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Inclusion of Adverse Outcome Pathways in the Safety Assessment of Nanomaterials

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OECD WPMN Meeting on Advanced Materials
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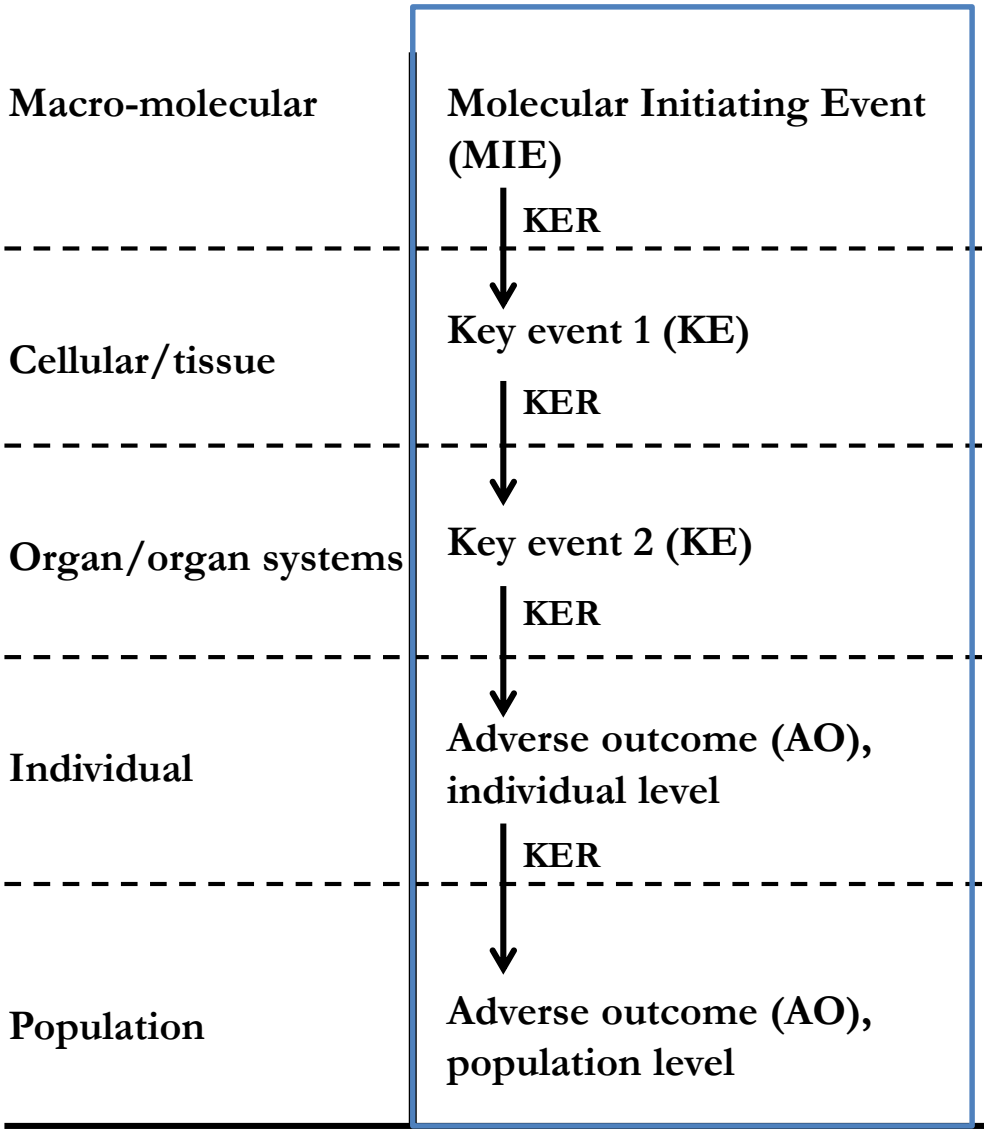
Adverse outcome pathway – general outline and relevant terminology



MIE defines the initial point of substance interaction at the molecular level in an organism, responsible for initiating the toxicity cascade.

KEs represent the change in the biological state that is both measurable and essential for the progression of a specified perturbation towards a specific adverse outcome.

Key Event Relationship (KER) defines the direct relationship shared between the two KEs (an upstream and a downstream KE), supported by the scientific evidence.

AO represents a KE of regulatory significance, used for establishing protection goal and is equivalent to an apical endpoint in an accepted regulatory guideline toxicity test.

From OECD AOP handbook - https://aopkb.oecd.org/howToContribute.html

The process of AOP development

- Top-down (MIE→forward), bottoms-up (backward←AO), middle way-out
- (←KEs→ either direction), case study approach, high-content/high-throughput data
- Putative, qualitative, semi-quantitative, quantitative
  - AOP synthesis
  - Proposal, collaboration, communication and sharing
  - Submission – AOP knowledgebase/AOPwiki
  - Review of AOPs – transparent process
    - Adherence to AOP principles and guidelines
    - Biology presented
  - Endorsement
    - Working party of National Coordinators for the Test Guidelines Programme and the Working Party for Hazard Assessment
    - Publication of the AOPs
- Validation, Quantitative support

Potential applications – regulatory context
Advanced materials, nanomaterials

- Hazard profiling
- Ranking toxicity, prioritisation for the assessment
- Chemical read-across, categorisation, grouping
- Weight of evidence- highly diverse sets of data
- Research data-gap filling, new data generation
- Safety assessment – keeping up with the technology development
  - Detailed mechanistic understanding
  - Reduce animal testing –
    - Selection of sensitive targeted tests/endpoints
    - Design *in vitro*/*in silico* tests
  - Predictive approaches
- Dose-response, biological points of departure
- Reduction of uncertainty
- Chemical mixture effects - AOP networks
- Ranking of advanced materials, material substitutes - safe substitution of hazardous materials or safe(r) by design approach

Why NanoAOPs?

- AOPs are chemical agnostics; however, toxicological mechanisms of chemicals have been the primary focus (~225 AOPs and thousands of KEs)
- Similarities exist between chemical and nanomaterial induced toxicity pathways and adverse outcomes
- Similarities in the KEs leading to adverse outcomes

But for nanomaterials

- MIEs are non-specific
- A change in the structural feature of a nanomaterial may trigger a different MIE and thus, a different AO
- Nanomaterial induced adverse outcomes involve immune responses; redundant and pleiotropic nature of the immune response mediators makes it difficult to establish KERs
- Specific consideration to physical, chemical and structural features is a must
- Networks of AOPs may be more suitable for incorporating property-specific deviations in the pathway

The challenges

- Not all AOs induced by nanomaterials are known
- Quality data supporting AOP development is lacking
- The process of AOP development is onerous
- Misconception that a full quantitative AOP is a must for decision making or there is lack of understanding of how individual units of AOPs can inform different layers of regulatory decision making process
- A systematic methodology to identify KEs from a sea of biological events reported in the literature and relating them to a potential AO is lacking

Current state-of-the-art - advancing the development of AOPs for application in nanotoxicology

OECD WPMN PROJECT

ADVANCING ADVERSE OUTCOME PATHWAY DEVELOPMENT FOR NANOMATERIAL RISK ASSESSMENT AND CATEGORIZATION (NANOAOP)

Vireo Advisors, LLC
Raising the Bar on Sustainability in Innovation

Health Canada Santé Canada

Environment and Climate Change Canada
Environnement et Changement climatique Canada

NanoCASE

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Confederation

Federal Department of Home Affairs FDHA
Federal Office of Public Health FOPH

National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

OECD

NATIONAL INSTITUTE FOR OCCUPATIONAL HEALTH

ALBERTA INNOVATES

UNIVERSITY OF BIRMINGHAM

National Institute for Occupational Safety and Health
NIOSH

SmartNanoTox
Smart Tools for Gauging Nano Hazards

OECD WPMN NanoAOP project

Goal: Case study to assess utility and advance the adoption of AOP frameworks relevant to nanomaterials for risk assessment

- **Sub-Objectives**
  - To establish a database of nano-relevant key events information data
  - To identify and develop nanomaterial relevant key events for potential AOP development

Focus on inflammation

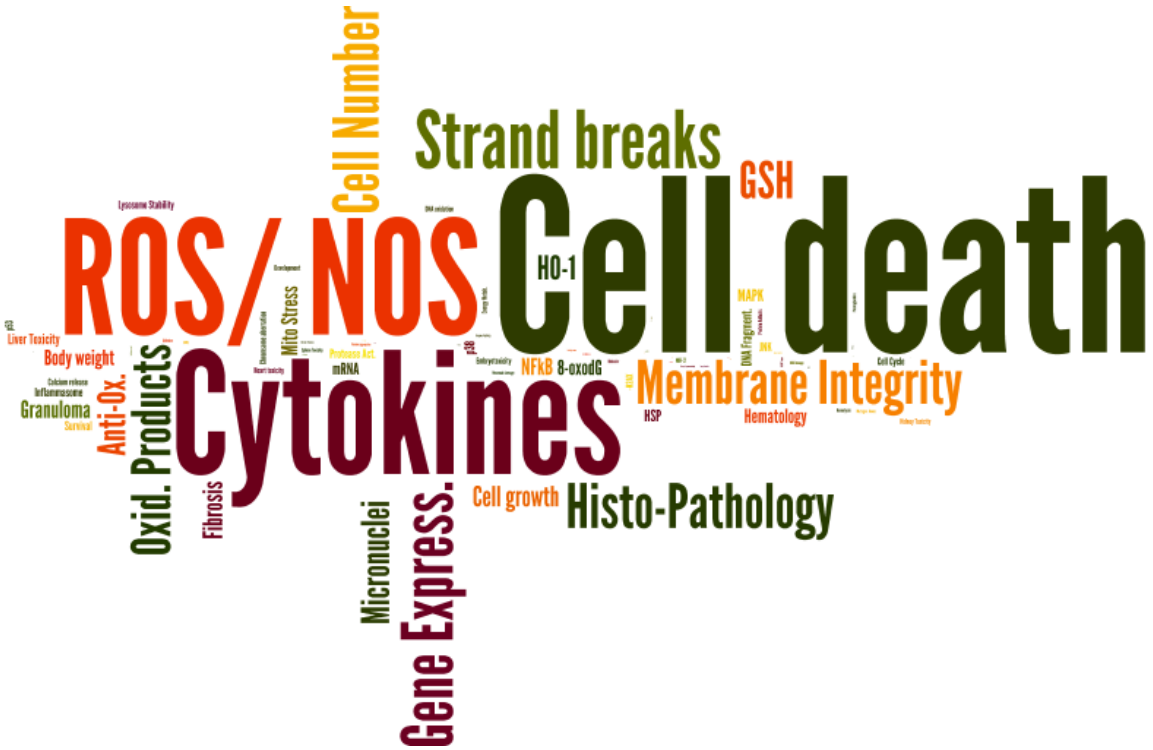
- Inflammation is one of the most consistently observed and reported response following exposure to nanomaterials

- Nanomaterial-induced inflammation is assessed in target and non-target tissues and in different species

- Inflammation is a precursor event for many diseases

OECD WPMN NanoAOP project – the approach and results

VCI database (co-financed by the SWISS Fed. Office of Public Health) ~30,000 papers - **Word clouding of endpoints with weighting**)



~11,000 quality assessed publications from the period of 2000-2013

~200 manuscripts
Reporting on inflammation

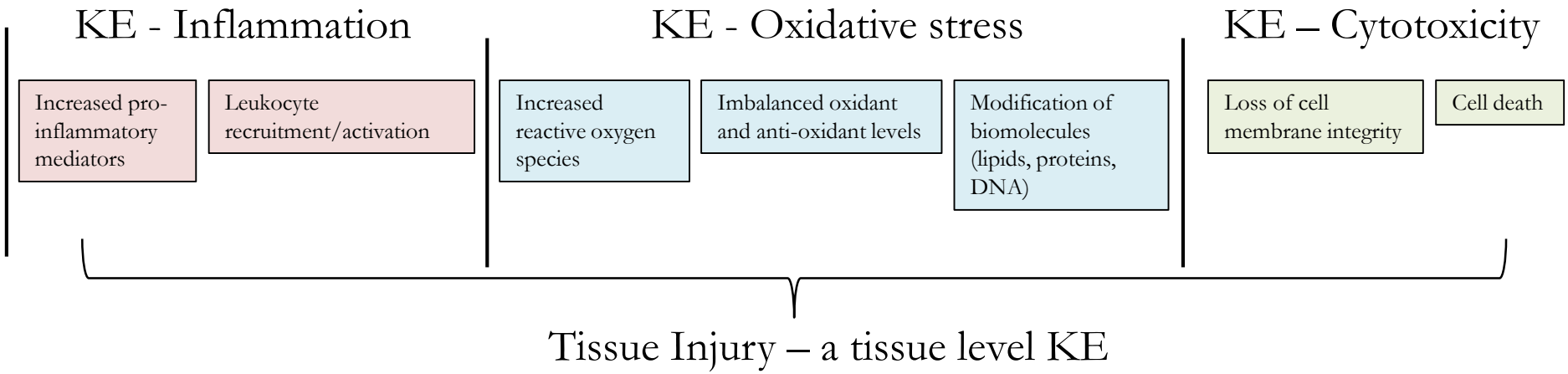
447 datasets

- ~ 60 endpoints
- 45 different materials
- Mice, Rats
- ~ 20 different cell types

Harald F. Krug hfk@nanocase.ch,
unpublished

OECD WPMN NanoAOP project – the approach and results

- Established a methodology for quality assessment of nanotoxicology literature (OECD WPMN expert workshop report – Sept 2019)
- Established a systematic process for identifying nanomaterial-induced KEs from the existing literature (Halappanavar et al. 2019; OECD WPMN expert workshop report – Sept 2019)



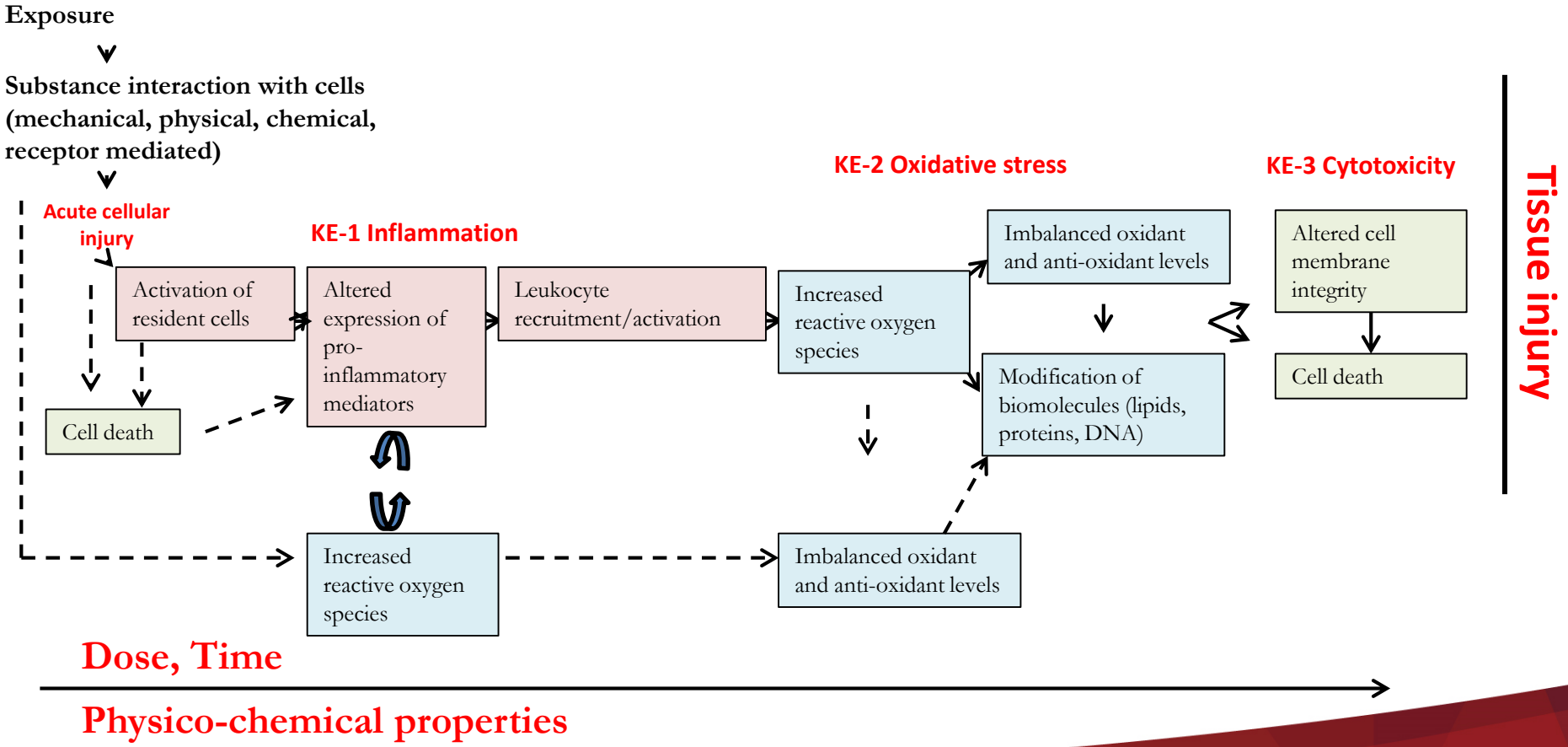
- Nanotoxicity is the interplay between these three key events -inflammation, oxidative stress and cytotoxicity

- 'Tissue injury' is the downstream KE or outcome of this interplay

Halappanavar et al., 2019

OECD WPMN NanoAOP project – the approach and results

- Inflammation, oxidative stress and cytotoxicity may represent different events in a specific AOP but when persist together, they are causal and represent tissue injury
- Tissue injury precedes tissue dysfunction
- Tissue injury is a point-of-no return KE in the path leading to several AOs of relevance to nano



Unclassified ENV/JM/MONO(2016)63

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development 30-Jan-2017

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

2017_OECD_ENV-JM-MONO63.pdf

ENV/JM/MONO(2016)63
Unclassified

59% of studies report on oxidative stress, immunology and cytotoxicity, with inference to cell/tissue injury

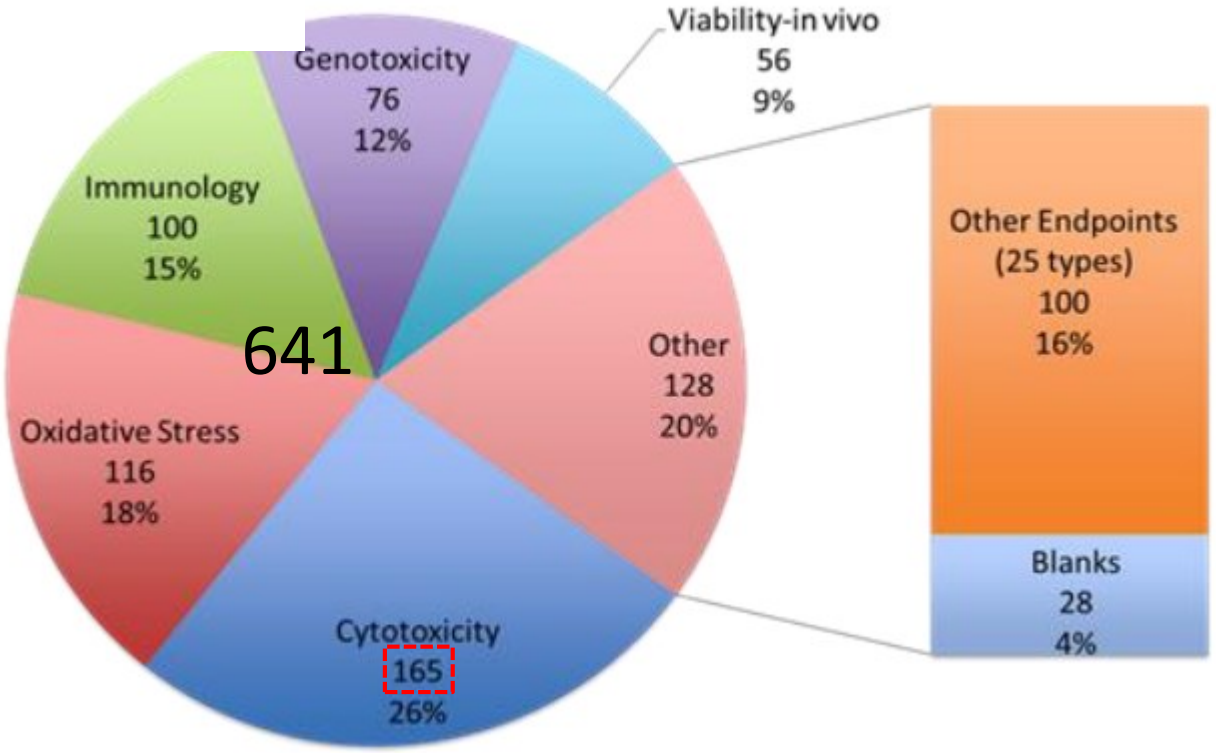
ALTERNATIVE TESTING STRATEGIES IN RISK ASSESSMENT OF MANUFACTURED NANOMATERIALS: CURRENT STATE OF KNOWLEDGE AND RESEARCH NEEDS TO ADVANCE THEIR USE

Series on the Safety of Manufactured Nanomaterials
No. 80

641 datasets evaluated for biological endpoints induced by ENM:
Figure taken from an OECD Monograph (Reference see above)

Krug HF, unpublished data

Figure 1. Distribution of endpoints analysed



Nano-relevant AOPs currently under development and associated research activities

- Inhalation route is the focus
- AOPs relevant to other routes of exposure are under consideration

API XML

https://aopwiki.org/aops/173

Aop: 173

AOP Title

OECD EAGMST internal review completed

OECD external review underway

Substance interaction with the lung resident cell membrane components leading to lung fibrosis

Short name:

- **Lead: Sabina Halappanavar, Environmental Health Science and Research Bureau, Health Canada, Ottawa, Canada**
- **Monita Sharma; Amy J. Clippinger** (PETA International Science Consortium Ltd. / International Council on Animal Protection in OECD Programmes, USA)
- **Hakan Wallin; Ulla Vogel** (National Research Centre for the Working Environment, Copenhagen, Denmark)
- **Kristie Sullivan** (Physicians committee for Responsible Medicine, USA)

Snapshots | All AOPs | View history | Discussion

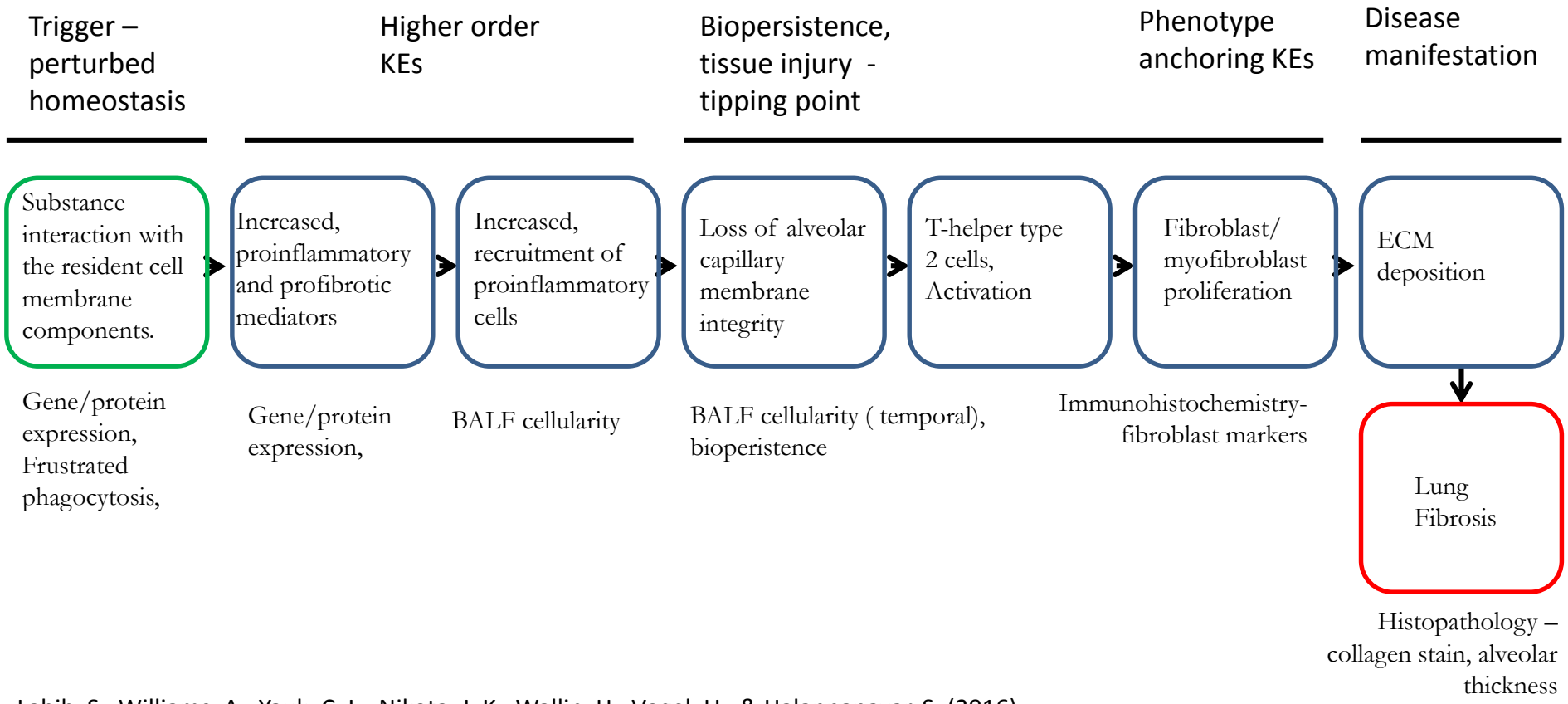
1. AOP Title
2. Graphical Representation
3. Abstract
4. Background
5. Summary of the AOP
   1. Molecular Initiating Event
   2. Key Events
   3. Adverse Outcome
   4. Relationships Between Two Key Events
   5. Network View

Labib et al., 2016; Nikota et al., 2017; Sharma et al., 2016

A putative/qualitative AOP for lung fibrosis

AOP 173: Substance interaction with the resident cell membrane components leading to lung fibrosis - under OECD external review

Sabina Halappanavar, Monita Sharma, Hakan Wallin, Ulla Vogel, Kristie Sullivan, Amy J. Clippinger
Halappanavar et al., manuscript in preparation



Labib, S., Williams, A., Yauk, C. L., Nikota, J. K., Wallin, H., Vogel, U., & Halappanavar, S. (2016). *Particle and Fibre Toxicology*, *13*, 15.

Nikota, J., Banville, A., Goodwin, L. R., Wu, D., Williams, A., Yauk, C. L., … Halappanavar, S. (2017).

SmartNanoTox
Smart Tools for Gauging Nano Hazards

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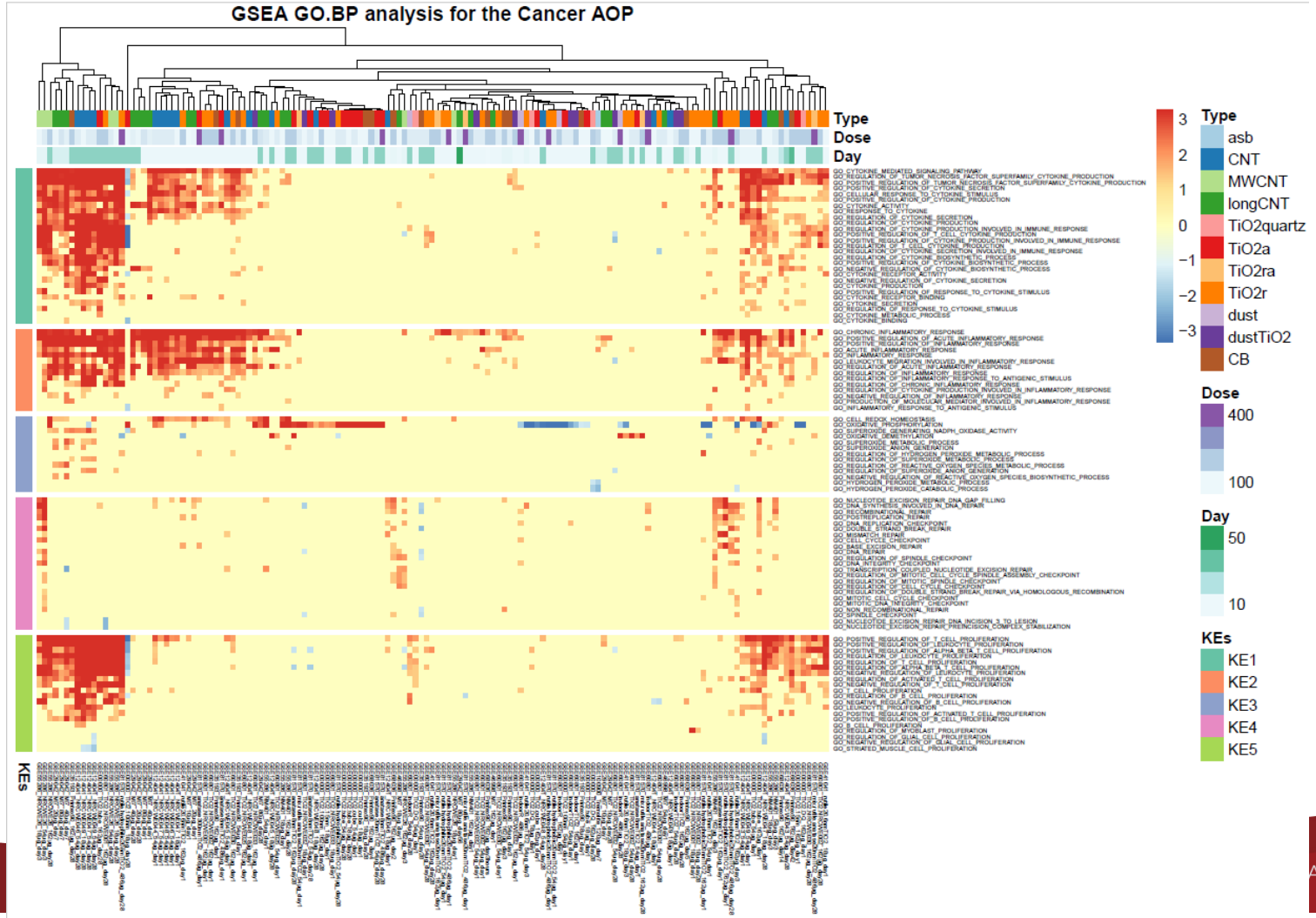
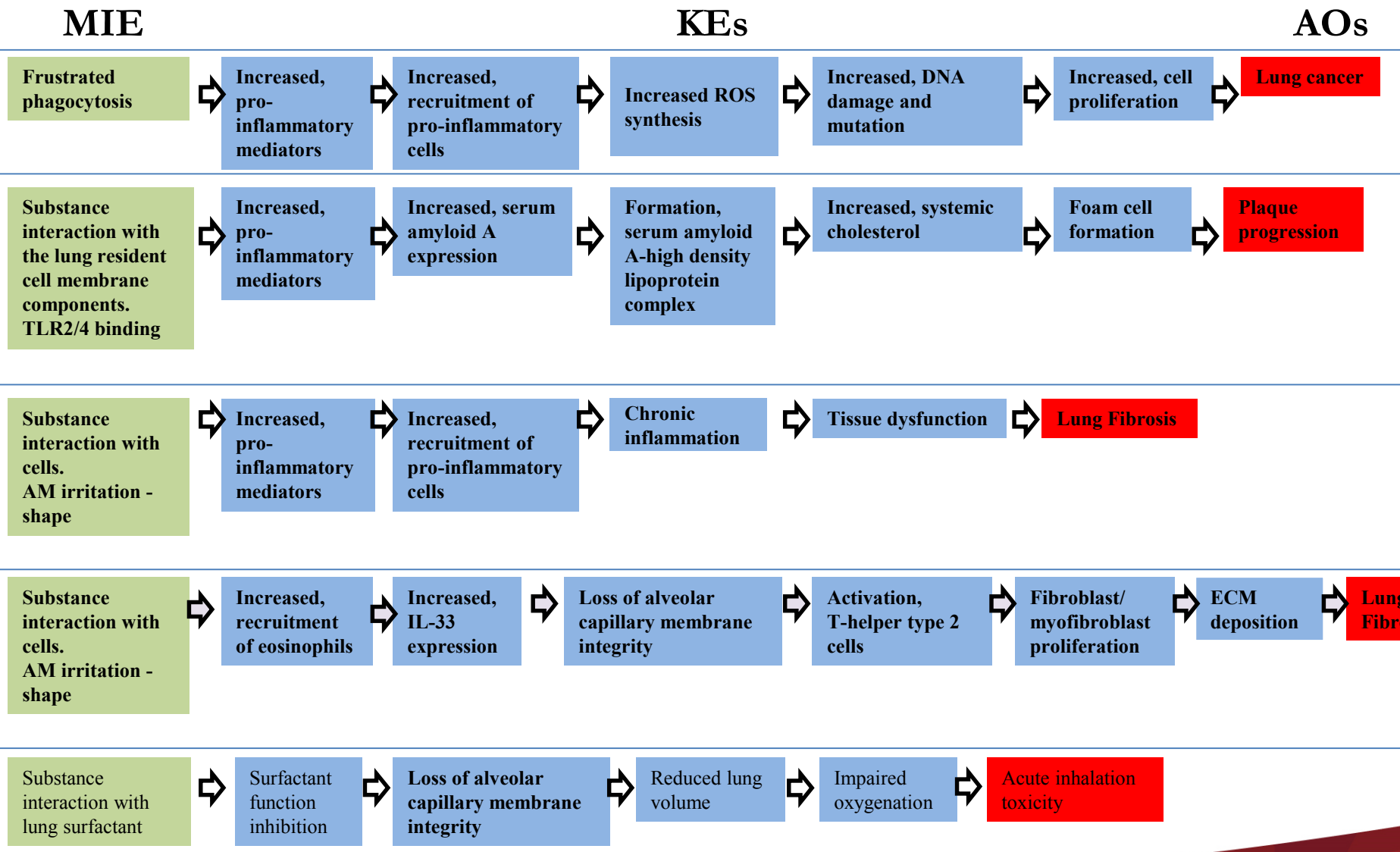
Data mining approach to identify KEs and targeted markers for KE measurement
Very valuable for application to advanced or next generation materials



Nano-relevant AOPs that are currently under development by SmartNanoTox

Laurent Gate; Jorid Birkelund Sørli (JBS); Tobias Stöger; Wolff Henrik; Carole Seidel; Ulla Vogel



Halappanavar and Vogel et al., in preparation

PATROLS
Advanced Tools for NanoSafety Testing

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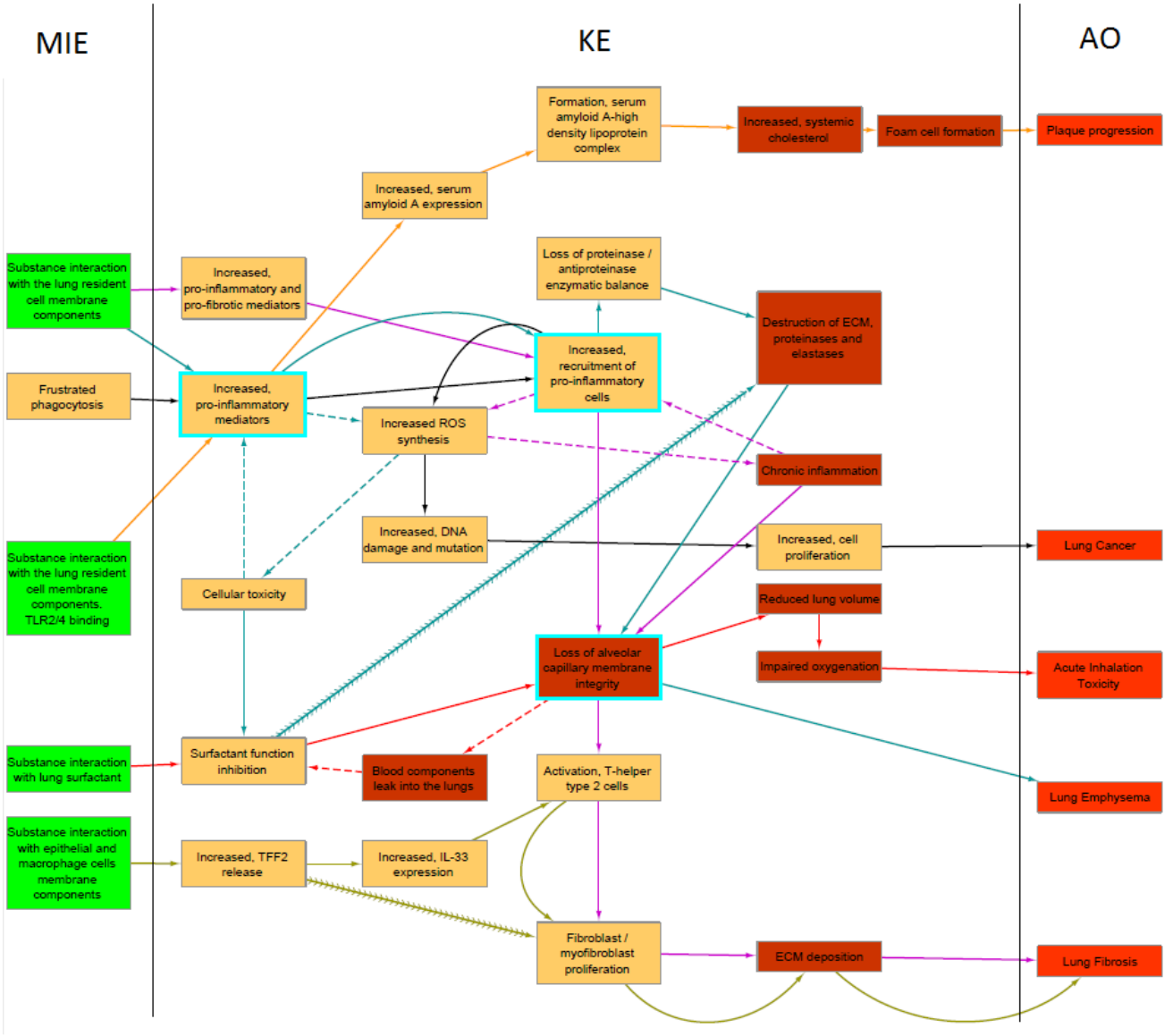
- Design and develop targeted *In vitro* and *in silico* tools to assess in vivo toxicity
- Networks of AOPs – overlapping KEs
- Defined approaches



Halappanavar and Vogel et al., in preparation

PATROLS
Advanced Tools for NanoSafety Testing

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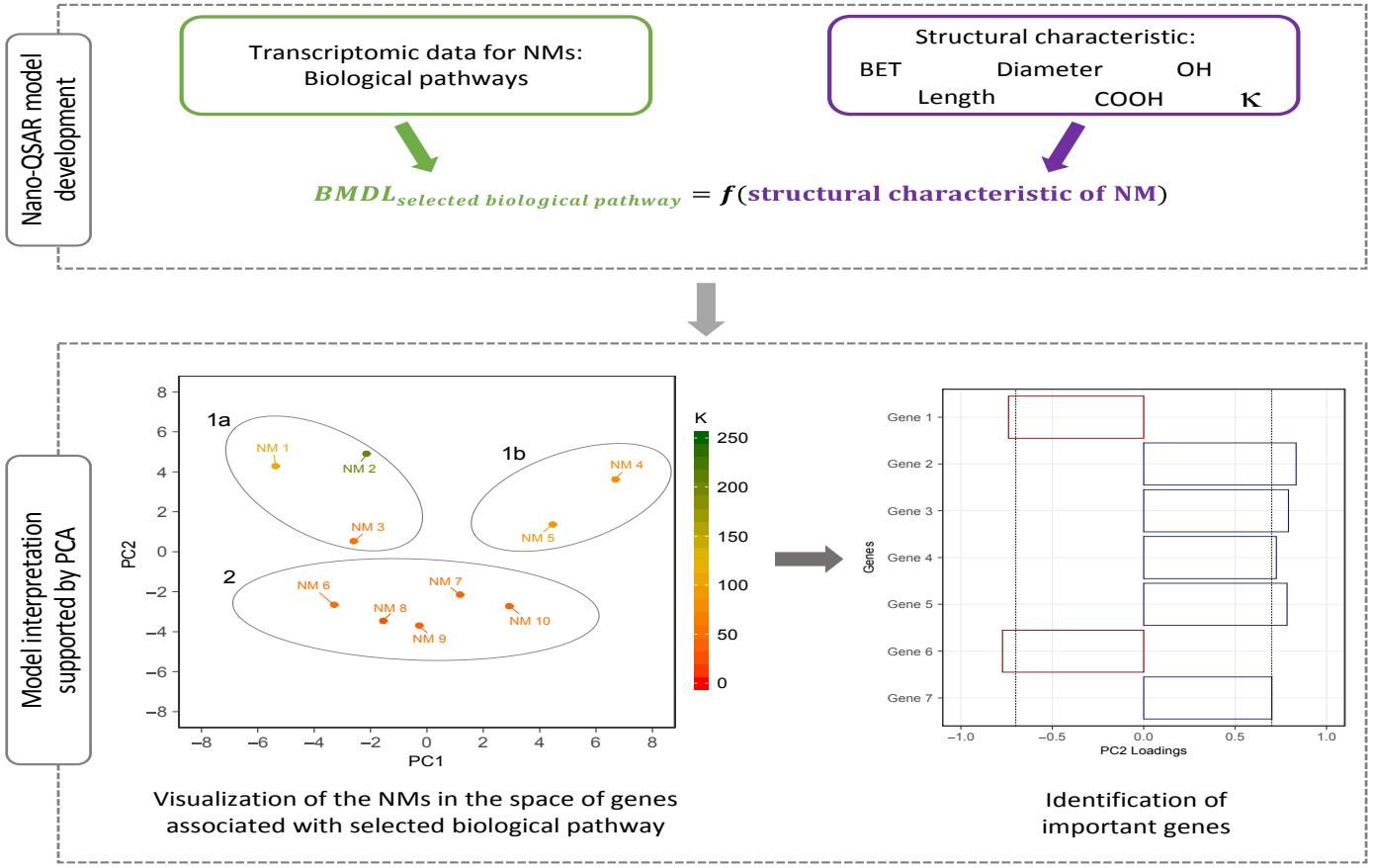
- High-content omics data and AOP knowledge to build QSAR models
- Structural features of nanomaterials responsible for triggering the MIE in AOP173



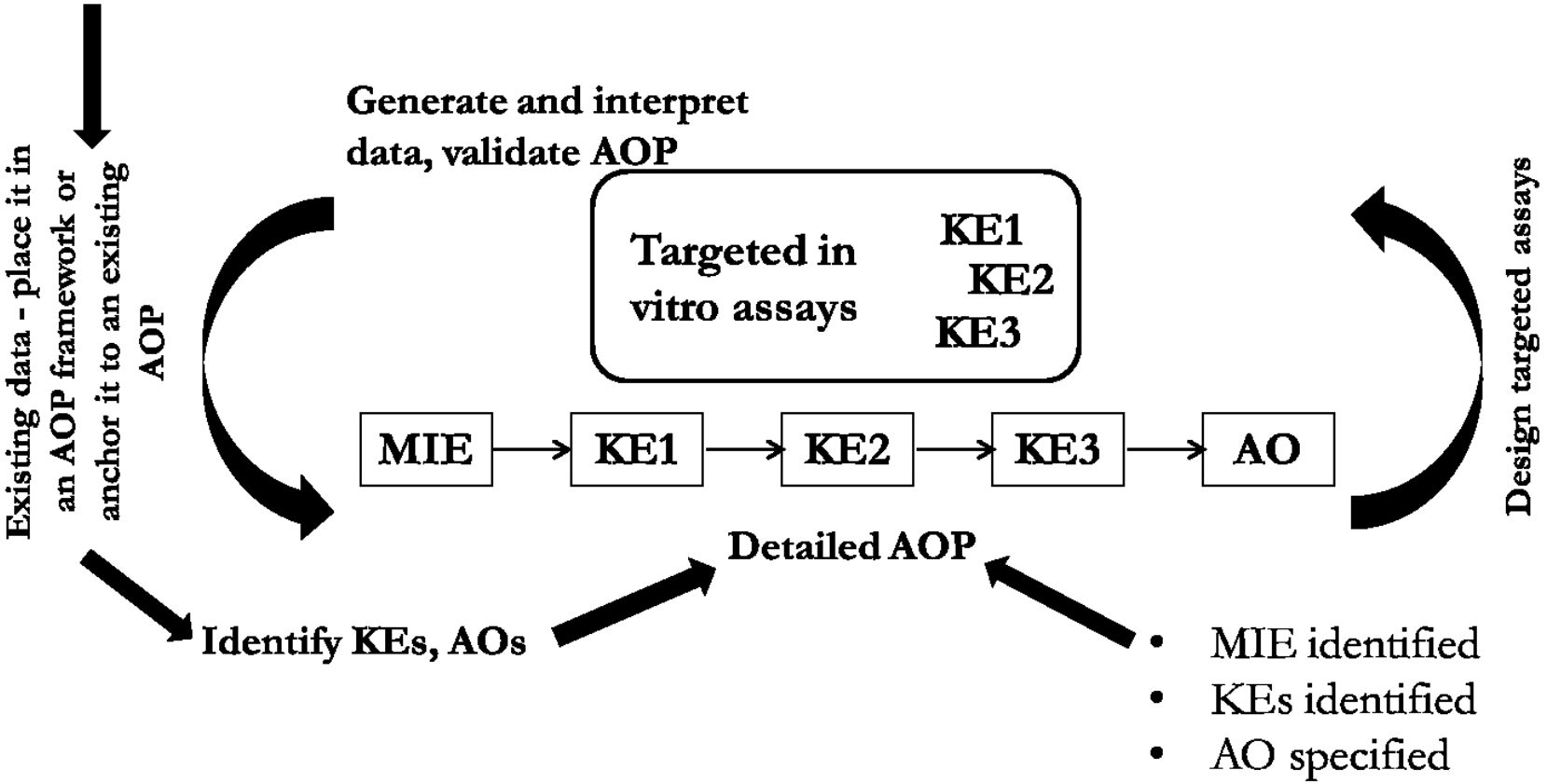
Thomas Putzyn and Halappanavar et al., submitted

Conclusions: plenty of opportunities for AOPs in nanotoxicology

Detailed mechanistic understanding

AOP-informed design *in vitro*/*in silico* tests and predictive approaches - which can then be applicable to diverse set of simple and advanced materials

- MIE non-specific
- KEs not identified
- AO not specified
- Plenty of data available



Conclusions: plenty of opportunities for AOPs in nanotoxicology

Moving forward ….the focus should be on

- **OECD WPMN NanoAOP database enrichment and suitability to support AOP development and to support quantitative analysis**
- **Quantitative AOPs**
- **Test guidelines**
- **Establish criteria for the validation of in vitro assays (non-traditional thinking is required)**
- **Guidance on how to use data derived from the alternative toxicity testing methods in risk assessment**

Acknowledgements

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Genomics, Nanotoxicology and Alternative Methods Laboratory

Andrew Williams
Andrey Boyadziev

OECD WPMN PROJECT
ADVANCING ADVERSE OUTCOME PATHWAY DEVELOPMENT FOR NANOMATERIAL RISK ASSESSMENT AND CATEGORIZATION (NANOAOP)

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Environnement et Changement climatique Canada
National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport
OECD
Swiss Confederation
Federal Department of Home Affairs FDHA
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Vadim Zernovkov

Tomasz Puzyn
Karolina Jagiełło