COMPREHENSIVE ANALYSIS OF ORGANIC COMPOUNDS IN HUMAN SERUM BY GAS CHROMATOGRAPHY COUPLED WITH HIGH RESOLUTION MASS SPECTROMETRY

Palát Jiří[1], Codling Garry[1], Schacht Veronika[1], Klánová Jana[1]

[1]Masaryk University, Research Centre for Toxic Compounds in the Environment, Brno, Czech Republic, 625 00 Brno, Czech Republic, e-mail: palat@recetox.muni.cz

Introduction:

The Stockholm Convention in 2001 on Persistent Organic Pollutants (POPs) created the dirty dozen, 12 compounds for global restriction that have properties of long range transport, bioaccumulation and toxicity. These include pesticides, industrial compounds and by-products. The Convention has continued to add more chemicals of concern and lists more compounds that are suspect for investigation. Until recently, much of environmental and human monitoring was focused on these legacy compounds and their metabolites. These chemicals are of vital global importance for human and environmental protection and require monitoring but too great a focus upon these may omit emerging chemicals.

POPs compounds are now restricted or removed from global production however; these compounds were produced because they have a use. For each compound restricted one or more replacement chemical is manufactured and yet more compounds are being created. The United States Environmental Protection Agency (US-EPA) has at any one time 300 new compounds under review for license. The European Union's Registration, Evaluation, Authorization and Restriction of chemicals (REACH) lists around twelve thousand substances used in Europe and 520 of these substances at amounts greater than 100 000 tons per annum. The fate and behavior of all these chemicals need to be known, in addition parent compounds can undergo degradation and metabolic processes to form thousands of other compounds. As a result, the focus on POPs is vital to know what these known toxicants are doing while omitting emerging compounds is risking missing

Chromatographic instrumentation used in the past to detect compounds has in the last decade undergone a revolution. In the past full scan, methods were limited to low resolution and very poor sensitivity, such that most methods were target and restricted to a tens of compounds in an instrument run. Today the latest generation of ultra-high resolution mass spectrometers (UHRMS) are capable of high mass sensitivity and across a wide mass range. Thus, it is possible to identify legacy compounds for routine monitoring and look for emerging pollutants within the same run.

For the present work, polychlorinated biphenyls (PCBs) were chosen as a model POPs group of compounds because they are well defined legacy compounds of importance. PCBs are one chemical class of the initial dirty dozen. They are anthropogenic compounds produced in large quantities in the past for numerous industrial and commercial applications, including dielectric fluids for capacitors and transformers. Due to their physical chemical properties. PCBs are highly stable in the environment. Biodegradation processes and photolysis being the primary degradation routes for PCBs are slow and depending on various factors such as temperature, bacterial community, oxygen, etc. Hence, PCBs remain a global contaminant class of concern for population health.

Despite more than 50 years of research the extent to which PCBs are toxic remains controversial. They are known or suspected to have various adverse effect to both humans and wildlife, including endocrine disruption, neurotoxicity, thymic atrophy, dermal toxicity and carcinogenicity. Yet, human exposure to PCB concentrations potentially leading to adverse health effect are still reported. For example, populations locally exposed to PCB hotspots such as in eastern Slovakia have shown concerning internal PCB levels[1]. Evidently, monitoring programs are still of significant importance as the environment and humans are still impacted by PCBs.

The aim of this project is under the idea of comprehensive analysis initially a review of current exposure concentration in humans to PCBs across Europe and further to use a non-compound specific extraction technique for serum coupled with gas chromatography high-resolution mass spectrometry (GC-HRMS) to determine concentration of target PCBs. However, the information about selected PCB congeners is just snapshot of the whole picture. By using full scan acquisition far more exposure information can be collated not just the target PCBs but all 209 congeners can be investigated along with any chemical ionized above the detection limit on the instrument.

Method: Blood serum from oncology patients (<18 years of age) diagnosed with different types of cancer was used in this study. These samples were taken in the initial diagnosis and consent was given that the material may be used in additional investigation such as this study. In total, 170 samples were extracted by solid phase extraction (SPE), after fortifying with recovery standards (3 ^{13}C-PCB). All samples were eluted with methanol

and ethyl acetate, concentrated under N_2 and solvent exchanged to nonane containing the internal standard (PCB 121). Detection was performed by GC-Orbitrap-HRMS operating in full scan acquisition from 67-1000 amu.

Results: PCB concentrations found in humans across the EU have been found to vary greatly. In general, nanograms to hundreds of nanograms per gram of lipid are reported in human serum[1-3]. The exposure concentration depends upon location, diet and proximity to sources. In our oncology samples, PCB concentrations were generally low across samples compared to findings in the literature. For the most determined PCBs similar trends were observed between individuals. In full scan by GC and by LC thousands of additional features have been detected and using calibration compounds and library searches a more in-depth analysis of these human samples are possible.

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