## **Section 3 - Monitoring**

# 3.1 Lethality of Imidacloprid and Fipronil on *Apis mellifera*: a retrospective on the French case

#### Isaac Mestres Lóbez

Rue du Zéphyr, N°88 – 1200 Woluwé-Saint-Lambert (Brussels-Capital Region) – Belgium; +32473516197 E-Mail: isaac.mestres@solvaypostgrad.net DOI 10.5073/jka.2020.465.023

#### **Abstract**

The aim of this study is to draw a retrospective analysis on the lethality of imidacloprid (Gaucho<sup>\*</sup>) and fipronil (Régent<sup>\*</sup> TS) on *Apis mellifera* between 1992 and 2016 in France. Early monitoring reports in the 1992-2002 period notified these two embedded insecticides to be at the origin of massive colony collapse disorders. Ecotoxicological analyses based on the LD<sub>50</sub> of imidacloprid and fipronil highlighted their differential lethality by both contact (imidacloprid: 81 ng/honeybee vs fipronil: 5,9 ng/honeybee) and ingestion (imidacloprid: 3,7 ng/honeybee vs fipronil: 4,2 ng/honeybee) but failed to point imidacloprid's high solubility as a higher lethal agent. Chemical properties and action mode of these two insecticides originated neural disfunction in the case of imidacloprid, and honeybee brood immune depression for fipronil. Despite the conduction of these monitoring reports and laboratory researches, Fipronil was completely banned in 2005 but Imidacloprid only in 2016.

Keywords: Apis mellifera, Imidacloprid, Fipronil, Monitoring, Colony Collapse Disorder, LD<sub>50</sub>

#### Aim and context

This study draws a retrospective analysis on the lethality of imidacloprid (under commercial denomination Gaucho®) and fipronil (under the commercial denomination Régent® TS) on *Apis mellifera* between 1992 and 2016 in France. The aim is to fact per periods the succession of responses between stakeholders and analyse why even with significant and scientific conclusive proof of lethality, the outcome was a time-shifted ban of these pesticides well after damage occurred.

After the successive reforms of the EU Common Agricultural Policy (CAP) and a massive decrease of its budget, agricultural practices have been intensified, massively oriented towards monocultures. These practices have brought to a scarcity of available melliferous resources and ultimately a loss of entomological biodiversity. In this context, and in an effort to improve productivity and efficiency of monocultures, agrochemical multinationals found an opportunity so sell their pesticides.

## Early crisis: colony collapse disorder reports in the 1992-2002 period

Gaucho° on the market: the first devastating effects

Early monitoring reports reveal after July 1992 Gaucho® on market: first devastating effects the first marketing campaign of Gaucho®, an insecticide massively employed in sunflower cultures. This commercial product composed of Imidacloprid (IMI) targeted insects-suckers, beet predators, sunflower and maize crops. In a first time it was treated on seeds, in an effort to protect the seed envelope, later on the seedling in order to penetrate the whole plant through the sap. It will be later on extended to rice, fall cereals and maize.

Immediately after its use in July 1992, bee mortality in hives boosted from 40% in 1994 to 50% in some cases in 1997. Beekeepers declare themselves as psychologically devastated as they walk along a "carpet of dead bees". Beekeepers witnessed that honeybees "stay on the flower, as if stuck unable to extricate themselves and shake by ending in convulsions before dying". Such witnesses reinforced evidence of colony disorders.

## Régent<sup>®</sup>'s first shakes

First use of Régent come back to 1993 for the sunflower cultures and different mixed exploitations. This pesticide based on fipronil (FIP) was applied on seed coating and for soil treatment. FIP is a neurotoxic molecule applied in insecticides not only particularly in France but also in Europe. This product was brought to market and largely commercialized by BASF despite its neurotoxic effects and harm to environment.

The first local consequences felt by the exposition to Régent\* date back to April 2002, period during which use of Fipronil results in the direct colony collapse of local beehives. Furthermore, direct exposition to this substance lead to the intoxication of beekeepers with oedemas, cutaneous irritations and swelling when harvesting their honey.

## Proving and rooting the impacts: A race agains time and noise (2003-2007)

## INRA, CNRS and AFSSA assessments on IMI and FIP

In 2003 INRA CNRS and AFSSA demonstrated the high toxicity, persistence and long remanence of Gaucho\*, where both its active components and metabolites act on plants, non-target insects and environment. The released reports denounced Bayer's negligence and contested its ethics. Bayer had estimated the lethal doses to 5000 ppb; whereas. in reality they were at 0.1 ppb. In fact, with a budget of €150 million, Bayer created a more effective generation of pesticides and marketed it strongly, without sufficient accuracy on the analyses and ecotoxicological data reported.

In 2005, new INRA and CNRS studies confirmed extreme toxicity of FIP on pollinators and environment, as well as its induced risks on human health.

## Comparative analysis of IMI and FIP lethality

In order to measure the toxicity of a substance and its lethality,  $LD_{50}$  measures were conducted. IUT Professor J-P. Louvet in 2004 submitted an ecotoxicological report to compare the toxicity between IMI and FIP on honeybee *Apis mellifera*. On the one hand, IMI lethality was quantified at 3.7 ng/honeybee through ingestion, against 81 ng/honeybee through contact. On the other hand, FIP lethality was quantified at 4.2 ng/honeybee through ingestion, against 5.9 ng/honeybee per contact.

#### IMI and FIP action mode

In Nicolino and Veillerette study of 2007 also described IMI and FIP action mode. They qualified the disease process of honeybees exposed to IMI witnessed by beekeepers as due to a neurotoxic trigger. IMI's mode of action brings an over-excitation of the acetylcholine nicotinic receptors (nAChRs) inside insect's nervous system. Seeds treated with IMI diffuse the substance into the vascular system of the plant so that parasites such as aphids sucking the stems die by paralysis. Unfortunately, given the fact that the entire vascular system of plants is affected by the spread of IMI to the anthers, pollinators are de facto exposed to the harmful effects of the molecule.

Regarding FIP, it is important to highlight that when exposed to the sun (surface of the soil or plants), it undergoes a photo-degradation in desulfinyl-FIP which is clearly more toxic than FIP itself. In soil and water FIP is first degraded into other molecules, many of which are as active as FIP. Since it is very difficult to define the moment when a substance has completely disappeared from an environment, it is conventional to consider its half-life time, that is to say the duration after which half of the quantity initially produced has disappeared. Some results reported short half-lives (less than 1 month). Ecotoxicological studies were concerned only with the substance and not with its degradation product. But in reality, as a neurotoxic compound, this molecule acts specifically by completely altering the behaviour of bees resulting in a decrease in their foraging activity following exposure by contact or ingestion. In particular, it can lead to intoxication of the hive during the

brood of the fact that the nectar and pollen are in the hive. If the new bee comes to birth, it does so with great immune weaknesses and immunosuppression syndromes.

## Final outcome: Time-shifted resolutions (2005-2016)

The initial ban of FIP: April 2005

FIP is officially banned in France after 3 successive decrees:

- April 6, 2005 decree prohibiting the marketing of seeds treated with phytopharmaceutical products containing fipronil;
- April 15, 2005 decree prohibiting the placing on the market of phytopharmaceutical products containing fipronil and intended for soil treatment in the context of the fight against wireworms and weevils:
- April 19, 2005 decree prohibiting the use of phytopharmaceutical products containing fipronil as soil treatment in the fight against wireworms and weevils, and seeds treated with these products.

It is worth to mention that after this initial ban, further laws, regulations and directives were applied with exceptions, or restrictions to a specific context.

Final ban of IMI: France's 2016 Law on Biodiversity

According to the press journal *Le Monde*, the new France Law on Biodiversity, known as "*LOI* n° 2016-1087 du 8 août 2016 pour la reconquête de la biodiversité, de la nature et des paysages", passed on August 9, 2016, has served to draw a list of insecticides that were to be prohibited as of September 1<sup>st</sup> 2018. These insecticides are clothianidine, imidacloprid, thiamethoxam, thiacloprid and acetamiprid.

## **Attention points**

IMI and FIP chemical properties in the water cycle

According to the US National Pesticide Information Center, IMI and FIP have the following chemical properties. IMI is an insecticide that belongs to the family of the nicotinyls, being the first of today's known list of neonicotinoids. It is a synthetic derivative of nicotine, possesses a molar mass of 255.66 g/mol, a density of 1,54 g/cm³ and a water solubility of 610 mg/L at 20°C.

On the other side, FIP is a broad-spectrum insecticide that belongs to the phenylpyrazole chemical family. It possesses a molar mass of 437.14 g/mol and a density comprised between 1,477 g/cm<sup>3</sup> and 1.626 g/cm<sup>3</sup>. It has a 20°C solubility in water of 1.9 mg/L at pH 5 versus 2,4 mg/L at pH 9.

Solubility states that an agent with a higher solubility is more prone to saturate the solvent than a low solubility agent. Since the water cycle defines how water reaches plants and pollinators through the continuous movement of water, all chemical which is highly soluble in water will be more easily transported with water than a lower one. From these facts, since IMI solubility in water is much higher than FIP, its fit through both in and trough water poses it as a higher exposing factor to pollinators and in our case honeybees.

#### Conclusions and lessons learnt

From the retrospective study of the lethality of IMI and FIP on *Apis mellifera*, we can state the following conclusions.

In the first place, and despite thorough monitoring reports revealed by beekeepers and scientists on the one hand, and ecotoxicological assessments conducted by independent research centres on the other hand, this first group of stakeholders were trapped in a noise loop and time pressure in the effort to carry on scientific, objective and standardised methods and ultimately bring to the public conclusive and significant results, with limited resources. In this context, failure to highlight IMI higher solubility in water and therefore its spread in the water cycle, the lack of on the field

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ecotoxicological assessments in the first place or focussing uniquely on the lethality of FIP, instead of its by-products and degradation products, shadowed the scientific community from acquiring more data

On the second place, multinational agrochemical companies took advantage of a legal vacuum to fulfil their business objectives by large-scale marketing of Gaucho\* and Régent\* TS embedded pesticides. Colony collapse disorders and related disruptions caused by IMI and FIP to plants and non-target insects such as honeybees were a contingency non or poorly evaluated based on the current legislation in the moment of commercialisation. Low entry barriers were exploited as a business opportunity with incomplete focus on the consequences to the ecosystems.

The third group of stakeholder's worth mentioning are the decision poles and legal architects. This group designed, implemented, shifted and enforced the successive legal frameworks that went from absence of regulation to a shifted in time restrictions and bans according to the context and pressure to which they were exposed.

Finally, and as part of the responsibility the scientific community faced related to this topic, the following recommendations can be provided. The defence of universal interests towards sustainable, renewable and foundational sources of life, require accurate and effective strategy focus. In the aim to avoid tit for tat, risks of backfire and other crisis situations between and among all involved stakeholders, full resources and capabilities are of the essence. When confronted to disruptive events, such resources and capabilities need to be made fully available and communicated assertively. Only then an accurate root of choices and clear resolution path can be executed in order to secure the preservation of our common heritage and legacy.

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