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Section **Fields (of activity)**

TOX-SISTEM - Sensitive identification of toxic substances in complex mixtures by combining thin layer chromatography with effect-based tools and high resolution mass spectrometry

Project Leaders
Inge Werner[1], Juliane Hollender[2], Andreas Schönborn[3]

Investigators
Alan Bergmann[1], Eszter Simon[1], Etienne Vermierssen[1]

[1] Swiss Centre for Applied Ecotoxicology, Eawag, Überlandstrasse 133, 8600 Dübendorf. Switzerland
[2] Department of Environmental Chemistry, Eawag, Überlandstrasse 133, 8600 Dübendorf. Switzerland
[3] Institute of Natural Resource Sciences, Zürich University of Applied Sciences, Grüental / Postfach, 8820 Wädenswil, Switzerland

Key words
Effect-based tools; toxicity, chemical mixtures; HPTLC; LC-HRMS/MS; drinking water; migrates from food contact materials

Aim of the study
We aimed to advance, implement, and validate bioanalytical methods on HPTLC and combine them with high resolution mass spectrometry for toxicant identification. Specifically, we targeted improvement of HPTLC methods for detection of (1) estrogenicity, and (2) genotoxicity, and application of these toxic endpoints to diverse chemicals and samples (e.g. food contact chemicals, drinking water and migrates from food contact materials). We then aimed to perform non-target analysis with high resolution mass spectrometry toward the identification of toxicants in relevant samples.

Material and methods
Bioassays: We began with a yeast-based method for estrogenicity (P-YES) because it is the best established reporter gene assay on HPTLC plates. With 20 food contact chemicals related to plastic packaging materials, we evaluated the sensitivity and quantitation performance of the P-YES against a standardized version of the assay in microtiter plates (L-YES). In stages throughout the project, we adapted a genotoxicity test, the umuC assay to HPTLC plates (P-umuC). We ensured the functionality of P-umuC using several chemical standards. We prepared a protocol for incorporating metabolism into estrogenicity screening by incubating samples with rat liver (S9) enzymes before applying to HPTLC or microtiter plates. It is an important aspect to investigate chemicals that can become bioactive (so called “pro-estrogens” in this case) or inactive after enzymatic metabolism.

Samples: We collaborated with project partners to obtain a variety of relevant samples, including namely from Cantonal Laboratory Zürich, Cantonal Laboratory St. Gallen, Industrielle Werke Basel, and Swiss Quality Testing Services. The samples included coated metal cans, paper food contact articles, water from drinking water pilot treatment plant, wastewater samples, and surface water samples. Migration of food contact materials (FCM) was performed either by the Ecotox Center or by our project partners. Extraction of water samples was performed at the Ecotox Center. We tested samples as needed in P-YES, L-YES, and P-umuC.

Chemical analysis: We evaluated recovery of estrogenic chemicals from HPTLC plates with targeted HPLC-MS/MS. For non-target analysis, we optimized HPLC methods for representative compounds, explored workflows for data reduction, and strategies for structure elucidation. We confirmed success of the non-target analy-

sis workflow with a migrate of a coated metal can that was spiked with three estrogenic chemicals. Finally, we investigated (an) unknown estrogenic chemical(s) in the can to evaluate the non-target analysis workflow.

Results and significance

Bioassays: We established HPTLC-based and related assays at the Ecotox Center. Assays included the P-YES, umuC, P-umuC, and metabolic pre-incubation in the P-YES. The P-YES was more sensitive than the L-YES to most chemicals and similarly accurate when comparing potencies relative to the reference, 17β-estradiol. We observed that P-YES might be more susceptible to limits of water solubility than L-YES. These trends appear to be similar when comparing the umuC with P-umuC. Metabolic activation was successful for pro-estrogens and is ready to be adapted for diverse assays, most importantly genotoxicity assays such as umuC and P-umuC.

Samples: P-YES detected estrogenic activity in all three coated metal cans and several surface water samples that was not detected by the L-YES or masked by effects of the mixture on cell growth. Wastewater samples showed estrogenic signals typical, i.e. with same retention factors, of steroid hormones. No estrogenic activity was detected in drinking water samples. Genotoxic responses were detected in 3 of 6 migrates of FCM that were tested with umuC and P-umuC. P-umuC determined that there are at least two genotoxic chemicals in some of these paperboard samples.

Chemical analysis: We recovered up to about 80% by mass of target chemicals from extraction of the HPTLC plates for chemical analysis. A non-target analysis workflow was applied that successfully detected two estrogenic chemicals spiked in a coated metal can that were associated with plastic packaging. A steroid hormone was not detected with chemical analysis, matching expectations based on the higher sensitivity of P-YES than chemical analysis. Towards identifying unknown estrogenic chemical(s) with PYES, the developed peak prioritization, which relied heavily on HPTLC separation, reduced the number of chemicals from more than 600 in the whole migrate of a metal can to 46 features. Structural information is being assigned based on MS/MS data and prioritized based on chemical (e.g. retention time) and biological (e.g. predicted binding of estrogen receptor) plausibility. During toxicant confirmation, HPTLC-bioassays will be helpful by confirming both biological activity and retention factor on developed HPTLC plates.

This project evaluated the utility of HPTLC-bioassays for toxicant detection and identification in samples including FCM. Differences in detection of biological effect between microtiter- and HPTLC-bioassays show advantages of HPTLC-bioassays (sensitivity and accuracy) and possible limits of those advantages (for poorly soluble chemicals). The results also demonstrate the ability of HPTLC-bioassays to illuminate effects in complex mixtures such as FCM. With microtiter and HPTLC-bioassays, we detected estrogenic and genotoxic effects in FCM and water samples. Finally, we confirmed that HPTLC methods were advantageous in non-target analysis. HPTLC-bioassays therefore are promising for use in evaluating toxicity of food packaging and drinking water and provide a good foundation for toxicant identification studies.

Publications, posters and presentations

Manuscripts

Alan Bergmann, Eszter Simon, Andrea Schifferli, Andreas Schönborn, Etienne Vermeirssen. Estrogenic activity of food contact materials – evaluation of two yeast based screening tools, on HPTLC or 96-well plates. In preparation for resubmission to Analytical and Bioanalytical Chemistry.

Alan Bergmann, Eszter Simon, Andreas Schönborn, Etienne Vermeirssen. HPTLC methods in environmental effect directed analysis. Review paper in preparation.

Selected presentations

Alan J. Bergmann, Teresa Mairinger, Daniel Olbrich, Eszter Simon, Juliane Hollender, Andreas Schönborn, Etienne Vermeirssen. June 2019. HPTLC-bioassays for food packaging. Presentation to Cantonal Laboratory St. Gallen. St. Gallen, Switzerland.

Alan J. Bergmann, Teresa Mairinger, Daniel Olbrich, Eszter Simon, Juliane Hollender, Andreas Schönborn, Etienne Vermeirssen. November 2018. Effect-directed identification of estrogenic substances in food contact materials with HPTLC-bioassays and HRMS. International Symposium for High Performance Thin Layer Chromatography. Bangkok, Thailand.

Selected posters

Alissa Tophinke, Alan Bergmann. 2019. Entwicklung der metabolischen Aktivierung auf Bioassay mit Dünnschichtchromatographie zum Nachweis Pro-östrogener Chemikalien. Society for Environmental Toxicology and Chemistry: German Language Branch. Landau, Germany.

Alan J. Bergmann, Eszter Simon, Andrea Schifferli, Andreas Schoenborn, Etienne Vermeirssen. 2019. Performance of measuring estrogenicity with planar-YES compared to 96-well plate YES. Swiss Food Sciences Meeting. Neuchâtel, Switzerland.

Alan J. Bergmann, Eszter Simon, Andrea Schifferli, Etienne Vermeirssen, Andreas Schoenborn. 2018. Performance of measuring estrogenicity with planar-YES compared to 96-well plate YES. **Best Poster**: International Symposium for High Performance Thin Layer Chromatography. Bangkok, Thailand.

Alan J. Bergmann, Eszter Simon, Andrea Schifferli, Etienne Vermeirssen, Andreas Schoenborn. 2018. Performance of measuring estrogenicity with planar-YES compared to 96-well plate YES. Biodetectors Symposium. Aachen, Germany.

Alan J. Bergmann, Eszter Simon, Andrea Schifferli, Etienne Vermeirssen, Andreas Schoenborn. 2018. Performance of measuring estrogenicity with planar-YES compared to 96-well plate YES. Society of Environmental Toxicology and Chemistry Europe. Rome, Italy.

Students advised

Pravin Ganesamoorthy (Bachelor's Semester project, April – July 2019). Development of umuC assay on HPTLC plates.

Alissa Tophinke (Bachelor's Thesis, Feb - July 2019). Development of metabolic activation for bioassays on thin-layer chromatography for the detection of pro-estrogenic chemicals.

Collaborations

Cantonal Laboratory Zürich. Food contact material samples provided by Can. Laboratory. Three-day bioassay training provided to technician to assess feasibility of transferring techniques to Can. Laboratory.

Cantonal Laboratory St. Gallen. Food contact material samples provided by Can. Laboratory. Post-doctor gave invited talk at Can. Laboratory in St. Gallen.

Industrielle Werk Basel. Post-doctor visited Basel IWB facility to collect samples from drinking water treatment plant.

Swiss Quality Testing Services. Provided food contact material samples.

Paul Böhm, student at RWTH Aachen. Mr. Böhm learned about P-YES and provided surface water samples from India.

Univ. Offenburg, BfG Koblenz, and Zweckverband Landeswasserversorgung Langenau. Introductory visits. Exchange with analyst (Tobias Bader) from Langenau to Eawag Chemistry department.

Project 4.17.05

Project duration *October 2017 – October 2019*