**Aim 1:**

To investigate and compare the cytotoxicity of Diesel Exhaust Particles, Ox66™, and the combination of both on four different cell lines belonging to two different organs in two different model systems (Human astroglial and type II alveolar cells and rat astrolgilal and type II alveolar cells).

Materials and methods:

All cell lines will be cultured in well-plates and then exposed to DEP, Ox66, and a mixture of both for 24 and 48 hours in normoxic (21% oxygen) incubation conditions at 37 degrees C and 5% CO2. DEP will be acquired from NIST’s standard reference materials library (SRM 2975). Ox66™ will be acquired from Hemotek LLC. Cytotoxicity will be measured using the LDH colorimetric assay to determine cell damage and the MTT colorimetric assay to determine cell viability post-exposure. Furthermore, proteomic analysis using the MagPix instrument will be performed to search for differences in inflammatory chemokines and cytokines between exposure scenarios.

Statistical Analysis:

A mixed linear model will be performed on the resulting data in order to understand the difference in dose-response between the 8 different exposure scenarios. If any significant differences exist, a post-hoc Wilcoxon-method will be performed on the pairs which exhibited differences in the linear model to parse out the pair-wise non-parametric significance.

**Aim 2:**

To investigate the effect of hypoxia on the toxicity of diesel exhaust particles, Ox66™, and the mixture on the 4 cell-lines, and whether the addition of Ox66™ to DEP in hypoxic conditions ameliorates the effects of hypoxia on DEP toxicity.

Materials and methods:

All cell lines will be cultured in well-plates and then exposed to DEP and Ox66 for 24 and 48 hours in hypoxic (2% oxygen) incubation conditions at 37 degrees C and 5% CO2. Cells will also be exposed to a mixture of DEP and Ox66 in hypoxic conditions. Cytotoxicity will be measured using the LDH colorimetric assay to determine cell damage and the MTT colorimetric assay to determine cell viability post-exposure. Furthermore, proteomic analysis using the MagPix instrument will be performed to search for differences in inflammatory chemokines and cytokines between exposure scenarios.

Statistical Analysis:

A mixed linear model will also be performed on the resulting exposure scenario data, followed by a post-hoc non-parametric Wilcoxon-method for pairs which show difference in the linear model. The *in vitro* oxygenating effects of Ox66™ will be determined by looking at the non-parametric significance between exposure to DEP alone and exposure to a mixture of Ox66™ and DEP in hypoxia. A statistically-significant reduction of toxicity would indicate an amelioration of hypoxia-induced toxicity.

**Aim 3:**

To compare the difference between the effects of Ox66™ and a perfluorocarbon emulsion on DEP toxicity in hypoxic conditions

Materials and methods:

The cell line which experiences the largest effect of hypoxia on DEP toxicity will be cultured and placed in hypoxic (2% oxygen) incubation conditions at 37 degrees C and 5% CO2 for 12 hours. During the last 4 hours of exposure, Ox66™ will be added to one set of plates while the perfluorocarbon emulsion will be added to another set. The perfluorocarbon emulsion will be acquired from NuVox Pharma. After exposure, Cytotoxicity will be measured using the LDH colorimetric assay to determine cell damage and the MTT colorimetric assay to determine cell viability post-exposure.

Statistical Analysis:

A mixed linear model will also be performed on the resulting exposure scenario data, followed by a post-hoc non-parametric Wilcoxon-method for pairs which show difference in the linear model. The *in vitro* oxygenating effects of Ox66™ will be compared to those of the perfluorocarbon emulsions by comparing the dose-response for cytotoxicity between the set of plates containing DEP and Ox66™ and those containing DEP and perfluorocarbon emulsions. The compound with the strongest oxygenating properties would exhibit the larger reduction in hypoxia-induced toxicity.