Nestlé Good food, Good life

AOAC Virtual Session

AOAC INTERNATIONAL EUROPE SECTION

Follow-up of the Workshop "Best Practices for Bioassay Testing of Food and other Complex Mixtures"

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AOAC WORKING GROUP PROPOSAL

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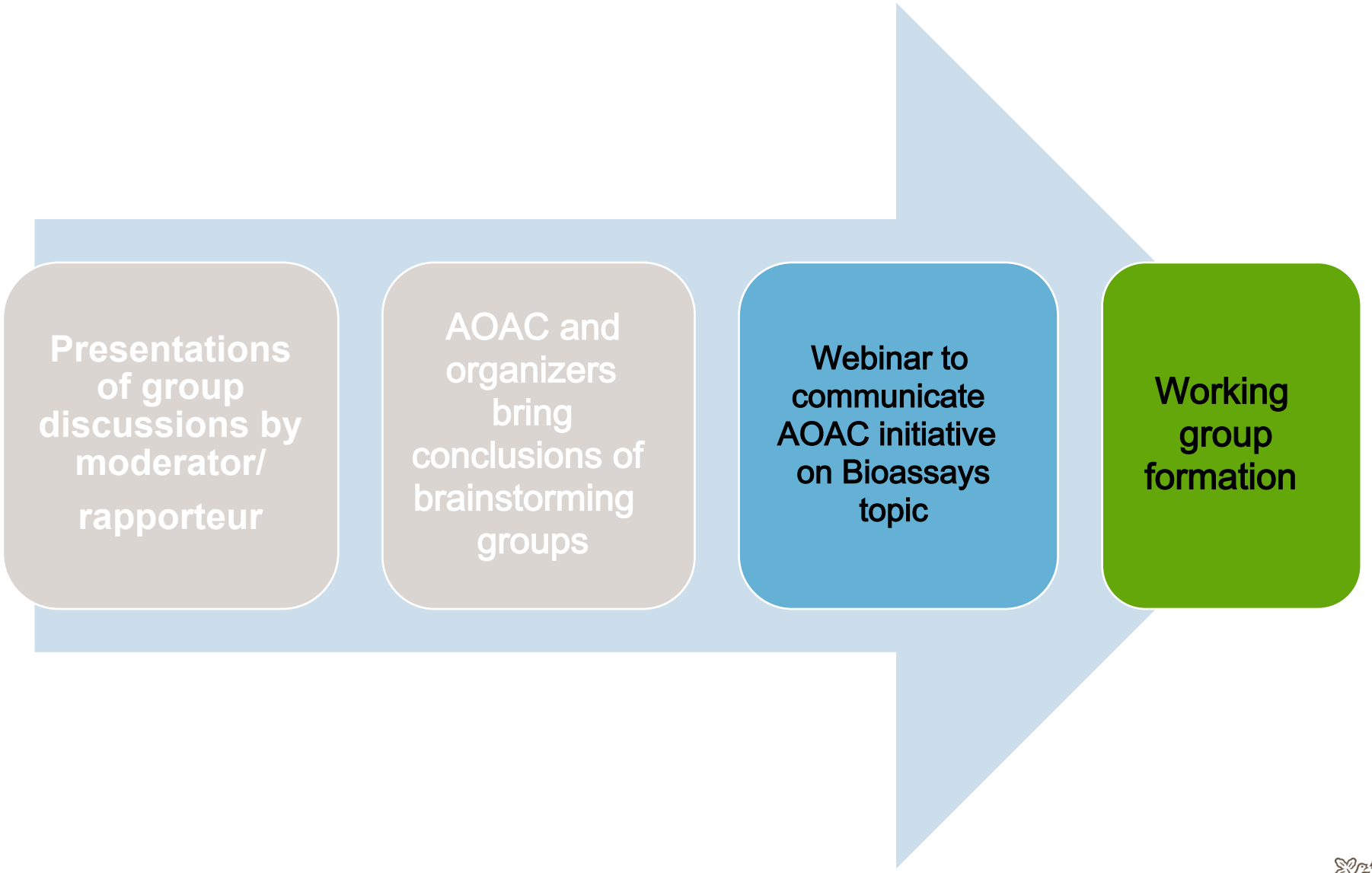
Rational of open discussion on “Best Practices for Bioassay Testing of Food and other Complex Mixtures”

- Transition of the use of *in vitro* methods as fundamental tools to drive the shift to the Next Generation Risk Assessment (NGRA) paradigm
- NGRA recommend to apply 3R’s principle of Reduction, Replacement and Refinement to face-out animal experimentation
- As a requirement, New Approach Methodologies (NAMs) (in vitro methods) need to be accepted by regulatory entities
- **Underlying issues in the *in vitro* field for food and other mixtures need further attention for best practices and therefore regulatory recognition (e.g; accuracy, reliability, reproducibility)**

Today's discussion

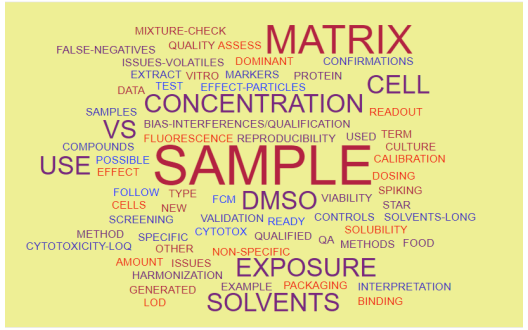


Research and Development

Outcome of workshop on bioassays and challenges for best practices of bioassays for mixtures applications

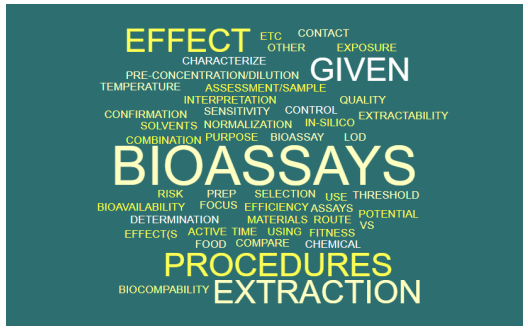
Guidance Document on Good In Vitro Method Practices (GIVIMP) use

Critical factors for accurate testing



Current extraction practices

Key topics for future WGs



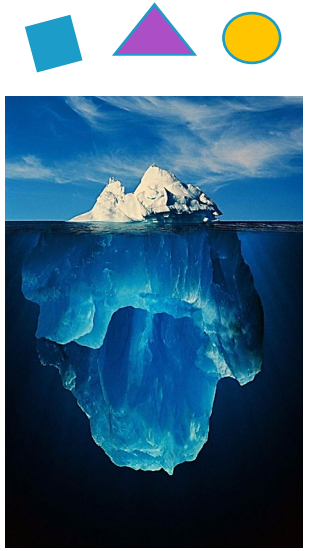
Research and Development

A framework for the assessment and harmonization of the validity of *in vitro* studies is needed

Assessment of Qualification Parameters

Important considerations for data quality when performing effect-based bioassays

- Distance between Negative and Positive Controls
- Complete Axis Shown on Graph
- Sample Activity within Quantifiable Range
- Reproducibility Over Time
- Reproducibility Between Labs
- Limit of Biological Detection/Quantitation
- Appropriate Controls in Each Experiment
- Proficiency with Known Substances
- Low Variance between Replicates
- Triplicate Sample Extraction and Testing

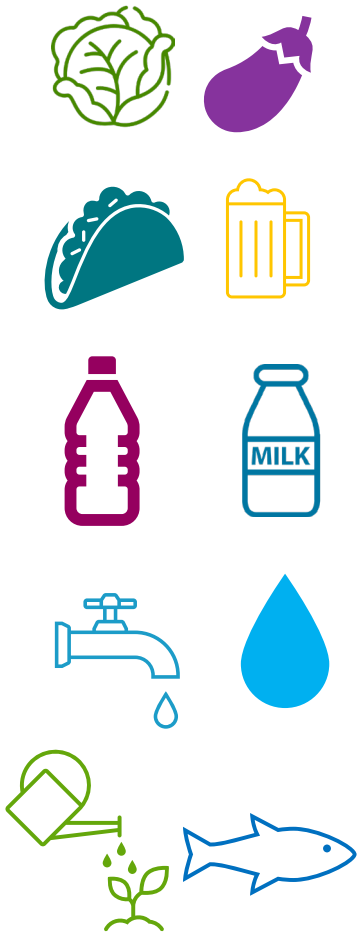


Fussell K et al; Limitations of currently available in vitro oestrogenicity bioassays for effect-based testing of whole foods as the basis for decision making. Food Additives and Contaminants. Part A (Volume 38, 2021 - Issue 11)

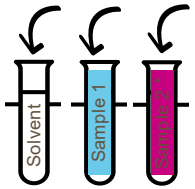
Research and Development

Critical limiting factors for a reliable approach to characterize complex mixtures

Complex mixtures (diversity) → Sample Preparation → Bioassays & chemical analysis → Concordance analysis



Extraction methods



spiking

Standards

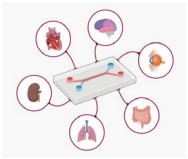
QUESTIONS:

- Are guidelines appropriate for complex mixtures?
- Suitable and validated methods for mixtures available? recovery test?

Biological tools examples

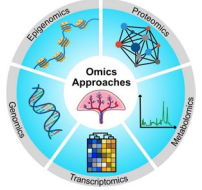
Multiwell -based

Organ-in-a-chip



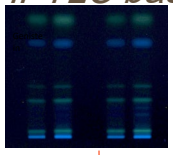
faCellitate.com

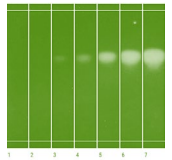
Omics technologies



*Jaremek 2021

HPTLC-based



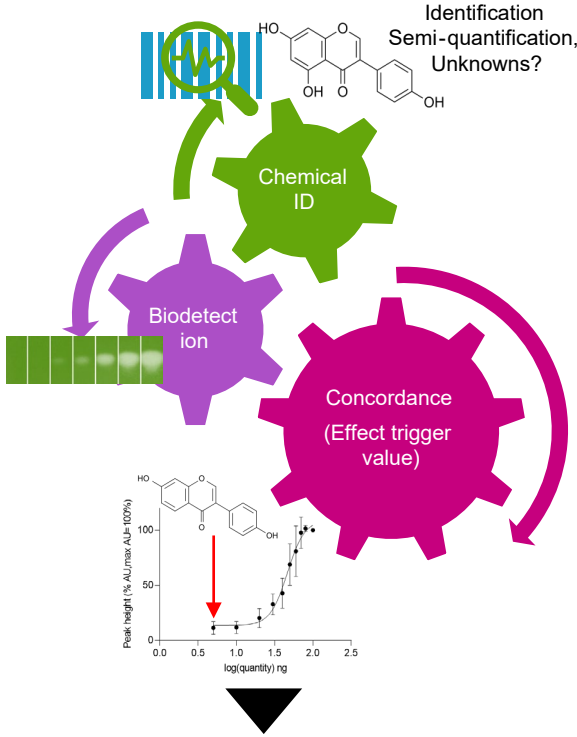


QUESTIONS:

Qualification criteria (data quality & internal validation) for screening including considerations on?

- *Systemic in vivo screening vs fit -for-purpose in vitro screening? biological endpoints?*
- *Strategy for exposure and data interpretation?*
- *e.g; matrix effect, effect -based trigger value*
- *Concordance analysis : identified compound(s) and biological activity?*
- *Insight on extrapolation of in vitro to in vivo ; concepts for pure compounds compatibles with mixtures?*

Toxicological assessment



Insights towards the acceptance of *in vitro* testing for mixtures testing is needed

Jaremek A, et al (2021) Front. Cell Dev. Biol. 9:674162. doi: 10.3389/fcell.2021.674162

Research and Development

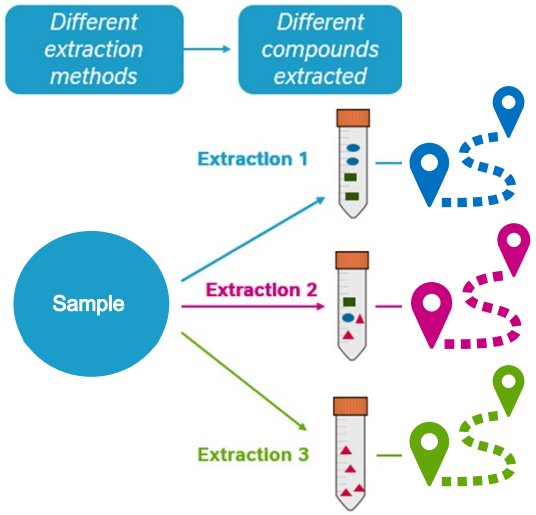
Broader harmonization needed at each stage to reduce bias/misinterpretation?

Complex mixtures (diversity) → Sample Preparation → Bioassays & chemical analysis → Concordance analysis

Diversity samples

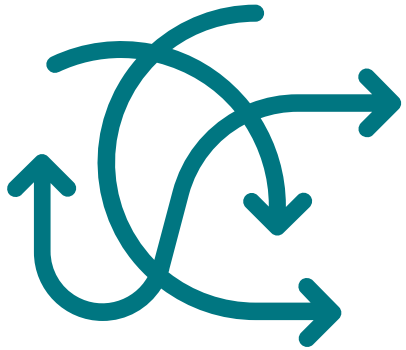
Variability factor?

Extraction methods



Variability factor?

Bioassays practice



- Qualified and validated methods?
- Exposure?
- Point of Departure assessment?

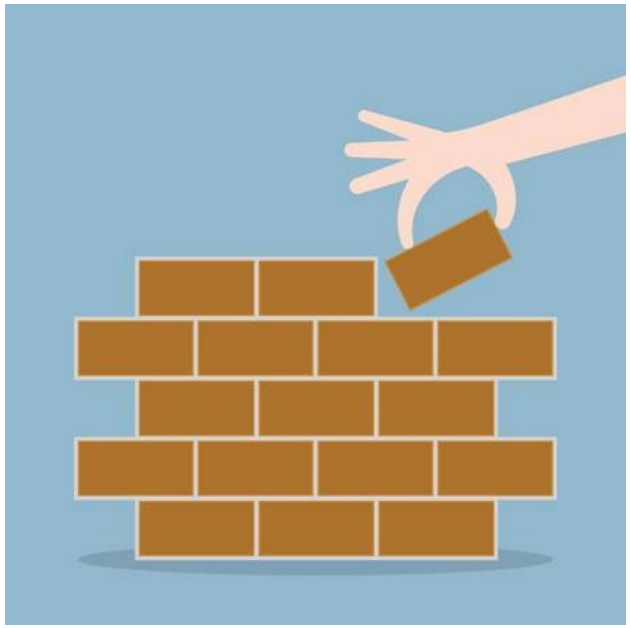
Variability/bias factor(s)?

Toxicological assessment

Jaremek A, et al (2021) Front. Cell Dev. Biol. 9:674162. doi: 10.3389/fcell.2021.674162

Research and Development

Fondations needed for transition towards acceptance requirements of best practices of bioassays testing for complex mixtures beyond QA?



1. **State-of-the-art** of bioassays testing tools and actual guidelines: which are the opportunities and limitations?

2. **Integration** of fundamental upstream boundaries including sample preparation and best laboratory practices for use of chemical and bioassays analysis

3. **Define qualification parameters requirements** of bioassays methods considering acceptance and achievable regulatory criteria for mixtures

4. Need of a **framework/guidelines** for the assessment and harmonization of the validity of bioassays methods to build robust ground for reliable data analyses and therefore **biological interpretation** . *(Effect-based trigger value concept (benchmark and highlight current issues with certain endpoints e.g; thyroid?)*

Research and Development

WORKING GROUP:
Best Practices for Bioassays testing of Food and other Complex Mixtures

Project objective : Consolidation of guidelines defining the requirements for qualification & validation of bioassays testing on food and other complex mixtures towards the harmonization and the acceptance of in vitro practices by regulatory authorities

TOPICS (to refine)

State-of-the-art on Best Practices Bioassays application for complex mixtures:

1. Sample preparation & characterization
2. Extraction methods & issues (matrix effect, interferences, recovery)
3. Integration Bioassays and Analytical methods: identification of qualifications criteria (method validity according to existing guidelines, suitability for matrix testing, bias effect)
4. Framework for qualifications requirements for use of bioassays usable across entities and regulatory sectors
5. Certification of bioassays application needed?
6. Introduction to biological data interpretation (Effect-based trigger value concept extrapolation to human situation)

Key deliverables / Achievements

- Working document for *in vitro* practice for mixtures through a scientific review publication including "recommendation guidelines"
- Definition of risk and opportunities on complex mixtures testing and propose consensus/limitations discussions with authorities
- Proposal for building robust ground for reliable *in vitro* data and therefore, biological interpretation
- Transfer of guidelines to CRO's and interested entities
- Identify key topics in the area for future working groups for refinements (e.g; in vitro to in vivo extrapolation, key biological endpoints (ED), AOP's for mixtures?)

Organization

- Working group participants
- Steering committee (4-6) members AOAC Europe & AOAC International
- Networking (projects and regulatory entities) for alignment on the topic e.g;

PANORAMIX PARC ecetoc ECHA EUROPEAN CHEMICALS AGENCY efsa EPA United States Environmental Protection Agency

- Monthly meetings for progress follow-up
- Alignment meeting(s) during AOAC INTERNATIONAL event(s)

Interested parties

Experts and stakeholders from packaging, environmental (water, soil, agro), food & bev., regulators & government, academia, contract research laboratories, raw material suppliers

Research and Development

Need of framework is confirmed by initiatives on this direction: PARC project with «INVITES-IN» (internal validity of *in vitro* studies)

Evidence-Based Toxicology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tebt20

Protocol for designing INVITES-IN, a tool for assessing the internal validity of *in vitro* studies

Camilla Svendsen, Paul Whaley, Gunn E. Vist, Trine Husøy, Anna Beronius, Emma Di Consiglio, Ingrid Druwe, Thomas Hartung, Vasiliki I. Hatzi, Sebastian Hoffmann, Carlijn R. Hooijmans, Kyriaki Machera, Joshua F. Robinson, Erwin Roggen, Andrew A. Rooney, Nicolas Roth, Eliana Spilioti, Anastasia Spyropoulou, Olga Tcheremenskaia, Emanuela Testai, Mathieu Vinken & Gro H. Mathisen

Framework for evidence-based use of NaMs in toxicological research and chemical risk assessment.

Required principles proposed list:

1. Result in identification of all relevant NaM-generated evidence relating to the research question addressed in a systematic review or risk assessment.

2. **Provide for the evaluation of the internal validity of NaM studies (propensity for systematic error due to how the study is designed and conducted).**

3. **Provide for the evaluation of the external validity of NaM studies (the degree to which results of a study can be translated/generalised to human adverse health effects).**

4. Contribute to objectivity, robustness, transparency and reproducibility in the hazard identification and characterisation process.

5. In its approach to normalising and structuring the description and analysis of NaMs, contribute to progress in the extent to which research data conform to FaiR (Findable, accessible, interoperable and Reusable) principles of open science

Svendsen, C., Whaley, P., Vist, G. E., Husøy, T., Beronius, A., Di Consiglio, E., Druwe, I., Hartung, T., Hatzi, V. I., Hoffmann, S., Hooijmans, C. R., Kass, G., Machera, K., Robinson, J. F., Roggen, E., Rooney, A. A., Roth, N., Spilioti, E., Spyropoulou, A., … Mathisen, G. H. (2023). ***Protocol for designing INVITES-IN, a tool for assessing the internal validity of in vitro studies (Version 2)*** *. Zenodo. https://doi.org/10.5281/zenodo.8315091*

Research and Development

Opportunities to align during 2024 AOAC INTERNATIONAL events

AOAC Annual Meeting & Exposition

138th Annual Meeting • August 23-28, 2024 • Baltimore, MD

- Report out to and engagement with the AOAC INTERNATIONAL annual meeting attendees
- Possibility of having a scientific session focused on bioassays

11th International Symposium on Recent Advances in Food Analysis

November 5-8, 2024; Prague, Czech Republic

- Report out during the AOAC INTERNATIONAL session at 2024 RAFA
- Possibility of having a face-to-face AOAC Europe Section meeting

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