

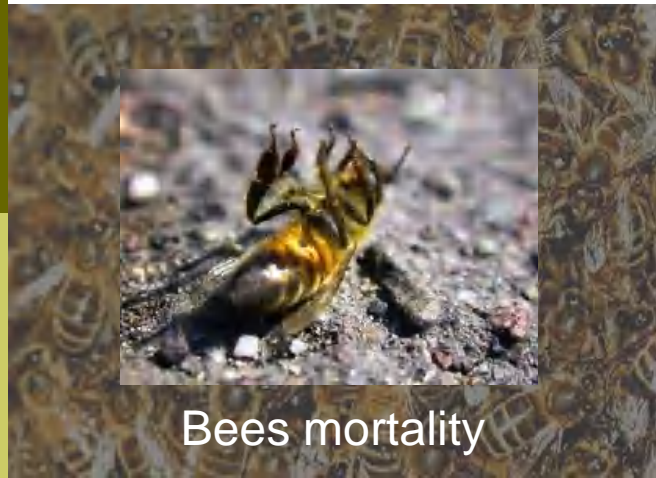
Development of a multi-residue routine analysis of 80 pesticides in honeys, honeybees and pollens

Apimondia 2011, Buenos Aires



Barbara Giroud
Audrey Buleté
Cédric Fratta
Carine Arnaudguilhem
Laure Wiest

Introduction

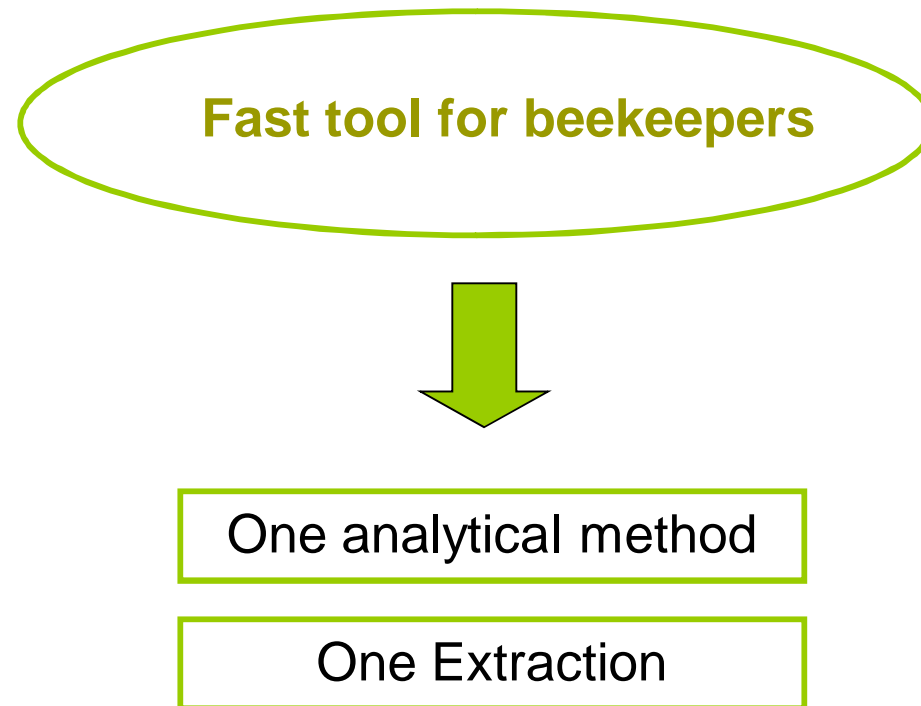


Fast tool for beekeepers

Global view of pesticide presence



Objectives



Objectives



Global view of pesticide presence



80 targeted compounds
More than 10 categories
At low concentrations
(ng/g)

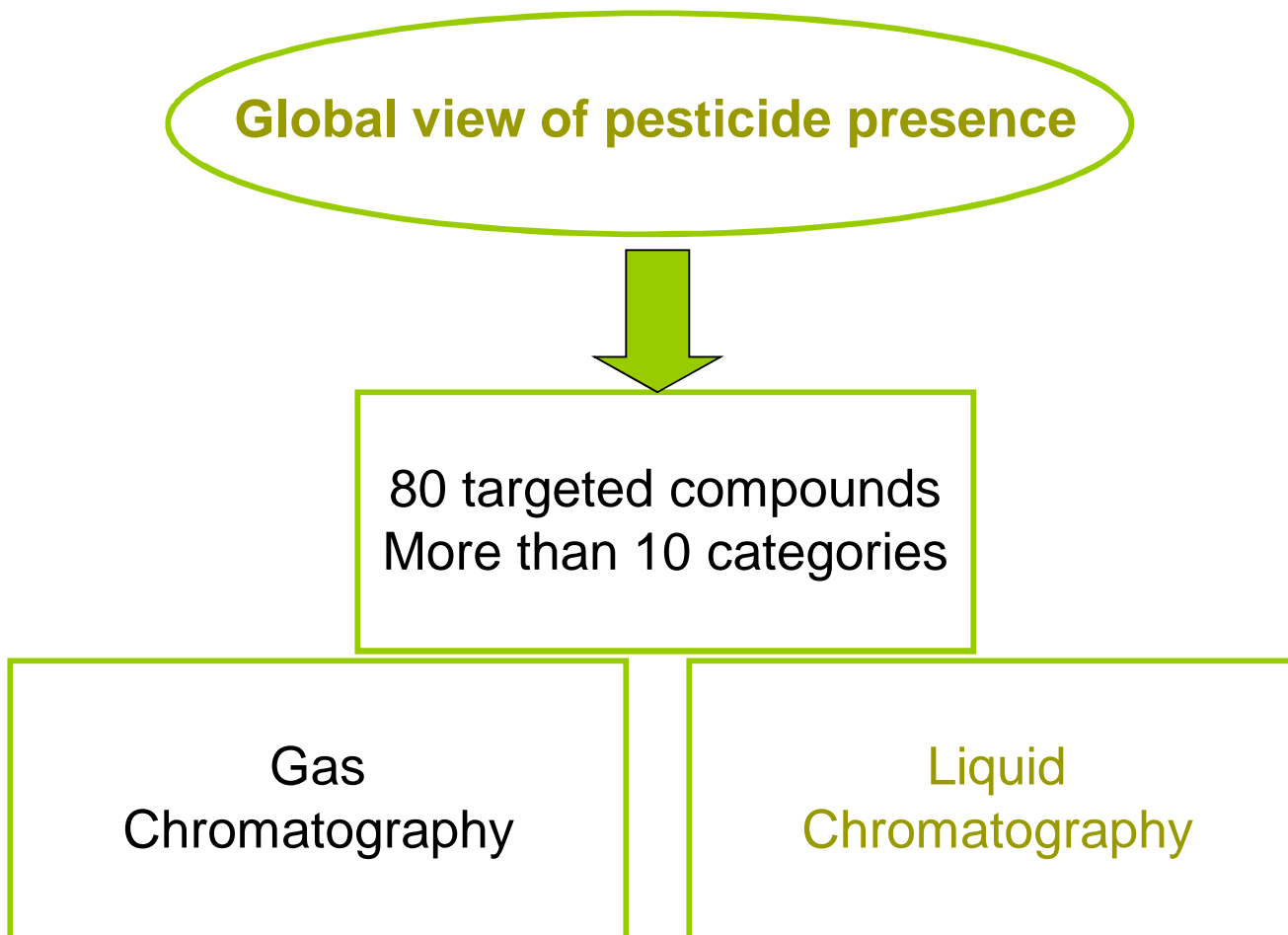
Objectives



□ Analysis of 80 compounds



Objectives



Objectives



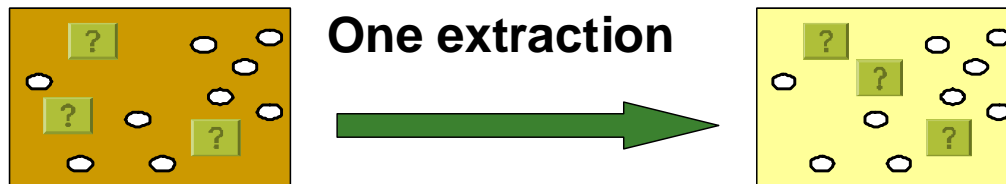
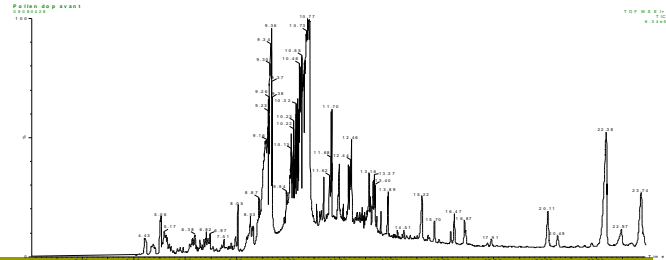
Global view of pesticide presence



80 targeted compounds
More than 10 categories

Untargeted
Compounds

Analytical strategy

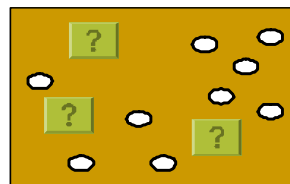
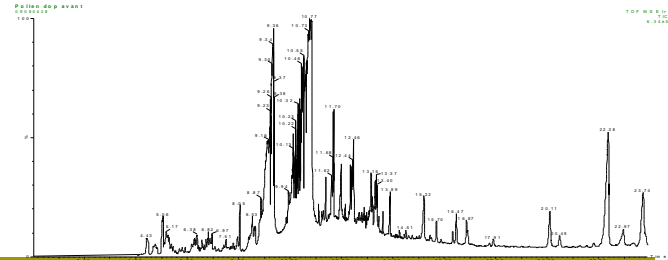


Salting-out liquid liquid extraction with acetonitrile*

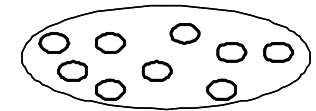
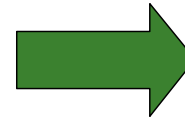
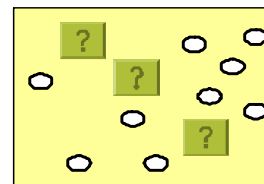
Purification by dispersive Solid Phase Extraction

* « QuEChERS method » (Quick, Easy, Cheap, Efficient, Rugged and Safe)

Analytical strategy



One extraction



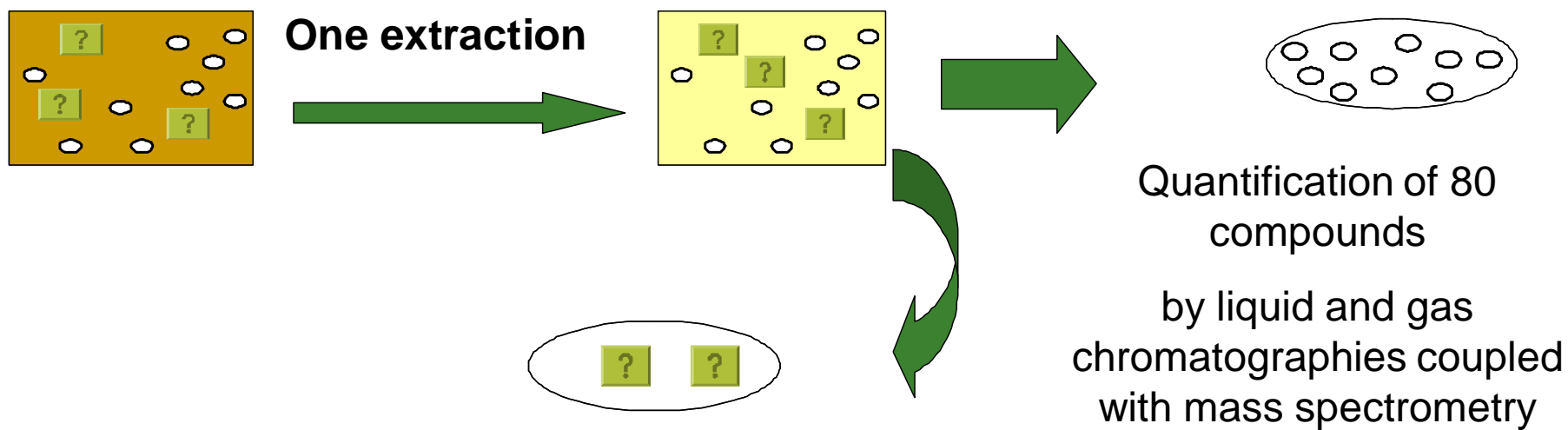
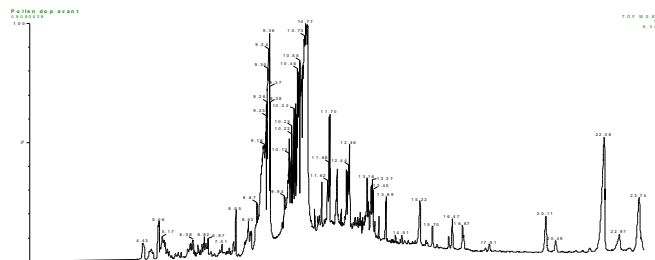
Quantification of 80
targeted compounds

by liquid and gas
chromatographies coupled
with mass spectrometry

by **Liquid Chromatography coupled with Tandem Mass spectrometry (LC-MS/MS)**

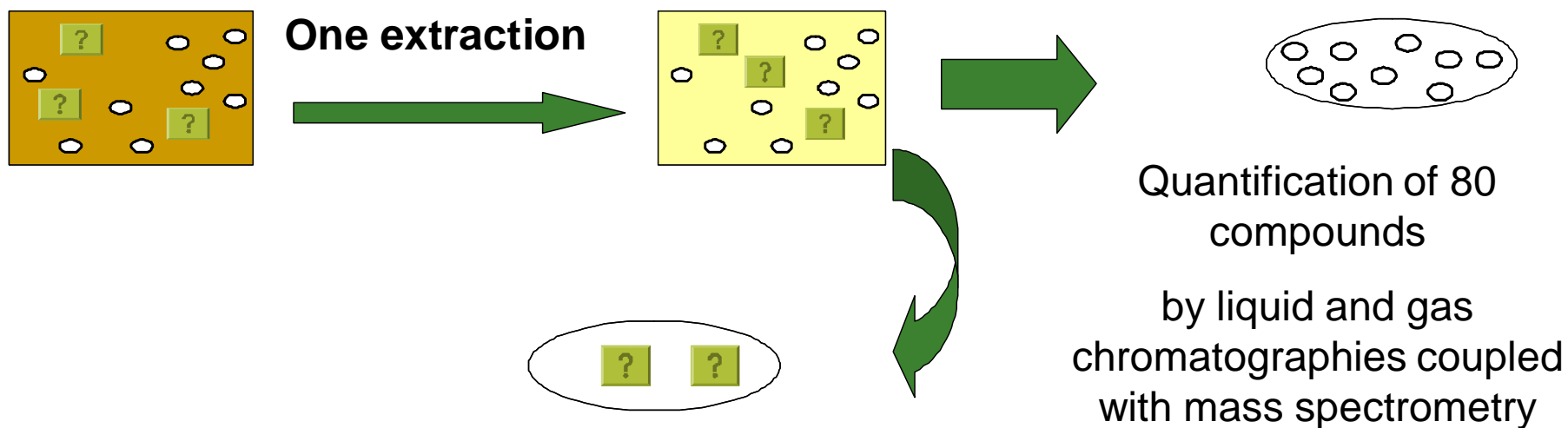
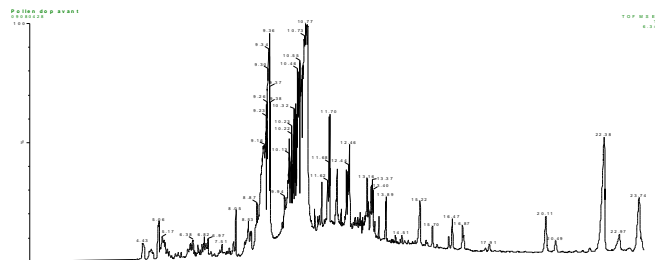
by **Gas Chromatography coupled with Time of Flight Mass spectrometry (GC-ToF)**

Analytical strategy



Unknown compounds possible to identify

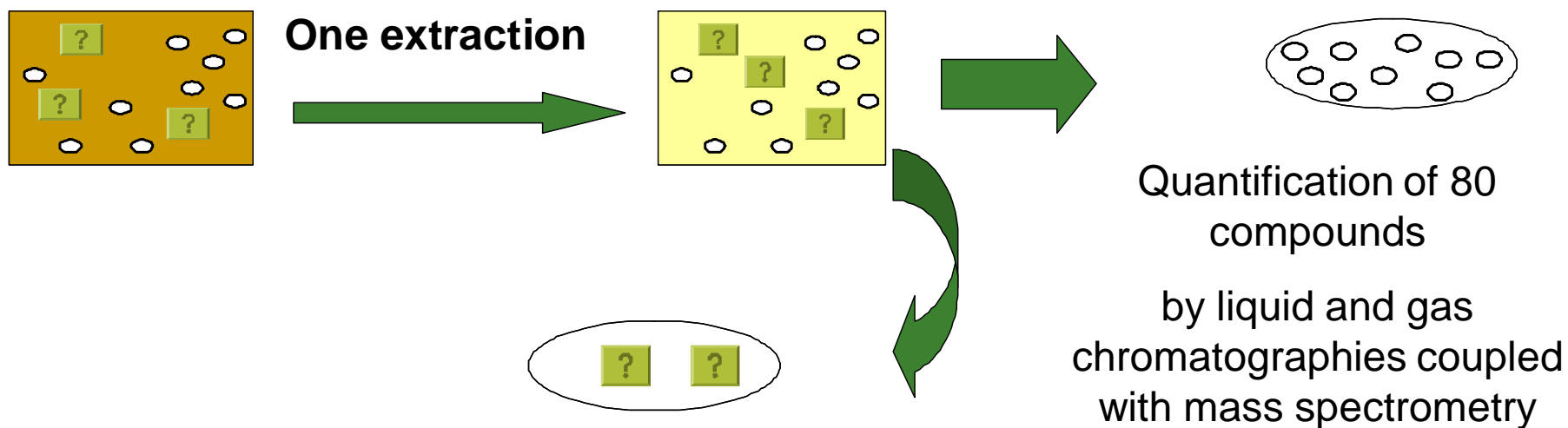
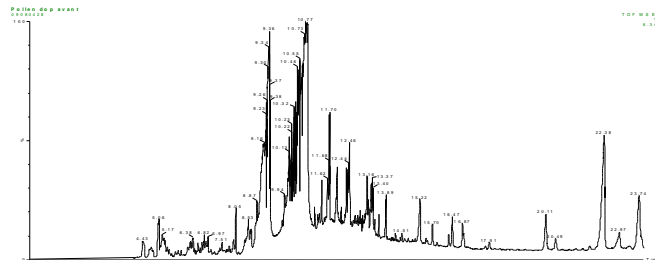
Analytical strategy



Unknown compounds possible to identify

by **Gas Chromatography coupled with Time of Flight Mass spectrometry (GC-ToF)**

Analytical strategy



Unknown compounds possible to identify

Global view of pesticide presence

Application to more than 300 samples

Results

One extraction method for all compounds



✓ 5 grams



✓ 5 grams



✓ 2 grams

L. Wiest et al. / Journal of Chromatography A, 1218 (2011) 5743-5756

Results

Detected compounds



✓ 80
/80



✓ 78
/80

Procymidone
Chlorothalonil



✓ 75
/80

Dichloran
4 avermectins

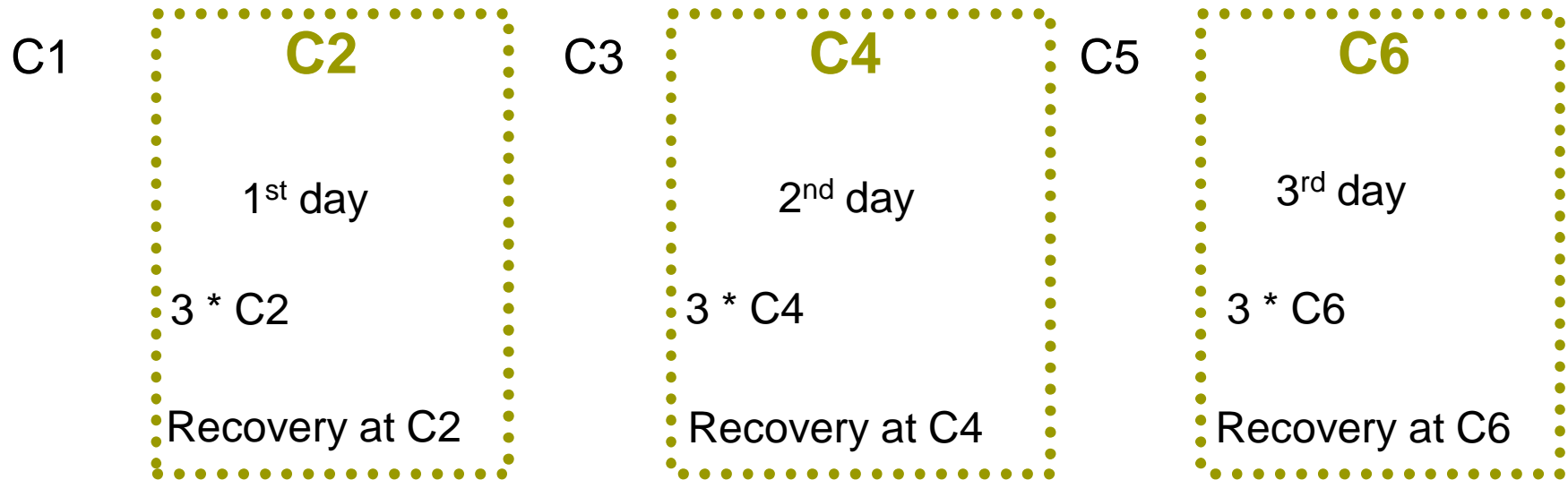
Validation



- Recovery
- Repeatability
- Inter-day precision
- Linearity

Validation

3 days – Concentration levels (4 – 60 ng/g)
(10 – 150 ng/g)



Validation



- Recovery
- Repeatability
- Inter-day precision
- Linearity ✓

Between 60 and 120%

Relative Standard Deviation (RSD) < 20%

RSD < 25%

Regression coefficient > 0.99

Honeys Validation



✓ **80 detected compounds**

✓ **Recoveries 60% - 120 %** for all compounds

✓ **Repeatability/ Inter-day precision**

RSD < 20 % for 62 compounds

RSD < 25 % for 64 compounds

Honeybees Validation



✓ **78 compounds detected**

✓ **Recoveries 60% - 120 %** for 77 compounds

✓ **Repeatability/ Inter-day precision**

RSD < 20 % for 62 compounds

RSD < 25 % for 62 compounds

Pollens Validation



✓ **75 compounds detected**

✓ **Recoveries 60% - 120 % for 74 compounds**

✓ **Repeatability/ Inter-day precision**

RSD < 20 % for 71 compounds

RSD < 25 % for 57 compounds

Honeys



✓ Limits Of Detection (LOD) and Quantification (LOQ)

LOD < 7 ng/g for 65 compounds

(LOD_{max} = 24 ng/g)

4 < LOQ < 20 ng/g for 67 compounds

(LOQ_{max} = 70 ng/g)

✓ Inferior to Maximum Residue Level (MRL) of the European Union except 5 compounds

Honeybees



✓ Limits Of Detection (LOD) et Quantification (LOQ)

LOD < 7 ng/g for 70 compounds

(LOD_{max} = 38 ng/g)

4 < LOQ < 22 ng/g for 68 compounds

(LOQ_{max} = 72 ng/g)

Pollens Validation



✓ Limits Of Detection (LOD) et Quantification (LOQ)

LOD < 15 ng/g for 64 compounds

(LOD_{max} = 77 ng/g)

10 < LOQ < 50 ng/g for 67 compounds

(LOQ_{max} = 230 ng/g)

✓ Inferior to MRL of the European Union

except 1 compound (LC-MS/MS)

Pollens Validation



✓ Limits Of Detection (LOD) et Quantification (LOQ)

LOD < 15 ng/g for 64 compounds

(LOD_{max} = 77 ng/g)

10 < LOQ < 50 ng/g for 67 compounds

(LOQ_{max} = 230 ng/g)

✓ Inferior to MRL of the European Union

except 11 compounds (GC-ToF)

Application to 300 samples

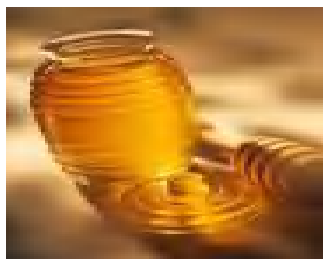
- Matrix-matched calibration
 - 6 calibration points in blank matrix

- Method performance:
 - Instrumental: Injection standard
 - Extraction: spiking with 3 standards

- Application
 - 141 honeys
 - 145 honeybees
 - 130 pollens

Application to 300 samples

Compounds detected



In 2008	23 /80	18 /78	20 /75
In 2009	21 /80	13 /78	14 /75

Application to 300 samples

Compounds quantified



In 2008

7
/80

9
/78

13
/75

In 2009

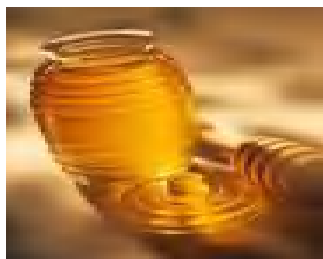
9
/80

10
/78

9
/75

Application to 300 samples

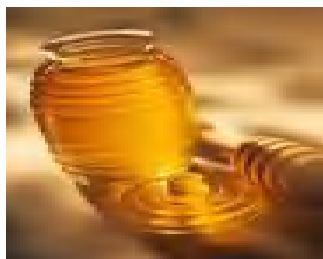
Samples contaminated



In 2008	60 /61	52 /62	49 /63
In 2009	75 /80	54 /83	27 /67

Application to 300 samples

Maximum number of compounds detected
In one sample



8



6



5

Compounds detected in the 3 matrices

Compound	Honey	Honeybee	Pollen
Triphenylphosphate	<LOQ	62	<LOQ
Flusilazole	<LOQ	<LOQ	52
Phosmet	42	62	78
Coumaphos	56	47	40
Carbaryl	4	<LOQ	15
Tau-Fluvalinate	30	53	85
Amitraze I	26	30	115
Amitraze II	116	17	129
Carbendazime	88	66	2595
Thiophanate-méthyl	5	2419	3674

Maximum Concentrations in ng/g

To conclude

Frequency of
detection



Concentrations

Perspectives

□ Tool for beekeepers

- Apiarian matrices: royal jelly, propolis , bee bread
- The number of searched compounds
 - GC-ToF data treatment
 - Study of pesticides metabolites

Perspectives

□ Tool for beekeepers

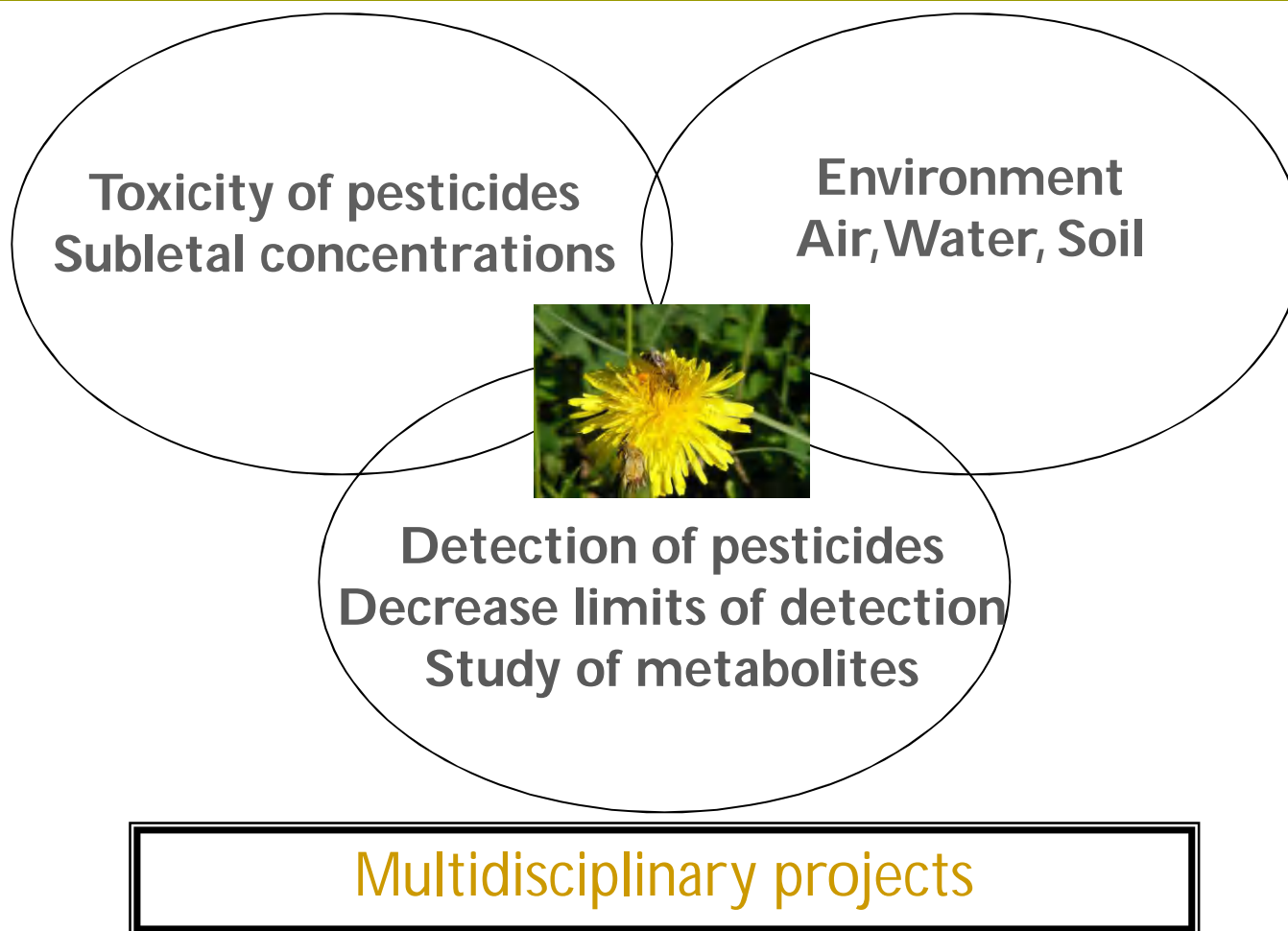
- Apiarian matrices: royal jelly, propolis, bee bread
- The number of searched compounds
- Specific analysis of the most toxic compounds

Perspectives

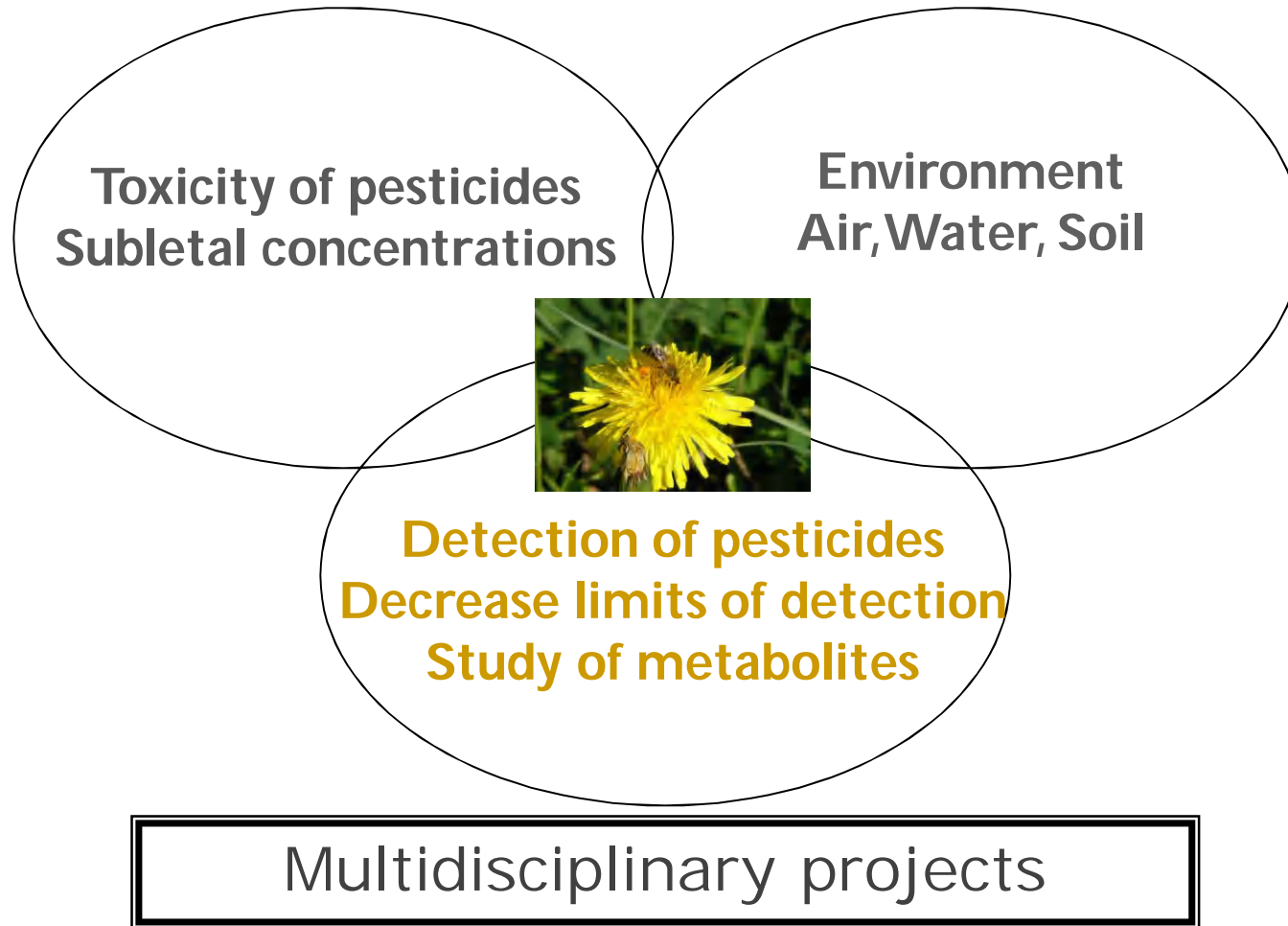
- Tool for beekeepers
 - Apiarian matrices: royal jelly, propolis, bee bread
 - The number of searched compounds

- Understanding bee's mortality

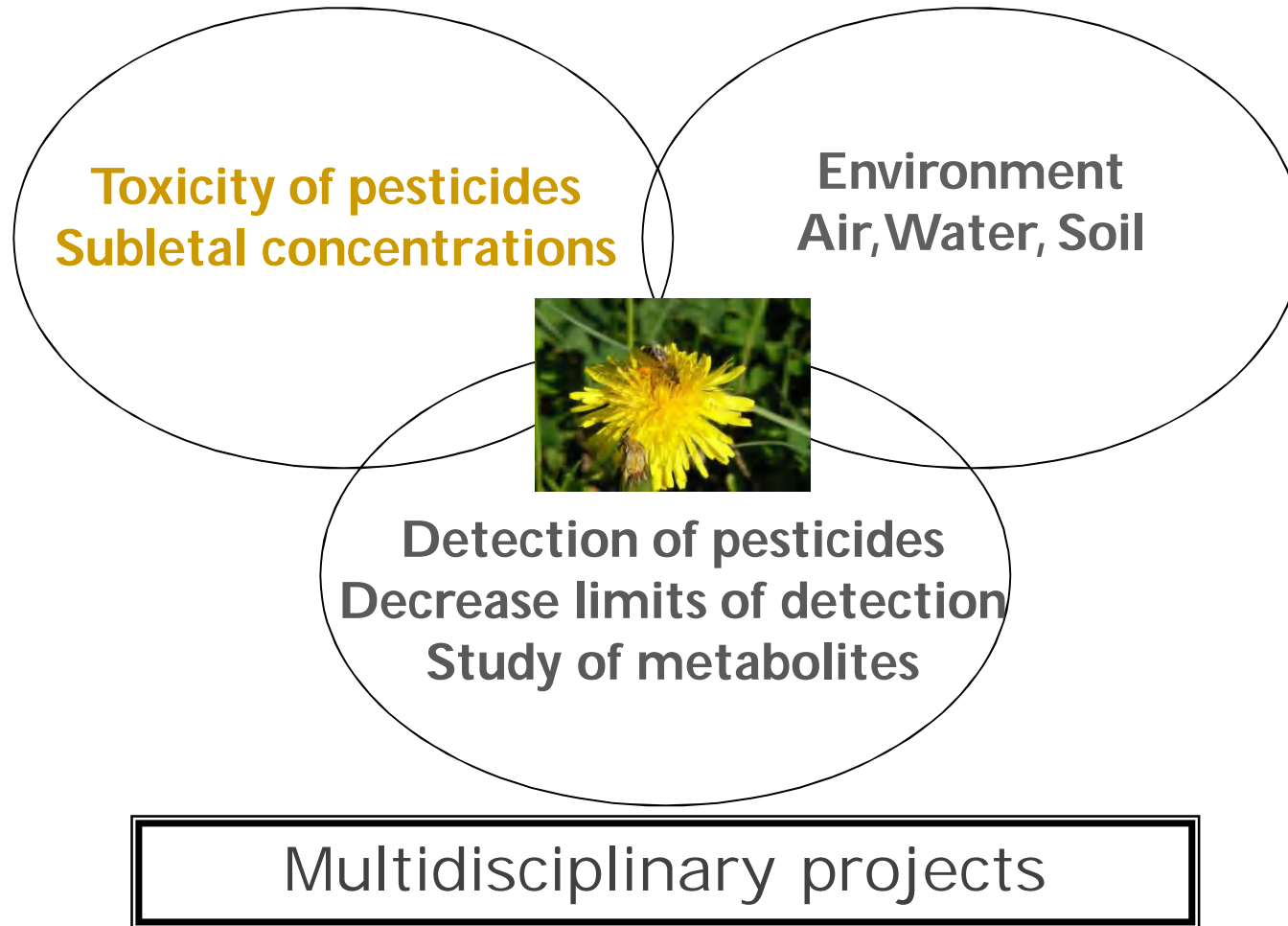
Perspectives & Conclusion



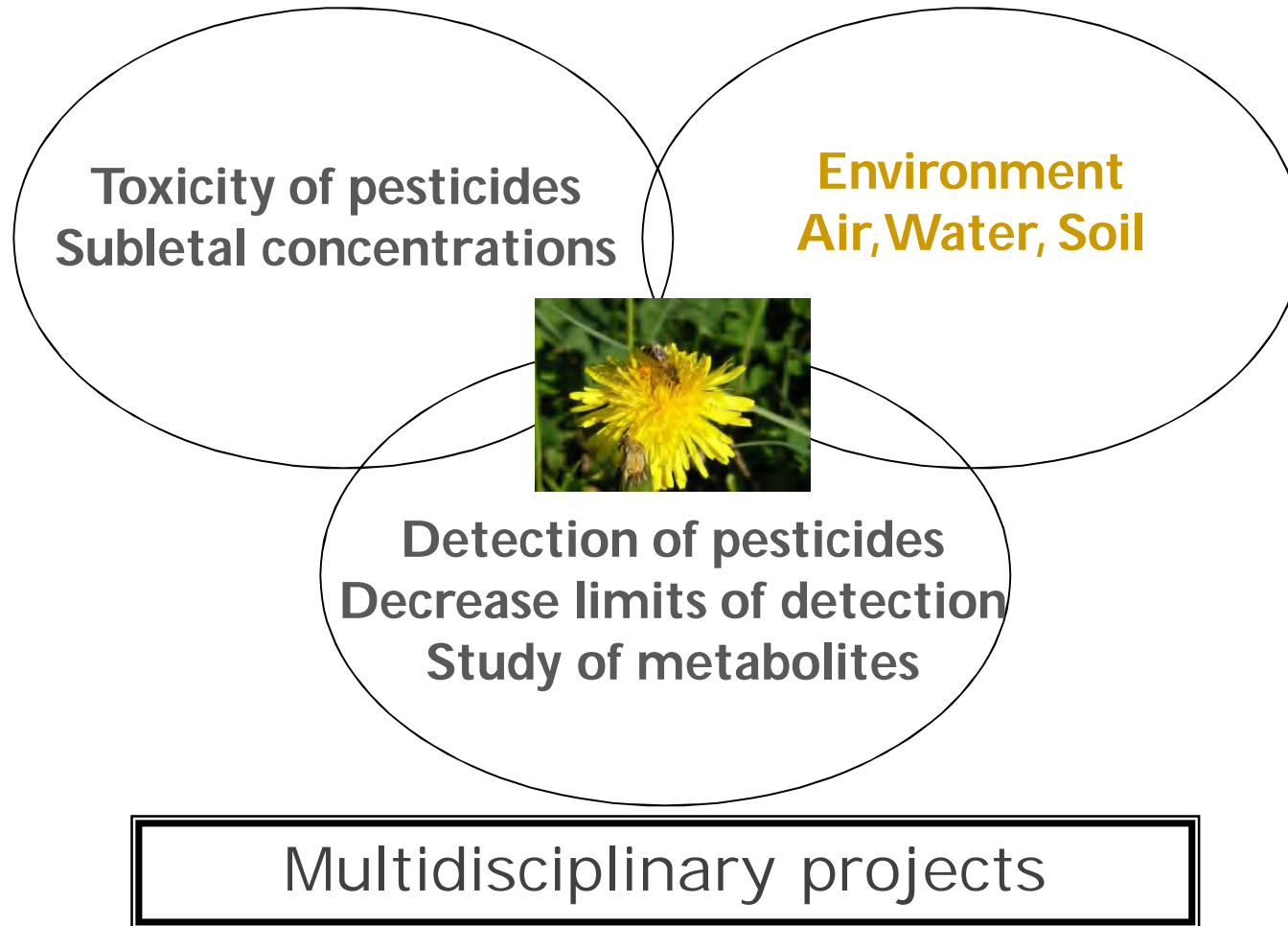
Perspectives & Conclusion



Perspectives & Conclusion



Perspectives & Conclusion



Acknowledgements



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Gracias por su atención



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