

Pesticide residue monitoring and investigation of intoxications in honeybees in Spain (2012-2016)

Iratxe Pérez Cobo.

Coordinator of the veterinary animal health network

Ministry of Agriculture, Food and Environment Affairs (Spain)

Email: iperezco@magrama.es

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FRAMEWORK OF THE SURVEILLANCE

- ▶ **SURVEILLANCE PROGRAM OF HONEYBEE COLONY MORTALITY (2012-2016)**
 - ▶ EPILOBEE: EUROPEAN PILOT PROGRAM (2012-2014).
 - ▶ SPANISH PROGRAM (2014-2016)

- ▶ **INVESTIGATION OF HIGH MORTALITIES OUT OF THE PROGRAM**
 - ▶ INTOXICATIONS
 - ▶ OTHERS

OBJETIVES OF THE SURVEILLANCE PROGRAM ON HONEYBEE COLONY MORTALITY

- **Harmonization** of national and EU active epidemiological surveillance systems
- **Implementation of prevalence studies** on major bee diseases
- Estimate the **mortality of honeybee colonies** during winter and spring
- **Systematic monitoring of pesticide residues for assessment of the potential risks (acute and accumulated) to bee health and investigation of clinical suspicion of intoxication.**

EVALUATED DISEASES

- *Varroa destructor* INFESTATION RATES AND VARROOSIS
- *Nosema spp.* INFESTATION RATES AND NOSEMOSIS
- AMERICAN FOOLBROOD (AFB)
- EUROPEAN FOOLBROOD (EFB)
- CHRONIC PARALYSIS VIRUS (CBPV)
- OTHER VIRUSES: (WING DEFORMED VIRUS (DWV), ACUTE PARALYSIS VIRUS (APV))
- EXOTIC PARASITES (Small hive beetle -*Aethina tumida*-, *Tropilaelaps spp.*)
- **INTOXICATIONS BY PESTICIDES**
- **RISK ASSESSMENT BY INTOXICATIONS**

➔ SURVEILLANCE PROTOCOL

➔ DATA PROCESSING AND ANALYSIS OF INFORMATION

➔ RESULTS



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COLLECTING AND DATA PROCESSING



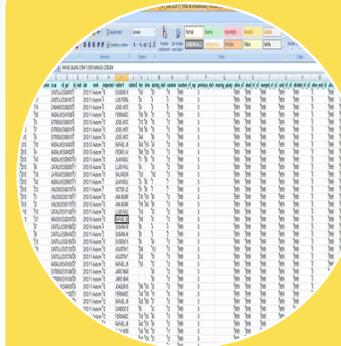
VISITS:
Autumn
Spring
Summer



Laboratory
analysis



Recordin
gin APINET



Data
processing
and
analysis





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SURVEILLANCE PROTOCOL

Systematic sampling/ Mortality

➤ AUTUMN

- INFESTATION RATES OF *Varroa spp*
- INFESTATION RATES OF *Nosema spp*
- DWV y ABPV (2012)
- PESTICIDES RESIDUES (2012)

➤ SPRING

- WINTER MORTALITY

➤ SUMMER

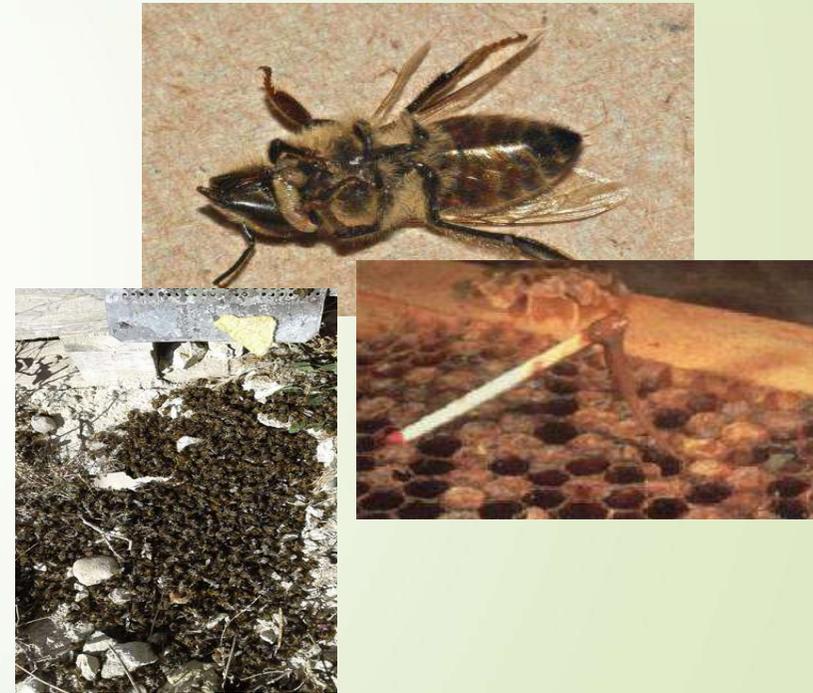
- SPRING MORTALITY
- PESTICIDES RESIDUES (2013 y 2016)
- CBPV (2013)



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SIMTOMATIC SAMPLING: searching of clinical symptoms

- AUTUMN
- SPRING
- SUMMMER



NETWORK OF PARTICIPATING LABORATORIES

EU-RL PARA LA SALUD DE LAS ABEJAS

LABORATORIO CENTRAL DE VETERINARIA DE ALGETE (MAGRAMA)

nesto de enfermedades
Confirmación de *Aethina tumida* y *Tropilaelaps spp*

LABORATORIO ARBITRAL AGROALIMENTARIO DE ARAVACA (MAGRAMA).

LABORATORIO EUROPEO DE REFERENCIA PARA EL ANÁLISIS DE RESIDUOS DE PESTICIDAS EN FRUTAS Y HORTALIZAS (UNIVERSIDAD DE ALMERÍA).

Pesticides residues

LABORATORIOS OFICIALES DE LAS CCAA.

*Tasas de parasitación por *Varroa destructor**

*Búsqueda de la presencia de parásitos sospechosos. *Aethina tumida* y *Tropilaelaps spp.**

TYPES OF SAMPLES

- **SYSTEMATIC MONITORING OF EXPOSURE TO PESTICIDES RESIDUES (RISK ASSESMENT):**
 - **BEEBREED HONEYBEE-COMB:**
 - Autumn 2012 and Summer 2013
 - **BEEBREED HONEYBEE-COMB AND EXTERNAL BEES** (2015-2016). In progress
 - Summer 2016
- **INVESTIGATION OF INTOXICATIONS:**
 - **BEEBREED HONEYBEE-COMB AND BEES** (dead or with clinical symptoms during the three visits every year)



análisis de la información



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DATA PROCESSING AND ANALYSIS

PESTICIDE

They are a type of chemical or mixture of substances intended to kill, repel, attract, regulate or stop the growth of living organisms considered as a pest.

In this definition we have also included those substances used as veterinary treatment for Varroa mite control.

TOXICOLOGICAL INFORMATION USED

- **European pesticide database of EFSA**
(http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database-redirect/index_en.htm)
- Study conducted by the Faculty of Agriculture and Environment of Sydney (Australia) that has been compiled and compared information from various sources (Sanchez Bayo, F. et al, 2014).
 - **Pesticide manual** (Tomlin, CDS. 2009), **ECOTOX** (database of U.S Environment Agency (<http://cfpub.epa.gov/ecotox/>))
 - **Agri-Tox Database** of the Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail in France (<http://www.agritox.anses.fr/index.php>)

RISKS ASSESSMENT: METHODOLOGY (Sanchez Bayo, F. et al, 2014)

- **TWO LEVELS:**
 - NATIONAL
 - PER APIARY
- **TWO PARAMETERS:**
 - Risk of acute intoxication (probabilistic parameter) (%)
 - Risk of accumulate intoxication (T50 contact) (nº days)
- **CONTACT RISK BY BEEBREED COMB**
- **85 pesticides evaluated**
- **IT IS DIFERENT FROM EFSA EVALUATIONS for the evaluation, authorization and registration of pesticides**

WHY WE USE CONTACT RISK BY BEEBREED COMB?



- **Beebreed honeybee-comb is a mixture of two matrices (wax and beebreed), being a nice biological marker of acute and chronic exposure to pesticides in honeybee colonies, not only from environmental exposure but also that derived from beekeeping management practices. The bio-accumulation of pesticides residues in wax and their transference to hive-stored pollen may play an important role in the survival of the honeybee colonies.**
- **Wax:**
 - It is a significant route of contact exposure not only for bees (nurses, cleaners, food storers, foragers) but also for larvae.
 - The lipophilic compounds are the major exposure risk to honeybees and are highly persistent in this matrix, so this feature may be used to assess in-hive chemical exposure history (EFSA workshop, april 2016)
- **Beebreed** is a route of contact exposure (also oral) to nurses and larvae, as it is a relevant nutrition source for bees and larvae during the Winter. (EFSA Journal, 2012).
- **Foragers** are good indicators of acute exposure to pesticides, but they are metabolized in a few days.

Pesticide Risk Assessment

ACUTE INTOXICATION RISK:

- ✓ **Probability of causing a 50% mortality of bees** from a colony in contact with contaminated beebree honey-comb during a short exposure period (two days)

$$Riesgo = \frac{Frecuencia (\%) \times Dosis \text{ de residuo } [\mu g]}{DL50 \left[\frac{\mu g}{abeja} \right]}$$

- ✓ **Contact with 1 gr of beebreed/bee/day**
- ✓ **Maximun 2 days of contact**
- ✓ **National level: average and maximum concentrations (worst case scenario)**

ACCUMULATED TOXICITY RISK (T50contacto):

- ✓ Time to reach the DL50 for each detected residue, in a bee in contact to 1 gr of beebreed honeybee-comb per day.

$$T50c \text{ (días)} = \frac{DL50c [\mu g \text{ abeja}^{-1}]}{Dosis \text{ diaria } [\mu g \text{ día}^{-1}]}$$

- ✓ **National level: average and maximum concentrations (worst case scenario)**
- ✓ **Per apiary**

RISK LEVELS

- ✓ **High risk of intoxication:** when the risk estimation is **> 5% probability**, usually corresponding to a **T50c <2 days**.
- ✓ **Moderate risk of intoxication:** when the risk estimation is between **1 to 5% of probability**, usually corresponding to **T50c between 2 and 7 days**.
- ✓ **Low risk of intoxication:** when the risk estimation is below **1% of probability**, usually corresponding to **T50c higher than 7 days** (range from 7 to 60 days or more), covering the life-span of foragers in summer and most of the life-span of Winter bees.



RESULTS

- Descriptive analysis
- Risk assessment
- Investigation of suspected intoxications

Systematic surveillance of pesticide residues (Autumn 2012 and Summer 2013)

➤ ANALYSIS PERFORMED: 353

- Autumn 2012 samples : 176
- Summer 2013 samples: 177

➤ Type of sample: panal de polen

➤ Pesticides analysed: 306

➤ Risk assessment included 85 pesticides

➤ Extracción QuEChERS modificado y posterior análisis GC-MS/MS y micro-LC-MS/MS

➤ Laboratories involved:

- Agrifood Laboratory of Aravaca (MAGRAMA)
- European Union Reference Laboratory for Pesticide Residues in Fruit & Vegetables (University of Almería)



Neonicotinoids subject to restrictions and fipronil

- ✓ **Directiva 2010/21/UE de la Comisión de 12 de marzo de 2010**, por la que se modifica el anexo I de la Directiva 91/414/CEE por lo que respecta a las disposiciones específicas relativas a la clotianidina, el tiametoxam, el fipronil y el imidacloprid.
- ✓ **Clotianidina, Tiametoxam:** Not detected.
- ✓ **Imidacloprid:** frequency (3,4%). Only in one case its concentration could pose a risk of intoxication.
- ✓ **Fipronil:** frequency (0,3%) at nontoxic concentrations.

AUTUMN 2012: Acute intoxication risk and time-cumulative toxicity

OTOÑO 2012				Riesgo por intoxicación aguda (% probabilidad)		Riesgo por toxicidad acumulada T 50 contacto (días)	
PESTICIDA	USO AGRÍCOLA	AUTORIZACIÓN UE	DL50 (µg/abeja)	Concentración Promedio	Concentración Máxima	Concentración Promedio	Concentración Máxima
Coumaphos	I-A	NO	20	17,974**	1,057*	8,472	1,078**
Acrinathrin	I-A	SÍ	0,17	15,642**	2,002*	7,555	0,569**
Chlorpyrifos	I	SÍ	0,072	14,429**	1,391*	7,009	0,819**
Cypermethrin	I-A	SÍ	0,034	8,932**	6,213**	0,891**	0,183**
Chlorfenvinphos	I-A	NO	4,1	8,750**	1,118*	22,079	1,019**

- ****High risk of intoxication:** when the risk estimation is > 5% probability, usually corresponding to a T50c <2 days.
- ***Moderate risk of intoxication:** when the risk estimation is between 1 to 5% of probability, usually corresponding to T50c between 2 and 7 days.
- **Low risk of intoxication:** when the risk estimation is below 1% of probability, usually corresponding to T50c higher than 7 days (range from 7 to 60 days or more).

SUMMER 2013: Acute intoxication risk and time-cumulative toxicity

PESTICIDA	VERANO 2013			Riesgo de intoxicación aguda (% probabilidad)		Riesgo por toxicidad acumulada T 50 contacto (días)	
	USO AGRÍCOLA	AUTORIZACIÓN UE	DL50 (µg/abeja)	Concentración Promedio	Concentración Máxima	Concentración Promedio	Concentración Máxima
Acrinathrin (1)	I-A	SI	0,17	23,592** 10,751**	12,848** 2,038*	4,215* 9,249	0,087** 0,549**
Ethofenprox	I	SÍ	0,015	18,860**	1,357*	2,217*	0,826**
Bifenthrin	I-A	SÍ	0,015	15,961**	3,386*	1,416**	0,331**
Chlorpyrifos	I	SÍ	0,072	15,144**	0,890	5,894*	1,258**
Coumaphos	I-A	NO	20	9,197**	0,626	17,937	1,788**
Cypermethrin	I-A	SÍ	0,034	8,589**	4,797*	1,842**	0,233**
Chlorfenvinphos	I-A	NO	4,1	5,955**	0,729	29,601	1,536**

- **** High risk of intoxication:** when the risk estimation is > 5% probability, usually corresponding to a T50c <2 days.
- *** Moderate risk of intoxication:** when the risk estimation is between 1 to 5% of probability, usually corresponding to T50c between 2 and 7 days.
- **Low risk of intoxication:** when the risk estimation is below 1% of probability, usually corresponding to T50c higher than 7 days (range from 7 to 60 days or more).

Analysis of time-cumulative toxicity per apiary

Apiaries having lower time-cumulative toxicity per contact (less than 7 days-T50 <7 days) showed a significantly LOWER STRENGTH($p < 0,05$).

No significant differences were found in relation to mortality.

Investigation of pesticides intoxication suspicions 2012-2015

INTOXICACIONES	Otoño	Primavera	Verano	Total casos campaña	Prevalencia intoxicaciones campaña
2012-13	1	3	7	11	5,4%
2013-14	1	2	5	8	4,2%
2014-15	3	2	1	6	5,4%
TOTAL	5	7	13	25	5,0%

- ✓ 47 suspicions investigated. Only 53% confirmed.
- ✓ Pesticides appeared, in 93% of confirmed cases, at concentrations that showed **time-cumulative toxicity (T50c) by contact lower than two days.**
- ✓ **1 or more pesticided involved:**
 - Chlorpyriphos (78,6%)
 - Coumaphos (64,3%)
 - Achrinatrina (42,9%)
 - Others: Bifenthrin, Dimethoate, Spinosad, Chlorfenvinphos, Sulfotep, Etophenprox

Relationship between intoxications and Chronic Paralysis Virus

- **28% confirmed intoxications** during the three campaigns (considered those with $T50 < 7$ days) showed **CBPV virus infection rates higher than 10^6 viral particles per bee.**
- **However, in summer 2013 systematic sampling, it has not been able to establish any statistically significant correlation ($p > 0,05$) between CBPV virus rates and:**
 - total concentration of pesticides
 - total concentration of highly toxic pesticides to bees
 - time-cumulative risk of toxicity ($T50 < 7$ days)

MONITORING PESTICIDES CONCLUSIONS

- It is **the first time in Spain** that is carried out a risk assessment of acute intoxication and time-cumulative toxicity.
- **Wide and comprehensive assessment:** broad spectrum of pesticides (306) and very low levels of quantitation (0.1-10 mg / kg).
- **The prevalence of acute intoxications was 5%**
- **For period 2012-2013, 7 pesticides were associated with a high risk of acute intoxication (> 5%): Coumaphos, Acrinathrina, Chlorpyrifos, Etophenprox, Bifenthrin, Cypermethrin, Chlorfenvinphos**
- Situation regarding to **neonicotinoids and Fipronil** does not seem a worrying, with low or undetected risks. **Clothianidin and thiamethoxam were not detected in any case.**
- **Next evaluation will provide us the evolution of the situation.**



¡¡Thank you very much for your attention!!