Metabolic & Epigenetic Responses of Human Liver Cells to DEET and Fipronil

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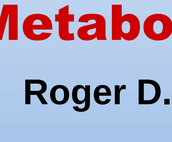
Introduction

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 (fluocyanobenpyrazole), 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 toxicants. 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 RNA's (lncRNA's) associated with these xenobiotic metabolic transcripts.

LncRNAs are RNA transcripts greater than 200 nucleotides long which rarely code for protein. While lncRNAs 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 lncRNAs 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.



DEET

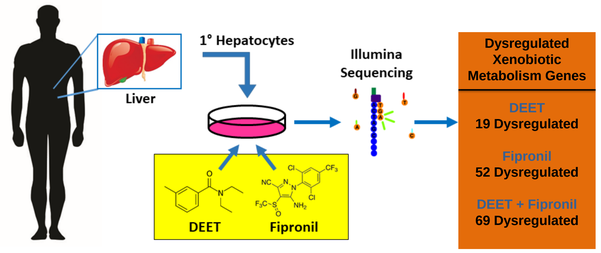
Fipronil

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.

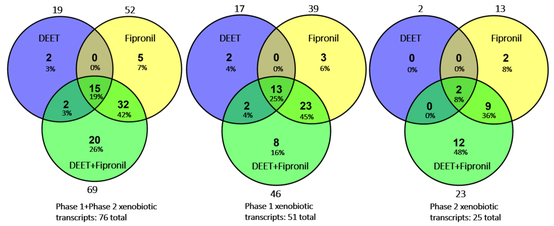
Methods

Xenobiotic metabolism enzyme gene expression affected by DEET and Fipronil

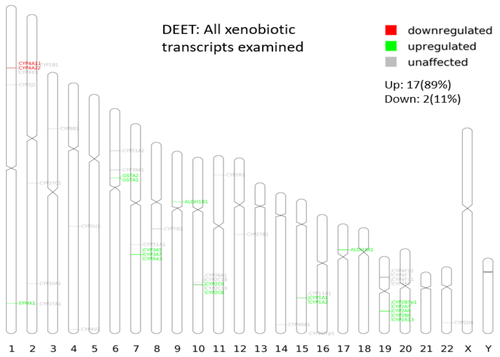


Results

- 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.



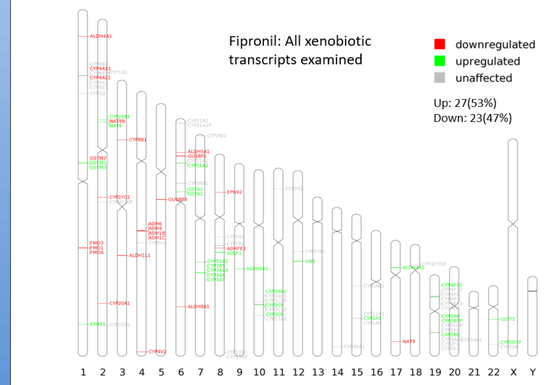
- Less than additive effect in xenobiotic metabolic transcript levels



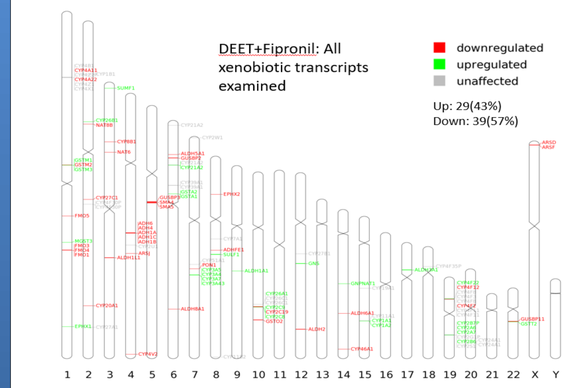
- Chromosomal mapping (ideogram) displays the 19 genes that were dysregulated by 100 µM DEET after 72 hours. 17 are phase 1 genes and the additional 2 genes are involved in phase 2. The table below details gene family & fold change data for each gene in the above chromosomal map.

Gene Symbol	Family & Gene Name	Chromosome	Log 2 Fold Change
Phase 1			
CYP4A11	cytochrome P450, family 4, subfamily a, polypeptide 11	chr1:47394845-47407156	-1.11
CYP4A22	cytochrome P450, family 4, subfamily a, polypeptide 22	chr1:47403106-47614526	-1.07
EPHX1	epoxide hydrolase 1	chr1:225897796-226070420	+2.30
CYP3A5	cytochrome P450, family 3, subfamily a, polypeptide 5	chr7:99245812-99277621	+1.15
CYP3A7	cytochrome P450, family 3, subfamily a, polypeptide 7	chr7:99282301-99332819	+3.39
CYP3A43	cytochrome P450, family 3, subfamily a, polypeptide 43	chr7:99425635-99464173	+3.25
ALDH1B1	aldehyde dehydrogenase 1 family, member B1	chr9:38392660-38398662	+0.66
CYP2C9	cytochrome P450, family 2, subfamily C, polypeptide 9	chr10:96698414-96749148	+0.93
CYP2C8	cytochrome P450, family 2, subfamily C, polypeptide 8	chr10:96796528-96829254	+2.29
CYP1A1	cytochrome P450, family 1, subfamily A, polypeptide 1	chr15:75011882-75017877	+1.82
CYP1A2	cytochrome P450, family 1, subfamily A, polypeptide 2	chr15:75041183-75048941	+1.23
ALDH3A1	aldehyde dehydrogenase 3 family, member A1	chr17:19641297-19651746	+2.76
CYP2B6	cytochrome P450, family 2, subfamily B, polypeptide 6	chr19:41497203-41524301	+3.82
CYP2B7P1	cytochrome P450, family 2, subfamily B, polypeptide 7 pseudogene 1	chr19:41430169-41456565	+2.97
CYP2A6	cytochrome P450, family 2, subfamily A, polypeptide 6	chr19:41349442-41356352	+2.21
CYP2A7	cytochrome P450, family 2, subfamily A, polypeptide 7	chr19:41381343-41388657	+2.44
CYP2A13	cytochrome P450, family 2, subfamily A, polypeptide 13	chr19:41594355-41602100	+2.34
Phase 2			
GSTA2	glutathione S-transferase alpha 2	chr6:52614884-52628361	+2.51
GSTA1	glutathione S-transferase alpha 1	chr6:52656177-52668664	+1.90

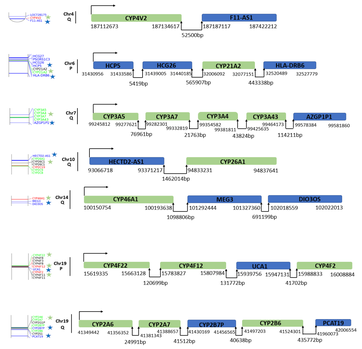
[a]Gene symbol defined by NCBI Database.
[b] Fold change was statistically significant (α=0.05).



- 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.

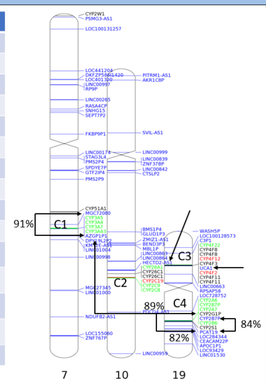


- Chromosomal mapping (ideogram) displays the 69 genes that were dysregulated by 100 µM DEET & 10 µM fipronil after 72 hours. 46 are phase 1 genes and the additional 23 genes are involved in phase 2.



- 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 when treated with fipronil and DEET + fipronil respectively.

lncRNA Alignment	Percent Identity
MGC72080 x AZGP1P1	91%
CYP2B7P x PCAT19	84%
AZGP1P1 x PCAT19	82%
AZGP1P1 x CYP2B7P	89%
MGC72080 x PCAT19	84%
MGC72080 x CYP2B7P	90%
HECTD2-AS1 x AZGP1P1	91%
HECTD2-AS1 x MGC72080	91%
HECTD2-AS1 x PCAT19	94%
HECTD2-AS1 x CYP2B7P	90%
UCA1 x CYP2B7P	No significant alignment
UCA1 x PCAT19	No significant alignment
UCA1 x AZGP1P1	No significant alignment
UCA1 x MGC72080	No significant alignment
UCA1 x HECTD2-AS1	No significant alignment



- Blast alignment of lncRNA's associated with groups of CYP genes under DEET + fipronil treatment. We found ≥82% identity between lncRNA's associated with groups primarily or only up-regulated. No significant association was found with the lncRNA UCA1 which was associated with a primarily down-regulated or unaffected group of CYP's.

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 lncRNA's as well. A number of these being in close proximity to metabolic transcripts (Phase 1 &2 genes) which indicates a regulatory role.
4. Several lncRNA's associated with groups of CYP's have a high percent identity which indicates regulation by these specific lncRNA's on CYP groupings via a similar mechanism. This leads to the potential future investigation of long non-coding RNA's as a global "switch" for xenobiotic metabolic genes.

Acknowledgements

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