OP-095 [Bee Health]
Exploring RNA methylation as a promising biomarker for assessing risk of pesticide on honeybees

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Honeybees play a crucial role as pollinators for crops and are regarded as sensitive bioindicators of environmental health. The widespread use of pesticides poses a severe threat to honeybee survival. It is imperative to assess the toxic effects of pesticides on honeybees to facilitate informed scientific application and management of these compounds, ultimately reducing their potential ecological risks. Exposure to toxic components, even at low concentrations can induce epigenetic alterations in organisms, and various epigenetic marks are recognized as indicators of environmental disturbances. In recent years, RNA methylation has emerged as a growing field of research in toxicological studies of xenobiotic compounds. However, the study on RNA methylation in honeybees is still in its early stages, and no demonstrated examples exist thus far illustrating changes in RNA methylation levels in response to external environmental factors. Recognizing the significance of RNA methylations across diverseorganisms and the lack of relevant information regarding honeybees, our study aimed to investigate the effects of phenylimidazole pesticides on various RNA methylations in honeybee larvae and adults at environmentally relevant doses. We found the response patterns of RNA methylation upon exposure to these pesticides. Furthermore, we detected significant alterations in the expression levels of genes associated with RNA methylation in both honeybee larvae and newly emerged adults. Notably, we identified potential biomarkers derived from RNA methylation and their related genes for pesticide risk assessment purposes. This pioneering study provides valuable insights into the impact of phenylimidazole pesticides on RNA methylations within honeybees while exploring the feasibility of utilizing RNA methylation as a tool for assessing pesticide risks among this vital pollinator species. These findings offer new perspectives on strategies for protecting honeybees and advancing toxicity evaluation systems for pesticides.

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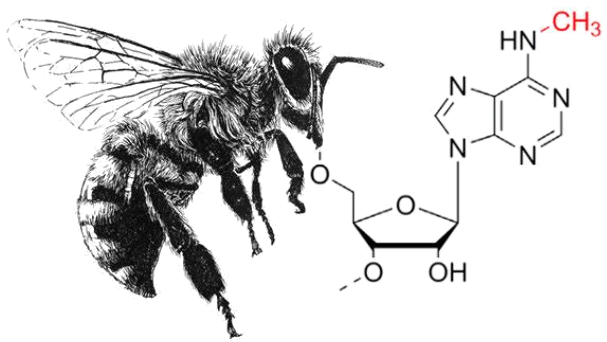
Exploring RNA methylation as a promising biomarker for assessing risk of pesticide on honeybees



Miao Wang
Ph.D, Associate Professor

Institute of Apicultural Research
Chinese Academy of Agricultural Sciences

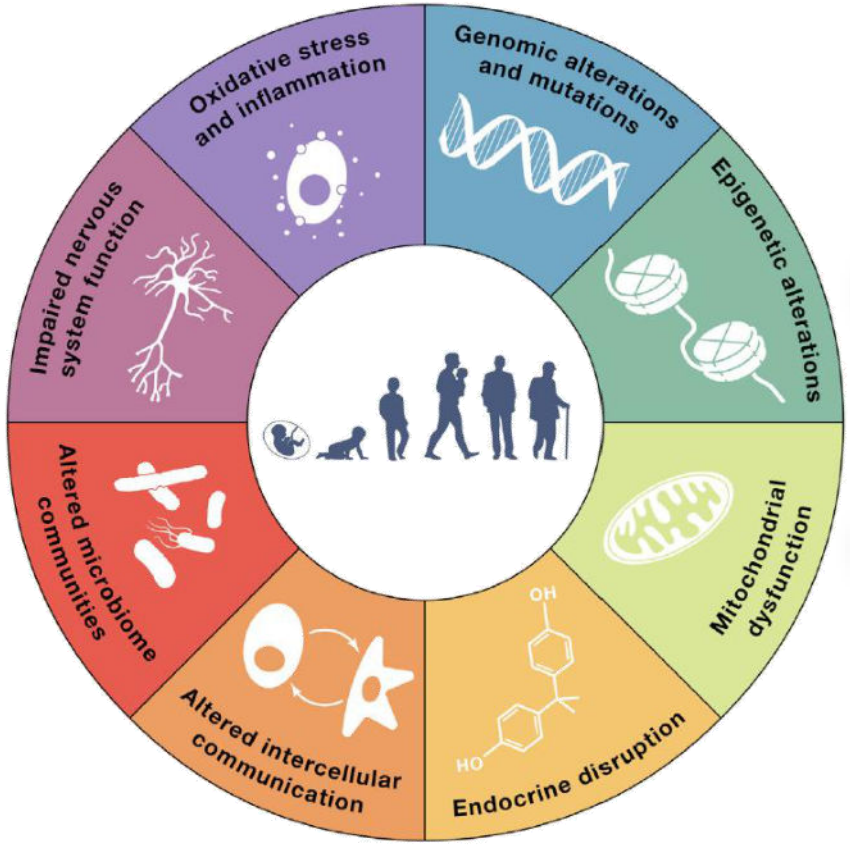
Pesticides endanger honeybee survival and health

Honeybees:
pollinators
bioindicators of environment

Pesticide risk assessment for honeybees

Rapid stress response via epigenetic mechanisms

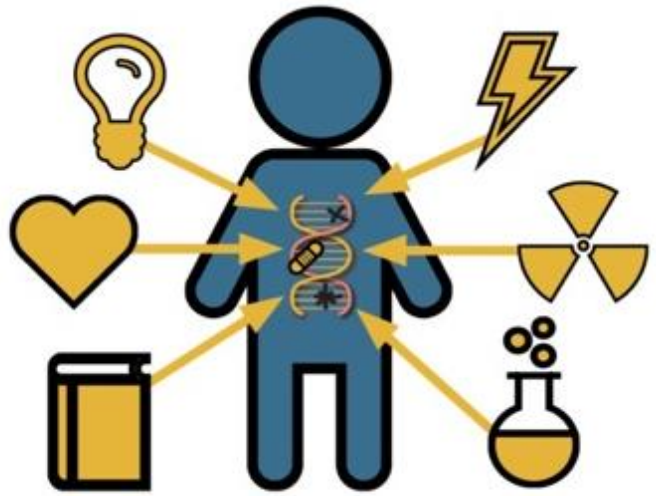
Epigenetic modifications serve as hallmarks of environmental insults



Annette Peters *et. al*, Cell, 2021

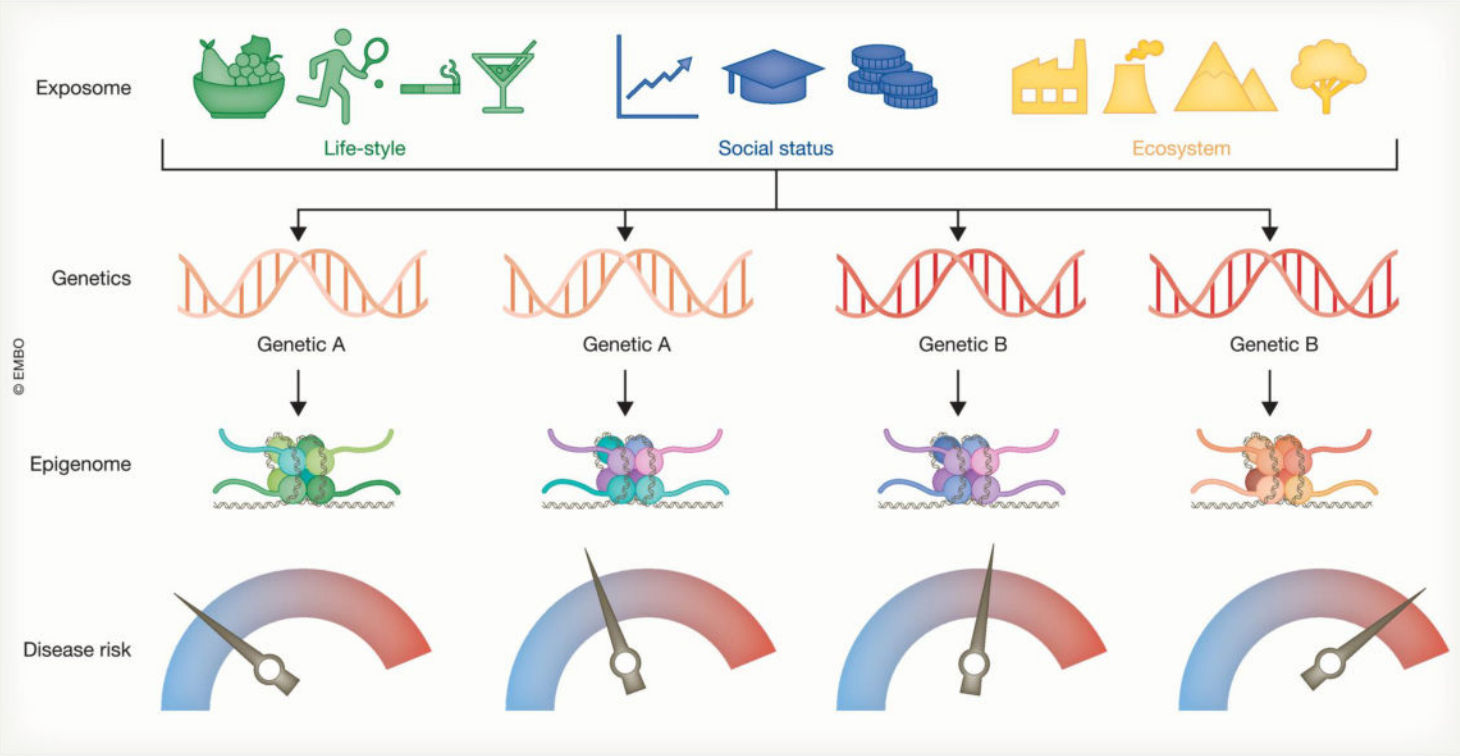
Epigenetic modifications

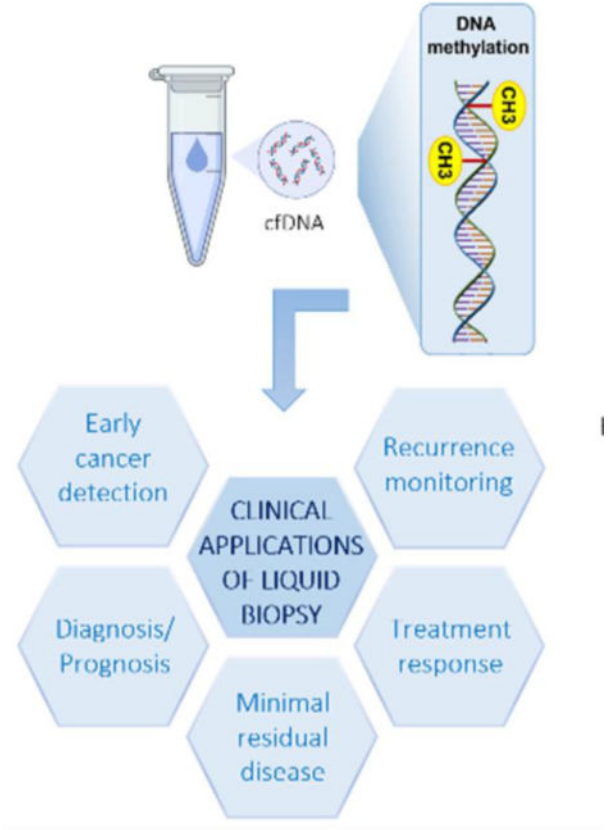
- A rapid response mechanism to environmental stress
- Promising early-warning biomarkers for environmental risks



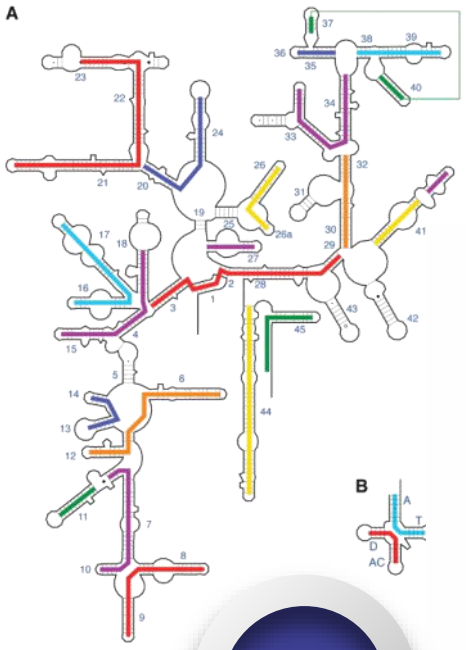
DNA methylation-based biomarkers

DNA methylation biomarkers applied in cancer detection and exposure monitoring





RNA methylation: the untapped promise of an exposure biomarker



rRNA

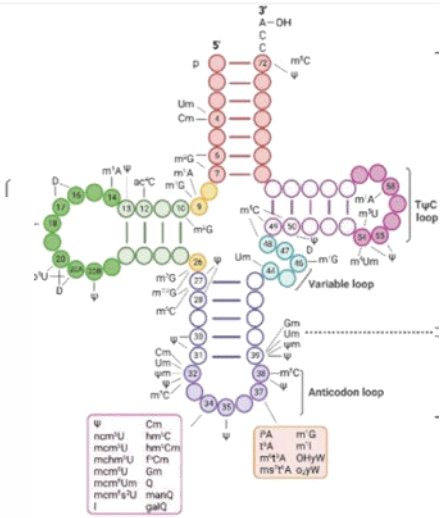
mRNA

5-methycytosine (m^5C)

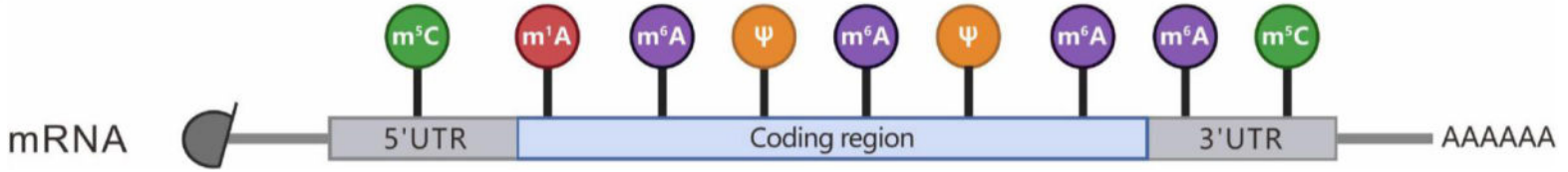
N^1-methyladenosine (m^1A)

N^6-methyladenosine (m^6A)

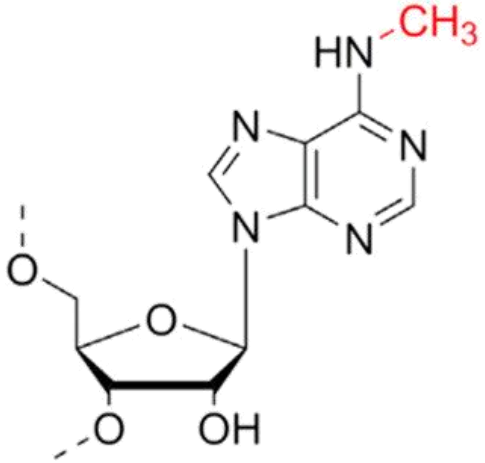
Pseudouridine (ψ)



tRNA

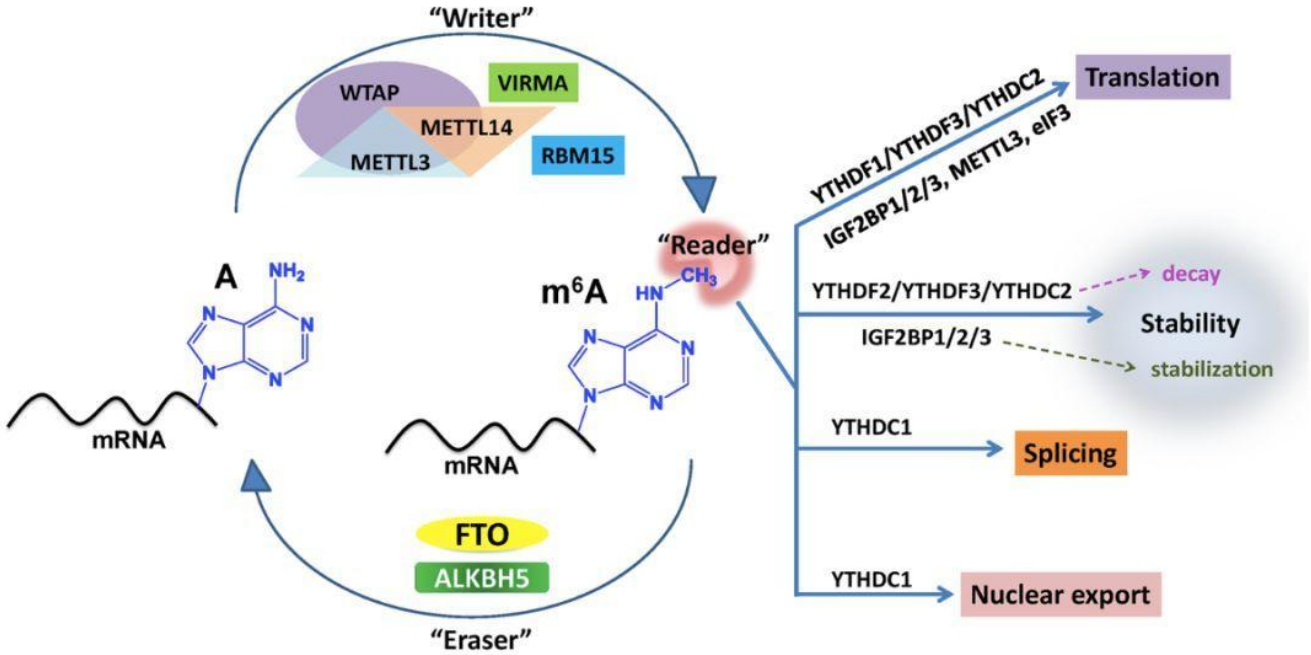


RNA m^{6}A modification



m^{6}A

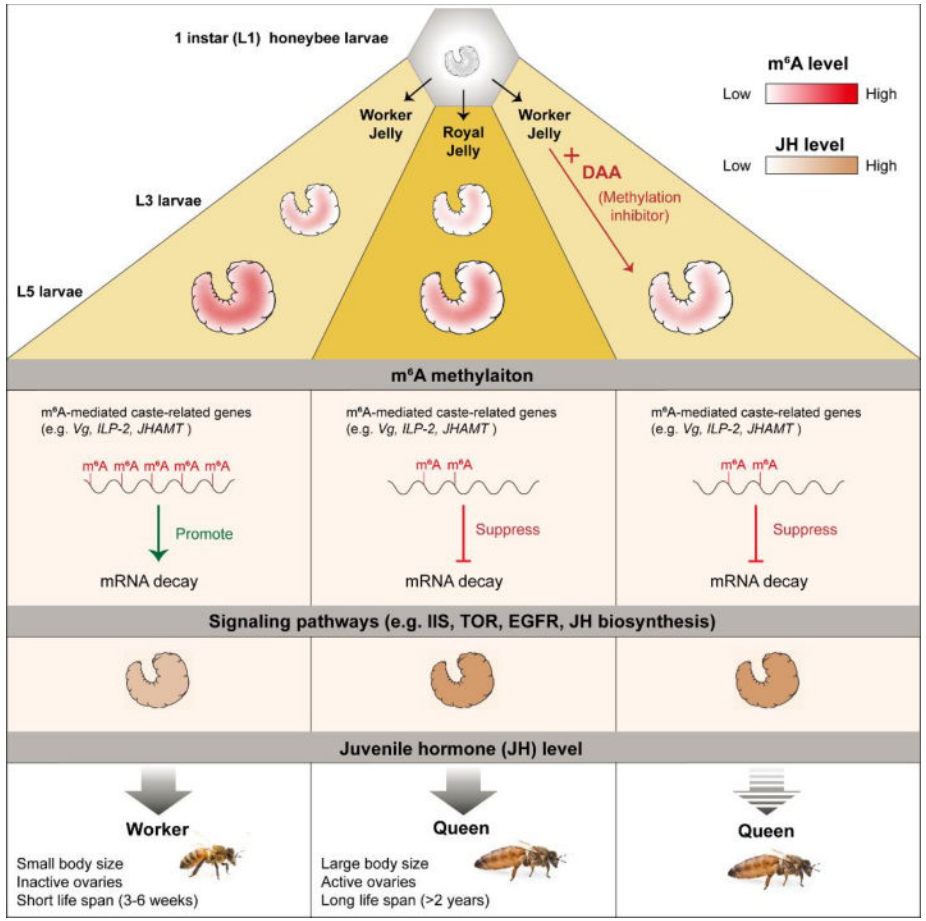
***N*^{6}-methyladenosine**



- Prevalent
- Reversible
- Post-transcriptional regulation

Cell Reports

RNA m^{6}A Modification Functions in Larval Development and Caste Differentiation in Honeybee (*Apis mellifera*)



Highlights

- m^{6}A marks can be differentially altered by nutritional input
- m^{6}A marks are increased and regulate gene expression during larval development
- Worker larvae contain more hypermethylated m^{6}A marks than do queen larvae
- Chemical suppression of m^{6}A marks in worker larvae induces queen caste features

Wang *et. al*, Cell Reports, 2021

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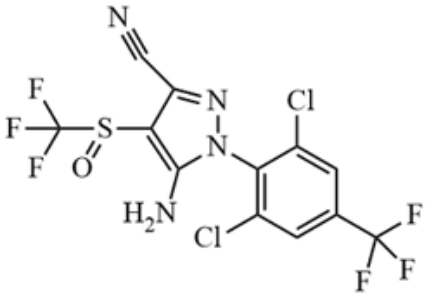
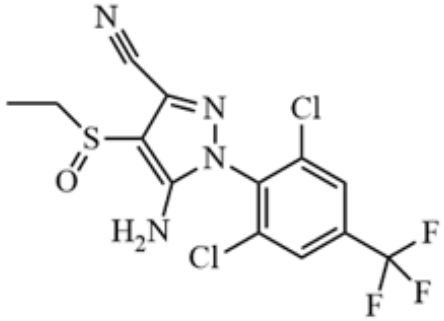
Is RNA m^6A Modification Altered upon Pesticide Exposure?

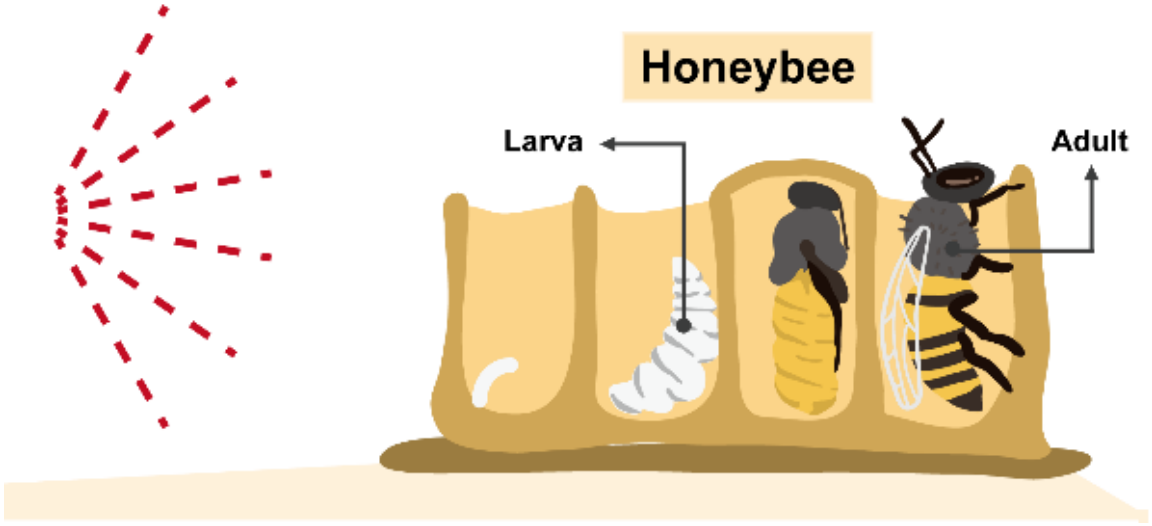
Phenylpyrazole insecticide



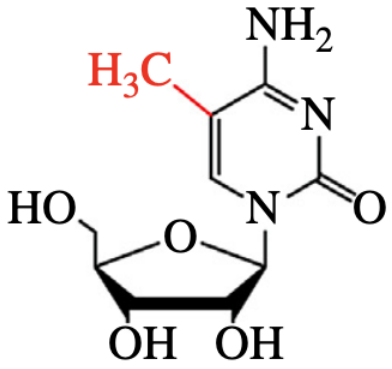
Fipronil



Ethiprole

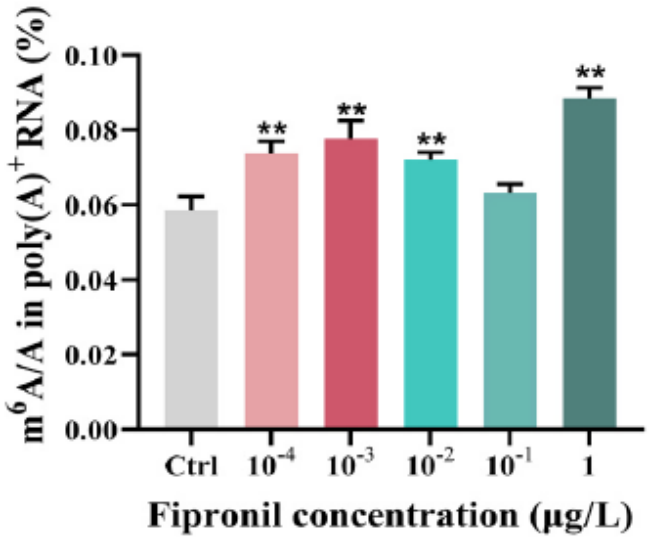


Fipronil exposure

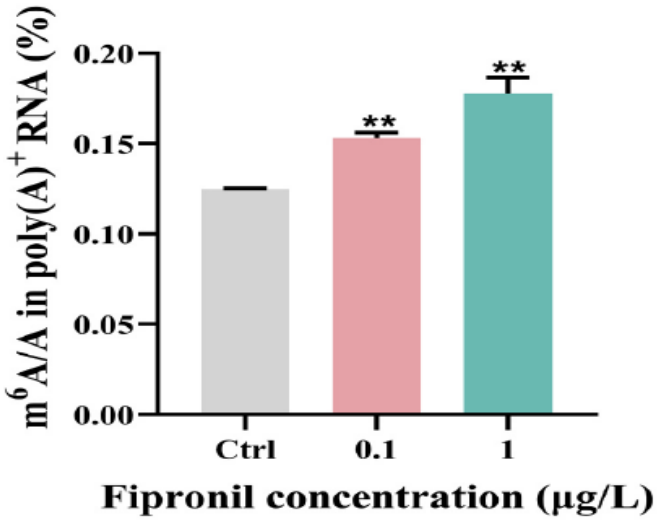


N^6-methyladenosine
(m^6A)

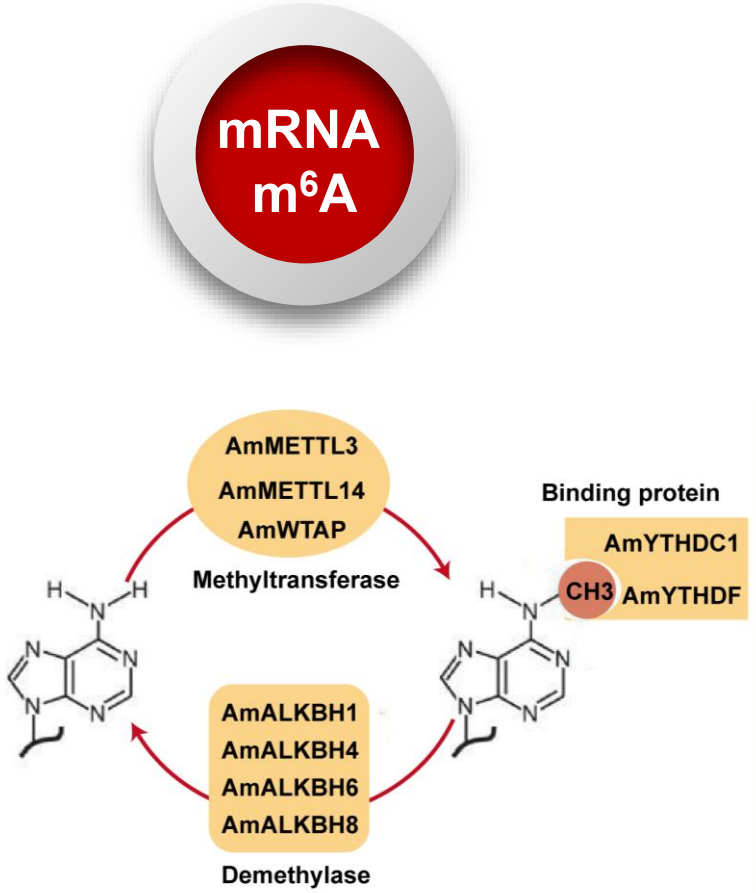
Larvae



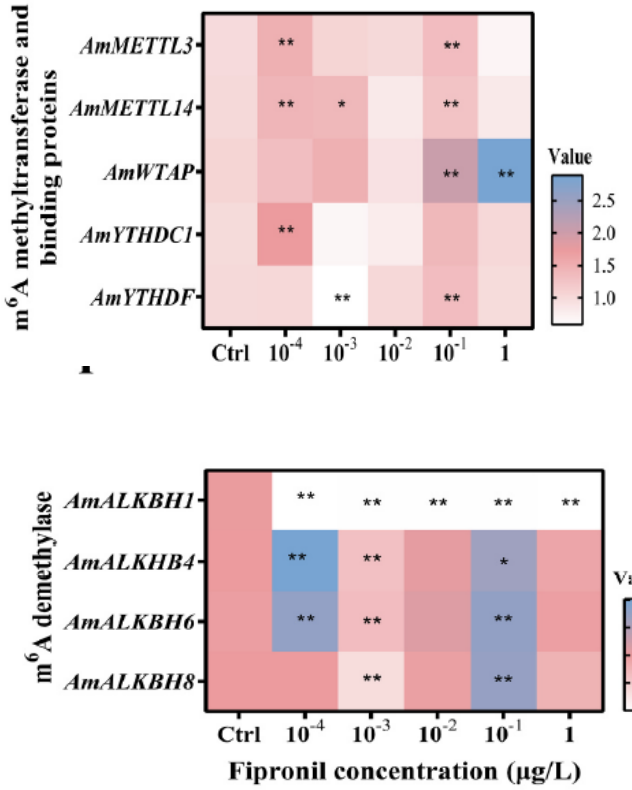
Adult brain



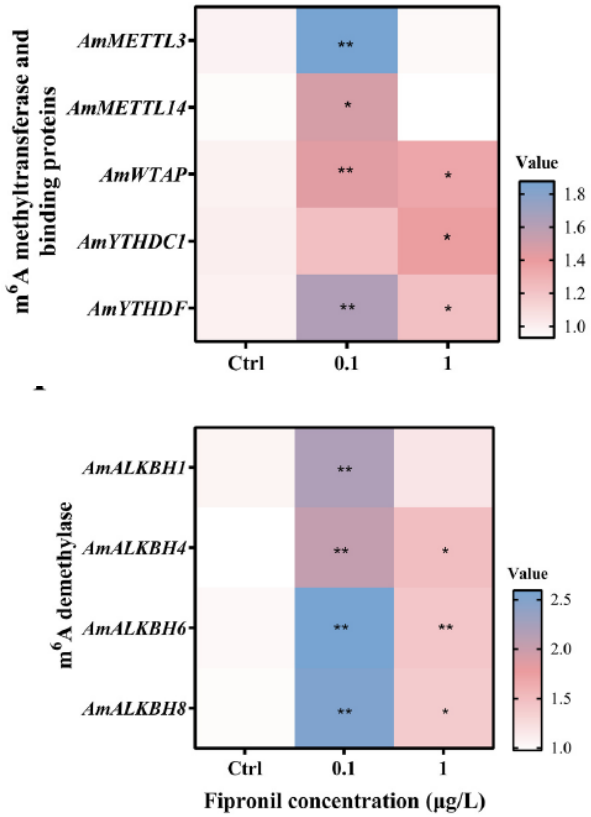
Fipronil exposure



Larvae



Adult brain

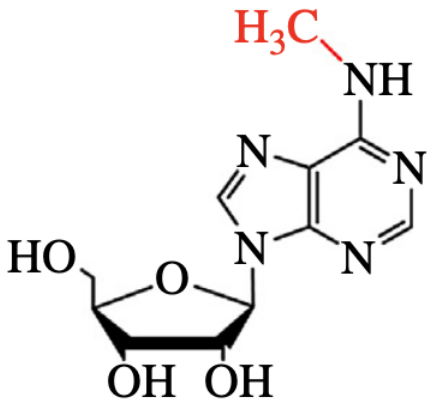


Fipronil exposure

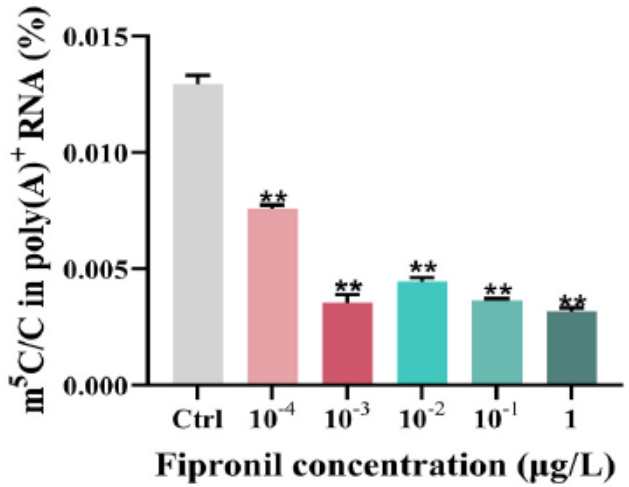
mRNA
m^5C

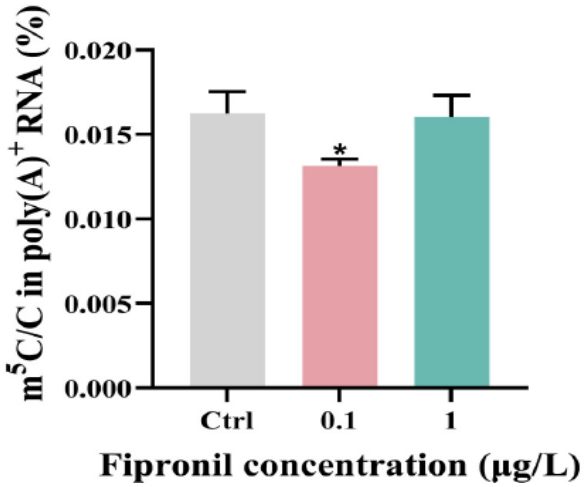
Larvae

Adult brain

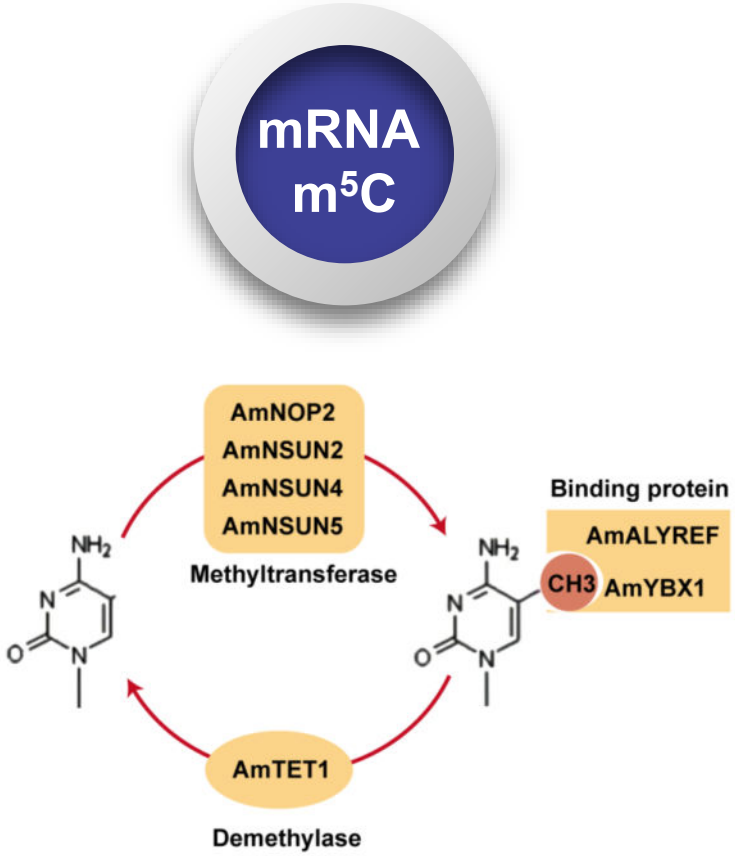


5-methylcytosine
(m^5C)

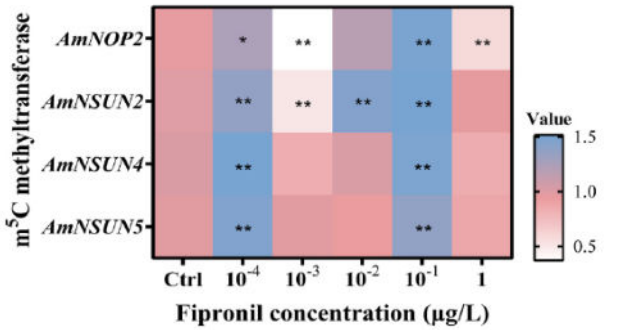


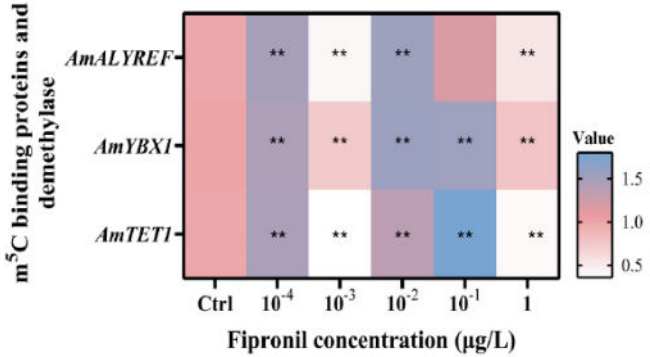


Fipronil exposure

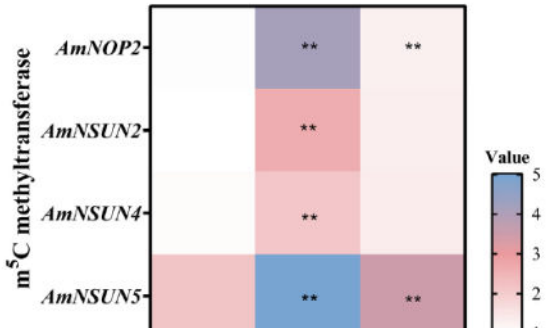


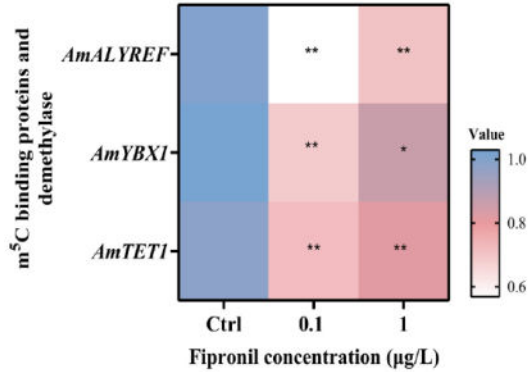
Larvae



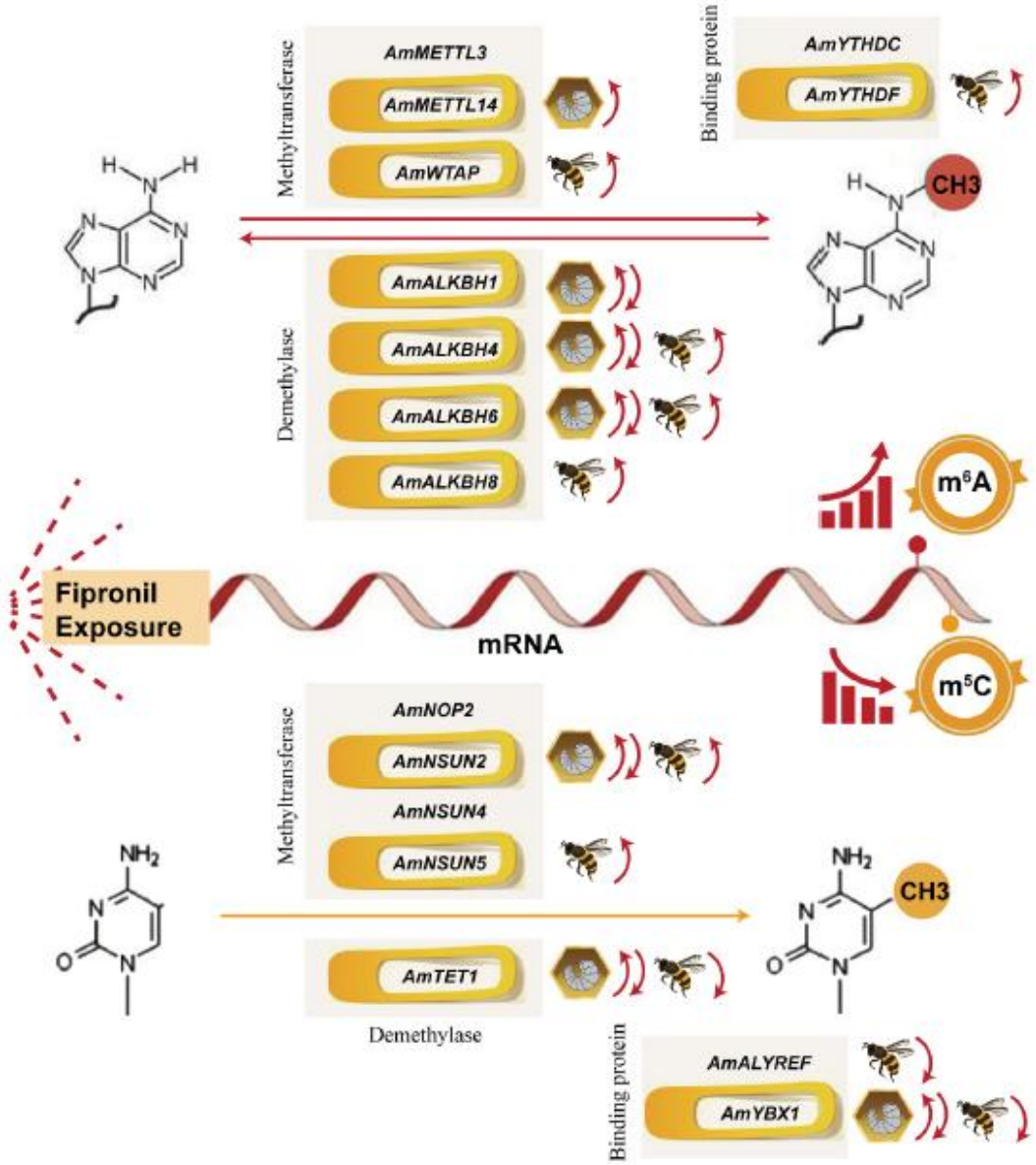


Adult brain





Promising biomarkers for fipronil exposure risk assessment



RNA m^{6}A methylation and related genes

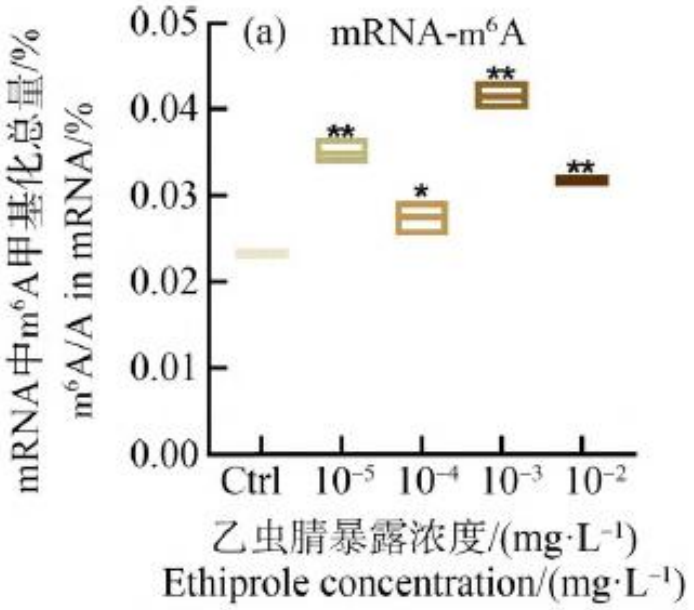
RNA m^{5}C methylation and related genes

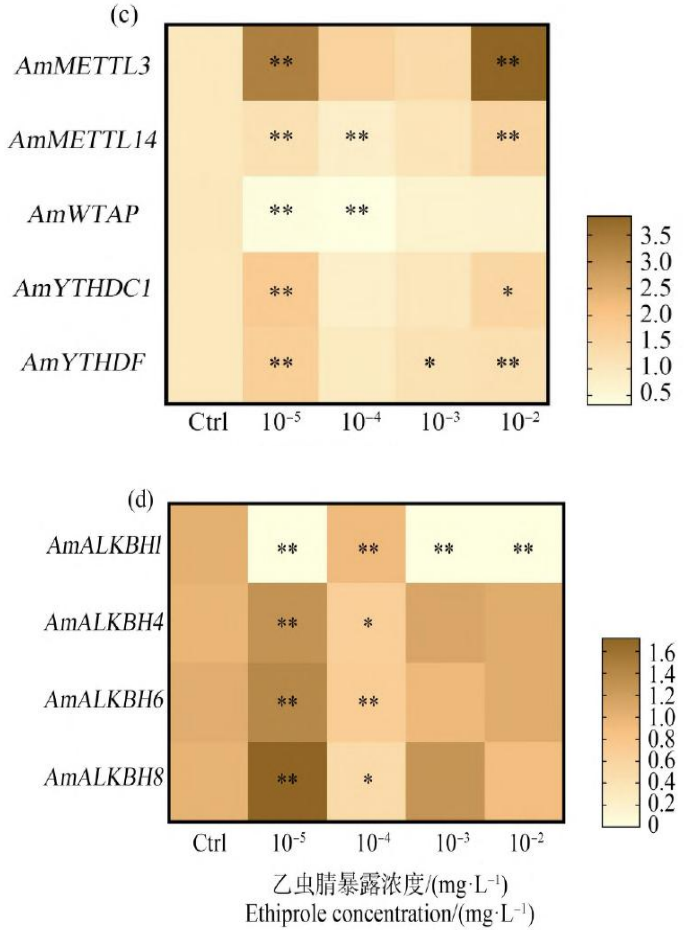
Fan *et. al*, Ecotoxicology and Environmental Safety. 2023

Ethiprole exposure

Larvae

mRNA
m^{6}A

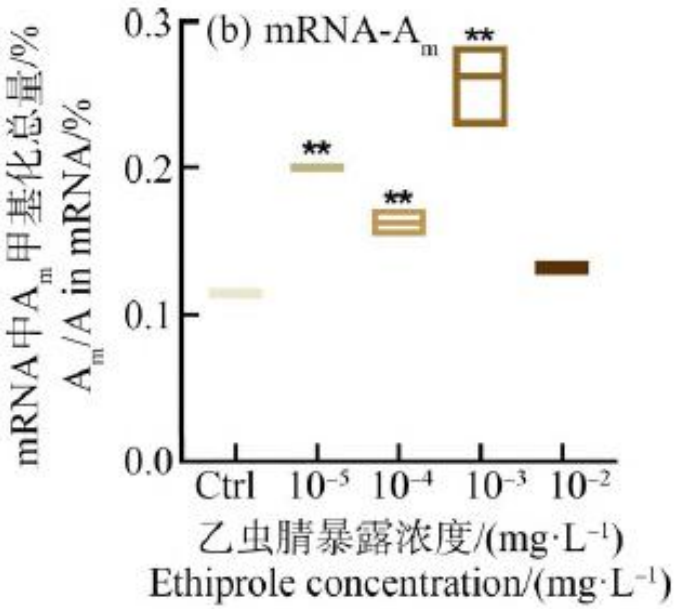


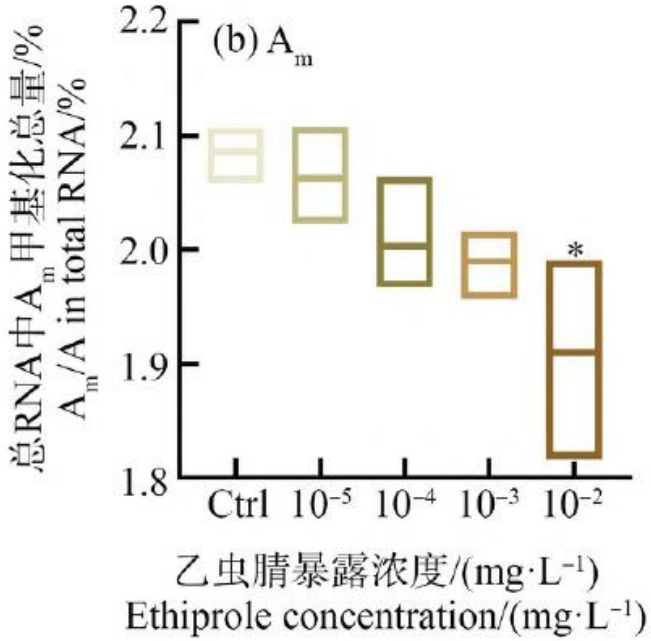


Ethiprole exposure

Larvae

mRNA
A_m

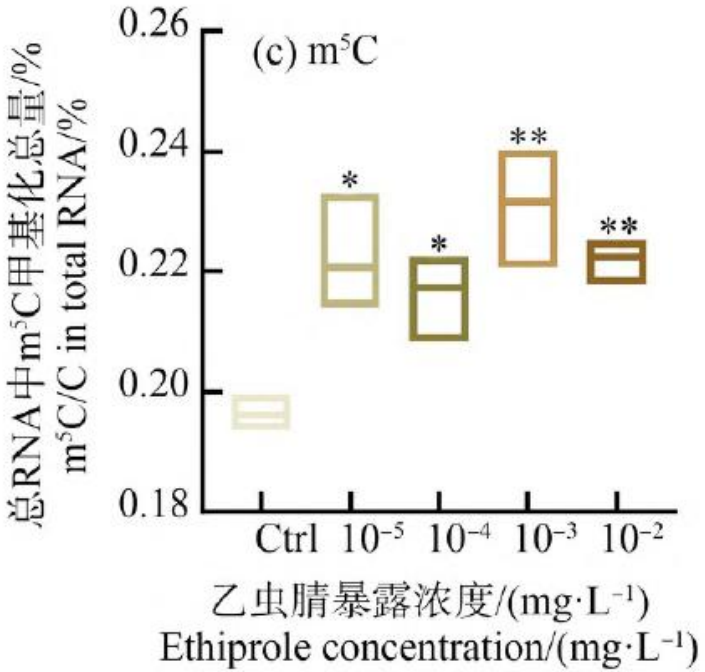


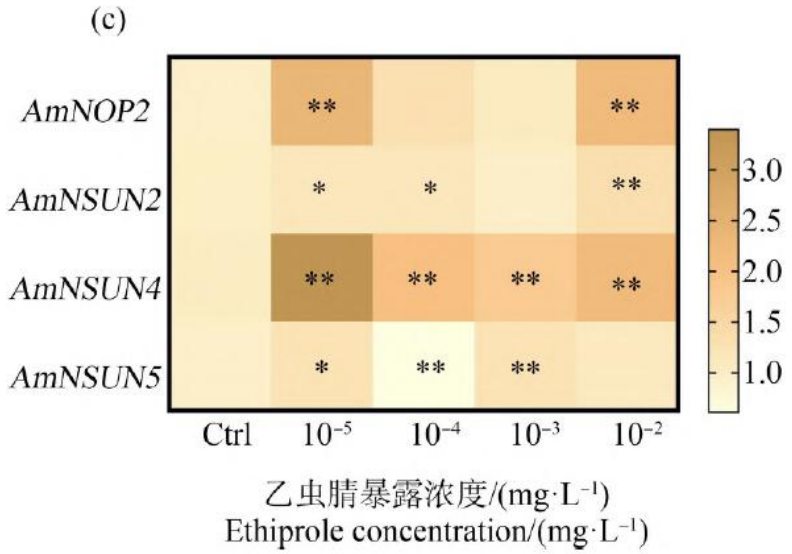


Ethiprole exposure

Larvae

mRNA
m^{5}C

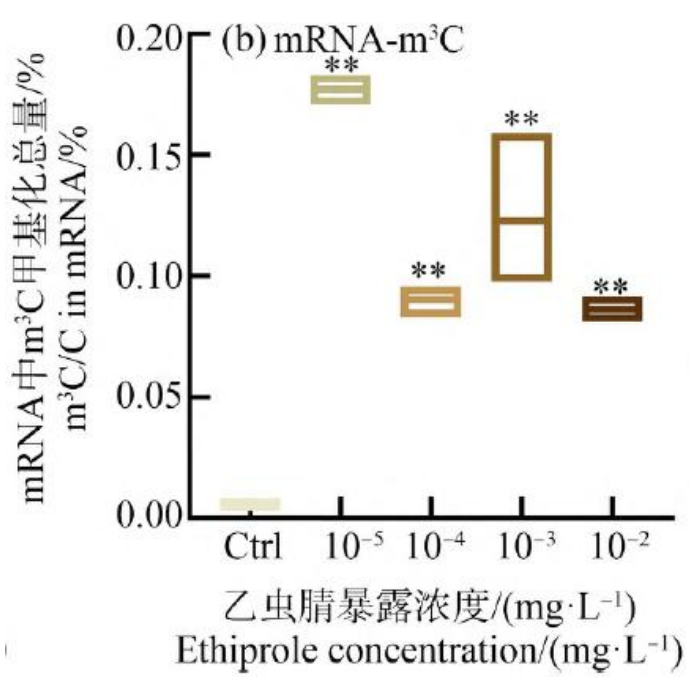


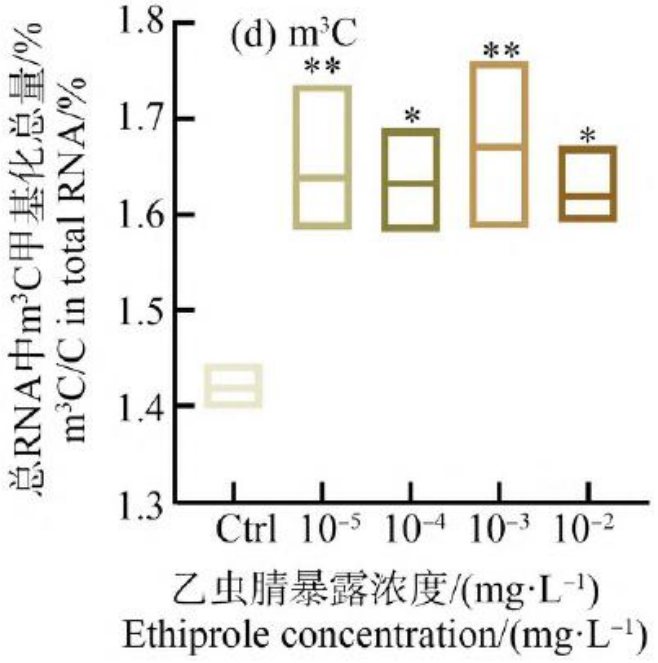


Ethiprole exposure

Larvae

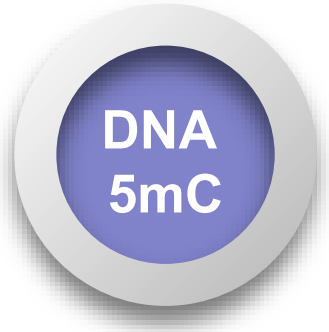
mRNA
m³C

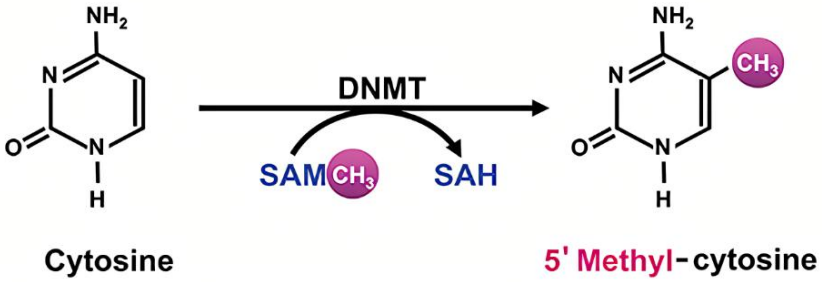


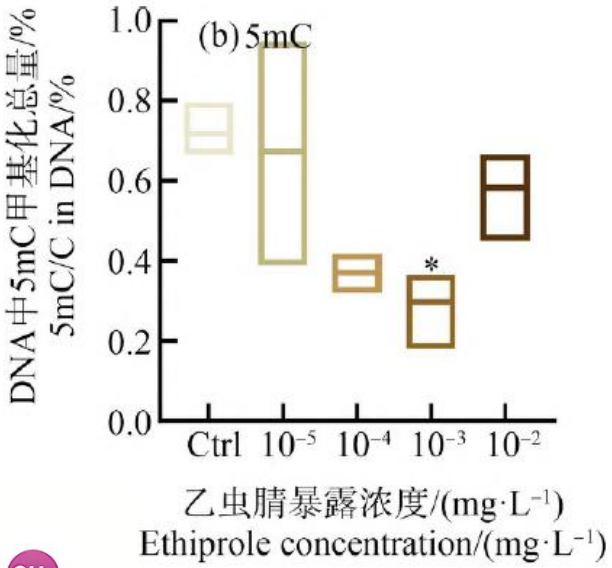


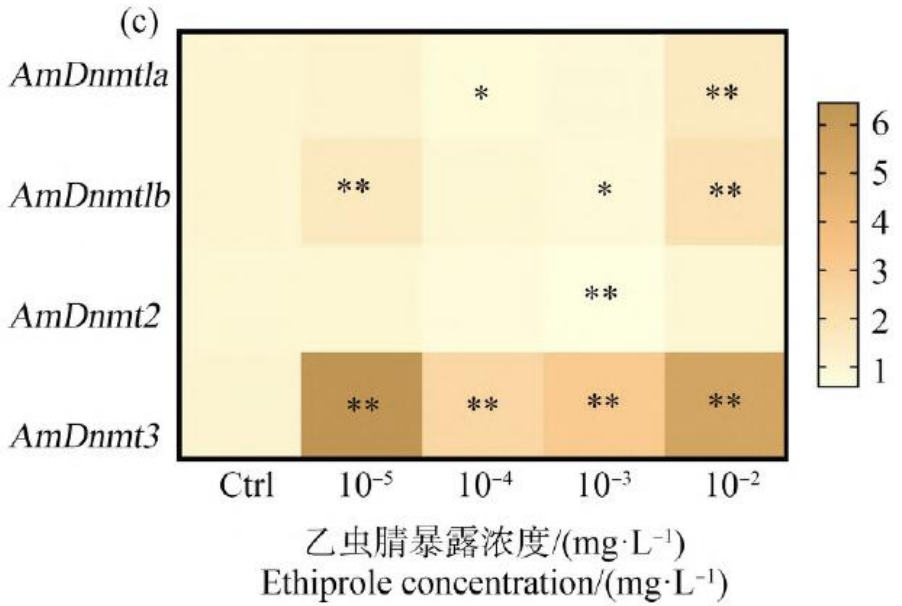
Ethiprole exposure

Larvae

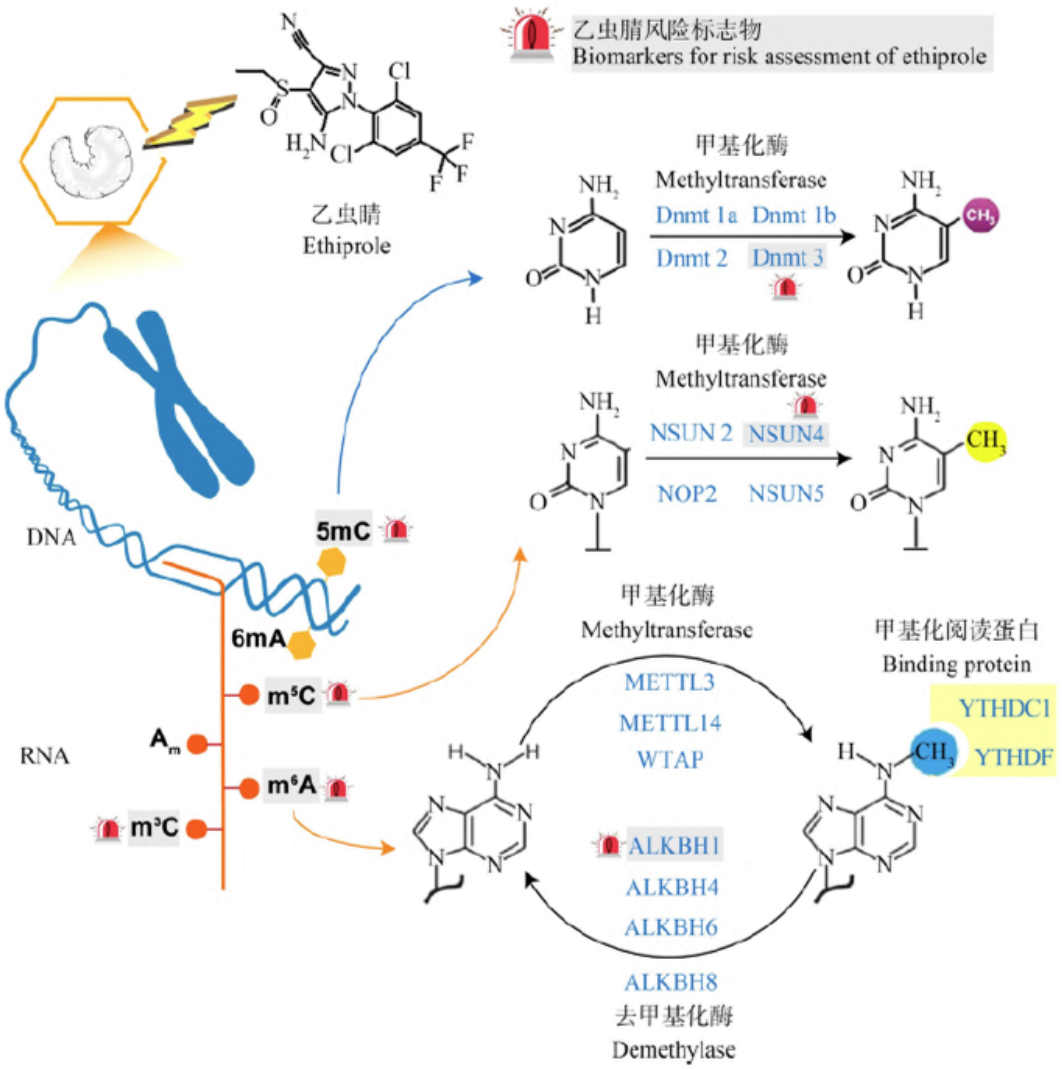








Promising biomarkers for ethiprole exposure risk assessment



DNA 5mC methylation

RNA m^6A, m^5C, and m^3C methylation

Dnmt3, NSUN4, ALKBH1

Fan *et. al*, Asian Journal of Ecotoxicology. 2023

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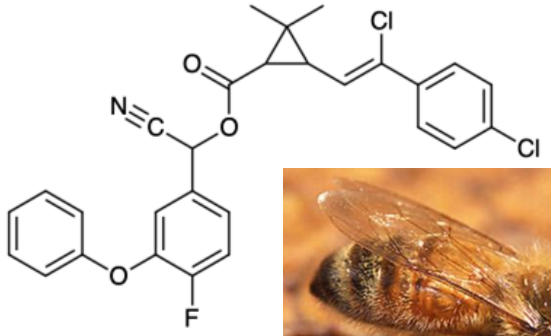
RNA m^{6}A Methylation in Specific Transcripts as Biomarkers for Pesticide Exposure

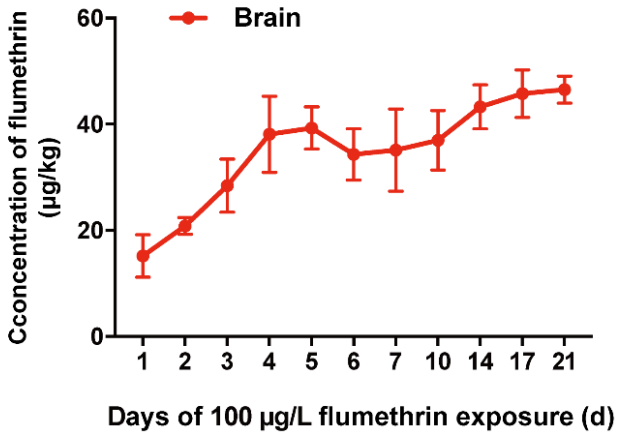
Flumethrin exposure

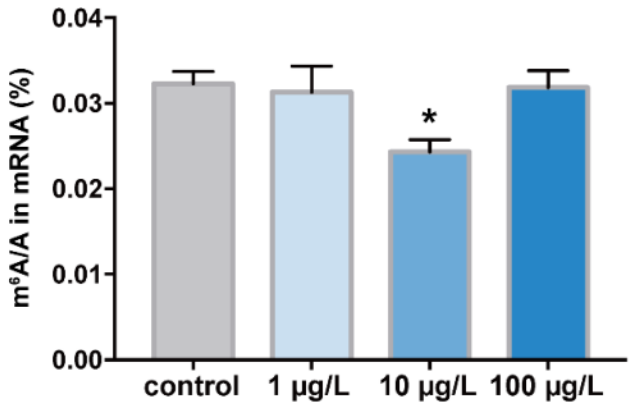


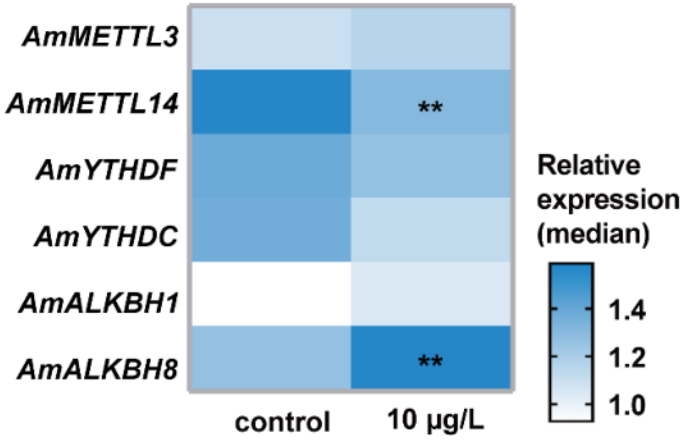
Flumethrin
an acaricide used to control honeybee mites

Adult brain

Flumethrin accumulated in the brain through oral intake



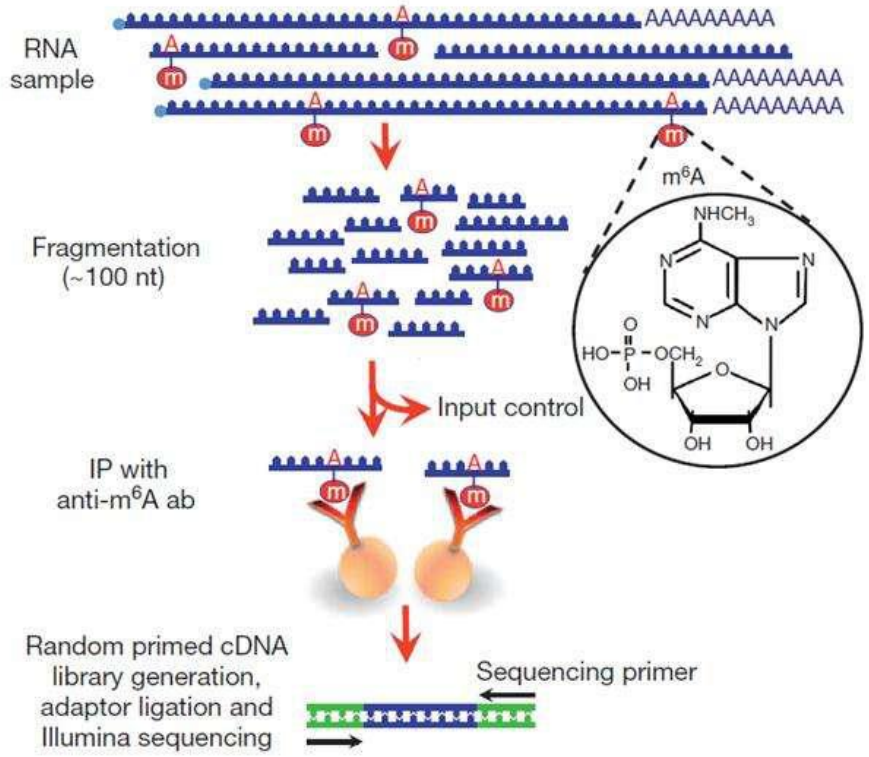


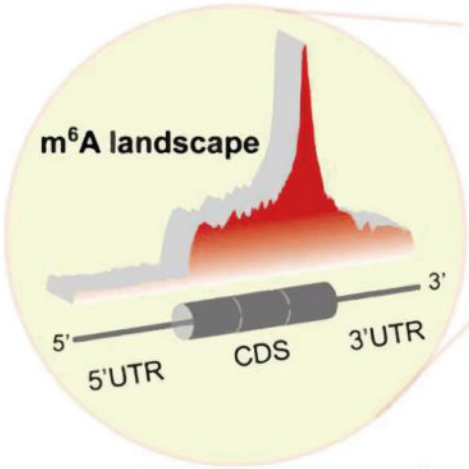


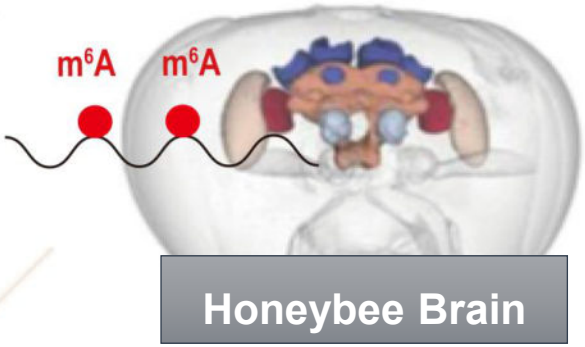
Flumethrin exposure

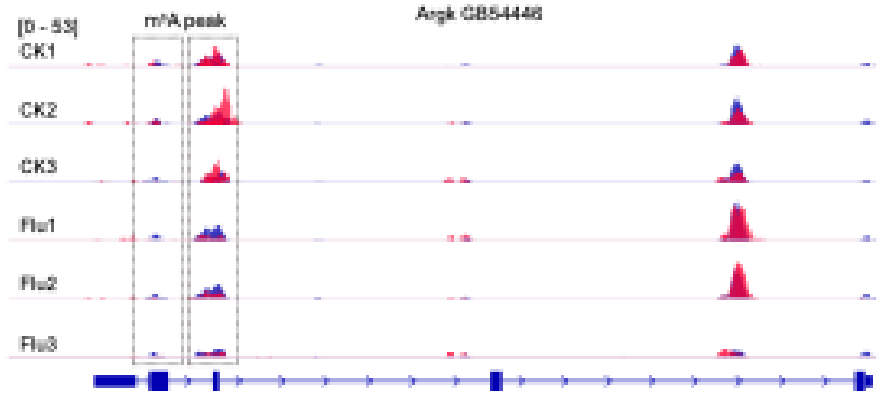
m^{6}A-seq

m^{6}A-seq enables identification of m^{6}A-mediated transcripts



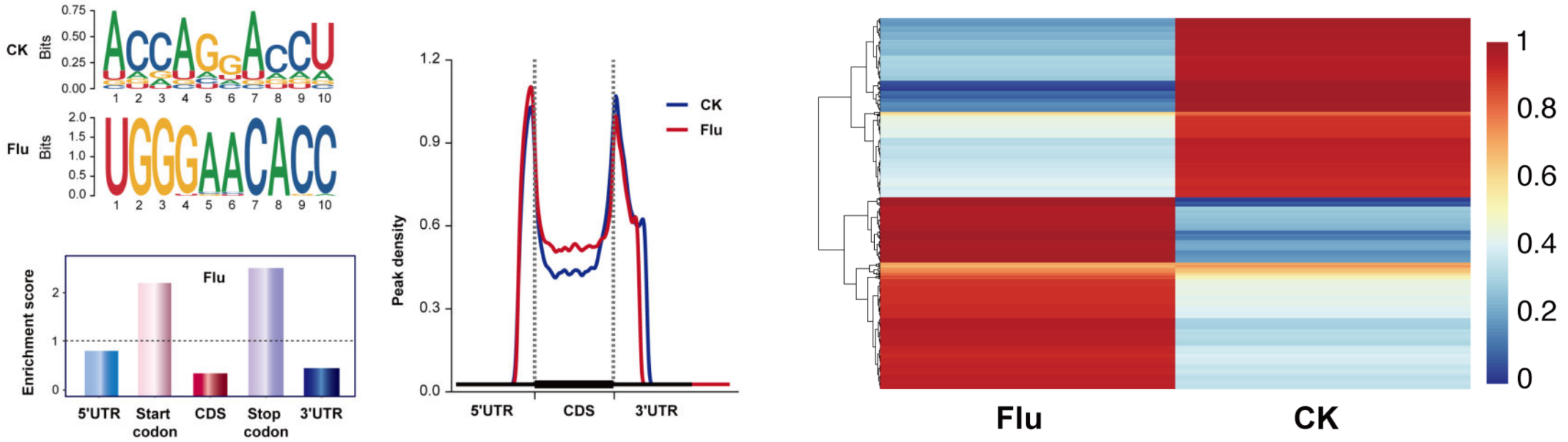






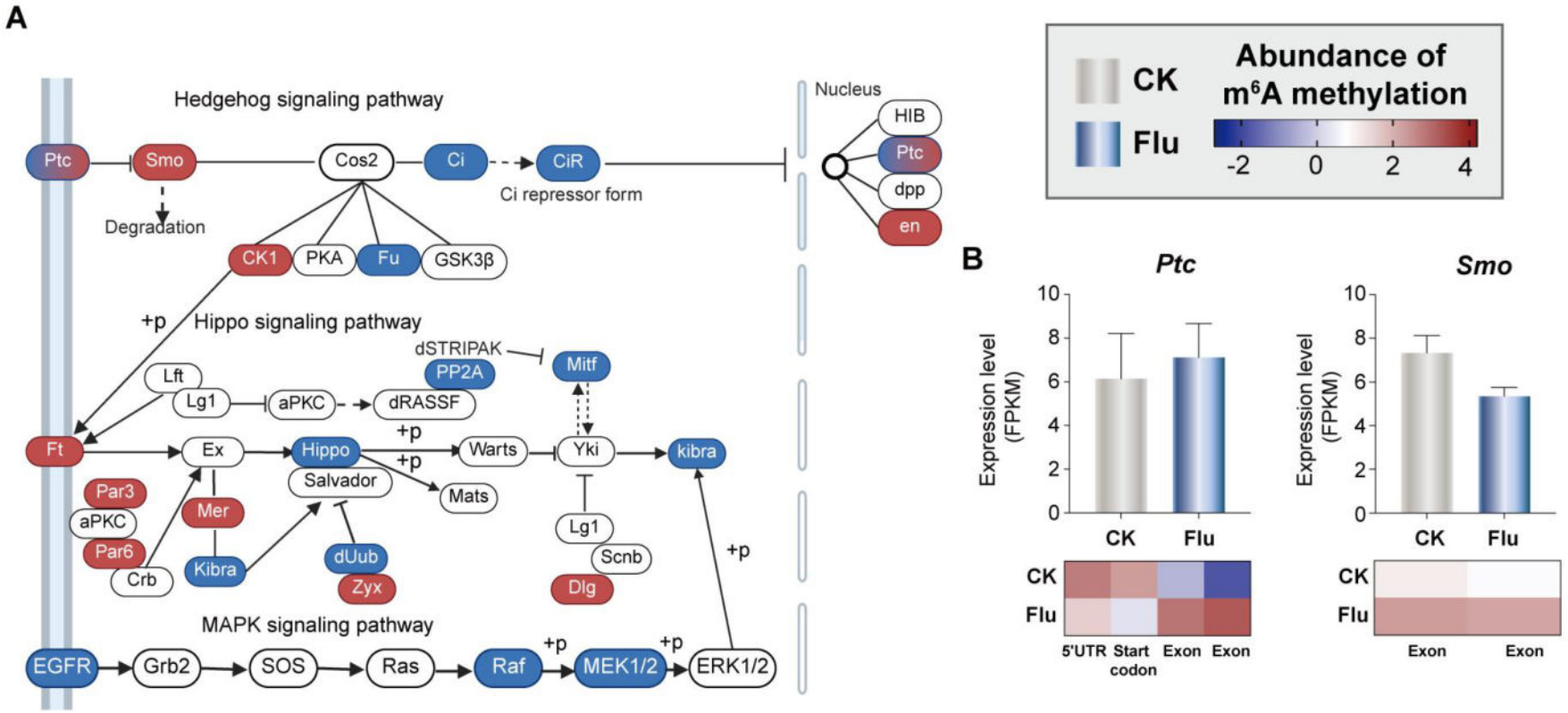
Flumethrin exposure

Flumethrin exposure alter the m^{6}A modification and the expression of m^{6}A-mediated genes

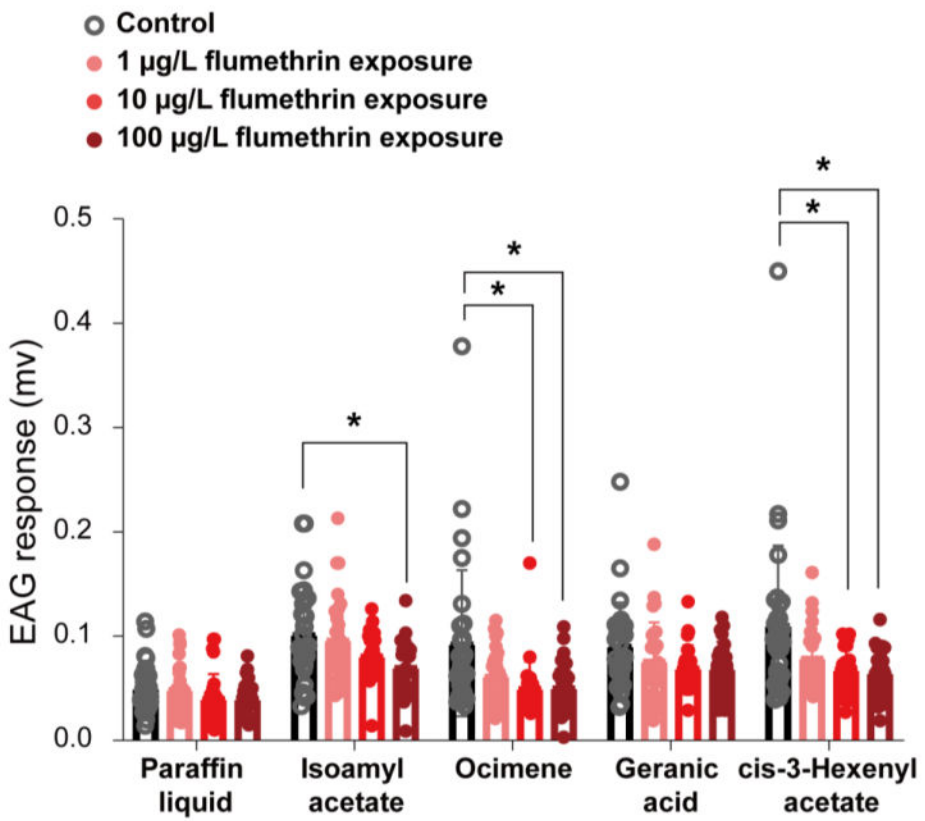


Promising biomarkers for flumethrin exposure risk assessment

Flumethrin significantly altered m^{6}A methylation on transcripts crucial for neuronal activity and memory within Hedgehog and Hippo signaling pathways.

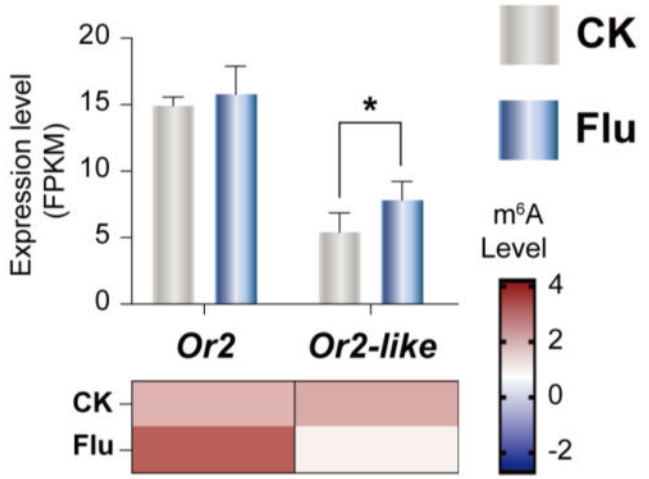


Promising biomarkers for flumethrin exposure risk assessment



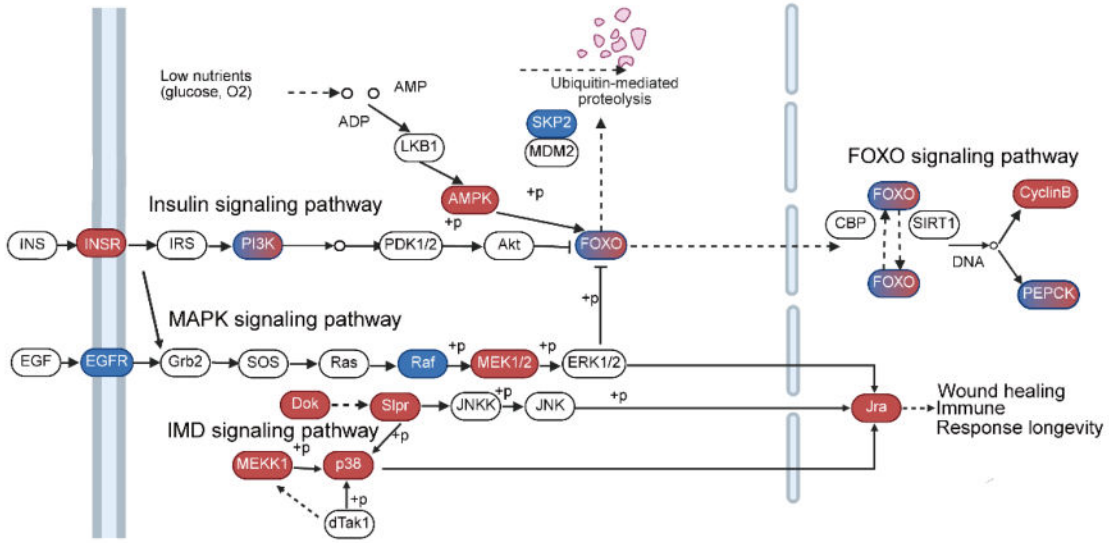
Flumethrin exposure cause olfactory sensitivity deficits

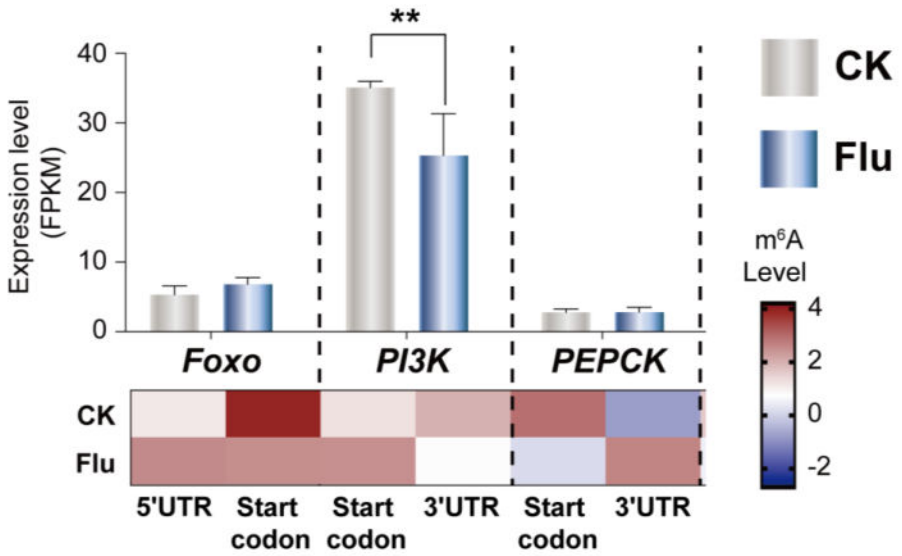
Flumethrin exposure altered m^{6}A modifications in 12 odorant receptor transcripts



Promising biomarkers for flumethrin exposure risk assessment

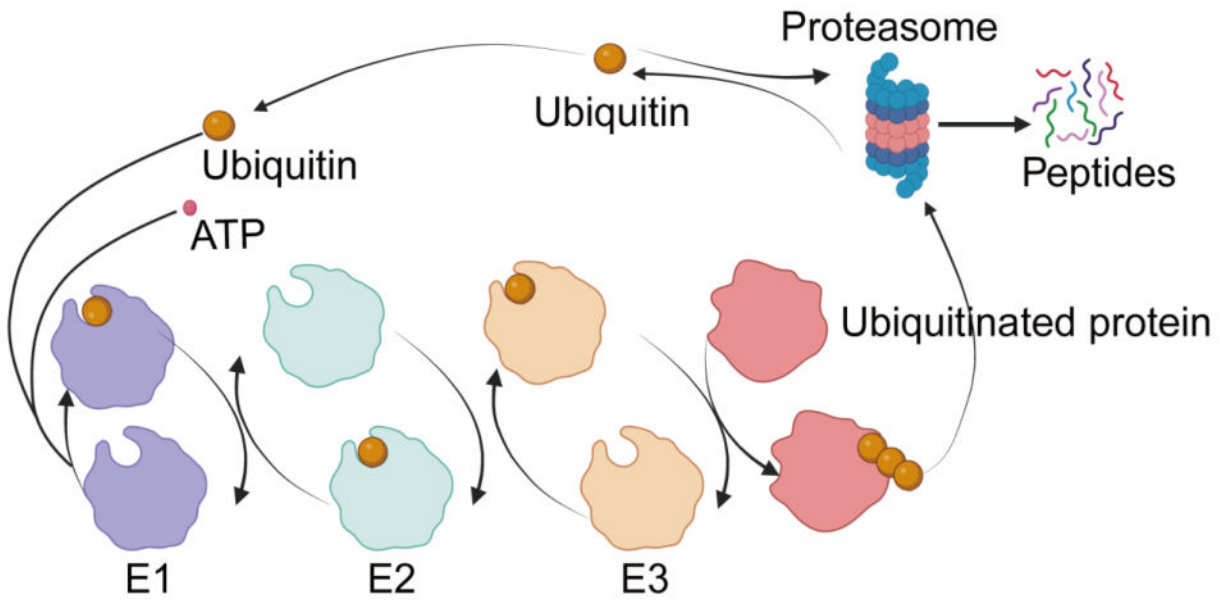
Flumethrin altered methylation on transcripts encoding stress sensors and immune modulators in FoxO and MAPK signaling pathways

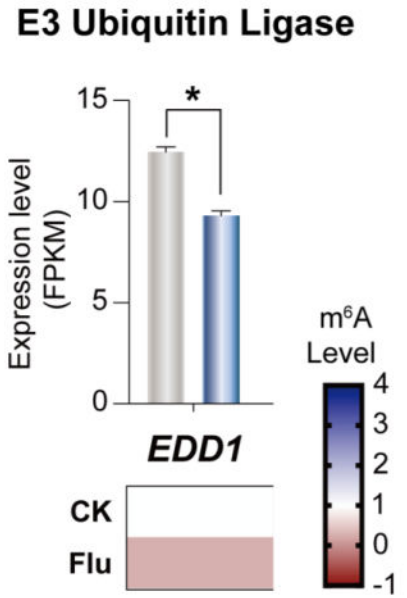




Promising biomarkers for flumethrin exposure risk assessment

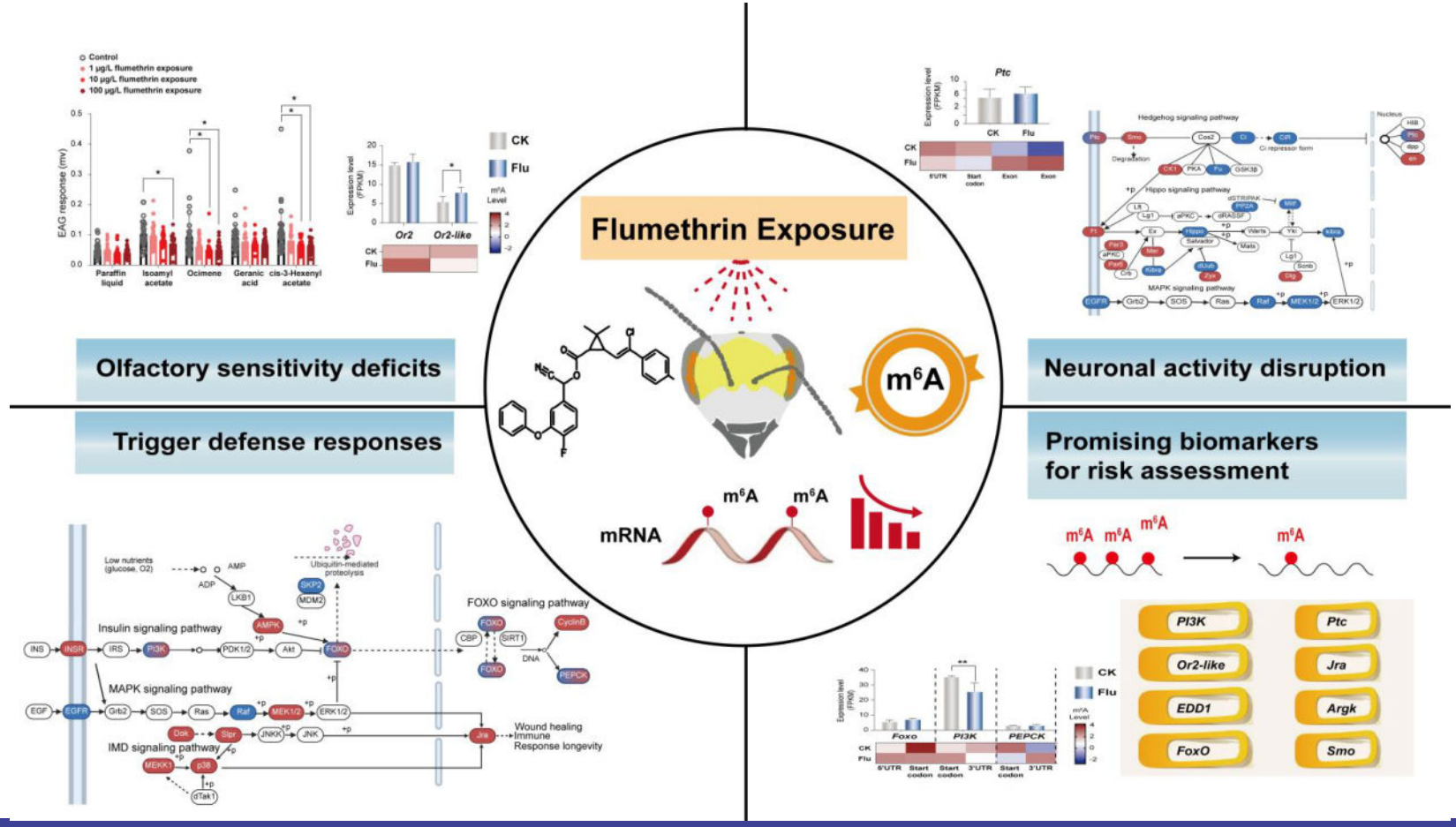
Flumethrin altered m^{6}A modifications in ubiquitin mediated proteolysis





Promising biomarkers for flumethrin exposure risk assessment

All these transcripts, with their flumethrin-specific m^{6}A modifications, were selected as potential biomarkers for flumethrin risk assessment.



The manuscript is under review

Outlook

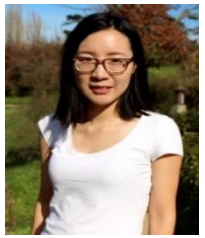
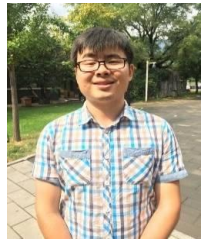
Our future research will focus on

- standardizing and expanding the applications of RNA methylation-based biomarkers
- developing rapid detection methods using these biomarkers to evaluate pesticide risks to honeybees
- artificially modulating m^6A methylation levels using chemical inhibitors/enhancers to improve honeybees' pesticide tolerance

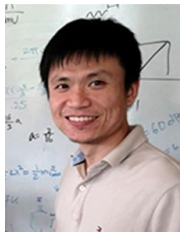
ACKNOWLEDGEMENT

Our lab

State Key Laboratory of Resource Insects
Quality and Risk Assessment of Bee Products

Cooperation



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Fundings

NSFC
CAAS
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THANKS