Development of a Fit-For-Purpose *In Vitro* Model of Lung Toxicity

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Government regulations will require the testing of many chemicals for inhalation toxicity, and it is well recognized that there is a need for the development of in vitro testing methods to replace traditional in vivo inhalation studies. However, typical high-throughput assays that rely on immortalized cell lines and in-solution exposures, such as those found in the ToxCast panel, do not accurately mimic in vivo physiology or exposure scenarios, and they have frequently failed to respond even to known respiratory toxicants. Improved approaches utilizing exposure of organotypic cultures to gases, vapors, or aerosols at the air-liquid interface (ALI) should be more fully developed.

The goal of this project is to develop a fit-for-purpose *in vitro* pulmonary assay that is useful for dose- response studies and chemical risk assessment. Prevalent *in vitro* approaches employ pulmonary models lacking the complexity needed to model the *in vivo* lung biology and *in vivo* exposures, typically using only one cell type with chemical treatments in media. The use of individual cell types does not allow for cell-cell signaling necessary to recapitulate human responses, and media treatments with volatile chemicals or aerosols cannot be easily translated into real-life human exposures. An improved *in vitro* assay approach has been developed that better represents the human lung and allows for complex air-liquid-interface exposures to various chemical types (gases, vapors, aerosols) using commercially available 3D organotypic co-culture models (e.g., SmallAir-HF (Epithelix) and EpiAirway (MatTek)) and the VitroCell exposure system. To date, culture and exposure models have been integrated with assays measuring viability, cytotoxicity, glutathione depletion, epithelial barrier integrity, and transcriptomic changes, and initial validation has been conducted for sensitivity and reproducibility. Investigations have also compared simpler approaches using monocultures of immortalized human lung epithelial cell line (BEAS-2B) and/or treatment in media under various testing scenarios to the complex cell culture model and the more complex exposure system. The initial hypothesis is that the complex system will add value when accurate risk assessment is needed or when complex adverse outcome pathways are investigated, but that it may not add value, or could even be counter-productive, for hazard identification scenarios involving acute responses with simple adverse outcome pathways, particularly when high- throughput screening is desirable.

Scientists at Syngenta have recently proposed a New Approach Methodology (NAM) for the assessment of the point-of-contact inhalation toxicity of a respiratory irritant using the pesticide chlorothalonil as a test compound. Their results were presented to and discussed by the FIFRA Scientific Advisory Panel in December of 2018 at a meeting entitled “Peer Review of Evaluation of a Proposed Approach to Refine the Inhalation Risk Assessment for Point of Contact Toxicity: Case Study Using a NAM”. The suggested approach took advantage of human organotypic in vitro models of the airway epithelium and computational models of airway dosimetry. The organotypic model was treated with test substance in solution, and points of departure were assessed for several assays indicative of cytotoxicity. Those points of departure were then extrapolated to external exposure concentrations using a computational flow model of the human airway.

While this approach was well-received by the peer review panel and recognized as a much-needed advance, the panel also offered several constructive critiques and suggestions for further advancement of the technique before it could be reliably used for risk assessment in the place of animal studies. Among these were:

- Media-borne exposure of submerged cultures may not faithfully recapitulate the effects of exposure to a gas, vapor, or aerosol at the ALI. Use of airborne exposure was recommended.
- The response of the single organotypic cell culture model that was used in the study may not accurately represent the response of all of the diverse epithelia that are present throughout the airway (e.g., olfactory, nasal respiratory, tracheal respiratory, bronchial respiratory, bronchiolar, alveolar). Multiple

models should be used in order to capture any diversity of response.
- Cytotoxicity endpoints alone may not capture more subtle adverse effects. Additional assays should be considered that capture broader effects.
- No in vivo data was available for comparison of the calculated human equivalent concentration for validation purposes. Using a model compound with thoroughly characterized in vivo toxicity would be of value for validation purposes.

In 2020, ScitoVation will design and initiate a case study that is of similar design, but which addresses the recommendations of the FIFRA Scientific Advisory Panel, using two prototypical lung effects chemicals. Experimental work on the first compound will be completed in 2020. In the outyears, testing of the second compound will conclude and the case examples will be completed.

Implications: It is imperative for chemical innovators that risk decisions be made using human-relevant models capable of accurately evaluating quantitative dose-response. Our goal is to replace primitive classification and hazard identification methods with methods that support risk-based decisions for safety assessments. High-throughput assays often fail to identify respiratory toxicants due to their volatile nature, and an *in vitro* cell-based assay would provide significant benefit for chemical safety testing for respiratory effects.

Collaborations: NIEHS NICEATM, EPA NHERL, PETA

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Peer-reviewed publication(s):

McMullen, P. D., Andersen, M. E.; Cholewa, B., Clewell III, H. J., Dunnick, K. M., Hartman, J. K., Mansouri, K., Minto, M. S., Nicolas, C. I., Phillips, M., Slattery, S., Yoon, M., Clewell, R. A. (2018). Evaluating opportunities for advancing the use of alternative methods in risk assessment through the development of fit-for-purpose in vitro assays. Toxicology In Vitro. 2018, 48, 310-317.

Slattery, S. D., Bowen, L. A., Mistry, A., Dzierlenga, M., McMullen, P., Hartman, J. K. Development of an in vitro approach to point-of-contact inhalation toxicity testing of volatile compounds, using organotypic culture and air-liquid interface exposure. *Manuscript in preparation.*

Presentation(s):

Slattery, S. D., Bowen, L., Balbuena-Venancio, P., Phillips, M. B., Dzierlenga, M., Gullick, D., Smeltz, M., Norini, R., and Hartman, J. (2019). Development of a fit-for-purpose in vitro model of lung toxicity. Poster presented at the Society of Toxicology Annual Meeting, Baltimore, MD, March 10–14, 2019.

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