

HARMLESS



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HARMLESS

AOP training

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23/6/2022



Agenda

Part 1:

- Introduction to AOPs
- AOP example “From research to AOPs”

Part 2:

- Introduction to the AOPwiki
- How to submit an AOPwiki

Agenda

Part 1:

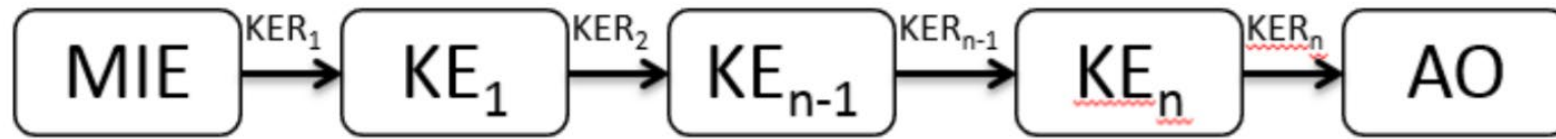
- **Introduction to AOPs**
- AOP example “From research to AOPs”

Part 2:

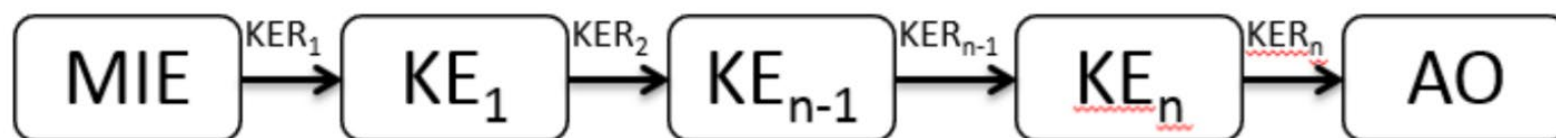
- Introduction to the AOPwiki
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Part 1: Introduction to AOPs

AOP structure



AOP structure

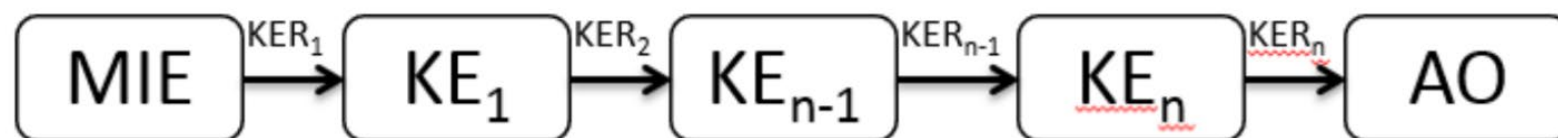


Molecular initiating event (MIE): A specialized type of key event that represents the initial point of chemical/stressor interaction at the molecular level within the organism that results in a perturbation that starts the AOP.

Key event (KE): A change in biological or physiological state that is both **measurable** and **essential** to the progression of a defined biological perturbation leading to a specific adverse outcome.

- Just because a particular KE is observed, does not mean the perturbation will necessarily progress all the way to the AO.

AOP structure



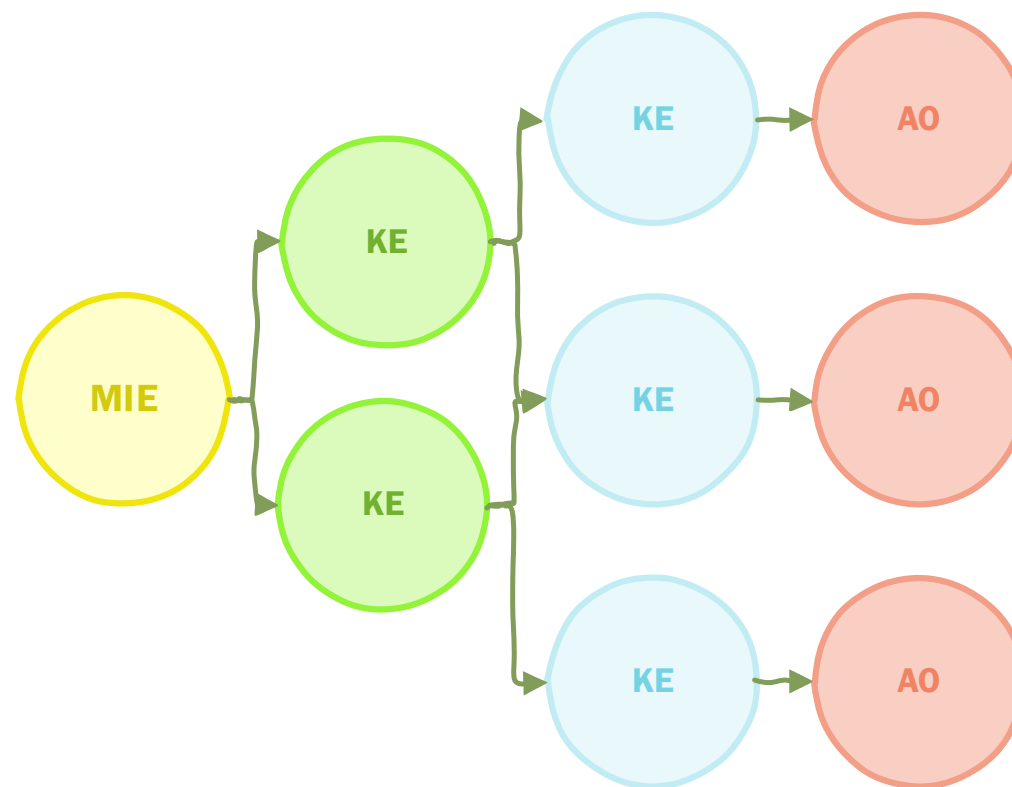
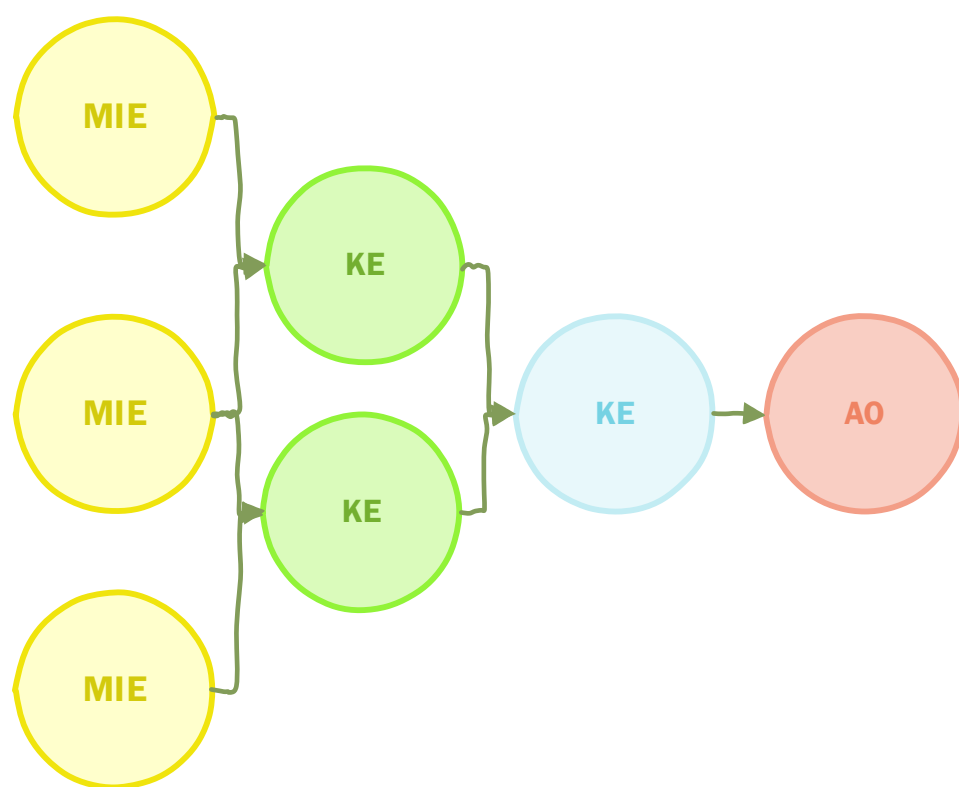
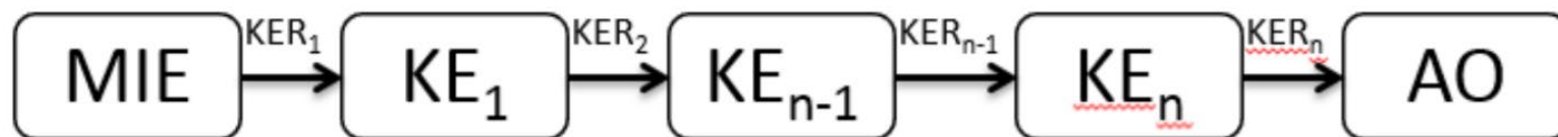
Key event relationship (KER): A scientifically-based relationship that connects one key event to another.

KER defines a causal and predictive relationship between the upstream and downstream event.

This facilitates inference or extrapolation of the state of the downstream key event from the known, measured, or predicted state of the upstream key event.

Adverse outcome (AO): A specialized type of key event that is generally accepted as being of **regulatory significance** on the basis of correspondence to an established protection goal or equivalence to an apical endpoint in an accepted regulatory guideline toxicity test.

AOP networks

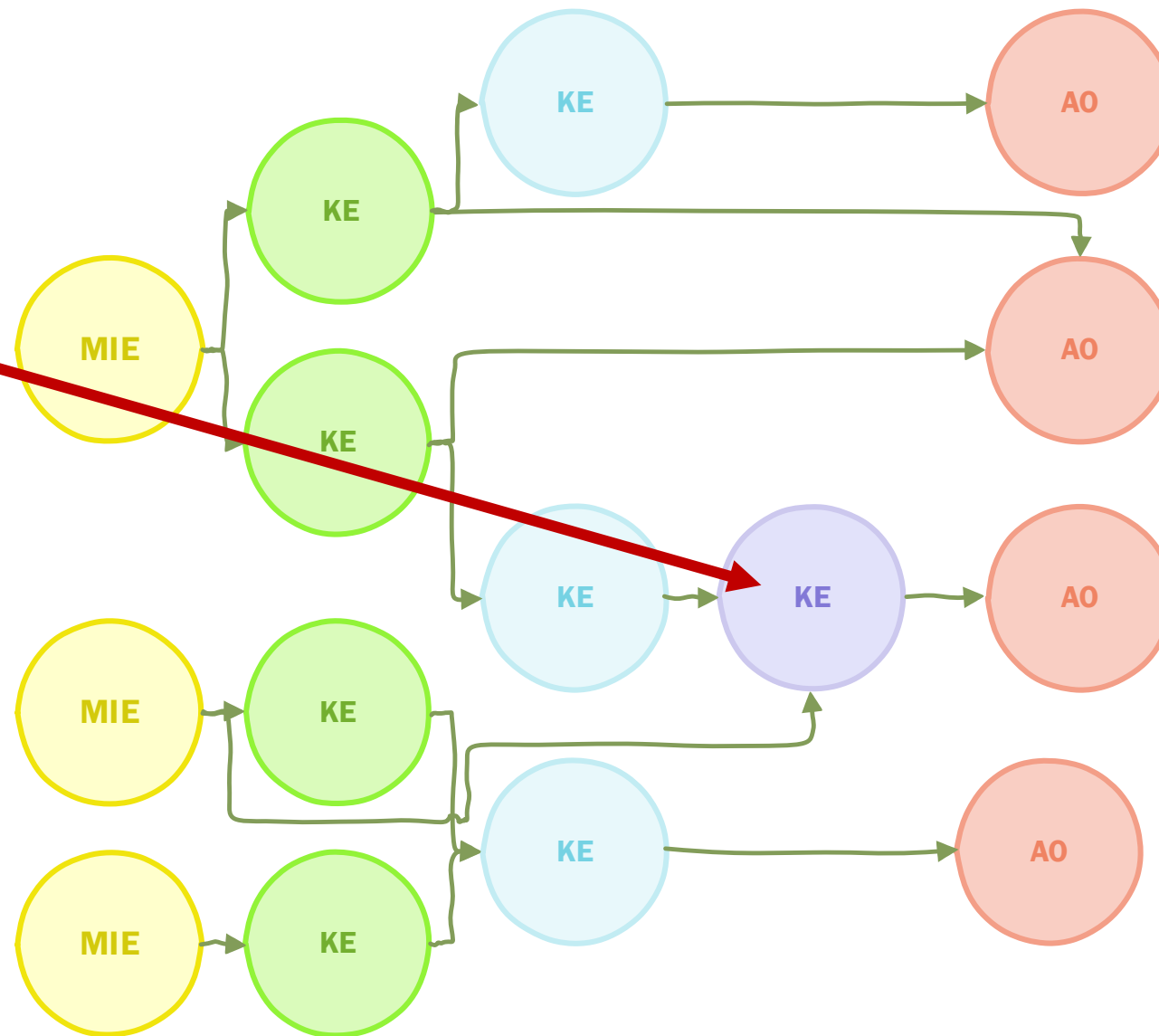


AOP networks

Identification of KE nodes.

Provide focus points for *in vitro* assays.

Presentation Tuesday.



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Part 1: “From research to AOPs”

AOP #237: Secretion of inflammatory cytokines after cellular sensing of the stressor leading to plaque progression

What's the story?

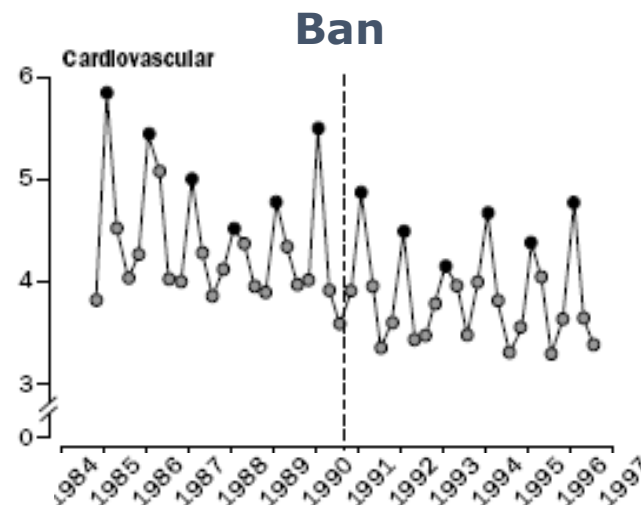
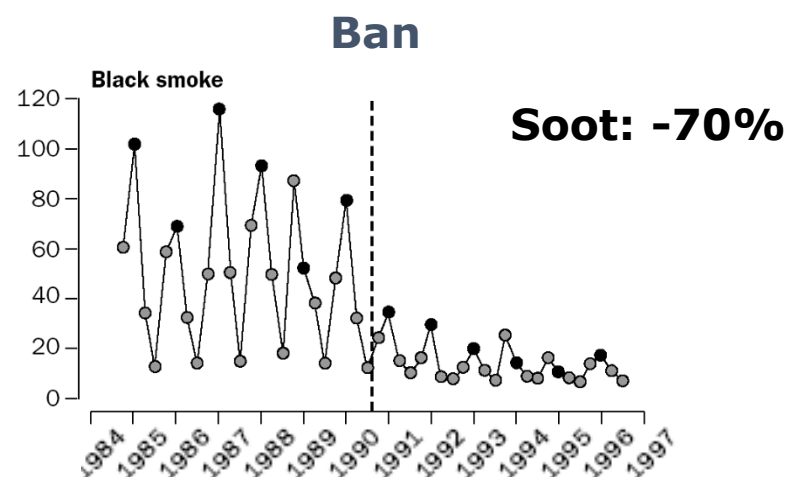
Part 1: “From research to AOPs”

Inhalation of particles increases risk of cardiovascular disease

Direct link between mortality and concentration of fine particles (PM_{2.5}) in the air.

Inhalation of particles affect mortality:

Soot in the air and heart-related mortality per 1000 persons in the years before and after coal was banned in Dublin:



**Heart-related
mortality: -10%**

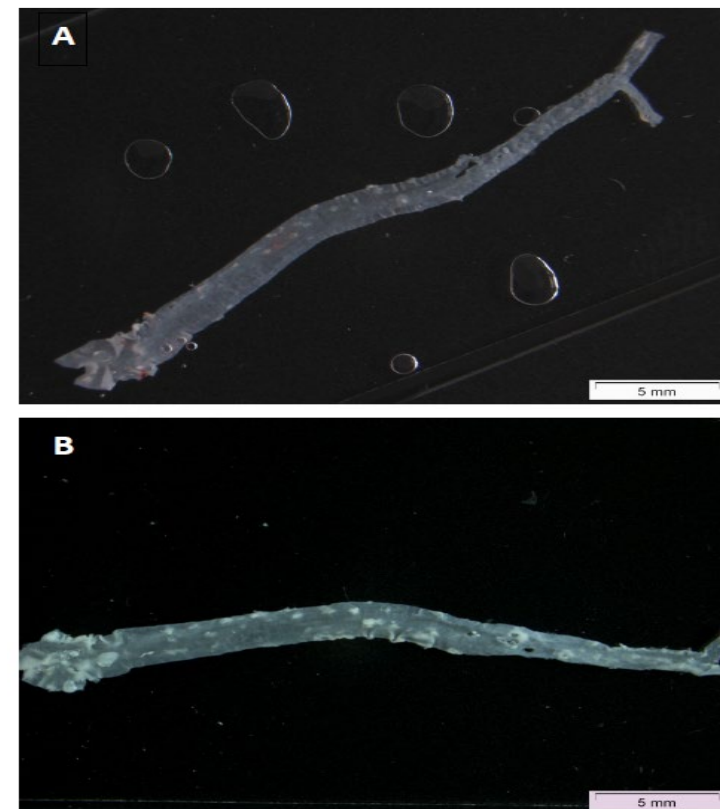
Clancy et al. Lancet
360: 1210–14, 2002

Inhalation of particles increases risk of cardiovascular disease

Inhalation of nanomaterials induces the risk:

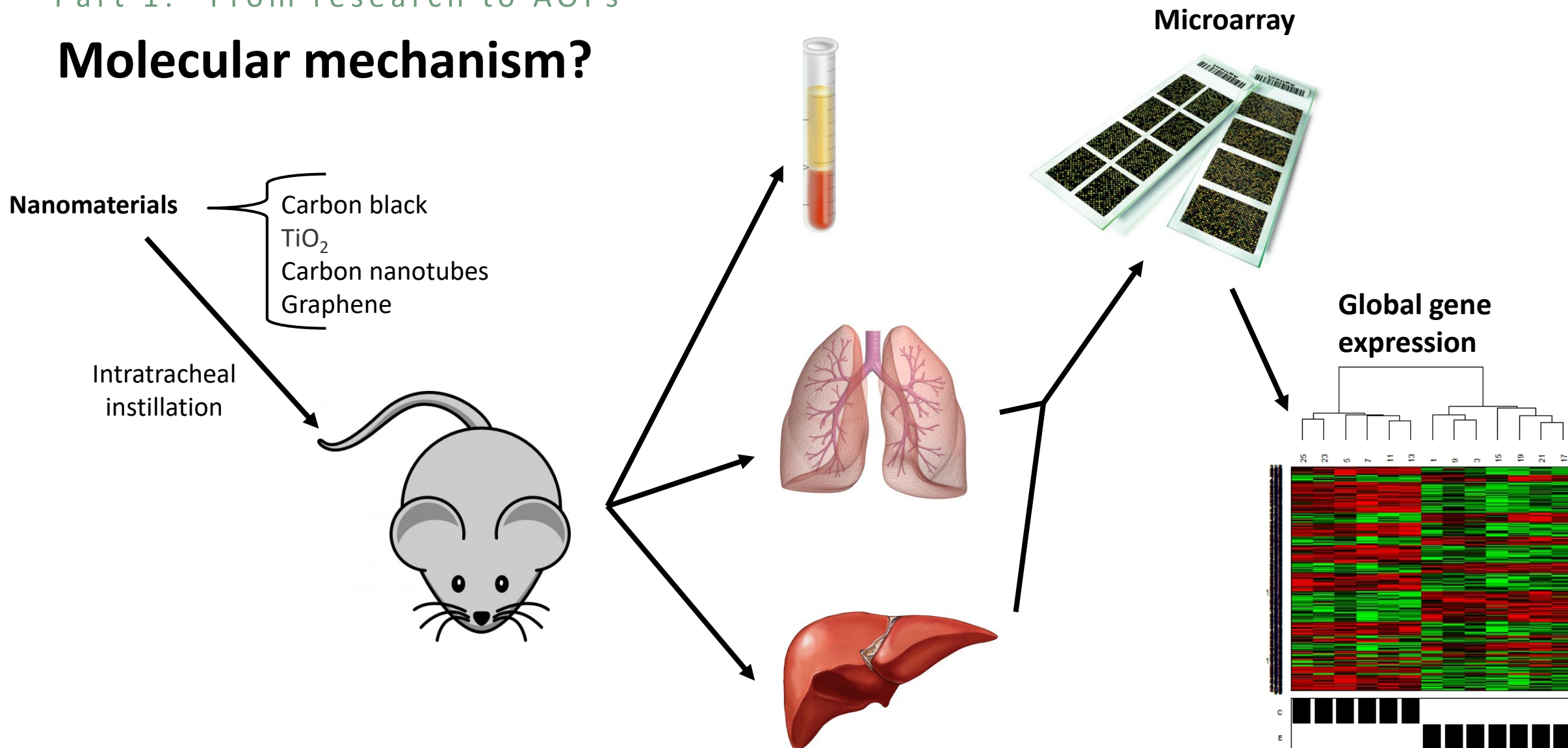
Effects are additive.

Could we possibly identify a molecular mechanism behind nanomaterial-induced increased risk of cardiovascular disease?



Mikkelsen et al. *Particle and Fibre Toxicology*
2011, 8:32

Molecular mechanism?



Global gene expression:

All nanomaterials tested induced a pulmonary acute phase response.

- The most differentially regulated gene was *Saa3*.
- Dose response.
- Sustained response.

Close correlation between *Saa3* and inflammation

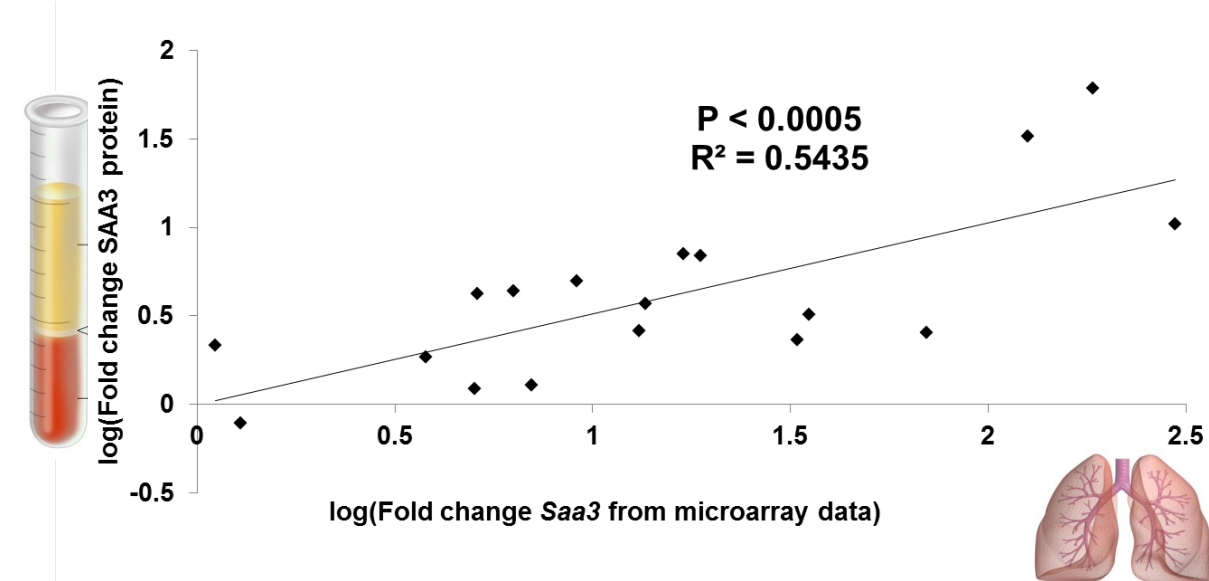
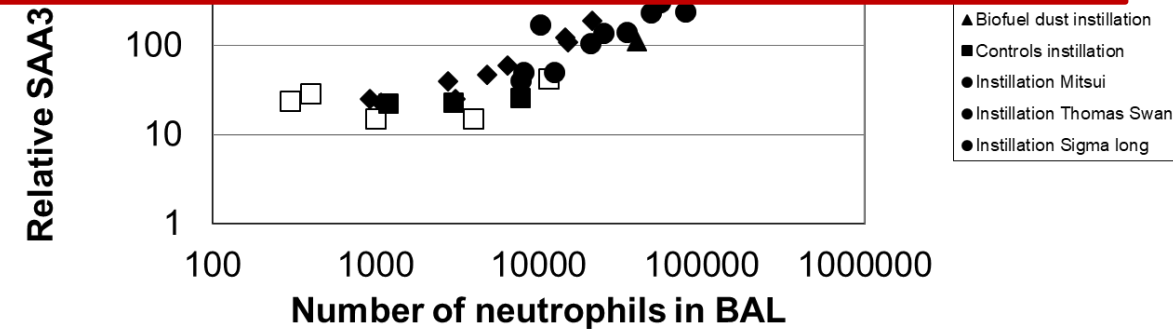
Mice have 3 inducible SAA isogenes (*Saa1*, *Saa2*, *Saa3*).

Increased SAA1/2 og SAA3 levels were also identified in the blood after lung exposure to nanomaterials.

We observed a significant correlation between *Saa3* mRNA levels and plasma levels of SAA3 protein.

Increased SAA plasma levels also seen in humans.

- After ZnO inhalation, pulp and paper mills and welding fumes.



Part 1: “From research to AOPs”

SAA: an acute phase protein

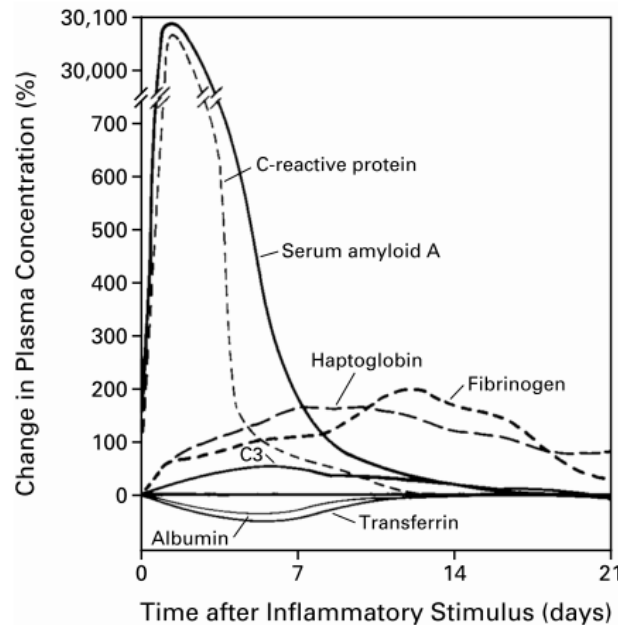


Figure 1. Characteristic Patterns of Change in Plasma Concentrations of Some Acute-Phase Proteins after a Moderate Inflammatory Stimulus.

Modified from Gitlin and Colten⁵ with the permission of the publisher.

Gabay and Kushner 1999, NEJM

The acute phase response is the systemic response to acute and chronic inflammatory states caused by eg. bacterial infection or trauma.

Conditions that induce acute phase response are associated with risk of cardiovascular disease.

It is traditionally considered a liver response.

SAA: an acute phase protein

This inhibits HDLs role in reverse cholesterol transport.



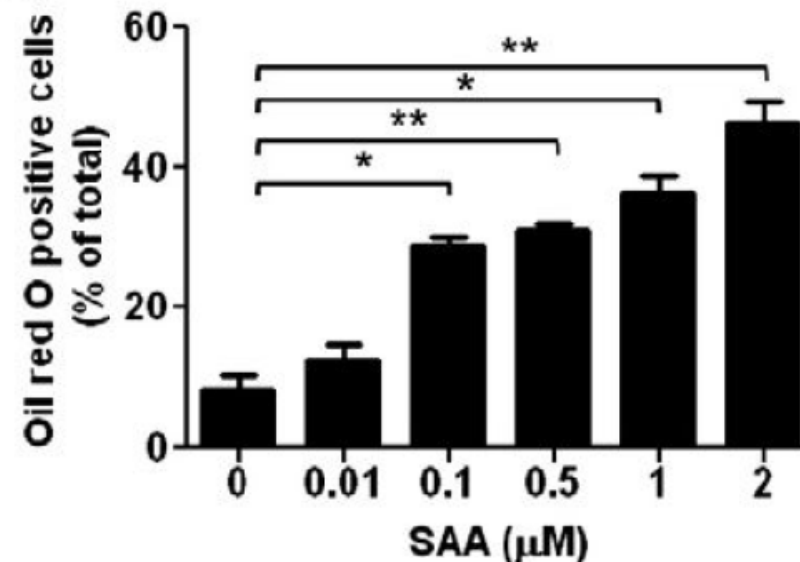
Part 1: “From research to AOPs”

SAA: an acute phase protein

SAA can replace ApoA-1 as the major HDL protein.

This inhibits HDLs role in reverse cholesterol transport.

SAA induces foam cell formation in macrophages.

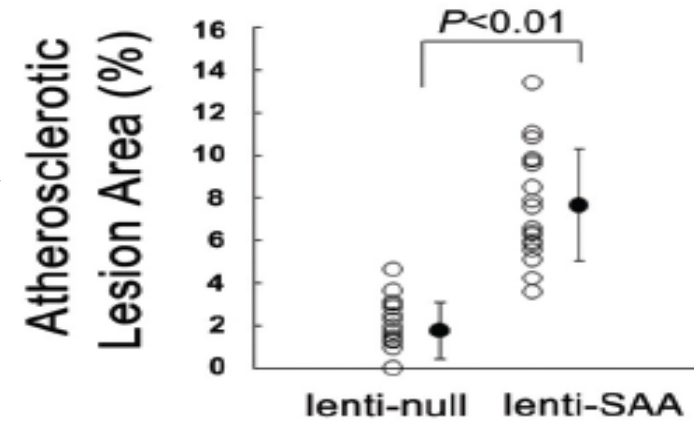


Lee et al, 2013, BBRC

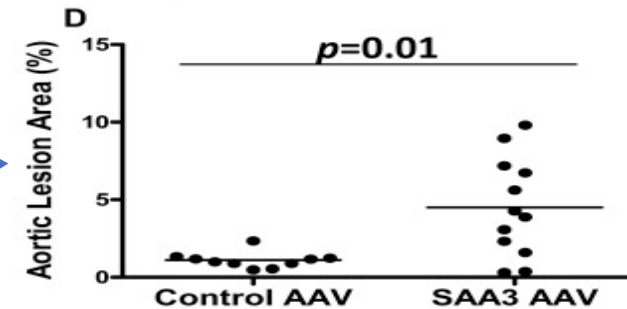
Part 1: “From research to AOPs”

SAA: an acute phase protein

- Overexpression of SAA1 protein accelerates plaque progression.
- Overexpression of SAA3 protein accelerates plaque progression.
- Knock out of all SAA isogenes resulted in reduced plaque progression



Dong et al, 2011, Mol. Med



Thompson et al, 2018, Atherosclerosis

Acute phase proteins CRP & SAA are associated with risk of CVD in prospective epidemiological studies

Nurses’ Health Study

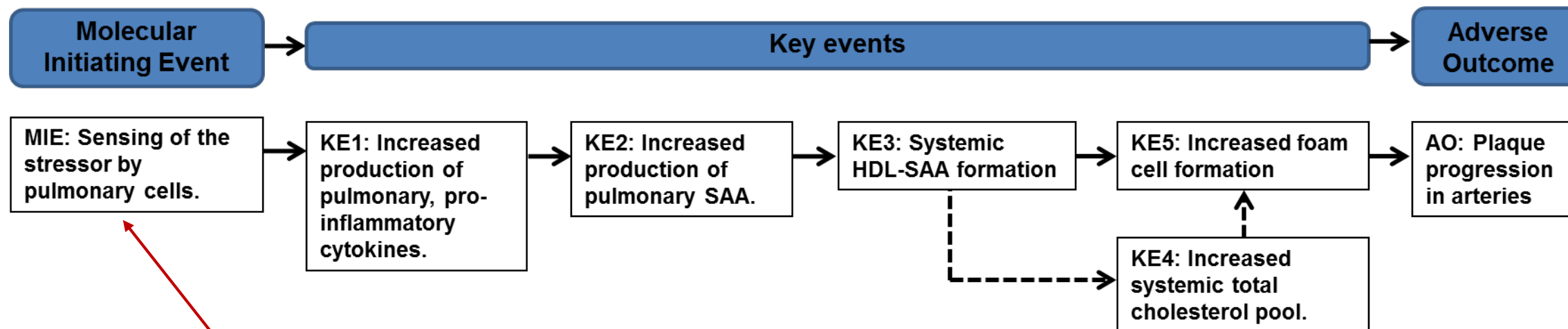
TABLE 3. RELATIVE RISK OF CARDIOVASCULAR EVENTS ACCORDING TO BASE-LINE PLASMA LEVELS OF MARKERS OF INFLAMMATION AND LIPIDS.*

VARIABLE	QUARTILE OF PLASMA LEVEL				P VALUE FOR TREND
	1	2	3	4	
High-sensitivity C-reactive protein					
Median — mg/dl	0.06	0.19	0.38	0.85	
Relative risk (95% CI)	1.0	2.1 (1.0–4.5)	2.1 (1.0–4.4)	4.4 (2.2–8.9)	<0.001
Serum amyloid A					
Median — mg/dl	0.25	0.43	0.62	1.17	
Relative risk (95% CI)	1.0	1.8 (0.9–3.6)	1.9 (0.9–3.8)	3.0 (1.5–6.0)	0.002

Ridker *et al.* 2000, NEJM

Part 1: “From research to AOPs”

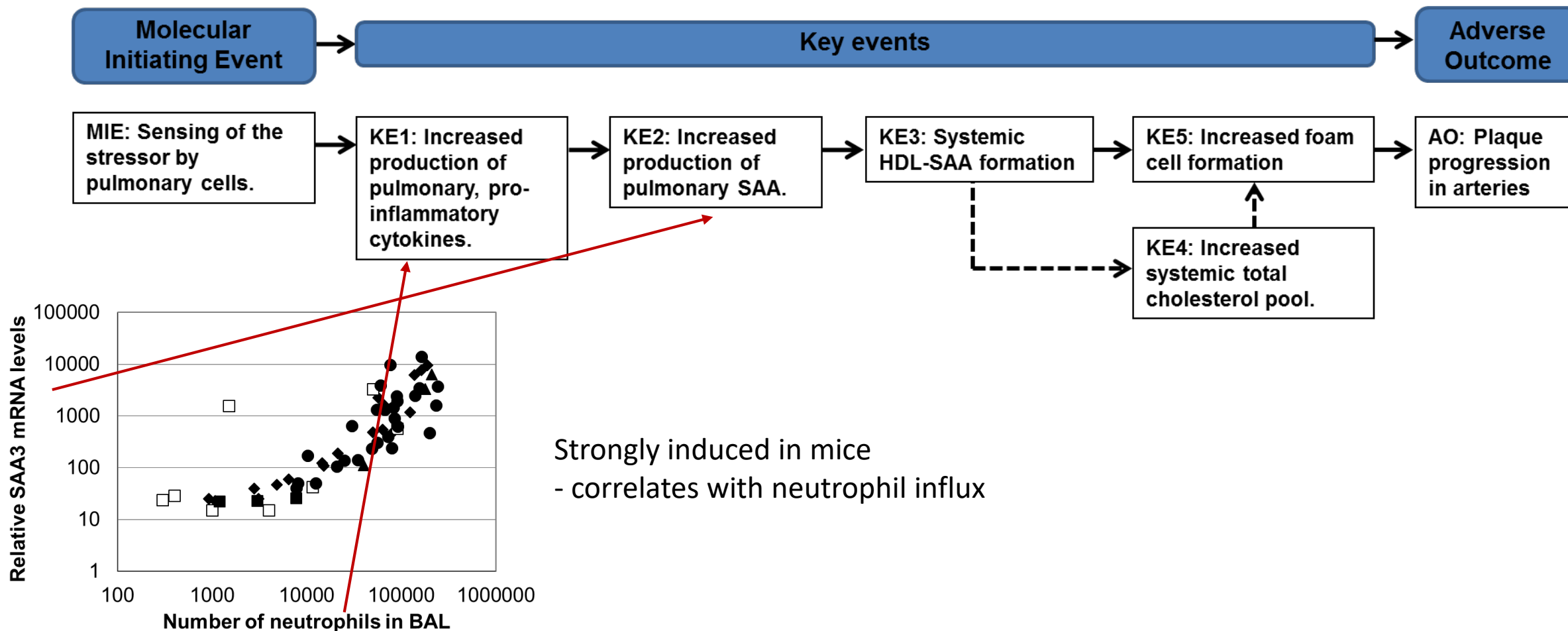
AOP #237



Largely undetermined.
T. Stöger lab: single-cell transcriptomics

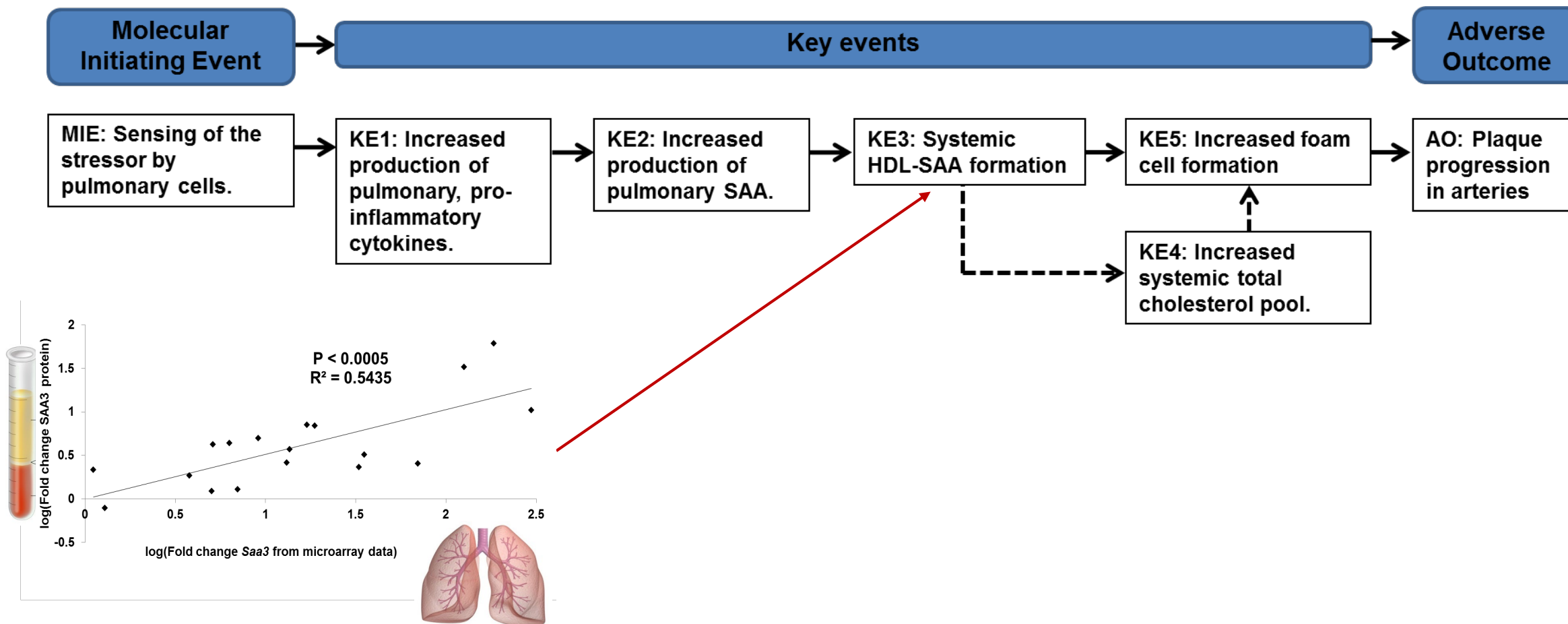
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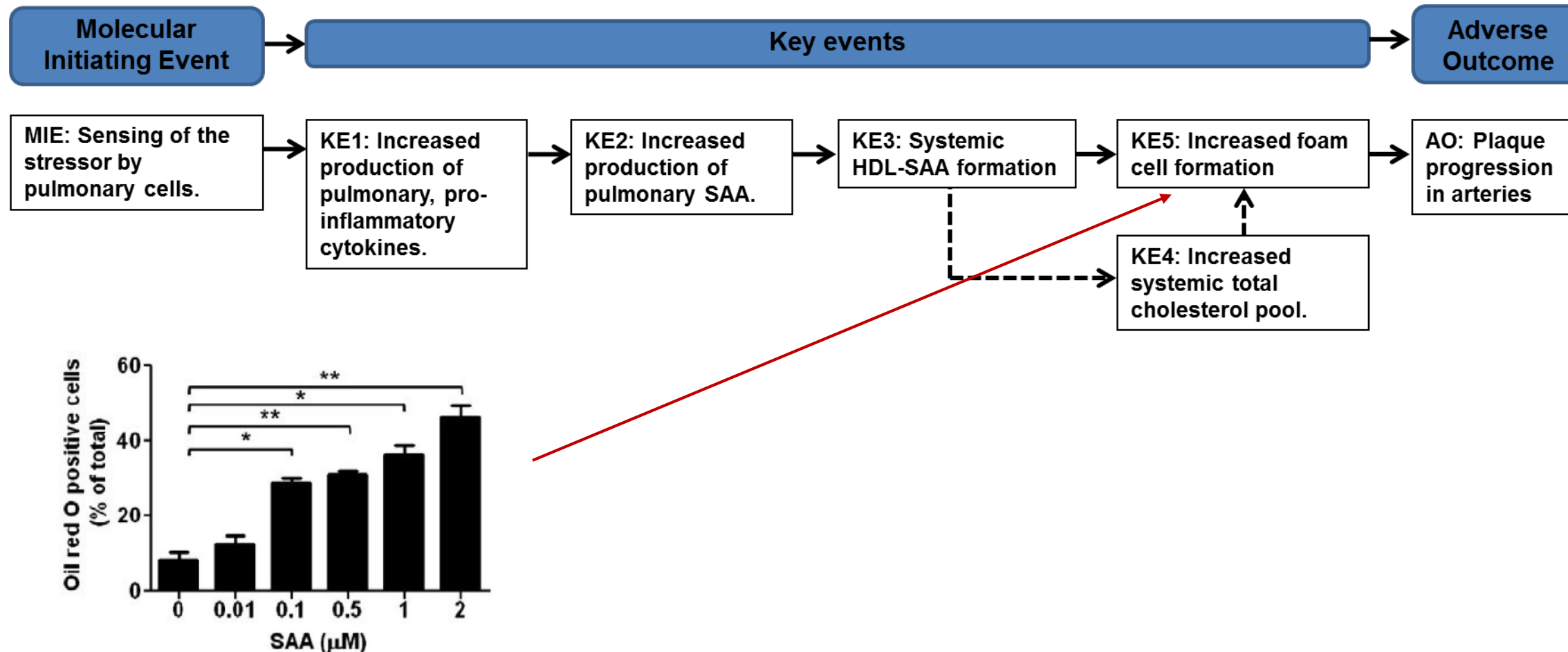
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AOP #237



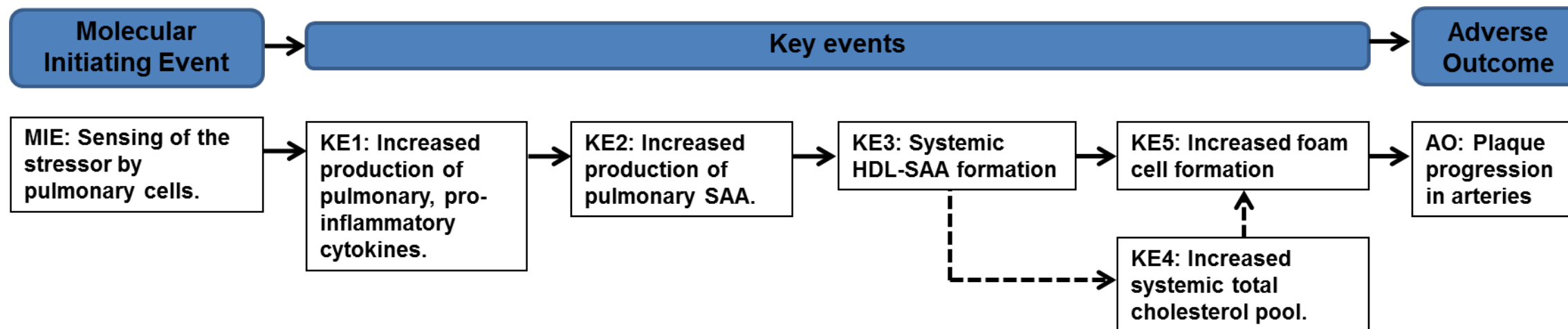
Part 1: “From research to AOPs”

AOP #237



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AOP #237



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- How to submit an AOPwiki

Part 2: “Introduction to the AOPwiki”

“Tour the AOPwiki”

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Part 2: “How to submit an AOPwiki”

Fill in the form

<https://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm>

Template

OECD ADVERSE OUTCOME PATHWAY

Project Submission Form

If you require further information please contact the OECD Secretariat
Return completed forms to our generic account (env.tgcontact@oecd.org), and
Nathalie Delrue (Nathalie.delrue@oecd.org)

PROJECT TITLE

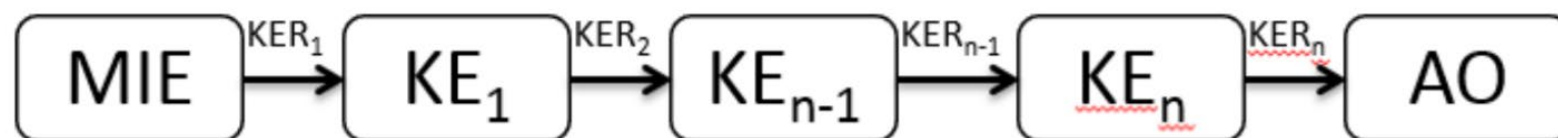
SUBMITTED BY (organisation/consortium/ agency,...)

DATE OF SUBMISSION TO THE SECRETARIAT

DETAILS OF PROJECT PROPONENT(S)

Country/Organisation:	
Agency/ministry/Other:	

Construct your AOP



Important things to consider:

MIE should be unrelated to nano.
-Nanomaterials may be stressors.

Use existing KEs when possible.
-Many close to identical KEs in the wiki.

KEs and KERs should be described as discrete units without reference to a specific MIE, AO, or other KEs.

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The screenshot displays the AOP-Wiki interface for a specific Key Event (Event: 1495). The page includes a navigation bar at the top with links to AOPs, Key Events, KE Relationships, and Stressors, along with a domain dropdown and user options (Login, Register). The main content area is divided into sections: Key Event Title, Substance interaction with the lung resident cell membrane components, Short name, Biological Context, Cell term, Organ term, and Key Event Components. The 'Key Event Components' section is highlighted with a red box and contains a table with three columns: Process, Object, and Action. The table lists three components: 'pattern recognition receptor signaling pathway', 'toll-like receptor signaling pathway', and 'toll-like receptor 4 signaling pathway', all with the action 'increased'. A 'Table of Contents' sidebar on the right lists various sections of the page, including 'Key Event Title', 'Biological context', 'Cell Term', 'Organ Term', 'Key Event Components', 'Key Event Overview', 'AOPs including This Key Event', 'Stressors', 'Taxonomic Applicability', 'Life Stages', 'Sex Applicability', 'Key Event Description', 'How it is Measured or Detected', 'Evidence Supporting the Domain of Applicability', 'Evidence for Perturbation of This Molecular Initiating Event by Stressor', and 'References'.

Process	Object	Action
pattern recognition receptor signaling pathway		increased
toll-like receptor signaling pathway	Toll-like receptor	increased
toll-like receptor 4 signaling pathway	Toll-like receptor 4	increased

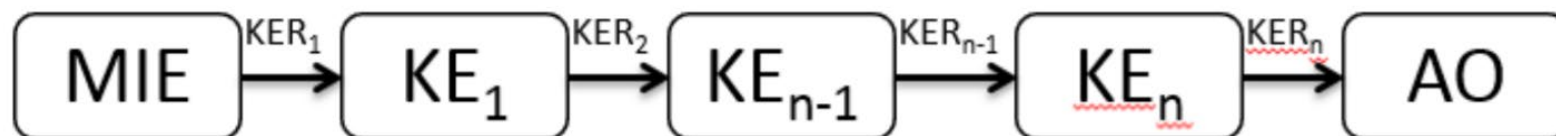
}

of biological

Key Event Components

-Important, as they make AOPs interoperable as they connect AOPs and their KEs to ontologies useful in e.g. bioinformatics analyses.

Construct your AOP



Important things to consider:

There will be an update of the whole AOPwiki in the beginning of July, which will include links to third party tools. These are valuable for exploring AOPs further. E.g. it will have links to the WikiPathways database, which presents bioinformatically employable AOPs.

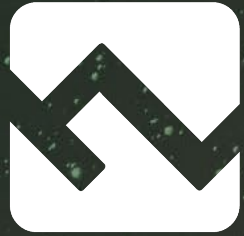
Thanks to:



Health
Canada

Santé
Canada

To you!



HARMLESS

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www.harmless-project.eu