Update for PPDC: Animal Alternatives

Reducing Animal Use in Pesticide Testing:
Update on avian retrospective analysis

Moving Towards In Vitro & Computational Approaches:
Skin sensitization & inhalation risk assessment

Anna Lowit, Ph.D.
703-308-4135 (w)
703-258-4209 (c)
Lowit.anna@epa.gov

Monique Perron, ScD.
703-347-0395 (w)
perron.monique@epa.gov

Melissa Panger, Ph.D
703-305-6136 (w)
panger.melissa@epa.gov

Background: Toxicity Testing in the 21st Century

- EPA's Office of Pesticide Programs has developed a Strategic Direction for New Pesticide Testing and Assessment Approaches
  - https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-21st-century-science
  - A broader suite of computer-aided methods to better predict potential hazards and exposures, and to focus testing on likely risks of concern;
  - Improved approaches to more traditional toxicity tests to minimize the number of animals used while expanding the amount of information obtained;
  - Improved understanding of toxicity pathways to allow development of non-animal tests that better predict how exposures relate to adverse effects.

Guiding Principles for Data Needs for Pesticides

- Guiding Principles for Data Requirements
  - Purpose: provide consistency in the identification of data needs, promote and optimize full use of existing knowledge, and focus on the critical data needed for risk assessment.
  - http://www.epa.gov/pesticides/regulating/data-require-guide-principle.pdf
- "…ensure there is sufficient information to reliably support registration decisions that are protective of public health and the environment while avoiding the generation and evaluation of data that does not materially influence the scientific certainty of a regulatory decision…."
- "…avoid unnecessary use of time and resources, data generation costs, and animal testing."

Data Needs for Pesticides

- Flexibility in implementing Part 158 data requirements (§158.30):
  - Waivers may be granted as permitted by 40 CFR Part 158.45;
  - Additional data beyond the 158 data requirements may be important to the risk management decision (§158.75), alternative approaches can be accepted, and other data can be used.

Reducing Animal Use in Pesticide Testing:
Update on avian retrospective analysis

Avian Subacute/Acute Risk Retrospective Comparison Project (Background and Questions Asked)

- Background
  - 40 *CFR* Section 158 outlines two requirements for avian acute effects testing
    - Two single oral dose LD50 studies (commonly quail or mallard and a songbird)
    - Two subacute dietary LC50 studies (commonly quail and mallard)
  - Pesticide risk assessments conduct estimation of risk quotients using BOTH lethal effects study types using the most sensitive endpoint from each type of study
  - EPA and PETA collaborated on a retrospective analysis of avian risk assessments
- Questions Asked: **Can we confidently assess acute risk for birds using a reduced suite of effects studies focusing on the single oral dose protocol?**
  - How often have subacute dietary risk quotients (RQs) quantitatively driven risk assessment conclusions?
  - How often have subacute dietary risks qualitatively altered the risk conclusions?

Avian Subacute/Acute Risk Retrospective Comparison Project (Methods)

- Focus on risk assessment outcomes not effects data
  - Integrates the effects of both toxic potency and exposure assessment
  - Allow for a differentiation (if any) in conclusions relative to surrogate bird size and exposure media (food type)
- Establishment of evaluation data set
  - Focused on pesticide actives newly registered through RD for the years 1998-2016
  - Most recent classes of pesticides
  - Review most recent publicly available risk assessment
  - Determine mode of action for each pesticide (publicly available sites)
- From each risk assessment:
  - Extract and compare the single oral dose- and dietary-based risk quotients
  - Summarize any risk characterization qualitative discussion of dietary-based risk estimates

Avian Subacute/Acute Risk Retrospective Comparison Project (Results)

- EPA identified **181** pesticides new to the Agency from the annual reports from 1998 to 2016.
- **119** chemicals had ecological risks assessments available to PETA for analysis.
  - **79** of the chemicals did not have RQ values calculated so a difference between dietary and oral RQs was moot (**dietary RQ had no impact**)
    - **70** of these were Limit test results for both diet and oral endpoints (**there was no difference in risk prediction for dietary or oral**),
    - **9** were non-standard assessments (indoor, greenhouse, or piggy back assessments)
  - **40** of the chemicals had RQ values presented for comparison
    - **37** cases **oral RQ dominated dietary and drove the assessment,**
    - **2** cases **RQs for dietary only as oral was at limit, but no concern for risk in any case**
    - **1** case dietary RQ> Oral RQ, it was a anticoagulant rodenticide
- **Bottom Line: In 99% of cases (118 of 119) the subacute dietary approach did not change risk conclusions already reached using oral, dose-based RQs**

Avian Subacute/Acute Risk Retrospective Comparison Project (Results cont.)

But what about those 62 cases not evaluated?

- Reviewed the MOA's for each case chemical: Was there coverage?
- An unevaluated chemical was reasoned important if its MOA was not represented by an analog's risk assessment comparison

Results

- Only 8 chemicals and their associated MOAs were not represented by analogs
- These 8 were all unique mechanisms
- **Bottom Line: In the majority of unevaluated cases, the subacute dietary approach was represented by chemical analogs; unique modes of action may be a category for establishing a base set of studies (and RQ comparisons) for future use.**

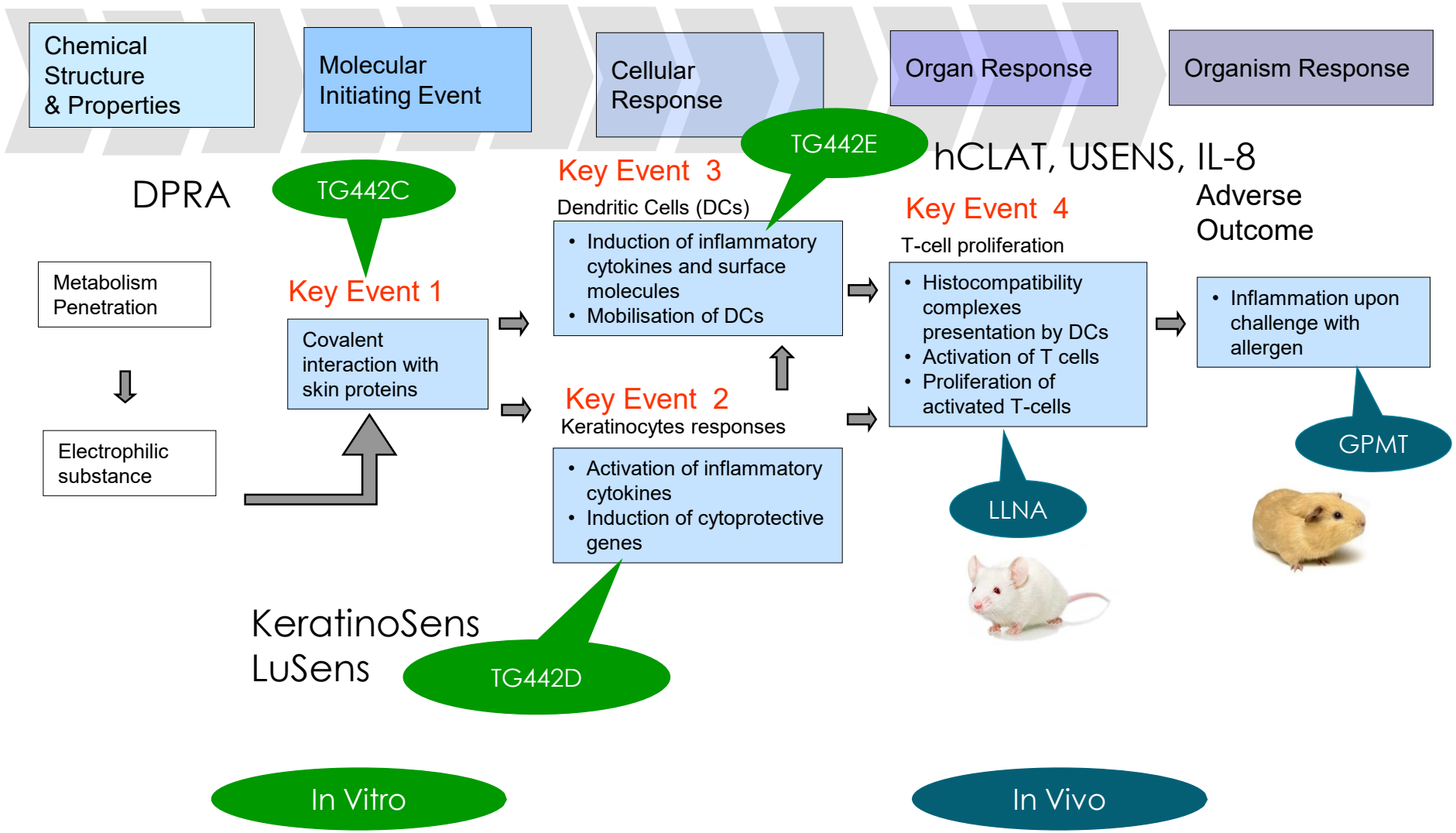
Avian Subacute/Acute Risk Retrospective Comparison Project (Next Steps)

- Peer-reviewed scientific journal publication (PETA lead, Agency coauthors): manuscript has been submitted
- Developing policy/guidance
  - Outlining comparison effort and its results by citation to journal article
  - Recommend, for new chemicals with mechanisms of action covered, a reliance on acute oral dose protocols, with dietary protocols held in reserve
  - Recommend an evidence-driven consideration of dietary testing for:
    - Unique modes of action
    - Cases where data on MOA suggest a mechanism for accumulative damage (e.g., anticoagulant rodenticides)
    - A high potential for bioaccumulation or a facilitated transport mechanism of absorption
      - High octanol-water partition coefficient and high molecular weight
      - High bioconcentration factor
      - Mammalian toxicity and animal residue studies
- Outreach to international and other partners
- Release draft policy for public comment

Moving Towards In Vitro & Computational Approaches:

Skin sensitization & inhalation risk assessment

Skin Sensitization Adverse Outcome Pathway



Skin Sensitization: International Activities

- 2016 International Cooperation on Alternative Test Methods (ICATM) Workshop: USA, EU, Japan, Korea, Canada, Brazil, China
- OECD proposal (SPSF) co-led by US, EU, and Canada
  - Create a performance based test guideline for non-animal defined approaches to skin sensitization testing
  - Included in OECD workplan April 2017
  - Expert group is actively moving forward on developing guideline for defined approaches
- *Multiple non-animal testing strategies - in vitro, in chemico & in silico inputs demonstrate **comparable or superior performance** to the mouse LLNA*

BETTER POLICIES FOR BETTER LIVES

Draft Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing

- Announced April 10, 2018 & describes the science that supports a policy to accept alternative (*in vitro, in silico, in chemico*) approaches for identifying skin sensitization hazard in place of animal studies.
- The interim policy is the result of collaboration between
  - Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)
  - NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
  - European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)
  - Health Canada (PMRA).

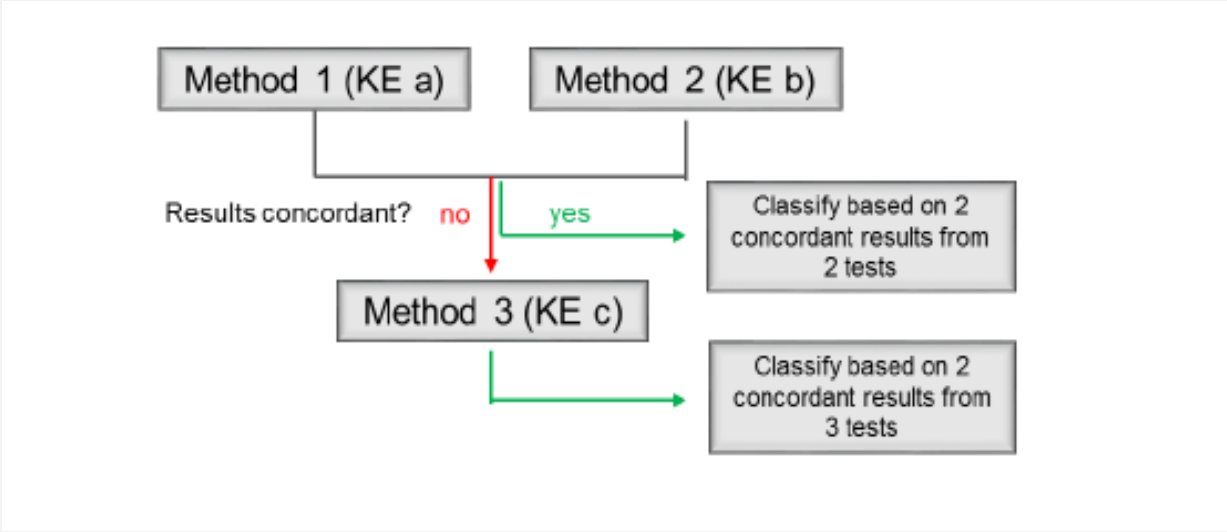
Draft Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing

- EPA's OPP & OPPT will begin accepting these approaches immediately under conditions described in the interim policy.
  - https://www.epa.gov/newsreleases/epa-releases-draft-policy-reduce-animal-testing-skin-sensitization
  - Existing OECD guidelines for determining hazard (only)
  - Approaches for combining results of 2 or 3 assays described in the draft, interim policy
  - Active or inert ingredients (not formulations yet)

Draft Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing

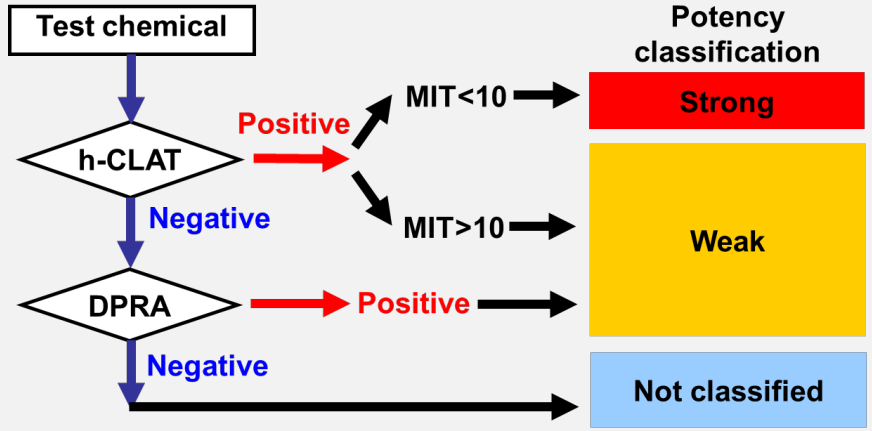
2 out of 3

- No differential weighting of individual test methods, or defined sequential order of testing



Sequential Testing Strategy

- Prediction can be derived after first tier
- Depends on KE3 (e.g. hCLAT) and KE1 (e.g. DPRA)



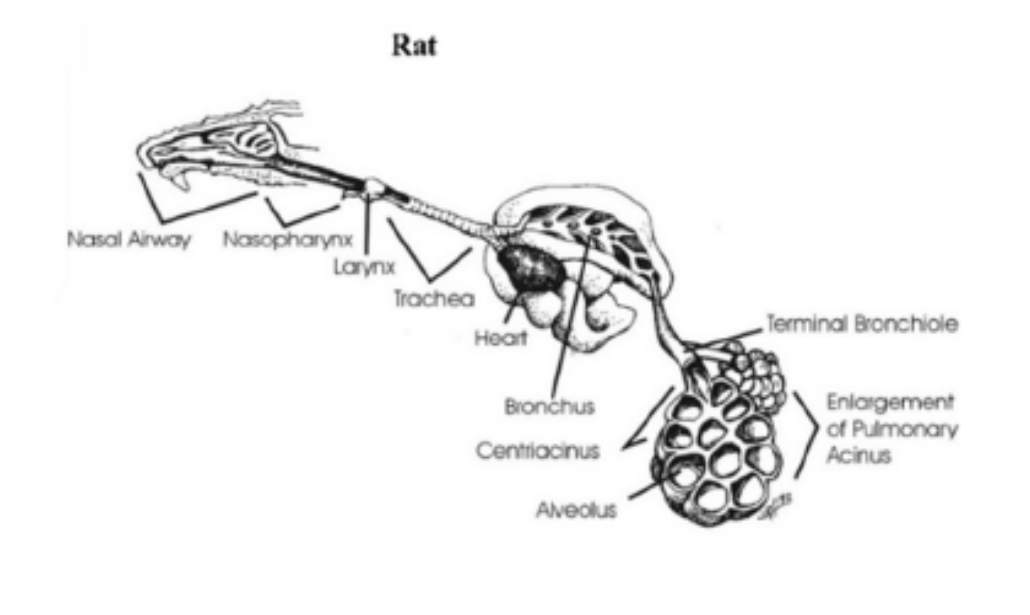
Expanding Substance Space Coverage

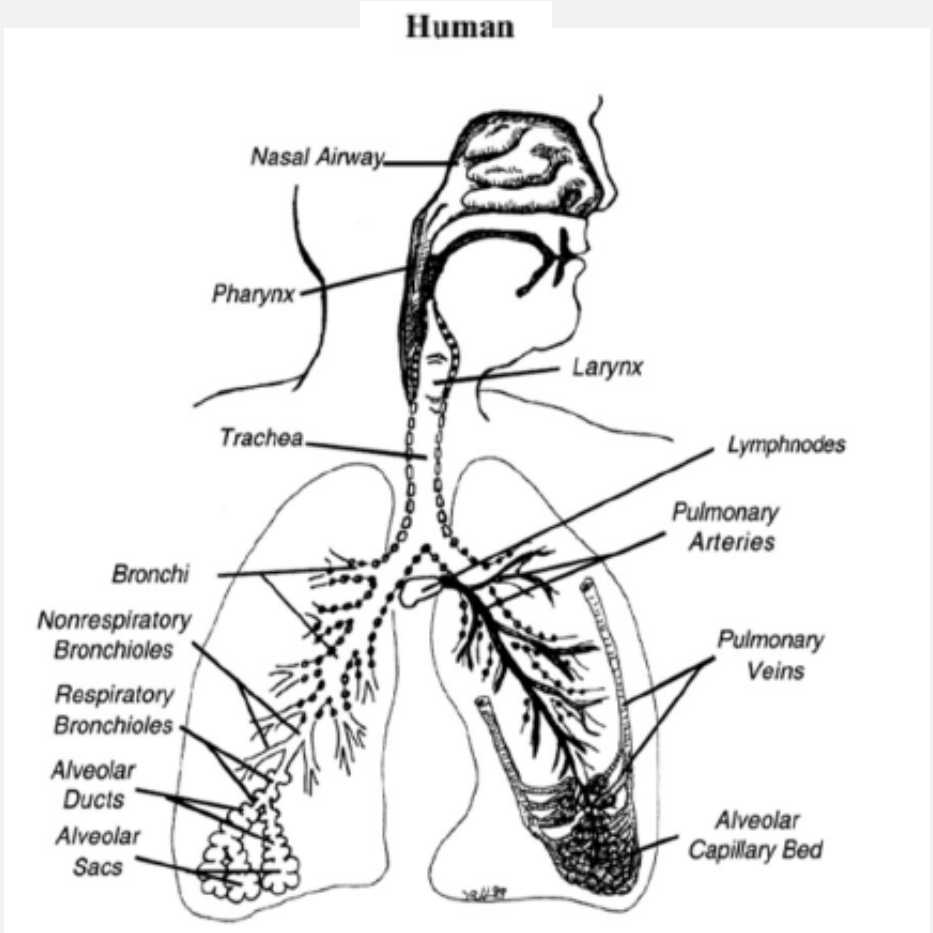
- NTP (D. Germolec) is supporting testing of additional substances in three alternative test methods: *DPRA, KeratinoSens, hCLAT*
- Expanded substance space includes: pesticide/agrochemical formulations, dermal excipients, personal care product products, "challenge" chemicals
- Total of 266 substances nominated from multiple ICCVAM agencies/partners
  - EPA's OPP, OPPT, ORD
  - CPSC, FDA, NTP, etc
  - ICATM partners
- NTP has procured 135 substances for initial testing phase, testing began in late 2017

Rat vs Human Respiratory Tract

Differences lead to changes in airflow and deposition of inhaled substances

- Airway size and surface area
- Nasal turbinate systems
- Branching patterns
- Cell composition/distribution of surface epithelium
- Anatomy of larynx





Images taken from Clippinger et al. (2018)

Challenges associated with irritants

- Traditional *in vivo* inhalation studies are resource intensive in terms of animal use, expense, and time
- Respiratory irritants can elicit damage at very low doses
  - Clear no observed adverse effect concentration (NOAEC) may not be established
  - Animal welfare concerns
- Efforts to develop new approach methodologies (NAMs)

Available *In Vitro* Test Systems

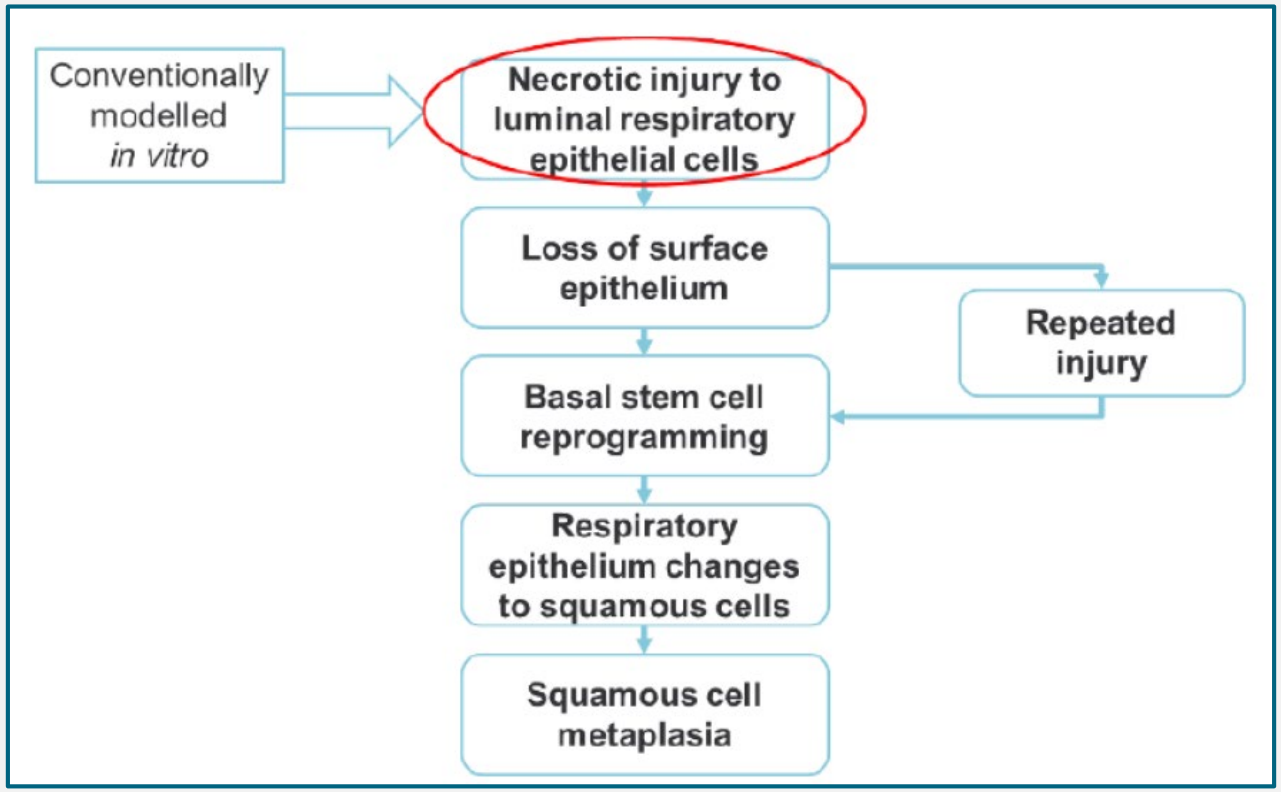
- Lung-on-a-chip: replicates microarchitecture of tracheobronchial airways and alveoli
- *Ex vivo* lung slices: reflect natural microanatomy and functional response to an inhaled chemical
- *In vitro* cell cultures
  - Simple cell cultures: overlying medium in submerged cell cultures does not allow for direct exposure to air-liquid interface
  - Three-dimensional models: cultured from airway epithelial cells and can mimic particular regions of the human respiratory tract, including barrier function, mucous production, and cilia function

Case Study Using a NAM to Refine Inhalation Risk Assessment for Point of Contact Toxicity

- Submitted by Syngenta Crop Protection, one of the registrants for products containing the contact irritant, chlorothalonil
- Proposal for refining inhalation risk assessment using an *in vitro* model initially presented to EPA in 2014
- Agency recognized the value of the proposal for chlorothalonil, as well as other respiratory contact irritants and encouraged further development
- Collaborated with NICEATM early in the process for review of the proposed approach

Case Study for Point of Contact Toxicity

- Epithelial cell damage occurs from initial inhalation exposure to chlorothalonil and causes cell death
- Following repeated exposure, repeated cell death results in metaplastic response and transformation of respiratory epithelium into stratified squamous epithelium
- Sufficient amount of chlorothalonil is needed at the cell surface to result in cell death in pathway



Extracted from MRID 50610402 (Flack et al. 2018)

Proposed Approach

- Syngenta considered available in vitro models for assessing damage to respiratory epithelial cells and identified MucilAir™ as optimal model
- MucilAir™ is a three-dimensional in vitro test system derived from human epithelial cells from nasal, tracheal, or bronchial tissues
  - Proposed approach used nasal tissue since it was the only model available at the time and cellular composition is similar to tracheal and bronchial epithelia (i.e., similar responses expected across tissue types for evaluating cell damage from irritation)
- Site-specific deposition in human upper respiratory tract predicted by computational fluid dynamic (CFD) modeling

Peer Review: FIFRA Scientific Advisory Panel

- December 4-7, 2018
- Charge questions regarding:
  - How the biological understanding informs the applicability of the *in vitro* testing
  - Use of *in vitro* system (study design, methods, selected measurements, robustness of data, data reporting)
  - Assumptions and calculations using CFD model to calculate cumulative deposition
  - Calculation of human equivalent concentrations
  - Strengths and limitations of using approach for other contact irritants, as well as potential for use with other chemicals that cause portal of entry respiratory tract effects

https://www.epa.gov/sap/fifra-scientific-advisory-panel-meetings

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Thank you!