A metabolomic approach to assess neurotoxic effects of Imidacloprid on the freshwater snail *Lymnaea Stagnalis*
Background - Imidacloprid

- **Neonicotinoids - New generation of neuro-active insecticides**
- **High water solubility**
- **High environmental occurrence**
- **Evidence of a connection to honey-bee colony collapse disorder**
- **EU imposed a number of use restrictions**
  
  but its use it is still allowed in greenhouses

In the Netherlands:

- > 1 µg/L
- < 1 µg/L
- < 0.1 µg/L

Picture taken from Atlas Bestrijdingsmiddelen in Oppervlaktewater

http://www.bestrijdingsmiddelenatlas.nl/
Objective

- Imidacloprid affects nicotinid acetylcholine esterase (nAchRs) receptors of insects
- Low affinity for mammals nAChRs has been shown, but what for invertebrates and aquatic species?

Macro-Invertebrate Decline in Surface Water Polluted with Imidacloprid

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Aim of the study

Study sub-lethal neurotoxic effects in non-target invertebrate specimen exposed at environmentally relevant concentrations of Imidacloprid

Lymnaea Stagnalis  CNS
Method - Chemical Analysis

SNAIL CNS

EXTRACTION

MeOH/H$_2$O fraction

Chloroform fraction

TARGETED ANALYSIS
neurotransmitters

UNTARGETED ANALYSIS
Polar metabolites

UNTARGETED ANALYSIS
Non Polar Metabolites

ANALYTICAL STRATEGY

ANALYTICAL METHOD

LC-QQQ

HILIC - (ESI) TOF

GC – (APCI) TOF

IIVM Institute for Environmental Studies
Data Analysis workflow - PathwayScreener

- Targeted metabolomics list based on MSMLS library (e.g. IROA Technologies)
- Batch processing for target analysis in PathwayScreener
- t-test with FDR correction in ProfileAnalysis

BIOMARKER DISCOVERY

METABOLIC NETWORK
Data Analysis workflow - Pathway Screener

Target list in CSV format based on metabolite library of standards

Pathway screener – Targeted metabolomics batch processing

Profile Analysis - t-test with FDR p values
PathwayScreener – Review Screening results
ProfileAnalysis – Volcano Plot and multiple t-test

Sample Table

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<tr>
<th>File Name</th>
<th>Include</th>
<th>Exposure Concentrations</th>
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<td>Indistinguishable tug/tugL</td>
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<tr>
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Bucket Table

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T-Test Exposure Concentrations

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Data Interpretation of the snail exposure is ongoing

The results will be published soon in a peer-reviewed journal
Conclusions

**Pathway Screener**

- The Bruker PathwayScreener software is very useful in the data analysis workflow in combination with Profile Analysis.
- Important aspects are the assessment of identified peaks, use of target list of metabolites.
- Important package to assess metabolomic data and to discover biomarkers.
Thanks

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Sara Tufi

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Novel tools in Effect Directed Analysis for identifying & monitoring emerging toxicants on a European scale