UNIVERSITÁ DEGLI STUDI DI TERAMO

Chemicals compounds in beeswax samples collected from the Italian hives during the years 2013 – 2015

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HONEYBEES

During the foraging activity, honeybees carry out 8000 sampling from different environmental matrices: water, soil and air, covering an area of 7 Km^2

THE IDEAL BIOINDICATOR

(Stöcker 1980)

HONEYBEES PRODUCTS: not only foods…

- **HONEY**

  Honeybees strainer

- **PROPOLIS**

  Honeybees strainer

- **ROYAL JELLY**

  Unfavorable composition

- **POLLEN**

  Unfavorable composition

- **BEESWAX**

  Long-term accumulation

BEESWAX

Beeswax is a natural substance consisting primarily of a mixture of esters of fatty acids and fatty alcohols, paraffinic hydrocarbons and free fatty acids.

Most of the chemicals used in agricultural and beekeeping activities are fat soluble, non-volatile and persistent, and then they can accumulate and persist in this matrix long after treatment has ceased.

BEEKEEPING

FOODS

COSMETICS

PHARMACEUTICALS

Beeswax is listed in the Pharmacopeia of different countries and in Europe is authorized as food preservative under the name of E 901.

Actually in Europe does not exist beeswax quality control, because this product is not considered a food.

POLLUTANTS IN BEESWAX

Pollution of beeswax can be divided into environmental and apicultural sources although some products can occur from both origins as they are used in both activities.

Acaricides

Fungicides

Herbicides

Insecticides

POLLUTANTS IN BEESWAX

In Italy the limits of contaminants in beeswax were set only for amitraz, coumaphos, chlorfenvinphos, *tau*-fluvalinate and cymiazole by the **Technical Guidelines RT-16 of 2009 (RT-16 rev.04, 2009)**. This document considers the chemical quality of beeswax but take care off only the organic beekeeping practices.

Amitraz
Chlorphenvinphos
Coumaphos
Cymiazole
Tau-Fluvalinate

ACCREDIA
L'ENTE ITALIANO DI ACCREDITAMENTO

LIMITI

L'uso degli **antibiotici** in apicoltura non è consentito, pertanto gli stessi devono risultare non rilevabili[12] nei prodotti dell'alveare.

Il metodo di produzione di apicoltura biologica, non prevede l'utilizzo di **acaricidi**; tuttavia, data l'elevata persistenza degli acaricidi nella cera, occorre definire una quantità massima di residui. La persistenza degli acaricidi nella cera è stata oggetto di studio da parte di un gruppo di lavoro coordinato dall'Istituto Nazionale di Apicoltura di Bologna, che ha portato alla definizione dei limiti temporanei riportati in tabella.

PRODOTTI	**Quantità di Residui di acaricidi [mg/kg]**
Miele, Propoli e Pappa reale	δ 0,010
Cera [13]	Somma dei residui totali dei 5 principi attivi[14] (coumaphos, fluvalinate, Clorfenvinphos, cimiazolo, amitraz): **≤0,30**, con le seguenti limitazioni: Coumaphos: ≤ 0,20 Fluvalinate: ≤ 0,10 Clorfenvinphos ≤ 0,010

11 Residui inferiori al limite di rilevabilità del metodo analitico;
12 Residui inferiori al limite di rilevabilità del metodo analitico.

13 Tutta la cera (da nido e da melario) deve risultare conforme ai limiti critici su definiti.

14 Considerato che sempre più si utilizza cera proveniente da paesi terzi (Sudafrica e Australia) è necessario ampliare la ricerca con altri principi attivi utilizzati in tali paesi.

The lacking of beeswax quality parameters, and of the legal obligations to submitted it to chemical analyses, makes possible:

- the circulation in European countries of **not authorized molecules** through the purchase of beeswax or comb foundations from foreign countries;

- the **illegal use of homemade preparations**, difficult to monitor if the analytical matrix is selected not considering the capacity of long-term accumulation;

- **recycling** of contaminated beeswax;

- **the absence of validated method of analysis and of a reference laboratory**

PROBLEM ON RESULTS INTERPRETATION

What about the accuracy and the error???

Chemicals compounds in beeswax samples collected from the Italian hives during the years 2013 – 2015

Considering that a long-term accumulation of the lipophilic miticides in the wax can become a source of contamination of bees, honey and pollen, the aim of the present work was to obtain an overview of chemical molecules present in the Italian beeswax.

Italian regions	2012	2013	2014	Total samples
Abruzzi	3	4	3	10
Aosta Valley	1	1	1	3
Apulia	2	2	2	6
Basilicata	1	1	1	3
Calabria	2	1	3	6
Campania	4	4	3	11
Emilia-Romagna	3	2	4	9
Friuli-Venezia Giulia	1	1	2	4
Latium	3	5	5	13
Ligury	4	3	3	10
Lombardy	5	5	6	16
Marches	3	2	3	8
Molise	1	2	2	5
Piedmont	5	3	5	13
Sardinia	3	4	3	10
Sicily	3	3	2	8
Trentino-Alto Adige	4	3	4	11
Tuscany	7	7	6	20
Umbria	2	1	2	5
Veneto	3	2	2	7
Total samples	60	56	62	178

Analytical Method:

The extractive step was performed with the **Quechers Method** and the multiresidue analysis was performed with:

➢**Triple-quadrupole GC-MS/MS (30 m × 0.25 mm × 0.002 mm).**

MODE	INJECTOR TEMPERATURE	LOQ (mg/kg)	LOD (mg/kg)	RECOVERY	SD
Splitless	280°C	0.01	0.005	70 - 110 %	1 - 13 %

➢**Triple-quadrupole LC-MS/MS (Zorbax SB-C18, 2.1×150 mm i.d. and 3.5 µm)**

PHASE A	PHASE B	LOQ (mg/kg)	LOD (mg/kg)	RECOVERY	SD
water and 5 % of formic acid	methanol and acetonitrile (3:2) added with 5 mM of ammonium formate	0.01	0.005	80 - 110 %	1 - 10 %

Chemicals compounds in beeswax samples collected from the Italian hives during the years 2013 – 2015

	Positive molecules	LOQ (mg/kg)
Acaricides	Amitraz, Bromopropylate, Chlorobenzilate, Chloropropylat, Cymiazole, Tetradifon	0.01
Herbicides	Pendimethalin, Terbuthylazine	0.01
Fungicides	Chlorthalonil, Cyprodinil, Fludioxinil, Iprodione, Penconazole, Pyrimethanil, Spiroxamine, Tebuconazole, Tetraconazole, Tolifluanide	0.01
Insecticides	α-HCH, Acrinathrin, Chlordane, Chlorfenvinphos, Chlorpyriphos-ethyl, Coumaphos, Cypermethrin, Diazinon, Flumethrin, Heptachlor, Lindane, *op'*-DDD, *op'*-DDT, Permethrin, Piperonil butoxide, *pp'*-DDD, *pp*'-DDE, *pp'*-DDT, Pyrethrin, Rotenone, Spirodiclofen, Tau-fluvalinate, Tetramethrin	0.01

Regard to the 178 samples analyzed for the present investigation, only 47 (26.4 %) reported concentrations of residues below the detection limit.

Within 247 molecules searched, we detected 41 molecules with different frequencies.

Chemicals compounds in beeswax samples collected from the Italian hives during the years 2013 – 2015

The samples showed positive values of concentration for a minimum of 1 compound to a maximum of 14 compounds, with an average of 3 different pesticide residues each.

ORGANIC

69,5 % (91 samples)

- 25 detected molecules
- 2,9 molecules for sample (average)
- Lower average concentrations

CONVENTIONAL

30,5% (40 samples)

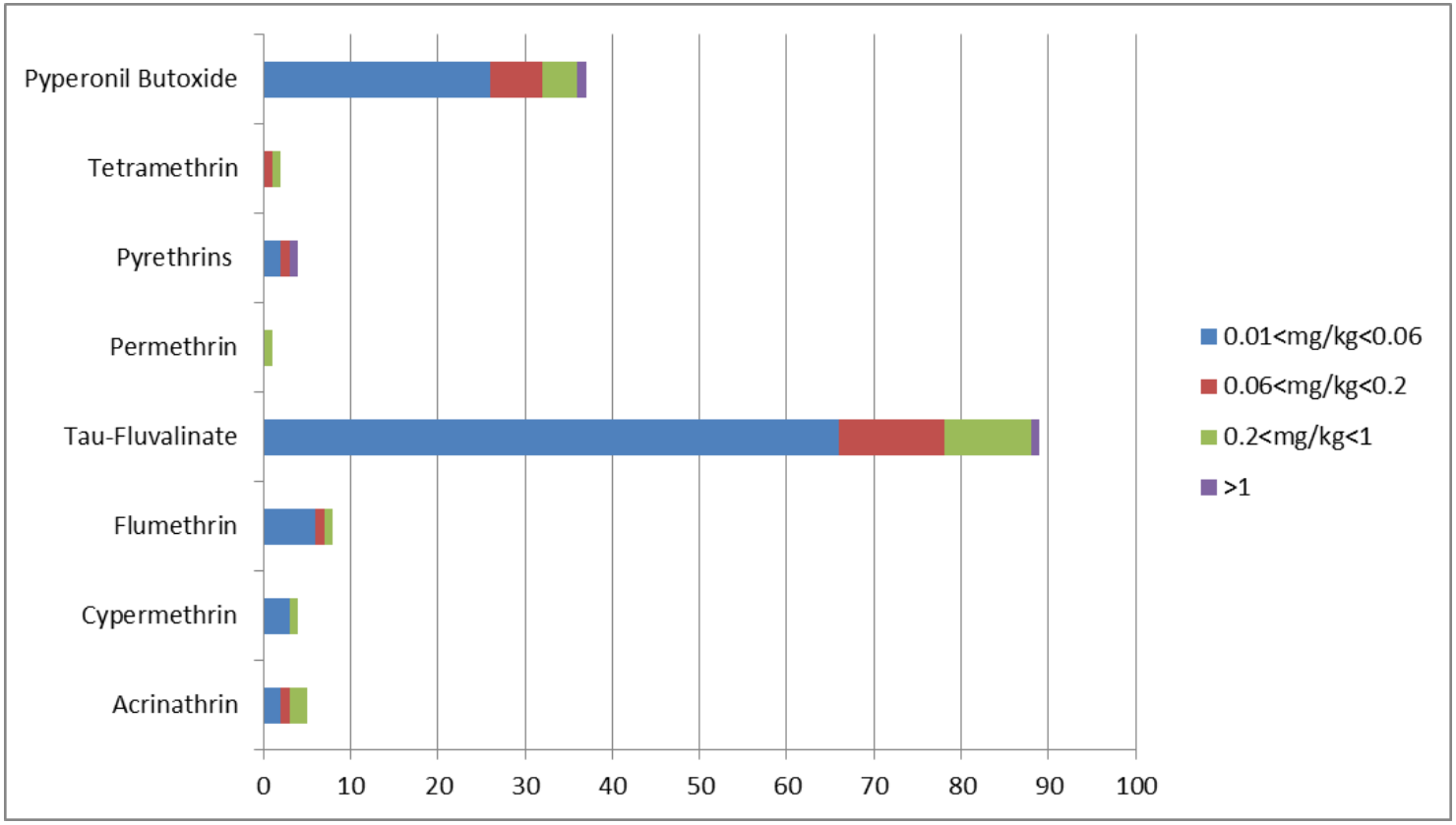
- 32 detected molecules
- 4,3 molecules for samples (average)
- Higher average concentrations

Chemicals for sample					
≤3		4 ≥ **X** ≤ 6		≥7	
ORGANIC	CONVENTIONAL	ORGANIC	CONVENTIONAL	ORGANIC	CONVENTIONAL
66	18	24	17	1	5

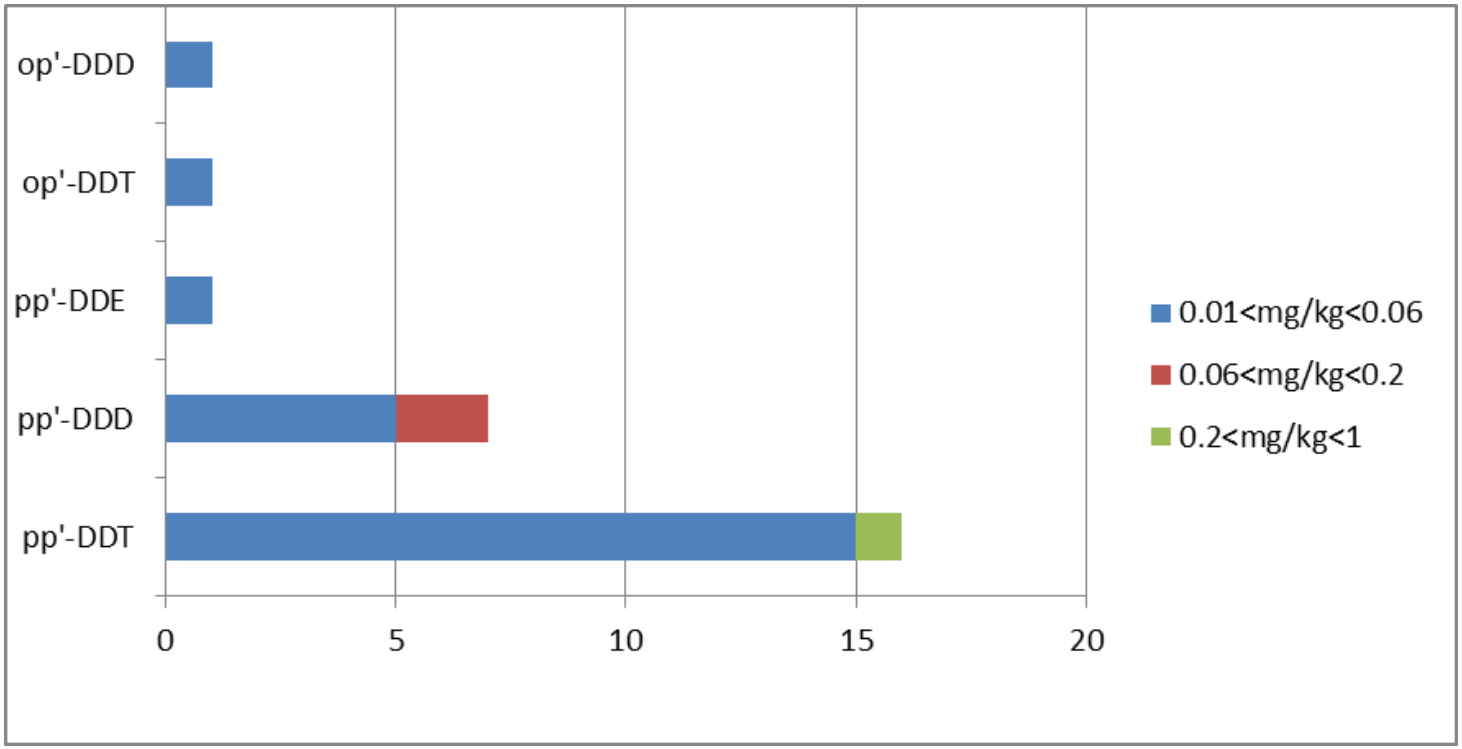
10 and 14 molecules are detected in conventional samples

The samples collected in 2014/2015 reported a higher number of chemicals compounds compared to those of 2013.

Molecole	positive samples	%	lowest concentration	highest concentration	average concentration
α-HCH	1	0,56			0,04
acrinathrin	5	2,81	0,01	0,23	0,1
amitraz	27	15,17			0,01
bromopropylate	6	3,37	0,01	0,03	0,02
chlordane	2	1,12	0,01	0,03	0,02
chlorfenvinphos	63	35,39	0,01	0,63	0,06
chlorobenzilate	1	0,56			0,02
chloropropylat	3	1,68	0,01	0,02	0,03
chlorothalonil	1	0,56			0,03
chlorpyriphos ethyl	2	1,12	0,01	0,02	0,01
coumaphos	108	60,67	0,01	0,99	0,1
cymiazole	1	0,56			0,02
cypermethrin	4	2,25	0,01	0,67	0,18
cyprodinil	1	0,56			0,02
DDD-*op*	1	0,56			0,01
DDD-*pp*	7	3,93	0,01	0,08	0,03
DDE-*pp*	1	0,56			0,02
DDT-*op*	1	0,56			0,01
DDT-*pp*	16	8,99	0,01	0,43	0,05
diazinon	1	0,56			0,04
fludioxonil	1	0,56			0,01
flumethrin	8	4,49	0,01	0,11	0,04
fluvalinate-*tau*	89	50	0,01	1,07	0 ,09
heptachlor	3	1,68	0,01	0,44	0,16
iprodione	1	0,56			0,01
lindane	1	0,56			0,02
penconazole	1	0,56			0,01
pendimethalin	2	1,12	0,01	0,02	0,02
permethrin	1	0,56			0,28
pirethrin	4	2,25	0,02	4,42	1,14
piperonyl butoxide	37	20,79	0,01	2,3	0,16
pyrimethanil	1	0,56			0,01
rotenone	24	13,48	0,01	0,23	0,02
spirodiclofen	1	0,56			0,03
spiroxamine	1	0,56			0,02
tebuconazole	2	1,12	0,01	0,05	0,03
terbuthylazine	1	0,56			0,01
tetrachonazole	1	0,56			0,03
tetradifon	1	0,56			0,01
tetramethrin	2	1,12	0,16	0,37	0,26
tolifluanide	1	0,56			0,09



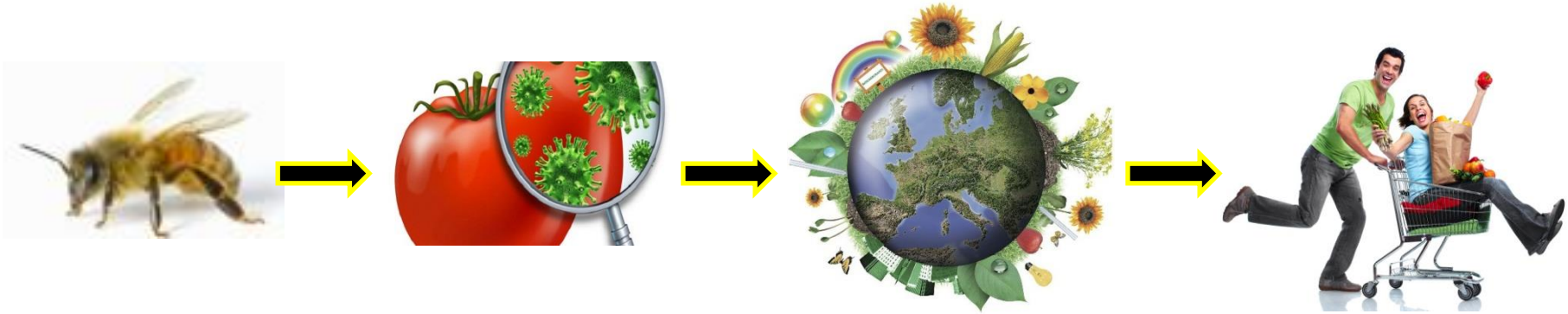
Particularly interesting is the presence in the samples collected in the last years (2014/2015) of some compounds as acrinathrin, a new generation pyrethroid authorized in products for agricultural practice from 2011. Pyrethrins are associated to the higher concentrations (4.42 mg/kg)



The higher levels of *pp'*-DDT compared to its metabolites (*pp'*-DDD or DDE) points to a more recent use of these insecticides. Their presence could be due to the increased use, from beekeeping of foreign foundations. It is known that the African wax is almost clean from new generations molecules as pyrethroids and fungicides but presents high levels of organochlorine insecticides.

Also if a recent opinion of the European Food Safety Authority (EFSA, 2007) concluded that the use of beeswax as an additive for the existing food uses and the proposed new food use is not of safety concern, given the contamination of beeswax, it should be advantageous consider to take actions aimed at lowering the pesticide contamination of beeswax due to the countless uses of this natural product.

Actually, even if several molecules dangerous for honeybees, have been banned, beeswax remains the best matrix to study the historic contamination coming from the agricultural practices or, such as highlight by others authors, the illegal use of these forbidden molecules by homemade preparations.



Grazie per l'attenzione...

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