

# USING HUMAN-DERIVED NEURAL CELLS AS AN *IN VITRO* MODEL FOR DEVELOPMENTAL NEUROTOXICITY FOLLOWING EXPOSURE TO PESTICIDES

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## Abstract

Agricultural, industrial and commercial use of pesticides continues to increase with an estimated usage nearing a billion lbs/year. Many of these compounds target the nervous system of nuisance animals and due to their lack of selectivity cause adverse effects in non-target species. Several classes of pesticides such as the organophosphates, carbamates and organochlorines are known to elicit neurotoxic effects in mammals. However, current testing and safety requirements do not require developmental neurotoxicity (DNT) tests for these chemicals. Understanding the consequence of pesticide exposure on fetal brain development specifically during critical windows of susceptibility is necessary to accurately predict risk. Thus, there is a critical need for *in vitro* models to aid in DNT screening and chemical prioritization. The objective of this study was to develop a metabolomics-based DNT assay that includes stages of neural development using progenitor (hNP) and post-mitotic neuronal cells (hN2) to delineate the adverse outcome pathways (AOP) associated with pesticide exposure. Assays were initially validated with known neurotoxic chemicals. In this study, cells were exposed to 0, 0.1, 0.3, 1, 3, 10, 30 and 100  $\mu$ M of chlorpyrifos, aldicarb, and lindane for 48 hrs. Following exposure, media and cells were separated and biological reactions were quenched prior to extraction, derivatization, and analysis by GC-MS. Metabolomic profiling and subsequent multivariate analysis demonstrated separation for each pesticide class and dose dependent responses were observed at concentrations lower than those eliciting effects in cytotoxicity assays. Understanding the biochemical metabolites associated with these responses and mapping them to critical pathways of DNT will aid in predicting the risks due to pesticide exposures.

## Introduction

Evidence suggests that environmental toxins play a role in the origination and progression of a host of central nervous system disorders. Such chemicals may adversely affect the nervous system in adults as well as early in development through maternal exposure. Yet no human model system can adequately predict neurotoxicity associated with toxin exposure, especially at early stages of nervous system development. More predictive human model systems must be developed to address the sheer multitude of potentially toxic chemicals that go completely unscreened or insufficiently evaluated before they reach the consumer.

## Objectives

- Compare the sensitivity of a traditional viability assay to metabolomic profile analysis for detecting responses of neural progenitor cells after pesticide exposure.
- Expose early neural progenitor cells (hNP) to pesticides, and use GC-MS analysis to generate metabolomic profiles, identify specific metabolites disrupted and predict which pathways are affected when pesticide treated cells are compared to control cells.

## Results

### Cellular Responses of hNP cells to Aldicarb, Lindane, or Chlorpyrifos

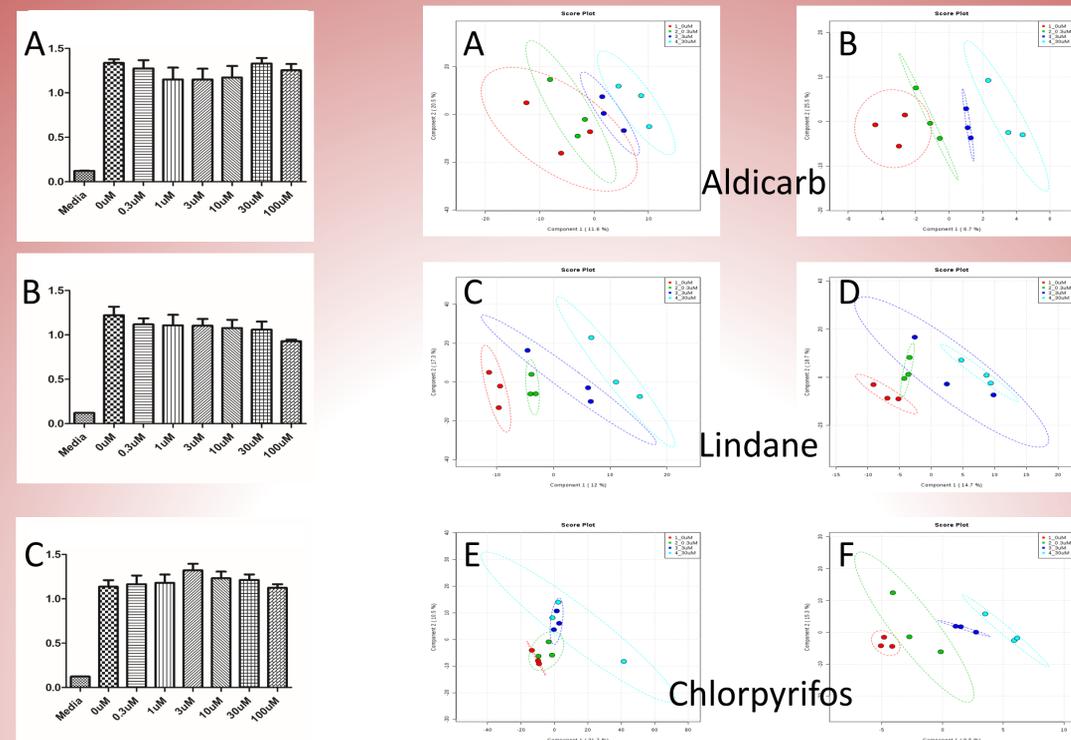


Figure 1A-C: Results from viability assays following 48 hr exposure to aldicarb (A), lindane (B), or chlorpyrifos (C)

Figure 2A-F: PLS-DA scores plots from the spectra of cellular metabolites (A,C,E) and media (B,D,F) after hNP cells were exposed to aldicarb, lindane or chlorpyrifos for 48 hrs.

### Understanding the Response of hNP cells to Chlorpyrifos Exposure

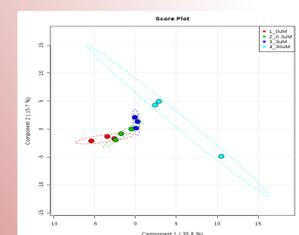


Figure 3: PLS-DA model constructed from the top 30 metabolites identified as significant in chlorpyrifos exposure.

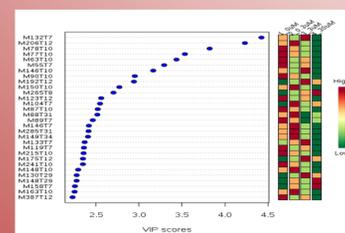


Figure 4: Variable importance plot of the important features for differentiating between control and treated cells in our assay.

Table 1A-B: List of metabolites that were significantly altered in cellular extracts (A) and media (B)

A	B
Glutamic acid	Methionine
Hexadecanoic acid	Valine
Octadecanoic acid	Isoleucine
Heptadecanoic acid	Leucine
2-Monostearoylglycerol	Alanine
Cholesterol	Hexadecanoic acid
Proline	Octadecanoic acid
Tetradecanoic acid	Benzenecetic acid
Eicosanoic acid	Proline
Butanoic acid	Stearic acid
Benzoic acid	
Myo-Inositol	
Phosphoric acid	
carbamate	

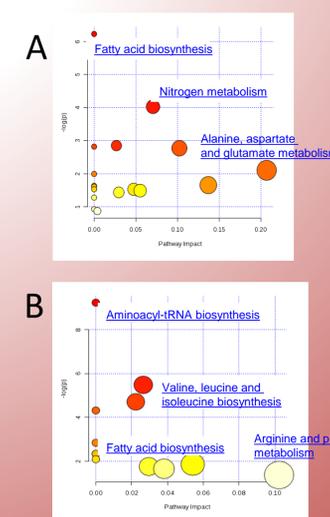
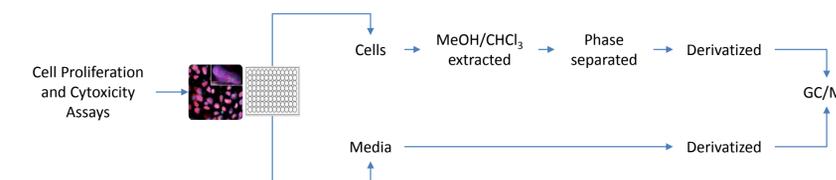


Figure 5A-B: Cloud plot from the pathway analysis of significantly altered metabolites in cell extracts (A) and media (B).

## Materials and Methods



## Conclusions

- Based on traditional viability assays, little to no differences were observed in hNP cells exposed to pesticides for 48 hrs (Figure 1)
- Lindane caused the greatest reduction in cell viability compared to aldicarb and chlorpyrifos and statistically decreased viability at 100  $\mu$ M (Figure 1B)
- Compared to viability data, metabolomic profiling of hNP cells was more sensitive and class separation was observed following exposure to 30  $\mu$ M aldicarb, 0.3  $\mu$ M lindane and 3  $\mu$ M chlorpyrifos (Figure 2A,C,E).
- Interestingly, analysis of the GC/MS spectra from the analysis of extracellular metabolites was more sensitive than cellular profiles in the current study (Figure 2B,D,F) and class separation was observed at 3  $\mu$ M for aldicarb and lindane and 0.3  $\mu$ M chlorpyrifos.
- Using the important features identified as responsible for differentiating control and chlorpyrifos treated cells, a linear dose response is observed and class separation is seen at all doses.
- Pathways identified as significant in both cellular and media analysis after chlorpyrifos exposure included amino acid and fatty acid metabolism (Figure 5)

## Future Directions

- Compare the data from hNP cells to the profiles of hN2 cells to identify pathways that may adversely affect the nervous system at various stages of susceptibility.
- Add metabolic capacity to our assay using co-cultures with C3A cells.

## Acknowledgements

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