14th BioDetectors Conference 2024

ABSTRACT BOOK

Amsterdam
The Netherlands
27-28 June 2024

Sponsored by:

BDS BioDetection Systems

BERTHOLD

AQUA SCIENCE

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Program
Thursday 27 June

General introduction session

10.00-10.15 Welcome by organizers 14th BioDetectors
10.15-10.45 State-of-the-art BioDetectors (B. Brouwer, BDS)
10.45-11.15 BlueWater (M. de Baat, UvA)

Application of bioassays for PFAS in water

11.15-11.35 PFAS in Dutch waters (J.K.H. de Schepper, HWL)
11.35-11.55 PFAS TOX Profiling & water (C. van der Wielen, BDS)
11.55-12.15 AquaConnect – case studies (J. Specker, UvA)
12.15-12.30 Fluorescence-based assay for PFOA toxicity after plasma treatment (Y. Topalova, Uni Sofia)
12.30-13.30 Lunch and Poster Session

Application of bioassays in water safety context

13.30-13.50 Effect- and EDA-based water testing (C. Houtman, HWL)
13.50-14.10 TR/TTR-TR in surface waters (P. Sauer, Uni Bohemia)
14.10-14.30 EDCs profiling & regulatory purposes (M. Lukas, UBA)
14.30-14.50 h- and zf-NR activities (B. Kyei Amankwah, Uni Bohemia)
14.50-15.10 Coffee Break
15.10-15.30 EBM performance analysis (T. Pronk, KWR)
15.30-15.50 Receptomic biosensors (M. Henquet, WUR)
15.50-16.10 EDA on Human Serum Samples (M. Margalef, VU Amsterdam)
16.10-16.30 Acute Chemical Toxicity Testing (K. Miklas, Microlan)
16.30-17.00 Aperitif
19.00-……. Social event

Social event - dinner cruise on the Amsterdam canals

Program
Friday 28 June

Contribution of biodetection for global issues

09.00-09.10 Introduction (T. de Boer, BDS)
09.10-09.30 What is EATS? How about metabolism? (C. Budin, BDS)
09.30-09.50 EU project SAFFI: Safe infant food (H. Besselink, BDS)
09.50-10.10 City Monitoring (A. Arkenbout, Toxicowatch)
10.10-10.30 Effect- and EDA- soil testing (M. Larsson, Örebro Uni)
10.30-11.00 Coffee Break and Poster Session
11.00-11.20 Effect-based indoor plastic testing (N. Struwe, Örebro Uni)
11.20-11.40 EDCs in packaging materials (M. Vrolijk, Maastricht Uni)
11.40-12.00 Bioassays for risk assessment of micro & nanoplastics (A. Fernández Ramos, Aimplas)
12.00-12.20 Evaluation of the effects of phytoestrogens on 3D skin models: friend or foe? (F. Rispo, Uni Genoa)
12.20-13.30 Lunch and Poster Session

Science Cafë

13.30-14.00 PFAS – how to move effect-based analysis forward (T1)
14.00-14.30 BLUE WATER – how to use effect-based trigger values (T2)
14.30-15.00 Plastics – safe & sustainable (T3)
15.00-15.15 Wrap-up conference

Lab-Tour and City Walking Tour

15.15-16.00 Lab Tour of High-Through-Put Screening facilities
16.00-18.00 City Walking Tour

Organizing committee:
Milo de Baat (University of Amsterdam)
Peter Behnisch (BioDetection Systems)

For any questions, please refer to :
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Posters

Poster 1	Optimization of L/L-extraction for AhR-CALUX screening PAHs in water samples (Kinrooi) and validation by HPLC (Yigi Su et al.; VU Brussels, Brussels, Belgium)
Poster 2	In vitro assessment of thyroid peroxidase and thyroid hormone receptor-disrupting activities (Hyunki Cho et al.; KIST Europe, Saarbrücken, Germany)
Poster 3	Changes in complex toxicity of leachate, spiked with PFOA as a result of direct plasma treatment (Mihaela Kirilova et al.; Sofia University, Sofia, Bulgaria)
Poster 4	Evaluation of the endocrine disruptor potential of the fishing gears from the Mar Ligure area (Giulia De Negri Atanasio, University of Genoa, Genoa, Italy)
Poster 5	Can human thyroid bioassays evaluate endocrine pressures in wildlife taxa? (Tom Nolte et al.; Naturalis Biodiversity Center, Leiden, The Netherlands)
Poster 6	Incorporating metabolizing system from different tissue sources to improve the value of in vitro endocrine disruption assays: a comparative study (Sylvie Emery et al. ; L'Oréal Research and Innovation, Aulnay-sous-Bois, France)
Poster 7	An Estrogen, androgen, thyroid and steroidogenesis (EATS) assay panel to predict endocrine disruption of chemicals and chemical mixtures - I (Bart van der Burg et al.; BioDetection Systems BV, Amsterdam, the Netherlands)
Poster 8	Monitoring of toxicity of plastic recyclates from low- and middle-income countries by bioassay panel to support the global management of chemicals in plastics (Peter Behnisch et al.; BioDetection Systems BV, Amsterdam, the Netherlands)
Poster 9	An Estrogen, androgen, thyroid and steroidogenesis (EATS) assay panel to predict endocrine disruption of chemicals and chemical mixtures - II (Bart van der Burg et al.; BioDetection Systems BV, Amsterdam, the Netherlands)
Poster 10	Evaluation of thyroid hormone disruption by PFAS in WWTP influent/effluent and surface waters (Harrie Besselink et al.; BioDetection Systems BV, Amsterdam, the Netherlands)
Poster 11	In vitro toxicity profiling of PFAS on a tailored panel of effect-based CALUX bioassays Harrie Besselink et al.; BioDetection Systems BV, Amsterdam, the Netherlands)

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BIODETECTORS FUTURE

Abraham Brouwer

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Abstract:

In this presentation an overview on the use and the ***current and future*** potential of effect-based tools for several different applications (green chemicals, chemical & cosmetics safety, blue water, safe food and healthy people) will be given.

Linking human health status, and better covering the safety of our food and water from exposure to complex mixtures of chemicals strongly calls for complementing chemical analysis with high throughput ***biodetectors*** covering all kinds of known/unknown chemicals and their mixtures.

In vitro cell-based bioassays can cover a wide range of ***disease and toxicity related molecular pathways*** and therefore can predict a range of different toxic and adverse health endpoints, such as acute toxicity, oxidative stress, dioxin-like, endocrine-like effects, reproductive, genotoxic and carcinogenic effects of compounds.

Key ***benefits*** of bioassays are high predictive of health-related effects, good estimate of total effect from mixtures, predicting unknown effects of chemicals, high precision, low cost and high capacity.

In this presentation I will show some examples of our latest developments involving a panel of thyroid related bioassays (TRβ, TTR-TR, TPO, DIO, NIS) allows us now also to detect again ***new classes of emerging pollutants*** such as PFAS, SCCPs and BFRs.

Furthermore, we will show developments on integrating phase I and II ***metabolic steps*** in the bioassays, allowing to better predict, and align with in vivo effects of chemicals & cosmetics.

Finally we combine and compare our AOP-based CALUX read-outs to ***in silico*** predicted transcriptome-based activity profiling of signal transduction pathways (STAP-STP) that are linked to disease outcome pathways for e.g., cancer, and immune-related effects, with an aim to find better treatment solutions.

In conclusion, application of AOP-based bioassays, like our CALUX® panel are very useful to know more about the ***KNOWN as well as UNKNOWN*** biological effects in complex mixtures of wide range of matrices,and are very useful to be applied in ***non-animal testing*** for Green Chemicals, healthier Water & Food as well as in prediction, monitoring, ***diagnosis and scope for treatment*** of chronic diseases in humans.

Keywords:
BioDetectors, Non-animal testing, Complex mixtures, Green Chemistry, Cosmetics safety, Human health & biomonitoring, CALUX

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LINKING BIOANALYTICAL RESPONSES TO WATER CYCLE CHEMICAL HEALTH

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As the development, production, and release of new chemicals outpace the rate at which we can assess their hazards and risks, the planetary boundary for novel entities is being exceeded. Many novel and legacy contaminants end up in the water cycle,

constituting variable, complex, and low-level chemical mixtures. The combined toxic effects of these mixtures are increasingly recognized as a threat to human and environmental health. However, traditional target chemical analyses are unfit to assess the risks of complex chemical mixtures, and new monitoring approaches are required for human and environmental chemical risk assessment in the water cycle.

Combining effect-based methods (EBM) with advanced chemical suspect and non-target screening offers a much broader assessment of the environmental chemical universe, and especially provides risk-scaled insights into the biological activities of complex mixtures. Downstream analyses can subsequently identify harmful (combinations of) causative chemicals to allow targeted mitigation methods for water quality improvement.

EBMs and their applications in water quality assessment have seen substantial developments over the past two decades.

The availability of high-throughput bio and chemical analyses can now generate large datasets that are required to quantify and understand the effects of chemical pollution at different stages of the water cycle. Ultimately, this can provide insights into the relationships between aquatic chemical pollution, negative health outcomes, and biodiversity loss.

This presentation will provide an overview of the present availability of EBMs and their interpretation for chemical water quality assessment. Examples will be provided of current applications, to illustrate the state of science and identify knowledge gaps and the need for future research efforts. Potential technological and analytical improvements will be discussed and new avenues for the applications of EBMs for a better understanding of the relationship between chemical pollution and water quality will be explored.

The final part of the presentation will provide room for discussion and pose questions that may help to identify where and how we can collectively advance the field of biodetection in the coming years.

Keywords:
Water quality, effect-based methods, ecotoxicology, biodiversity, human health

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THE CONTRIBUTION OF 29 PFAS TO THE WIDESPREAD OCCURRENCE OF THYROID HORMONE DISPLACING ACTIVITY IN WATER

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Per- and polyfluoroalkyl substances (PFAS) are a group of xenobiotics that are widely distributed throughout the aquatic environment. Many PFAS are possible thyroid hormone (TH) disruptors because -amongst other effects- these compounds are able to inhibit the binding of the TH thyroxine (T4) to its transport protein transthyretin (TTR).

Our previous study investigated the occurrence of TH-displacing activity in the Dutch (drinking)water cycle, and more specifically, the contribution of PFAS to this effect. [1]

Monitoring data from chemical target analysis of 29 PFAS (November 2021 – January 2023) revealed that PFAS were continuously present in drinking water (DW) and surface water (SW) used as sources for the production of DW.

A field study in 2022 therefore investigated whether TH-displacing activity might also be present in these samples, while extending the scope to include samples from PFAS hotspots and wastewater treatment plants (WWTPs).

Two bioassays -a TTR binding assay (FITC-T4) 2 and the TTR-TRβ-CALUX®[3] - detected TH-displacing activity in each of the samples and mutually showed a positive correlation (R^2 0.85). However, target PFAS ($n \leq 20$) could only explain ≤4.1 % of this effect.

This indicated that as yet unknown compounds contributed to the majority of the measured TH-displacing activity in these samples.

The former field study was recently extended in by:

1. determining the occurrence of TH-displacing activity (FITC-T4 assay) in SW and DW for a period of half a year,
2. studying a variety of PFAS hotspots to assess the contribution of target PFAS.

Aim 1 was performed by 4-weekly sampling of six locations between November 2023 and February 2024. The locations comprised 3 DWs and the 3 SWs that were used for their production, these were also included in the previous study.

TH-displacing activity was detected in all SW samples, but the 20 analyzed target PFAS were not the main effect drivers (≤0.7 %).

Regarding aim 2, 7 locations known to be PFAS hotspots (NL, BE) were sampled in 2023-2024. PFAS levels in these samples spanned multiple orders of magnitude (300 ng/L to 10,000+ ng/L).

Preliminary results indicate that 20 target PFAS -for which relative potency factors were available- contributed 6 up to 25 % to TH-displacing activity in 6 out of 7 hotspot samples.

TH-displacing activity in 1 sample might even be explained fully by the presence of target PFAS (~100 %). Current work focuses on characterizing the part of TH-displacing activity in water that is not explained by the analyzed PFAS.

We pursue to identify other (bioactive) compounds that may act as mixture effect drivers. This is done by developing a targeted and nontarget effect-directed analysis (EDA) workflow to explain the currently missing gap of TH-displacing activity measured in water samples.

References:

1. de Schepper, J. K. H., van Oorschot, Y., Jaspers, R. J., Hamers, T., Lamoree, M. H., Behnisch, P., Besselink, H. & Houtman, C. J. The contribution of PFAS to thyroid hormone-displacing activity in Dutch waters: A comparison between two in vitro bioassays with chemical analysis. Environ Int 181, 108256 (2023) doi: 10.1016/j.envint.2023.108256.
2. Leusch, F. D. L., Aneck-Hahn, N. H., Cavanagh, J.-A. E., Du Pasquier, D., Hamers, T., Hebert, A., Neale, P. A., Scheurer, M., Simmons, S. O. & Schriks, M. Comparison of in vitro and in vivo bioassays to measure thyroid hormone disrupting activity in water extracts. Chemosphere 191, 868–875(2018) doi: 10.1016/j.chemosphere.2017.10.109.
3. Behnisch, P. A., Besselink, H., Weber, R., Willand, W., Huang, J. & Brouwer, A. Developing potency factors for thyroid hormone disruption by PFASs using TTR-TRβ CALUX® bioassay and assessment of PFASs mixtures in technical products. Environ Int 157, 106791 (2021), doi: 10.1016/j.envint.2021.106791

4
PFAS TOXICITY PROFILING

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Per- and polyfluoralkyl substances (PFAS) are a group of synthetic chemicals with widespread industrial and consumer applications, presenting significant environmental and public health challenges due to their persistence and extensive use. Although attempts were made to phase out certain PFAS compounds, many remain in use, with regulatory measures varying significantly.

The detection of PFAS is complicated because of the numerous congeners, with the standard Liquid Chromatography-Mass Spectrometry (LC-MS) methods limited to analysing a maximum of 30 congeners. Bioassays offer a relevant alternative for monitoring a broader range of PFAS.

BioDetection Systems tested the relevance of various CALUX bioassays for detecting PFAS through a wide panel screening with 10 selected PFAS compounds. The results identified a smaller panel of CALUX bioassays suitable for toxicity profiling. This refined panel was subsequently employed to profile the toxicity of 45 different PFAS compounds, providing a comprehensive understanding of their toxicological impacts.

The screening of 45 congeners revealed that the TTR-TRβ CALUX assay was the most responsive. Utilizing the screening results, relative potencies were determined, providing insight into the toxicological profiles of the various PFAS congeners.

To utilize the PFAS CALUX assay for measuring water samples, a method was developed to specifically extract PFAS from water. The WAX-SPE technique was employed, given that PFAS generally have a lower pKa value compared to other persistent mobile toxicants. This method was tested and a good recovery PFOA was found, while all the tested persistent mobile toxicants were effectively eluted from the column.

To demonstrate the applicability of the CALUX bioassays for quantitative monitoring of PFAS in water, surface water and influent/effluent samples were processed and analysed using both PFAS CALUX bioanalysis and LC-MS analysis. Following conversion of chemical analysis results, the data was expressed in ng PFOA equivalents per litre water, making it possible to directly compare the results. A good correlation between the detection methods was found; however, the results from the PFAS CALUX are 1000 times higher than those from LC-MS.

In addition to measuring PFAS levels in water, measurements were also conducted on PFAS in food. In experiments involving spiked infant food, PFAS CALUX measurements indicated a recovery of 61**%,** contrasting sharply with 0.1**%** reported by other partners, suggesting variations in detection efficiency between methods**.**

In conclusion there was demonstrated that CALUX bioassays, particularly the TTR-TRβ CALUX, are effective for the detection and toxicity profiling of PFAS, offering an alternative to LC-MS with the ability to detect a broader range of congeners. The developed WAX-SPE extraction method proved successful for PFAS recovery from water samples, with a notable recovery rate for PFOA. While CALUX bioassays showed a strong correlation with LC-MS results, they reported significantly higher PFAS levels.

References

1 Behnisch, P. A., Besselink, H., Weber, R., Willand, W., Huang, J., & Brouwer, A. (2021). Developing potency factors for thyroid hormone disruption by PFASs using TTR-TRβ CALUX® bioassay and assessment of PFASs mixtures in technical products. *Environment International*, *157*, 106791. https://doi.org/10.1016/j.envint.2021.106791

2 Behnisch, P. A., Besselink, H., ten Hulscher, D., Jans, A., Hogendoorn, C., Hin, J., van der Wielen, C., Brouwer, A. (2022). Evaluation of Thyroid Hormone Disruption by PFAS in WWTP Influent/Effluent and Surface Waters in the Netherlands. Organohalogen compd. 83.

5
AQUACONNECT - EFFECT-BASED ANALYSIS FOR TREATED WASTEWATER REUSE

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Abstract:
Water scarcity is expected to increase in the future due to climate change and increased urbanization. Therefore, to ensure water supply for the public and environment of sufficient quantity and quality, alternative water management options are required, such as reusing (waste)water. However, the presence of micropollutants (MPs) and their transformation products (TPs) in wastewater raises safety concerns for both human and environmental health. In this instance, effect-based methods offer an excellent option for water quality assessment due to their ability to assess the entire mixture.

Complemented with chemical target and non-target screening, water quality can be comprehensively assessed. Thus, potential hazards stemming from MPs and TPs during water reuse can be identified, which will ultimately help to design water reuse in a safe and sustainable manner.

This work will present preliminary results for three different water reuse field studies in the Netherlands.

These include the advanced treatment of treated wastewater to assess reuse possibilities in Wervershoof, the reuse of treated wastewater for agriculture by sub-surface irrigation in Haaksbergen, and the indirect-potable reuse of treated wastewater affected surface water during soil-aquifer treatment in Eibergen.

Each field study applied a different set of bioassays such as the Nrf2-, E2-, PAH-, Cytotox-, PXR-, TTR-, or MicroGLO-CALUX assay.

This was furthermore supported by chemical target analysis for different classes of MPs such as pharmaceuticals, pesticides, or per- and polyfluoroalkyl substances (PFASs).

For the advanced treatment in Wervershoof, clear water quality improvements could be identified by the applied bioassay battery, which is supported by chemical target analysis.

In Haaksbergen, the MicroGLO assay was able to clearly identify several classes of antibiotics present during and after water reuse for agricultural purposes.

Regarding the indirect potable reuse in Eibergen, a special emphasis was put on the analysis and detection of PFASs, which proved to be persistent throughout the treatment chain.

However, PFAS concentrations in produced drinking water were below corresponding regulatory limits and the TTR bioassay was able to identify additional effects in the soil-aquifer system which were not detected by chemical target screening.

Overall, this talk will emphasize the advantages of coupling chemical and effect-based analysis for the analysis of complex mixtures. Furthermore, it highlights the potential of treated wastewater reuse and potential reuse options based on the applied treatment and water source.

Keywords:
Water Reuse, Chemicals of Emerging Concern, Mixture Assessment

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FLUORESCENCE-BASED ASSAY FOR ASSESSMENT OF PFOA TOXICITY REDUCTION AFTER PLASMA TREATMENT

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Abstract:
PFAS (per- and polyfluoroalkyl substances) are one of the most toxic environmental pollutants. Their widespread distribution leads to their accumulation in natural, wastewater, sediments, sediments, landfill leachate and other natural resources. Unlike most of the other compounds from the group of xenobiotics, these compounds are extremely difficult to biodegrade and bioremediation technologies are ineffective and inapplicable in lab, in situ and on site.

On the other hand, this group of xenobiotics is extremely diverse and the influence/elimination of combinations of these compounds is a difficult task for engineers, biotechnologists, biochemists and chemists.

The question of "how to quickly, effectively and efficiently determine the course of intoxication/detoxification processes" remains unresolved, and the determination of the complex toxicity of PFAS group xenobiotics is a scientific challenge of great importance for future applied research and technologies that involve the participation of PFAS.

An opportunity to determine the complex toxicity, both of individual representatives of PFAS, as well as of combinations and unknown mixtures of them, are fluorescence techniques for tracking metabolism and morphological changes in test biological objects - microorganisms. In this regard, the use of CTC (5-cyano-2,3-ditolyl tetrazolium chloride)/DAPI (4',6-diamidino-2-phenylindole) staining, in which the strength of the fluorescence signal is proportional to the metabolic activity and the degree of preservation of the vitality of the cells affected by the toxicant, is a reliable mechanism for monitoring the degree of intoxication/detoxification.

The treatment with plasma methods of waters contaminated with hard-to-degradable or almost non-degradable xenobiotics is a modern alternative that can be used to create innovative modules for pre-treatment or post-treatment of wastewater or specialized modules for the recovery of resources damaged by the presence of PFAS.

The present study consists of two parts. In the first part, the toxicity of different concentrations of PFOA (perfluorooctanoic acid), as a representative of PFAS, was investigated with fluorescence techniques after staining the test object *Escherichia coli* ATCC 700728 with CTC and DAPI (4',6-diamidino-2-phenylindole). The following parameters were monitored – fluorescence signal intensity, mean perimeter and circularity of microbial cells, % area occupied by bacteria and % viable cells after exposure to PFOA. Mathematical dependencies between the impact concentration and the above parameters are derived. They allow us to predict and calculate the toxicity depending on the concentration of the toxicant.

In the second part of the study, the influence of treatment with microwave plasma in flow mode and DBD (dielectric barrier discharge) on the reduction of PFOA toxicity in increasing concentrations was tracked. Again, a wide range of the indicated parameters were tracked and mathematical dependencies of the interrelationships - PFOA concentration and the given parameter of the biological characteristic of the microbial indicator *Escherichia coli* ATCC 700728 - were deduced.

The data confirm that fluorescence intensity, cell roundness, % viable cells are the most suitable indicators to determine the toxicity of PFOA and residual components from plasma treatment. Plasma treatment in flow mode was also found to reduce the toxicity of PFOA but preserve to a higher degree the metabolism and the number of surviving *Escherichia coli* ATCC 700728 cells after plasma treatment. This type of treatment is more suitable for pretreatment modules in water treatment technologies. DBD plasma treatment has a more significant effect on the physiological and biological parameters of the indicator mechanism. This type of plasma treatment would be more suitable in post-treatment modules for treated waters, where residual amounts of xenobiotics and pathogenic and opportunistic microorganisms must be removed simultaneously.

These results can be used both in experiments to determine the toxicity of PFAS and other chemically related xenobiotics and for the planning of specialized modules for the elimination of PFAS, antibiotics, microplastics, together with the presence of pathogenic and opportunistic microorganisms.

Keywords:

Toxicity, fluorescence techniques, CTC staining, Escherichia coli ATCC 700728*, PFOA concentration-parameters metabolism parameters and indicator morphology, treatment with flow plasma and DBD*

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BIOASSAYS AND EFFECT-DIRECTED ANALYSIS IN THE CONTEXT OF THE EUROPEAN DRINKING WATER DIRECTIVE

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Chemical pollutants in the EU Drinking Water Directive

The recast EU Drinking Water Directive (DWD, Directive (EU) 2020/2184[1]) became law in EU countries in January 2023. It aims to protect human health by ensuring the quality of water intended for human consumption. An important aspect of water quality is the possible contamination with harmful chemical compounds.

Regarding chemical pollutants, three main new features in the recast DWD are[2]:

1. The DWD contains **updated quality standards** (more restrictive or permissive) and introduced new chemical parameters that have to be monitored. The new quality standards (QS) are in line, or even more strict, than the than the World Health Organisation recommendations. In addition, they aim to include contaminants of emerging concern, such as PFAS and bisphenol A. The monitoring of the chemical parameters is to be performed by quantitative targeted chemical analysis.
2. The adoption of a **risk-based approach**, in accordance with the principles of the Water Safety Plan, along the entire supply chain (sources, treatment, distribution network);
3. The **identification of possible emerging pollutants present in supply sources** (catchment areas);

As such, the DWD provides that Member States (MS) protect human health far beyond the chemical parameters with a QS (feature 1).

The risk-based approach allows monitoring (and subsequent risk management) that is tailor-made to a specific catchment with an abstraction point. If a chemical compound is not detectably present (anymore) in a catchment, its monitoring frequency can be reduced (even to zero/y). This is possible as, on the same time, a safety net is provided that enables the detection of new and other compounds that are relevant at the site (feature 2). MS therefore have to proactively seek for emerging compounds at the catchment of the abstraction points in order to ensure that the water treatment is sufficient to protect human health against harmful effects of chemicals (feature 3). This enables MS and water supplies to respond to

growing public concern about the effects on human health of emerging compounds in drinking water.

Bioassays and Effect-directed Analysis

Bioassays are effect-based monitoring tools (effect-based methods; EBM) that directly assess biological activities of chemicals using responses of biological (sub)cellular systems. They detect the combined effect of all chemicals to which they are exposed. As such, they respond to both known and unknown chemicals and their mixture effects in a tested (water) sample.

Many bioassays are specific for one mechanism of action, such as activation of a certain cellular receptor, or activation of a specific transcription factor. In addition, some cases bioassays are able to respond to very low concentrations of compounds (such as steroidal estrogens in bioassays for activation of the estrogen receptor[3], f.i. the ER CALUX®) that are difficult to detect by chemical analysis.

Because of these characteristics, EBM are increasingly used as monitoring tool complementary to chemical analysis. Often, the cause of bioactivity response is unknown. In such cases, Effect-Directed Analysis (EDA) can help to characterize and identify the responsible compounds. EDA combines bioassay analysis with chromatographic separation (fractionation) and chemical analysis of active fractions. The contribution of candidate compounds identified in the active fractions is confirmed (or rejected) by testing pure standards of the candidate compounds in the bioassay[4].

Bioassays and EDA in the context of the DWD

Although the updated DWD does not oblige MS to apply EBM, bioassays and EDA certainly can be very valuable to comply with the goals of the DWD, especially in the setting of the risk-based approach.

The DWD allows some flexibility in monitoring plans and the most appropriate monitoring methods and approaches in the risk-based approach. EBM can thus be included into monitoring programs in DW, its sources and supply chain as a valuable complement to chemical analysis. Companies are allowed to reduce monitoring frequencies of compounds that are (no longer) relevant in their catchment. EBM - in combination with chemical screening for compounds, to prevent (new) chemicals that do not give a response in the selected in vitro bioassays from being overlooked - can serve a safety net to detect new emerging compounds and as a way to get informed in case a compound labelled as 'no longer relevant' might emerge again.

Bioassays indicate the presence of compounds with biological activities; i.e. they pinpoint to those emerging compounds deemed most relevant in terms of health hazards. As such, bioassays, in combination with EDA, are important tools for the compulsory identification of possible emerging pollutants present in supply sources (feature 3).

Bioassays are a fixed part of the monitoring programs of several Dutch DW companies, especially those relying on surface waters as their sources.

Sources, critical process steps and produced DW are monitored frequently with a panel of bioassays for those endpoints considered the most relevant for human health.

The measured responses are benchmarked against Effect-Based Trigger values (EBT)[5]. If EBT are exceeded repeatedly at a certain location and the causative compounds are unknown, EDA is performed to identify the drivers of the bioactivity. The identity of the driving compounds is essential to make a proper risk assessment, assess the fate of the compound in water treatment and to initiate action to reduce the pollution at the source.

Keywords:
Drinking Water Directive, Bioanalytical tools, Effect-based monitoring, Risk-based Approach

References:

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8

UNVEILING HOTSPOTS AND CO-OCCURRENCE OF ACTIVITIES ON THYROID HORMONE RECEPTOR AND TRANSTHYRETIN BINDING IN PASSIVE SAMPLERS FROM CZECH SURFACE WATERS

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Abstract:

Well-functioning of the hypothalamus-pituitary-thyroid (HPT) axis is essential for many physiological processes, such as metabolism, growth, and energy regulation. One of the endpoints on the HPT axis that can be affected, followed by exposure to environmental pollutants, is disruption of thyroid hormone receptor beta (TRβ) or the transport protein transthyretin (TTR).

TRβ agonistic and antagonistic activities and binding to TTR are less frequently studied *in vitro* biological effects in the aquatic environment, compared to for example, estrogenic, androgenic or dioxin-like activity. TRβ-mediated activities and TTR binding have been predominantly assessed in in extracts collected using active sampling methods (such as grab or composite sampling), though they have not been so frequently found. What remains unclear is

1) whether these activities co-occur,
2) if they can also be found in extracts from passive samplers, and
3) what drives the (anti-)TRβ activity in the aquatic environment.

The main aims were to determine (anti-)TRβ activities and TTR binding in passive samplers from Czech surface waters, assess if they can co-occur and attempt to identify the drivers of anti-TRβ activity using a combination of *in vitro* bioassays and instrumental analysis.

Passive samplers (polar organic chemical integrative samplers - POCIS) were deployed at twenty-one sites across Czech rivers. These sites comprised so-called closure profiles (ends of watersheds) and some important monitoring points along the Elbe River.

We assessed (anti-)TRβ and TTR binding activity using bioassays—(anti-)TRβ-CALUX and TTR-TRβ-CALUX, respectively. Lists of known compounds active on (anti-)TRβ and ligands of TTR were compiled and further used as a list of suspect chemicals.

The compounds were analyzed using liquid chromatography-high resolution mass spectrometry (LC-HRMS) analysis via suspect screening.

Surprisingly, we found no TRβ agonistic activity. The anti-TRβ activity was found at eight sites. Six of them were associated with large municipalities. The three most burdened sites were associated with industrial pollution, and the two most affected sites can be polluted by either brown or black coal mining-related chemicals.

Interestingly, out of the eight sites with anti-TRβ activity, six also exhibited TTR binding, and all were associated with municipalities. Since the binding sites of TRβ and TTR are specific to the molecular structure of thyroid hormones, it is probable that similar chemicals have driven the anti-TRβ and TTR binding activities observed in extracts in this study.

Further, we focused on the extract from the Bílina River, which displayed the highest anti-TRβ activity. After fractionation of the extract into five fractions, the anti-TRβ activity was retained in the fraction eluted only with organic solvent, and thus, this fraction should predominantly contain mid-polar compounds. However, despite our efforts, the suspect LC-HRMS analysis did not reveal the chemical drivers of the anti-TRβ effects.

Nevertheless, our findings show the efficacy of passive sampling in detecting anti-TRβ activity in surface waters, often alongside TTR binding activity.

Additionally, our fractionation technique and non-target data acquisition methodology lay a foundation for future investigations into identifying the drivers behind these effects.

Keywords:
In vitro effects, passive sampling, thyroid hormone receptor, surface water

Acknowledgement:
This work was financially supported by the Czech Science Foundation (project No. 20-04676X). We thank Marie Šandová and Tereza Směšná for their technical support.

9
ENDOCRINE DISRUPTORS (AND OTHERS) – THEIR POTENTIAL FUTURE RELEVANCE IN DIRECTIVES FROM LAB POINT OF VIEW

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Abstract:
The European Union (EU) has established strict guidelines to manage harmful substances, including endocrine-disrupting chemicals (EDCs) and others. These guidelines aim to protect ecosystems and public health. EU regulations like the drinking water directive, waste framework directive, and urban waste water treatment directive have been or are being revised. These revisions include watch lists with substances such as pharmaceuticals and endocrine-disrupting compounds. In this context, bioassays for the detection of endocrine disruptors and other ecotoxicologically relevant effects, e.g. by dioxins or perfluorinated alkyl substances (PFAS), can be applicable. Such (mostly in-vitro) bioassays can be considered as sum parameters for substance groups or effects and, in addition to the usually used chemical parameters, can also provide essential data for regulatory decision-making.

This presentation will address three different questions for the applications of biotests in different stages of development that have or will have a need in regulation from the perspective of a lab and will highlight the capability of such bio-analytical methods.

i) Dioxins are well known contaminants in water and are regulated in various documents, such as the list of priority substances of the water framework directive. The instrumental analysis of the broad spectrum of dioxins is generally carried out using chromatographic methods coupled with mass spectrometry. A useful alternative is cell- and reporter gene-based method, such as the DR CALUX, which detect aryl hydrocarbon receptor (AhR) active compounds, such as dioxins and PCBs.
Technical details of EU directives are generally referenced by harmonized european standards, i.e. ISO or CEN standards. Without such standards, objectives of the respective EU directive would not be applicable, as the directives only contain basic requirements. Accordingly, the participation in an international laboratory testing following an ISO draft for the standard procedure, e.g. of DR CALUX for dioxin like substances, generate precision data for the development of the full ISO-standard.

ii) The assessment of plastic containing waste, especially with complex and unknown composition, poses major challenges for chemistry, because of lots of substances, the need of adaptation of analytic workflows and potentially missing standards. Useful alternatives are in-vitro bioassay, such as the Yeast Estrogen Screen (YES test) or the ER CALUX, which can be used for the hazard assessment of estrogenic active compounds, as for instance ER-CALUX data and chemical analyses confirm estrogenic potencies of plastic containing waste. However, a suitable method for the elution of potential pollutants is crucial.

iii) Waste water surveillance currently focuses on the epidemiological assessment of infectious diseases such as SARS-CoV-2 or influenza. However, this municipal waste water monitoring allows samples analyses also of other, well-known and ubiquitous pollutants. By this, knowledge about specific and diffuse entries can be achieved. The presentation will highlight examples from monitoring approaches at first steps.

In conclusion, analytical approaches, particularly with in-vitro bioassays, are useful to collect necessary information regarding requirements from EU directives such as monitoring data for watch list substances.

Keywords:
EU regulations, standards, estrogenic compounds, dioxins, PFAS, waste water surveillance

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ASSESSING DIFFERENCES BETWEEN ZEBRAFISH AND HUMAN NUCLEAR RECEPTOR ACTIVATION BY ENVIRONMENTAL WATER EXTRACTS

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Abstract:

Recently, there have been many concerns about the presence of man-made and naturally occurring compounds in the environment that can cause endocrine disruption in exposed animals.

In vitro bioassays have developed into time and cost-effective screening tools, and they are now widely used to detect endocrine-disrupting activities in various environmental matrices, including surface waters, groundwater, drinking water, and wastewater.

In the aquatic environment, bioassays can be utilized to assess better the combined impacts of endocrine active chemicals. Furthermore, a combination of bioassays and instrumental analysis may identify significant contributors to endocrine-disrupting activities in environmental samples.

To date, most of the available *in vitro* bioassays are based on mammalian receptors. However, the mammalian receptors may not reflect accurately what happens in fish. Recently, some studies have proven that some environmentally relevant chemicals and environmental water extracts differentially activate the human and zebrafish nuclear receptors. The aim of this study was to explore the species-specific differences in the activation or inhibition of several human and zebrafish nuclear receptors by environmental water extracts. To do this, a novel battery of human and zebrafish nuclear receptors indicative of activation of the estrogen receptor-alpha, androgen, progesterone, glucocorticoid, mineralocorticoid, and pregnane X receptors were employed. Samples were also tested on the parental cell lines without the nuclear receptors to ensure that the detected activities did not result from non-specific luciferase expression (false positives).

All samples tested in this study were collected from the Czech Republic. This study employed two sampling methods (grab sampling and polar organic chemical integrative samplers – POCIS). Two wastewater treatment plant (WWTP) influents, effluents and their respective

receiving surface waters and three ponds were sampled by grab sampling, while three rivers (2 samples from each sampled during the fall and spring seasons) were sampled using POCIS. Samples were analysed both in agonist and antagonist mode on both the human and zebrafish estrogen receptor α (ERα), androgen receptor (AR), progesterone receptor (PR), glucocorticoid receptor (GR), mineralocorticoid receptor (MR), and pregnane X receptor (PXR).

The results from the test on the parent cell lines indicated that none of the obtained results were false positives. For both human and zebrafish nuclear receptors, no antagonistic activities were detected. Also, no (anti-)glucocorticoid activities were detected for both receptors. None of the samples were cytotoxic.

Our study detected both human and zebrafish ERα agonistic activities in influent samples. For influent of WWTP Protivín, similar human and zebrafish ERα activities were detected (0.28 and 0.23 ng/L E2 equivalents, respectively). However, in the influent of WWTP Vodňany, a slightly stronger human ERα agonistic activity (0.70 ng/L E2 equivalents) was detected as compared to the zebrafish ERα agonistic activity (0.18 ng/L E2 equivalents).

Androgenic activities were detected in only influents, and the detected activities were similar for both human and zebrafish AR receptors (in the range of 0.45-0.50 and 0.29-0.37 ng/L R1881 equivalents, respectively).

Progestogenic activities were detected in only influent samples. In the influent of WWTP Protivín, both human and zebrafish PR activities were detected (2.45 ng/L R5020 equivalents and 2.66 ng/L DHP equivalents, respectively). For the purpose of comparison, the activity on the human PR (R5020 equivalents) was recalculated to get it's DHP equivalents. We found that the activity on the human and zebrafish PR were similar (2.37 and 2.66 ng/L DHP equivalents, respectively). However, in the influent of the WWTP Vodňany, only zebrafish PR activity (reaching 34 ng/L DHP equivalents) was detected.

In the case of mineralocorticoid activities, no human mineralocorticoid activity was detected; however, zebrafish mineralocorticoid activities were detected in influents of Vodňany and Protivín WWTP (0.32 and 0.12 ng/L aldosterone equivalents, respectively).

Zebrafish PXR agonistic activities were detected in both influents and surface waters, whereas human PXR agonistic activities were detected only in influents of WWTPs. In the influents, human PXR activities ranged from 115 to 256 ng/L SR12813 equivalents and zebrafish PXR activities from 28 to 69 ng/L clotrimazole equivalents.

The results from this study indicate that, for ERα and AR activities in the aquatic environment, human-based nuclear receptor assays may be suitable for testing. However, in the case of PR, MR, and PXR activities in the aquatic environment, the zebrafish-based nuclear receptor assays should be prefered.

Keywords:
In vitro bioassays, nuclear receptors, endocrine disruptors, human, zebrafish.

Acknowledgement
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11
BIOASSAY PERFORMANCE STATISTICS

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Abstract:

Water managers are confronted with micropollutant concentration mixtures that can be harmful for human health when ingested via drinking water. Bioassays are a means to measure effects of such unknown mixtures. However, bioassays are not unfailing in identifying risks. In this study we provide a framework to gain insight into the performance of bioassays to correctly signal chemicals at harmful concentrations, with techniques from the medical domain to calculate positive and negative predictive values.

This analysis, based on Bayesian statistics, has not been applied to bioassays before. It provides a very relevant metric for the performance of bioassays.

The study provides insight in **why water managers in many cases may not find actual harmful concentrations in follow up research** after a response of a bioassay above the effect-based trigger value, **especially in highly diverse low level concentration mixtures of chemicals in water**.

Highlights of this study, applied to publicly available bioactivity data of several *in-vitro* assays, include:

- A framework for bioassay predictive values was developed and evaluated.
- Negative predictive values were 100% for all assays.
- The positive predictive value increased with increasing chemical concentrations.
- Health-risk predictive values were lowest for diverse, low-concentration mixtures.
- Values can be used to optimize thresholds in effect-based water quality assessment.

We feel this theoretical framework adds considerable new and useful insight into water quality monitoring with bioassays.

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Pronk TE, Hoondert RPJ, Kools SAE, Kumar V, de Baat M. Bioassay predictive values for chemical health risks in drinking water. Environment International (2024) (under review).

Keywords:

Bioassays, Performance, water quality, predictive values

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MULTIPLEX BIOSENSOR FOR WATER QUALITY MONITORING

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Abstract:

In this presentation, we will introduce a novel biosensor technology called Receptomics, developed by Wageningen Plant Research. This technology enables the simultaneous and repetitive measurement of the activation of hundreds of receptors in a flow cell format.

The procedure involves printing a grid pattern of several hundred spots on a square centimeter of a microscope slide. Each spot contains DNA encoding different human receptors and a calcium sensor protein, with each spot hosting a unique receptor.

Cells growing over these spots absorb the DNA, expressing the receptor on the cell membrane and the sensor in the cytoplasm.

This results in the formation of a cell layer on the slide featuring an array of diverse receptors on its surface. This receptor array is then encapsulated in a compact flow cell, equipped with an inlet and outlet for the repeated exposure of receptors to pure compounds or complex extracts.

The accompanying calcium sensor emits a fluorescent or luminescent signal, facilitating microscopic observation to determine whether any of the receptors reacts to substances in the passing microfluidic sample.

To date, the platform has demonstrated compatibility with different taste receptors (bitter, sweet), hormone and cytokine receptors, and ion channels (TRPs). Several experiments also highlight the ability to measure receptor activities in complex mixtures such as plant and mushroom extracts.

Beyond screening the taste and health of plant extracts and other samples, Receptomics holds great potential for broader applications. This year, a new Public-Private Partnership (PPP) will begin, with our goal being to adapt existing in vitro cell-based methods for measuring water quality to the Receptomics format and to expand these methods to detect new water contaminants.

Depending on the type of test, dozens of water samples can be analyzed per chip, offering opportunities for the development of online monitoring systems. Our primary objective is to enhance the usability, accessibility, and cost-effectiveness of water quality bioassays.

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Keywords:

Biosensor, GPCR, Ion channels, Receptomics, Complex mixtures

13
CONSIDERATIONS WHEN DOING EFFECT-DIRECTED ANALYSIS (EDA) ON HUMAN SERUM SAMPLES

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Abstract

Effect-directed analysis (EDA) is a multifaceted approach integrating biological and chemical analysis to identify bioactive compounds in complex matrices[1, 2].

Generally, EDA involves assessing the biological activity of the original extract, followed by its fractionation and subsequent biological and chemical analysis [2]. Effective sample preparation is critical for successful EDA.

Typical steps involve protein precipitation and solid-phase extraction, aiming to reduce sample complexity while preserving bioactivity. Selection of strategic extraction approaches fully aligned with the final goal of the study, together with the enrichment factor used for fractionation are fundamental to obtain insightful results via EDA.

On the other hand, the bioassay choice is also influenced by its sensitivity, and specificity. When performing EDA, the limits of applicability of bioassays must be considered, as some assays may not detect all bioactive compounds or may yield false positives/negatives due to matrix effects or interfering substances. Finally, chemical identification is essential to elucidate which chemicals may trigger an effect in the bioassay.

In this study, a high-throughput EDA approach was utilized to evaluate chemical mixtures found in human serum samples to compete for the binding to thyroid hormone (TH) serum protein—an essential molecular initiating event linked to TH system disruption [3]. For this, different strategies of sample extraction and concentration are compared. In addition a workflow for data acquisition, annotation, mass spectra (MS) alignment and evaluation is used to assess the chemical composition of the active fractions. In conclusion, performing EDA on human serum samples requires meticulous attention to sample preparation, enrichment strategies, bioassay selection, and chemical analysis to effectively identify bioactive compounds and understand their potential health impacts.

Keywords:

Complex mixtures, High-throughput effect-directed analysis, human serum samples, Transthyretin binding competition assay

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14
ACUTE CHEMICAL TOXICITY TESTING

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BioLight® Toxy

AQUA SCIENCE

Acute Chemical Toxicity Testing

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Abstract:

In this presentation, there is an overview on the use and future potential of bioluminescent toxicity testing for many applications in water quality protection and early warning will be discussed.

Understanding the levels of chemicals and their mixtures in water, soil, and sediments is vital in making decisions on environmental clean-ups and protection of the water supply. Everything can be toxic in the right amount. Often there are compounds present that alone are not toxic but combined with others present toxicity. The acute chemical toxicity is the only test to provide information on this synergistic effect.

Our BioLight Toxy along with BioLight bioluminescent reagent (using Aliivibrio fischeri) provides detection to over 3,600 chemicals simultaneously. This acute toxicity test offers a level of testing of multiple and mixed compounds not available by other chemical test protocols.

The short list of key benefits to the use of the BioLight Toxy and reagents is early warning, rapid results, no need to do multiple chemical tests, cost savings, repeatability plus multiple protocols and applications.

There are more and more countries around the world which are adding regulations for toxicity testing using bioluminescence. Our BioLight Toxy is used globally to meet these regulatory requirements as well as in other areas where water quality is a concern.

There are many research papers written related to the use of bioluminescent toxicity testing for wastewater, drinking water, mining run off, oil and gas, soils, sediments, and more. In addition to the positive test outcomes for these areas, the technology is also being studied for other new chemicals of concern.

In conclusion, this bioluminescent bacterium used with our BioLight Toxy is the test of the future for understanding the single and synergistic effects of chemicals causing toxicity in our waters, soils, and sediments. The protection it provides to water facilities, humans, and animals is unmatched by any chemical lab testing available.

Keywords: bioluminescence, acute chemical toxicity, Aliivibrio fischeri

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WHAT IS EATS? HOW ABOUT METABOLISM?

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Abstract:
The endocrine system is a network of signaling pathways, hormones and organ systems that regulates diverse physiological systems. This network is vulnerable to disruption by endocrine disrupting compounds (EDCs), potentially impacting health. Endocrine disruption (ED) has been recognized as a priority endpoint in safety evaluation of chemicals and EDCs are classified as substances of very high concern.

In the EU, the current regulatory assessment of EDC focuses on substances that interact with the **estrogen, androgen, thyroid or steroidogenesis (EATS)** modalities.

The reason for that is that EATS modalities are the pathways for which we have the best mechanistic and causal knowledge with respect to adverse outcomes caused by ED. EATS are also the modalities, for which standardized test guidelines (OECD and US EPA) exist within a tiered approached framework for the *in vitro* (and *in vivo*) investigation of EATS-related mechanisms and EATS-mediated adverse effects.

To be able, to test these EATS modalities in a regulatory context, we have designed a (validated) panel of *in vitro* bioassays.

For the estrogen (TG455), androgen (TG458) and steroidogenesis (TG456 ; Nikopaschou et al., 2023) modalities OECD validated assays are available.

For the evaluation of thyroid interference, many relevant assays targeting key points are available, although no official test guideline exists yet.

We selected several thyroid-targeted bioassays to be implemented (or developed) to complete our EAT bioassay panel. Some of these bioassays are being considered for official test guidelines.

Another important point of chemical safety evaluation is the **consideration of metabolism** and associated biotransformation, since some chemicals can be metabolized *in vivo* and yield bioactivated or inactivated metabolites.

Metabolism is generally not accounted for by *in vitro* methods such as reporter gene assays.

While this is often a deliberate assay design choice to ensure stable readouts of the activation of receptors or transcription factors, it can hamper the extrapolation of the results to *in vivo* situations.

To counter that, methods employing the application of exogenous S9 (with associated phase I and phase II metabolism cofactors) to *in vitro* assays have been developed, aiming at better capturing metabolism and decrease the uncertainty associated with the prediction human health hazard (van Vugt-Lussenburg et al. 2018).

Such methods rely on the use of induced rat liver S9 and we recently explored the possibility of incorporating human liver S9 to derive data from human metabolism systems.

Keywords:
EATS, Non-animal testing, In-vitro methods, Bioassays

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HIGH THROUGHPUT SCREENING AND SAFETY ASSESSMENT OF BABYFOOD USING EFFECT-BASED BIOANALYSIS

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Humans, including infants, are exposed to complex mixtures of anthropogenic chemicals, with food being a major route of exposure. Food safety assessment of chemicals traditionally focuses on the analysis of single prioritised chemicals of known structure and toxicity (targeted approach) that may pose significant human health risk. In contrast, only limited attention is given to potential safety issues of chemical mixtures and of unknown/undetected contaminants (Carvalho et al., 2014; Kienzler et al., 2016). Nevertheless, there is ample evidence that combined exposures to chemicals is involved in the aetiology of major human chronic diseases and focus is shifting towards potential adverse health effects of complex chemical mixtures and to the analysis of so-called unknown contaminants (Drakvik et al., 2019).

The EU-SAFFI project aims to develop an integrated approach to enhance the identification, assessment, detection and mitigation of safety risks raised by chemical hazards along the infant food chain. Here we report on the selection of most relevant CALUX bioassays to capture the potential risks associated with complex mixtures and/or unknown contaminants in babyfood.

In addition, generic and dedicated processing methods were developed and evaluated. Finally, an innovative model for safety assessment of babyfood was postulated based on effect-based CALUX bioanalysis results.

An extensive panel of more than 30 CALUX bioassays has been developed including assays adressing interference of pollutants with a specific type of nuclear hormone receptors (Sonneveld et al., 2005; Van der Burg et al., 2013) and assays assessing influences of chemicals on pathways involved in basic cellular signalling which are relevant for e.g. acute toxicity and carcinogenesis (Van der Linden et al., 2014). For assessing babyfood safety, this wide panel of CALUX bioassays was trimmed down by selecting a set of 10 bioassays based on:

1 a priori indicated toxic endpoint (such as endocrine disruption and genotoxicity.

2 responsiveness of bioassays towards specific and prioritized analytes (e.g. POPs, packaging migrants and perfluorinated compounds)

3 initial screening of babyfood samples on a wide-panel of CALUX bioassays

Three processing methods were developed and/or evaluated:

1 a dedicated extraction/processing method for dioxins and dioxin-like compounds (in-house method used for monitoring PCDD/F/dlPCBs in food according EU Regulations (Commission Regulation (EU), 2017)
2 a dedicated extraction/processing method for PFAS
3 an easy to apply, high-throughput, cost-effective generic extraction/cleanup method applicable for a wide range of chemicals

To intepretate the analysis results of 10 different effect-based CALUX bioassays, a safety assessment model was postulated. The model is based on exceedance of CALUX bioassay-specific effect-based trigger values, assigning Safety Weighing Factors (SFW) to the various CALUX bioassays and calculating a single Aggregated Exceedance Level AEL allowing assessment of babyfood samples to be based on a single value. This model requires the availablility of bioassay-specific effect-based trigger values (EBTs). To this end, we also started to build a database of babyfood bioassay analysis results from which we intend to derive pragmatic EBTs. The distribution of all bioassay-specific analysis results are evaluated and the concentration below which 80% of the analysis were observed, is chosen as the pragmatic derived effect-based trigger value.

In brief, processing methods and a panel non-targeted CALUX bioassay have been selected for monitoring babyfood safety. In addition, pragmatic EBTs are being derived and will be applied in an easy to use innovative model for safety assessment of babyfood.

Keywords:
babyfood; infant formula, bioanalytical tools, effect-based monitoring, safety assessment, non-targeted screening

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EMERGING ISSUES & BIOASSAYS

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Abstract:
People concerned about possible harmful health effects in their living area due to local industrial activities, approach ToxicoWatch for a scientific study on substances of very high concern (SVHC). ToxicoWatch does (multi-year) biomonitoring studies of dioxins, PAH and PFAS mostly with bioassays. The research starts with a well-considered sampling procedure. It all starts with a secure sampling procedure.

The second step is the application of an analysis program, which is flexible. We set up (long-term) environmental programs with a wide range of CALUX bioassays.

Sample preparation for the CALUX analysis allowed a wide range of matrices so the environment can be explored on multiple levels, from the finest grain of

dust to large waste deposits, from the bark of a tree to the blood serum of human population or mother milk.

This broad applicability also means space for matrices like application of sheep wool or specific vegetation (leaves of evergreen trees/plants) as indicator of pollution.

The CALUX bioassays used in our biomonitoring research are DR CALUX, PAH CALUX, ER CALUX and PFAS CALUX.

Results of our biomonitoring research could have implications for governments, enforcement authorities, and industry.

The application of CALUX shows the discrepancies between the limited chemical analyses and the total toxicity approach of bioassays.

Following our scientific studies, it may be important to review conventional sampling and analysis techniques for the extended group of substances of very high concern, like the whole PFAS class.

Differences in outcome with CALUX compared to conventional regulation methods (like 4 PAHs, EFSA 4 PFAS and 17 dioxin congeners) show clearly these discrepancies. This strongly suggests that CALUX results give a more realistic figure of the actual toxic load of POPs in the environment.

If the CALUX measurement method were to be applied more prominently in various measurements for the regulation of POPs, the actual toxic load of POPs in our environment could be mapped more realistic.

An example is the measurement of emissions of waste incinerators never have been performed by bioassays.

We stand for a transparent research program to make research more applicable to governments, industry, and the local population.

ToxicoWatch wants to be a bridging function for people government and industry.

Our aim is to keep the quality of our ecosystem viable and protect environmental health.

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EFFECT- AND EDA- SOIL TESTING

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Abstract:
The composition of hazardous chemicals at contaminated sites tends to be highly complex; for instance, at sites contaminated with polycyclic aromatic hydrocarbon (PAH), hundreds of PAHs, oxy-, nitro-, and alkyl-substituted PAHs and heterocyclic compounds (NSO-PACs) may be present, collectively referred to as polycyclic aromatic compounds (PACs). The chemical composition differs widely due to the source and age of contamination.

There is an urgent need for improved and applicable analytical methods for assessing the occurrence and likely impact of PACs on humans and the environment. The current risk assessment method suffers from insufficient tools to give a comprehensive picture of the chemical risks of PACs contaminated environments. Despite the highly complex mixtures of PACs, the current approach is based on chemical analysis of 16 US EPA PAHs. Over the past 40 years, these 16 PAHs have become widely accepted as representatives for all PACs and routinely analyzed in environmental monitoring programs and risk assessments. There are PACs that have considerably higher toxicity than the priority PAHs, but many of them have not been studied enough with respect to frequency of occurrence in the environment and toxic effects in different organisms to be included in routine measurements. Consequently, many PACs are unanalyzed/unknown and thereby the risk.

In this presentation an overview on the use of effect-based analysis of soils will be given. Soil samples from industrial, city park and arable land areas were investigated by use of CALUX in vitro bioassays for aryl hydrocarbon (Ah), estrogen and androgen receptors, and GC-MS analysis of 90 PACs (PAHs, oxy-PAHs, alkylated PAHs, and NSO-PACs). Potency (mass) balance estimations were used to determine relative contributions of quantified PACs to the measured activities in the bioassays.

This presentation discusses the results from the bioassay characterization of soils with different contamination degree of PACs, relationship between targeted PAC concentrations and the observed in vitro bioassay activities. The use of a high-throughput effect-directed analysis (EDA) approach with a high-resolution fractionation GC-MS workflow to identify bioassay active PACs in soils will also be presented.

Keywords:
Polyaromatic compounds, Soil, Complex mixtures, EDA

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EFFECT-BASED CHARACTERIZATION OF INDOOR PLASTIC MATERIALS USING IN VITRO METHODS

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Abstract:

In vitro bioassays are a useful tool when it comes to the risk assessment of different materials. Through bioassays, the combined effect of all compounds present in a sample can be measured, allowing a broader understanding of what types of analytes are present and what potential risk that they might pose. There are a variety of different endpoints that can be tested, for example endocrine disruptive effects and oxidative stress. The receptor-specific assays can either be run in agonistic or antagonistic mode.

By utilizing chemical analysis, either target or non-target, the analytes in a sample can be identified. However, chemical analysis is often limited to what standards are available and can result in a lot of unidentified peaks with unknown toxicity. The chemical data can often not explain all the activity observed in the bioassays.

By combining chemical and biological analyses, a broader understanding of the chemical composition in a sample can be obtained.

This presentation will give an overview of different projects at Örebro University that applies this combined approach in characterization of different materials.

The in vitro assays of choice are all Chemical Activated LUciferase gene eXpression (CALUX®) bioreporter assays.

Indoor environments consist of a broad mixture of chemicals, originating from different materials and from the outside air. Plastic is a very commonly used material for *e.g.*, furniture, consumer products, interior decorations, electronics and building materials.

Since plastic additives are often not bound to the plastic polymer, migration to surrounding materials and the air is a possibility. The effect of these additives and other chemicals in plastic is not well explored but could potentially be harmful to human health. The car cabin can be considered as an indoor environment and is of special interest do to the high material-to-space ratio, the large amounts of plastic used in the interior design, as well as high temperatures that can be reached, especially in the summer on a sunny day.

In this presentation, I will present results from chemical and bioassay characterization of compounds present in the car cabin air and plastic material parts used inside the interior

design of cars. The characterization of the samples was done using a target chemical method for polycyclic aromatic hydrocarbons (PAHs) and *in vitro* bioassays.

The bioassays used were the ER- (estrogen receptor), Nrf2- (oxidative stress), anti-AR- (antagonistic androgen receptor) and DR- (dioxin response) CALUX.

Additionally, results from bioassay characterization of post-consumer plastics will be presented.

The plan is to perform effect-directed analysis on materials that give high effects in the bioassays to identify the chemicals responsible for the effect.

Results will also be presented from a study where the bioassay activities of different compound mixtures found in human blood have been investigated.

A screening for a variety of potentially hazardous compounds was performed, from which six different mixtures have been prepared, representing the concentrations found in human blood. The mixes consisted of the following compound groups: PFAS (per- and polyfluoroalkyl substances), phthalates, phenols, BDE (brominated diphenyl ethers), pesticides and a mix of PCB (polychlorinated biphenyls) and dioxins.

In addition, a master mix containing all compounds was made. The mixes were tested in DR-, ER-, anti-AR- and nrf2-CALUX® in concentrations ranging from 0.01 times human blood levels (HBL) up to 1000 times HBL.

The identification of compounds responsible for endocrine disruptive effects and other toxicological endpoints is important since it provides the basis for restrictions and regulations against the production and usage of them.

The long-term goal with these projects is to help manufacturers to create materials that are safe for the consumer and the environment. By doing so, steps are being taken towards implementing the UN sustainable development goal of *sustainable consumption and production* as well as the Swedish green goal of having a *non-toxic environment*.

Keywords:
Polycyclic aromatic hydrocarbons, CALUX®, in vitro bioassays, chemical analysis, GC-MS

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HAZARD ASSESSMENT OF RECYCLED PAPER AND CARDBOARD FOOD CONTACT MATERIALS: AN EFFECT-DIRECTED APPROACH

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Abstract:
In this presentation, the use of **effect-based** tools for the safety assessment of paper and cardboard food contact materials will be given.

In the European Union (EU), Regulation (EC) 1935/2004 provides a harmonized legal EU framework and sets out the general principles for safety for all **Food Contact Materials** (FCMs). From a **food safety** point of view, however, specific EU legislation for paper and cardboard FCMs is lacking. More than 11,000 **chemicals** have been identified in all types of FCMs, most of them without any information on **toxicity** or **migration** potential from FCM to food.

The current hazard assessment employs two primary sample preparation strategies. **Migration** approaches seek to replicate realistic conditions of contact between food and FCMs, selectively recovering migrating chemicals relevant to specific food categories. In contrast**, exhaustive extraction** approaches aim to retrieve the whole spectrum of chemicals present in the FCM.

This study aims to investigate the impact of migration and exhaustive extraction approaches on the chemical profile of extracts and the subsequent **hazard assessment**. FCM samples underwent a 10-day incubation at 40°C while immersed in food simulants for dry, acidic, aqueous, and fatty foods.

Additionally, the same samples underwent exhaustive extraction using Soxhlet Extraction. The recovered extracts were assessed for **endocrine-disrupting, dioxin-like, and genotoxic responses**.

The toxic response profile of the extracts exhibited dependence on **the choice of food simulant** during sample preparation. Furthermore, samples subjected to exhaustive extraction displayed **toxicity** at lower concentrations in comparison to those prepared through migration, wherein food simulants were employed.

Our data highlight the potential of using an **effect-directed approach** for assessing the safety of FCMs. The findings furthermore underscore the crucial role of aligning the **selection** of food simulants and sample preparation conditions with the anticipated real-

world use of FCM. The direct implication is that a well-informed choice in these parameters is pivotal for an accurate representation of migration from FCM to food. Failing to consider foreseen usage conditions may result in intentionally selected conditions producing chemical profiles in the extracts that lack relevance to real migration. Thus, our study highlights the imperative of informed decision-making in ensuring the integrity **of hazard assessments** for FCMs.

Keywords:
hazard assessment; effect-directed approach; food safety; food contact chemicals; recycled paper and cardboard; food contact materials; food packaging; bioassays

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IN VITRO BIOASSAYS AS A TOOL TO EVALUATE RISK ASSESSMENT OF MICRO AND NANOPLASTICS

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Abstract:

The concern regarding nano and microplastics has significantly increased as they can be present in food, water and environment. Once the particles enter the food chain, they can cross the biological barriers, as well as cell membranes, leading to different molecular effects.

In this sense, **some key challenges** must be overcome to carry out a risk assessment of micro and nanoplastics.

On one hand, the availability of **standards of micro and nanoplastics that allow robust and reproducible results to be obtained; and on the other hand, a standardised battery of tests to assess the risk at the different trophic levels of the food chain.**

Non-animal approaches as *in vitro* bioassays based in cell culture assays have been used for the evaluation of complex mixtures of chemicals present at a very low level.

For particles, specifically, some properties must be considered to design a suitable battery of bioassays, such as characterization, impurities, surfaces, dissolution ratio or stability under biological conditions.

The aim of the study is to develop standards of micro and nanoplastics (PLA, PET and PE) to be used, first of all, in an *in vitro* gastrointestinal digestion to evaluate their stability and dissolution under biological conditions and, finally, to assess cytotoxicity (Alamar Blue), oxidative stress (ROS) and genotoxicity (Micronucleus and Comet assay) in different cell lines.

The study demonstrates the importance of standardization in the manufacturing of micro and nanoplastics and the necessity of developing a standardized *in vitro* gastrointestinal digestion and a battery of *in vitro* bioassays for the evaluation of hazards related to these particles and guarantee consumer safety.

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EVALUATION OF THE EFFECTS OF PHYTOESTROGENS ON 3D SKIN MODELS: FRIENDS OR FOE?

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Abstract

Increasing scientific concern exists about the nature and the safety of the ingredients used by the cosmetics industry regarding their endocrine-disrupting effects.

Due to their daily use, the presence of an endocrine disruptor (ED) in a cosmetic can strongly impact human health. A "cosmetic product" shall mean any substance or mixture intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odors and/or protecting them or keeping them in good condition.

People use an average of seven different products *per* day, demonstrating that cosmetics are used substantially by everyone.

In the EU's published call for data on concern for the presence of EDs in cosmetic formulations (https://ec.europa.eu/newsroom/growth/items/651201/en), the only two naturally occurring compounds, genistein, and daidzein, were included in the 14 substances, which should be given higher priority for evaluation because of concern for their effects on humans.

These two natural compounds present in soy are considered the most powerful phytoestrogens due to their ability to interact with estrogen receptors. Phytoestrogens have a structural similarity to 17β-estradiol. The beneficial or harmful effects are still under debate.

We have designed a tiered approach aimed to investigate the effects of potential ED that involves exposure of the target organ (skin) and evaluation of multiple parameters. Phytoestrogens were tested on 3D skin models (EpiDerm) that mimic the physiology of the skin, in order to study the percutaneous permeation in comparison with the estradiol to evaluate the safety. The amount of estrogen compound ED present in the body will be determined through the CALUX bioassay.

In conclusion, the major challenge was to estimate the relevant human safety levels of phytoestrogens from human-relevant *in vitro* test models. The iterative strategy involves computational modeling and *in silico* tools.

Keywords
Phytoestrogens, cosmetics, endocrine disruptors, percutaneous permeation

POSTER 1

OPTIMIZATION OF L/L-EXTRACTION FOR AhR-CALUX SCREENING PACS IN WATER SAMPLES (KINROOI) AND VALIDATION BY HPLC

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Introduction:
Polycyclic aromatic compounds (PACs) pose significant environmental and health risks, including carcinogenic effects and disruptions to developmental systems.
Effect-based bioassays, such as the aryl hydrocarbon receptor-mediated Chemical Activated LUciferase gene eXpression (AhR-CALUX), are promising tools for monitoring PACs in the environment.
However, extraction methods suitable for instrumental analysis may not be directly applicable to bioassays. This study aims to optimize a liquid-liquid extraction (LLE) method for screening PACs in water samples using AhR-CALUX.

Methods:
The preliminary experiments were conducted using 1 L of water sample, concentrating the extract in 5 mL of n-hexane. Serial dilutions (50, 4, 0.3, 0.03, and 0.002 mL sample/well) were prepared to identify a dilution range yielding a response greater than 50% relative light units (RLUs) of benzo[a]pyrene (BaP).
The optimization parameters include extraction solvent type, solvent volume, and extraction time. Spiked MQ water were extracted with 10, 20, and 40 mL of n-hexane or n-hexane/dichloromethane (DCM) (1:1, v/v) in three cycles, each lasting for 5, 10, or 20 min.

Results:
Results preliminary study showed that that a 250 mL water sample to 3 mL of n-hexane provided the optimal dilution. Subsequently, the highest concentration for the experiment was established at 21 mL sample/well, followed by ten serial dilutions prepared using a dilution factor of 3.5.
The optimization study showed that spiked MQ water extracted using 20 mL of n-hexane/DCM for 10 min yielded results closer to the predicted BaP equivalent. The addition of 5 g of NaCl can avoid emulsification in effluent water.

Validation:
The LLE-AhR-CALUX method showed an uncertainty of 17% CV. HPLC determined the recovery of 18 PACs (20, 100, and 400 ng/L), with recovery ranging from 41% to 110%, except for naphthalene and acenaphthylene. According to the method described above, the LOD and LOQ for LLE-AhR-CALUX is 0.2 and 0.5 ng/L, respectively.

Application:
The optimized LLE for PACs extraction using AhR-CALUX analysis was applied to seven different water samples. In addition, these samples were quantitatively analyzed by HPLC, where the BaP equivalent was calculated by multiplying the concentration of each PAC by its respective REP value.

The results showed that the AhR-CALUX derived BEQ values are highly comparable to the BaP equivalent values calculated from HPLC, indicating that the LLE using AhR-CALUX bioassay can be a reliable and efficient tool for screening complex environmental samples for AhR-activity.

POSTER 2

IN VITRO ASSESSMENT OF THYROID PEROXIDASE AND THYROID HORMONE RECEPTOR-DISRUPTING ACTIVITIES

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Abstract:
Thyroid peroxidase (TPO) is an enzyme crucial for the biosynthesis of thyroid hormones, catalyzing the iodination of tyrosine residues in thyroglobulin and the coupling of iodotyrosines to produce the thyroid hormones thyroxine (T4) and triiodothyronine (T3). Thyroid hormone receptors (THRs), including hTHR-α and hTHR-β, are nuclear receptors that mediate the biological effects of T3 by regulating the transcription of target genes. Proper functioning of TPO and THRs is essential for maintaining normal thyroid hormone levels and ensuring proper physiological development and metabolic regulation. Disruption of the thyroid hormone system by chemicals can perturb these delicate developmental processes, potentially leading to neurodevelopmental disorders, growth abnormalities, and reproductive impairments. Thus, it is important to develop and validate in vitro assays to identify toxicants affecting thyroid hormone systems to reduce risks from chemical use.

In this study, we assessed human thyroid peroxidase (hTPO) and thyroid hormone receptor (hTHR) activities upon exposure to antimicrobial agents, flame retardants, and bisphenols & phenylphenols. hTPO expression was transiently induced in 293FT cells, and the Amplex UltraRed reagent (AUR) was applied for activity measurement. Methimazole (MMI), used as a positive control, showed a dose-response decrease in hTPO activity with an IC_{50} value of 0.5299 μM. Among the chemical groups, only bisphenol A (BPA) and bisphenol F (BPF) showed inhibitory activity, with IC_{50} values of 26.90 and 46.55 μM, respectively.

hTHR activity was measured using a luciferase reporter cell line (HEK293-TRE-hTHR-α/β).Luciferase activities were induced approximately 10-fold and 14-fold (for hTHR-α and hTHR-β, respectively) at 3.16 nM T3 exposure compared to basal levels and were inhibited upon exposure to the THR antagonist 1-850 in a dose-response manner in both hTHRs (IC_{50} for hTHR-α/β: 26.51/29.59 μM). In the chemical exposure, none of the groups showed an agonistic effect on either hTHR-α or hTHR-β. However, decabromodiphenyl ether (DBDPEther; IC_{50} for hTHR-α/β: 15.56/18.97 μM) and decabromodiphenyl ethane (DBDPEthane; IC_{50} for hTHR-α/β: -/0.8676 μM), which are flame retardants, and tetramethyl bisphenol F (TMBPF), which is a bisphenol, showed an antagonistic effect.

These results underscore the necessity for thorough screening and regulation of such chemicals to safeguard human health and development.

Keywords:
In vitro assay, Thyroid peroxidase, Thyroid hormone receptor, Amplex UltraRed, luciferase assay

POSTER 3

CHANGES IN COMPLEX TOXICITY OF LEACHATE, SPIKED WITH PFOA AS A RESULT OF DIRECT PLASMA TREATMENT

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Abstract:
The presented case study concerns one serious environmental problem – **leachate** formation from solid waste disposal in landfills. This dark-colored, hardly biodegradable effluent contains high concentrations of organics, ammonium nitrogen, and a huge variety of recalcitrant, extremely toxic compounds such as polyaromatic hydrocarbons, metals, phenols, phthalates, pesticides, microplastics, plasticizers, halogenated organic chemicals, per- and polyfluoroalkyl substances (PFAS). Waste management and conventional technologies face a challenge in treating this **complex mixture of emerging contaminants** and reducing their **toxicity**.

In this regard, the development of **innovations** is needed in two directions:

(1) advanced methods and modules of hybrid technologies for the treatment of leachate and removal of hazardous compounds;

(2) new methods for assessment of leachate toxicity and its reduction after treatment with developed modules. The standard components in the leachate give rise to significant ecotoxicological concerns and have a synergistic effect - the complex toxicity of the leachate is not easy to assess simultaneously from the point of view of the risk for the treatment technology itself and the environment.

In the first direction, treatment with **plasma-assisted methods** is considered a promising advanced approach for the complete removal/detoxification of hazardous chemicals. In the second – the **fluorescence-based assays are** assessed with a high potential for generating reliable and meaningful data for monitoring toxicity changes.

The data presented are for the reduction of complex leachate toxicity, spiked with **PFOA** (perfluorooctanoic acid) as the target recalcitrant compound and treated with non-thermal plasma. The leachate samples were real and taken from the Sofia Waste Treatment Plant (Bulgaria). PFOA was added to samples at three high concentrations (2, 5, 10 mg/L). The plasma was produced by a dielectric barrier discharge (DBD).

The toxicity of treated and untreated samples was studied by **fluorescence staining** with **CTC** (5-cyano-2,3-ditolyl tetrazolium chloride) and **DAPI** (4',6-diamidino-2-phenylindole) on a test bacterial culture *E. coli* ATCC 700728 at an incubation time of 1 hour. The method assesses the **intensity of metabolic processes** in bacterial cells and their inhibition in the presence of toxic agents. The obtained images were processed with a digital image analysis (using the software *daime*).

The changes in the complex toxicity due to plasma treatment were assessed by comparing the effect of treated and untreated samples by two endpoints: (1) the **share of viable cells**; and (2) the **intensity** of bacterial fluorescence as an **indicator of metabolic activity** inhibition.

The addition of PFOA increased the complex toxicity of leachate samples – the share of viable cells was 10-fold lower and the metabolic activity of test bacteria decreased by 30%. The treatment with plasma dramatically reduced the complex toxicity – the share of viable cells after incubation with treated samples was more than 50% compared to 1-4% for untreated samples. The intensity of fluorescence showed high metabolic activity in the plasma-treated samples.

The study results show that plasma treatment can successfully **reduce the landfill leachate's toxicity.** This reduction can be measured reliably and quickly by CTC/DAPI staining and fluorescence analyses. The plasma-based technologies have the potential to improve the efficiency of the removal of recalcitrant compounds.

Keywords:
Complex toxicity, Leachate, Plasma treatment, Fluorescence analysis

POSTER 4

EVALUATION OF THE ENDOCRINE DISRUPTOR POTENTIAL OF THE FISHING GEARS FROM THE MAR LIGURE AREA

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Abstract:
The impact of fishing gear on the marine environment is multifaceted and can vary depending on factors such as the type of gear used, the intensity of fishing activities, and the characteristics of the local ecosystem.

Moreover, the leaching from abandoned, lost, and discarded fishing gear into the seawater carries out the release of substances from the gears into the marine environment. This process can result in diverse ecological consequences, influenced by the characteristics of the substances released.

Fishing gears are often made of synthetic materials such as nylon, polyethylene, and polypropylene. Through mechanisms like abrasion, and degradation, these plastics can emit small particles into the water. Marine organisms may ingest microplastics, posing potential risks to their well-being and the ecosystems they populate.

These compounds can also interfere with the endocrine systems of organisms, including fish, invertebrates, and mammals. These compounds, often found in materials like plastics and chemical additives, can interfere with the endocrine systems of marine organisms, including fish, invertebrates, and mammals.

The aim of the project is to investigate the leaching in seawater of polyethylene plastic fishing gear. Two different samples were tested, a new and used fishing gear for 14 days.

The seawater was tested through the CALUX assay to investigate the presence and the amount of estrogen compound that could be leached by the fishing gear.

This can have an impact on analyzing the different impacts of a new and exhausted fishing gear on the release of endocrine disruptor molecules.

Keywords:

Fishing gear, seawater, leaching, endocrine disruptor

POSTER 5

CAN HUMAN THYROID BIOASSAYS EVALUATE ENDOCRINE PRESSURES IN WILDLIFE TAXA?

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Abstract:
This presentation explores predicting pollutant toxicity using assays on thyroid metabolism. Though chemical pollutants affect health, monitoring can be cumbersome; linking pollutants to health may be facilitated by complementing chemical analysis with (cheaper) bioassays. Still, bioassay data does not always validate existing models on mixture toxicity, hampering risk assessment. Recent developments in thyroid assays allow better detection of pollutant mixtures. Thyroid hormones are key to energy metabolism – connected to many vital organs. First goal of this research was to develop calculus that unifies Modes of Actions in mixtures to evaluate endocrine disruption (ED) via thyroid (stimulating) hormones.

To this end, epidemiological data from the open literature was collected for health effects on human liver, kidney, bone, heart and brain. Least Squares, Pearson and normalization were applied to obtain exposure relationships: pollutants vs. human plasma biomarkers like TSH. This subsequently links to the effect: sigmoid distribution curves between TSH and health, using natural growth factors (1.618..). The calculus covers various modes of action and pollutants including dioxins, PCBs, heavy metals and iodine mimickants (CrO_4, etc.) to predict a range of effects. While aforementioned calculus was validated on humans, there is a lack of such tools for wildlife, related to costly sampling in the field.

Thyroid metabolism is key to functional diversity in ecosystems as TSH/TH interrelate and associate with e.g. circadian rhythms, behavior and sex hormones, reproduction homeostasis, etc. Given the universality in endocrinal growth across taxa and conservation of biochemical targets among apex species, we will explore the validity of the aforementioned calculus for ED in wildlife. We aim to make use of data from the Horizon Europe project 'TerraChem' which involves case studies on terrestrial apex species (e.g. barn owl, red fox) food chains across Europe. As exposure data, we take uptake of pollutants via feeding (prey) and (their) contact with soil. We take accumulated concentrations in predator species' livers as markers for effect. We also test the calculus using TH bioassay results from the case studies, and benchmark results with wildlife data from the open literature and closely related projects (Posthuma et al., Barmentlo et al.).

Keywords:
Endocrine disruption, wildlife, mixtures, modeling, non-animal testing

POSTER 6

European Society of Toxicology In Vitro (ESTIV) – PRAGUE 2024

INCORPORATING METABOLIZING SYSTEM FROM DIFFERENT TISSUE SOURCES TO IMPROVE THE VALUE OF *IN VITRO* ENDOCRINE DISRUPTION ASSAYS : A COMPARATIVE STUDY

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1 CONTEXT

- Metabolically competent *in vitro* cell-based assays are required for the efficacy and safety assessment of xenobiotics, including the assessment of potential endocrine active substances (EAS) [1, 2]
- We developed new metabolically competent *in vitro* assays for EAS, based on current gene reporter transactivation (TA) assays for estrogenic and androgenic activities, by incorporating S9 fractions metabolic system, using S9 fractions from different tissues and different species, to identify potentially toxic metabolites and to determine if the parent compound is detoxified
- The method incorporating hepatic S9 fractions with phase I cofactors in TA assays has shown the ability of more estrogenic metabolite formation from parent compound with less estrogenic activity such as methoxychlor and its well-described metabolite HPTE [3]

2 MATERIALS AND METHODS

❑ CHEMICALS

We used well-known estrogenic and androgenic active compounds :

*Bisphenol A (BPA), CAS 80-05-7

*Flutamide (FLU), CAS 13311-84-7

❑ S9 FRACTIONS

*Liver rS9 : Arochlor induced Rat liver (MetTA)

*liver rS9: PB/BNF induced Rat liver (CALUX)

*Liver hS9: Human liver mixed pool, 200 donors

*Skin hS9: Human Skin, T-Skin™

❑ Results interpretation

Increased activity : EC50 Ratio TA / TA+S9 > 2

Decreased activity: EC50 Ratio TA / TA+S9 < 0,5

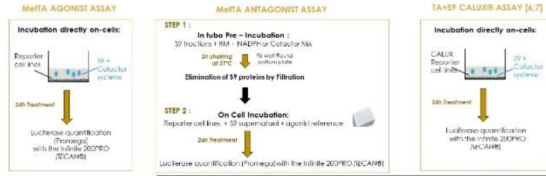
❑ ANALYTICAL QUANTIFICATION

TA ASSAY → *24 hours incubation of cells at 37°C* → *Cell medium collected* + Acetonitrile Vortex (protein precipitation)

➡ **Quantification of BPA disappearance + Metabolite formation by LC/fluo** HPLC: NEXERA AD30 (Shimadzu)

➡ **Quantification of FLU disappearance + metabolite formation by LC/MS** HPLC: NEXERA AD40 (Shimadzu) MS: exploris 120 (Thermo)

❑ TRANSACTIVATION (TA) ASSAY WITH S9 PROTOCOLS



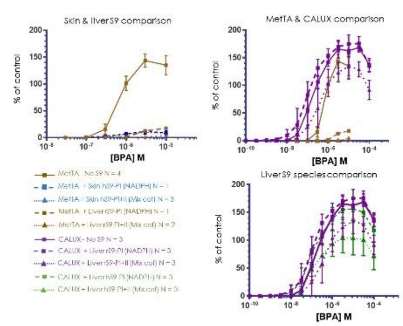
		ER Agonist MetTA assay	AR Antagonist MetTA assay	CALUX® + S9 assay	CALUX® + 10X S9 assay
Cells		T47D-KBluc (ERα) [4]	MDA-kb2 (AR) [5]	U2-OS (ERα and AR)[5]	
Metabolic system incubation time (hours)		24h with cells	2h without cells	24h with cells	
S9 Fractions concentration		0.5mg/mL	1mg/mL	0.01mg/mL	0.1mg/mL
Cofactor Phase I (PI)	NADPH (CYP450)	135µM		200µM	
NADPH Regenerating system (RS)	G6P	-		3mM	
	G6P-DH	-		0.3units/mL	
Cofactor Phase II (PII)	UDPGA (UGT)	200µM		1mM	
	A-CoA (NAT)	260µM		-	
	PAPS (SULT)	75µM		20µM	
	GSH (GST)	260µM		2mM	

3 RESULTS

I. ESTROGENIC (ER) AGONIST ASSAYS ± S9 fractions

		BPA: ER MetTA Skin hS9	BPA: ER MetTA Liver rS9	BPA: ERα CALUX Liver rS9	BPA: ERα CALUX Liver hS9
TA - No S9	ER Activity	EC50 = 4,0.10⁻⁷ M		EC50 = 1,8.10⁻⁷ M	
	Quantification	No BPA metabolization		No BPA metabolization	
TA + S9 PI	ER Activity	Not Detected (ND)	ND	EC50 = 1,0.10⁻⁷ M	EC50 = 1,1.10⁻⁷ M
	Parent Quanti* Metabolite quanti**	No Data	Remaining BPA (50%) None	Remaining BPA (100%) None	Remaining BPA (100%) None
TA + S9 PI+II	ER Activity	ND	ND	EC50 = 3,7.10⁻⁷ M	EC50 = 1,1.10⁻⁷ M
	Parent Quanti* Metabolite quanti*	No Data	Remaining BPA (30%) BPA-Glu	Remaining BPA (40%) BPA-Glu	Remaining BPA (100%) BPA-Glu

* Analytical quantification preliminary results (n=1) # BPA Glucuronide and BPA Sulfate quantification, no phase I metabolites monitored



Comparison of skin and liver S9 in MetTA assay : BPA undergoes into glucuronide-BPA formation after 24h of incubation with skin or liver rS9 in MetTA leading to reduced BPA estrogenic activity. The decreased Estrogenic activity of BPA using liver rS9 with PI cofactors could be due to the ipso reaction resulting in BPA breakdown [9], leading to metabolites with no ER activity in TA assay.
MetTA & CALUX+S9 assays comparison: MetTA assay induced more metabolism of BPA than CALUX+S9 assay, with no change in BPA EC50 using PI cofactors or a slight decreased ER activity using PI+II cofactors, despite the 40% remaining BPA and BPA-Glu formation after 24h incubation. Differences in S9 concentration (50 times less concentrated in CALUX assay) and the kinetic of BPA biotransformation could explain these outcomes.
Liver S9 species comparison : Incubation using induced liver rS9 led to stronger biotransformation of BPA than human liver S9, with unchanged BPA ER activity than TA assay without S9

II. ANDROGENIC ANTAGONIST (ANTI-AR) ASSAYS ± S9 fractions

		FLUTAMIDE: ANTI-AR MetTA Liver rS9	FLUTAMIDE: ANTI-AR CALUX Liver rS9	FLUTAMIDE: ANTI-AR CALUX Liver hS9
TA - No S9	Anti-AR Activity	IC50 ~ 2,0.10⁻⁶ M	IC50 = 2,3.10⁻⁷ M	
	Quantification		No FLU consumption / No OH-FLU formation	
TA + S9 PI	Anti-AR Activity	IC50 < 3,0.10⁻⁷ M	IC50 = 5,8.10⁻⁸ M	IC50 = 3,7.10⁻⁷ M
	Parent quanti* Metabolite formed**	Remaining FLU (40%) OH-FLU	Remaining FLU (80%) OH-FLU / dealkylated metab	Remaining FLU (> 70%) dealkylated metab
TA + S9 PI+II	Anti-AR Activity	IC50 < 3,0.10⁻⁷ M	IC50 = 6,2.10⁻⁷ M	IC50 = 6,4.10⁻⁷ M
	Parent quanti* Metabolite formed**	Remaining FLU (40%) OH-FLU	Remaining FLU (85%) dealkylated metab	Remaining FLU (> 50%) dealkylated metab

* Analytical quantification preliminary results (n=1) ** OH-FLU quantification and dealkylated metabolites semi quantification, no phase II metabolites monitored

Liver S9 + PI cofactors :
- Increased Anti-AR activity of FLU using Rat liver S9 in both MetTA and CALUX assays with a 6,6 and 3,9 times IC50 decrease, respectively, mainly due to OH-FLU formation observed.
- slight FLU metabolization observed with dealkylated metabolite observed in Anti-AR Calux assay with human liver S9 resulting in similar IC50 than anti-AR Calux assay without S9

Liver S9 + PI+II cofactors:
- similar outcomes are observed using PI or PI+II cofactors in MetTA assay
- a 2,7 decrease of FLU anti-AR activity in CALUX assay using both liver S9, with dealkylated metabolite formed

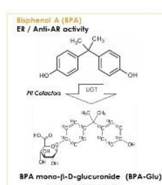
Liver S9 species comparison: stronger FLU biotransformation into dealkylated metabolite with no OH-FLU formation using liver human S9

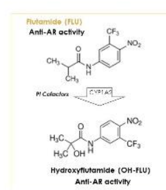
III. EFFECT OF S9 CONCENTRATION IN CALUX ASSAYS

Liver rS9 (0,1mg/mL)		BPA: ERα CALUX	FLU: Anti-AR CALUX
S9 10X – PI	Activity	EC50 7,3.10⁻⁷M	IC50 ND
	Parent quanti* Metabolite formed	Remaining BPA (80%) none#	Remaining FLU (70%) OH-FLU/dealkylated metab**
S9 10X – PI+II	Activity	EC50 1,1.10⁻⁶M	IC50 ND
	Parent quanti* Metabolite formed	Remaining BPA (<10%) BPA-Glu# ++	Remaining FLU (80%) OH-FLU/dealkylated metab**
S9 10X	Cytotoxicity	No cytotoxicity	No cytotoxicity

* Analytical quantification preliminary results (n=1)
** OH-FLU quantification and dealkylated metabolites semi-quantification, no phase II metabolites monitored
\# BPA Glucuronide and BPA Sulfate quantification, no phase I metabolites monitored

Increased S9 concentration in CALUX assays led to:
- A decrease of BPA and FLU EAS activities
- An increase of BPA metabolization (BPA consumption and BPA-Glu formation)
- Increased protein concentration may interfere with CALUX readout due to changes in compound uptake dynamics
- Contrary to BPA, more FLU metabolism was observed using liver hS9 fractions than liver rS9





4 CONCLUSIONS

This study demonstrated the feasibility and the relevance of incorporating metabolic systems into classical *in vitro* TA assays (OECD guidelines). Such experimental design leading, mainly, to reduction of estrogenic and androgenic activity in TA assays, relying on the following points:

- ➢ Biotransformation of BPA to BPA Glucuronide with Liver S9 and Phase I+II cofactors.
- ➢ Biotransformation of FLU to OH-FLU with phase I metabolism using rat Liver S9 fractions
- ➢ Weaker BPA detoxification but increased FLU detoxification using Human liver S9 fractions.
- ➢ Difference of sensibility between protocols, depending on target cells, S9 concentration. Additional study would lead to optimize the most relevant protocol
- ➢ No impact of Regenerating system of NADPH on metabolic system efficiency
- ➢ S9 fractions concentration being a critical parameter in such assays to be able to capture both reactive metabolites and no active metabolites

Ask us anything !

L'ORÉAL RESEARCH & INNOVATION

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C1 - Internal use

BDS BioDetection Systems

POSTER 7

BioDetection Systems

Evaluation of disinfection by-products (DBPs) resulting from different water sources using a panel of effect-based bioassays

safe CREW

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Introduction

Water systems worldwide are confronted with a complex mixture of thousands of known and unknown (unregulated) emerging compounds. Furthermore, water systems and treatment technologies face a major challenge and are under great pressure to deliver safe and affordable water services to a growing population. The SafeCREW project catalyzes innovations in several European water treatment sites through improved combinations of natural and engineered treatment systems.

Water quality and treatment performance is generally assessed for a limited set of individual parameters, possibly resulting in an incomplete quality assessment. Room is now given in the Drinking Water Directive to develop a risk-based monitoring program.

In the SafeCREW project we applied a comprehensive panel of human cell-based biological detection methods (i.e. CALUX® assay) to assess the impact of disinfection by-products (DBPs), related chemicals and chemical mixtures on a range of key types of toxicity pathways (e.g. cytotoxicity, genotoxicity, oxidative stress, endocrine effects, PAH and PFAS-like properties and obesity) in different model and real demonstration site waters.

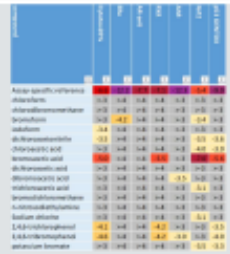


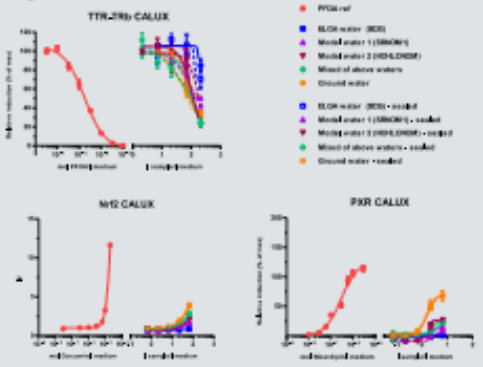
Table 1:

- Most relevant and regulated DBPs were selected and tested by the CALUX panel to obtain relative potency factors (RPFs).
- Stress pathways activated:
  Cytotoxicity
  Nrf2 (oxidative stress)
  p53 (DNA damage)
- 11 of 16 compounds are active
- 6 of 7 assays are activated

Approach

For this first round of analyses, model and real ground water samples with varying character of natural organic matter (NOM) at various DOC (3 mg/L for model water; 3.4 mg/l for real groundwater) as matrices for ground and surface waters were selected and disinfected by sodiumhypochlorite (NaClO) (dosage ar 5 mg/L free chlorine) to evaluate the response of the panel of effect-based CALUX bioassays (see Figure 1):

Figure 1:



Our here applied effect-based trigger values (EBTs) for the assessment of water quality and implementation of effect-based bioassays in regulatory water frameworks for risk assessment is discussed (see **Table 2** below):

Sample	cytotox CALUX activity (eq TBT) sample)	P53 CALUX activity (eq Actinomycin D) sample)	Nrf2 CALUX activity (eq Curcumin) sample)	PXR CALUX activity (eq Nisoldipine) sample)	ERα CALUX activity (eq Diethylstilbestrol) sample)	Anti-AR CALUX activity (eq Flutamide) sample)	TTR-TRb CALUX activity (eq PFOA) sample)
EBT range (literature 1,2,3)			10 - 21	3 - 54	0,18 - 0,28	14 - 25	0,56 - 22
LOQ	0,8	0,01	31	1,2	0,04	13	1,1
Model water 1 (SRNOM)	LOQ vs LOQ	LOQ vs LOQ	37 vs 46	3,1 vs 1,8	LOQ vs LOQ	LOQ vs LOQ	3,2 vs 4,0
Model water 2 (HEHLONEM)	LOQ vs LOQ	LOQ vs LOQ	52 vs 61	4,3 vs 3,9	LOQ vs LOQ	LOQ vs LOQ	3,4 vs 2,8
Mixed of above waters	LOQ vs LOQ	LOQ vs 0,02	81 vs 95	4,8 vs 4,4	LOQ vs LOQ	LOQ vs LOQ	4,9 vs 6,9
Ground water	LOQ vs LOQ	LOQ vs LOQ	120 vs 180	18 vs 12	LOQ vs LOQ	LOQ vs LOQ	8,4 vs 3,3
ELGA water (BDS)	LOQ vs LOQ	LOQ vs LOQ	LOQ vs LOQ	LOQ vs LOQ	LOQ vs LOQ	LOQ vs LOQ	LOQ vs 1,4

Summary

- ✓ Assessment of several DBPs with general in vitro toxicity testing (cytotox, genotoxicity p53, oxidative stress Nrf2) and early warning (PXR) as well as estrogen-, anti-androgen- and thyroid hormone competition (TTR-TRb) have been carried out using quantitative human CALUX® reporter gene technology (see Table 1).
- ✓ 11 of 16 compounds are active and 6 of 7 assays are activated
- ✓ Most active CALUX bioassays have been related to stress pathways with Nrf2 (oxidative stress) and PXR (early warning)
  - ✓ in the selected DBPs as well as
  - ✓ in the model and real ground water samples.
- ✓ Effect-based trigger values (EBTs) have been relevant also for TTR-TR CALUX bioassay

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POSTER 8

Monitoring of Toxicity of Plastic Recyclates from Low- and Middle-Income Countries by Bioassay Panel to Support the Global Management of Chemicals in Plastics

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BioDetection Systems

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Introduction

More than 13,000 chemicals are associated with plastics with more than 3200 having hazardous properties [1]. The complexity of the plastic composition, which include additives, unreacted monomers, processing aids, and non-intentionally added substances (NIAS), raises concerns about their potential environmental and health impacts [1,2].

NIAS are particularly prevalent in products made from recycled materials, as recycling processes can introduce a variety of contaminants including banned hazardous chemical additives, such as POPs or brominated dioxins [1,3].

Therefore, it is important to investigate the potential toxicity of recycled plastics in a more comprehensive way than just measuring a few selected chemicals in them. While a range of studies have been published on POPs in major products, there is a lack of knowledge on POPs in plastic recyclates in particular in low- and middle-income countries (LMICs) having limited analytical capacities.

Therefore, activities were conducted under the UNEP/GEF Global Monitoring Plan (GMP) projects to collect plastic pellets and shreds from selected LMICs in Africa, Asia, and the GRULAC region from February to June 2023.

In addition to chemical compounds analysis of target POPs, it was decided that also the screening of toxic effects of selected plastic recyclates would be beneficial. For this screening we applied a panel of human cell-based biological detection methods (i.e. CALUX assay) to assess the impact of plastic and plastic recyclate mixtures on a range of key types of toxicity pathways (e.g. cytotoxicity, genotoxicity, selected endocrine effects, and PAH toxicity).

Materials and Methods

A) THF/hexane:	B) 50% Ethanol/Water:	C) 20% Ethanol/Water:
• 0.5 gr plastic, milling,	• 0.5 gr plastic, milling,	• 0.5 gr plastic, milling,
• add 10 ml THF, shake 20 min,	• add 10 ml EtOH/Water (50%),	• add 10 ml EtOH/Water (20%),
• add dropwise 2 x 10 ml hexane, combine,	• put in oven at 60 ° C for 3 days	• put in oven at 40 ° C for 1 day
• add 50 ul DMSO,	• add 50 ul DMSO,	• add 50 ul DMSO, evaporate to a final volume of 50 ul.
• evaporate to a final volume of 50 ul.	• evaporate to a final volume of 50 ul.	

Take home messages

- ✓ Highest bioassay activities were found in PVC samples. This exemplifies that additives and NIAS in a plastic are the driver of toxicity of plastics and that a polymer like soft PVC with high share of additives can have several orders of magnitude higher potential to cause adverse effects compared to a polymer with low additive content.
- ✓ Also recycled plastic used for food contact and skin contact showed several in vitro toxicity effects.
- ✓ High PAH toxicity in the PAH CALUX (45.000 ng BaP-EQ/g material) was detected in a black PVC sample from Nigeria in the extraction precipitation experiment indicating that carbon black containing PAHs might have been present.
- ✓ Bioassays reviled multiple in vitro toxicity effects including cell death, PAH-like, estrogen activity and anti-androgen toxic in the samples tested.
- ✓ None of the samples tested showed any genotoxic effect (no p53 DNA repair activation by p53 CALUX).
- ✓ In most cases the extraction with THF/hexane resulted in higher toxicity compared to the two other applied extraction methods (20% and 50% ethanol/water).

Results of effect-based CALUX monitoring

- With the most aggressive extraction method (dissolution in THF and precipitation with hexane) all analysed recycled plastic showed at least an effect in one of the bioassays.
- The assessment of genotoxicity (p53 CALUX) showed that none of the 20 plastic extracts exhibited any genotoxicity (≤ LOQ (0.01) µg Actinomycin D eq./g).
- The cytotoxicity of the samples were in the range of ≤0.34 to 41 µg TBT eq./g. 4 of the 10 plastic samples showed cytotoxicity. The highest cytotoxicity was observed with the THF extraction combined with hexane precipitation and detected in a recycled HDPE (41 µg TBT eq./g) followed by a LDPE (32 µg TBT eq./g), a PP recyclate (16 µg TBT eq./g) and a PVC recyclate sample (1.6. µg TBT eq./g).
- The estrogen activities of the plastic extracts measured by the ERa CALUX ranged from ≤0.075 to 5.7 ng 17β-Estradiol eq./g. 5 of the 10 plastic samples showed an estrogenic effect in at least one of the extracts.
- The anti-androgen activities of the plastic extracts measured by the Anti-AR CALUX ranged from ≤ LOQ (0.34) to 390 µg Flutamide eq./g. 5 of the 10 samples showed an anti-androgen activities at least in one of the tests.
- The PAH toxicity (B[a]P eq./g) of the plastic extracts measured by the PAH CALUX ranged from ≤0.85 to 45,000 ng B[a]P eq./g. 8 of 10 plastic extracts using THF/hexane showed PAH toxicity
- In most of the tests, the activity in the migration tests (ethanol/water) were lower compared to the THF/hexane extraction. However, in a few cases the activity in the 50% ethanol/water were higher indicating that there are substances in the polymers which were better extracted by the ethanol/water migration test compared to THF/hexane or which were adsorbed in the hexane precipitation step (**see table 1 and figure 1**).

Table 1: Plastic pellet results of effect-based CALUX bioassay panel

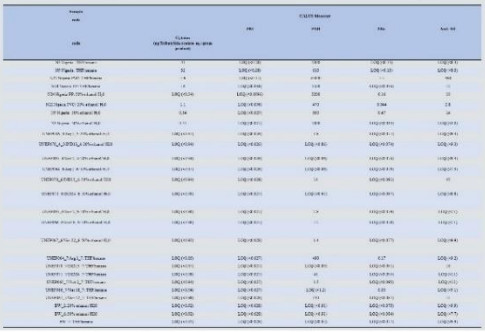
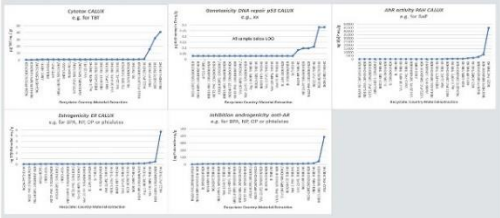


Figure 1: In vitro toxicity CALUX profiling



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POSTER 9

An Estrogen, Androgen, Thyroid and Steroidogenesis (EATS) assay panel to predict endocrine disruption of chemicals and chemical mixtures

BioDetection Systems

Bart van der Burg, Barbara van Vugt-Lussenburg, Harrie Besselink, Bram Brouwer

Summary

Endocrine disrupting compounds (EDCs) are being classified as substances of very high concern, and regulations to restrict their use are being installed world-wide. Yet there has been very limited testing of chemicals and chemical mixtures to which we are exposed to at a daily base. EDC effects are hard to predict and are best tested using bioassays. To avoid animal studies BDS has now developed a range of reliable and validated assays that allow screening and safety assessment of chemicals and consumer products in a rapid and cost-effective manner.

Background

Endocrine disruption has been recognized as a priority endpoint in safety evaluation of chemicals and consumer products. Yet, there has been very limited testing so far due to limited clarity on the testing approach, and the reliability of methods proposed. Based on established modes of action of EDCs, recently interferences with *Estrogen, Androgen, Thyroid and Steroidogenesis (EATS)* pathways have been selected by OECD,ECHA,EFSA and JRC as the focus of regulatory testing approaches [1,2]. Because of ethics, capacity, speed and relevance animal models are not very suitable to assess EDC effects. Therefore alternative methods are needed, particularly those that are validated and incorporated in relevant international guidelines, thus promoting regulatory acceptance of the safety data generated.

The unique CALUX®-based test panel includes

E: (anti)Estrogens: ERalpha CALUX (OECD TG455)

A: (anti)Androgens: AR CALUX (ECVAM validation, OECD TG458)

T: Thyroid interference*: TRbeta CALUX, TTR and TPO assay (TG in preparation)

S: H295R steroidogenesis (OECD TG456)

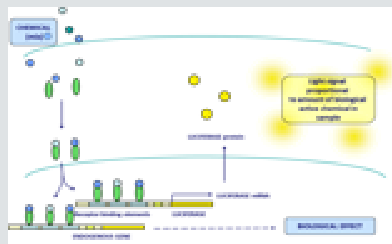
+/- modular phase 1 and 2 metabolic steps

Characteristics

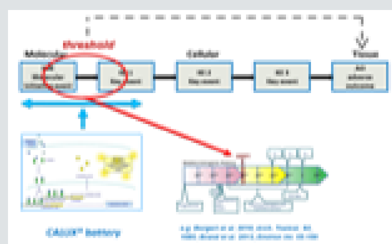
- Assessment of interferences with estrogen-, androgen- and thyroid pathways and steroidogenesis (EATS) can be carried out using robust and quantitative human CALUX® reporter gene technology, and complementary assays
- Methods to measure in a wide variety of products and applications
- Used world-wide in major chemical-, pharmaceutical-, cosmetics-, food- and feed companies, and others
- Extensively validated to demonstrate robustness and predictivity
- Available through (ISO 17025/GMP+ accredited) contract service or transferred to your laboratory via licensing and training

Approach

Based on its CALUX reporter gene technology and focusing on the EATS mode of action of EDCs BDS has developed a range of specific assays that allow screening and safety assessment of chemicals and consumer products in a rapid and cost-effective manner. Complementary assays have been selected to complete the comprehensive panel.



These assays have been extensively validated and most of them already incorporated in relevant OECD test guidelines. These OECD guidelines define thresholds based on the threshold between negative and positive compounds. In addition thresholds have been defined for specific applications such as water quality monitoring, thereby assessing the effects of complex mixtures. In addition, methods have been used for safe design of new chemical entities. Methods can be used with and without modular metabolic systems.



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POSTER 10

BioDetection Systems

Evaluation of thyroid hormone disruption by PFAS in WWTP influent/effluent and surface waters

the international water association

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Introduction

- PFAS, a mixture of thousands of synthetic compounds, are widely used in industrial applications and consumer products because of their physical/chemical characteristics.
- PFAS are widely distributed in the environment and are a potential risk for human and animal health.
- Monitoring PFAS is currently based on chemical-analysis of <30 target PFAS substances.
- An alternative approach to monitor the total contribution of PFAS in the environment is based on common toxic effects elicited by compounds sharing the same biological mode-of-action.
- **PFAS CALUX**: quantitative analysis of PFAS, based on their common property to bind to specific thyroid hormone transport proteins and thereby interfering with the thyroid-hormone system.

To demonstrate the applicability of the PFAS CALUX bioassay for quantitative monitoring of total PFAS in water samples, surface water and WWTP influent and effluent samples were processed and analysed using both PFAS CALUX bioanalysis and targeted LC-MS analysis. Following conversion of chemical data using individual response factors, both chemical analysis results and biological analysis results were expressed as ng total PFAS equivalents per liter of processed water (ng PFOA eq./l water).

Material and Methods

Sample processing: WAX-SPE (Oasis WAX, Waters 186002493) columns were conditioned (4 ml MeOH/0.1% NH_4OH; 4 mL MeOH; 4 mL super-demi water) after which 0.5 - 1 liter of filtered surface water or WWTP influent/effluent was loaded on the columns. Columns were washed (4 mL 25 mM NH_4Ac pH 4; 8 ml THF:MeOH (75:25)), after which PFAS were eluted using 4 ml MeOH/0.1% NH_4OH. Eluates were evaporated (N_2; 45 °C) and reconstituted in 15 µl of DMSO.

PFAS CALUX:

Step 1: Incubation (TTR competition)
Step 2: Separation TTR-bound and free T4 / compound (Bio-Gel P-6DG)
Step 3: TRβ CALUX analysis

References

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Results

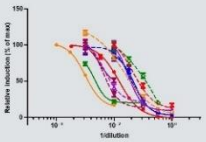

Figure 1 PFAS CALUX bioanalysis results of dilution series of 11 WWTP influent/effluent samples. PFAS CALUX activity is expressed as induction relative to maximum induction of the PFOA reference series (●; relative induction RI%).

Table 1 *In vitro* (REP) and *in vivo* (RPF) derived relative potencies of individual PFAS substances.

PFAS substance	REP[1] (in vitro)	RPF[2] (in vivo)
PFBA	0.0018	0.05
PFPeA	0.08	0.01
PFHxA	0.19	0.01
PFHpA	1.4	0.01
PFOA	1	1
PFNA	0.32	10
PFDA	0.12	4
PFUdA		4
PFDoA		3
PFTDA		0.3
PFTeDA		0.3
PFBS	0.052	0.001
PFPeS		0.001
PFHxS	1.6	0.6
PFHpS	1	0.6
PFOS	2	2
PFDS		2
DONA		0.0300
6:2 FTS	0.019	0.02
8:2 FTS		0.04

REP- Relative Potency; in vitro derived
RPF- Relative Potency Factor; in vivo derived
1: Behnisch et al. (2021) Environm. Int. 157, 106791-106798
2: Bil et al. (2021) Environ. Toxicol. Chem., 40, 859-87

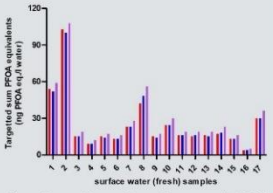

Figure 2 Comparison of converted LC-MS data (PFAS-13) using in vitro derived REP-values (Behnisch et al., 2021) or in vivo derived lower and higher relative potency factor (RPFs) (Bil et al., 2021).

PFAS CALUX REP
In vivo RPF – lower potency
In vivo RPF – higher potency

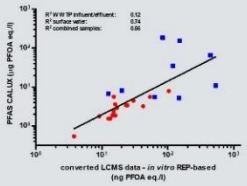

Figure 3 Correlation between the sum PFOA content using converted LC-MS data (in vitro REPs; Behnisch et al., 2021) and PFAS CALUX (Total PFAS) analysis results of fresh surface waters (●) and WWTP influent/effluent samples (■).

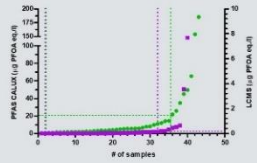

Figure 4 Comparison between the sum PFOA content using converted LC-MS data (*in vitro* REPs; ■) and PFAS CALUX bioanalysis results (●) of surface waters and WWTP influent/effluent samples lined-up from low to high values.

Table 2 Comparison of 5% and 80% percentile values measured by PFAS CALUX bioanalysis and by converted LC-MS data (PFAS-13) using *in vitro* derived REP-values for combined surface waters and WWTP influent/effluent samples.

Percentile (%)	PFAS CALUX (ug PFOA eq./l water)	LCMS (PFAS 13; upperbound) (ug PFOA eq./l water)
5	0.71	0.0094
80	22	0.19

Conclusions

Effect-based PFAS CALUX bioassay analysis results correlate well with LC-MS-derived converted data showing that in vitro toxicity analysis of total PFAS content in water samples using the PFAS CALUX reporter gene assay is a promising and suitable strategy to cover complex mixtures of PFAS and to assess total PFAS in water and the environment in general.

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POSTER 11

BioDetection Systems

PROMISCES

In vitro toxicity profiling of PFAS on a tailored panel of effect-based CALUX bioassays

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Introduction

- PFAS, a mixture of thousands of synthetic compounds, are widely used in industrial applications and consumer products because of their physical/chemical characteristics.
- PFAS are widely distributed in the environment and are a potential risk for human and animal health.
- Monitoring PFAS is currently based on chemical-analysis of <30 target PFAS substances.
- An alternative approach to monitor and rank toxicity of PFAS is based on common toxic effects elicited by compounds sharing the same biological mode-of-action.
- The toxic potency of >45 PFAS was evaluated on a tailored-set of 6 CALUX bioassays:

Cytotoxicity	- cytotox CALUX
Oxidative stress	- Nrf2 CALUX
Obesity / fat metabolism	- PPARα / anti-PPARγ CALUX
Thyroid hormone disruption	- anti-TRβ / PFAS CALUX

Toxicity profiling and ranking allows for establishing relative potency factors (**RPFs**) that can be used to convert chemical concentrations into **total PFOA equivalents** and compare direct to e.g. biological derived PFAS CALUX results.

Material and Methods

Following wide-panel CALUX screening (n=12) of the most common PFAS, the most potent bioassays (n=6) have been selected for PFAS-45 toxicity profiling. CALUX bioassays have been automated using a compact liquid handling system (see figure 1). Serial dilution series of PFAS have been analysed in duplicate and each analysis consisted of triplicate well-plate testing. *In vitro* toxicity was quantified using the lowest concentration showing activity in the concentration-response curves (PC5 and PC80 agonistic and antagonistic activity; IF-1.5 for non-receptor mediated activity; see table 1).

For sampling, transport and *in vitro* analysis of PFAS and PMTs in aquatic samples, specific SOPs has been developed: p-promisces-001.doc to p-promisces-003.doc

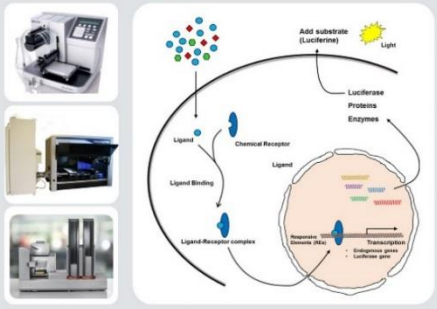

Figure 1 Principle of the *in vitro* CALUX effect-based bioassay. The bioassay has been automated to allow for high-throughput screening.

Results

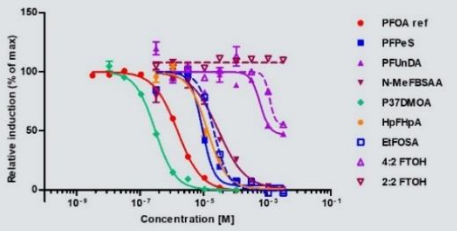

Figure 2 Typical example of concentration-response curves of various PFAS tested on the PFAS CALUX bioassay. Results are mean of triplicate analysis.

Table 1 Quantified activities (PC80 / PC5 for antagonistic and agonistic assays; IF-1.5 for non-receptor mediated assays) of PFAS on a tailored-set of CALUX bioassays for thyroid hormone disruption, obesity / fat metabolism, oxidative stress and cytotoxicity.

		CALUX bioassay						
		PFAS	anti-TRb	PPARa	anti-PPARg	Nrf2	cytotox	
Compounds	CAS	PFOA	diiodothyronine	GW7647	GW9662	curcumin	TBT	
Reference		-6.5	-7.2	-9.9	-9.3	-5.8	-7.0	
P37DMOA	172155-07-6	-6.8	-5.3	-5.3	-5.3	-5.3	>-4	
PFOS	1763-23-1	-6.8	>-4.4	-5.3	>-4.4	>-4.4	>-4.4	
PFHxS	355-46-4	-6.7	>-4	-5.3	>-4	>-4	>-4	
PFHpA	375-85-9	-6.6	>-4	-5.3	-5.3	>-4	>-4	
PFHpS	375-92-8	-6.5	>-4	-5.3	>-4	>-4	>-4	
PFOA	**335-67-1**	-6.5	-5.3	-5.3	-5.3	>-4	>-4	
PFOSA	754-91-6	-6.3	-5.4	-5.3	>-4	>-4	>-4	
ADONA	919005-14-4	-6.2	-5.4	>-4.5	>-4.5	>-4.5	>-4.5	
PFNA	375-95-1	-6.0	-5.4	-5.3	-5.3	>-4	>-4	
PFHxA	307-24-4	-5.7	-5.4	-5.3	>-4	>-4	>-4	
PFDcA	335-76-2	-5.6	-5.3	-5.3	>-4	>-4	>-4	
PFPeA	2706-90-3	-5.4	>-4	-5.3	>-4	>-4	>-4	
PFPeS	2706-91-4	-5.4	>-4	>-4	>-4	>-4	>-4	
HFPHpA	1546-95-8	-5.3	>-4	-5.3	>-4	>-4	>-4	
EtFOSA	4151-50-2	-5.2	>-4	>-4	>-4	-5.3	>-4	
PFBS	375-73-5	-5.1	>-4	-5.3	>-4	>-4	>-4	
MeFBSA	68298-12-4	-5.1	>-4	-5.3	>-4	>-4	>-4	
PFMOBA	863090-89-5	-5.0	>-4	-5.3	>-4	>-4	>-4	
N-MeFBSAA	159381-10-9	-5.0	>-4	-5.3	>-4	>-4	>-4	
8:2 FTUCA	70887-84-2	-4.9	-5.4	>-4	-5.3	-5.3	>-4	
PFDoDA	307-55-1	-4.8	-5.4	>-4.4	>-4.4	>-4.4	>-4.4	
6:2 FTSA / H4-PFOS	27619-97-2	-4.8	-5.4	>-4	-5.3	>-4	>-4	
H4-PFUnDA	34598-33-9	-4.8	-5.4	>-4	-5.3	>-4	-5.3	
MeFOSA	31506-32-8	-4.8	>-4.5	>-4.5	>-4.5	>-4.5	>-4.5	
PFBSA	30334-69-1	-4.7	>-4	>-4	>-4	>-4	>-4	
PFMOPrA	377-73-1	-4.7	>-4	-5.3	>-4	>-4	>-4	
PFNS	98789-57-2	-4.6	>-4.4	-5.3	>-4.4	-5.3	>-4.4	
PFHOPrA	13252-13-6	-4.6	>-4	>-4	>-4	>-4	>-4	
PFBA	375-22-4	-4.5	>-4	-5.3	>-4	>-4	>-4	
6:2 diPAP	57677-95-9	-4.4	-5.4	>-4.7	>-4.7	>-4.7	>-4.7	
4:2 FTOH	2043-47-2	-2.9	>-4	>-4	>-4	>-4	>-4	
6:2 FTAB	34455-29-3	-2.6	>-4	>-4	>-4	>-4	>-4	
4:2 FTSA / H4-PFHxS	757124-72-4	TBD	-5.4	>-4	>-4	>-4	>-4	
PFDS	335-77-3	>-3.2	>-4.7	>-4.7	>-4.7	>-4.7	>-4.7	
10:2 FTOH	865-86-1	>-2.5	>-4	>-4	>-4	>-4	>-4	
8:2 FTOH	678-39-7	>-2.5	>-4	>-4	-5.3	-5.3	>-4	
6:2 FTOH	647-42-7	>-2.5	>-4	>-4	>-4	>-4	>-4	
2:2 FTOH	54949-74-5	>-2.5	>-4	>-4	>-4	>-4	>-4	
6:2 FTSAM / DPOSA	80475-32-7	>-2.9	>-4.4	>-4.4	>-4.4	>-4.4	>-4.4	
MeFOSAA	2355-31-9	>-3	>-4.6	>-4.6	>-4.6	>-4.6	>-4.6	
FOSAA	2806-24-8	>-3	>-4.5	>-4.5	>-4.5	>-4.5	>-4.5	
BFOSAA	2991-50-6	>-3	-5.4	>-4.5	>-4.5	>-4.5	>-4.5	
Tech mix - 1	Tech Mix	>0.5	>-1	>-1	>-1	>-1	>-1	
Tech mix - 2	Tech Mix	>0.5	>-1	>-1	>-1	>-1	>-1	
GenX (*)	62037-80-3	>-5	>-8.5	>-8.5	>-8.5	>-8.5	>-8.5	

Note (*): GenX will be re-analysed at higher concentrations

Conclusions

Toxicity profiling of PFAS-45 using a tailored set of *in vitro* CALUX bioassays showed that the PFAS CALUX bioassay (TTR-T4 competition) is the most responsive and sensitive bioassay as compared to the other mode of actions evaluated. The RPF-values of the tested PFAS relative to PFOA (RPF = 1) varied between 0.00015 (6:2 FTAB) and 2.4 (P37DMOA).

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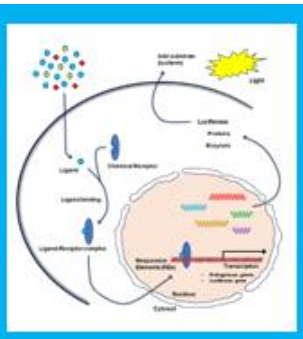

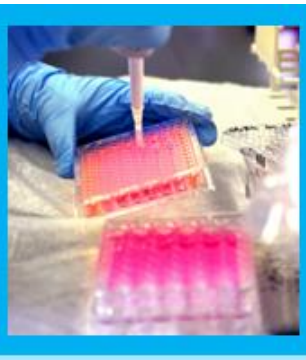

Venue

Auditorium University of Amsterdam (UvA)
Science Park 301 (Matrix ONE)
1098 XH Amsterdam

How to get there:

Amsterdam: - by plane (Amsterdam Schiphol Airport)
- by train (Amsterdam Central Station)

Auditorium UvA: (Matrix ONE) - bus (line 40) from A'dam Amstel Railway Station to Science Park (stop Terra)
- from Amsterdam Science Park Railway Station; appr 10 min.