# Use of MALDI Imaging to assess the distribution of pesticides in the honeybee

Original thinking... applied

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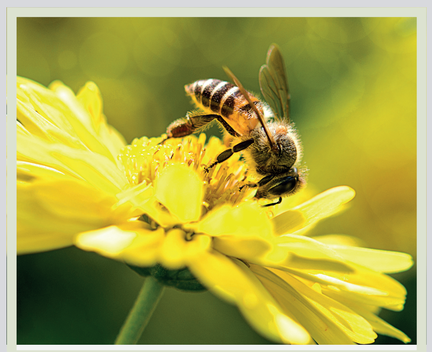
## ABSTRACT

**The direct analysis of tissue sections using matrix assisted laser desorption ionisation (MALDI) imaging mass spectrometry (IMS) is an emerging technology based on a surface sampling process. The technique allows analysis and visualisation of endogenous proteins and peptides along with exogenous molecular species, such as pesticides, within the same tissue section with high molecular specificity.**

**In order to provide further information on the distribution and transformation of neonicotinoids in vivo, honeybees were dosed orally with acetamiprid and sections taken using a Leica CM3600 microtome (Leica Microsystems), section thickness was 30 µm. The distribution of acetamiprid and its major metabolites (IM-2-1 and IM-1-4) was then studied using MALDI techniques. The data obtained in this study demonstrated that high quality tissue distribution and biotransformation data can be obtained using MALDI techniques.**



## INTRODUCTION

Neonicotinoids (neonics) are neuro-active insecticides chemically similar to nicotine that were developed in the 1980s and, when first introduced, were thought to have low toxicity to beneficial insects. Around 2006 a dramatic rise in the number of annual beehive losses spurred interest in factors that could affect bee health. Neonic use was linked in several studies to adverse ecological effects, although the findings have often been conflicting and therefore controversial. Recent studies have investigated the distribution and metabolism of neonicotinoids in the bee to elucidate the pathways of detoxification and elimination[1].

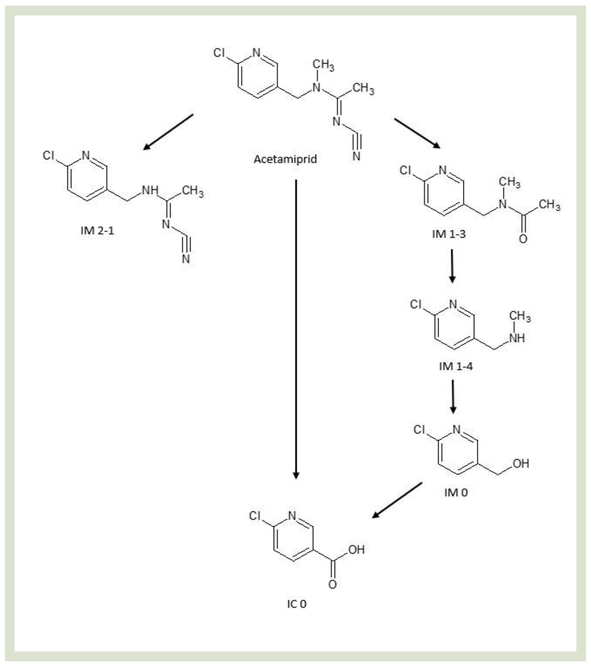



Figure 1 Proposed biotransformation of acetamiprid in the honeybee

A metabolic pathway for acetamiprid in the honey bee has been proposed[2]:

The aim of the current study was to determine the distribution of acetamiprid and it's major metabolites in the honeybee using MALDI imaging techniques.

## METHODS

(i) Dosing

Ten bees were offered a dose of 8 µg of acetamiprid per bee for a period of 3.5 hours. The bees consumed 67.14% of the feed offered and the mean dose consumed was 5.37 µg of acetamiprid per bee. After 3.5 h, the bees were frozen, transported on dry ice and kept in a -80°C freezer until the embedding and sectioning process.

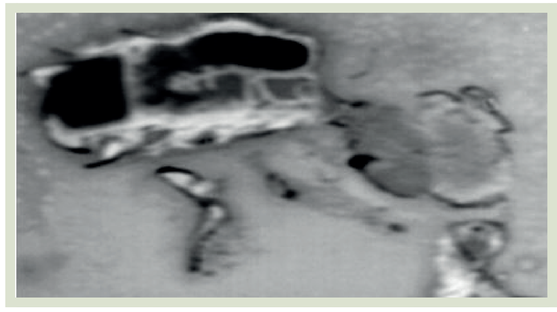
(ii) MALDI Imaging

Bees were dipped in 1% DDM and embedded in 5% gelatine followed by sectioning and drying in a vacuum desiccator. MALDI matrix (CHCA) was applied by sublimation, recrystallized and analysed by MALDI-MSI on an Applied Biosystems QStar Pulsar-I with spatial resolution set to 75 µm and 80% power of laser (8.0 µJ).

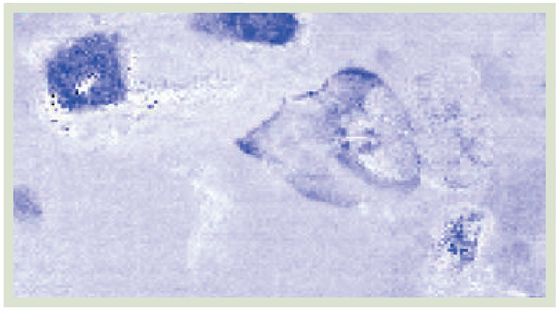
## RESULTS

Figure 1 shows the proposed biotransformation routes for acetamiprid in honey bees. Figure 2 (a-f) shows MALDI-MSI images obtained for the distribution of acetamiprid and metabolites in a honey bee following dosing with ~6.7 µg of acetamiprid over a 3.5 hour period. Figure 2a) Optical image of a cryosection of a dosed honey bee; Figure 2b) acetamiprid image showing distribution of m/z 223; Figure 2c) acetamiprid signal m/z 223 overlaid on an endogenous lipid signal at m/z 786. In this section acetamiprid can be observed externally on the legs of the bee (from feeding) and inside the eyes, crop and rectum of the bee; Figure 2d) Distribution of IM 2-1 signal m/z = 209; ; Figure 2e) Distribution of IM 1-3 signal m/z = 197 showing distribution in the abdomen; Figure 2f) Distribution of IM-1-4 signal m/z 155.

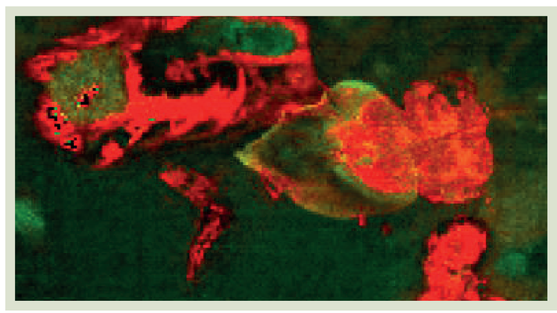
Figure 2 Distribution of Acetamiprid and metabolites in the honeybee



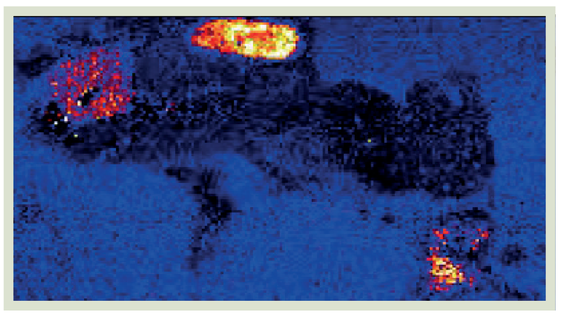
a) Optical image



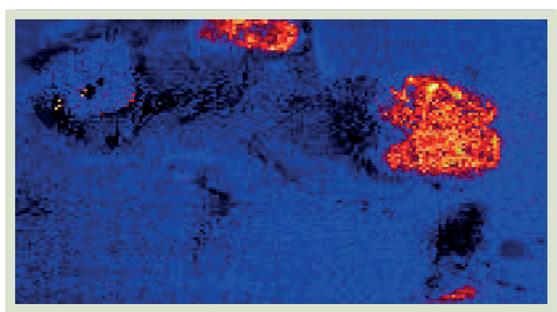
b) Acetamiprid - m/z 223



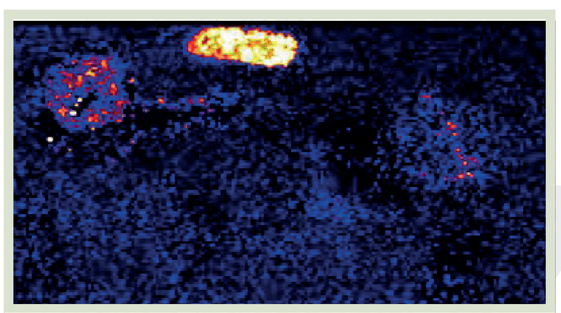
c) m/z 223 Acetamiprid (green) overlaid onto lipid m/z 786 (red)



d) Metabolite IM2-1 m/z 209



e) Metabolite IM1-3 m/z 197



f) Metabolite IM1-4 m/z 155

## CONCLUSIONS

MALDI-MSI has been successfully employed to study the distribution and metabolism of acetamiprid in a honey bee orally dosed with ~5.3 µg of acetamiprid (LD50 = 14.5 µg).

Acetamiprid and its major metabolites were easily detected and showed distinct and different distributions. The distribution of components was consistent with that previously reported using radiolabeled material.

Distribution can be related to the to the internal structure of the honey bee and further investigations will investigate effects on endogenous components. (lipid/protein).

## REFERENCES

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[2] Jean-Luc Brunet, Alexandra Badiou and Luc P Belzunces Pest Manag Sci 61:742–748 (2005) In vivo metabolic fate of [14C]-acetamipridin six biological compartments of the honeybee, Apis mellifera L

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