Original thinking... applied

Fera's Bee Ecotoxicology Testing Services

Trust the internationally recognised bee experts for your PPP safety testing needs

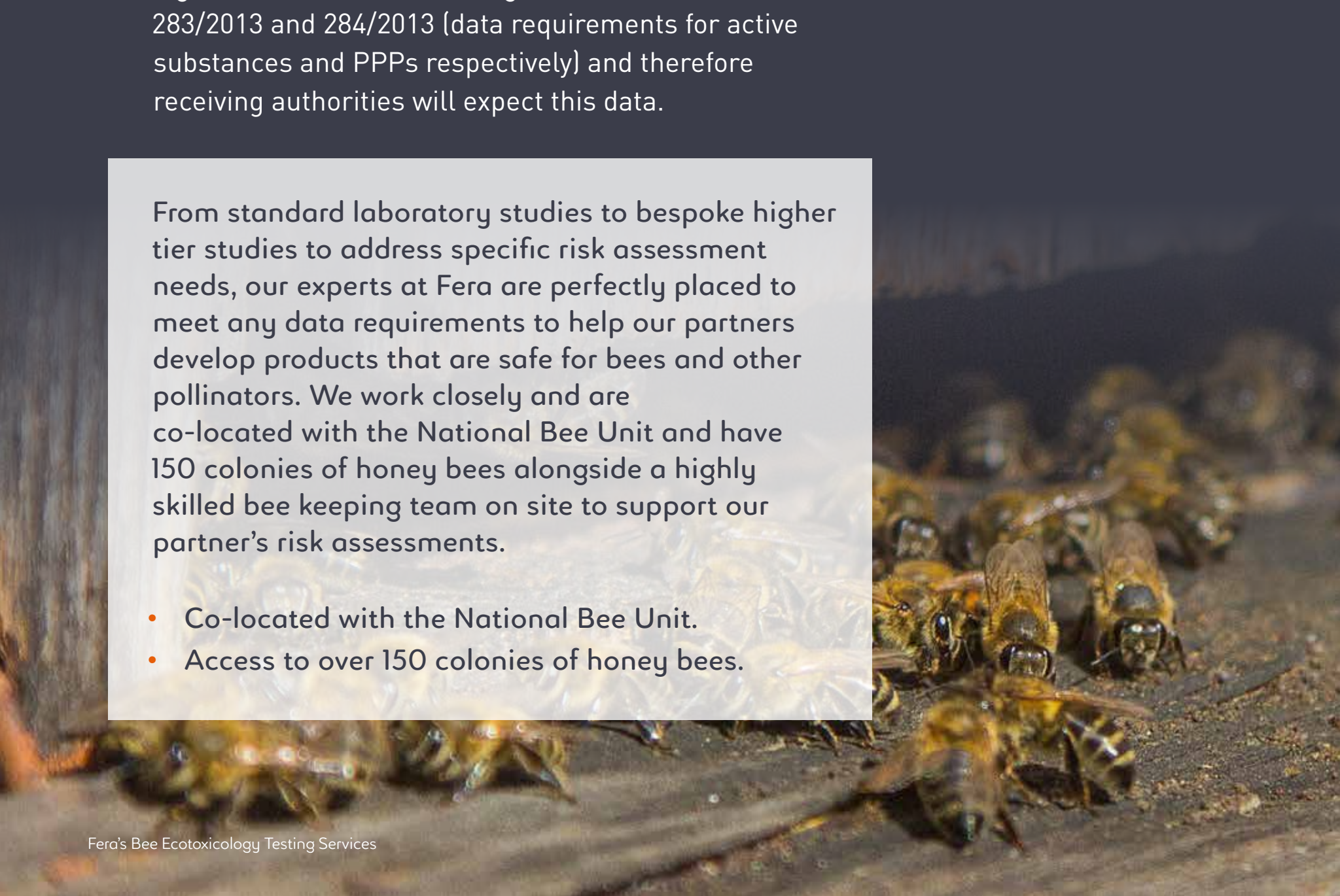
At the forefront of bee ecotoxicology testing

Bees provide a host of ecosystem services; most importantly they are key pollinators of agricultural crops and wild plants. In addition to this honey bees also provide products such as honey, wax, pollen, propolis, royal jelly and venom.

The most recent EFSA Guidance Document proposes a tiered risk assessment scheme including non-*Apis* species (*Bombus* spp. (Bumble bees) and solitary bees) as well as honey bees (*Apis mellifera*). This guidance will likely be followed by new regulations making the tiered risk assessment scheme mandatory. Although this guidance document has not yet been formally accepted into the legislative framework the data requirements are clearly set out in the current legislation – Commission Regulations (EU) Nos 283/2013 and 284/2013 (data requirements for active substances and PPPs respectively) and therefore receiving authorities will expect this data.

From standard laboratory studies to bespoke higher tier studies to address specific risk assessment needs, our experts at Fera are perfectly placed to meet any data requirements to help our partners develop products that are safe for bees and other pollinators. We work closely and are co-located with the National Bee Unit and have 150 colonies of honey bees alongside a highly skilled bee keeping team on site to support our partner's risk assessments.

- Co-located with the National Bee Unit.
- Access to over 150 colonies of honey bees.



Fera's Bee Ecotoxicology Testing Services

Case Study
Radio **F**requency **ID**entification tagging – Field Experiment

Over the last 30 years, Fera have amassed a wealth of expertise in testing the safety of Plant Protection Products (PPPs), using cutting edge or newly emerging techniques to help our partners.

Under laboratory conditions it has been demonstrated that some chemicals may have the potential to produce sub-lethal effects in insects exposed to them, for example affecting the ability of bees to return to the hive/nest through effects on learning, memory, or flight. However, one of the problems encountered when considering such laboratory studies is the relevance of these experiments compared to what may happen under field conditions using actual field application rates.

Because of our experience in designing bespoke studies, we were approached by a partner who wanted to look at such a real-life exposure situation at the field level to determine if there was any effect on the homing behaviour or lifespan of honey bees (*Apis mellifera* L.) foraging on oilseed rape (OSR) grown from pesticide treated seed.

Using our wide ranging practical experience of handling bees and willingness to embrace emerging technology Fera designed a large-scale **R**adio **F**requency **ID**entification (RFID) tagging and monitoring study to investigate this scenario.

There has been interest in using this technology as a tool for assessing flight and foraging behaviour of bees over recent years. The system involves fixing small transponders to free flying bees allowing their activity to be monitored by fixing readers to the front of the hive. Most of these studies have involved artificial feeding of the bees either by feeding the bees directly or by giving them access to dosed feed from artificial feeders.

The experiment designed at Fera was carried out under natural conditions of exposure i.e. open foraging of free flying bees on crops grown from treated seed under field conditions.

There were three treatment groups; one treated field grown from Thiamethoxam treated OSR seed and 2 control fields grown from untreated seed (each approximately 5 km apart). A total of 12 honeybee colonies were allocated to each treatment group field (4 located at the field edge and 4 each at approximately 500m and 1000m from the field). As part of the study a staggering total of nearly 10,000 newly emerged bees (approximately 300 per colony) were tagged with Mic3 RFID transponders.

These bees were then introduced to the test colonies. RFID readers fitted to the entrances of the test colonies were used to monitor the activity of the tagged bees for the exposure phase (i.e. the flowering period which in this case was 5 weeks). These activity data were analysed to assess any impact on flight activity of bees foraging on the treated compared to untreated crops*

*A full description of the study can be seen in the paper Thompson, H., Coulson, M., Ruddle, N., Wilkins, S. and Harkin, S. (2016), Thiamethoxam: Assessing flight activity of honeybees foraging on treated oilseed rape using radio frequency identification technology. Environ Toxicol Chem, 35: 385–393. doi:10.1002/etc.3183.

This field study remains one of the largest scale tagging open field studies to have been carried out on bees. As well as helping to understand the possible effects of the test item on the foraging behaviour and life-span of the bees this study gave a fantastic insight into how such data may be used to look at the behaviour of bees for future work.

Our active membership on a number of international panels and working groups means that we will continue to expand our expertise in bee testing methods, ensuring we remain at the forefront of the industry and help protect our important pollinators.

Laboratory Tests

Aged residue tests (extended laboratory test)
This test is designed to assess the toxicity of residues of PPPs on plant material post application. Test item applications are made to a crop and are aged on the crop. The crop is collected at different time points and bees are exposed to the foliage. This test is performed according to **EPA OCSPP 850.3030: Honey Bee Toxicity of Residues on Foliage.**

In vitro honey bee larval toxicity
These tests assess the toxic effects of PPPs on honey bee larvae. The larvae are grafted from healthy colonies into artificial cells where they are fed an artificial diet (royal jelly and sugars) and reared in the laboratory. The test item is combined with the feed and fed directly to the larvae. These tests can be run as a single dose test or with repeated dosing **(OECD Test Guideline 237: Honey bee larval toxicity test – Single exposure and OECD Guidance Document 239 Honey bee larval toxicity test – Repeated exposure).**

Adult bee acute toxicity
First tier tests designed to look at the worst-case scenario via the two main routes of exposure: ingestion and direct contact. These tests are short term and are designed to generate LD_{50} and LOEC or NOEC values. Honey bee tests are according to the **OECD Guidelines 213 & 214**: **Honey bees Acute Oral & Contact Toxicity Tests**. We also test bumble bees according to **OECD Test guidelines 246 and 246 Bumble Bee Acute Contact & Oral Toxicity.** Unlike honey bees we are not restricted to testing during the beekeeping season – bumble bee laboratory tests can be performed earlier and later in the year.

Bee metabolism studies
Fera has recently been developing protocols to investigate the honey bee metabolism of PPPs and toxicity of the metabolites to adult bees. These studies rely on our experience with bees backed up with high-end analytical chemistry capabilities on site.

Adult bee chronic toxicity
Cage tests designed to assess possible longer-term effects of feeding on PPPs to honey bees. Test units of bees are dosed with a treated sucrose solution over a 10 day period; the feed uptake per day is calculated to determine the daily dose of the test item (according to **OECD Test Guideline 245: Honey bee (*Apis mellifera* L.), chronic oral toxicity test (10 day feeding test in the laboratory) 2016.** (Accepted in April 2016 at the OECD National Representatives meeting).

Semi-Field Tests

Honey bee brood tests
These tests are run under tunnel conditions using a bee-attractive crop. A single colony is placed into each tunnel. Using photographic methods and bee brood monitoring software the development of different brood stages (eggs, young/old larvae) are monitored for any effects and the brood termination rate calculated (**OECD 75: Honey bee brood test under semi-field conditions (tunnel test**).

Open Field Studies

Honey bee brood feeding tests
Similar to the bee brood test. However, the test item can be fed directly to free-flying honey bee colonies in sugar syrup. Additional measurements of mortality and overall colony development are also taken. Tests run according to **Oomen, de Ruijter & Van der Steen (1992)**.

Honey Bee Effects Tests
These are conducted under open field conditions, to give realistic exposure scenarios (**EPPO 170**) and can be designed to look at a number of aspects including: spray applications, seed treatments (e.g. dust) and risks from guttation. Residue analysis can be incorporated to look at residues within the crop and returning foragers (and also from within hive matrices). We are also experienced with the use of **R**adio **F**requency **Id**entification studies (RFID) in open field situations as recommended in the EFSA GD to assess potential effects of PPPs on the foraging behaviour and mortality of worker bees. This RFID technology can also be used for dose/release/homing flight tests and we are part of the current ring test group assessing these methods.

Bumble Bee Effects Tests
Although there are no recognised guidelines for bumble bee field studies, we have a great deal of experience of developing bespoke protocols so that we can work with bumble bees under field conditions.

Solitary Bee Testing
Fera are members of the International Commission for Plant Pollinator Relationships (ICPPR) non-*Apis* working group helping to develop new test methods. We are also collaborating with Atlantic Pollination Ltd to extend our capabilities. **Contact us directly about the latest developments.**

Analytical Capability

With increased regulatory requirements for the analysis of dosing solutions, diets and residue analysis of other matrices we are very well placed to meet your particular needs. Fera has a team of experienced analytical chemists with access to state-of-the-art equipment, facilitating both quantitative and qualitative analysis. Our team has over 25 years of bee testing experience with our partners in Industry, to ensure regulatory compliance of new and existing products. This, combined with over 50 years of experience in dealing with the collection and analysis of a vast array of bee related matrices keeps us at the front of research and in regulatory issues related to bee health.

Our research expertise, scientific resources and bespoke capabilities help our partners test plant-protection products for their effects on bee survival, development and behaviour – helping them to develop products that are safe for bees and other pollinators with the highest quality data.

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