Method Development for Toxicity Screening with Exposure Route Relevance: Initial Progress

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Introduction

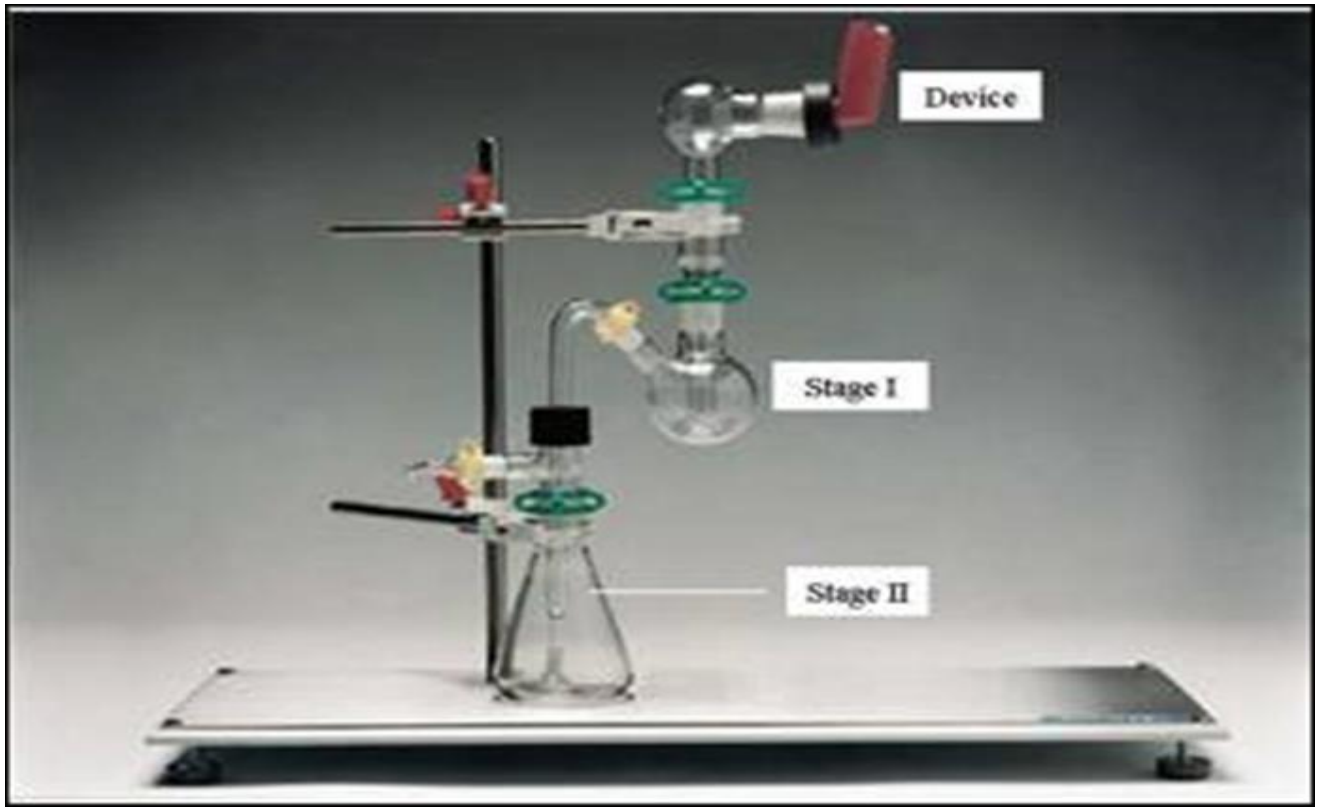
- Inhalation is an important route of exposure to particulates

- There is an increased need for bio-relevant exposure methods
  - Use of tissue cultures that reflect lung biology (e.g. air-liquid interface models)
  - Deposition of particles as an aerosol onto those cells
  - Appropriate size classification of particles reflecting deposition sites in the lungs

- The toxicity endpoints should be unaffected by the particulates themselves

- Aim: To examine the appropriateness of employing pharmaceutical aerosol characterization apparatus for bio-relevant exposure assessments of environmental particles

Twin Stage Impinger (TSI)



- Stage I has a cut off of particles 6.4 µm at 60 L/min.
- Stage II collects particles <6.4 µm.
- Stage II represents the bronchial region.
- Transwells can be attached to the coupling tube in stages I or II.

Andersen Cascade Impactor (ACI)

Diameter cut off µm at different flow rates

	28.3 L/min	60 L/min	90 L/min
0	9	8.6	8
1	5.8	6.5	6.5
2	4.7	4.4	5.2
3	3.3	3.3	3.5
4	2.1	2	2.6
5	1.1	1.1	1.7
6	0.7	0.54	1
7	0.4	0.25	0.43

Next Generation Impactor (NGI)

Diameter cut off µm at different flow rates

	15 L/min	30 L/min	60 L/min	100 L/min
1	14.1	11.7	8.06	6.12
2	8.61	6.4	4.46	3.42
3	5.39	3.99	2.82	2.18
4	3.3	2.3	1.66	1.31
5	2.08	1.36	0.94	0.72
6	1.36	0.83	0.55	0.4
7	0.98	0.54	0.34	0.24

Experimental work

Study 1 – Weight Loss Experiment

- Typically employ an air-liquid interface model in transwells/snapwells
- Potential effects of airflow need to be assessed with deposition systems
    - If evaporation occurs, this would lead to a higher concentration of aerosol exposure after deposition
    - Aim: to determine if evaporation of the cell-lining fluid occurs since this may be a cause of cell stress

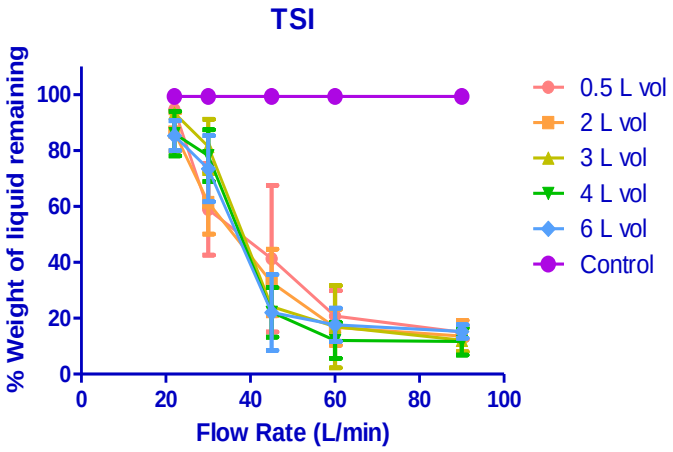
Study 2 – Fluorescein recovery from transwells/snapwells

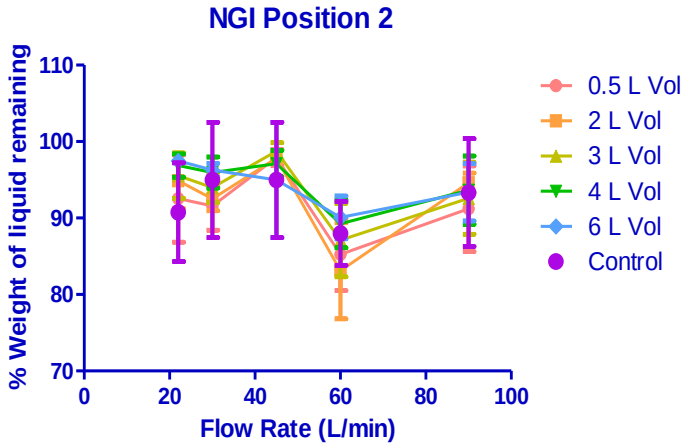
- Aim: To determine loss of cell lining fluid under air flow onto the following impinger/impactor stage

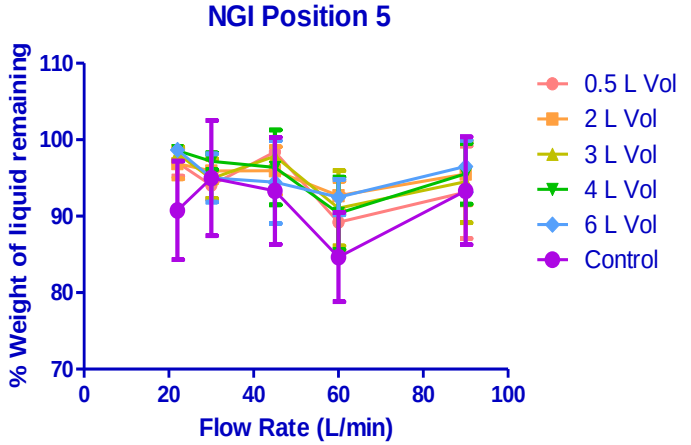
Study 3 – particle/assay interaction study

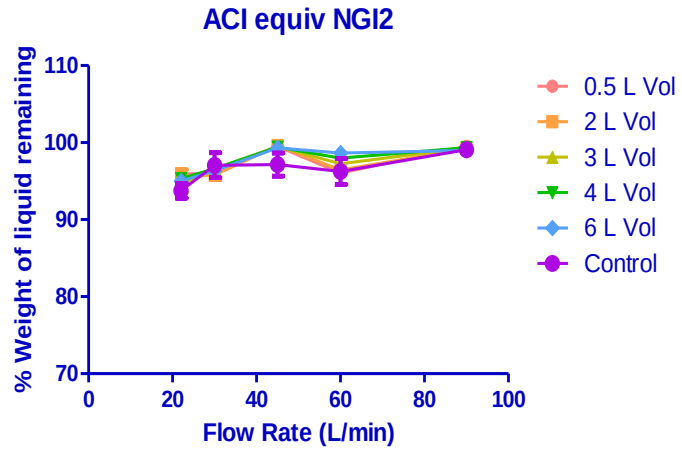
- Validation that toxicity endpoints that are unaffected by particulates is needed
- Aim: To determine if the environmental particles interact with any cell viability/proliferation assays

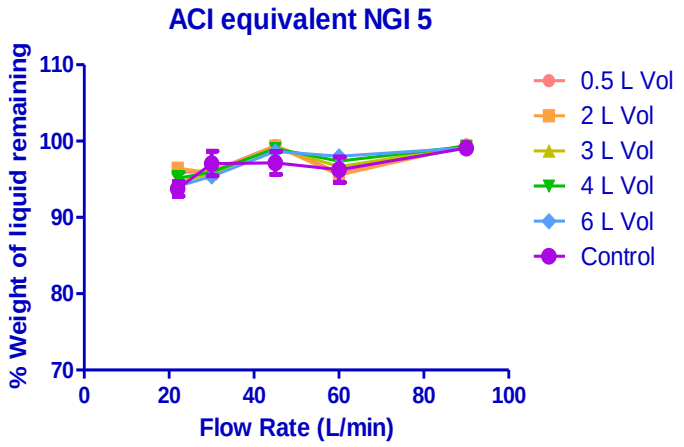
Weight Loss Experiment





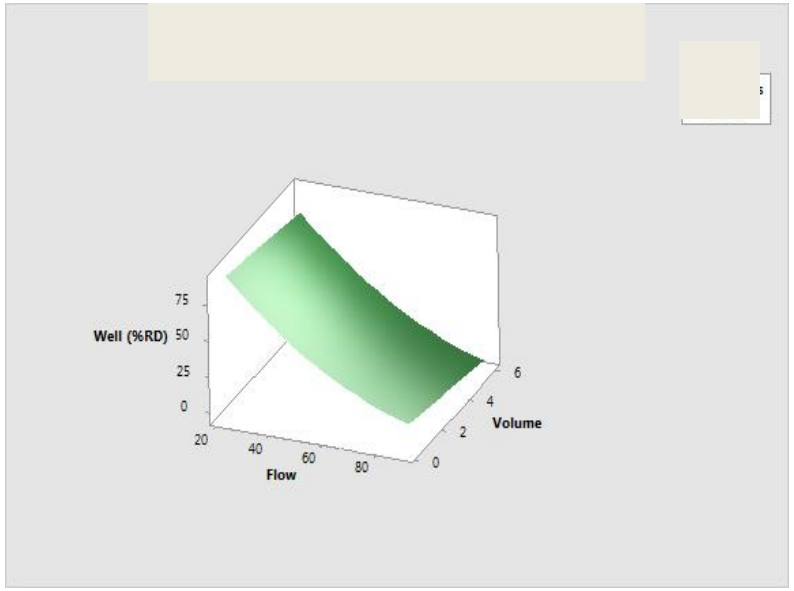






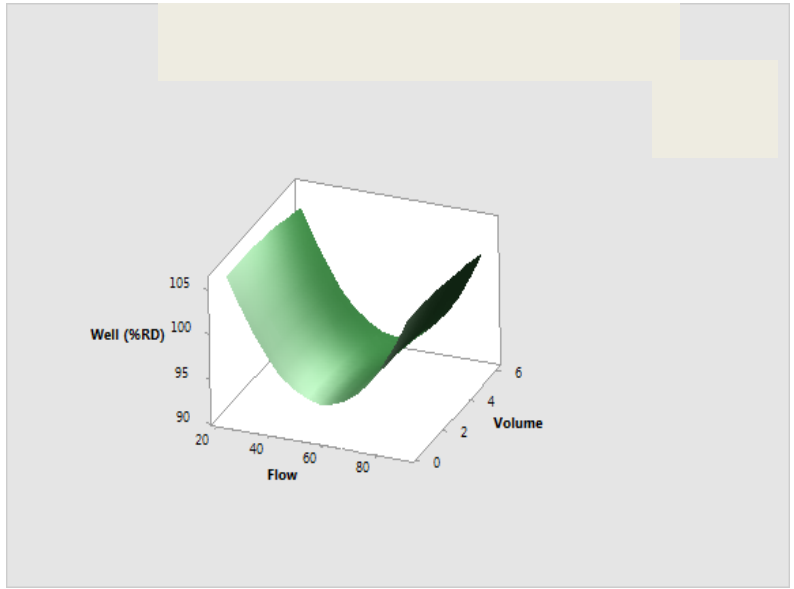
Fluorescein recovery from Trans/Snapwell

TSI



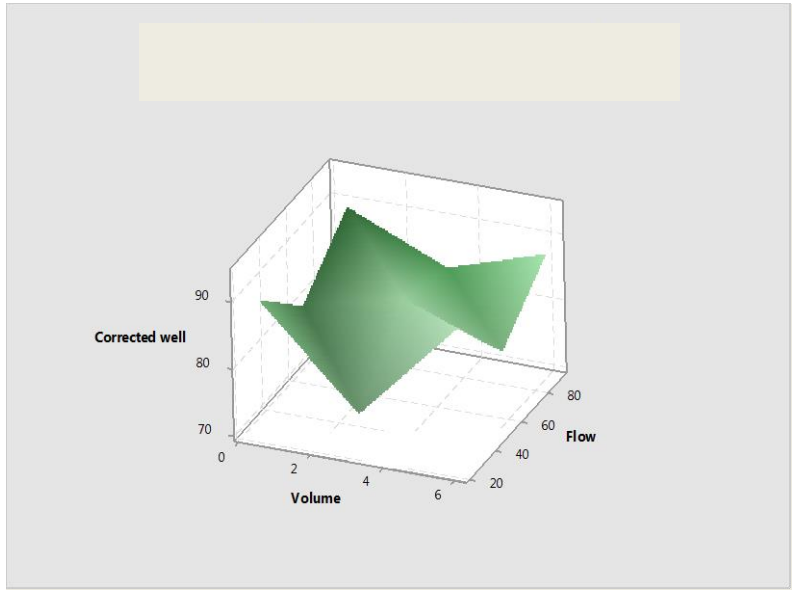
Significant loss

NGI



No significant loss

ACI



No significant loss

Particle/Assay Interaction Study

- A range of particles were chosen to include those with little or known toxicity
- Particles included different sizes and shapes
- The particles chosen were:
  - titanium dioxide powder, nanopowder and nanowires
  - Silicon carbide powder, nanopowder and nanowhiskers
  - DQ12
- The assays chosen were MTT, MTS, Prestoblue and WST-1

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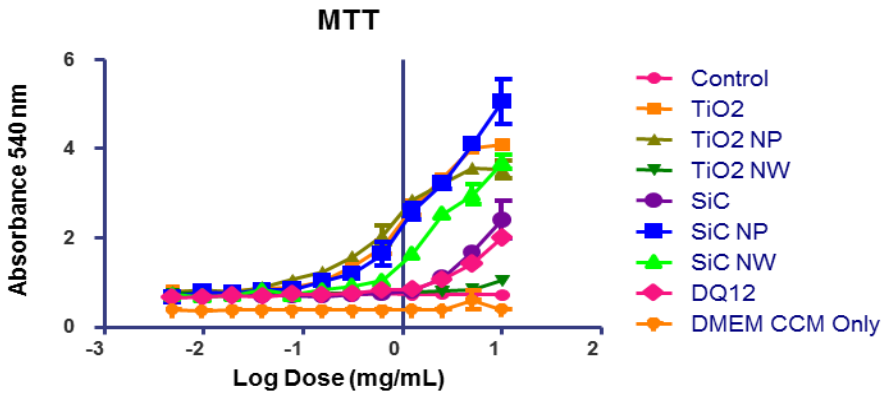
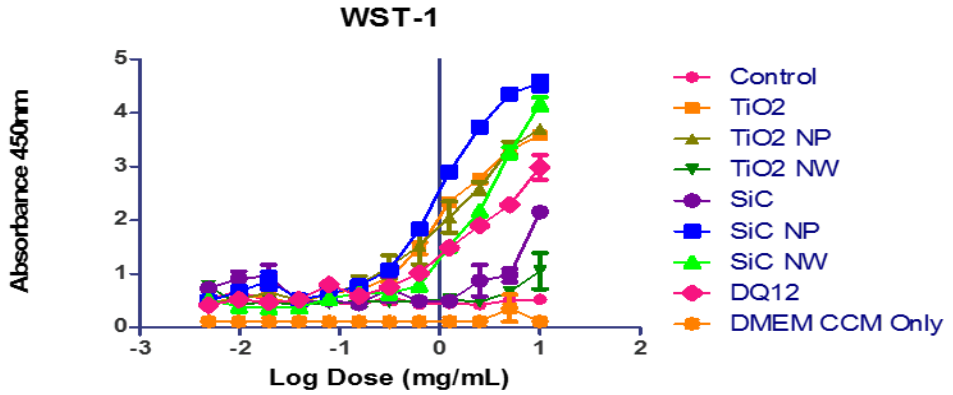
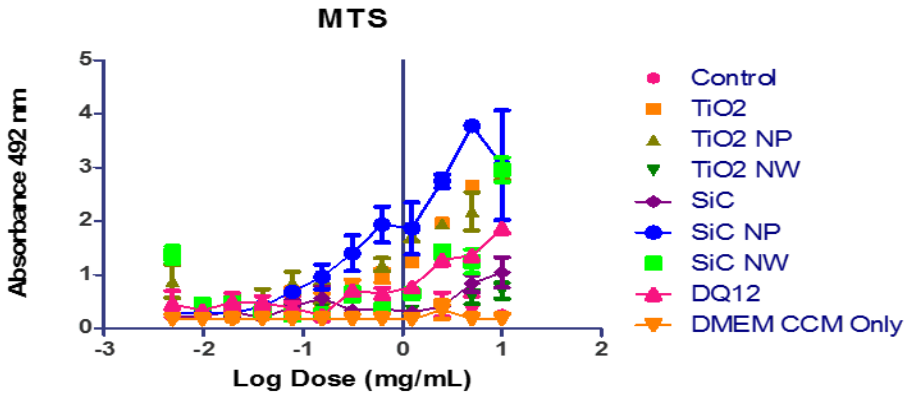
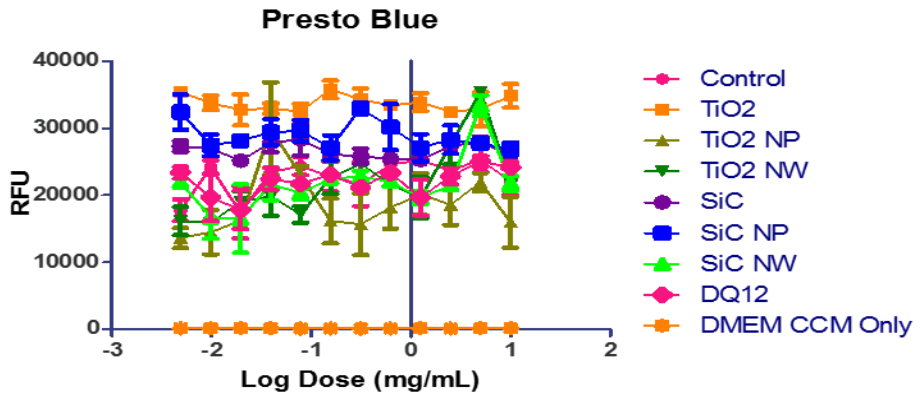
Particle/Assay Interaction Study









Future Work

- To repeat the airflow experiments using A549, Calu-3 and Alveolar type 1 cells to determine the effects of airflow on cell health.
- To develop an analytical method to determine the quantity of environmental particles (ICP-OES) or pharmaceutical particles (LC-MS) that are deposited into Transwells/Snapwells using these deposition systems.
- To test all particles using traditional 2D toxicity testing.
- To deposit particles onto cells using the deposition systems.

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Acknowledgements and References

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