Abstract

Polycyclic Aromatic Hydrocarbons (PAHs) are mainly emitted by human activities and are one of the main pollutant groups in air, soils, waters, and food. Therefore, they are subject to atmospheric, environmental and dietary monitoring. This study focuses on the particular case of exposure of pregnant women and their fetus, which are at risks. Indeed, PAH exposure can lead to fetal malformations, prematurity and even disorders in children and adult. To date, only a few PAHs regulated by US-EPA were searched and quantified in maternal or umbilical cord blood. Moreover, analytical performance data are often lacking. Hence, this study aims for the first time in France, at developing analytical and biological methods for the monitoring of PAH exposure during pregnancy.

In a first part, the simultaneous determination of the 24 regulated PAHs in sera from maternal and umbilical cord bloods was developed. First, the separation of the 24 PAHs by LC/UV-FD was optimized. Then a precipitation step of the proteins was optimized with a Design of Experiment to disrupt all the interactions between the PAHs and the proteins. A solid phase extraction (SPE) protocol, with a C18-based sorbent, was next optimized to extract and concentrate the PAHs. The nature and the proportion of an organic solvent in the sample was optimized to avoid the PAH adsorption on the cartridge and vial walls. The washing and elution steps were also optimized. To enrich again the elution fractions, an evaporation step was developed by testing 2 approaches: a total or a partial evaporation ("last drop" method). This sample pretreatment protocol was successfully applied to the analysis of spiked pooled sera from maternal and umbilical cord bloods.

After the automatization of the SPE step, the second part aimed at validating this analytical procedure by an accuracy profile approach with spiked maternal and umbilical cord sera and simultaneously developing and validating a confirmation method by GC/MS. Then, sera samples from maternal and umbilical cord bloods were analyzed by LC/UV-FD and GC/MS.

In a third part, after the validation of a spiked placental perfusion solution at 1 µM with 16 US-EPA PAHs, an *ex vivo* human cotyledon perfusion model was carried out to evaluate the PAH placental transfer. In addition, human trophoblast cell cultures with a spiked DMSO solution at 1 µM with 16 US-EPA PAHs were carried out to evaluate the impact of PAH exposure on placental functions.