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Adverse outcome pathways for grouping of nanomaterials

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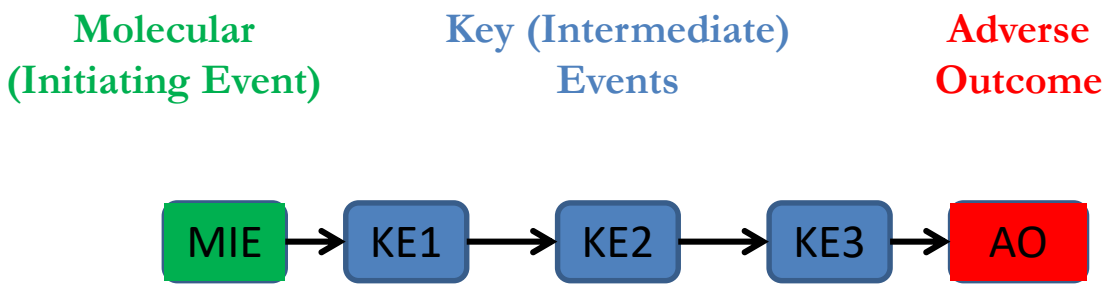
YOUR HEALTH AND SAFETY... OUR PRIORITY.

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, Environ. Toxicol. Chem., 29(3): 730-741).



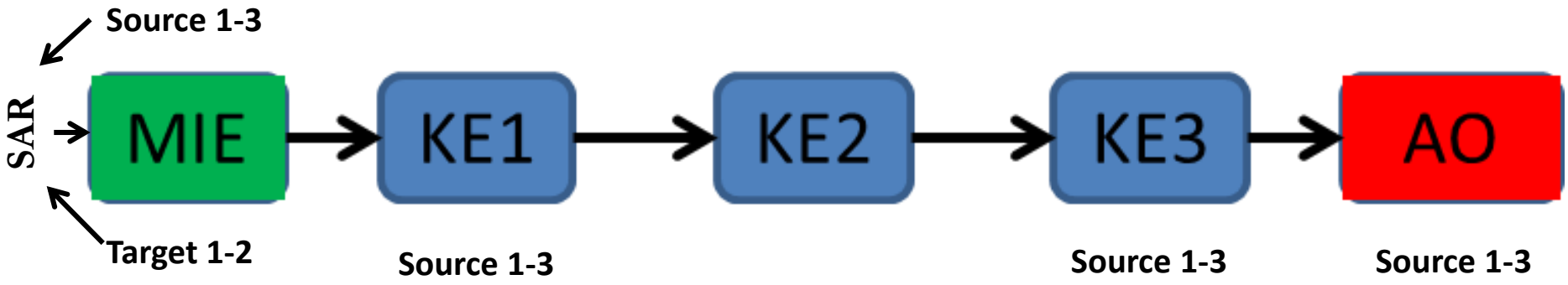
https://aopwiki.org/aops
AOP Knowledge Base
OECD's Series on Adverse Outcome Pathways
OECD AOP users' handbook

(Villeneuve et al 2014, Toxicological Sciences, 142(2), 312–320)

AOPs for categorising chemicals

Chemicals that activate the same AOP are grouped together

Example: Exposure to a group of (Source chemicals) chemicals will induce the same AO
A new chemical (Target chemical) that is structurally similar lacks data
An AOP is available, SAR is established for the MIE.
Both source and target chemicals induce SAR, thus assumed to induce the same AO



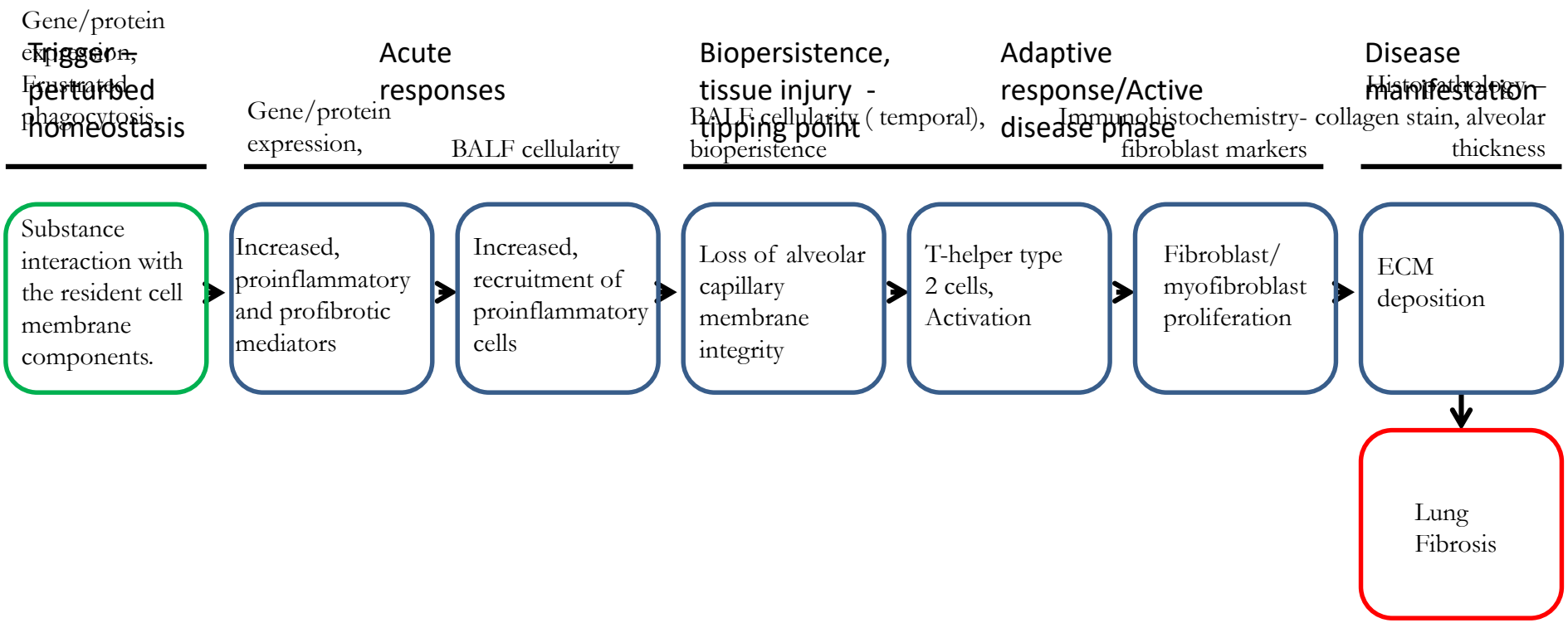
OECD(2017), Guidance document for the use of adverse outcome pathways in developing integrated approaches to testing and assessment (IATA), *Series on Testing & Assessment No. 260*, Environment, Health and Safety, Environment Directorate, OECD.

A putative/qualitative AOP for lung fibrosis

AOP 173: Substance interaction with the resident cell membrane components leading to lung fibrosis - <u>OECD EAGMS internal review completed</u>

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). Nano-risk Science: application of toxicogenomics in an adverse outcome pathway framework for risk assessment of multi-walled carbon nanotubes. *Particle and Fibre Toxicology*, *13*, 15.

Nikota, J., Banville, A., Goodwin, L. R., Wu, D., Williams, A., Yauk, C. L., … Halappanavar, S. (2017). Stat-6 signaling pathway and not Interleukin-1 mediates multi-walled carbon nanotube-induced lung fibrosis in mice: insights from an adverse outcome pathway framework. *Particle and Fibre Toxicology*, *14*, 37.

AOP for categorisation and read-across

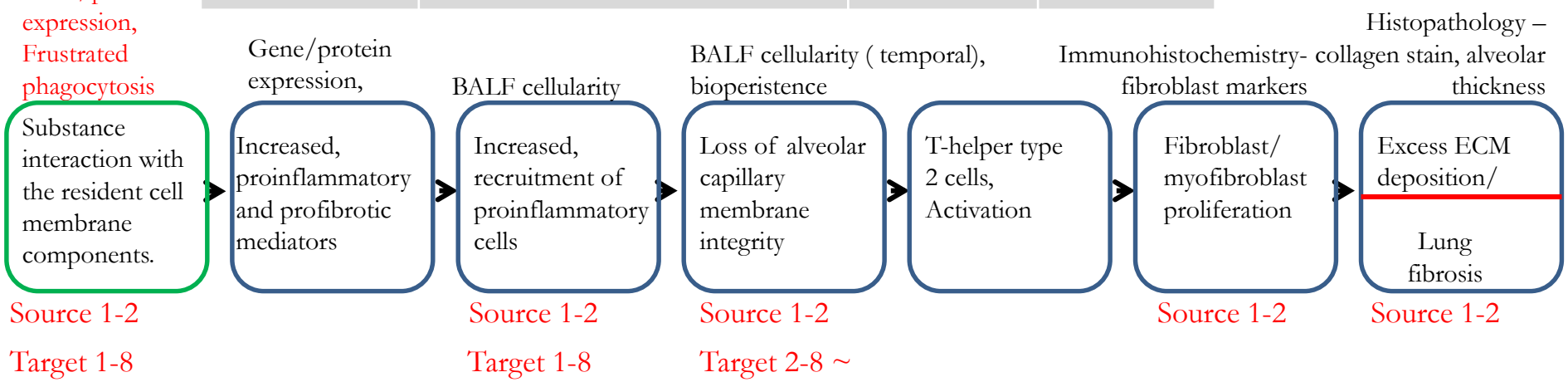
High aspect ratio materials (structural alert)

SAR CNTs, potentially fibrogenic to lungs upon inhalation

Experimental data available

Short → Thin

Target MWCNTs	Producer	CNT length (±SEM)	CNT diameter nm
1. NRCWE-026	Nanocyl (NC-7000)	847±102 nm	11
2. NRCWE-043	Cheap Tubes, Brattleboro, VT	771.3 (±3471)	26.73(±6.88)
3. NRCWE-044	Cheap Tubes, Brattleboro, VT	1330 (±2454)	32.55(±14.44)
4. NRCWE-045	Cheap Tubes, Brattleboro, VT	1553(±2954)	28.07(±13.85)
5. NRCWE-046	Cheap Tubes, Brattleboro, VT	717.2(±1214)	17.22(±5.77)
6. NRCWE-047	Cheap Tubes, Brattleboro, VT	532.5(±591.9)	12.96(±4.44)
7. NRCWE-048	Cheap Tubes, Brattleboro, VT	1604(±5609)	15.08(±4.69)
8. NRCWE-049	Cheap Tubes, Brattleboro, VT	731.1(±1473)	13.85(±6.09)



Source 1-2 Target 1-8

Source 1-2 Target 1-8

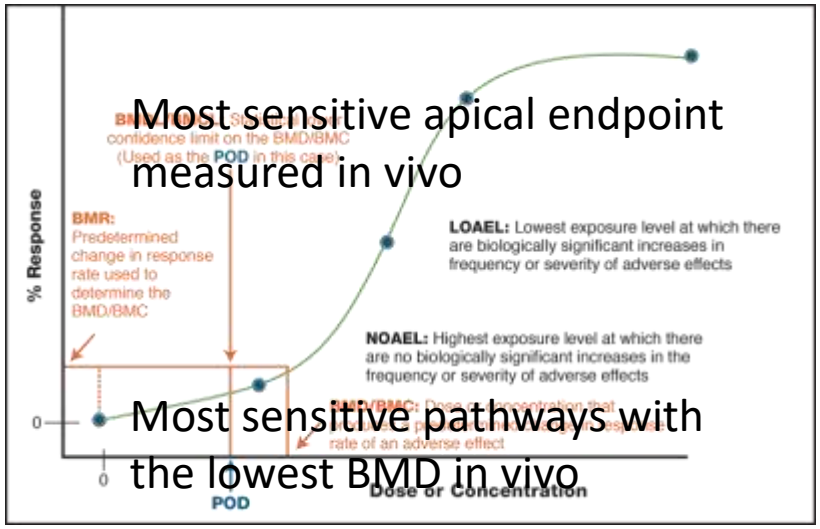
Source 1-2 Target 2-8 ~

Source 1-2

Source 1-2

Jackson et al, EMM, 56:183^203 (2015); Poulsen et al, Nanotoxicology, 2016; 10(9): 1263–1275

AOP for toxicity categorisation
Benchmark Dose (BMD)-response analysis



Most sensitive apical endpoint measured in vivo

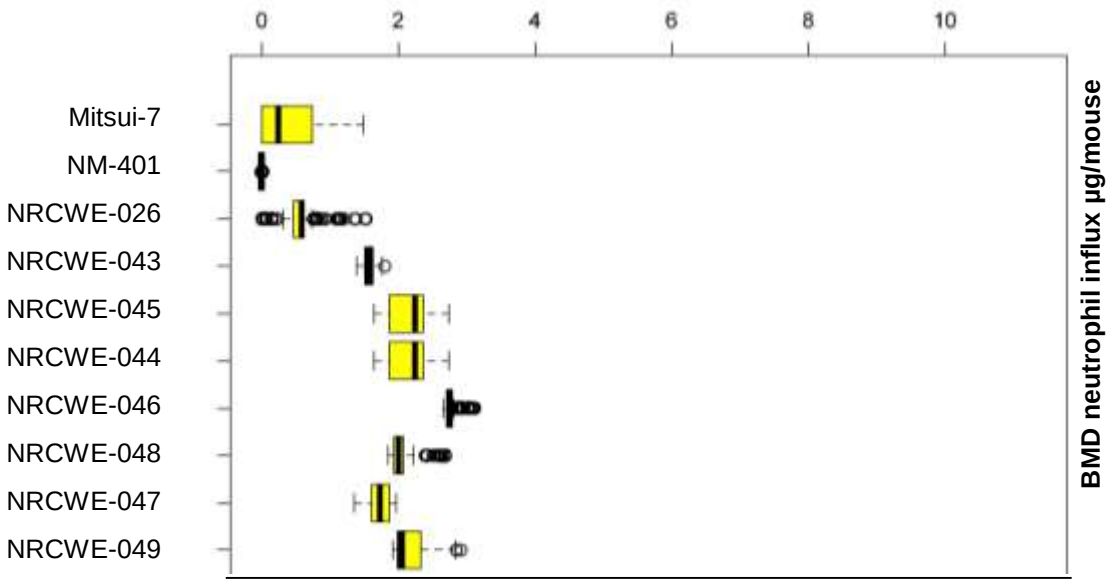
Most sensitive pathways with the lowest BMD in vivo

NCEA, USEPA (*USEPA 2010b*).

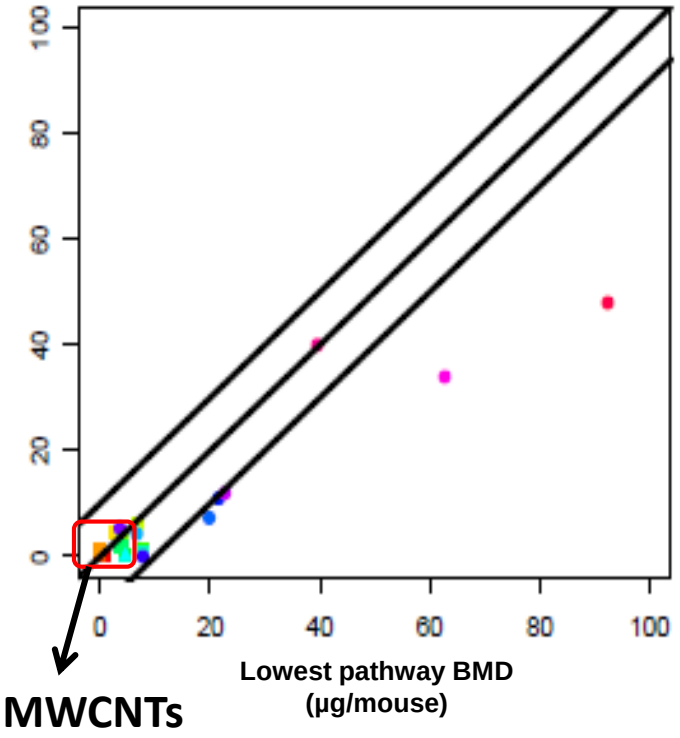
Apical endpoint BMD – pro-inflammatory cell influx in lung fluid – 24hr

Transcriptomics -pathway BMD 24hr (MIE)

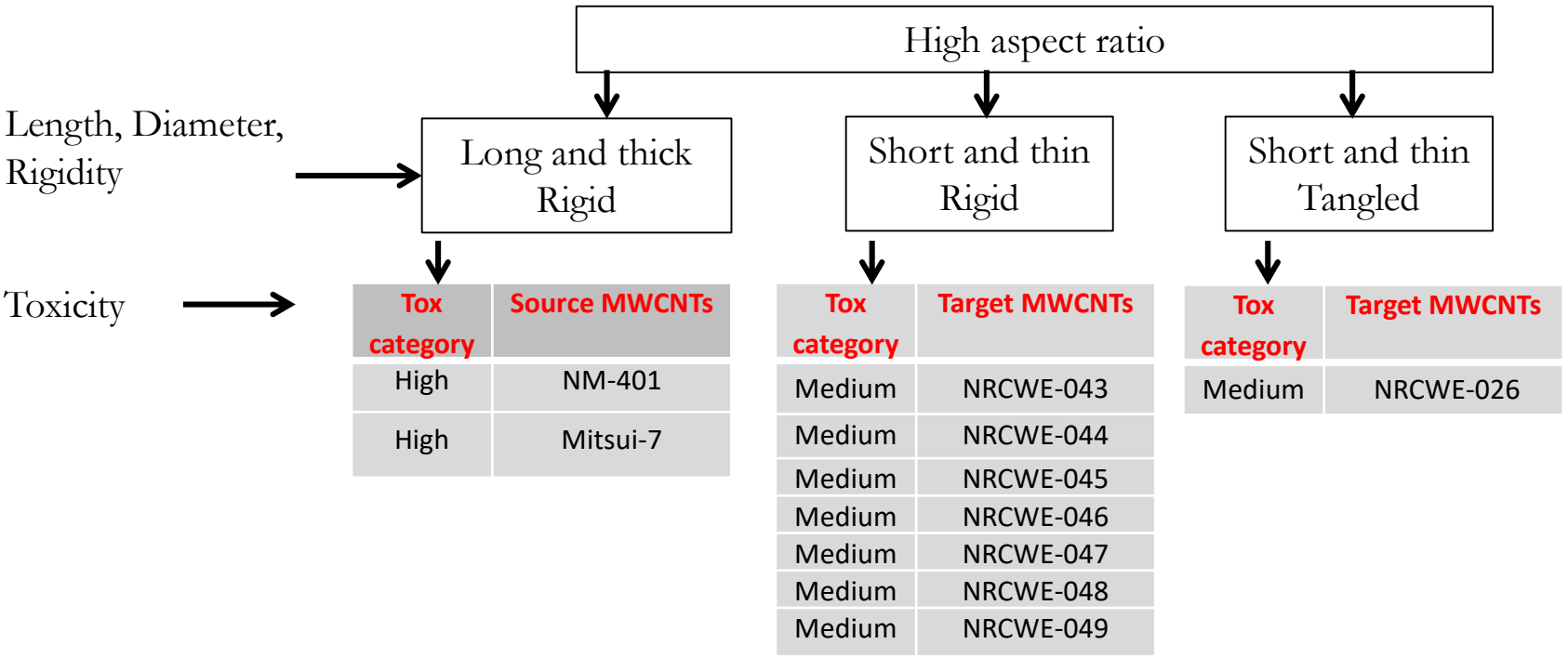
AOP for toxicity categorisation
Dose-response analysis



Transcriptional/pathway BMD
(Square Rooted)
Reflective of the MIE – substance interaction with the membrane components



AOP-guided categorisation or prioritisation (further testing)

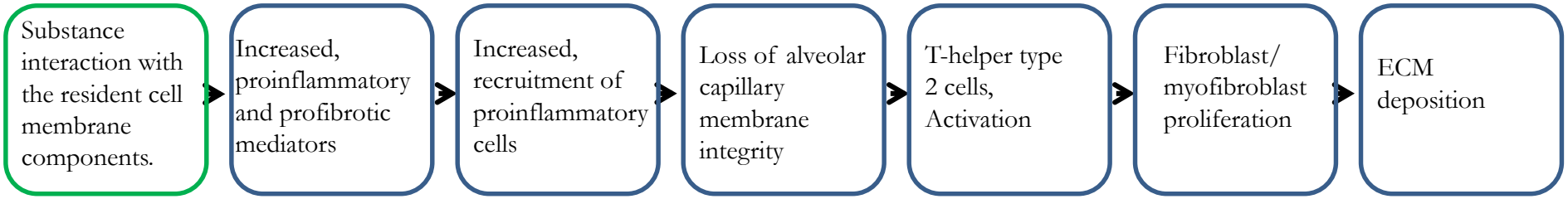


Tox category	Source MWCNTs
High	NM-401
High	Mitsui-7

Tox category	Target MWCNTs
Medium	NRCWE-043
Medium	NRCWE-044
Medium	NRCWE-045
Medium	NRCWE-046
Medium	NRCWE-047
Medium	NRCWE-048
Medium	NRCWE-049

Tox category	Target MWCNTs
Medium	NRCWE-026

AOP-aligned in vitro assays reflective of in vivo responses



Lysosomal uptake
DAMP release
Receptor signaling
Phys-chem
ROS synthesis

Pro-inflammatory cytokines
Fibrogenic factors
(Arg-1, IL1-β, TGFβ1, OPN)
Inflammosome activation

Th2 response genes/proteins

Fibroproliferation assays,
α-SMA
Sircol collagen assay
Hydroxyproline assay
Collagen genes I, III/proteins

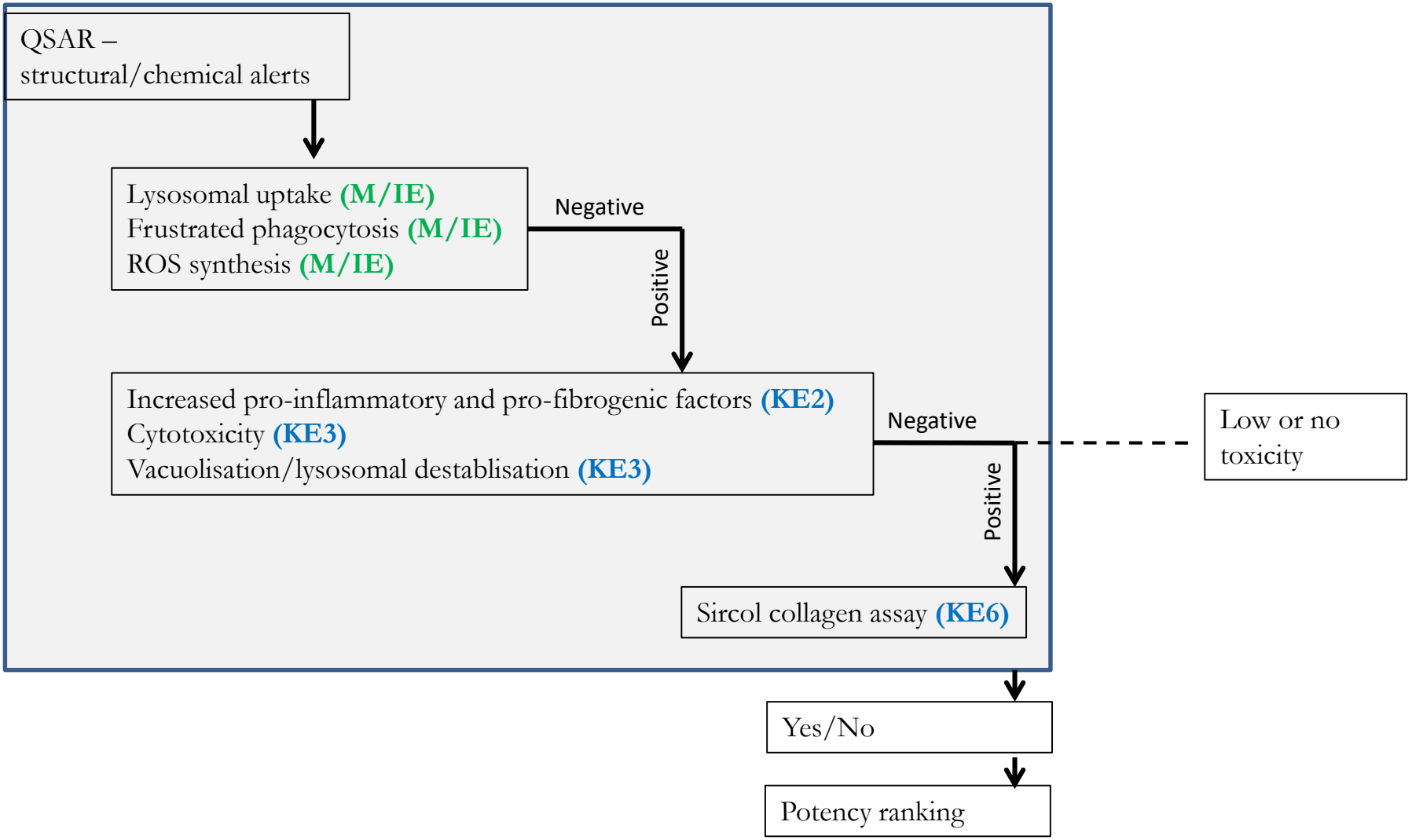
- Cell viability/cytotoxicity assays
- Colony forming/proliferation assay
- Loss of gap junctions
- Transepithelial electrical resistance– multi-cell type cultures
- Persistent ROS synthesis
- Lysosomal destabilisation
- Vacuolisation
- Imbalanced proteases/antiprotease
- TNF α, IL-1 β

Tiered -
Combinations -
Defined approaches -
–

Cell models
Cell types

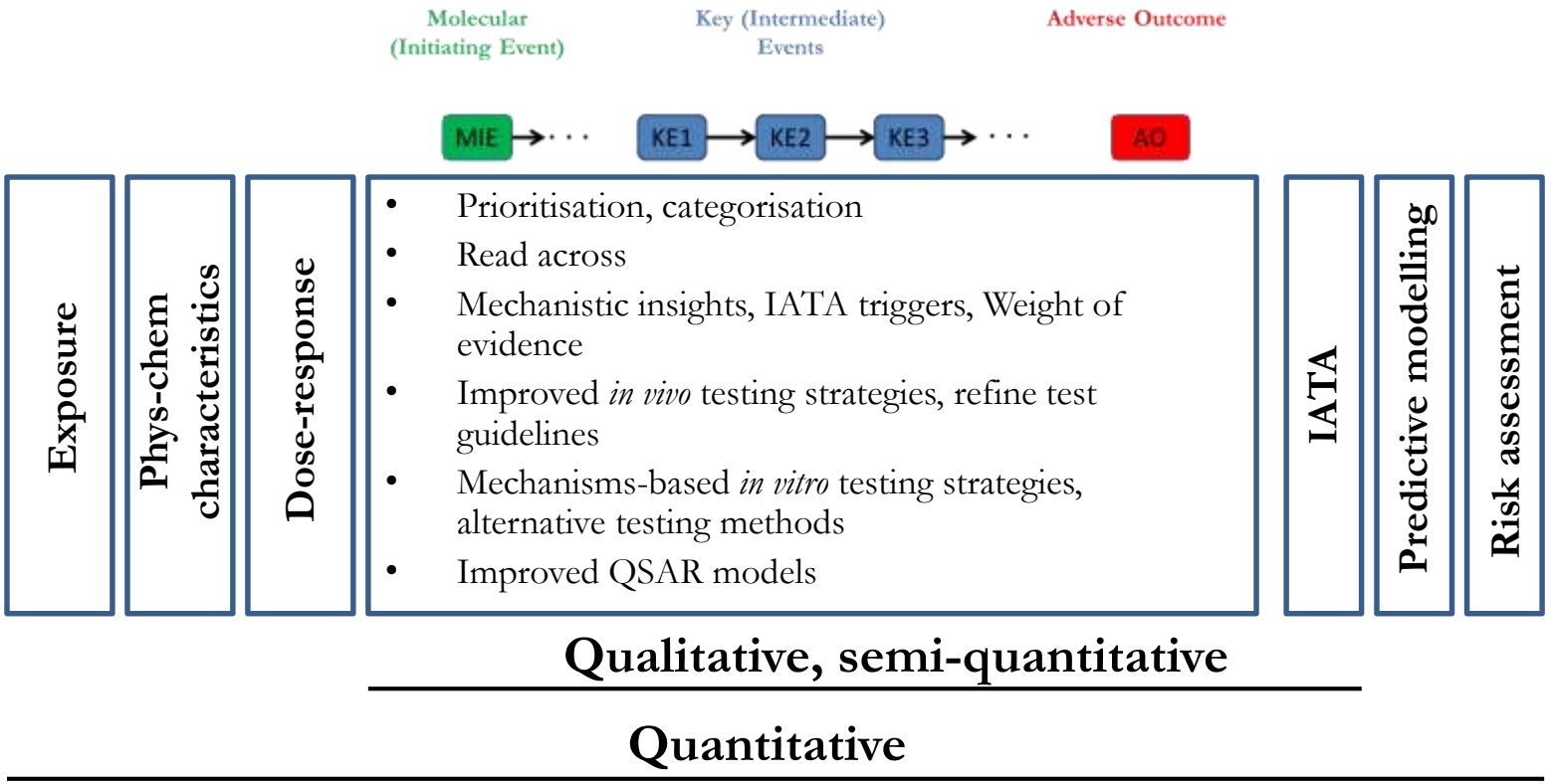
Halappanavar et al., in preparation

AOP-aligned in vitro assays reflective of in vivo fibrogenic responses in lungs
Decision tree
IATA



Halappanavar et al., in preparation

Conclusions



For further reading, refer to 'Validation of Alternative Methods for Toxicity Testing', Chantra Eskes, Maurice Whelan (eds). Advances in Experimental Medicine and Biology 856, Springer.

Acknowledgements

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