Introduction

While the synthesis and use of new chemical compounds is at an all-time high, the study of their potential impact on human health is quickly falling behind. We chose to examine the effects of two common household environmental chemicals, the insect repellent DEET (N, N-diethyl-m-toluamide) and the insecticide fipronil (fluocyanobenzylpyrazole), on transcript levels of xenobiotic metabolism enzymes in primary human hepatocytes. These are responsible for the processing and elimination of exogenous chemicals in the human body and are known to be susceptible to certain toxins. Metabolism is broken down into 3 phases; modification, conjugation, and elimination. This study focuses on those transcripts involved in phases 1 & 2. Additionally, we examined the role of long non-coding RNAs (lncRNA's) associated with these xenobiotic metabolic transcripts.

LncRNAs are RNA transcripts greater than 200 nucleotides long which rarely code for protein. While IncRNAs are believed to play a critical role in numerous important biological processes including regulatory roles, many still remain uncharacterized, and their functions and modes of action remain largely unclear, especially in relation to environmental chemicals. This research represents the first steps toward understanding the role of IncRNAs in DEET and fipronil metabolism. LncRNAs have the potential to serve as prognostic, diagnostic, or therapeutic tools for exposure to these (DEET and Fipronil) and other common exposures.

Results

**Objectives**

- Analyze the impact of DEET and Fipronil, both individually and in combination, on gene expression of transcripts involved in xenobiotic metabolism.
- To investigate the role of long non-coding RNA's associated with metabolic transcripts in primary human hepatocytes.
- Assess the consequences of combined pesticide exposure at the molecular level.

**Methods**

Xenobiotic metabolism enzyme gene expression affected by DEET and Fipronil

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- When primary human hepatocytes were treated with 100 µM DEET, 19 xenobiotic metabolism (XM) genes were differentially expressed (significance level of P ≤ 0.01), of which 17 transcripts are involved in phase 1 metabolism and 2 in phase 2.
- When treated with 10 µM fipronil, 52 XM genes were differentially expressed where 39 transcripts are involved in phase 1 and 13 in phase 2.
- When hepatocytes were treated with a combination of 100 µM DEET and 10 µM fipronil together, 69 XM genes were differentially expressed where 46 transcripts are involved in phase 1 and 23 in phase 2.

- Less than additive effect in xenobiotic metabolic transcript levels
- Chromosomal mapping (ideogram) displays the 52 genes that were dysregulated by 10 µM fipronil after 72 hours. 39 are phase 1 genes and the additional 13 genes are involved in phase 2.

- Chromosomal mapping (ideogram) displays the 69 genes that were dysregulated by 100 µM DEET & 10 µM fipronil after 72 hours. 46 are phase 1 genes and the additional 23 genes are involved in phase 2.

- Long non-coding RNA associations with CYP genes in the DEET + Fipronil treatment. 11 associations were found for this treatment. Not pictured are similar associations under the other 2 treatments and with other metabolic genes. There being 8 associations with CYP's in fipronil, 15 and 21 for other metabolic genes. The identity between lncRNA's associated with groups primarily indicates a regulatory role.

Conclusions

1. Xenobiotic metabolic transcript levels were significantly affected by exposure (over a period of 72 hours) to 100 µM DEET and 10 µM fipronil, either alone or in combination with cytochrome P450 (CYP) enzymes being particularly sensitive.
2. Unlike in global expression levels where a more than additive effect on transcription was seen, metabolic transcript levels did not meet this threshold. This implies that expression in metabolic genes is affected by DEET & fipronil exposure in a similar fashion.
3. Exposure to the two compounds elicited a response in IncRNAs as well. A number of these being in close proximity to metabolic transcripts (Phase 1 & 2 genes) which indicates a regulatory role.
4. Several IncRNAs associated with groups of CYP's have a high percent identity which indicates regulation by these specific IncRNAs on CYP groupings via a similar mechanism. This leads to the potential future investigation of long non-coding RNAs as a global "switch" for xenobiotic metabolic genes.

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