C) used in extraction and cleanup steps is also necessary to prevent background interferences in ultratrace PCB work.

The analysis for PCBs can, in theory, involve very simple approaches, such as the use of total chlorine (as chloride) and conversion to a PCB equivalent number, up to very complex analyses like HRGC/high-resolution mass spectrometry (HRMS).

As noted earlier, the use of PGC-ECD with Webb-McCall quantitation was the norm in the mid- to late-1980's. Burse has reported on some of the developments which culminated in the AOAC 990.7 method being finalized in 1992[2,13]. However, there are a number of disadvantages and limitations to use of Aroclor[tm] based analysis and, by the mid-1990's, biomonitoring studies carried out by CDC and others had moved away from the use of Aroclor[tm] analysis[14]. The last data reported by CDC in a population using Aroclor[tm] analysis appears to be the New Bedford Harbor study in the early 1990's. ATSDR has stated that Aroclor analyses of biological samples are prone to error due to the subjective manner of the peak selection and cannot be reliably compared to original technical mixtures[15]. Aroclor[tm] analysis often overestimates actual PCB concentration, with a factor of 2.5 being identified in some studies[16]. Perhaps the most significant shortcoming to the use of Aroclor[tm] analysis is the inability to provide information on specific PCB congeners, especially the dioxin-like PCBs (DL-PCBs) which are generally present at orders of magnitude lower concentrations than the most prevalent congeners. Aroclor[tm] data cannot be reliably compared to congener–specific data. Under no circumstances can Aroclor[tm] analytical results be used to compare to reference rangers produced from NHANES or other studies using HRGC with congener or homolog based quantitation.

DeCaprio has reported on the validation of a dual column HRGC-ECD method analyzing 101 specific congeners (in 83 peaks) for quantifying PCBs in human serum. The use of dual column gas chromatography (GC) allows for the reduction of the uncertainty associated with coeluting peaks, provided that initial method validation with primary congener standards has been performed. HRGC with low-resolution mass spectrometry (LRMS) allows for the determination of PCB concentrations on a homolog or congener specific basis. The ability to focus on specific masses of compounds of interest allows for the use of internal standards (IS) with different masses, such as fluorinated PCBs or isotopically related compounds, such as deuterium or carbon-13 labeled PCBs. The ultimate "gold standard" for PCB analysis is currently isotope-dilution mass spectrometry (IDMS) using carbon-13 labeled recovery, cleanup and internal standards and HRGC-HRMS. All 209 congeners can be analyzed for by this type of method. Although the uncertainty for coeluting congeners, especially for the same homolog group (i.e., isomers), is greater than for the fully resolved congener peaks. The NHANES currently reports up to 38 congeners by HRG-

HRMS by IDMS quantitation. The US Environmental Protection Agency (USEPA) Method 1668B is another example of such a method[17]. Finally, in addition to HRGC-HRMS, two-dimensional GC (2D-GC) also has the theoretical promise of complete PCB congener separation and analysis[18].

There are a number of QA/QC considerations for clinical laboratories performing PCB analysis. These include the sensitivity, the specificity, the GC column resolution, and the calibration range of the instrument. Important QC procedures that a laboratory should invoke include: i) validation of the method using standard reference material(s) (SRM) which now exist for serum[19], ii) ongoing precision evaluation using a QC serum sample, iii) the use of replicate sample preparation and injection, iv) the use of recovery, cleanup and injection IS, v) the use of blanks (solvent, instrument, container, etc.), and vi) the participation in interlaboratory studies, such as that run for the Arctic Monitoring and Assessment Programme (AMAP).

Recommended Best Practices

It is recommended that clinical laboratories utilize serum samples collected using red top, glass serological tube. Serum samples should be analyzed for triglycerides and total cholesterol using enzymatic methods to calculate TL. Appropriately validated sample extraction and cleanup procedure should be used including recovery IS. Routine analysis should use HRGC-ECD (with dual columns) and/or HRGC-LRMS calibrated for the latest NHANES congener list at a minimum. Where possible, some cross-validation of ECD or LRMS results using HRMS is recommended. The initial validation of the specific method used by a laboratory can use a National Institute of Standards and Technology (NIST) SRM (1589b, 1957, 1958); and ongoing precision can be evaluated through the use of a QC serum sample (a pooled sample, often from a blood bank, for example). The use of IS quantitation will improve analytical reliability. We do not believe there is any place for Aroclor^{tm} analysis of blood in a modern clinical chemical laboratory. Furthermore, data produced from Aroclor^{tm} analysis cannot be compared to reference population data (such as NHANES) produced by other methods.

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