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SPE-HPLC purification of endocrine disrupting compounds from human serum for assessment of xenoestrogenic activity

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Background: Assessment of xenoestrogenic activity in human serum samples requires the removal of endogenous sex hormones to assure that the activity measured originates from xenobiotic compounds only.

Methods: Serum samples representing high, medium and lower accumulation of persistent organic pollutants (POPs) were extracted using solid-phase extraction (SPE) followed by normal-phase high-performance liquid chromatography (NP-HPLC) for separation of POPs from endogenous hormones. The recovery of polychlorinated biphenyl (PCB) congeners in spiked serum samples was up to 86 % for certain compounds. MVLN cells, stably transfected with an estrogen receptor (ER) luciferase reporter vector, were exposed to the reconstituted SPE-HPLC extracts for determination of the integrated estrogenic activity. The effects of PCBs were analyzed by direct in vitro exposure of PCBs (138, 153 and 180) and by ex vivo analysis of SPE-HPLC extracts from serum spiked with the PCBs. Similar effects on ER transactivation were observed for the direct in vitro and the ex vivo analysis experiments.

Results: The ER transactivation responses determined for actual serum samples were in the linear range of the dose-response curve. 17β-estradiol titrations showed that the xenoestrogenic effects were mediated via ER. Moreover, our SPE-HPLC-MVLN cell-assay was demonstrated to elicit high interlaboratory correlation.

Conclusions: In the present study the combination of SPE-HPLC purification and the ex vivo estrogenic
responses measured by MVLN cells was validated and considered to be a valuable tool to assess the combined ER effect of lipophilic serum POPs where additive/synergistic and agonistic/antagonistic effects are integrated giving an overall estimate of exposure and bioactivity.