NTP
National Toxicology Program

Integrating Alternative Methods within a Regulatory Framework to Replace Animals in Inhalation Toxicity Testing

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SOT 2016

Outline

- Overview of the regulatory landscape
- Incorporating alternative methods within the current regulatory framework
- Using AOPs to design integrated approaches for inhalation toxicity testing

NICEATM

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), organized as an office under the NTP Division, part of NIEHS

ILS provides technical support for NICEATM under an NIEHS contract

- ICCVAM support
- Tox 21 validation support
- International harmonization efforts

Disclaimer

- *The views expressed in this presentation are those of the author and do not necessarily represent the views of any Federal Agency*.
- *No conflict of interest*

Inhalation Toxicology

- Need acute and repeated-dose inhalation toxicology studies
  - Complexity of the respiratory system and the diversity of local and systemic responses.
  - Often, lungs are the main route of exposure but not the main target (toxicity manifested in other organs; e.g., liver, spleen, kidney).
- Exposure to airborne particles is gaining more and more importance due to the ubiquitous application of these particles in the field of industry, pharmacy and in daily life.
- Products applied in form of sprays or powders considered as particularly harmful.
  - Industry and electrical, consumer, and medical applications.
  - Ex: nanosprays for shoe care, cleaning agents, antibacterial sprays, or during technical processing of plastics
- Hazard evaluation requires extensive, technically complex and expensive inhalation toxicology studies that are usually generated in animal experiments

Identifying Pulmonary Toxicants

- Human data are typically limited to accidental exposure.
  - Unpredictable; actual exposure uncertain
  - Pre-existing conditions?
  - Not representative of broader population
- Animal studies
  - EPA and OECD test guidelines
  - Large number of animals and $$$
- In vitro models
  - Many are human cell-based
  - Amenable to higher throughput evaluation
- In silico models
  - QSAR; read across

www.battelle.com

Test Guidelines for In Vivo Acute Inhalation Toxicology

	EPA OPPTS 870.1300	OECD TG 403	OECD TG 436	Draft OECD TG433
Limit test Concentration Duration	2 mg/L 4 hrs	20000 ppm (gas) 20 mg/L (vapor) 5 mg/L (aerosol) 4 hrs	20000 ppm (gas) 20 mg/L (vapor) 5 mg/L (dust/mist) 2 mg/L (aerosol) 4 hrs	5000 ppm (gas) 20 mg/L (vapor) 5 mg/L (dust/mist) 4 hrs
# Dose Groups/n (Main Study)	3/n=5 per sex	**Traditional**: 3/n=5 per sex **C x t**: 4 or 5 at multiple durations/n=1 per sex (or 2 of more susceptible sex)	≥1/n=3 per sex (or n=6 of more susceptible sex)	≥1/n=5 (most susceptible sex based on sighting study)
Recommended route (NOTE: not required)	Nose only	Nose only	Nose only	Nose only
Observation period	14 days	14 days	14 days	14 days
Observations	Daily clinical obs; weekly body weight; TOD; gross necropsy (optional histo)	Daily clinical obs; body weight on d0, 1, 3, 7, weekly; TOD; gross necropsy	Daily clinical obs; body weight on d0, 1, 3, 7, weekly; TOD; gross necropsy	Daily clinical obs; body weight on d0, 1, 3, 7, weekly; TOD; gross necropsy (optional histo); Evident toxicity
MMAD range	1-4 µM	1-4 µM	1-4 µM	1-4 µM
Notes	Covers entire range of the concentration-mortality relationship – LC50 point estimate	Covers entire range of the concentration-mortality relationship – LC50 point estimate; C x t can derive AEGLs; Better estimates of toxicity at upper and lower exposure concentration boundaries	Refinement and reduction – serial steps/fixed concentrations; LC50 range estimate	Refinement alternative by including evident toxicity TG not adopted yet

Acute Inhalation Toxicity Hazard Categories

GHS & OSHA	Category 1 DANGER	Category 2 DANGER	Category 3 DANGER	Category 4 WARNING
Gases (ppm/V)	**≤ 100**	**>100 ≤ 500**	**>500 ≤ 2500**	**>2500 ≤ 5000**
Vapors (mg/L)	**≤ 0.5**	**>0.5 ≤ 2.0**	**>2.0 ≤ 10**	**>10 ≤ 20**
Dusts and Mists (mg/L)	**≤ 0.05**	**>0.05 ≤ 0.5**	**>0.5 ≤ 1.0**	**>1.0 ≤ 5**

EPA	Category I	Category II	Category III	Category IV
Acute Inhalation	**≤ 0.05 mg/L**	**>0.05 thru 0.5 mg/L**	**>0.5 thru 2 mg/L**	**> 2 mg/L**

- Precautionary labeling based on hazard categories
  - First aid statement in case of accidental exposure
  - Identifies the precautions necessary to avoid exposure; personal protective equipment (PPE)

Sub-acute and Sub-chronic Inhalation Toxicity

- 28-Day Study (OECD): Determine the NOEL and any toxic effects associated with continuous or repeated (28-day) exposure to a test substance
- Provides information on target organs and possibilities of accumulation
- 6 hour/day exposure in rats for 28 days
- Recommend nose only for aerosols
- 3 concentrations, at least **5 rats/group/sex**
- Can be used for quantitative risk assessment (in absence of 90-day study)
- Dose selection for 90-day
- Limit concentration
  - OECD: 20000 ppm (gas); 20 mg/L (vapor); 5 mg/L (aerosol)

- 90-Day Study (**EPA & OECD**): Determine the NOEL and any toxic effects associated with continuous or repeated (90-day) exposure to a test substance
- Provides information on target organs and possibilities of accumulation
- 6 hour/day exposure in rats for 90 days
- Recommend nose only for aerosols
- 3 concentrations, at least **10 rats/group/sex**
- Can be used for quantitative risk assessment
- Dose selection for chronic study
- Limit concentration
  - EPA: 1 mg/L
  - OECD: 20000 ppm (gas); 20 mg/L (vapor); 5 mg/L (aerosol)

Possibilities for Waiving a Study

- Mixtures or formulated products
  - Substantially similar to well-characterized mixtures/products
- Severe local irritation and corrosivity
- Low volatility
  - Non-volatile actives not aerosolized or otherwise made inhalable as a gas or vapor.
  - Not typically for formulations - ultimately diluted/applied for potential inhalation exposure
- Particle size

Impact of Particle Size

- Inhalable Fraction
  - The fraction of total airborne particles that enter the body through the nose and/or mouth during breathing (≤ 100 µm)
    - Relevant to health effects anywhere in the respiratory tract and systemic effects
- Thoracic Fraction
  - Subfraction of inhalable particles that can penetrate into the tracheo-alveolar region (< 30 µm)
    - Important for asthma, bronchitis, and lung cancer
- Respirable Fraction
  - Subfraction of inhalable particles that penetrate into the alveolar region (≤ 10 µm)
    - Chronic respiratory diseases: e.g., emphysema
- Non-inhalable aerosol: ≥99% of the particles are >100 µm in diameter
- Friable material – attrition study?

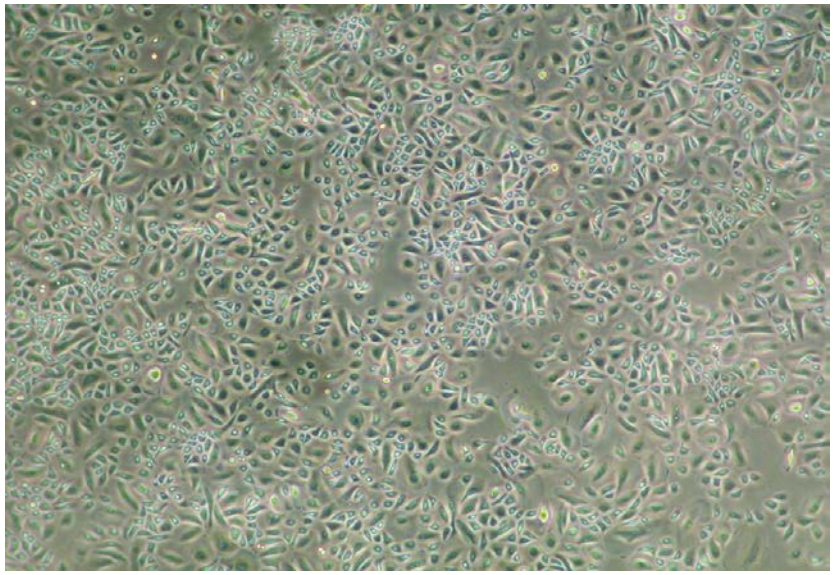
Slide courtesy of Jon Hotchkiss

Moving to Non-Animal Approaches

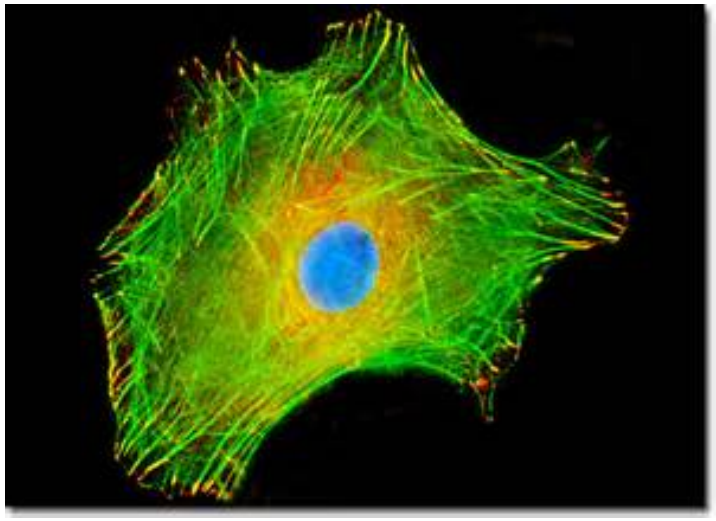
- Need fast and efficient screening tests
- Versatility, simplicity and still do the job
  - Realistic in simulating the human organ of interest
    - One challenge for inhalation is the fact that the target cell population (and therefore the cell types used in the in vitro model) changes along the length of the respiratory tract
  - Able to reproduce findings of in vivo studies
- Can create diseased models
- Impact on the 3R’s

Simple In Vitro Systems

- Monolayer cultures/co-cultures
- Primary cells and cell lines
- Homo- or hetero-geneity
- Variable life-span in culture
- Phenotypic differentiation varies



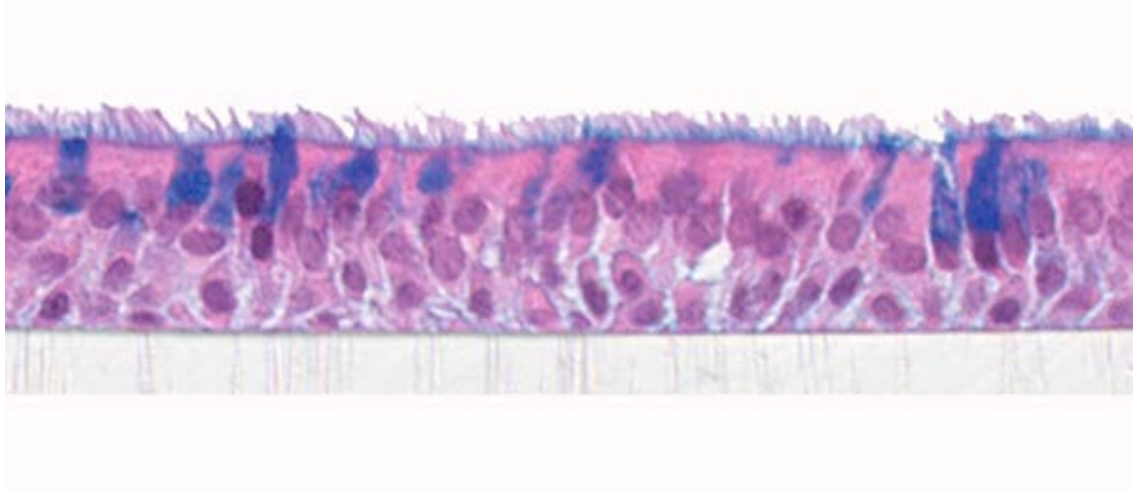
Primary human large airway epithelial cells (cellntec.com)



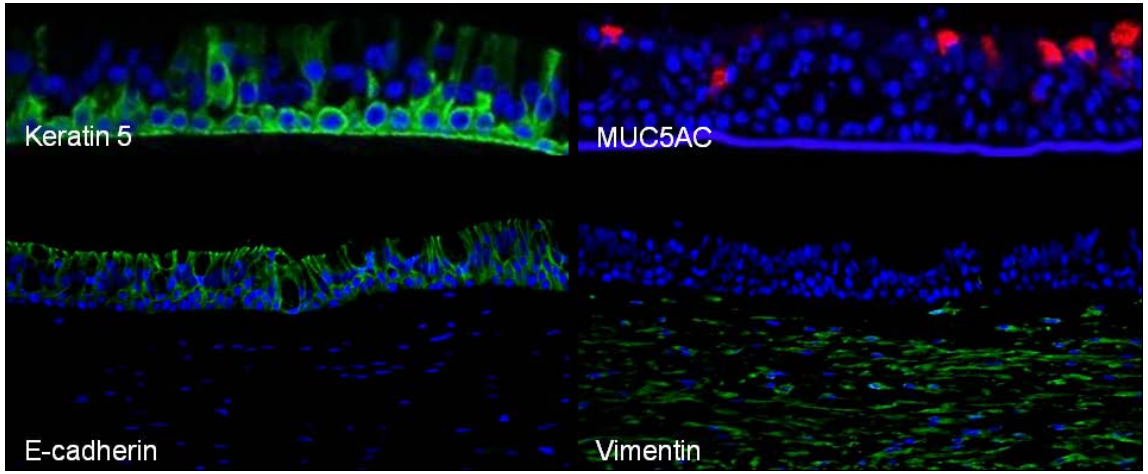
A549 cell (micro.magnet.fsu.edu)

3D Airway Epithelium Models

- Typically from primary cells
- Can be grown from normal or diseased tissues
- Harvested from multiple anatomical sites
- Human relevant tissue structure and cellular morphology
- Barrier function and mucociliary responses maintained



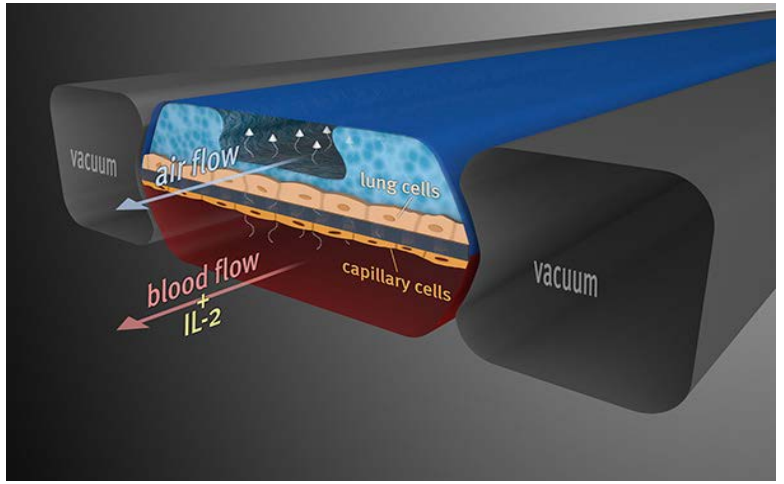
www.epithelix.com

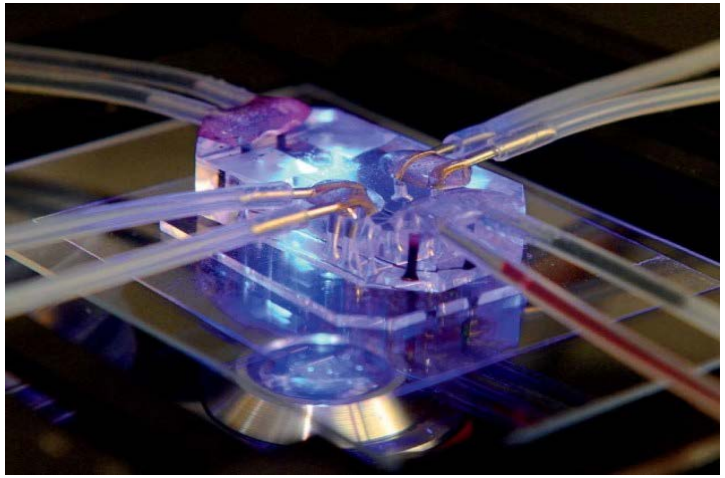


www.mattek.com

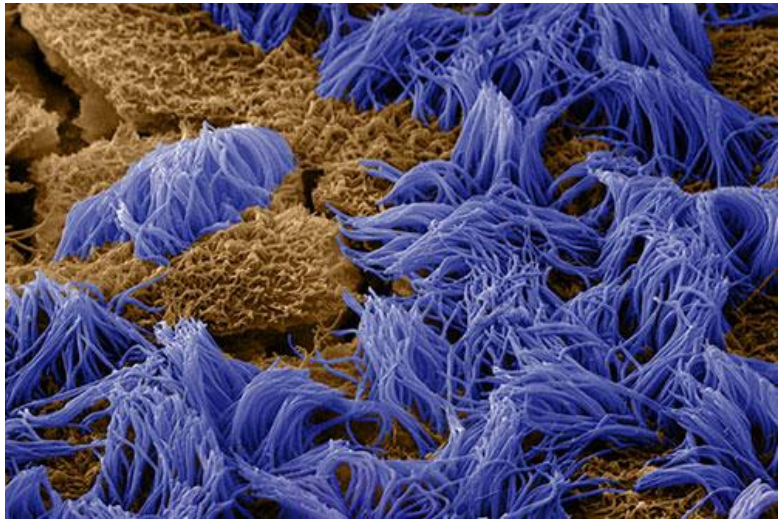
Microfluidic Systems

- Complex 3 dimensional multi-cellular models
- Interactions among multiple tissue types
- Translocation and distal target effects
- Diseased models
- Models based on sensitive subpopulations

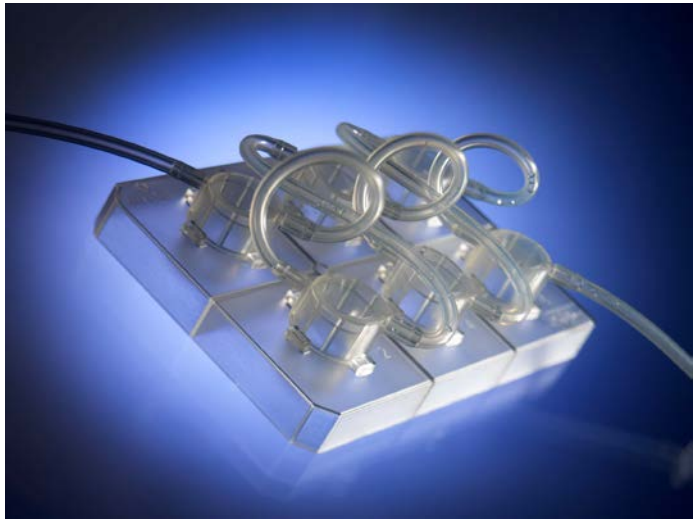




from Harvard Magazine, Jan-Feb 2016

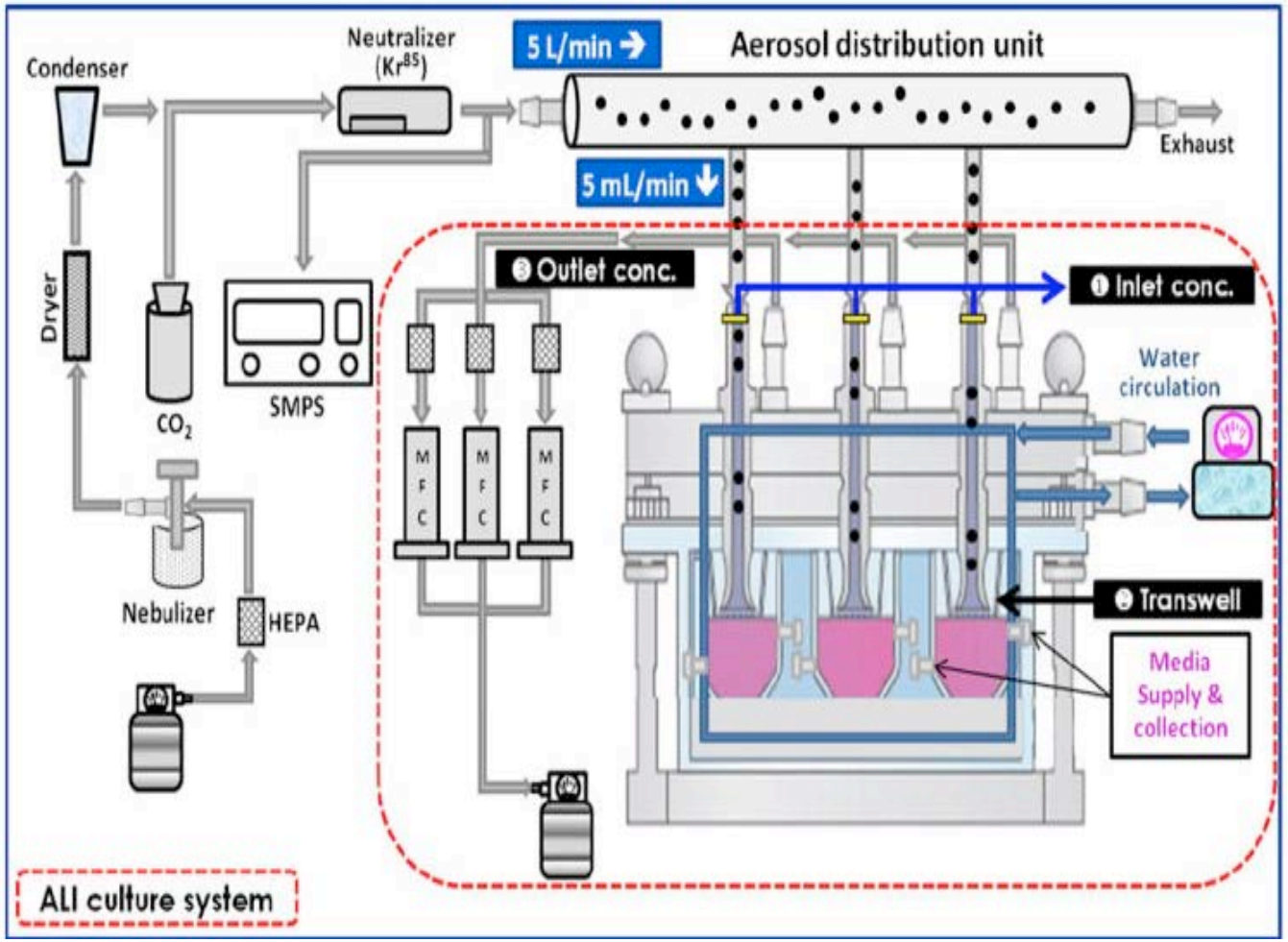


Wyss Institute at Harvard University



www.kirkstall.org

Exposure Systems



- Everything we know about generating/controlling/monitoring in vivo exposures is directly applicable to in vitro exposure system, **plus**:
  - May get better estimates of deposited dose of aerosols and vapors/gases
  - Can evaluate basic TK endpoints (e.g., parent concentration and any locally formed metabolites in the basolateral media)

Toxicol In Vitro. Feb 2013; 27(1): 164–173.

Exposures – System Feature Needs

Physics of Delivery

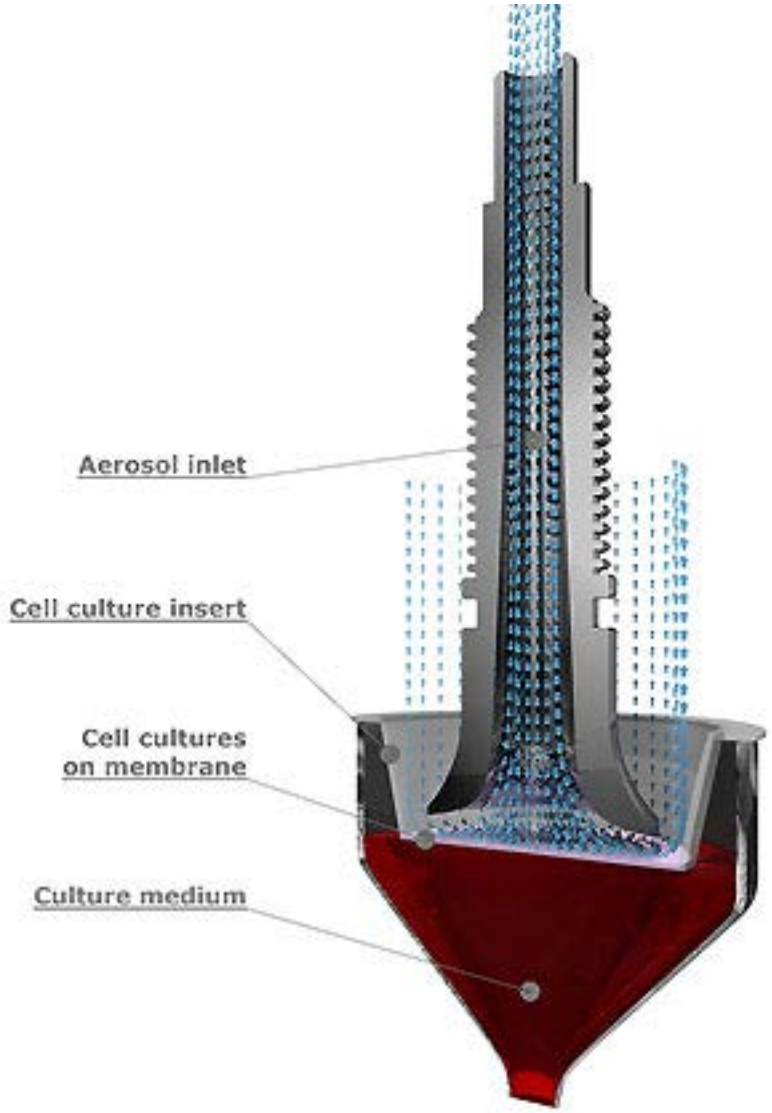
- Deposition of aerosol
- Accurate/Precise/Reproducible Delivery
  - To the chamber
  - To the cells
- Sampling Capability

Protocol Design

- # of replicates / doses needed
- QA/QC compatibility
- Containment of Hazards

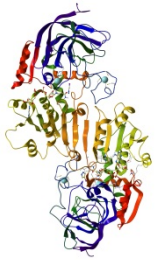
Biological Suitability

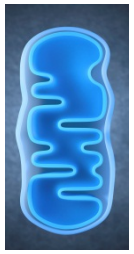
- Size of insert must generate sufficient sample
- Flexibility
- Maintenance of Cells – long term ALI culture
  - Sterility

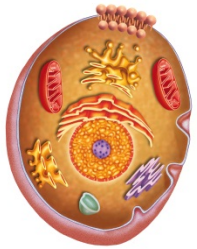


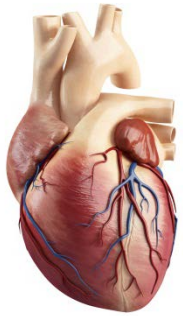
www.vitrocell.com

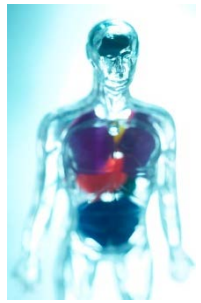
Adverse Outcome Pathway (AOP)







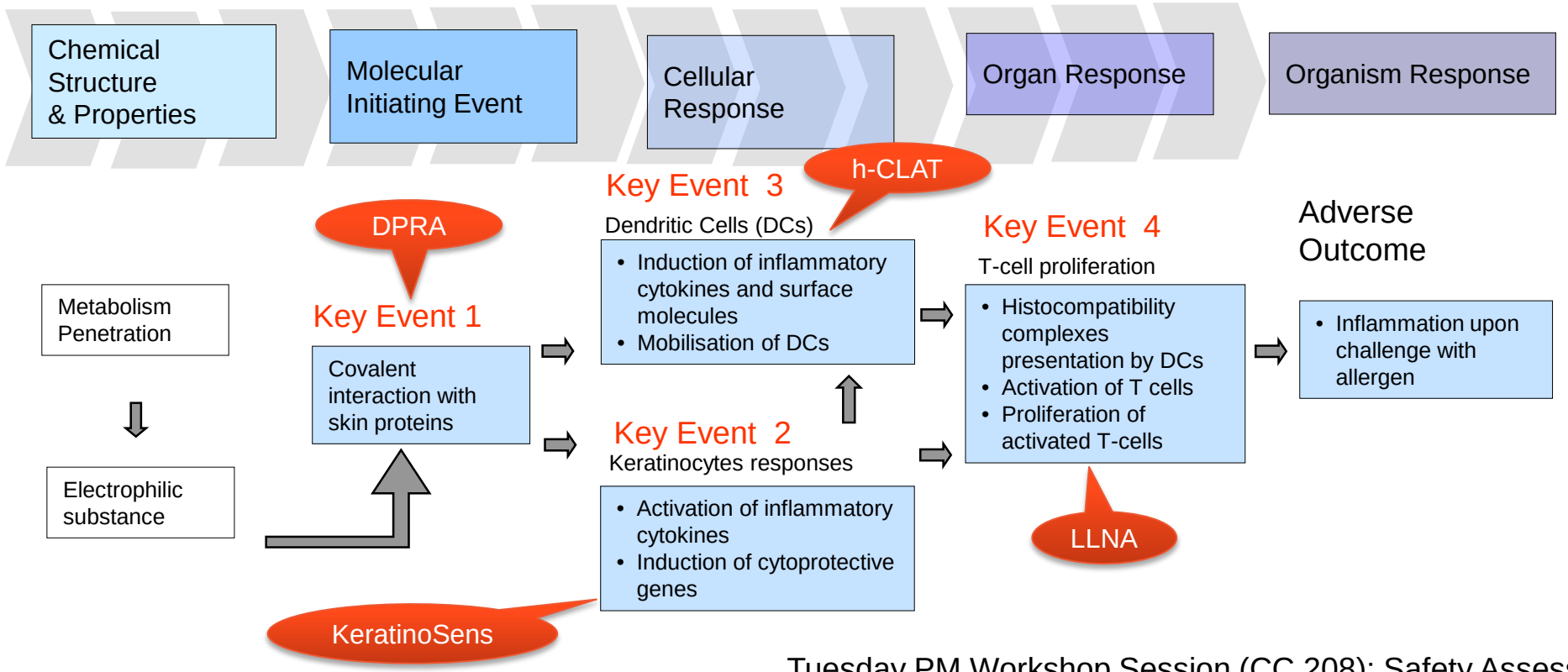




Exposure | Molecular Interaction | Organelle Effect | Cellular Effect | Organ Effect | Individual Effect | Population Effect

Slide courtesy of Donna Mendrick, FDA-NCTR

A Model of Success: OECD AOP for Skin Sensitization

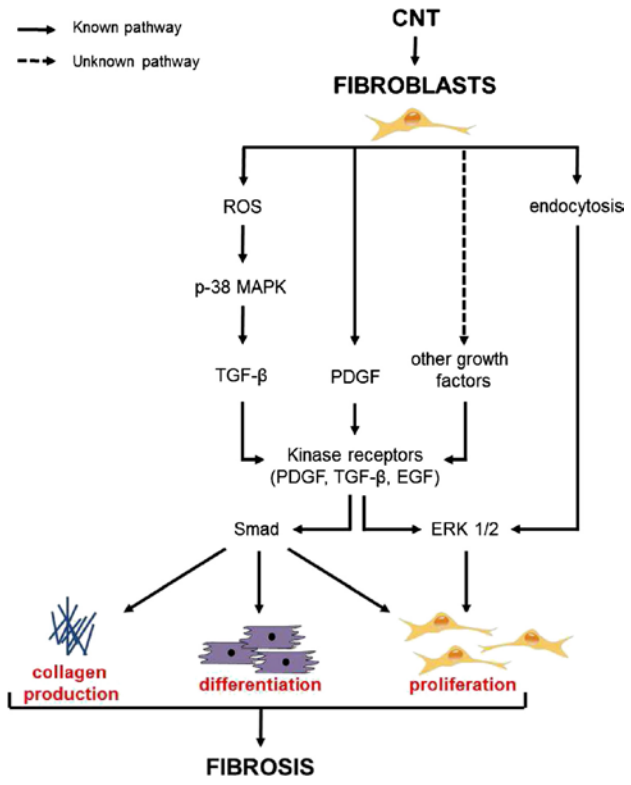


Tuesday PM Workshop Session (CC 208): Safety Assessment of Topically Exposed Cosmetic Ingredients: Lessons Learned
Abstract Number 2533: Kleinstreuer N, Skin Sensitization Testing Strategy Evaluation

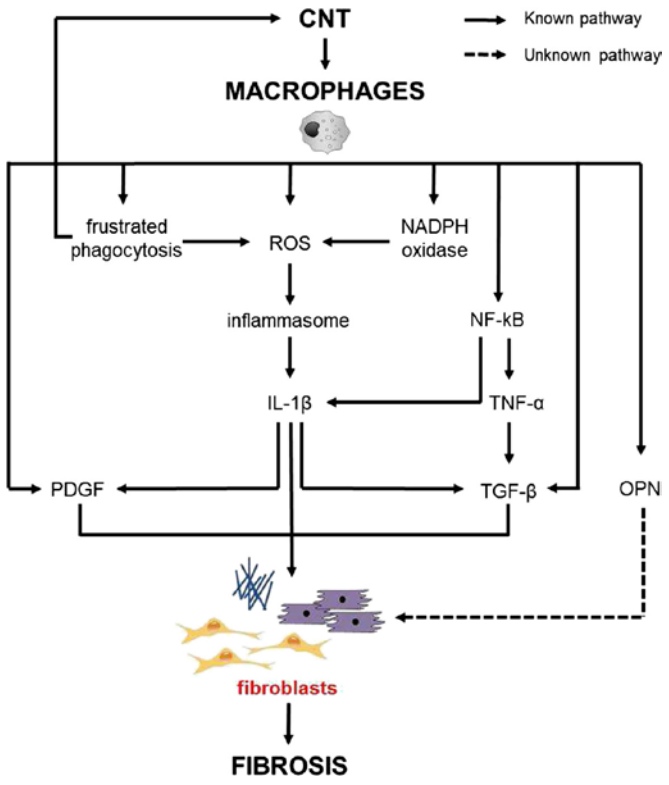
Tuesday PM Poster Session, **Abstract Number 2202**: Strickland et al., Multivariate Models for Prediction of Human Skin Sensitization Hazard
Poster Board Number P132

http://www.oecd.org/chemicalsafety/testing/seriesontestingandassessmentpublicationsbynumber.htm

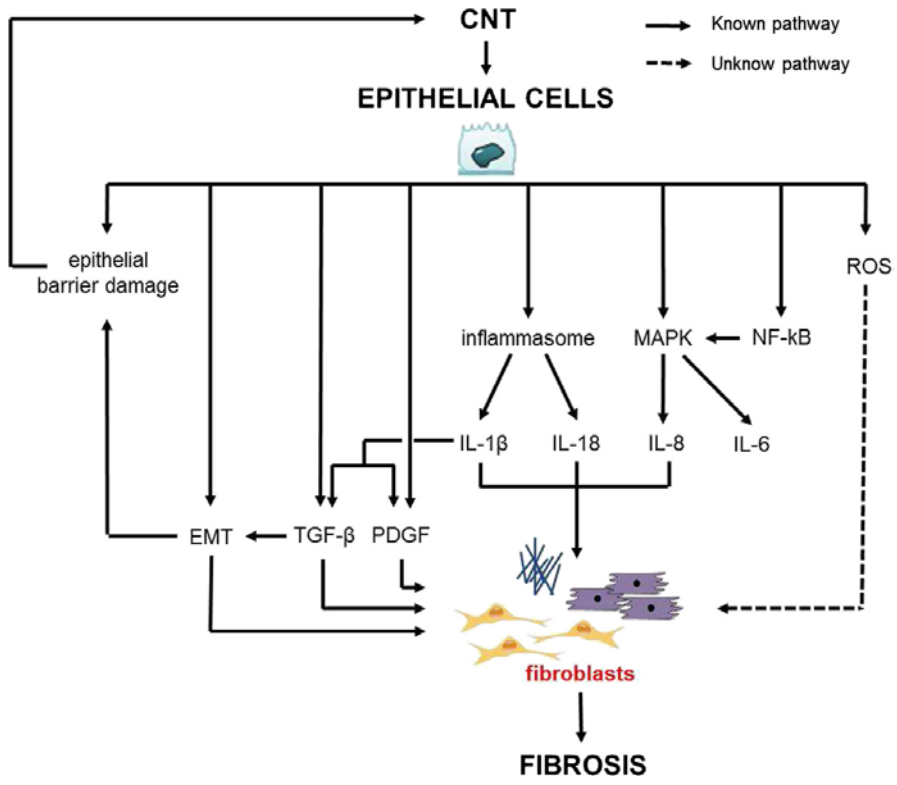
CNT and Lung Fibrosis: Multiple Mechanisms



Direct effects on fibroblasts



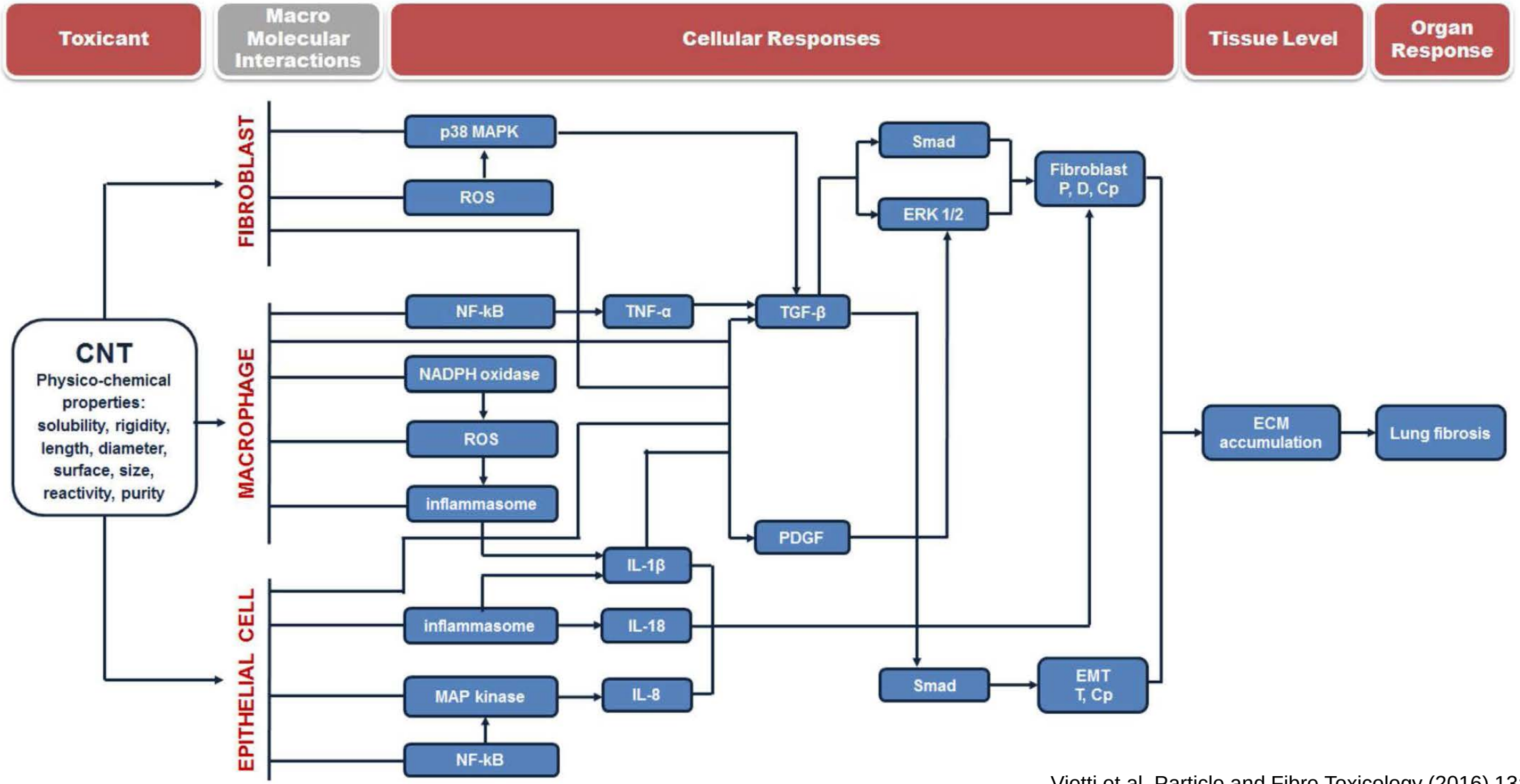
Indirect effects via macrophages



Indirect effects via epithelial cells

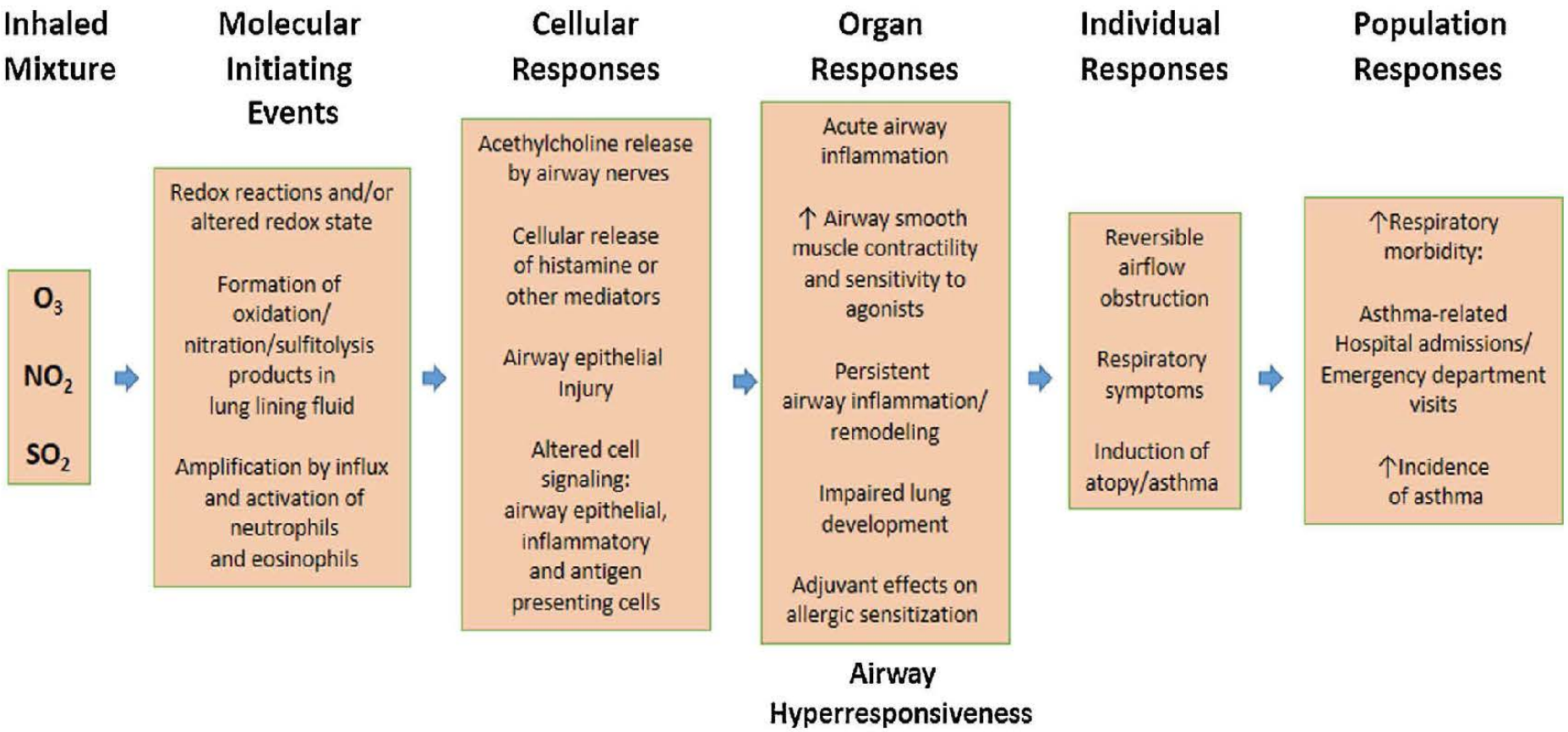
Vietti et al. Particle and Fibre Toxicology (2016) 13:11

AOP for CNT and Lung Fibrosis



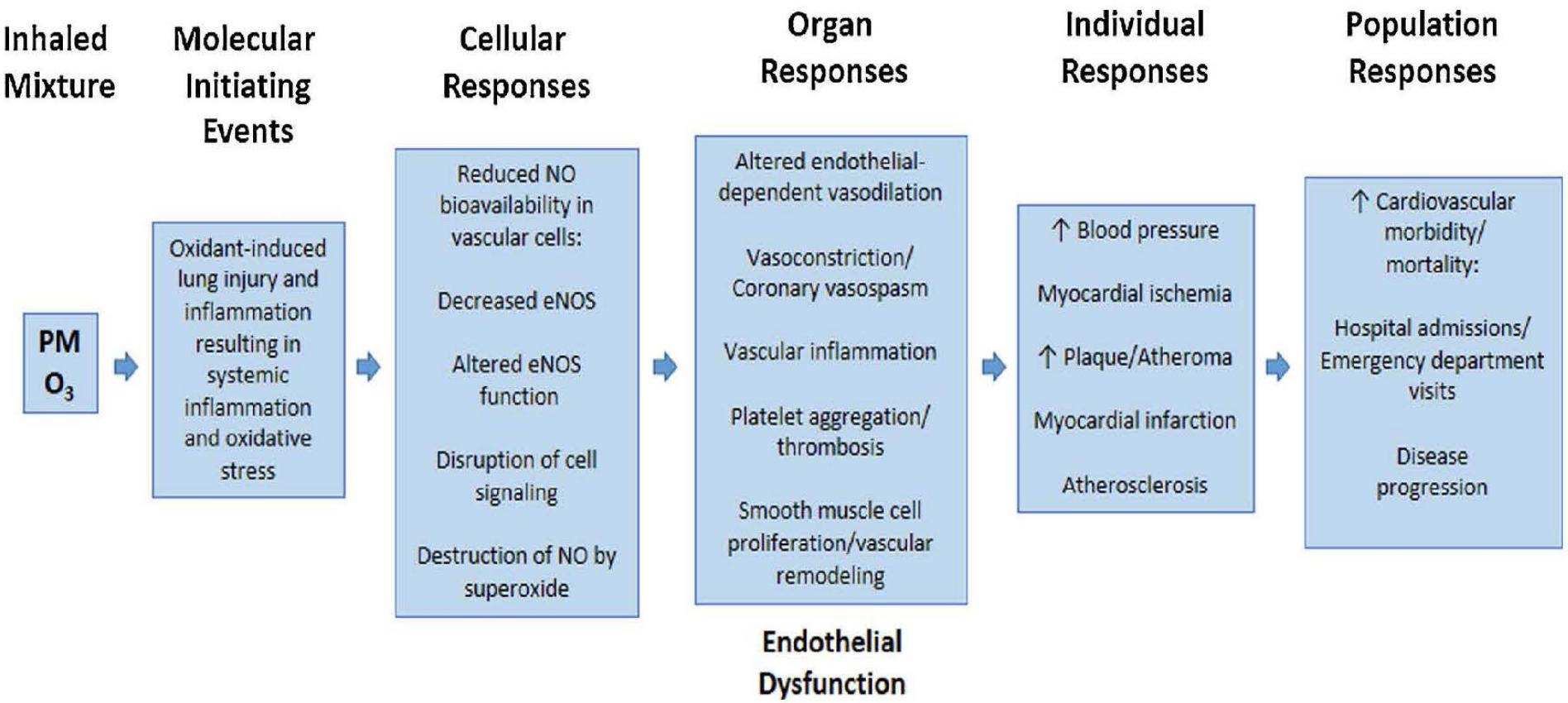
Vietti et al. Particle and Fibre Toxicology (2016) 13:11

AOP: Air Pollution Mixtures and Respiratory Outcomes



from Buckley and Farraj (2015). Toxicology 335:85–94

AOP: Air Pollution Mixtures and Cardiovasc. Outcomes



from Buckley and Farraj (2015). Toxicology 335:85–94

The Future is Bright

- Alternatives for inhalation toxicology are being developed and applied
  - In vitro systems recapitulate much of respiratory biology
  - In silico approaches leverage existing information to predict outcomes
  - Lung on a chip models may one day provide a complete solution
- AOPs can guide research to focus on relevant mechanisms of action or investigate underlined knowledge gaps
- AOPs can be used to systematically develop IATAs
  - Short-term goal: Limit the necessity of subsequent in vivo testing
  - Long-term goal: Stand alone to identify pulmonary toxicants without in vivo testing

Thank You!

- Jon Hotchkiss, Dow Chemical Company
- Amy Clippinger, PETA International Science Consortium
- John Redden, EPA-OPP
- Bill Polk, Olympus (formerly ILS-NICEATM)
- Warren Casey, NICEATM