Adverse outcome pathways: a conceptual framework to support the evaluation and extrapolation of toxicological hazards of nanomaterials.

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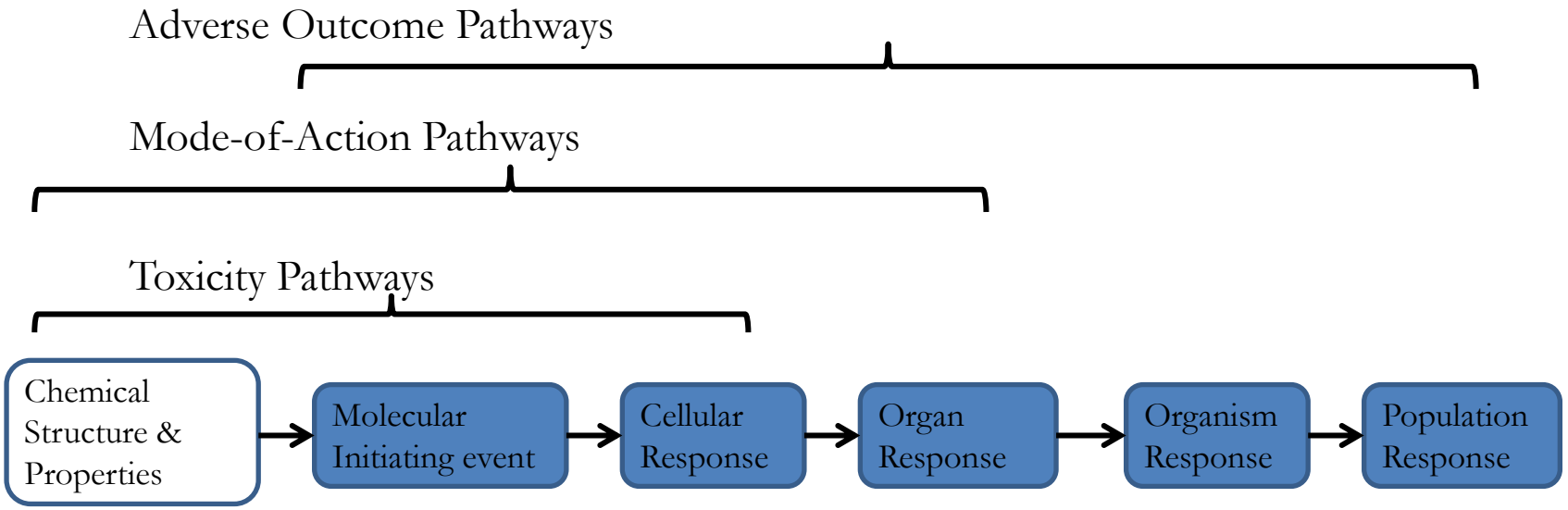
Toxicity Pathways, Mode-of-Action and Adverse Outcome Pathways

Knowledge driven approaches to hazard identification and risk assessment

Describe mechanistic basis of biological systems and ways in which toxic substances interfere with them leading to adverse outcome

Describe causal chain of biological and biochemical events across various levels of biological organization starting from an initiating event resulting in an adverse outcome



Adverse outcome pathways (AOPs)

- 'Conceptual constructs that portray existing knowledge concerning the linkages between a direct molecular initiating event and an adverse outcome at a biological level of organization relevant to risk assessment' (Ankley et al 2010).

-Systematic organization of existing knowledge concerning a toxicity pathway/MOA (simplified representation)

-Identification of measurable or observable biological/chemical changes that are essential for toxicity

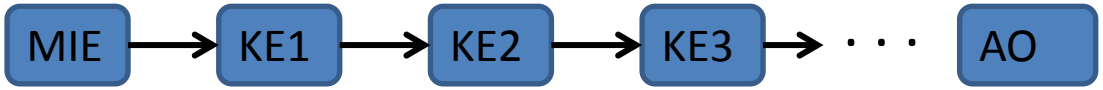
-Establish quantitative linkages between the essential biological events leading to an adverse outcome

-Support regulatory decision making – identification of key biological events or an adverse outcome of regulatory significance

Adverse outcome pathways (AOPs)

The main components of an AOP are

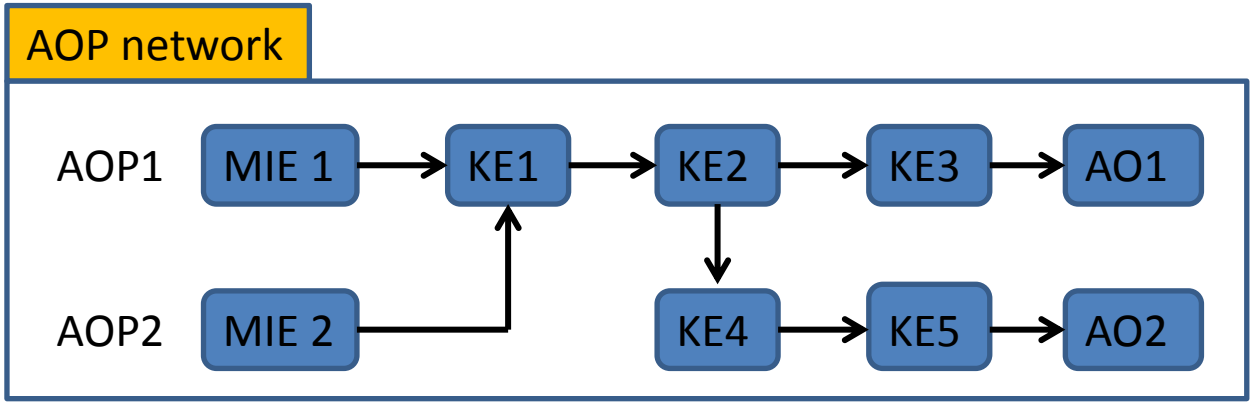
- Molecular Initiating Event (MIE) – a critical key event required to trigger the biological cascade leading to an adverse outcome – primary contact with a biomolecule
- Adverse Outcome (AO) – a toxic effect, phenotype – level of biological organization of concern and control – organ or higher level – a measurable apical endpoint
- Key Event (KE) – important and measurable intermediary biological events that connect the initial molecular event to the final adverse outcome. This is a necessary event but not sufficient on its own for an AO to occur
- Key Event Relationships (KER) – → supported by biological plausibility and empirical evidence



Villeneuve et al., TOXICOLOGICAL SCIENCES, 142(2), 2014, 312–320

Core principles of AOPs

- AOPs are not chemical specific
- AOPs are modular, components of AOPs (KEs and KERs) can be reused
- AOP is developed as an individual pathway composed of a single linear sequence of KEs and KERs
- One can develop multiple AOPs that share common KEs and KERs – networks of AOPs. These will be used to represent the complexity of the toxicity – have better predictive value
- AOPs are living documents



Phases of AOP development

- Putative AOP development

Hypothesized set of KEs and KERs supported by biological plausibility and/or statistical inference. Partial AOPs, incomplete linkages between MIE and AO.

- Qualitative AOP development

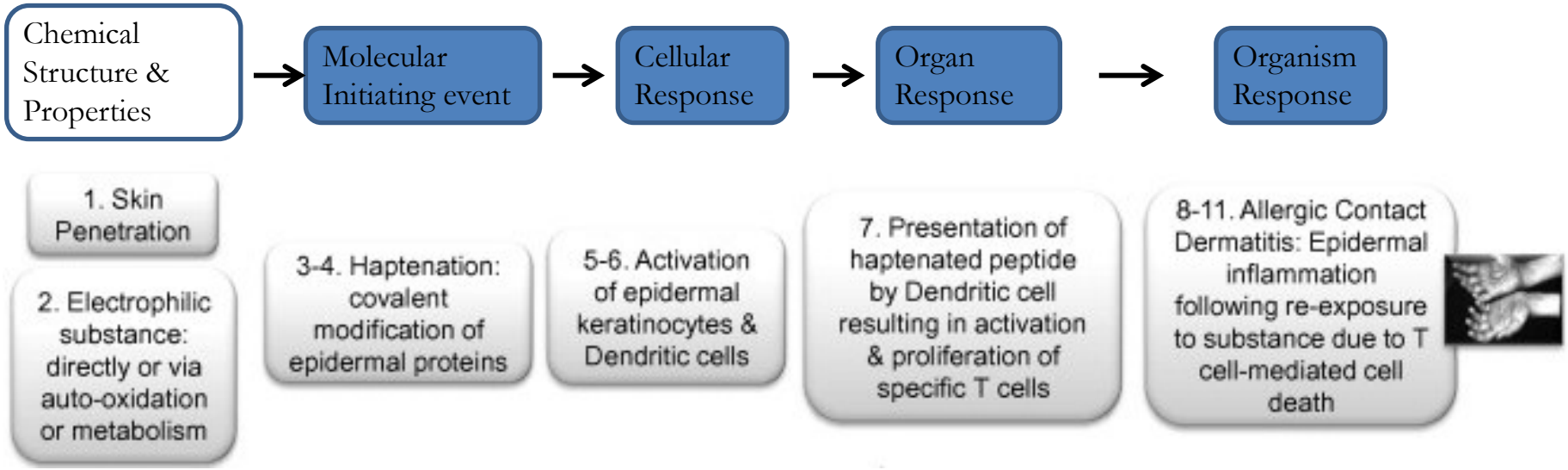
Well characterized KEs with information on how to measure a KE, qualitative evaluation of weight of evidence supporting the AOP. Information is consistent with OECD guidelines.

- Quantitative AOP development

Well defined KEs and KERs, accurate and precise ways of measuring KEs and KERs. Supported by quantitative understanding of what magnitude or duration of change in an upstream KE is required to induce change in a downstream KE.

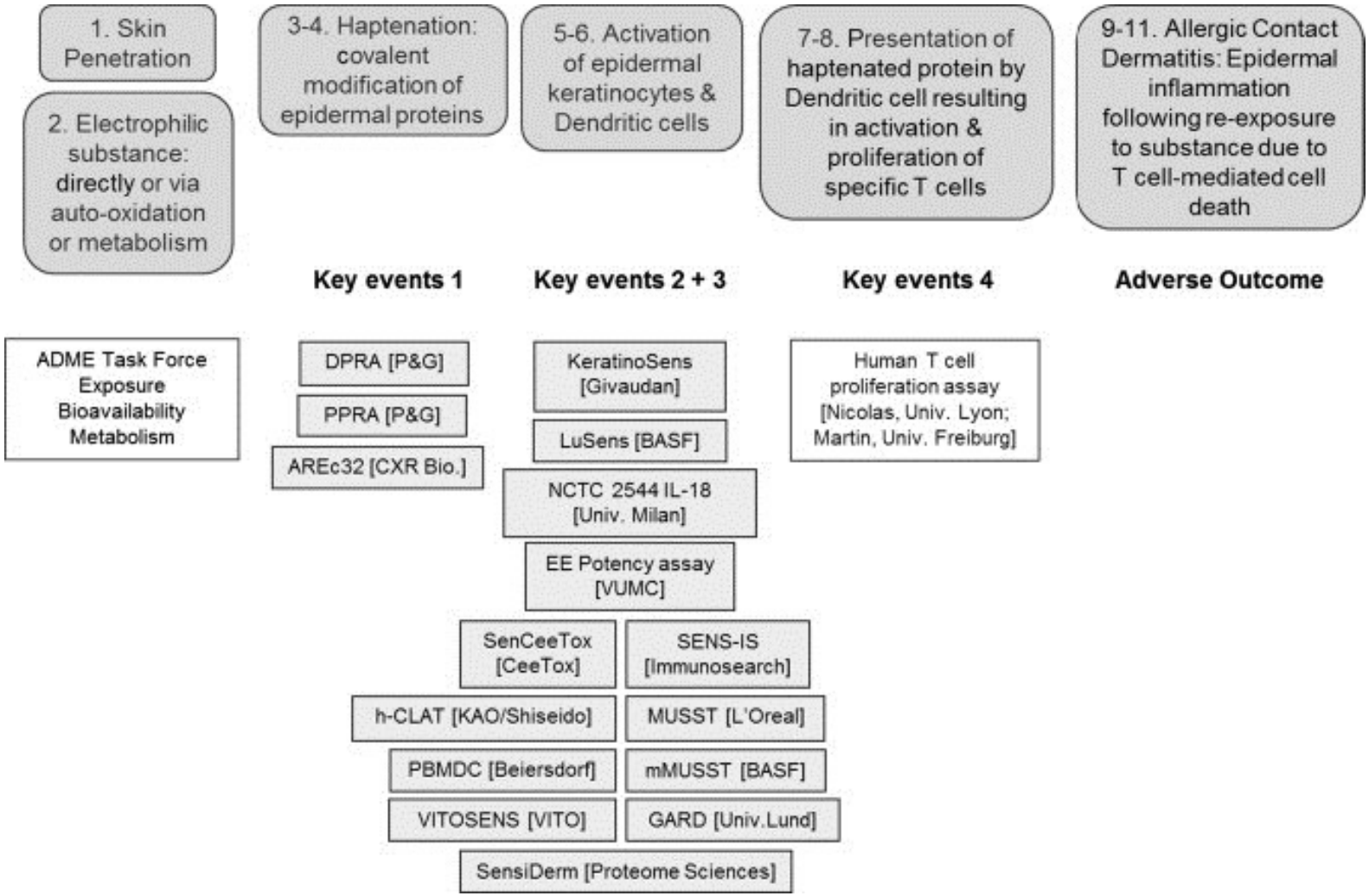
Villeneuve et al., TOXICOLOGICAL SCIENCES, 142(2), 2014, 312–320

Skin sensitization adverse outcome pathway – an example



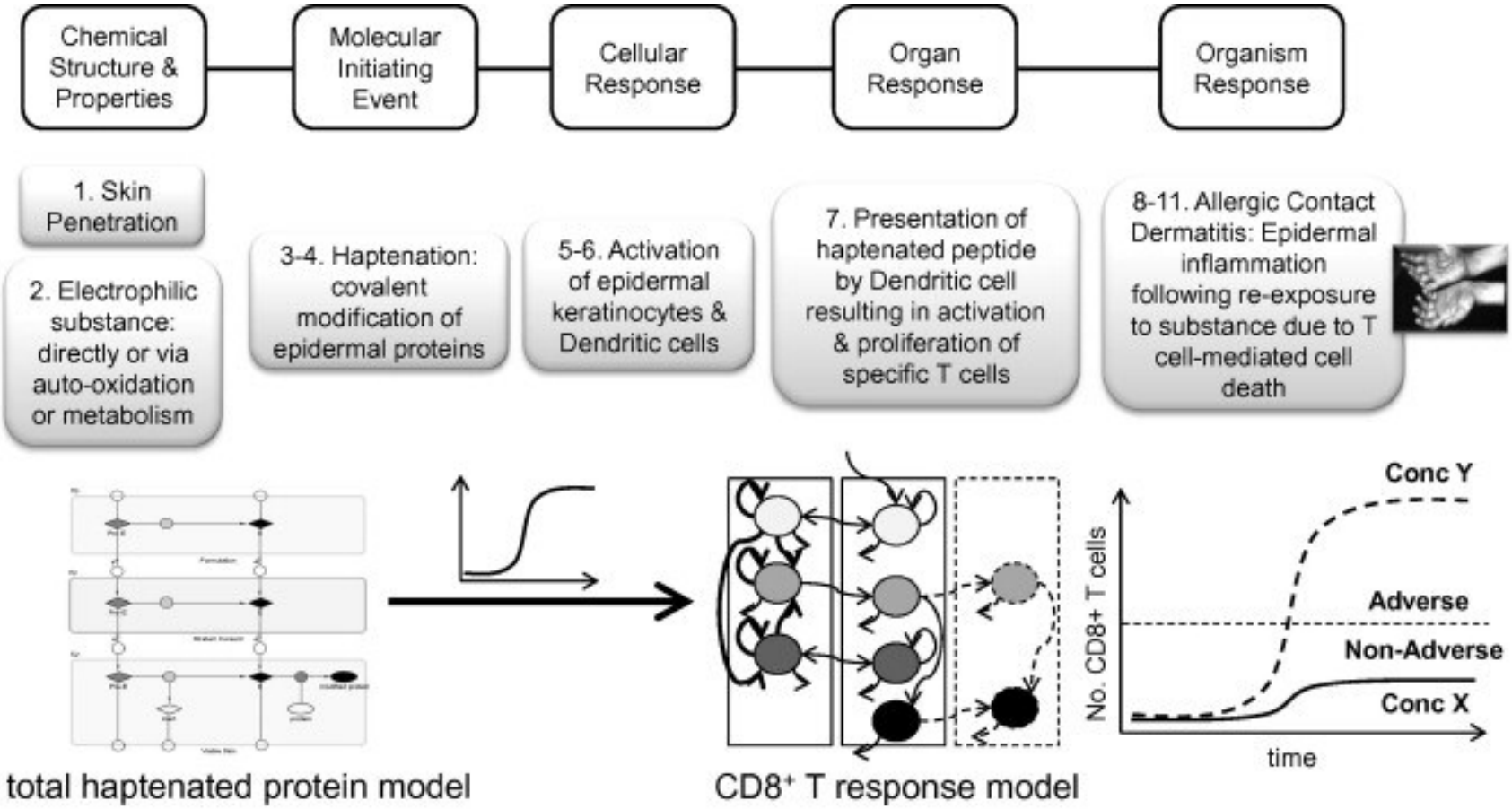
Maxwell et al, Toxicology in Vitro, Volume 28, Issue 1, 2014, 8 - 12

Non-animal test methods and their alignment to the skin sensitization AOP. Test methods analyzed during phase I of the Cosmetics Europe method evaluation study (grey boxes). Methods presented in white boxes represent Cosmetics Europe-funded studi...



Reisinger et al., Toxicology in Vitro, Volume 29, Issue 1, 2015, 259 - 270

Non-animal risk assessment approach for skin sensitization. Diagrammatic representation of non-animal risk assessment approach for skin sensitization demonstrating alignment with OECD AOP for skin sensitization



Maxwell et al, Toxicology in Vitro, Volume 28, Issue 1, 2014, 8 - 12

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Wiki authors: We patched the widget code again this weekend to fix all remaining known bugs. We will consider expanding the length of the short title for AOPs for the next release. If you notice any additional problems, please report them here: Bug_Reports.

Wiki users: To avoid spammers, we now require email confirmation before any user is allowed to edit. We apologize for any inconvenience this may cause. Instructions for confirming your email if you already have an account are given here: Confirm Email If you have any troubles confirming your email, please email us at aopwiki@googlegroups.com.

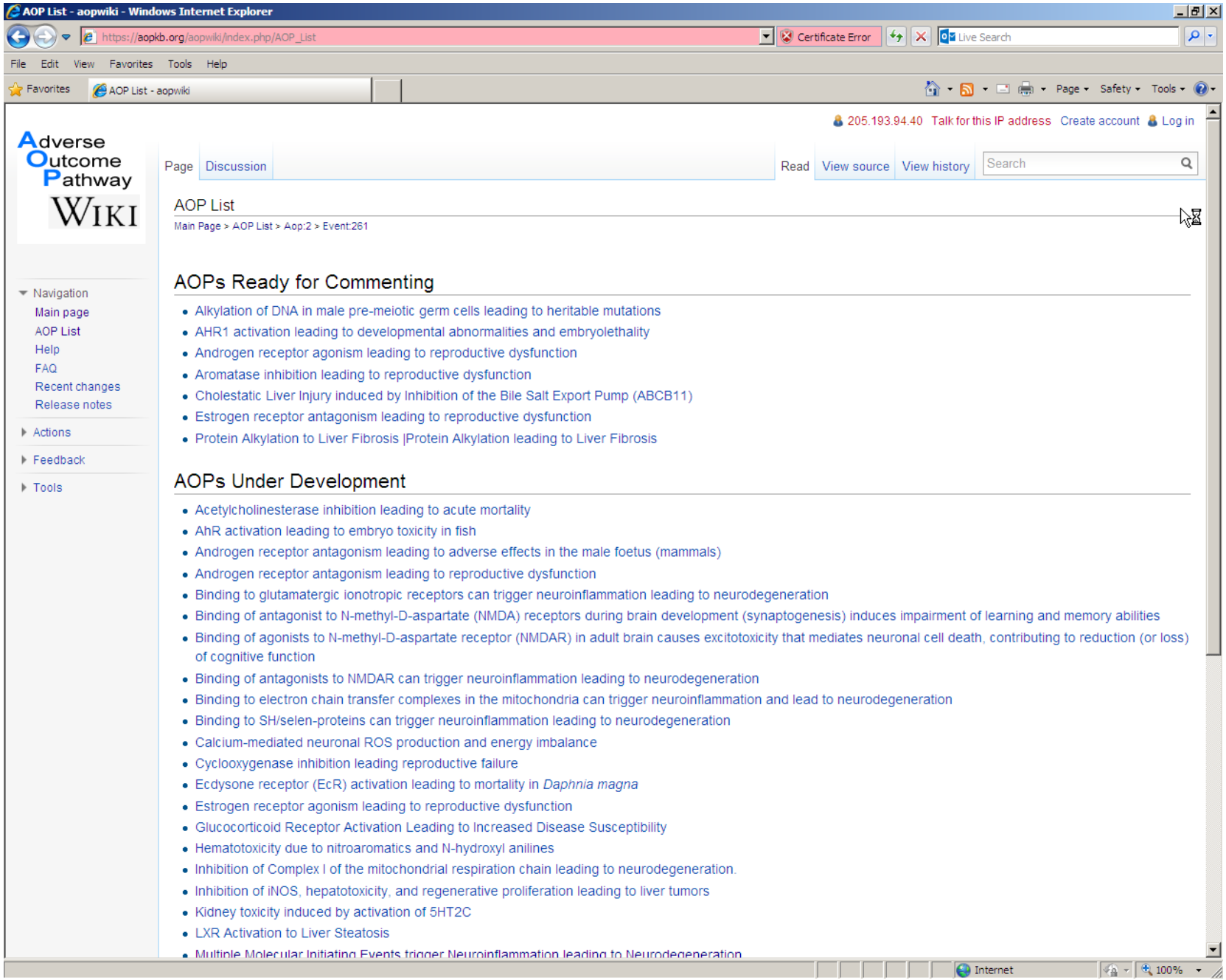
The Help documentation will be updated soon to provide more information about how to comment on existing AOPs. We ask that all users abide by the AOP Wiki Editing & Comments Policy. To author or edit AOPs still requires membership in an OECD AOP development project.

AOP-Wiki authors: We will be providing some additional help related to the new widget arrangement soon. Please see here for more information: Author Info.

Welcome to the Collaborative Adverse Outcome Pathway Wiki (AOP-Wiki)

This wiki represents a joint effort between the European Commission – DG Joint Research Centre (JRC) and U.S. Environmental Protection Agency (EPA). This serves as one component of a larger OECD-sponsored AOP Knowledgebase effort and represents the central repository for all AOPs developed as part of the OECD AOP Development Effort by the Extended Advisory Group on Molecular Screening and Toxicogenomics. The other major components of this knowledgebase are

Internet 100%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AOPs Ready for Commenting
Alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations
AHR1 activation leading to developmental abnormalities and embryolethality
Androgen receptor agonism leading to reproductive dysfunction
Aromatase inhibition leading to reproductive dysfunction
Cholestatic Liver Injury induced by Inhibition of the Bile Salt Export Pump (ABCB11)
Estrogen receptor antagonism leading to reproductive dysfunction
Protein Alkylation to Liver Fibrosis |Protein Alkylation leading to Liver Fibrosis
AOPs Under Development
Acetylcholinesterase inhibition leading to acute mortality
AhR activation leading to embryo toxicity in fish
Androgen receptor antagonism leading to adverse effects in the male foetus (mammals)
Androgen receptor antagonism leading to reproductive dysfunction
Binding to glutamatergic ionotropic receptors can trigger neuroinflammation leading to neurodegeneration
Binding of antagonist to N-methyl-D-aspartate (NMDA) receptors during brain development (synaptogenesis) induces impairment of learning and memory abilities
Binding of agonists to N-methyl-D-aspartate receptor (NMDAR) in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to reduction (or loss) of cognitive function
Binding of antagonists to NMDAR can trigger neuroinflammation leading to neurodegeneration
Binding to electron chain transfer complexes in the mitochondria can trigger neuroinflammation and lead to neurodegeneration
Binding to SH/selen-proteins can trigger neuroinflammation leading to neurodegeneration
Calcium-mediated neuronal ROS production and energy imbalance
Cyclooxygenase inhibition leading reproductive failure
Ecdysone receptor (EcR) activation leading to mortality in Daphnia magna
Estrogen receptor agonism leading to reproductive dysfunction
Glucocorticoid Receptor Activation Leading to Increased Disease Susceptibility
Hematotoxicity due to nitroaromatics and N-hydroxyl anilines
Inhibition of Complex I of the mitochondrial respiration chain leading to neurodegeneration.
Inhibition of iNOS, hepatotoxicity, and regenerative proliferation leading to liver tumors
Kidney toxicity induced by activation of 5HT2C
LXR Activation to Liver Steatosis
Multiple Molecular Initiating Events trigger Neuroinflammation leading to Neurodegeneration
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AOPs for nanomaterials – opportunities and challenges

- Underlying mechanisms of toxicity induced by some nanomaterials is relatively well characterized
- However, the applicability of the available data to support risk estimate derivation is not yet achieved
- AOPs will aid in the organization of basic knowledge and identification of gaps to inform testing strategies and design of the right assays that are immediately relevant to regulatory decision making
- Biomarker identification and development
- Global harmonization of efforts

Nanomaterial properties shape, size, surface modifications….

Aggregation, agglomeration before and after the exposure

Interaction with exposure stock components, Protein corona,

Dose metrics -heterogenous
Non-linear dose response – aggregation/agglomeration
Initial dose vs biologically available dose
Interference with assay components

AOPs for nanomaterials/chemicals under development

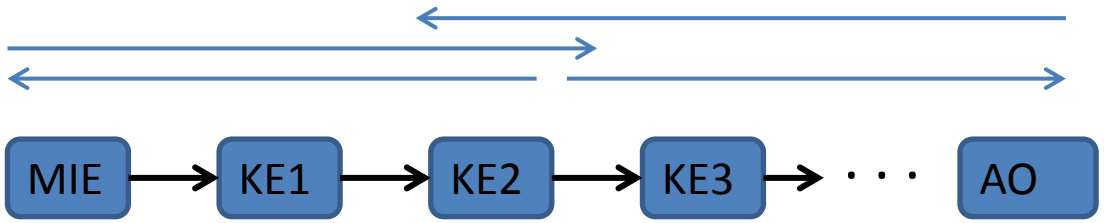
- Induction of secretion of inflammatory cytokines leading to lung emphysema – the proposal was accepted by OECD in June 2014. AOP development is underway.

- Pulmonary injury leading to fibrosis – the proposal is under development

lung is the target by inhalation, lung inflammation and lung fibrosis are the two well characterized responses

Working module

- Selection of the AOP (data-driven approach, high content – omics data)
- Identification of the Molecular Initiating Event
- Review and understanding of the underlying mechanisms (biology and physiology) pertinent to the AOP
- Selection of key events
- Characterization of key event relationships
- Modular representation of the AOP
- Internal and external evaluation
- Report/publish/AOP wiki



- Data-driven approach (high content – omics data)

Selection of the AOP (data-driven approach, high content – omics data)

Summary of the types of nanomaterials, toxicological models and biological endpoints investigated at HC in collaboration with NRCWE, Denmark and others

In vivo pulmonary and systemic response characterization in mice – global gene expression and other endpoints

	TiO_2			CNTs					CB	DEP	CNTs		Silver	
	Size	Surface coats	Matrix-bound	Lengths	Impurities	Wall numbers	Functionalization	Matrix-bound			Size	Functionalization	Size	Surface coats
In vivo														
Inflammation														
BAL cell counts	X	X	X	X	X	X	X	X	X	X				
Tissue cytokines	X	X	X	X	X	X	X	X	X	X				
Gene expression	X	X	X	X	X	X	X	X	X	X				
Genotoxicity														
Oxidative stress	X	X	X	X	X	X	X	X	X	X				
DNA breaks	X	X	X	X	X	X	X	X	X	X				
Mutation frequencies				X	X				X	X				
Systemic responses (liver and heart	X	X		X	X									
Histopathology	X	X	X	X	X	X	X	X	X	X				
In vitro														
Genotoxicity											X	X		
Cytotoxicity											X	X	X	X
Gene expression											X	X	X	X

Selection of AOPs

-Meta analysis of the in vivo pulmonary gene expression data

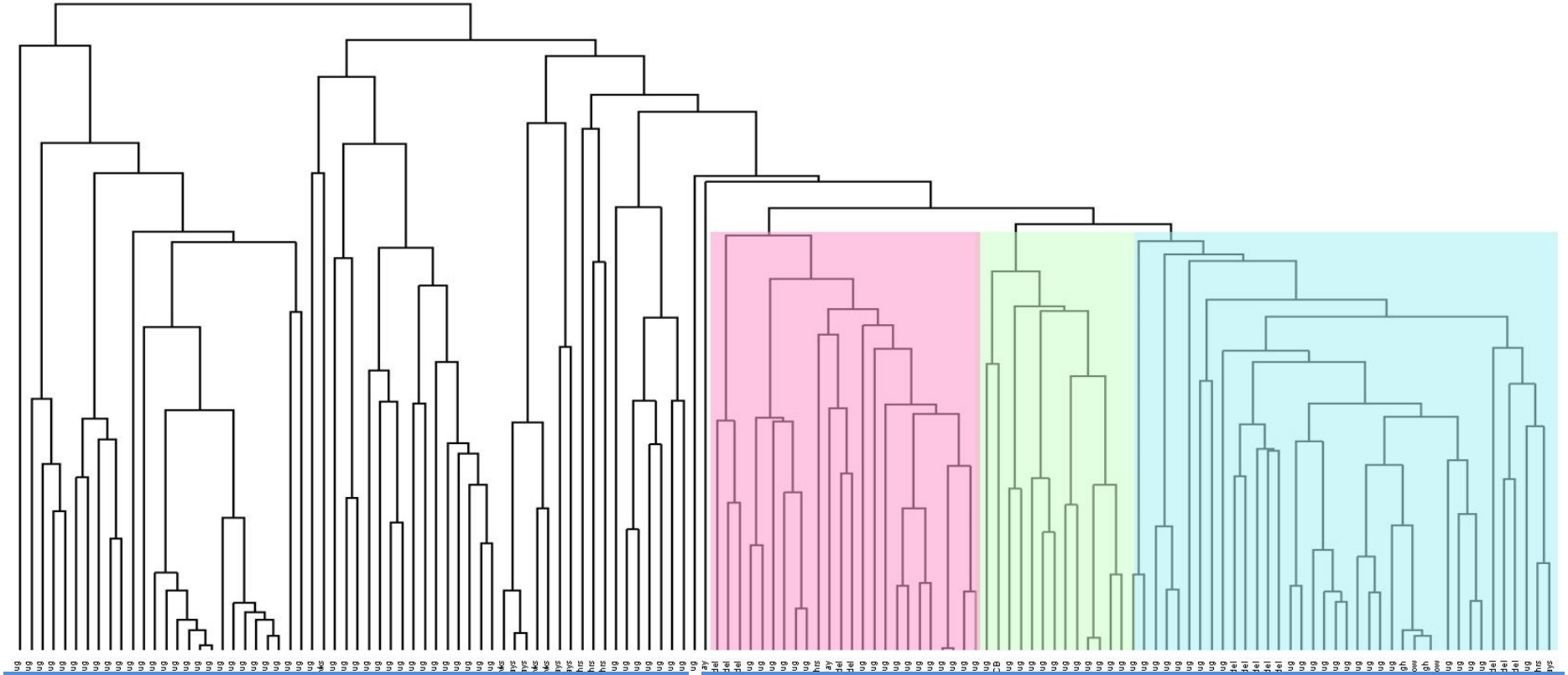
-Statistical and bioinformatics analysis

-Identification of disease causing nanomaterials

Literature review Nanomaterials → Investigation of pulmonary responses using microarrays → External data sets

Literature review Pulmonary diseases → Investigation of pulmonary diseases using microarrays → External data sets describing pulmonary diseases including fibrosis, bacterial infection, cytokine induced lung injury….

Meta analysis of the in vivo pulmonary gene expression data –identification of disease phenotypes



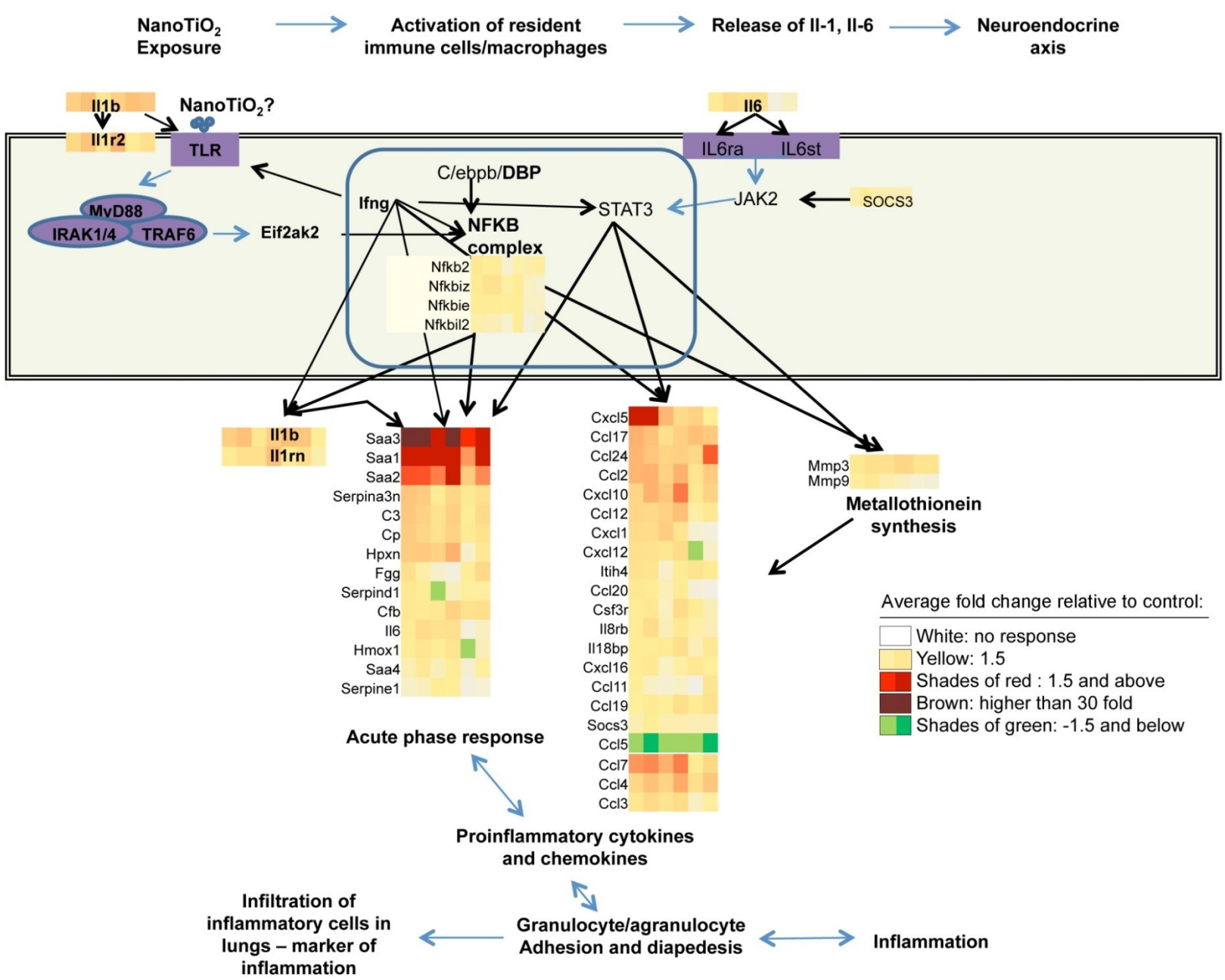
Lung inflammation - nanomaterials, metals, chemicals

Lung injury, Fibrosis – nanomaterials, chemicals, microbials

Halappanavar et al., Environ Mol Mutagen. 2014 Dec 11. doi: 10.1002/em.21936

Halappanavar et al., in preparation

Students and postdoctoral fellows : Mainul Husain, Jake Nikota, Sarah Labib, Luna Rahman, Charles Guo, Dongmei Wu, Katharine Baxter, Jacob Rogowski, Sos Poulsen…



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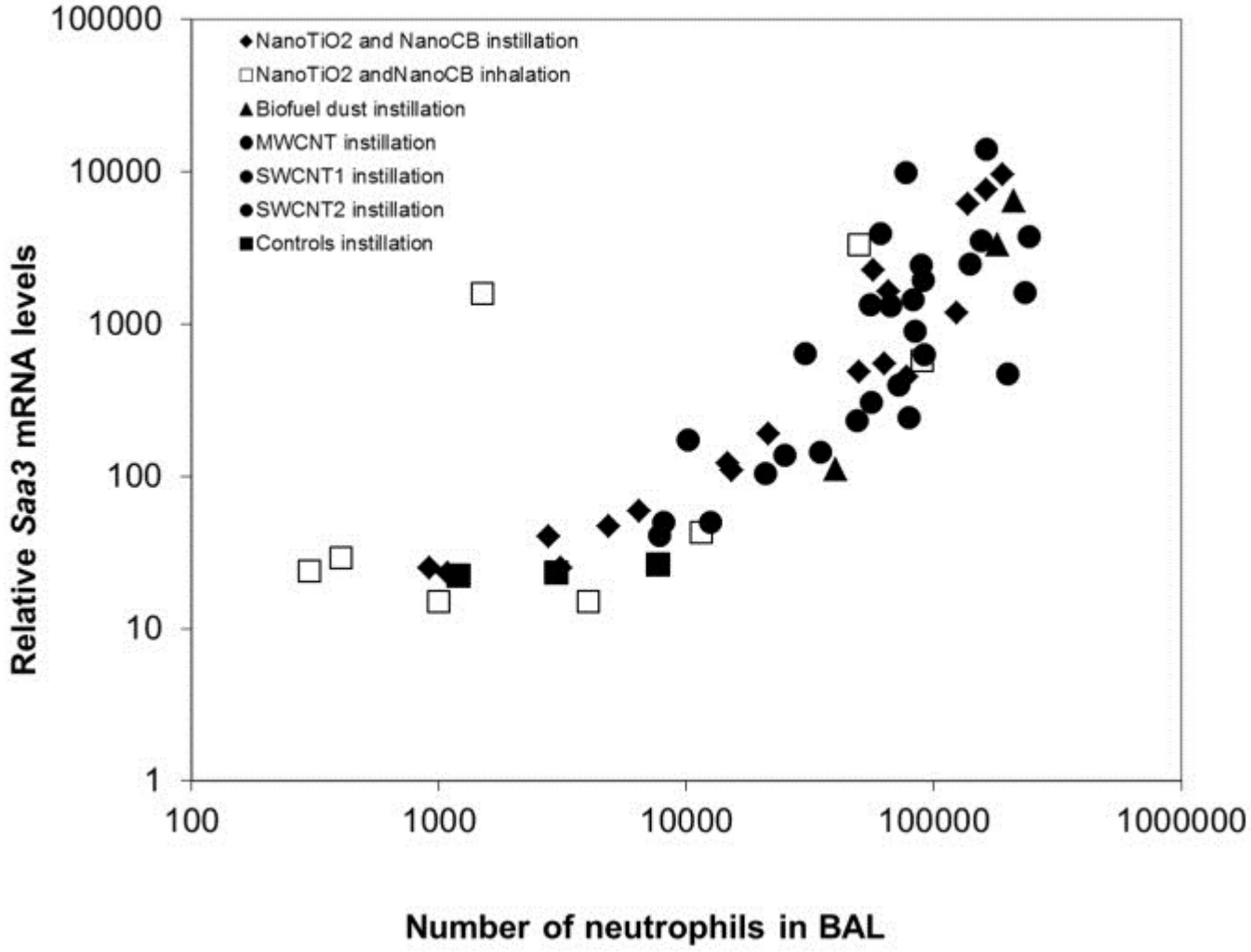
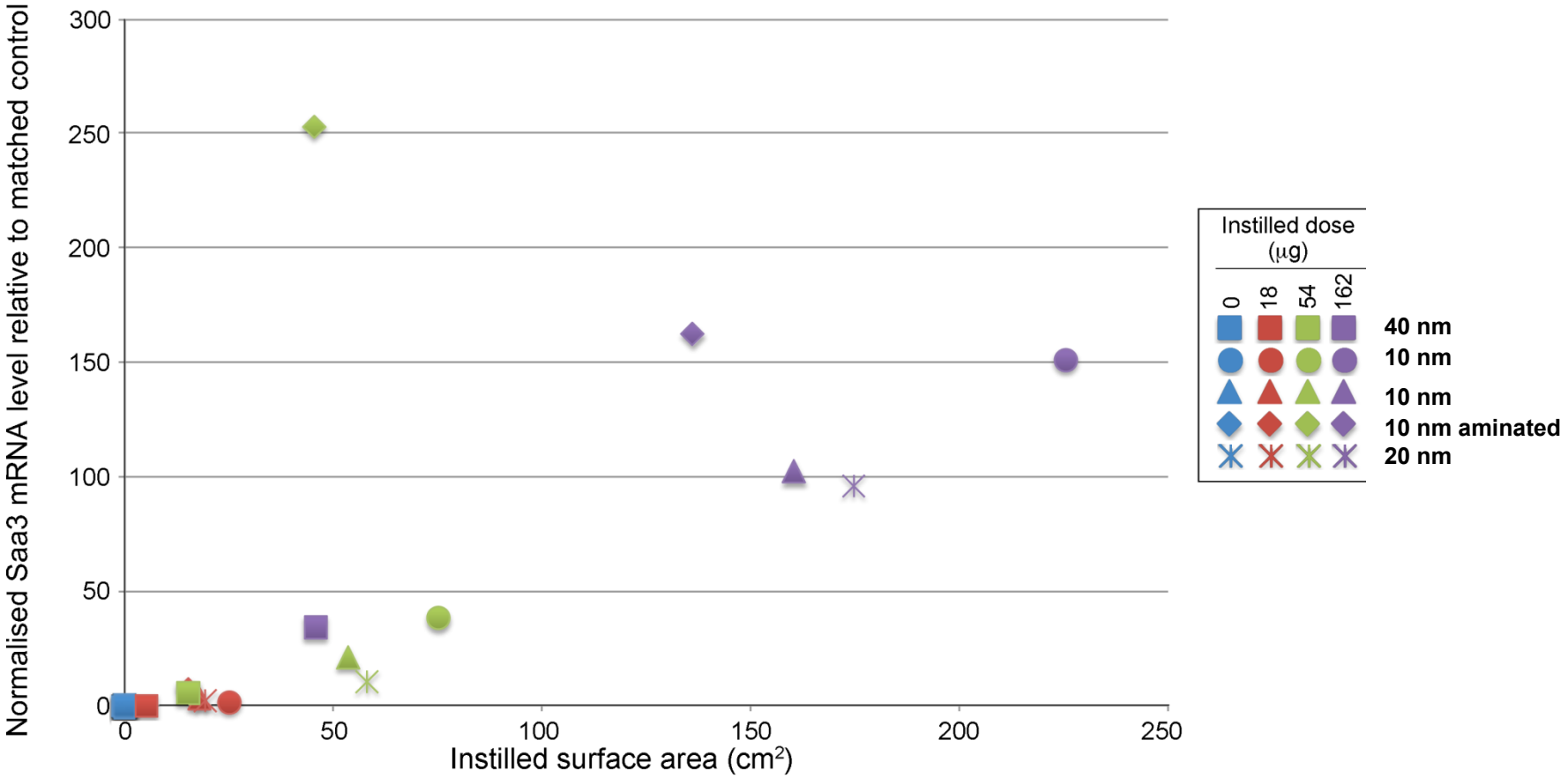
The correlation between the pulmonary mRNA expression of *Saa3* and the influx of neutrophils.



Saber et al., Plos One. 2013; 8(7):e69020

Saa3 mRNA levels against instilled surface area



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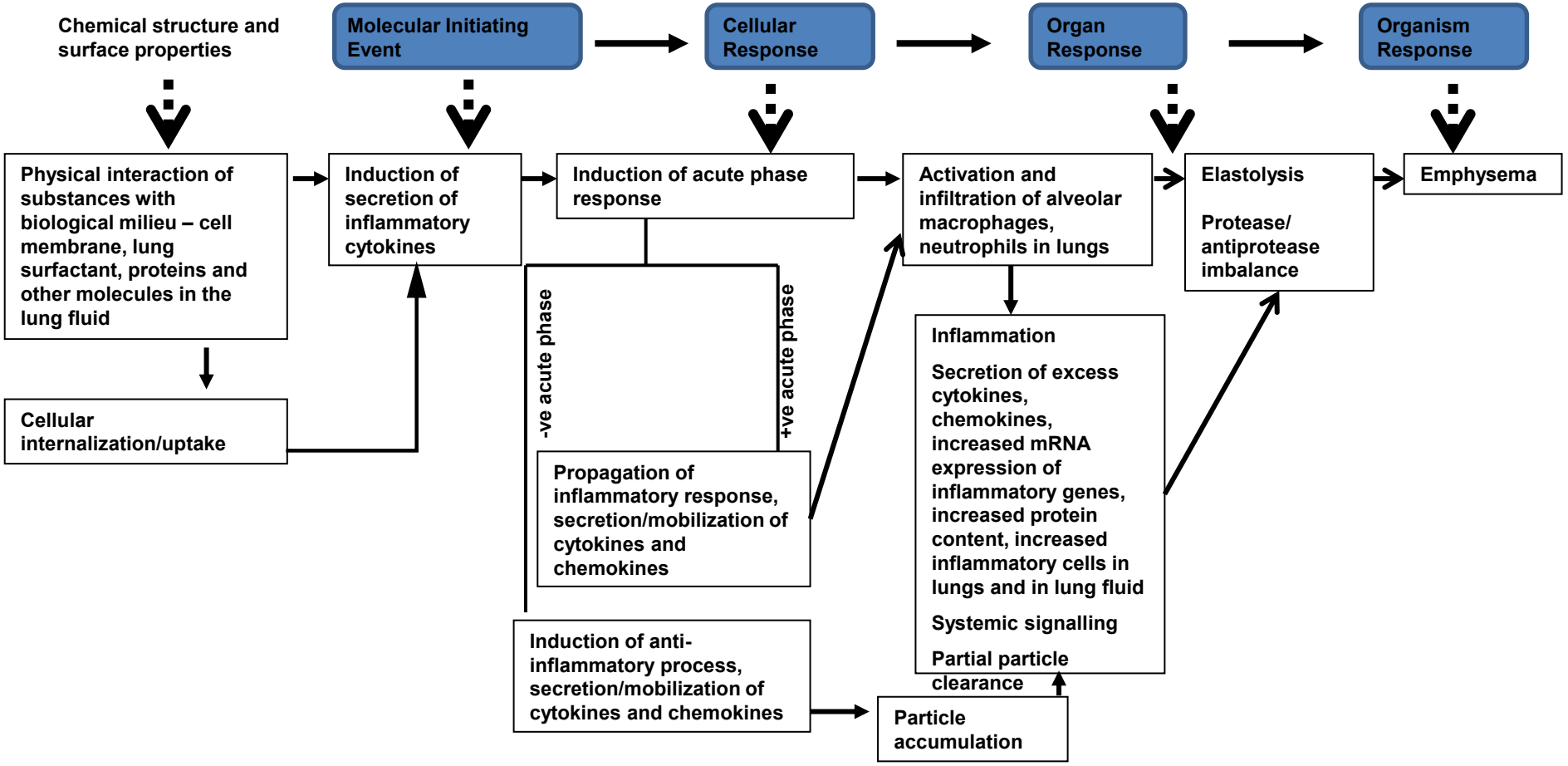
Induction of secretion of inflammatory cytokines leading to lung emphysema



Lead: Sabina Halappanavar, Health Canada, Ottawa, Canada
Collaborators: National Research Center for the Working Environment, Denmark; McMaster University, Hamilton, Canada

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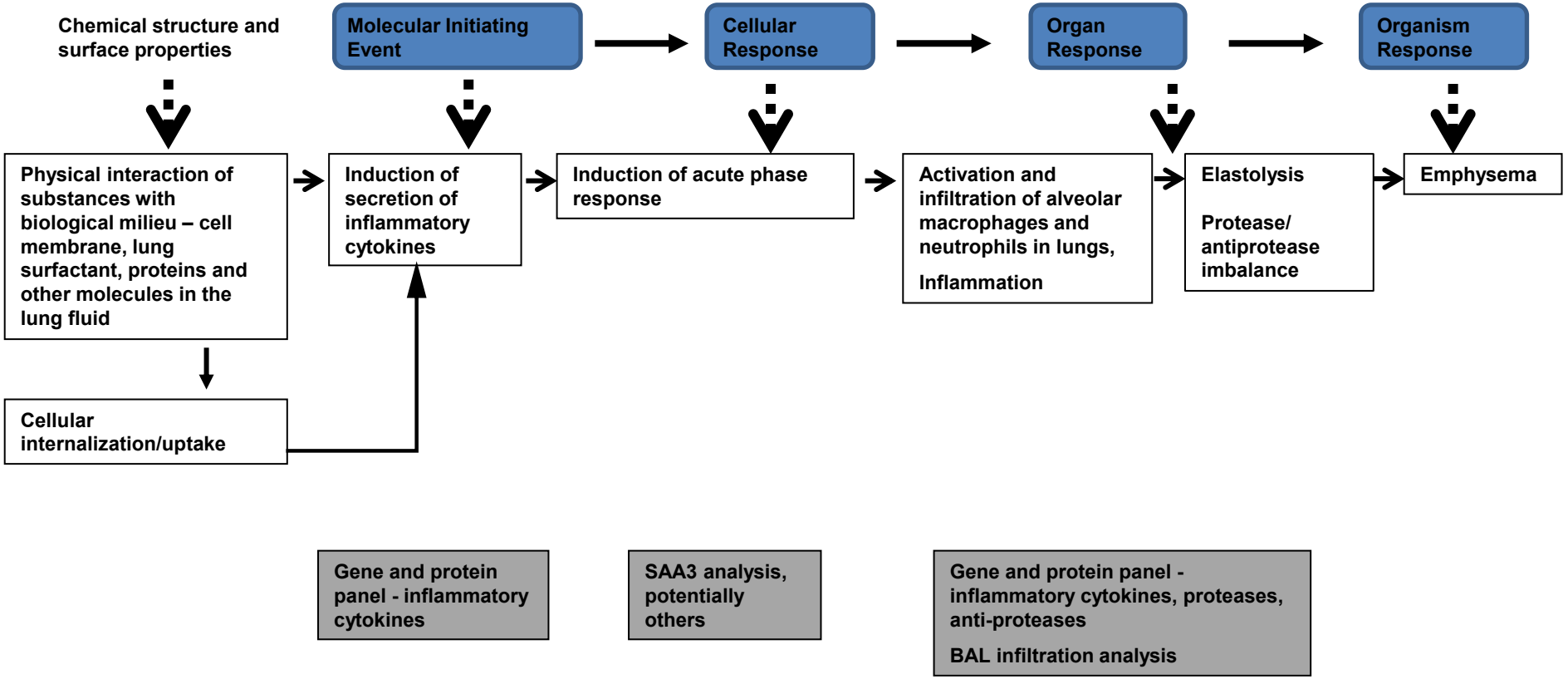

Non-animal test methods: in vitro monolayers, ex vivo lung tissue slices

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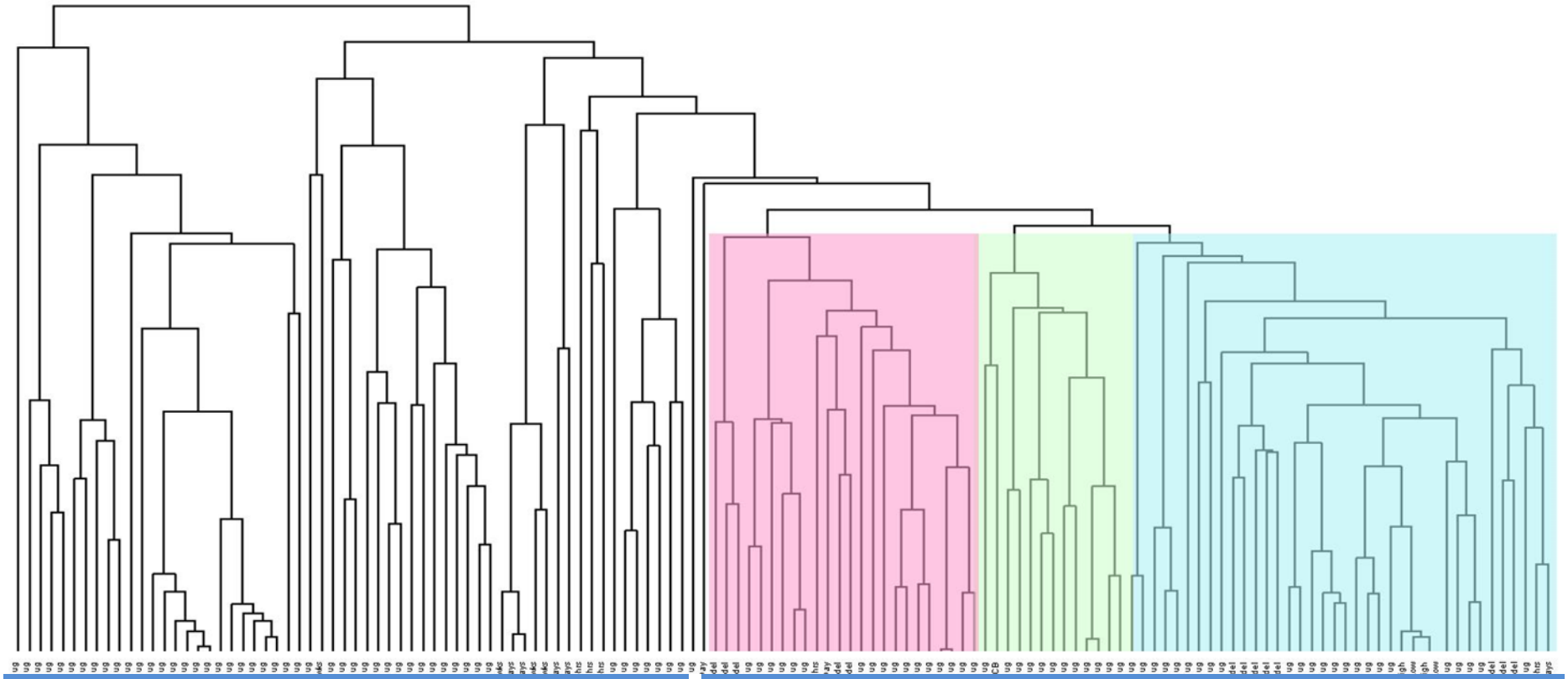

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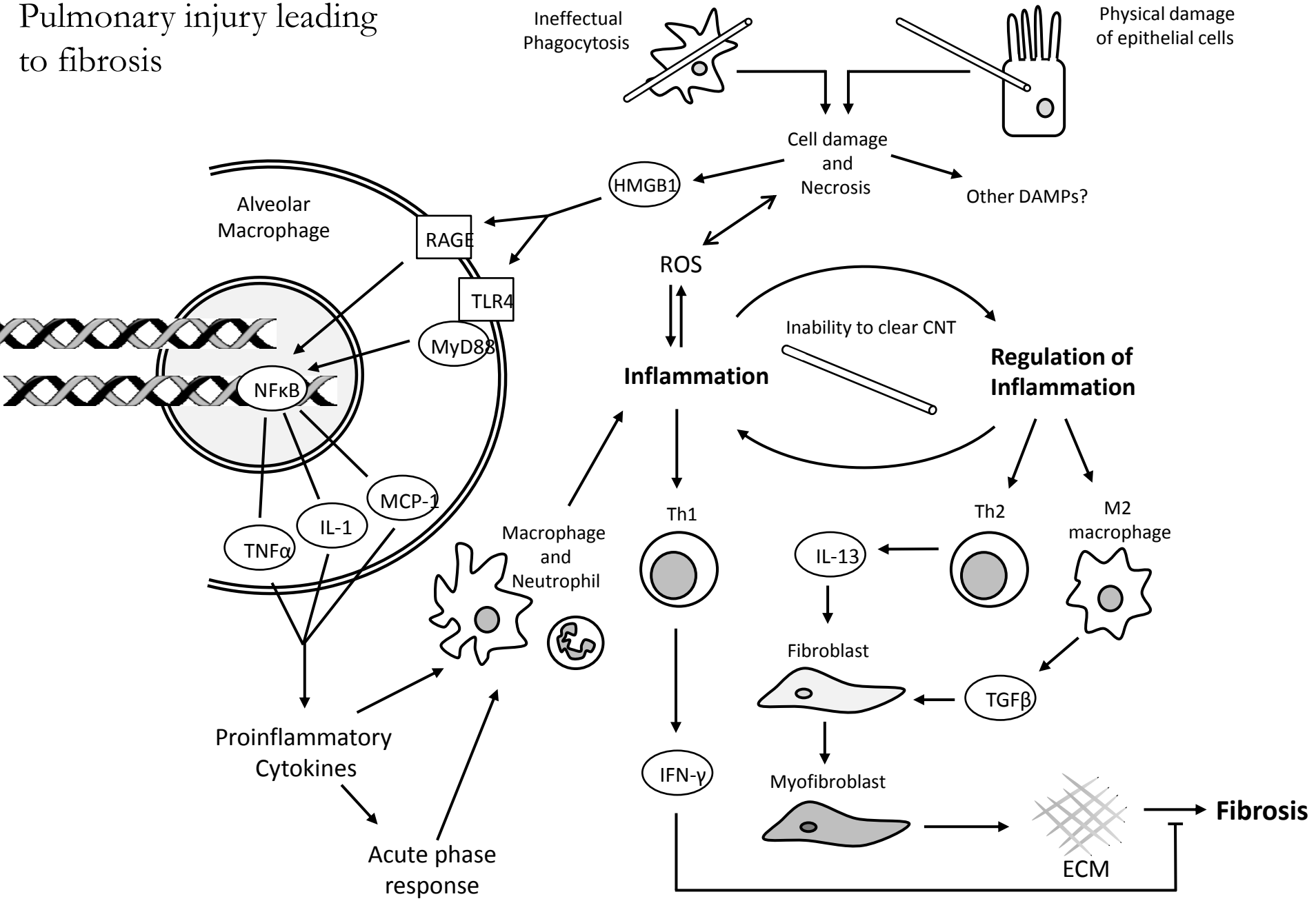
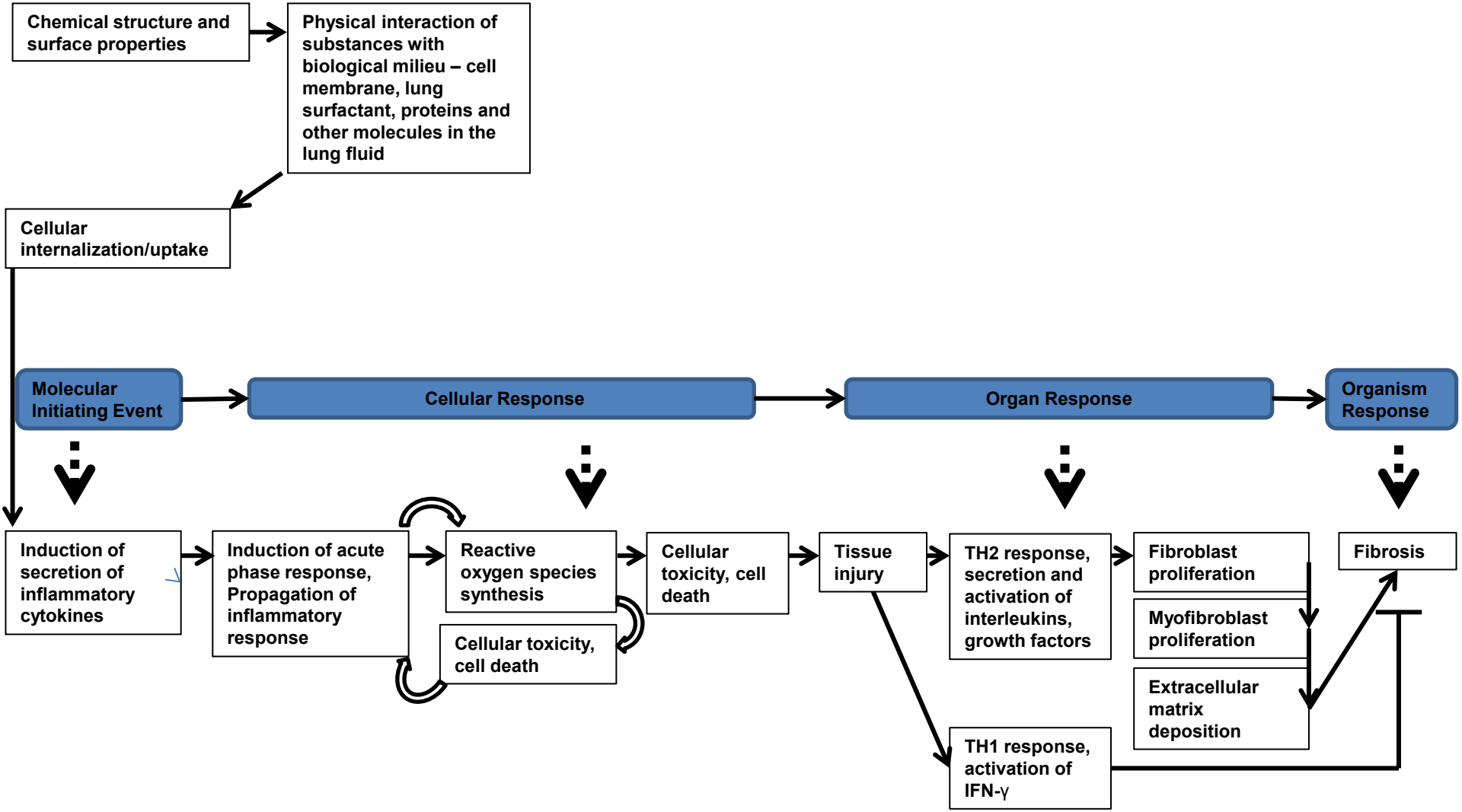
Pulmonary injury leading to fibrosis



Pulmonary injury leading to fibrosis



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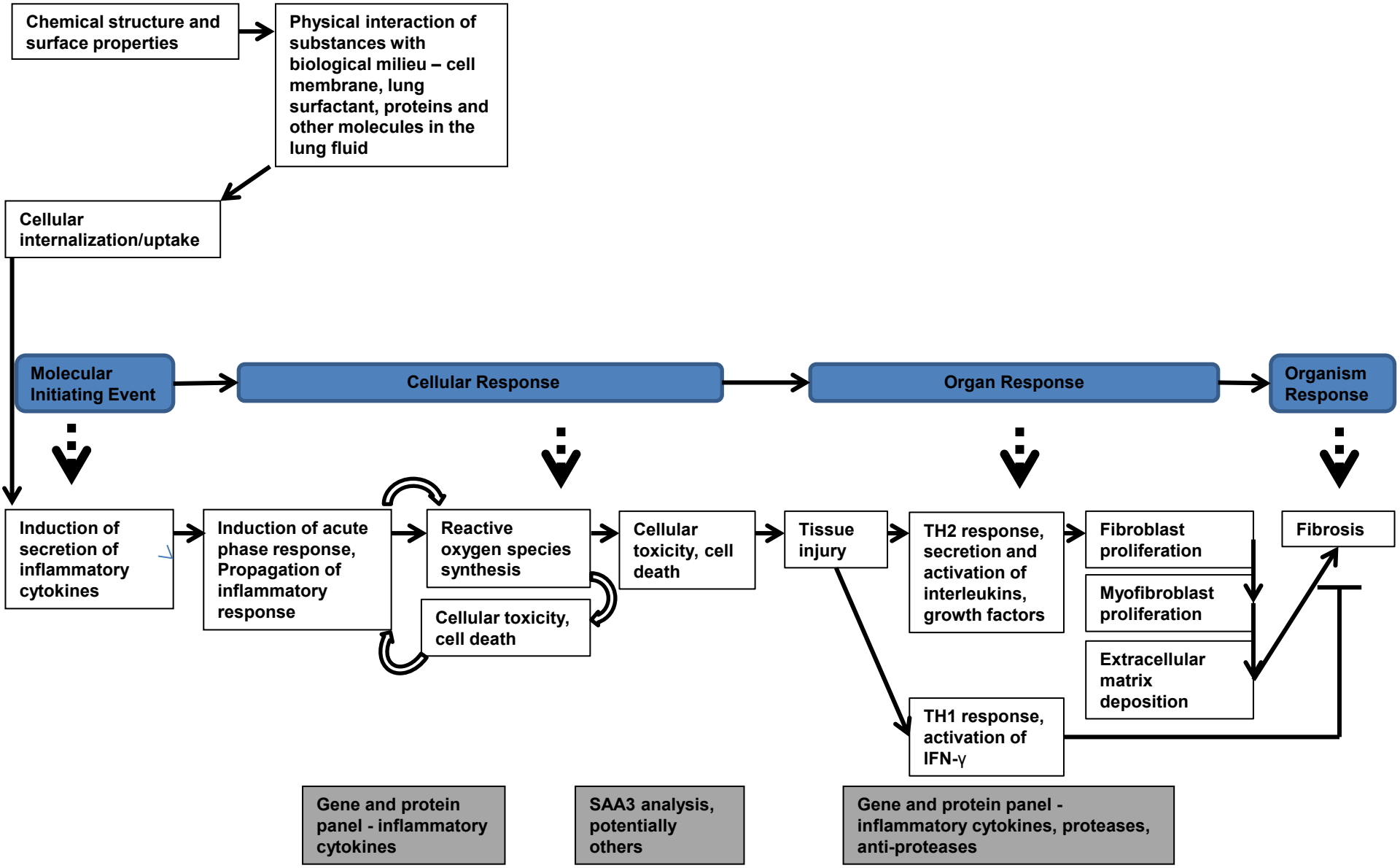

Non-animal test methods: in vitro monolayers, ex vivo lung tissue slices

Conclusions

AOPs address the needs of

- The OECD Test Guidelines Programme – new in vitro test methods
- The OECD QSAR project – new methods for grouping of chemicals
- The OECD Hazard Assessment activities – integrated testing approaches

AOPs will inform mechanism-based risk assessment approaches

AOPs will integrate systems biology thinking into risk assessment

AOPs are too simple – do not accurately reflect the complexity of underlying biology

Some aspects of risk assessment are not included - exposure

The success of AOPs will depend on the active participation of researchers around the world

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Mainul Husain
Sos Poulsen
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Jacob Rogowski

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