Comparison of Vapor and Liquid Phase Acrolein Exposures to Air Liquid Interface (ALI) Cell Cultures

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Abstract

The 3M Strategic Toxicology Laboratory (STL) is an internal corporate resource that emphasizes the use of *in vitro* methodology when providing support to 3M businesses. The STL is investigating vapor and liquid phase exposure to cell cultures at the air-liquid interface (ALI) as an animal alternative assay to help assess respiratory toxicity. This study describes the use of acrolein, a potent respiratory toxicant, as a model test substance. Vapor atmospheres were generated using a Vitrocell® 12/12 system. A549 cells were seeded into Transwell® inserts, grown submerged for 1 week then raised to the ALI for an additional 7-14 days, creating cultures robust to the clean-air negative control conditions of the Vitrocell apparatus. EpiAirway™ tissues were purchased from MatTek. All exposures were carried out for three hours and viability assessed immediately or 20 – 24 hours post exposure. At the vapor phase, A549 cell viability was reduced to 58% (14.6 ppm) and 91% (4.6 ppm) compared with the Vitrocell clean-air control group, with a further decrease in each case of approximately 20% following the post-exposure period. Cell viability was not affected at the 1.46 ppm level. In the liquid phase, EpiAirway cultures were exposed to acrolein in corn oil ranging from 0.01 - 3,000 ug/mL. Viability decreased at all concentrations greater than 10 ug/mL. At 1,000 ug/mL, viability was reduced to 48% immediately and 4% 20 hours post exposure. A549 cultures were also exposed to acrolein dilutions in DPBS in the same range as EpiAirway and followed a similar viability profile. Both modes of exposure, vapor and liquid, demonstrated dose dependent effects on viability. However, the concentrations of acrolein required to decrease viability were much lower with vapor exposure than with direct liquid exposure. The liquid exposure level (1000 ug/ml) which produced approximately 50% reduction in viability (EC50) converts to over 400,000 ppm in air, whereas 14.6 ppm vapor exposure reduced viability to approximately 56%. Further experiments are required to better understand the discrepancy in the liquid phase and vapor phase levels required to achieve the EC50 and to identify the most appropriate comparison method, such as concentration or mass/surface area. Understanding these differences will be critical to achieve the best utilization of these in vitro methods as a tool for respiratory toxicity assessment.

Introduction

The STL is working to develop an *in vitro* screening ALI model to assess the acute respiratory irritation potential for new chemicals. These experiments examined multiple aspects of the model, including different cell culture systems (A549 and EpiAirway), different exposure methods (dynamic vapor and liquid phase), and different post exposure periods, all using acrolein as a model respiratory irritant.

The goal was to better understand the critical parameters of the cell systems and exposure methods to enable the development of a consistent screening model, while gaining clarity of the dosimetry.

Materials and Methods

- A549 adenocarcinomic human alveolar basal epithelial cells (American Type Culture Collection, ATCC) grown at the air-liquid interface in Corning Polyester (PET) Membrane Transwell-Clear Inserts (0.33 and 1.12 cm^2).[1]
- EpiAirway AIR-100 and Air-100-PC12 tissues (MatTek Corp, Ashland, MA, USA)
- Vitrocell 12/12 dynamic *in vitro* vapor exposure test system creates three levels of exposure and constantly replenishes the test chemical atmosphere (Vitrocell Systems, Waldkrich, Germany) .
- Agilent MicroGC (thermal conductivity detector, PN G3581A) configured for periodic on-line sampling from each level of vapor exposure atmosphere.
- Liquid phase tests were run in two modes: static exposure (a single aliquot of acrolein dilution for the entire three-hour period) and dynamic exposure (replacing the acrolein solution every 30 minutes for the three-hour period).
- A549 air-liquid Interface (ALI) cultures and EpiAirway tissues were tested in both formats using a range of exposure concentrations. Viability was determined by MTT at 0- and 20/24-hours post exposure for each experiment.

Liquid and Vapor Phase Exposures

Liquid Phase Exposure – 3 Hours

A549 Cultures: Acrolein was diluted in DPBS and 100 uL aliquots applied to the apical surface. Groups labeled "single" had a single aliquot applied. Groups labeled "sequential" had exposure aliquots replaced every 30 minutes.

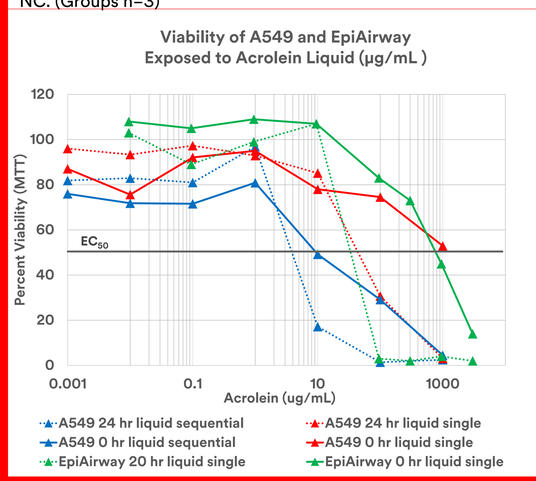
EpiAirway PC-100: Acrolein was diluted into corn oil and 100 uL aliquots applied.[2]

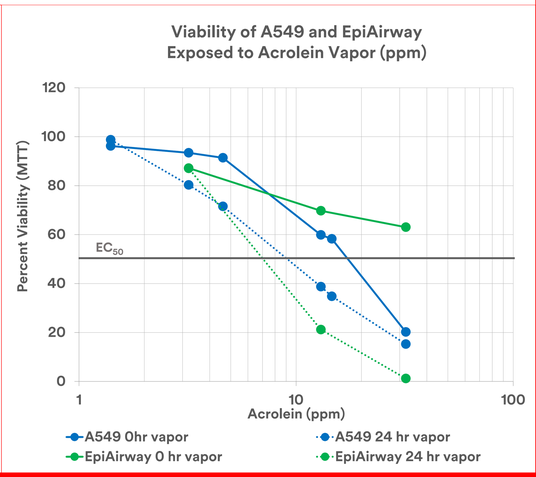
Both: The apical surface was washed with three rinses of DPBS at the end of exposure. Viability was measured either at 0- or 20/24-hours post exposure and presented as % of the vehicle NC. (Groups n=3)

Vapor Phase Exposure – 3 Hours

A549 and EpiAirway Air-100-PC12 cultures were exposed to acrolein vapor in the Vitrocell 12/12 system. A549 data from two experiments of overlapping exposure ranges is shown. Acrolein was vaporized, pre-diluted 1:3 into humidified air to achieve the high exposure level, and further diluted in ½ log steps to create the mid-and low-exposure levels.

Viability was measured either at 0- or 20/24-hours post exposure and presented as % of the clean-air exposure NC. (Groups n=3)





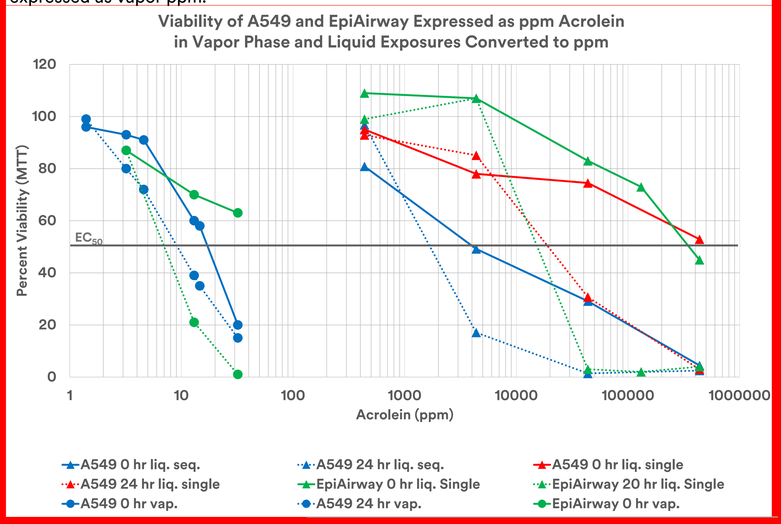
Exposure Estimates

Converting liquid concentrations to ppm increases the exposure estimate by many orders of magnitude, compared to vapor exposures.

This conversion was accomplished using the following series of calculations:

- Convert ug/mL to mg/m^3 by multiplying ug/mL by 1,000
- Use *[24.45 x concentration (mg/m^3)] / Molecular Weight = Concentration (ppm)* to solve for the concentration expressed as ppm.

For example, the 1000 µg/mL liquid exposure converts to over 400,000 ppm. Plotting these data on a log scale illustrates the large discrepancy between liquid and vapor data sets expressed as vapor ppm.



Critical Endpoint (EC_{50}) Comparison

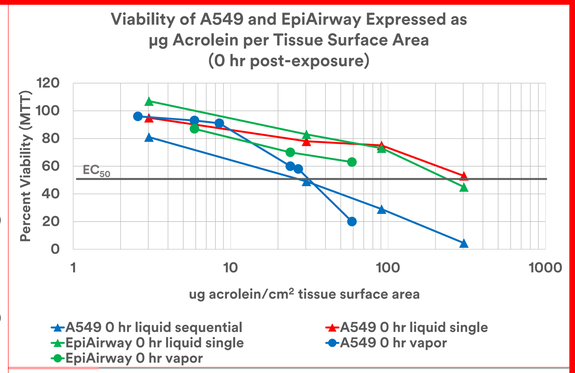
Comparing both modes of exposure on the basis of mass per surface area of tissue shows a very close relationship between the liquid and vapor phase results.

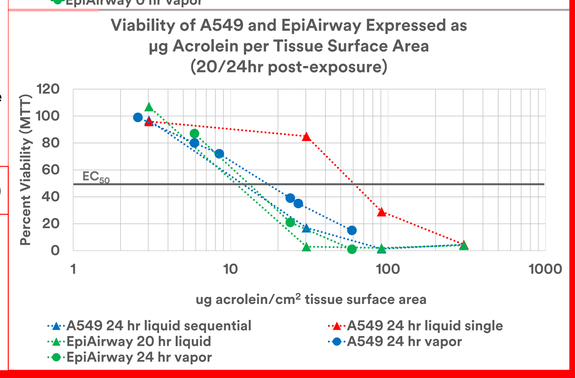
This is accomplished by using the following series of calculations:

- For vapor exposures:
  - Convert vapor ppm values into mg/m^3 using the previous formula (left)
  - Convert to ug/mL by diving by 1,000
  - Multiply by 900 mL (the total volume of air flowing over the tissue during a 3 hour exposure at a rate of 5 mL/min)
  - Divide by the surface area of the cell culture inserts (1.12 mm^2 large and 0.33 mm^2 small)
- For liquid exposures:
  - Multiply by 900 mL (the total volume of air flowing over the tissue during a 3 hour exposure at a rate of 5 mL/min)
  - Divide by the surface area of the cell culture inserts (1.12 mm^2 large and 0.33 mm^2 small)

The EC_{50} (the exposure level that produced a 50% reduction in cell viability) values listed below suggest that expressing the exposure estimate using mass exposed per unit surface area provides a consistent method of data evaluation for acrolein, regardless of the exposure conditions and provide clear evidence that both post-exposure periods and sequential liquid dosing applications are critical.

Exposure Condition	EC_{50} (ppm)	EC_{50} (ug/cm^2)
EpiAirway 24h Vapor	7	12
A549 24h Vapor	9	17
EpiAirway 20h Liquid Single	15000	11
A549 24h Liquid Sequential	17000	11
A549 24h Liquid Single	88000	60





Conclusions

- The two different ALI models, A549 and EpiAirway, showed similar responses, with cell viability decreasing as a function of acrolein exposure concentration, both during dynamic vapor exposure and direct liquid exposures.
- Post-exposure viability was a critical parameter for evaluation, as tissue viability decreased from immediate post-exposure to the 20/24-hour post exposure time-point, suggesting that evaluation of viability changes over a post-exposure period are necessary when setting up an *in vitro* model chemical exposure system.
- Sequential liquid exposures produced EC_{50} values well aligned with dynamic vapor exposures, compared to single liquid exposures.
- Real-time quantification of the vapor concentrations was very helpful to provide confidence in the actual exposure estimate.
- The method for comparing the relationship between liquid and vapor phase exposures is critical. Direct mathematical conversion from a liquid (ug/mL) into vapor (ppm in air) concentration results in thousands-fold higher exposure estimate compared to vapor concentrations that elicit the same effects on cellular viability.
- Calculation of exposure on a mass per surface area basis resulted in meaningful comparison between these two modes of exposure.
- These data provide a valuable step forward on the path to understanding and properly communicating the results of *in vitro* ALI respiratory irritation models using vapor and liquid phase toxicant exposure.

References

1. Bettmann et. al. *Exploring the Feasibility of A549 Cells for Use in a Vapor Exposure Test System for Acute Toxicity Evaluation.* The Toxicologist Abstracts 168(S1):2467, 2019.
2. Jackson et. al., Applied In Vitro Toxicology, June, 2018