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

Leslie Recio, Jamie Scaglione, Iru Paudel, Marjory Moreau *ScitoVation.*

Time, cost, ethical, and regulatory considerations surrounding in vivo testing methods render them insufficient to meet the current chemical testing demand. There is a need for the development of in vitro and in silico alternatives to replace traditional in vivo methods for inhalation toxicity assessment. High-throughput assays that rely on immortalized cell lines and in-solution exposures, such as those found in the ToxCast panel, do not fully mimic in vivo cellular physiology or exposure scenarios, and fail to respond to known respiratory toxicants.[1] Improved approaches utilizing exposure of organotypic cultures to gases, vapors, or aerosols at the air-liquid interface (ALI) better characterize in vivo responses to exposure and should be more fully developed.

We have recently demonstrated the predictive power of combining in vitro ALI exposure assays with in-vitro-to-in-vivo extrapolation (IVIVE) based on computational airway dosimetry models (Moreau et al., 2022). In this study, lung organotypic epithelial cell cultures were exposed acutely to 1,3-dichloropropene (1,3-DCP) vapors at the ALI, acute toxic responses were measured, and in vitro point of departure (POD) was determined. A PBPK model of 1,3-DCP airway dosimetry was then utilized to extrapolate the in vitro POD to a rat or human equivalent inhaled concentration (EIC), which served as an estimate of the in vivo POD. The rat estimate was found to closely match the empirically determined rat in vivo POD.

While these results were promising, further work in this proposal will generate additional confidence in estimates of in vivo toxicity determined via IVIVE from vapor and Multiple-Path Particle Dosimetry (MPPD) from aerosol exposures to lung organotypic cultures. Increasing the endpoint assays available with these cell culture models beyond the transepithelial electrical resistance (TEER), cytotoxicity assays to include transcriptomics will enable a broader interrogation of the biological responses related to one or more adverse outcome pathways. Understanding the relationship between these different endpoints will facilitate the design of an efficient strategy to assess respiratory toxicity using NAM based approaches to determine potential human risk. Assessing the effects of repeated exposures using in vitro models will help us understand whether lung organotypic models that measure in vitro responses can be linked to longer-term key events in adverse outcome pathways to reduce the need for animal repeat exposure studies. Understanding the kinetics of test article distribution within the in vitro exposure system would enable a more accurate determination of the true in vitro exposure and improve IVIVE and MPPD dosimetry for accurate estimates of human equivalent exposure scenarios.

Validation and optimization of more sensitive and descriptive endpoints

The assays used to assess response in our previous work included TEER to examine epithelial barrier integrity, LDH release to examine cytotoxicity, and ATP assays to examine viability. Additional assays that provide more information regarding genome and biochemical responses to inform mode-of-action can provide a better prediction of the in vivo point of departure and enable prediction of longer-term effects after repeated exposure. Additionally, we will also examine histopathological changes in organotypic cultures to provide more information regarding tissue-level effects that can enable a better connection to be made between the in vitro toxicity observed and in vivo tissue-level effects.

Transcriptomics

Next generation sequencing (NGS) RNASeq is used to determine the relative expression (number of gene-specific transcripts), in xenobiotic exposed samples relative to controls. This expression information can then be used to identify bioactivity, determine a point of departure, and elucidate mode of action (MOA).
With any sample type, efficient RNA extraction without degradation is a critical first step in a transcriptomics study. Procedures for disruption and homogenization of samples to extract high quality RNA required for NGS, differs for tissues or cell cultures, and is well established. However, organotypic lung cultures grown on the membranes of inserts for 12 - 24 well plates represent unique samples. In 2022, we established methods to produce high quality RNA from organotypic lung cultures. We have applied these methods to MucilAir Nasal

cultures treated at the ALI with 1,3-DCP vapor at sub-cytotoxic concentrations. Extracted RNA is undergoing NGS RNASeq to investigate MOA, determine in vitro POD, and these data will be extrapolated to rat and human EICs by IVIVE. These methods will be applied going forward to studies using organotypic lung cultures in ScitoVation's respiratory toxicology program.

Repeated exposure experiments

While identifying and understanding the toxicity of acute exposure is important, predicting the effects of sub-chronic or chronic repeated exposures in the home or workplace is also of concern for many conditions of use for chemical products. In vivo inhalation experiments lasting 90 days or more are a major and expensive undertaking that requires several test animals. Substituting repeated in vitro exposures to predict long-term in vivo toxicity could reduce costs and use of animals. Better yet would be a method that could computationally extrapolate from an acute or short-term repeated exposure in vitro study to sub-chronic or chronic in vivo effects. Repeat exposures will be evaluated in these studies.

In vitro dosimetry modeling

It is well-recognized that nominal cell culture media concentrations do not necessarily represent the actual exposure experienced by cells in submerged culture due to losses through adsorption, evaporation, and metabolism.[3] The concentration of compound free in the medium or the concentration within the target cells may be preferable dose-metrics for in vitro studies. Computational models have been developed to better understand how the test articles interact with and distribute within in vitro systems. These mass balance models simulate the distribution of the chemical among in vitro compartments using partition coefficients or rate constants. Armitage et al.[4],[5] developed a mass balance model that calculates the mass distribution, freely dissolved concentrations, and cell/tissue concentrations corresponding to the initial nominal concentration and experimental conditions specified by the user. Little has been done to model in vitro dosimetry in air-liquid interface exposure systems. ScitoVation proposes to investigate distribution of 1,3-DCP within the compartments of the Vitrocell exposure system and exposed cell culture models, and to build a computational model that is predictive of that distribution. We would begin this project with a feasibility study, in which we would assess approaches to modeling and to experimental validation of the model through analytical chemistry. Subsequently, we would build the model. Experiments employing analytical chemistry are being performed to determine model parameters and to measure actual concentrations at points within the exposure system to validate the model.

Increasing confidence in combined in vitro/in silico inhalation testing method

In our previous studies with 1,3-DCP, we used in vitro ALI acute exposures and in silico airway dosimetry modeling to predict an in vivo POD that was a reasonable approximation of the empirically determined in vivo POD. This result provided evidence that the general approach was well-suited for volatile chemical inhalation toxicity risk assessment. However, to build confidence in the approach, it will be necessary to demonstrate that this approach is an accurate predictor across a variety of volatile compounds. To that end, we propose to test a panel of 5 volatile compounds with in vitro acute ALI exposures, determine in vitro PODs, and perform IVIVE to predict HEICs by adapting our existing PBPK airway dosimetry model. We would select compounds that are (1) volatile, (2) known respiratory irritants, (3) have existing in vivo toxicity data for comparison, and (4) have associated pharmacokinetic data for adaptation of the model.

In our previous work with testing of 1,3-DCP in vitro at the ALI, we exposed 5 different cell culture models that represented 5 different epithelial tissues of the human airway to a dose range of 1,3-DCP vapor, and then determined in vitro PODs and predicted HEICs for each tissue. That was a thorough testing strategy, and it demonstrated that the combined in vitro/in silico approach could successfully identify the POD within the critical tissue type. However, that exhaustive approach is time- and cost-intensive. A tiered approach that can arrive at the same conclusion through a more efficient path will be valuable in the application of the in vitro/in silico approach to new compounds. It will also be a prerequisite to testing a panel of 5 compounds within this project. We propose to design such a tiered approach and to employ that design in testing our compound panel.

Aerosols exposures

In addition to vapor phase exposure, ScitoVation is developing methods for exposure of organotypic lung

cultures to aerosols using a VitroCell Cloud exposure system. VitroCell Cloud aerosol exposure system enables direct exposure of mammalian cells at the ALI to aerosols, particles, and semi-volatile compounds and includes a microbalance enabling quantification of deposited dose to each exposed culture. To expand our IVIVE expertise, we propose to extend studies using paraquat aerosols as an oxidative stressor to ALI and by using Multiple-Path Particle Dosimetry Model (MPPD) based IVIVE to calculate the deposition and clearance of paraquat aerosols in the respiratory tracts of laboratory animals, human adults, and children. Apical measures of cellular health studies and transcriptomics used in the vapor studies will be used in these studies as endpoints to determine POD and HEIC.

References

[1] McMullen, P. D., et al. (2018). "Evaluating opportunities for advancing the use of alternative methods in risk assessment through the development of fit-for-purpose in vitro assays." Toxicol In Vitro 48: 310-317.
[2] Stott WT, Kastl PE. 1986. Inhalation pharmacokinetics of technical grade 1,3-dichloropropene in rats. Toxicol Appl Pharmacol. 85(3):332-341.
[3] Groothuis, F. A., et al. (2015). "Dose metric considerations in in vitro assays to improve quantitative in vitro–in vivo dose extrapolations." Toxicology 332: 30-40.
[4] Armitage JM, Wania F, Arnot JA. Application of mass balance models and the chemical activity concept to facilitate the use of in vitro toxicity data for risk assessment. Environ Sci Technol. 2014 Aug 19;48(16):9770-9. doi: 10.1021/es501955g. Epub 2014 Jul 21. PMID: 25014875.
[5] Armitage JM, Sangion A, Parmar R, Looky AB, Arnot JA. Update and Evaluation of a High-Throughput In Vitro Mass Balance Distribution Model: IV-MBM EQP v2.0. Toxics. 2021; 9(11):315. https://doi.org/10.3390/toxics9110315.

Implications: Inhalation toxicity testing in laboratory animals can be technologically challenging, time consuming and costly, and because of anatomical differences between the respiratory tracts of laboratory animals and humans, it is challenging to extrapolate such toxicity studies in animals to humans. High-throughput in vitro assays cannot generally test volatile chemicals. Thus, an in *vitro cell*- based assay using human organotypic respiratory tract cells would provide significant benefit for chemical safety testing for assessing respiratory effects.

Current project start and end dates: January 2023 – December 2023

Other publication(s): None to date.

Abstract revision date: February 2023