Institution: University of Exeter		
Unit of Assessment: UoA 5 Biological Sciences		
Title of case study: BeeSafe – a toolkit to predict and avoid negative effects of current and future pesticides on bees		
Period when the underpinning research was undertaken: June 2014 - present		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Chris Bass	Professor of Applied Entomology	January 2016 - present
Kumar Suarabh Singh	Research fellow	January 2016 - present
Bartek Troczka	Research fellow	October 2018 - present
Period when the claimed impact occurred: 2016 - present		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact

Research by the University of Exeter (UoE) has established at the molecular level why managed bee pollinators, worth more than £650 million to the UK economy each year, are very sensitive to certain pesticides, such as the neonicotinoid imidacloprid, but highly tolerant to others. This knowledge has been **translated into tools** (the BeeSafe toolkit) which have been used by Bayer, a world-leading agrochemical company, to: (1) **rapidly screen for and accelerate the development of new insecticides that have low toxicity to bees**; (2) **predict and avoid harmful pesticide-pesticide interactions**; and (3) **support registration of specific pesticide combinations** that are safe for bees. The BeeSafe toolkit was integral to Bayer receiving **regulatory approval for a new insecticide** in Germany **with benefits to pollinators and oilseed rape production**.

2. Underpinning research

Pesticides play a key role in agriculture by controlling plant pests and diseases and securing quality and yield in plant production. However, there are growing concerns over the effects of plant protection products on the environment and non-target organisms such as bee pollinators. There is thus an urgent need to devise strategies to minimise harm from existing pesticides and develop new compounds that show high efficacy against crop pests but low toxicity to non-target beneficial insects such as bees. Research by the UoE has met this challenge by fundamentally advancing our understanding of the molecular determinants of bee sensitivity to insecticides and, in partnership with industry, leveraging this knowledge to develop bee-safe compounds.

Previous work has shown that bees can exhibit profound differences in their sensitivity to different members of the same insecticide class. For example, honey bees (*Apis mellifera*) are >1000-fold less sensitive to the neonicotinoid thiacloprid than the neonicotinoid imidacloprid in acute insecticide contact bioassays, with the former classified as 'highly toxic' but the latter categorised as 'practically non-toxic' according to the official categories of the U.S. Environmental Protection Agency. Understanding the molecular basis of this differential sensitivity is key to designing insecticides that have low toxicity to bees but retain efficacy against pest insects.

From 2016 to the present Chris Bass, Professor of Applied Entomology at Exeter, secured £1.5M funding from industry (Bayer AG) and the Biotechnology and Biological Sciences Research Council (BBSRC) to address this knowledge gap (see section 3). The research initially focussed on neonicotinoid insecticides and demonstrated that differential sensitivity to different neonicotinoids within the same class are also observed in other social bees such as bumblebees (*Bombus terrestris*) and solitary bees such as the red mason bee (*Osmia* *bicornis*). We hypothesised that the metabolic systems used by bees to detoxify the natural toxins encountered in their environment may be recruited to protect honey bees and bumblebees against certain synthetic insecticides. To investigate this we performed the most comprehensive functional characterisation of the primary bee phase I detoxification enzymes (P450s) ever conducted. The results revealed that in both honey bees and bumblebees, as well as in red mason bees, just one specific subfamily of P450s determine their sensitivity to neonicotionoids **[3.1, 3.2, 3.3]**. These enzymes metabolise *N*-cyanoamidine neonicotinoids with very high efficiency but show very limited activity against *N*-nitroguanidine neonicotinoids, explaining the differences in bee sensitivity to these compounds. Thus, as in humans where just a handful of the 57 functional P450s metabolise xenobiotics, important managed bee species possess key enzymes responsible for the biotransformation of toxins that are critically important in defining their sensitivity to pesticides. This is important as it means that simply by examining the capacity of this small number of P450 enzymes to metabolise (or be inhibited by) pesticides we can predict the pharmacological and toxicological outcomes of pesticide use on bee pollinators. Furthermore, it has provided underpinning data and tools to facilitate the development of new bee-safe compounds. Specifically, a key outcome of the research was the creation of a 'BeeSafe' toolkit.

This toolkit comprises two components. Firstly, a panel of purified bee P450 enzymes that we obtained from expressing bee P450 genes in an insect cell line in the laboratory. For each P450 enzyme we have identified a fluorescent model substrate that can be used to measure P450 activity in simple fluorescent assays **[3.1, 3.2]**. Together the enzymes and model substrates can be used in high-throughput screens of pesticides to examine if the P450 can metabolise the test compound (providing an early indication of its potential toxicity to bees and a predictor of a compound's likely development success). Secondly, we have created a series of transgenic fruit fly (Drosophila) lines each expressing a different bee P450 **[3.4]**. These genetically modified Drosophila lines are now resistant to the same pesticides as the native bee species, and their sensitivity to novel pesticides or pesticide combinations can be examined using simple pesticide bioassays. These lines have been made publicly available to facilitate research by scientists in both industry and academia (see section 4). Together, this toolkit can be used as a valuable screening tool to fast-track the development of new bee-safe insecticides (see section 4). They can also be used to identify negative pesticide-pesticide interactions. For example, to identify if a compound acts as an inhibitor of these key bee P450s, thus sensitising bees to other pesticides that are usually non-toxic **[3.1,3.2]**. This is useful as different pesticides are often co-applied to crops by growers (see section 4).

3. References to the research

Relevant peer-reviewed scientific publications in this area (Exeter authors highlighted in bold).

3.1 Manjon C, **Troczka BJ**, Zaworra M, Beadle K, Randall E, Hertlein G, **Singh KS**, Zimmer CT, Homem RA, Lueke B, Reid R, Kor L, Kohler M, Benting J, Williamson MS, Davies TGE, Field LM, **Bass C**, Nauen R (2018) Unravelling the molecular determinants of bee sensitivity to neonicotinoids. *Current Biology* 28, 1137-1143, DOI:https://doi.org/10.1016/j.cub.2018.02.045.

3.2 Beadle K, **Singh KS**, **Troczka BJ**, Randall E, Zaworra M, Zimmer CT, Hayward A, Reid R, Kor L, Kohler M, Buer B, Nelson DR, Williamson MS, Davies TGE, Field LM, Nauen R, **Bass C** (2018) Genomic insights into neonicotinoid sensitivity in the solitary bee *Osmia bicornis*. *Plos Genetics* 15 (2), e1007903, DOI: https://doi.org/10.1371/journal.pgen.1007903

3.3 Hayward A, Beadle K, **Singh KS**, Exeler N, Zaworra M, Almanza MT, Nikolakis A, Garside C, Glaubitz J, **Bass C**, Nauen R (2019) The leafcutter bee, *Megachile rotundata* is more sensitive to N-cyano neonicotinoid and butenolide insecticides than other managed bees. *Nature Ecology and Evolution* 3, 1521-1524, DOI: https://doi.org/10.1038/s41559-019-1011-2.

3.4 McLeman A, **Troczka BJ**, Homem RA, Duarte A, Zimmer CT, Garrood WT, Pym A, Beadle K, Reid RJ, Douris V, Vontas J, Davies TGE, ffrench Constant RF, Nauen R, **Bass C** (2020) Fly-Tox: A panel of transgenic flies expressing pest and pollinator cytochrome P450s. Pesticide Biochemistry and Physiology 169, 104674, DOI: https://doi.org/10.1016/j.pestbp.2020.104674.

These research outputs were co-authored and produced in partnership with colleagues at Bayer and Rothamsted Research and supported by ~£1.5 million of grant funding from Bayer and BBSRC.

4. Details of the impact

Research by the UoE has led to a step change in how insecticides are being developed and deployed by industry partners, such as Bayer (and more recently Syngenta), to: (1) rapidly screen for and develop new insecticides that have low toxicity to bees; (2) predict and avoid harmful pesticide-pesticide interactions; and (3) support the registration of specific pesticide combinations that are safe for bees.

Our BeeSafe toolkit **[3.1, 3.2, 3.4]** has met the urgent need for tools that can aid in the development process, and identify candidate insecticides with improved environmental and toxicological profiles (and those that do not have these attributes) at an early stage, by-passing the need to screen upward of 140,000 compounds per product. Before the availability of these tools, the safety of candidate insecticides was exclusively assessed by tests using live *A. mellifera*, *B. terrestris* and solitary bees such as *O. bicornis*. However, live bioassays on bees are expensive and time-consuming to perform, costing $10-$30K per test compound, dependent on bee species **[5.1]**. Furthermore, it is only possible to screen honey bees and solitary species for a few months of the year. In contrast, the BeeSafe toolkit is inexpensive and rapid to use, and can be employed year-round.

Rapid development of novel bee-safe insecticides

Pesticides remain an important component of modern agriculture with the global pesticide market worth $75 billion in 2017. Bayer AG's market cap as of October 26, 2018, was $70.34 billion, while its CropScience division produces pesticides for commercial and consumer uses, with pesticide sales of €9.57 billion in 2017, making them the largest Agrochemical company in the world (Statista, 2019). However, increased costs associated with a sharp escalation in the number of compounds that need to be screened/tested has led to a significant decline in the number of research-based companies in the US and Europe involved in the discovery of new insecticides. Indeed the current cost of developing a new insecticide now averages $250 million per active ingredient[1].

The BeeSafe toolkit has been used by Bayer (since 2016) to screen potential new insecticides; lead compounds that are metabolised by P450 enzymes are prioritised for development. The purified bee enzymes and associated model fluorescent substrates are being used by Bayer in *in vitro* high-throughput simple inhibitor assays to assess the metabolic liability of novel compounds. The transgenic fly lines are a useful complement to these assays and are being used to examine the ability of bee P450s to tolerate lead compounds *in vivo*. In contrast to assays on bees, tests using Drosophila are simple, inexpensive (costing <$0.5K per compound versus $30K per compound for *O. bicornis* **[5.1]**) and rapid (Drosophila has a two week life cycle, compared to 1 year for *O. bicornis*) **[3.4]**. Ralf Nauen (chief scientist at Bayer AG) noted that the new tools developed in the Bee Toxicogenomics project '*are now a vital part of Bayer's development pipeline, providing a filtering tool to screen new lead insecticides at an early stage, and are allowing us to more rapidly identify and develop new bee-safe compounds*' **[5.2]**. To allow these tools to be used more widely the transgenic Drosophila lines have been deposited with an open repository (Bloomington Drosophila Stock Center) **[5.3, 3.4]** to make the panel available to other companies. These tools and the underpinning knowledge were also advertised to scientists in other companies by holding a workshop in 2017 entitled 'Protecting Bee Pollinators with Comparative Toxicology and Functional

Genomics' attended by representatives from the majority of the five companies that dominate global pesticide sales **[5.1]**. Following this Syngenta, one of the 'big five' agrochemical companies, have also been supplied with the Drosophila BeeSafe Toolkit and are planning to use this as an integral component of their pesticide research and development pipeline, testifying that this work has '*fundamentally advanced our understanding of the molecular mechanisms that determine the sensitivity of bee pollinators to insecticides*' **[5.1]**.

Predicting and avoiding harmful pesticide-pesticide interactions
Previous research has shown that when certain pesticides are used in combination, they can be much more harmful to bees than when used individually. Our research has been used to predict and avoid these negative pesticide-pesticide interactions. For example, azole fungicides can profoundly alter the sensitivity of honey bees to insecticides, including those that are normally considered safe to bees[2]. Our research demonstrated that these azoles inhibit significantly the specific P450 enzymes that confer tolerance to certain insecticides, and thus provided a mechanistic understanding of why these compounds enhance the toxicity of insecticides to bees. Following this discovery, the BeeSafe toolkit has been used by Bayer to test the effects of non-insecticidal products used in crop protection (including fungicides) on bee P450 enzymes, in order to identify which products may be combined safely and which combinations should be avoided **[5.2, 5.4]**. This information has underpinned guidelines for growers and agronomists on safe use and has been used to support the registration of specific pesticide combinations that are safe (see below).

Supporting insecticide registration
The registration of insecticides is costly and time consuming, currently taking an average of 10 years before a product can reach market. The tools developed in our research have been used to facilitate and accelerate the registration of insecticides by providing evidence of their safety to bees.

In order to minimize the risk of negative environmental impacts of a new insecticide, any new product must pass a stringent registration process that includes assessing the product's safety to bee pollinators. Bayer went through this registration process in 2017/2018 with a product that combined an azole fungicide (prothioconazole) with the bee-safe neonicotinoid thiacloprid for application in oilseed rape crops. However, regulatory bodies in Germany initially rejected the submission due to concerns over possible effects of the azole fungicide sensitising bee pollinators to the insecticide component of the combination. By using the BeeSafe toolkit Bayer were able to demonstrate that, unlike other azole fungicides, prothioconazole does not inhibit the P450 enzymes that are key determinants of sensitivity to thiacloprid in bees. On receipt of this information the regulatory body accepted the registration dossier and allowed the product to come to market for use in Germany for the production of oilseed rape **[5.2]**.

In summary the knowledge generated by the UoE has had a significant impact on both insecticide production and on safeguarding bee health. It has facilitating the development and registration of bee-safe crop protection products and predicting negative pesticide-pesticide interactions. An article from The Scientist **[5.5]** quoting Professor May Berenbaum, editor-in-chief of the Proceedings of the National Academy of Science and National Medal of Science laureate, highlighted the following: *'They've provided an incredibly useful service in illuminating how honey bees process toxins.'* Given bees' routine exposures to pesticides as they forage for food and pollinate crops, *'it's incredibly important to know this.'*

[1] *https://doi.org/10.1016/j.pestbp.2013.05.012;* [2] *https://doi.org/10.1371/journal.pone.0054092*

5. Sources to corroborate the impact

5.1 Letter of support on the value of the BeeSafe toolkit for insecticide development at Syngenta from Dr Christoph Zimmer Insecticide Team Leader at Syngenta.

5.2 Letter of support on the impact of the research on insecticide development and registration at Bayer from Dr Ralf Nauen chief scientist at Bayer AG.

5.3 Evidence of fly lines supplied by Chris Bass, available at Bloomington Drosophila Stock Center, Indiana University Bloomington from June 2020 (to view, search for stock numbers 90811-90824); https://bdsc.indiana.edu/

5.4 Link to Bayer article outlining the research and application; https://cropscience.bayer.co.uk/blog/articles/2018/05/innovation-highlights-3-real-ways-of-improving-bee-health/ by Julian Little Head of Communications and Government Affairs, May 2018

5.5 Article by The Scientist on the findings of the research and applications, 22 March 2018 https://www.the-scientist.com/daily-news/bees-molecular-responses-to-neonicotinoids-determined-29922