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Innovative Approaches to Sampling for Clinical Toxicokinetic Studies

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Objective: Accidental or deliberate exposure to organophosphorus compounds (OPs; amongst the most widely used pesticides) account globally for around 20,000 deaths and 3 million non-fatal poisonings each year. Unfortunately not all poison victims make it to hospital, with a large number either dying in their villages or being cared for with no access to specialist or even basic equipment. Sampling in this situation is a major challenge with micro-sampling using the dried blood spot technique being a potential solution, allowing collection of valuable human toxicokinetic data and for accurate profiling of poisonings.

Methods: To address this we tested two Dried Blood Spot (DBS) commercially available specialist papers (Whatman® protein saver cards and Whatman® FTA® card technology, Whatman) and HemaSpot®-HF Blood Collection Device (Spot on Science, USA) for sample drying and recovery, accuracy and precision for phenthoate, quinalfos, chlorpyrifos and fenthion with terbufos as an internal standard (IS) following storage. From human whole blood spiked with 500 ng/mL of each OPs a range of concentrations (15.6 to 500 ng/mL each) were prepared and 80 µl transferred to the paper or device and allowed to dry (4 or 24 h). Quality controls were included. For analysis one blade or a 60 mm disc was extracted in 100% methanol, sonicated and centrifuged at 10,000g for 10 min at room temperature with the supernatant analysed. We developed a sensitive liquid chromatography mass spectrometer (LCMSMS) assay for the analysis using Shimadzu 8060 triple quadripole mass spectrometer in positive ion mode with turbo electrospray ionization sources (Shimadzu, Japan).

Results: The Whatman® protein saver card failed to dry after 4 h so was left for 24 h before storage and analysis. Precision (N=6) was acceptable with an area ratio of 2.67±0.06, CV=2.2%. Comparing Whatman® FTA® card technology and HemaSpot®-HF, linearity (N=3 sets of concentrations for each of the OPs) all gave r2 values >0.99 with %CV of 11.3 and 4.0 for phenthoate; 5.8 and 3.1 for quinalphos; 5.8 and 2.1 for chlorpyrifos and 5.1 and 1.3 for fenthion, respectively with statistical comparisons using line of identity showing no significant differences. For intraday variability using 200 ng/mL quality controls (N=6) accuracies of 4.22- 4.90 %CV for Whatman® FTA® card technology were no different to those for 3.60-8.59 %CV for HemaSpot®-HF.

Conclusion: Whatman® FTA® card technology and HemaSpot®-HF can be used reliably for micro-sampling. Further studies will include comparisons with and without haematocrit, field testing and cost comparison evaluation compared to conventional sampling.