EUROPE SECTION OF AOAC INTERNATIONAL

AOAC Bioassay Workshop

Program

01:00 PM **General Introduction** by AOAC Europe and to Workshop details

01:10 PM **Lecture 1: Prof. Beate Escher**
Department Cell Toxicology, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany

Title presentation: QA/QC during applications of in vitro assay for food monitoring

01:35 PM **Lecture 2: Prof. Bennard van Ravenzwaay**
Associate Professor of Reproduction Toxicity of the University of Wageningen, Chairman ECETOC

Title presentation: The use of metabolomics as a New Approach Methodology (NAM) in the context of the transition from in vivo to in vitro methods

02:00 PM **Lecture 3: Dr. Ir. Toine Bovee**
Senior scientist in vitro bioassays
Team Leader and Expertise group leader Bioassays and Biosensors
WFSR – Wageningen Food Safety Research, Wageningen UR
Business unit Authenticity and Veterinary Drugs (AV)

Title presentation: Development, validation and application of bioassays: their added value.

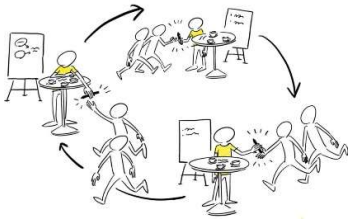
02:25 PM **Lecture 4: Dr. Peter A. Behnisch**
Director - Biodetection Systems BDS

Title presentation: Plastic and plastic additives testing by effect-based bioanalysis for endocrine disrupting chemicals – a CRO perspectives.

02:35 PM **Lecture 5: Dr. Maricel Marin-Kuan**
In vitro Toxicology Specialist
Food Safety Research Department, Société des Produits Nestlé S.A.
Workshop rational & introduction to brainstorm groups

02:35 PM Coffee Break

02:50 PM **Brainstorm Groups** organized in the World-Café format



We offer 4 groups rotating every 15 min with following questions:

Group 1:
Moderator: Prof. Bennard van Ravenzwaay
Rapporteurs: Aristeidis Tsagkaris and Csaba Boglari
Do you apply the OECD Guidance Document on Good In Vitro Method Practices (GIVIMP) and do you consider those as appropriate for complex mixtures? Why are or not appropriate? Other guidelines available to recommend?

Group 2:
Moderator: Dr. Ir. Toine Bovee
Rapporteurs: Harrie Besselink and Georg Braun
What consideration are you taking when extracting mixtures from water, food, food contact materials and other items to test with bioassays? (What is best practice and limitation of current approach?)

Group 3:
Moderator: Prof. Beate Escher
Rapporteurs: Peter Behnisch and Maricel Marin-Kuan
What are the most critical factors and source of errors for consideration to do accurate testing of complex undefined mixtures and extracts from water, food and related items using bioassays?

Group 4:
Moderator: Katerina Mastovska
Rapporteurs: Thomas Gude
There is a need for development of harmonized best practices (framework) when using bioassays for undefined mixtures.
a) to your opinion, which are the topics and key procedures that should be covered?
b) do you think those topics could be addressed through AOAC working groups?
c) would you be interested in participating in the working groups?

03:50 PM **Moderator/Rapporteur Preparation** – for all another coffee break

04:00 PM **Presentations of groups** by moderator/rapporteur (each 7.5 min)

04:30PM **General Discussion**

04:50 PM **Next Steps**

5:00 PM **Workshop Closure**

Abstract:

Prof. Beate Escher
Department Cell Toxicology, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany

QA/QC during applications of in vitro assay for food monitoring

The idea is simple: we extract mixtures of chemicals from different foodstuff and instead of a painstaking analysis of numerous target chemicals, we just let human cells tell us if the mixtures are toxic and pose a threat to human health. Reality is not that simple. Before we even dose the bioassay, the extraction is a crucial step. Exhaustive solvent extractions will coextract a lot of endogenous compounds and lipids, that disturb *in vitro* bioassays. If very selective extraction and clean-up steps are used, we lose a lot of potential toxicants. On the example of water quality testing, we will outline important steps of selecting and validating extraction methods for application of *in vitro* assays. We also discuss dosing, data evaluation and quality control of *in vitro* bioassays for the purpose of testing complex mixtures. It is not always the most sophisticated bioassay that is suitable but a robust one that is sensitive, robust, repeatable and fulfills quality criteria. Both extraction and bioassay application will be illustrated on the example of human blood and bovine/plant-based milk.

Abstract

Prof. Bennard van Ravenzwaay
Associate Professor of Reproduction Toxicity of the University of Wageningen, Chairman ECETOC

The use of metabolomics as a New Approach Methodology (NAM) in the context of the transition from in vivo to in vitro methods

Metabolomics in vitro is a powerful combination of a human relevant system with a multiparametric approach that allows assessing multiple endpoints in a single biological sample. Applying metabolomics in a cell-based system offers an alternative to both, the ethical concerns and relevance of animal testing and the restraining nature of single endpoint evaluations characteristic of conventional toxicological in vitro assays. Through the development and standardization of a targeted LC-MS/MS in vitro metabolomics platform characterization of hepatotoxicity was achieved. In addition, this platform is suitable for deriving dose- and time response metrics and the determination of Point of departure estimations for human risk assessment. Limitations of this system, which uses HepG2 cells, can potentially be overcome using human iPSCs-derived 3D liver organoid system. This work demonstrates the suitability of in vitro metabolomics for mechanistic-based hazard identification and its application in next generation risk assessment.

Abstract

Dr. Ir. Toine Bovee
Senior scientist in vitro bioassays
Team Leader and Expertise group leader Bioassays and Biosensors
WFSR – Wageningen Food Safety Research, Wageningen UR
Business unit Authenticity and Veterinary Drugs (AV)

Development, validation and application of bioassays: their added value

Most chemical analytical methods enable identification and quantification of targeted compounds at low concentrations. Although untargeted analysis, with e.g. hrMS, combined with mass spectra libraries theoretically allow the broad detection of all known hazardous compounds, in real practice the software is not able to correctly identify all the measured mass peaks. Moreover, real unknowns might be found, but if not present in a library, its relevance (hazard or not) cannot be addressed. Bioassays based on the mode of action are able to detect all compounds, both knowns and yet unknowns, that exhibit the cognate specific activity and directly flag suspected samples. In the past decades we have been introducing, developing, validating and applying several bioassays, e.g. for the detection of dioxins and PCBs in all kind of food and feed matrices, estrogens in supplements, feed and calf urine, androgens in supplements, feed and calf urine, and marine biotoxins in fish and shellfish. In addition, some compounds like sildenafil (Viagra) and its analogues, specifically inhibit a certain enzyme. In these cases, it is better and more easy to use an enzyme inhibition assay instead of a cell based bioassay. In our laboratory we use e.g., a PDE-5 enzyme inhibition assay to screen supplements for the presence of sildenafil-like compounds and explored the applicability of an AChE enzyme inhibition assay for the detection of carbamates and organophosphates and a COX 1 and 2 enzyme inhibition assays for NSAIDs. Their added value for monitoring and enforcement purposes will be shown as well as some new ideas for both cell and enzyme-based detection of other contaminants and drugs.

Abstract

Peter A. Behnisch
Director BioDetection Systems
Amsterdam, NL

Plastic and plastic additives testing by effect-based bioanalysis for endocrine disrupting chemicals – a CRO perspectives

In the EU, the main regulation in the chemical sector is the regulation on Registration, Evaluation, Authorisation and restriction of Chemicals (REACH, Regulation EC 1907/2006) with its Annexes requesting many new animal tests for chemical substances. An important revision of REACH is expected soon, introducing new features such as the registration of polymers (Annex VII). Many kinds of emerging compounds (e.g., endocrine concerns, POPs, PFAS) as well as unknown chemical features can cause critical issues in the life cycle of plastics (e.g. UNEP, Chemicals in

plastics, 2023). Increasing interest in addressing chemical mixture issues and how to take account of the combination effects in food additives, toys and food contact material proofs that there is a need for new innovative diagnostic tools to cover the handful regulated versus the huge amount of unknown not regulated chemicals. Regarding endocrine disrupting chemicals there is an urgent need to follow already existing guidelines for in vitro testing of chemicals (OECD TG455, 456, 458) and water (ISO 19040, and 24295) testing to evaluate them for other all kinds of plastic additives and materials. Such novel validated in vitro test systems are already ready for exploitation and industry uptake allowing effective, faster and cheaper toxicological testing for a safe & more sustainable design of novel plastic entities.

We will give a short overview about exciting ISO/OECD guidelines for in vitro EDC testing and will show results of several case studies with practical applications for emerging pollutants (e.g. BPA, phthalates, TBBPA, PBDD/F, PFAS) in various plastic (consumer products, food contact materials and plastic recyclates) and plastic additives.