EFFECTS OF PESTICIDE USE ON THE DEVELOPMENT OF BEE DISEASES-ANALYTICAL AND ECOTOXICOLOGICAL THREATS AND CHALLENGES
National Institute for Agricultural and Food Research and Technology, INIA 7th- 8th Novembrer 2016

Tracking the dangerous work of bees in agroecosystems

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GACT
Grupo de Analisis de Compuestos Traza
INCO
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Uruguay

Who we are?

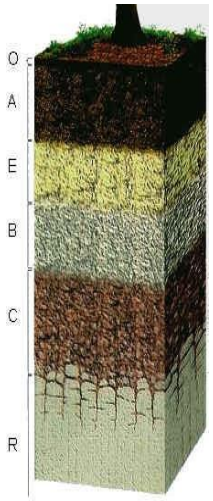
Polo Agroalimentario y Agroindustrial de Paysandú

400km

Facultad de Química

GACT

Who we are?



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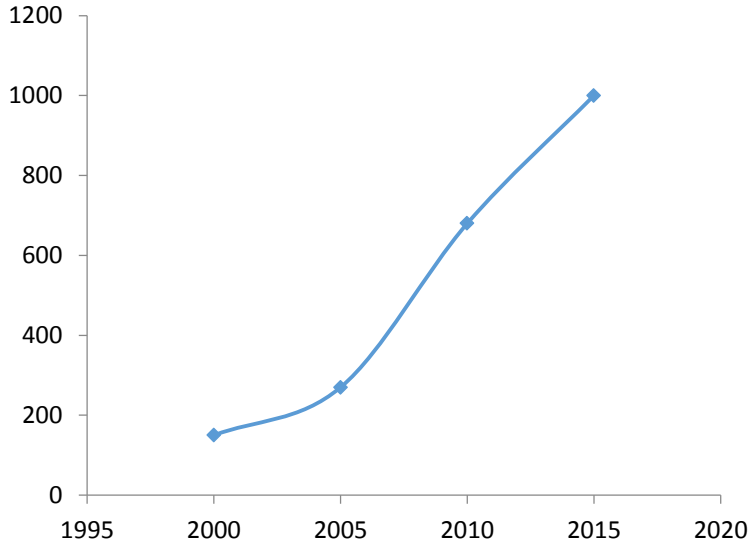
Overview

- The idea
- Development of analytical methods
- Application to monitoring programs
- Controlled semi-field studies
- Working on the data
- Proof of concept
- Atemps to model the classification of the environments

Agroecology considerations

- Agricultural activities boosted up in the last decade in the MERCOSUR region
- Agrochemical uses increased 250%
- Main agrochemicals used.
  - Fungicides.
  - Herbicides.
  - Insecticides

Agroecosystems sustainability?



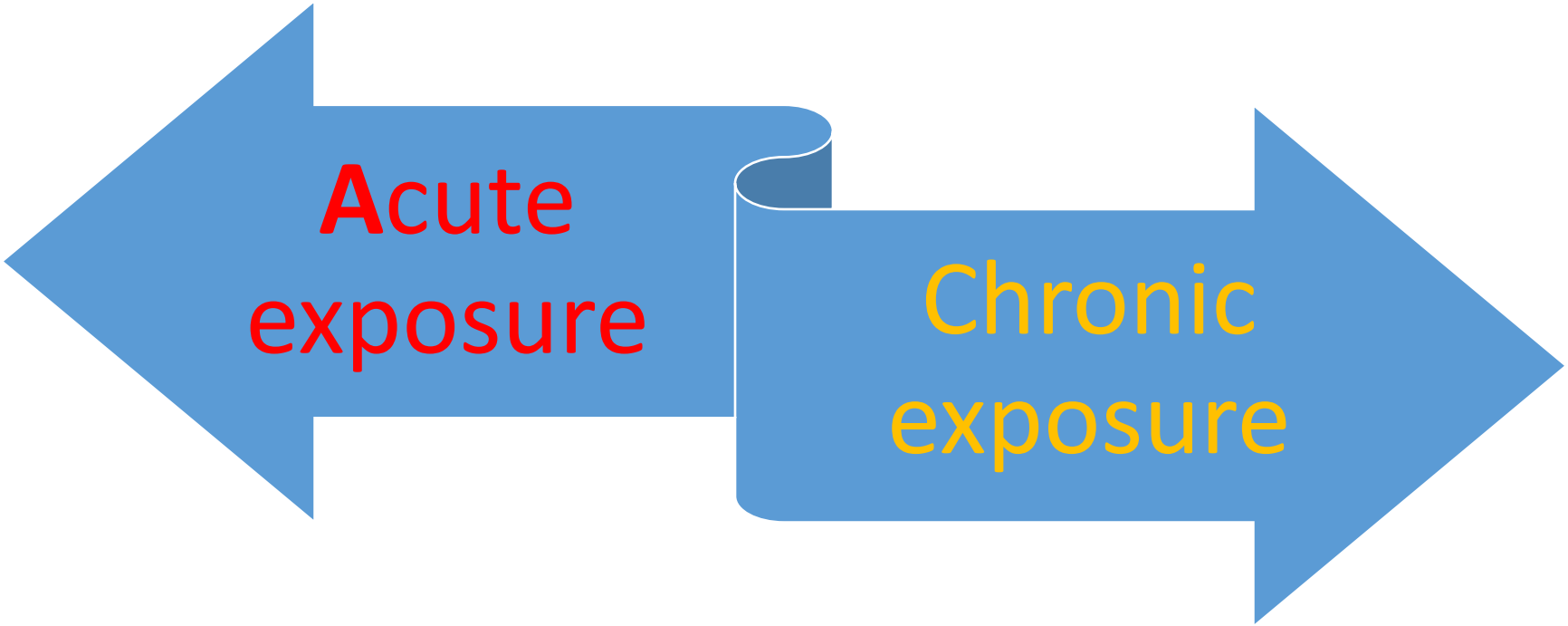
Bee and bee products as markers of the environmental status of an agroecosystem


Bees fly up to 2000m looking for food!!!

Beehive: enviromental biomonitor

- Hive products are reservoirs of environmental information

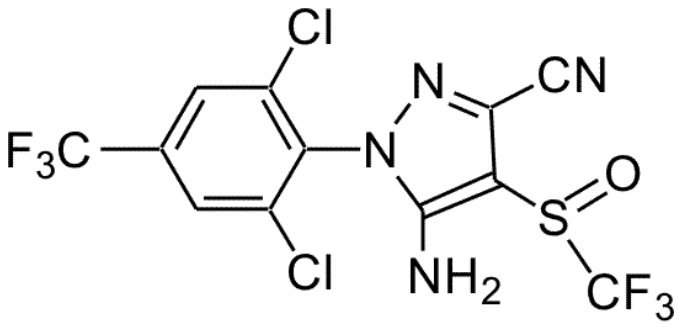
Porrini *et al*., 2002; Celli & Maccagnani, 2003; Ghini *et al*., 2004; Balayiannis & Balayiannis, 2008; Chauzat *et al*., 2011


Acute exposure
Chronic exposure

Acute toxicity

Death bees within the beehive due to fipronil misuse

Fipronil

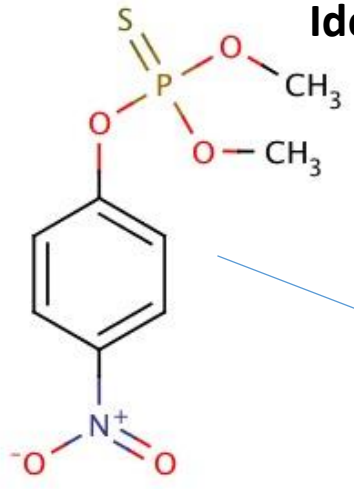


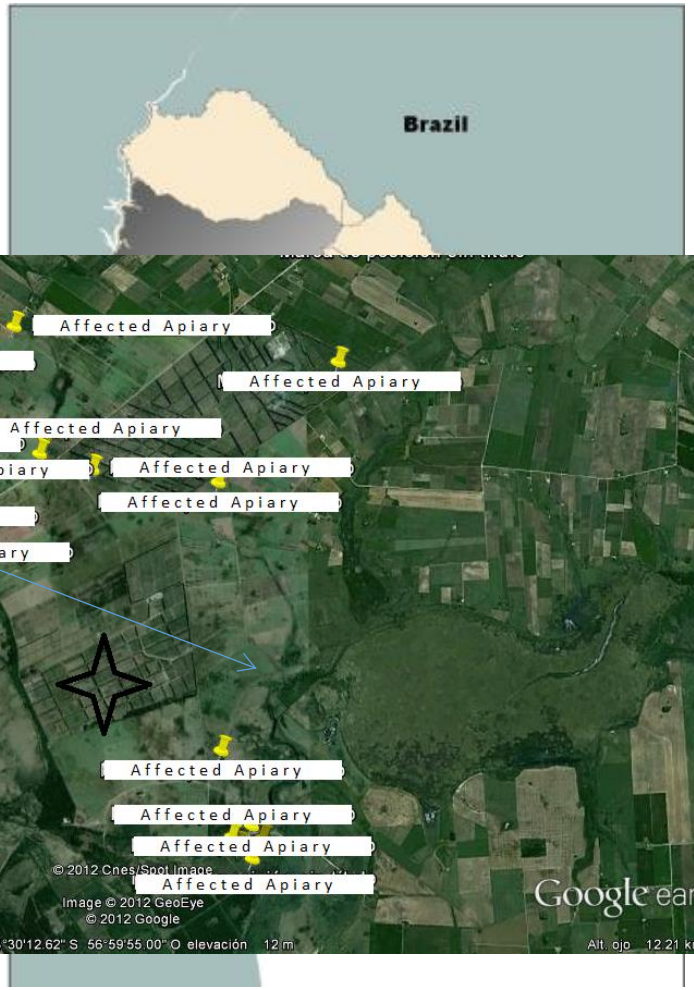
Identification by GC-MS

1.5-6.0 mg/kg

Parathion methyl

Identification by LC-MS/MS

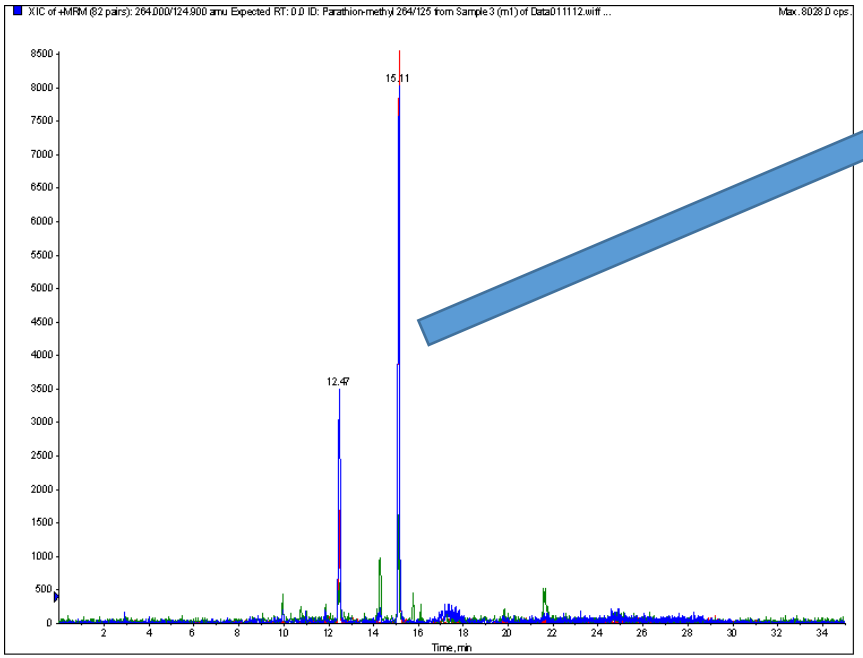


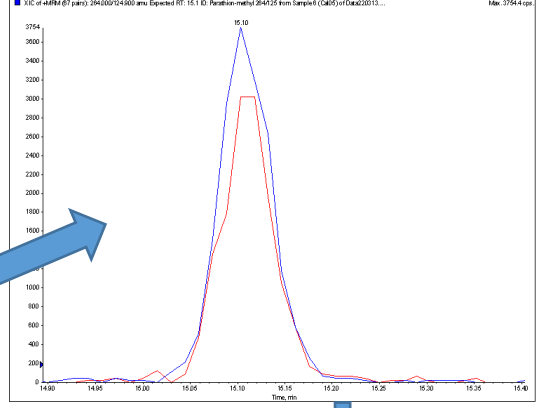


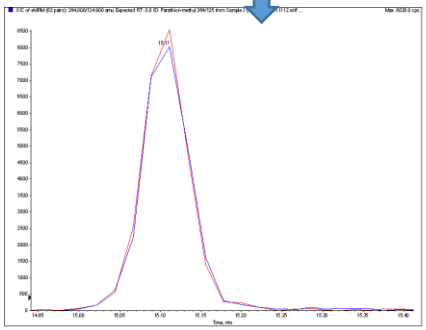
4.8mg/Kg

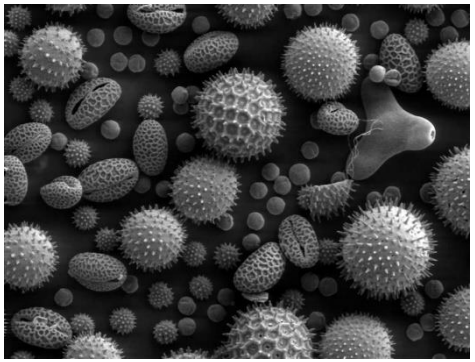
Niell et al Chromatographia 2016

LC-MS/MS Identification

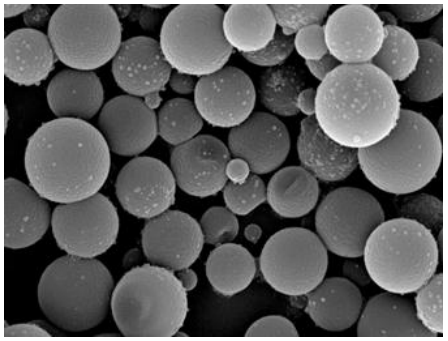




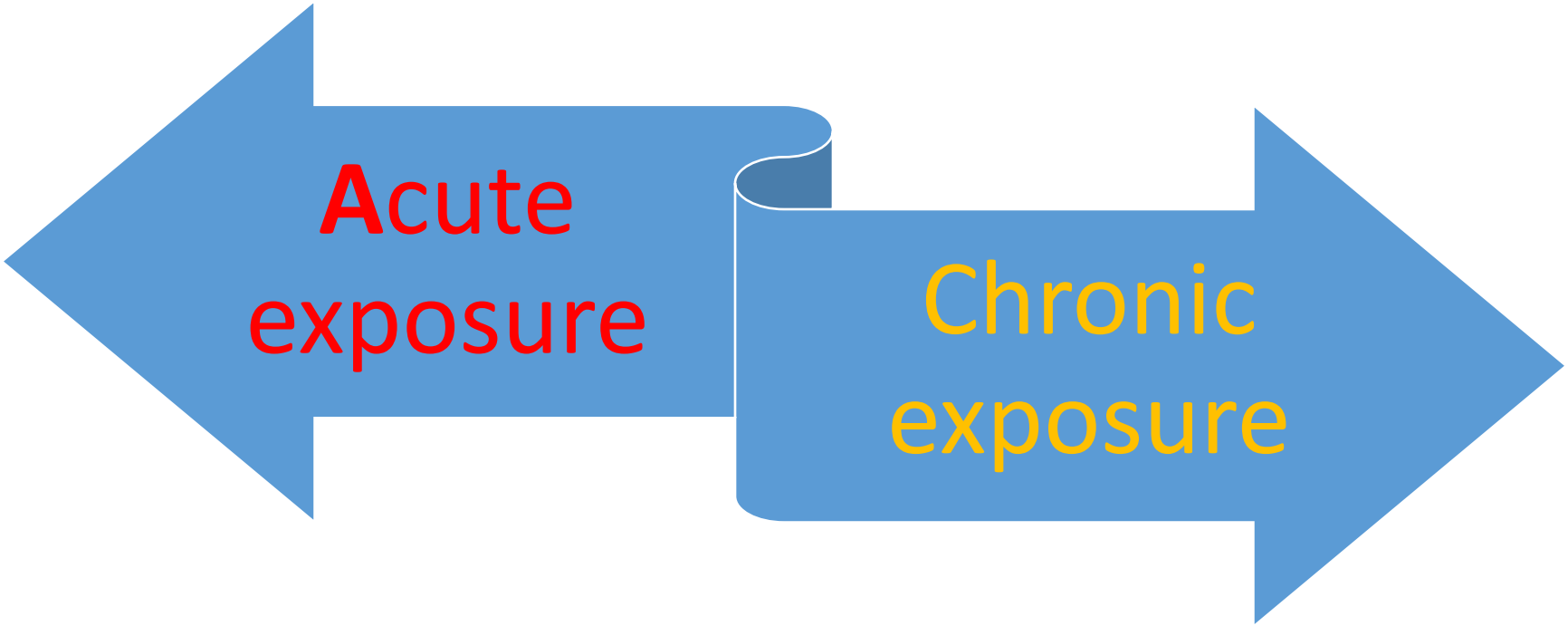




Polen Grains



Encapsulated Parathion methyl


Acute exposure
Chronic exposure

Previous work I (Honey)

Detected compounds	Chemical class	Beehive Status	Positive honey samples/ analyzed samples	Highest level detected (µg/kg)	Lowest level detected (µg/kg)	Average of detected concentration (µg/kg) (SD)	LD_{50} bees (µg/kg)*
Chlorpyrifos	Ops	A	13/31	80	30	46 (12)	1333
Coumaphos	Ops	A	1/31	60		60	51444
Cypermetrhin	Pys	A	6/31	80	< LOQ	61 (14)	655.6
Fipronil	Phenyl-pyrazole	D	2/31	100	40	70	44.4
α+β Endosulfan	OCs	A	2/31	< LOQ	< LOQ	–	87444

A: Active; D: Depopulated; S.D: standard deviation; * Assuming that a worker bee weighs 90 mg

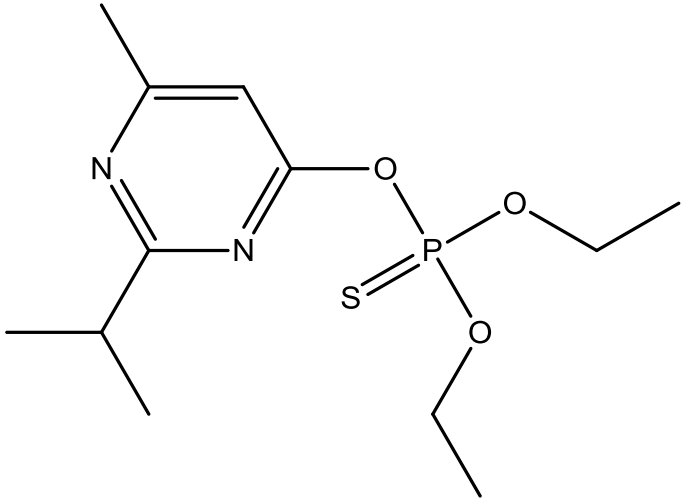
Int. J. Environ. Res. Public Health **2011**, *8*(10), 3844-3858; doi: 10.3390/ijerph8103844

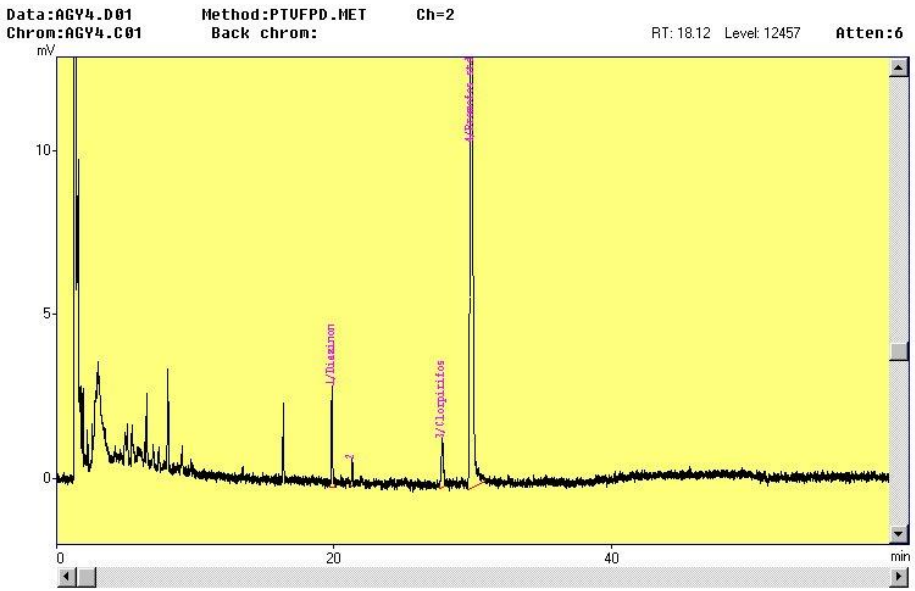
Previous work II (wax & propolis)

Honeycomb samples	Imidacloprid (µg/kg)	Propolis samples	Imidacloprid (µg/kg)
1a	N.D.	1b	N.D.
2a	N.D.	2b	N.D.
3a	N.D.	3b	N.D.
4a	240	4b	100
5a	440	5b	20

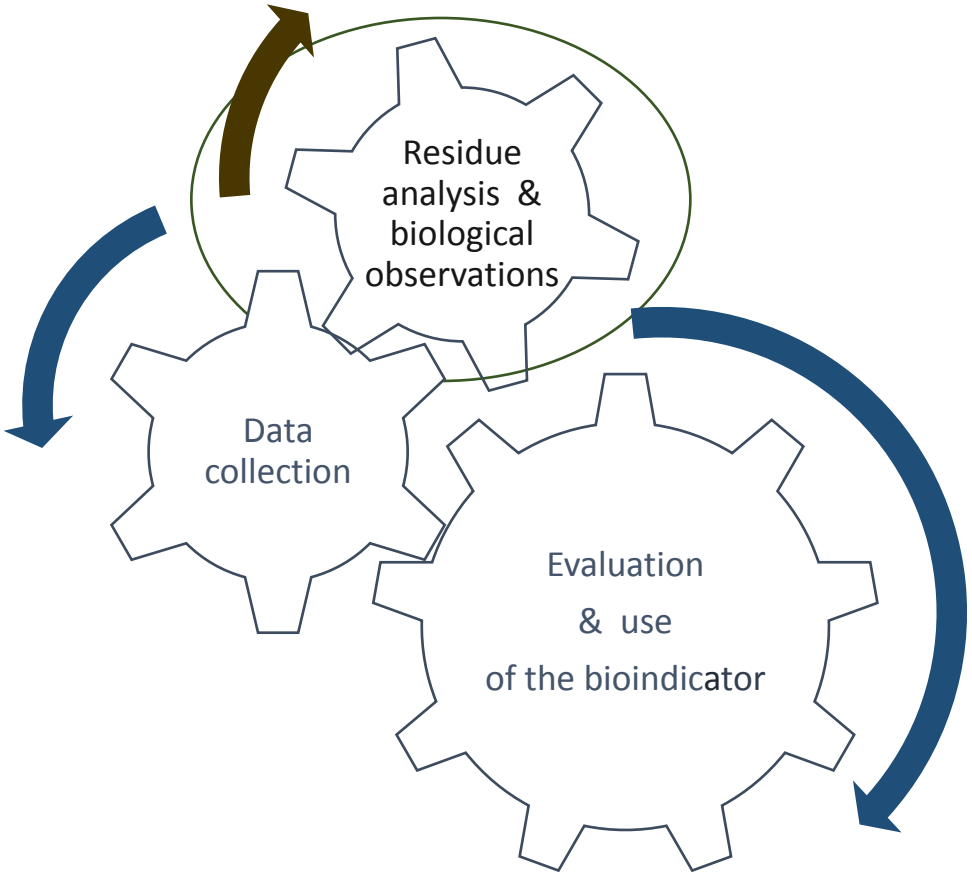
Int. J. Environ. Res. Public Health **2011**, *8*(10), 3844-3858; doi: 10.3390/ijerph8103844

Chronic toxicity





Is the beehive an indicator of the status of an agroecosystem ?



Beehive as biomonitor

biological evaluation

correlation

Chemical evaluation

Biological evaluación

- To estimate the population (adults, larvae)
- Behaviour in and out the hive
- Mortality
- Honey production
- Health condition of bees
- Larval survival

Porrini et al. 2002 CRC Press, ch11, pp 186-247

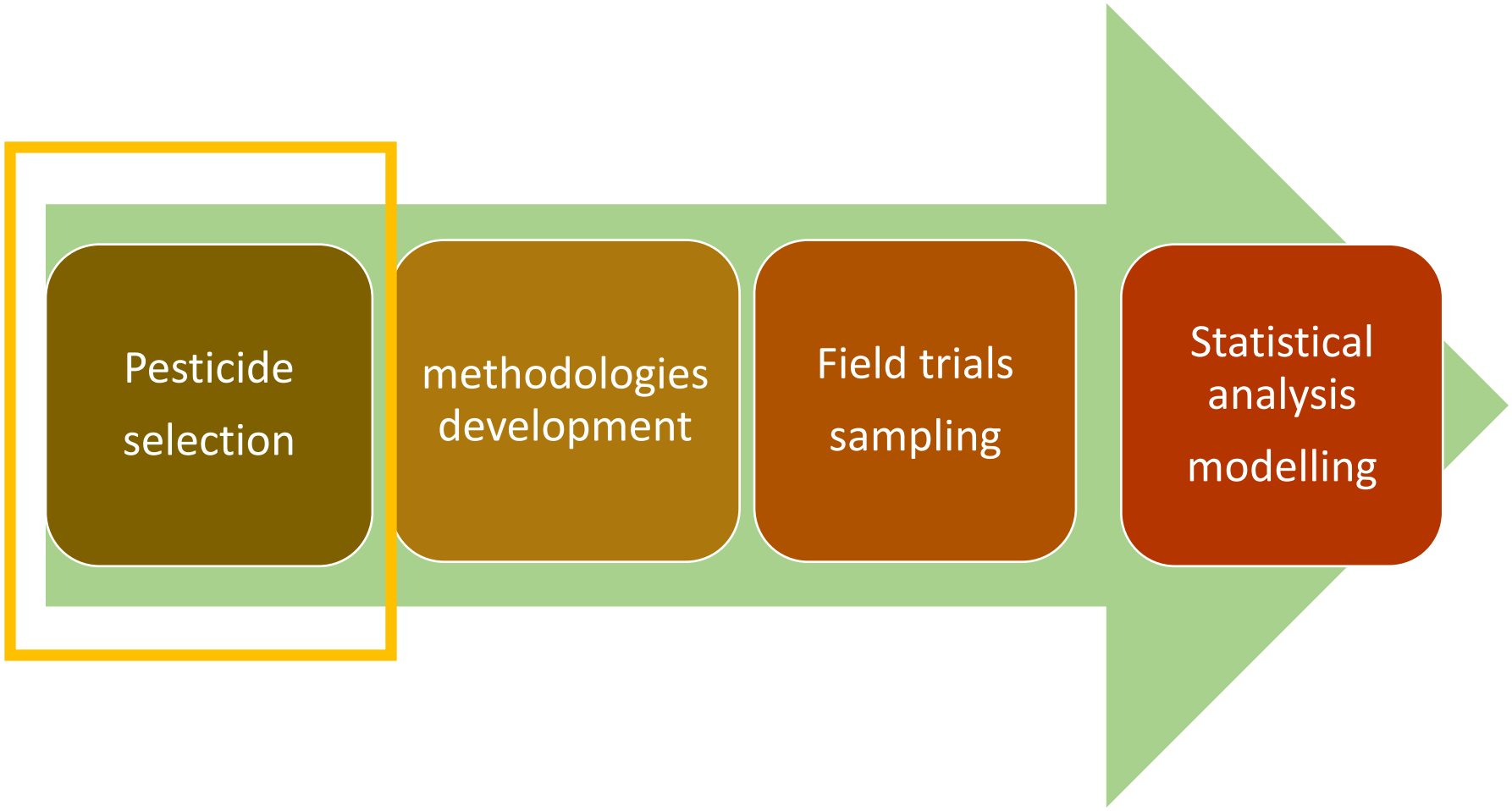
Pesticide residues analysis in beehive representative matrices

- Honey
- Bees
- Wax
- Pollen

Chemical evaluation

Multi Residue Methods (MRM)

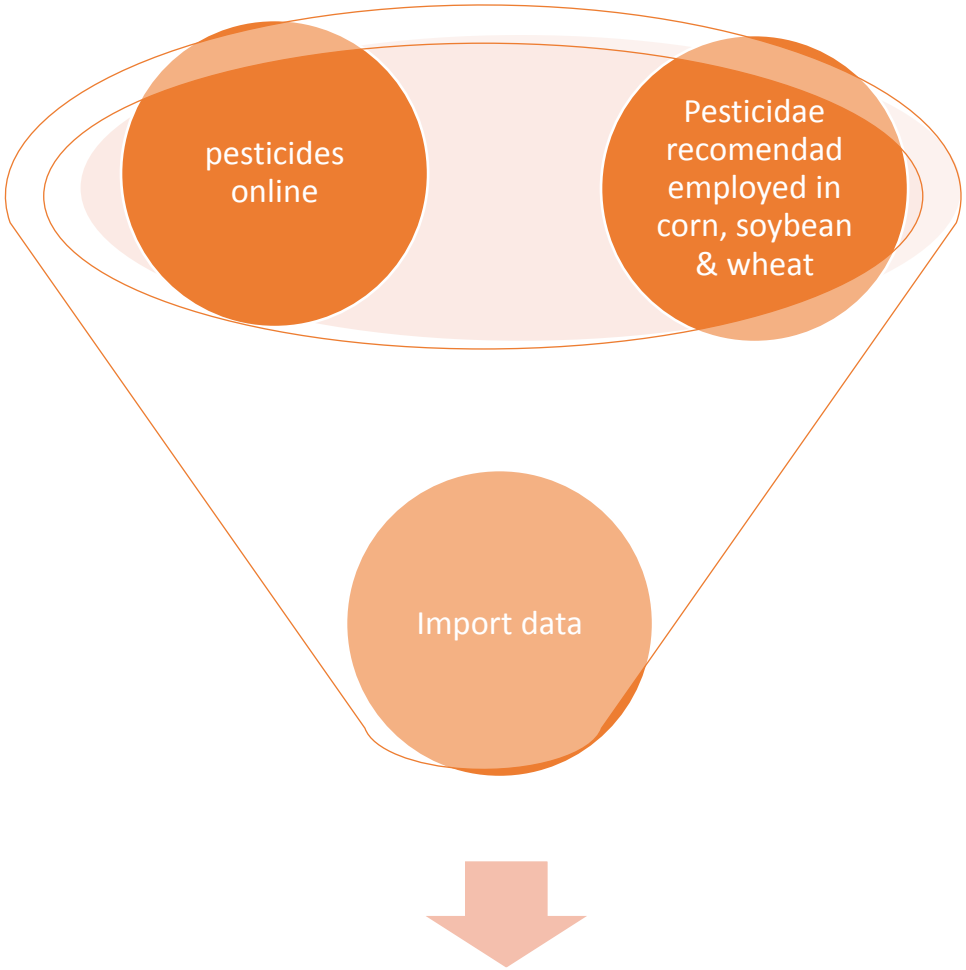
Strategy


Pesticide selection
methodologies development
Field trials sampling
Statistical analysis modelling

Distribution of the use of agrochemicals in Uruguay

Source: Ministry of Agriculture

Pesticides Selection



Pesticide Selection

LC-MS/MS

Carboxin
Clodinafop-propargil
Epoxiconazol
Flutriafol
Linuron
Metalxil
Metolaclor
Metoxifenozide
Metribuzin
Miclobutanil
Ometoato
Picloram
Profenofos
Tebufenozide
Teflubenzuron
Tetraconazole
Tiodicarb
Triflumuron

Pesticide	first Transsision m/z>m/z	DP	CE	Second Transsision m/z>m/z	DP	CE
Acetamiprid	223,2>126,1	55	25	223,2>99,2	55	47
Amitraz	294>163,1	100	17	294,3>122,1	100	37
Azoxistrobin	404,1>372,1	72	19	404,1>344	72	31
Boscalid	343,1>139,8	89	24	343,1>112,2	89	57
Carbaril	202,2>145	68	12	202,2>127,1	68	35
Carbendazim	192,1>160	80	23	192,1>132,1	80	42
Clorpirifos-etil	349,9>198,1	80	23	349,9>97	80	38
Clotianidin	250>169	56	17	250>132	56	19
Cumafos	363,1>227,1	100	33	363,1>307	100	22
Dimetoato	230,1>198,9	50	13	230,1>125,1	50	28
Haloxifop metil	376,1>316,1	85	22	376,1>288,1	85	36
Hexitiazox	353,1>228,1	70	23	353,1>168,1	70	34
Imazalil	297>159	130	32	298,9>161,1	130	30
Imazapir	262>217,2	137	28	262>220,1	137	24
Imidacloprid	256,1>209,1	86	22	256,1>175,1	86	23
Iprodione	330,1>245,1	64	21	330,1>288	64	16
Metomilo	163,1>88	44	12	163,1>105,9	44	14
Metsulfuron metil	382,1>167,1	62	21	382,1>140,9	62	29
Paration-metil	265>129,1	38	11	265>229,2	38	13
Piraclostrobin	388,1>194,2	67	17	388,1>163,1	67	39
Tebuconazol	308,1>70,3	85	40	308,1>125	85	45
Tiacloprid	253,1>126	98	28	255>128	98	25
Tiametoxam	292>211,1	88	15	292>181,2	88	43

Pesticides Selection

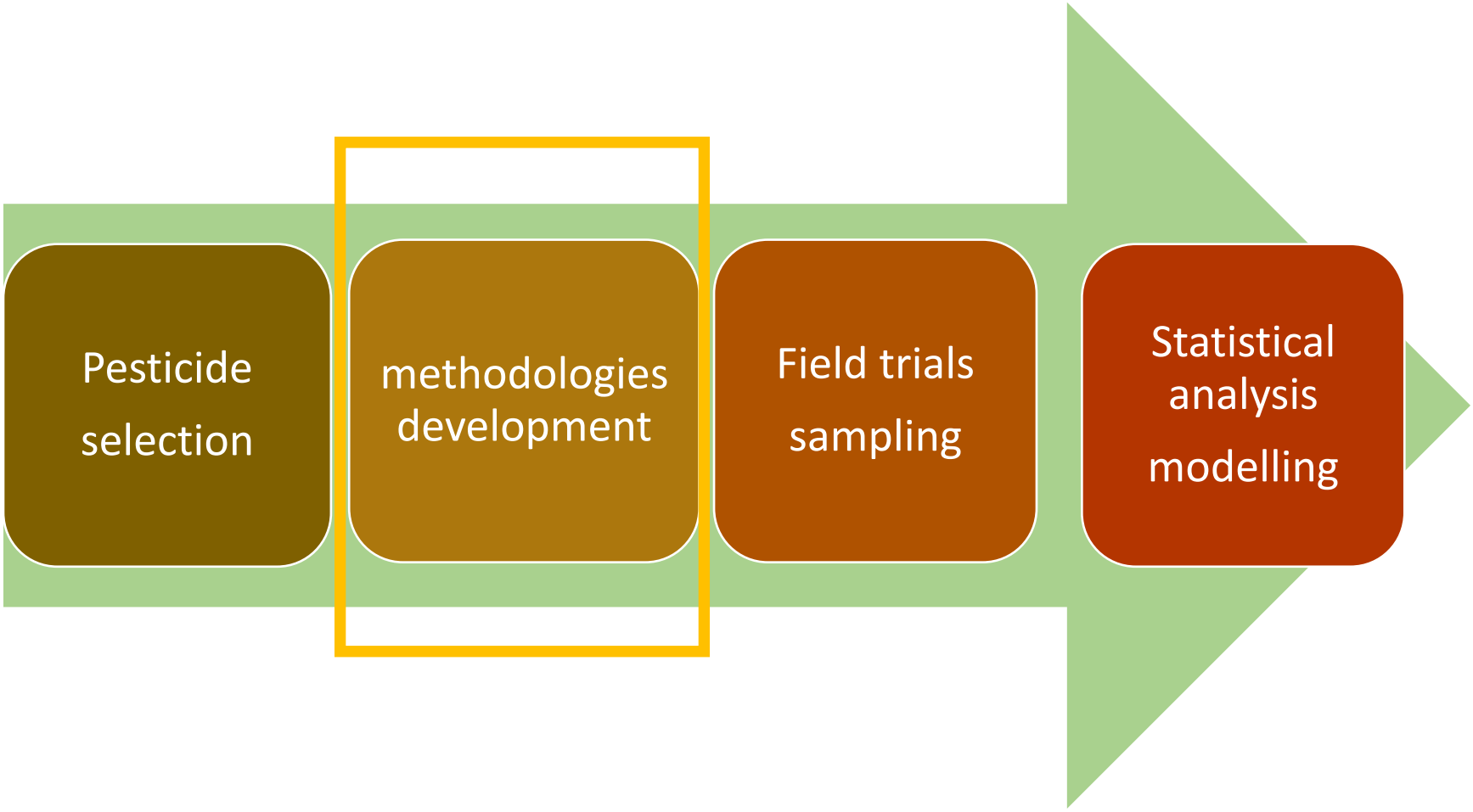
GCxGC-TOF

Bromopropilato
Clorfenvinfos
p,p-DDE
p,p-DDT
Diazinon
Endosulfan alfa
Endosulfan beta
Endosulfan sulfato
Lindano
Forato
Piperonilbutóxido*
Propargite*
Trifluralin
Tau-Fluvalinato*

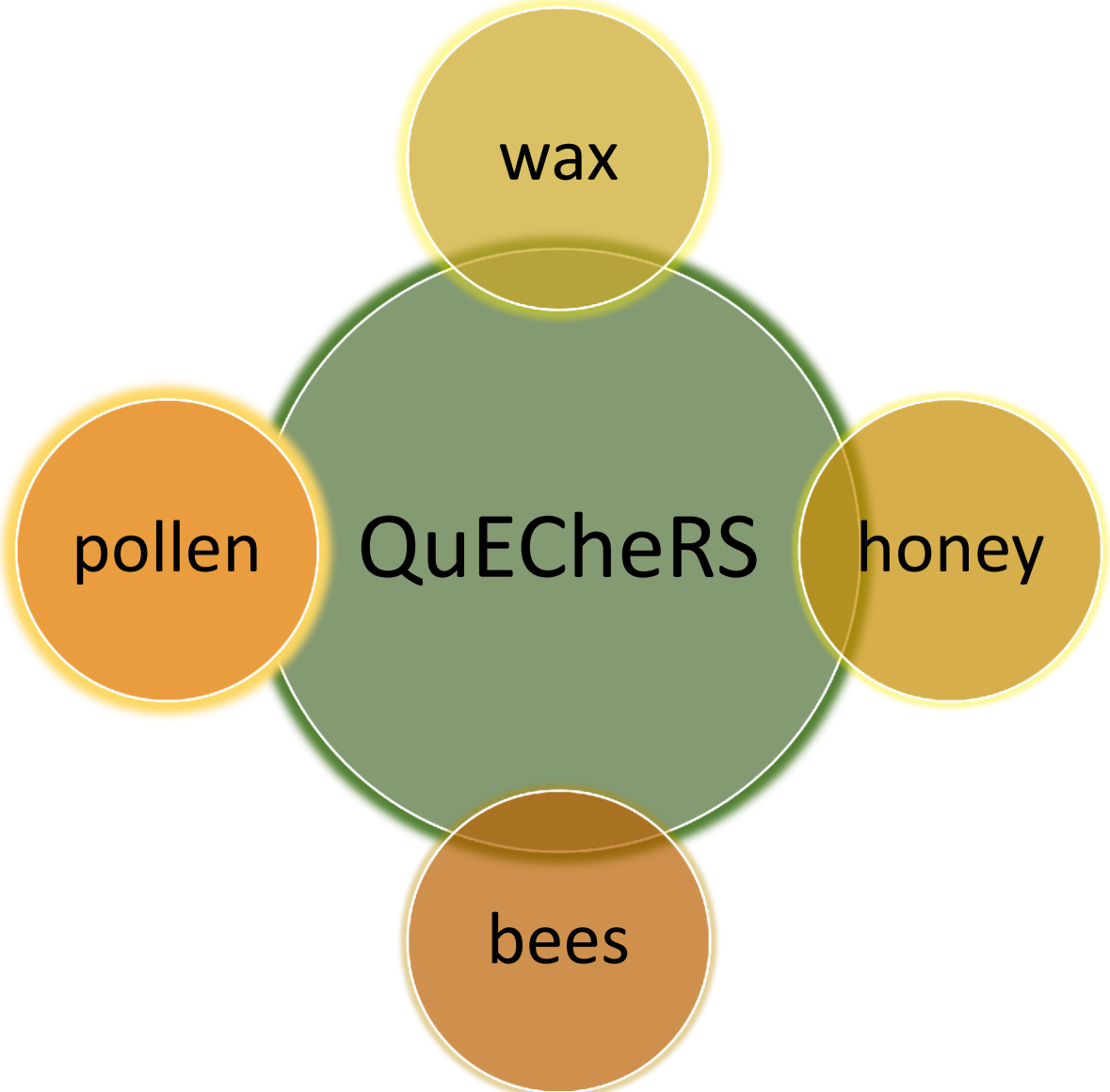
GC-MS

Pesticida	Iones seleccionados (m/z)	Ion de confirmación (m/z)	Ion de cuantificación (m/z)
Atrazina	200, 215,217	215	200
Azinfos-metil	160, 132,125	125	160
Bromopropilato	341, 343, 339, 183	343	183
Cipermetrina	163, 181, 165, 209	181	163
Clorfenvinfos	267, 269, 323	323	267
Clorotalonil	264, 266, 268, 231	264	266
Clorpirifos-metil	286, 288, 125	125	286
Ciflutrina	163, 165, 206, 226	226	163
Deltametrina	181, 253, 251, 255	251	181
Diazinon	179, 137, 304, 152	304	179
Endosulfan alfa	241, 237, 195, 239	237	195
Endosulfan beta	195, 237, 241, 207	241	195
Endosulfan sulfato	272, 274, 387, 237	387	272
Etion	231, 153, 384	384	231
Fipronil	367, 369, 213	213	367
Lambda Cialotrina	181, 197, 208	208	181
Malation	173, 125, 127, 93	127	173
Metidation	145, 85, 125, 302	302	145
p,p-DDE	246, 248, 318	318	246
p,p-DDT	235, 237, 165	165	235
Tau-Fluvalinato	250, 252, 181, 251	181	250
Trifluralin	306, 290, 335	335	306

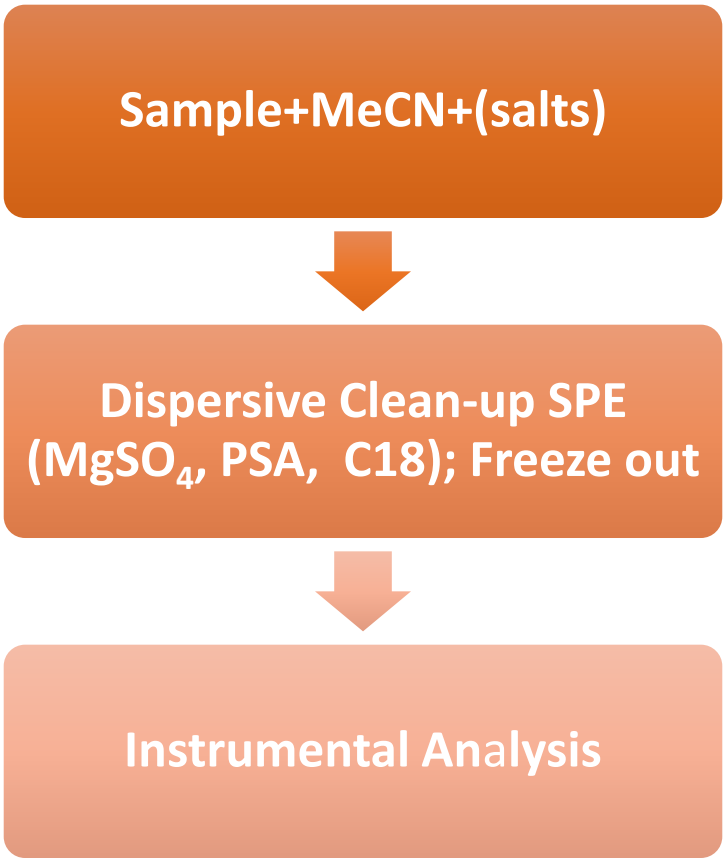
strategy


Pesticide selection
methodologies development
Field trials sampling
Statistical analysis modelling

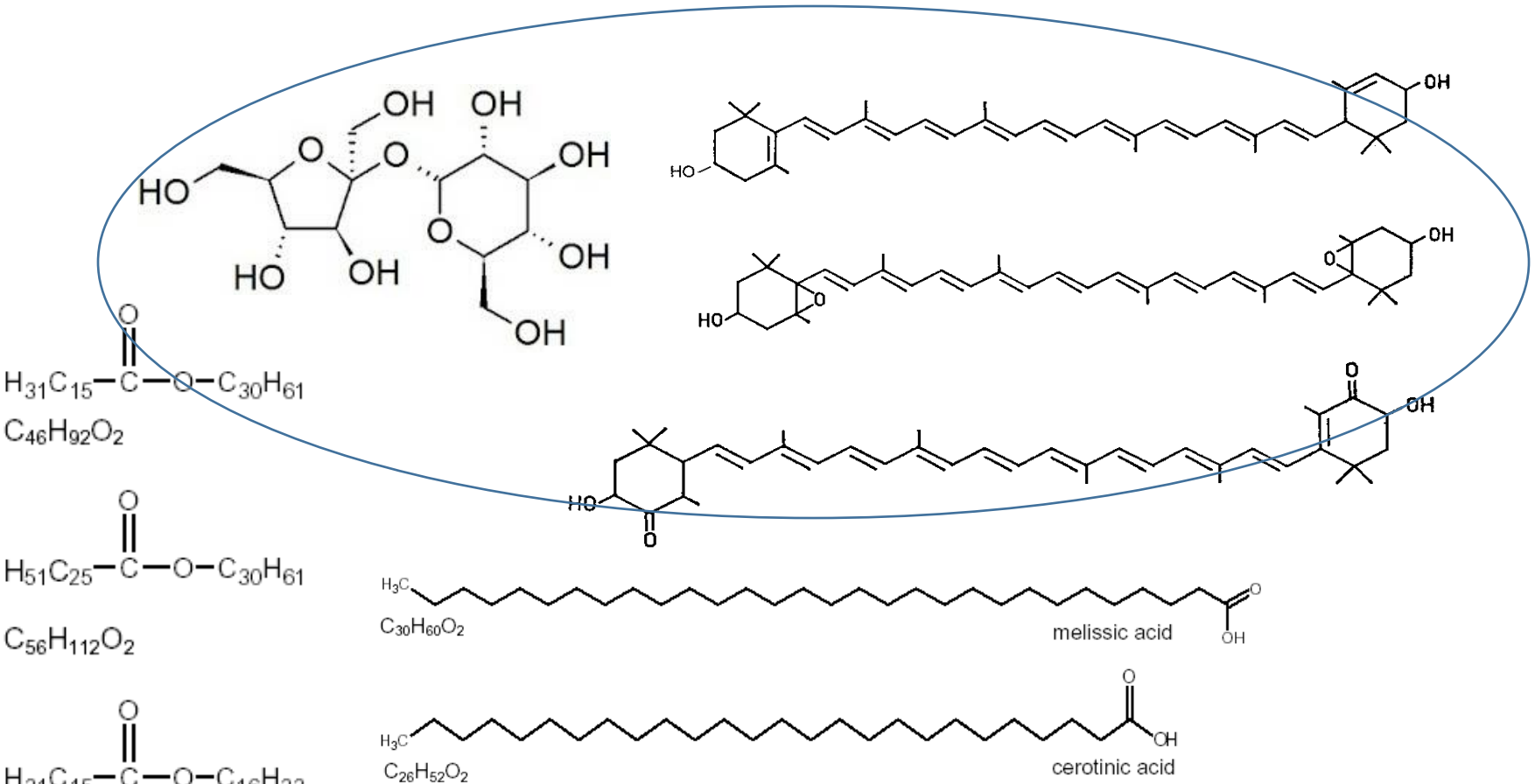
Analytical methodology



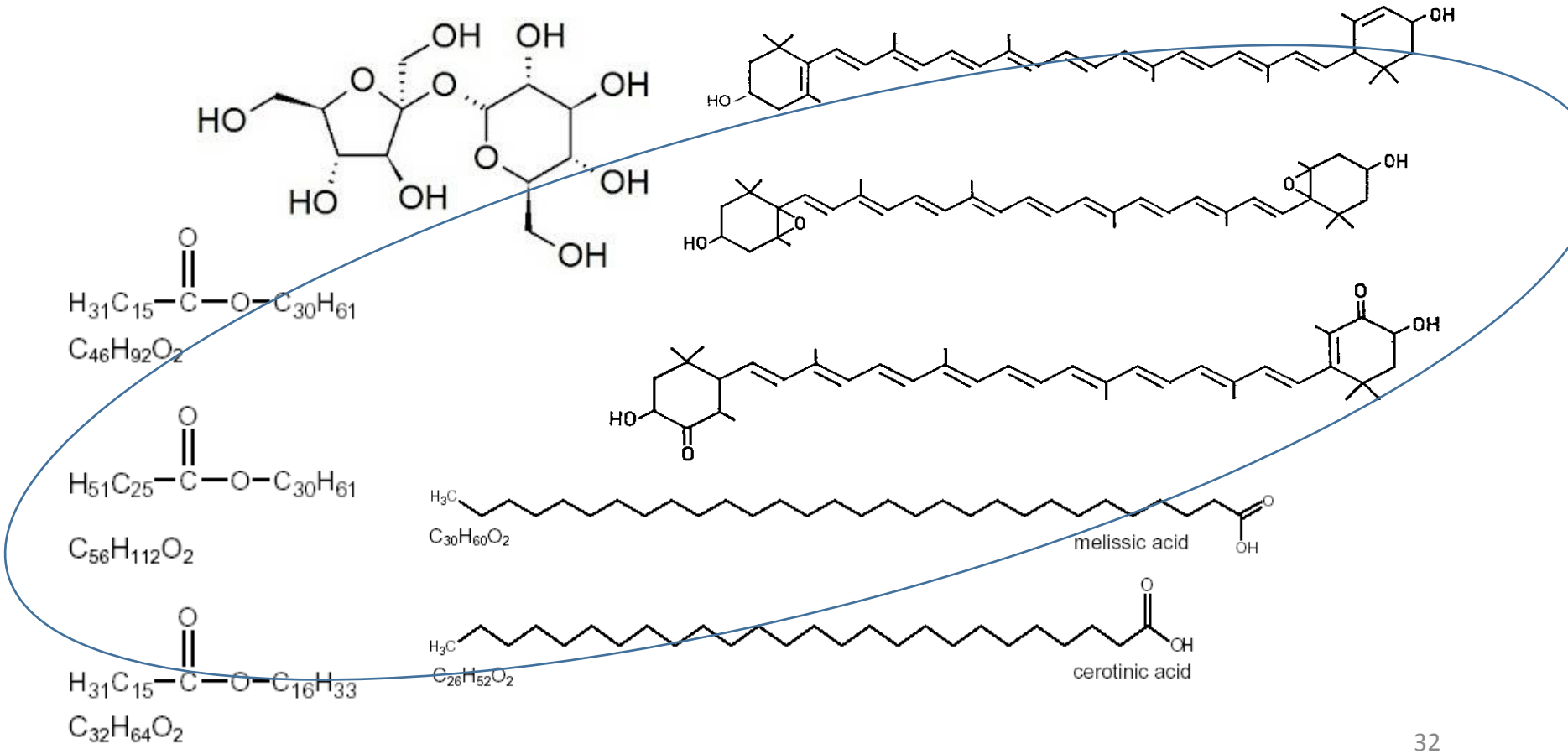
QuEChERS


Sample+MeCN+(salts)
Dispersive Clean-up SPE
($MgSO_4$, PSA, C18); Freeze out
Instrumental Analysis

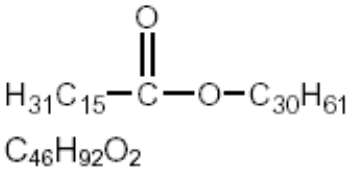
Chemical complexity of the wax and honey matrices



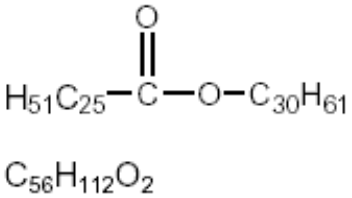
Chemical complexity of wax and honey matrices



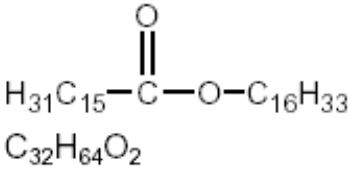
Chemical complexity of bees and pollen matrices



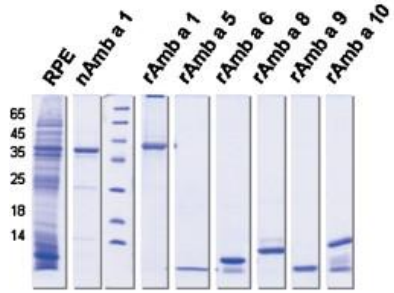
$C_{46}H_{92}O_2$

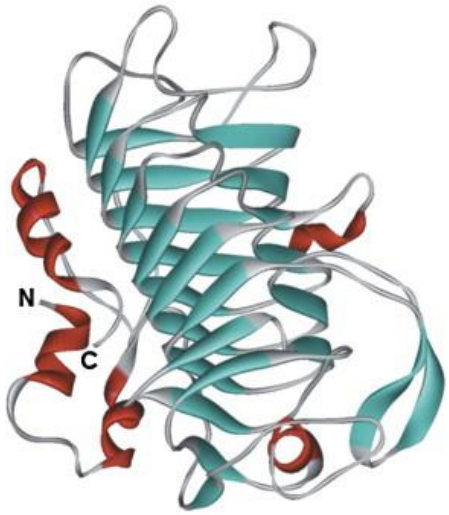


$C_{56}H_{112}O_2$

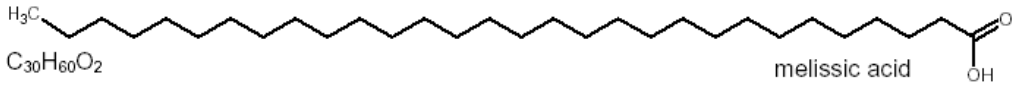


$C_{32}H_{64}O_2$

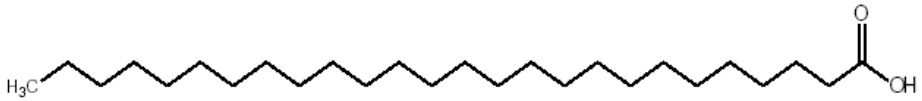




Chapman et al. 2007 The Journal of Allergy and Clinical Immunology 119,(2) 414-420



$C_{30}H_{60}O_2$ melissic acid

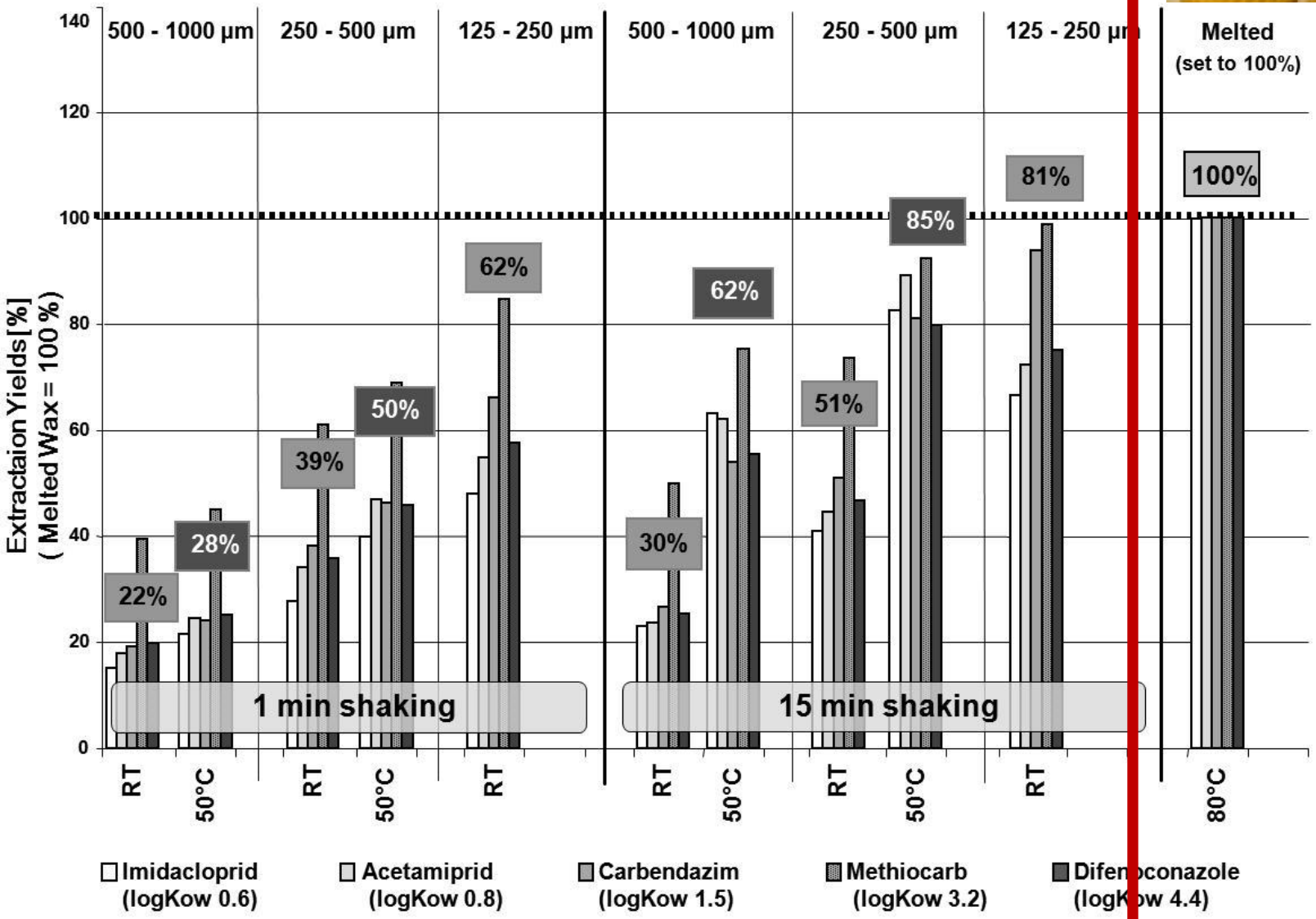


$C_{26}H_{52}O_2$ cerotinic acid

QuEChERS “incurred” residues in wax

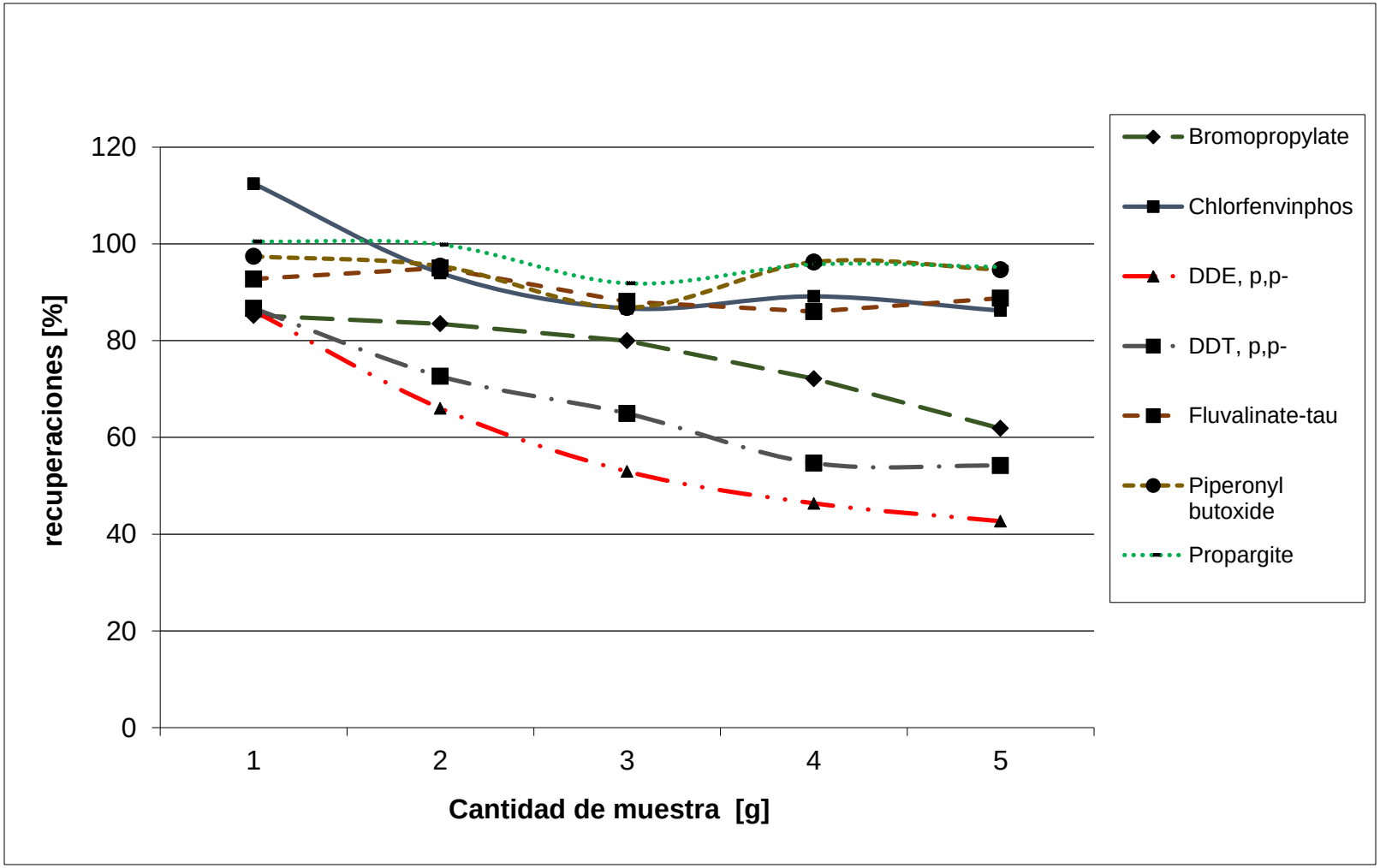
size/ pesticide	temp (~80°C)		small		medium		coarse	
	Conc (mg/kg)	RSD (%)	Conc (mg/kg)	RSD (%)	Conc (mg/kg)	RSD (%)	Conc (mg/kg)	RSD (%)
Tau-Fluvalinate	1,9	6	0,4	10	0,07	22	nd	-
Piperonil butóxide	1,9	10	0,5	12	0,2	3	0,1	4
Propargite	1,2	5	0,3	15	0,09	3	0,07	10
Coumaphos	0,07	4	0,03	6	0,02	3	0,02	5

How to extract the beeswax?



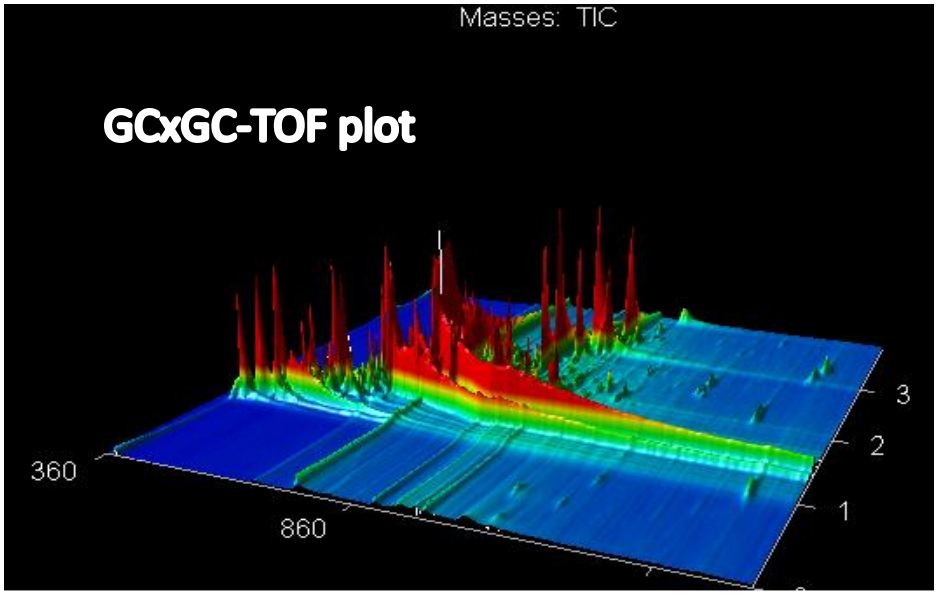
J. Agric. Food Chem., **2014**, *62* (17), pp 3675–3683

Optimization of sample amount

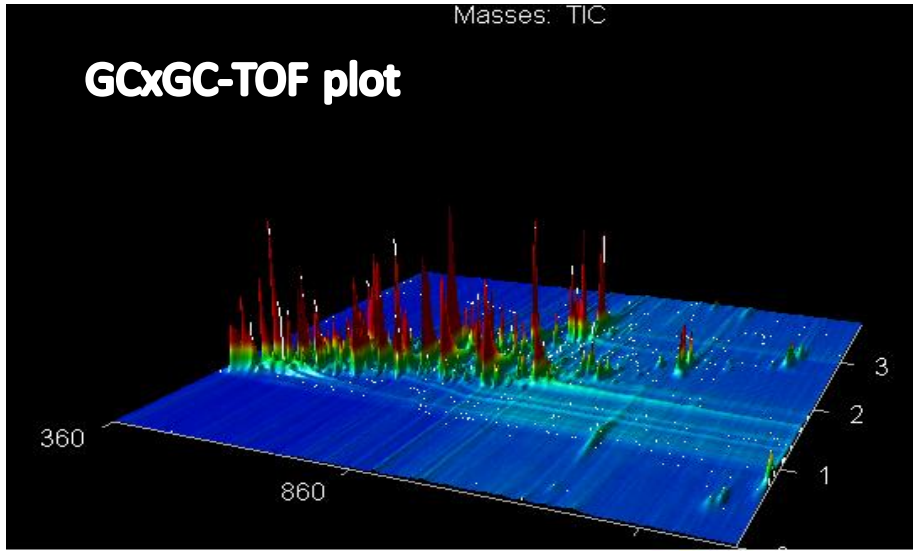


Wax Coextractives by GCxGC ToF

without clean-up



PSA clean up



Further clean up needed

	Residuo seco por mL extracto [mg]	Respecto extracto sin clean-up (=100%)	Respecto cantidad muestra inicial (200mg/mL=1000‰)
Sin cleanup	3,12	100%	15,6
freeze out 1h	2,80	90	14,0
freeze out 2 h	2,72	87	13,6
freeze out 4 h	2,60	83	13,0
+3 % H_2O freeze out 1h	2,75	88	13,7
+3 % H_2O freeze out 2h	2,78	89	13,9
+6 % H_2O freeze out 2h	2,60	83	13,0
+10 % H_2O freeze out 2h	2,40	77	12,0
PSA (25 mg/mL)	1,16	37	4,3
PSA (50 mg/mL)	1,15	37	4,1
+ 3% H_2O PSA (25 mg/mL)	1,00	32	5,0
+ 3% H_2O PSA (50 mg/mL)	0,85	27	4,2
+ 3% H_2O PSA (75 mg/mL)	0,77	25	3,9
+ 6% H_2O PSA (25 mg/mL)	0,97	31	4,9
C18 (25 mg/mL)	2,65	85	13,3
C18 (50 mg/mL)	2,55	82	12,7
+ 3% H_2O C18 (50 mg/mL)	2,40	77	12,0
+ 6% H_2O C18 (25 mg/mL)	2,32	74	11,6
+ 10% H_2O C18 (25 mg/mL)	2,17	70	10,9
+ 3% H_2O freeze out 2h/PSA (25 mg/mL)	0,80	26	4,0
PSA (25mg/mL)/C18 (25mg/mL)	0,85	27	4,3
Freeze out/ PSA (25mg/mL)	0,77	25	3,8
Freeze-out/PSA/C18 (25mg/mL)	0,7	23	3,6

2g Wax+MeCN
~80°C

Dispersive Clean-up ($MgSO_4$, PSA, C18); Freeze out

Instrumental Analysis

Nieli et al. Anal Bioanal Chem en elaboración

Final methodology for beeswax

2g Wax + 10 mL Acetonitrile

↓

Extract at ~80°C (use water bath)

↓

Freeze-out at ~-18°C (minimum 2 h) and decant extract to a separate vessel

↓

Mix aliquot w. PSA/C18 (25 mg/mL), centrifuge

↓

Take aliquot, add ISTD and acidify with 5% HCOOH in MeCN (10 µL/mL)

↓

Fill into autosampler vial and analyze by GC or LC techniques

Validation parameters

- Lineality
- % Recovery
- Repetibility (RSD_r)
- Intermediate precision (RSD_{wR})
- Reporting Límit
- Matrix effects

Method validation and quality control procedures for pesticide residues analysis in food and feed. SANTE Document

QuEChERS LC-MS/MS

Validation

	0,2 mg/kg		0,1 mg/kg		0,01 mg/kg		RL
	Rec(%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)	(mg/kg)
Amitraz	73	2	77	4	85	13	0,01
Atrazina	89	4	94	4	97	13	0,01
Azoxistrobin	110	3	111	4	112	14	0,01
Boscalid	98	6	103	8	105	24	0,1
Carbarilo	104	4	111	5	165	6	0,1
Carbendazim	72	4	78	6	100	15	0,01
Carboxin	104	4	108	6	107	9	0,01
Clorpirifos-etil	92	6	105	6	218	15	0,1
Clodinafop-propargil	104	1	105	5	104	14	0,01
Cumafos*	144	3	229	4	1679	8	0.1
Cihalotrina-lambda	92	16	100	9	nd		0,1
Dimetoato	102	6	106	6	107	7	0,01
Epoxiconazol	94	4	106	7	nd		0,1
Fenpropatrin	95	7	102	11	159	15	0,1
Flutriafol	101	11	114	15	nd		0,1
Hextiazox	182	4	323	5	2800	3	-
Imidacloprid	108	7	106	8	100	9	0,01

Wax: QuEChERS LC-MS/MS cont.

Validation data

	0,2 mg/kg		0,1 mg/kg		0,01 mg/kg		RL
	Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)	(mg/kg)
Iprodione	108	27	123	24	nd		0,2
Linuron	96	6	99	5	107	15	0,01
Metalaxil	107	3	109	5	113	15	0,01
Metomilo	111	8	127	4	113	12	0,01
Metoxifenozide	107	4	111	5	146	7	0,1
Metolaclor	96	3	103	2	120	17	0,01
Metribuzin	102	7	111	7	nd		0,1
Ometoato	96	11	104	8	111	6	0,01
Pendimetalin	81	19	83	19	nd		0,1
Pirimicarb	108	3	114	4	111	11	0,01
Profenofos	89	6	94	6	92	18	0,01
Piraclostrobin	102	4	106	5	112	14	0,01
Tebuconazol	98	11	99	5	103	27	0,1
Tebufenozide	96	4	99	5	104	15	0,01
Tetraconazol	103	7	104	6	111	23	0,1
Tiacloprid	113	7	120	5	108	18	0,01
Tiametoxam	103	14	103	7	106	15	0,01
Tiodicarb	90	5	85	7	107	12	0,01
Tiofanato-metil	94	5	91	7	97	15	0,01
Triflumuron	91	5	98	10	104	12	0,01

GC-MS QuEChERS + hexane partition

	0,1mg/kg		0,2 mg/kg	
	%rec	%RSD	%rec	%RSD
Trifluralin	96	18	71	18
Atrazina	76	9	71	8
Diazinon	87	11	71	14
Clorpirifos-metil	84	7	73	15
Malation	102	19	93	7
Clorpirifos	90	9	79	5
Clorfenvinfos	86	19	83	9
Fipronil	97	5	105	10
Metidation	77	17	88	8
p,p-DDE	57	9	47	12
Etion	100	6	88	9
Endosulfan sulfato	105	14	88	7
p,p-DDT	74	8	71	11
Bromopropilato	78	14	81	13
Azinfos-metil	111	12	94	10
Cumafos	110	9	100	11
Ciflutrina	108	14	91	2
Cipermetrina	116	5	106	4
Tau-Fluvalinato	119	10	105	8
Deltametrina	90	20	83	13

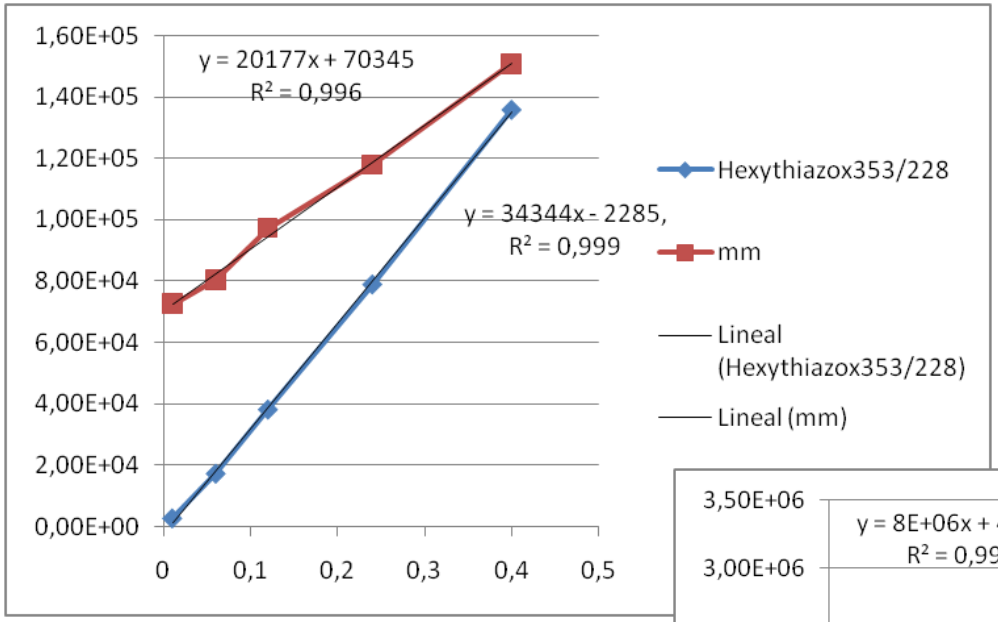
QuEChERS GCxGC-TOF

Validation

	0,2 mg/kg		0,1 mg/kg		RL
	Rec (%)	RSD (%)	Rec (%)	RSD (%)	(mg/kg)
Bromopropilato	83	4	102	9	0,1
Clorfenvinfos	91	10	80	7	0,1
p,p-DDE	61	21	73	4	0,1
p,p-DDT	103	3	108	17	0,1
Diazinon	81	4	94	12	0,1
Endosulfan alfa	120	10	158	18	0,2
Endosulfan beta	88	12	104	40	0,2
Endosulfan sulfato	71	11	84	8	0,1
Lindano	93	5	101	7	0,1
Forato	106	5	112	9	0,1
Piperonilbutóxido*	95	1	102	9	0,1
Propargite*	76	13	284	28	0,2
Trifluralin	79	13	85	16	0,1
Tau-Fluvalinato*	-	-	-	-	-

QuEChERS Wax Matrix Effect

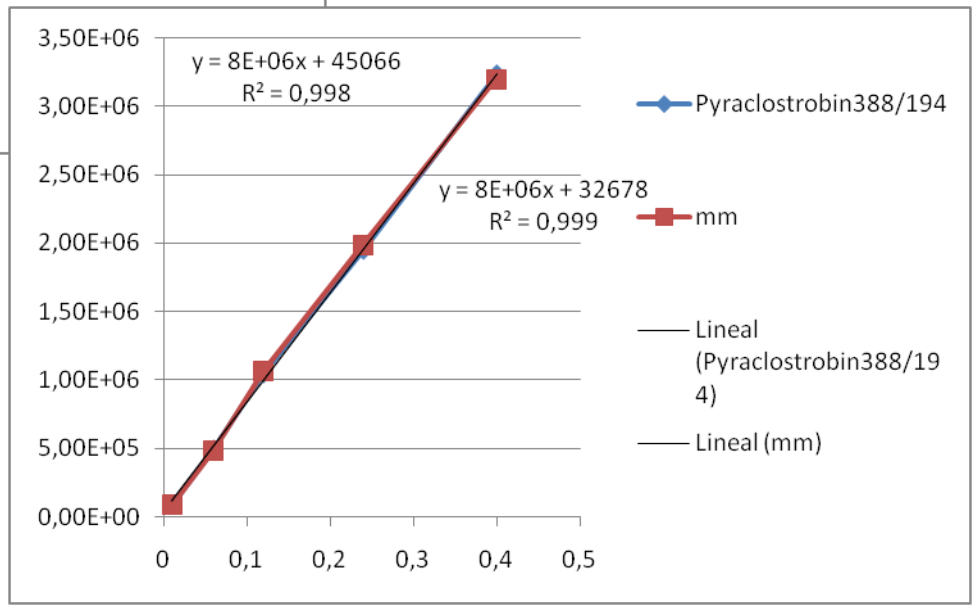
Validation



Lineal range
0,005-0,25 mg/kg LC
0,012-0,24 mg/kg GC

No matrix effects (<13%) except:

- azoxistrobin (24%)
- carbendazim (32%)
- imazapir (-55%)
- cumafos (24%)
- hexitiazox (-41%).



Honey QuEChERS citrate

Sample+H_2O+MeCN+
sales citrato

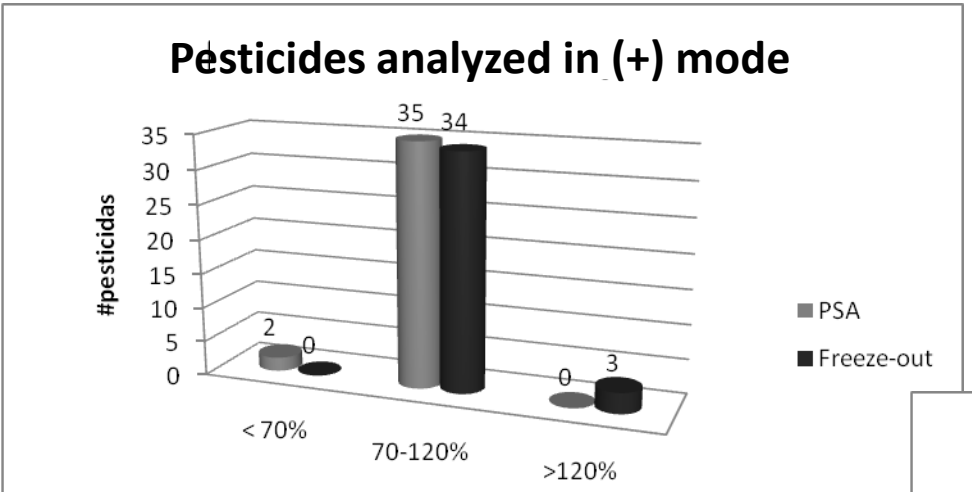
↓

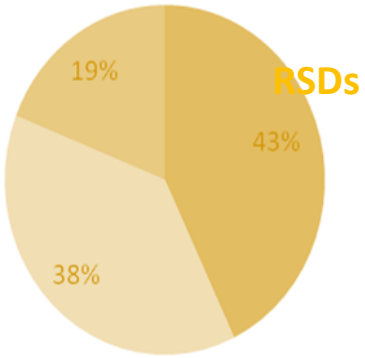
Dispersive Clean-up ($MgSO_4$, PSA);
Freeze out

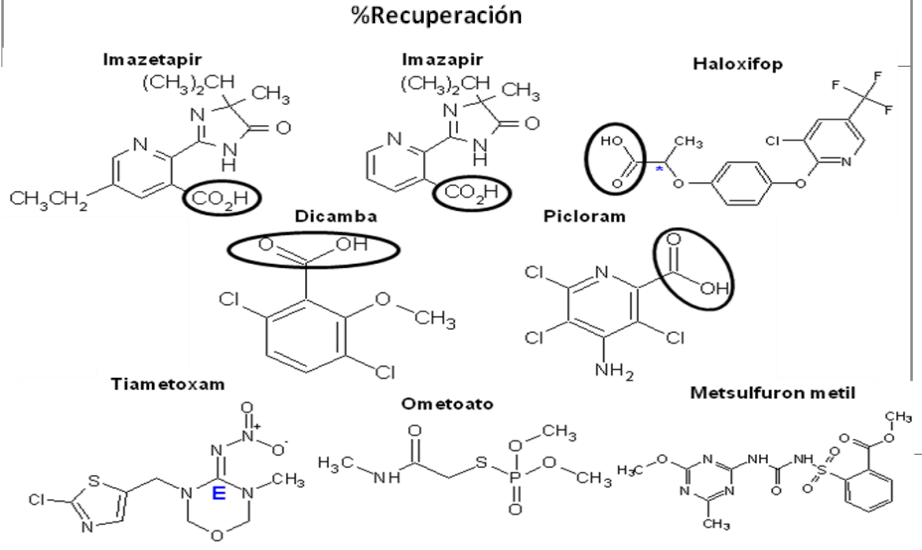
↓

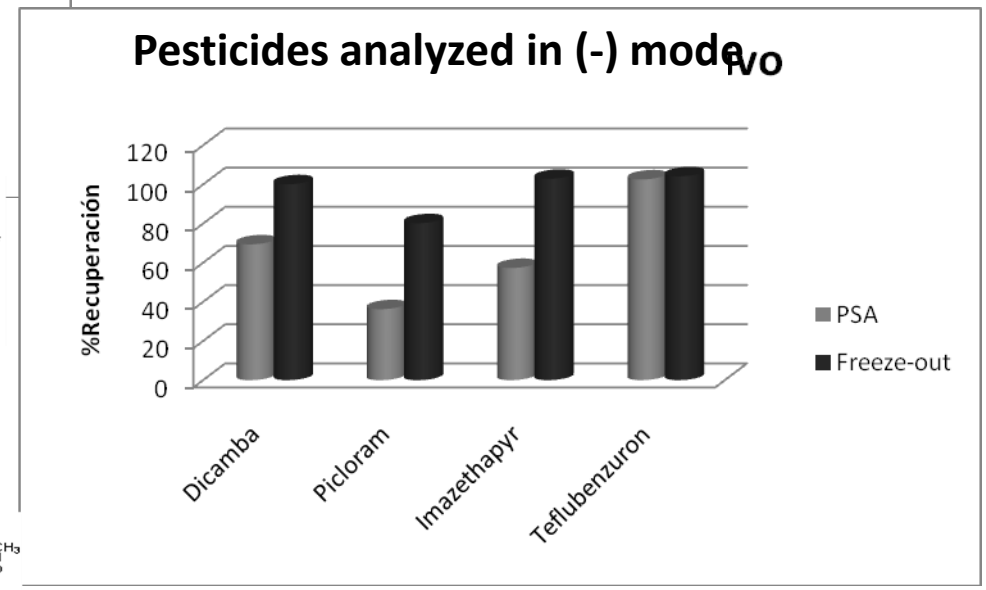
Instrumental Analysis

Honey: QuEChERS citrate



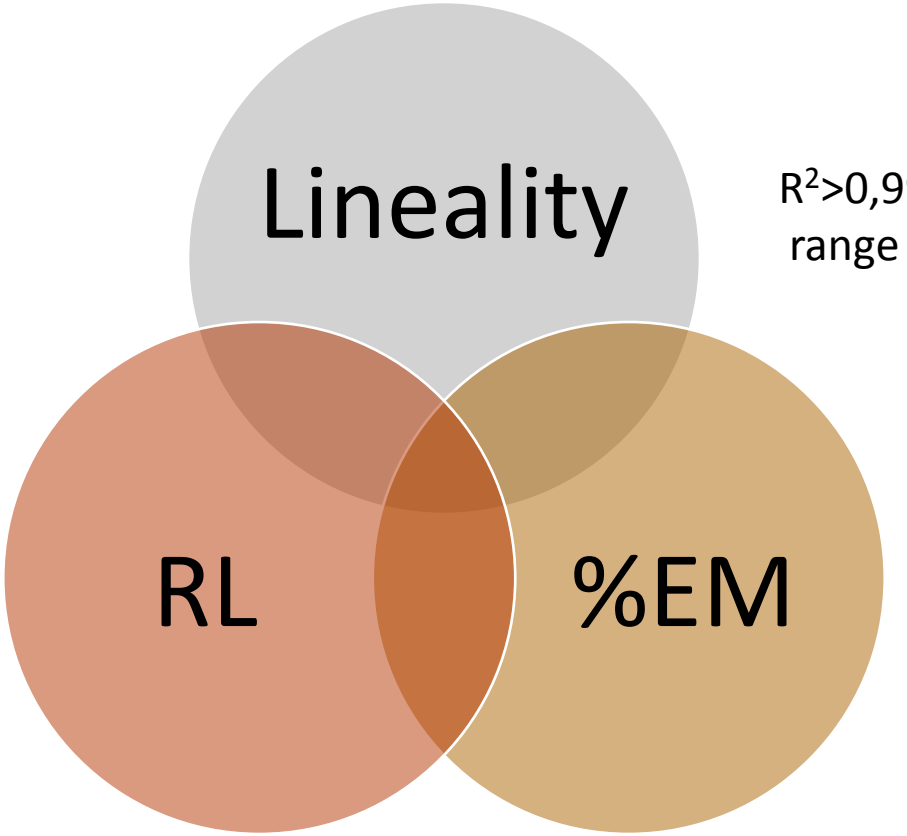






Honey: QuEChERS citrate

Validation



R^2>0,99
range 0,01 a 0,4 mg/L

0,01 mg/kg except:
•boscalid (0,05 mg/kg)
• metsulfuron metil, imazapir y amitraz (0,2 mg/kg)

Low to moderate (<11%) except amitraz (-48%), clorpirifos (17%) imazapir (43%)

Honey: QuEChERS citrate

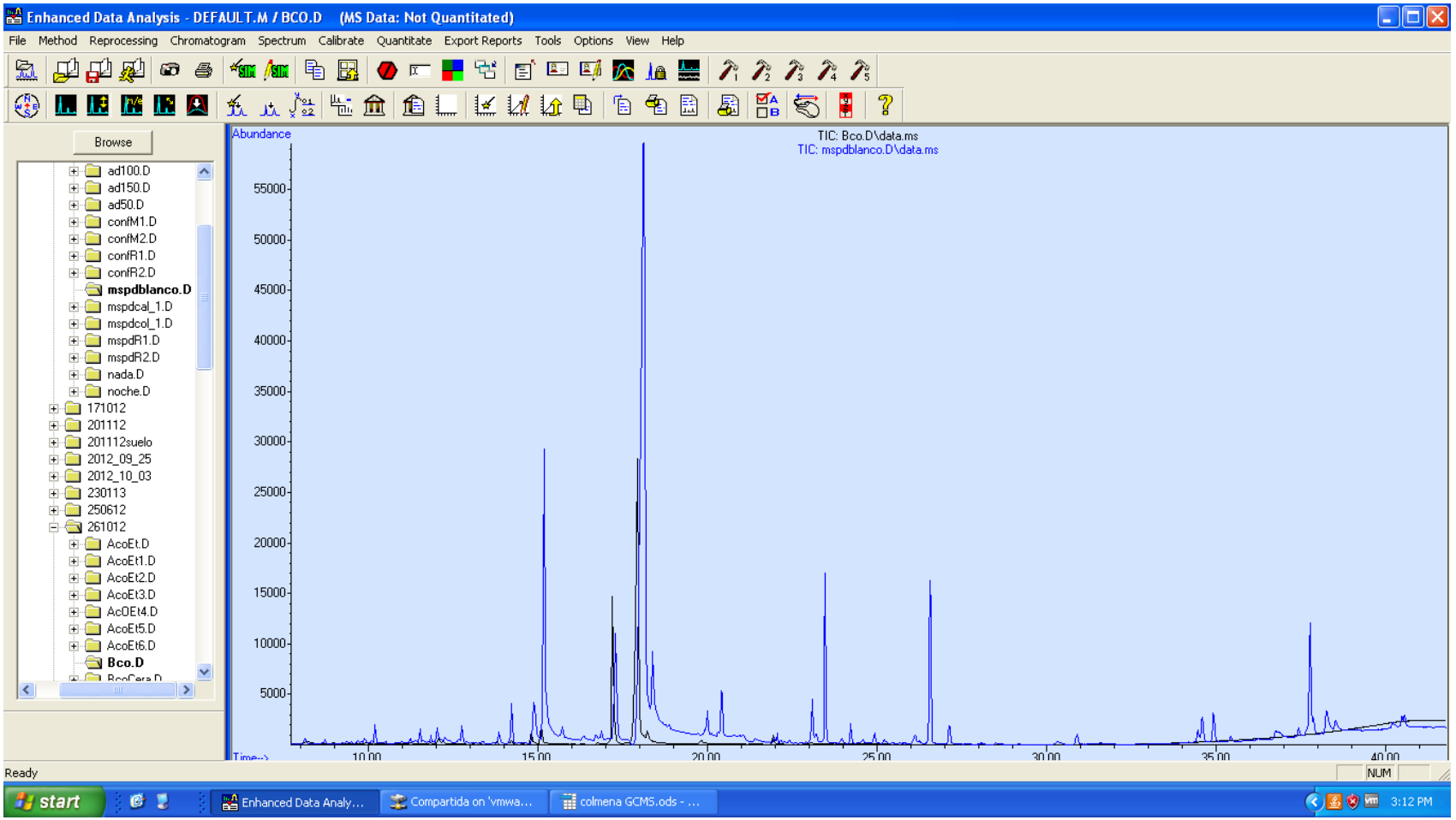
Validation

GC-MS	0,1 mg/kg	
	%rec	RSD
Trifluralin	89	19
Atrazina	87	11
Clorotalonil	78	10
Diazinon	95	11
Clorpirifos-metil	58	5
Malation	73	7
Clorpirifos	61	11
Clorfenvinfos	81	13
Fipronil	82	4
Metidation	93	11
Endosulfan	nd	nd
p,p-DDE	69	12
Etion	81	11
Endosulfan sulfato	81	6
p,p-DDT	76	13
Acetamiprid	75	4
Bromopropilato	78	8
Azinfos-metil	80	11
Cumafos	83	7
Ciflutrina	97	14
Cipermetrina	nd	nd
Tau-Fluvalinato	82	14
Deltametrina	84	13

GC-ECD

	0,1 mg/kg		0,05 mg/kg		0,01 mg/kg	
	% rec	RSD	% rec	RSD	% rec	RSD
Endosulfan a	131	3	109	14	85	11
Endosulfan b	81	4	97	3	54	10
Ciflutrina	77	5	96	3	76	6
Cipermetrina	215	5	301	4	127	3
Tau-Fluvalinato	87	4	74	4	92	5
Deltametrina	79	5	93	6	79	7

Bees: MSPD-QuEChERS coextractives comparasion



Bees: QuEChERS

	%recuperación a 0,5 mg/kg		
	Acetato	Citrato	Ac-Hexano
Acetamiprid	55%	96%	79%
Azinfos metil	103%	99%	67%
Bromopropilato	128%	95%	60%
Clorfenvinfos	83%	150%	100%
Cipermetrina	111%	73%	52%
Clorpyirifos	92%	91%	61%
Clorpirifos metil	93%	105%	65%
Cumafos	113%	97%	73%
Ciflutrina	106%	88%	66%
Diazinon	94%	105%	56%
Deltametrina	99%	85%	62%
Endosulfan sulfato	97%	95%	76%
Etion	120%	93%	65%
Fipronil	99%	87%	67%
Cihalotrina	109%	88%	61%
Malation	86%	94%	68%
DDE	86%	96%	40%
DDT	101%	84%	59%
Trifluralin	98%	129%	57%
Tau-fluvalinato	102%	85%	78%
Metidation	115%	97%	60%

Sample+MeCN+(sales)

↓

Dispersive Clean-up SPE ($MgSO_4$, PSA, C18); Freeze out

↓

Instrumental Analysis

Bees: QuEChERS citrate

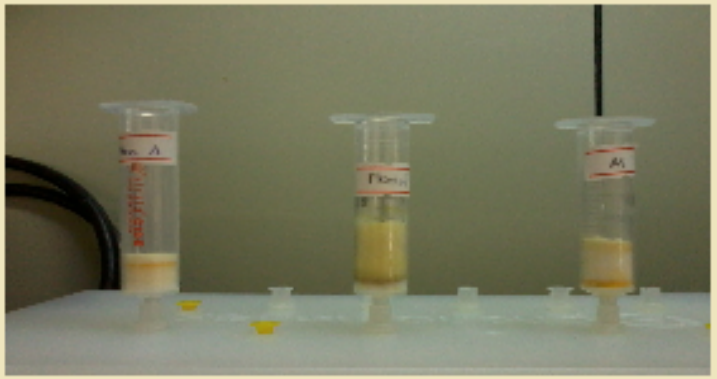
Validation

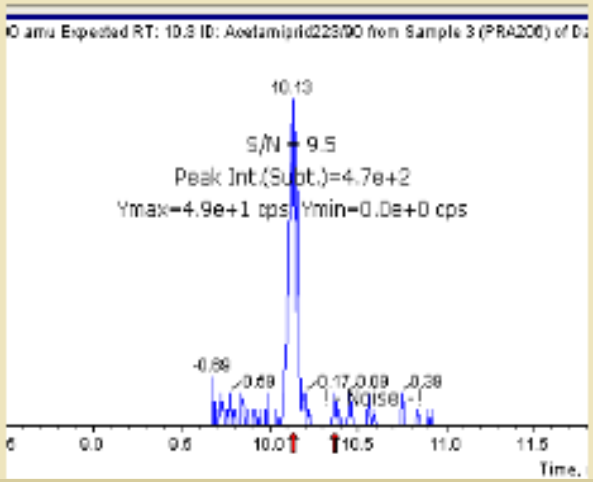
	0,2 mg/kg		0,1 mg/kg		0,05 mg/kg		0,01 mg/kg			
	% rec	RSD	% rec	RSD	% rec	RSD	% rec	RSD	LR µg/abeja	DL50 ug/abeja
Azoxystrobin	104	14	109	4	95	6	102	2	0,001	25
Boscalid	80	5	85	5	92	2	99	2	0,001	100
Carbendazim	73	4	73	6	46	7	69	19	0,01	50
Imidacloprid	94	5	103	4	88	5	105	14	0,001	0,0037
Acetamiprid	93	7	96	4	92	3	835	6	0,005	8
Tebuconazole	92	4	94	6	87	2	96	6	0,001	83
Iprodione	71	2	74	6	84	5	102	10	0,001	25
Metomilo	96	6	96	4	94	3	98	7	0,001	0,16
Dimetoato	95	6	99	4	97	3	97	3	0,001	0,12
Carbarilo	94	5	98	4	92	4	94	4	0,001	0,14
Clorpirifos etil	104	1	111	10	93	10	695	14	0,005	0,024
Clotianidin	92	6	94	4	99	6	118	24	0,005	0,004
Cumafos	98	3	102	8	77	7	350	4	0,005	-
Haloxifop metil	102	6	106	5	94	8	97	7	0,001	-
Hexitiazox	114	10	119	8	90	5	104	12	0,001	112
Imazalil	101	6	111	5	92	6	94	30	0,005	35,1
Piraclostrobin	103	4	107	5	90	11	97	3	0,001	73,1
Tiacloprid	92	5	96	5	97	5	101	7	0,001	17,3
Tiametoxam	97	8	106	7	91	10	106	10	0,001	0,005
Paration-metil	112	20	-	-	132	29	nd	nd	0,02	19,5

lineal range :0.002-0.1 mg/L; %EM <12% except amitraz, carbaryl, hexitiazox, imazapir

Pollen: QuEChERS acetate

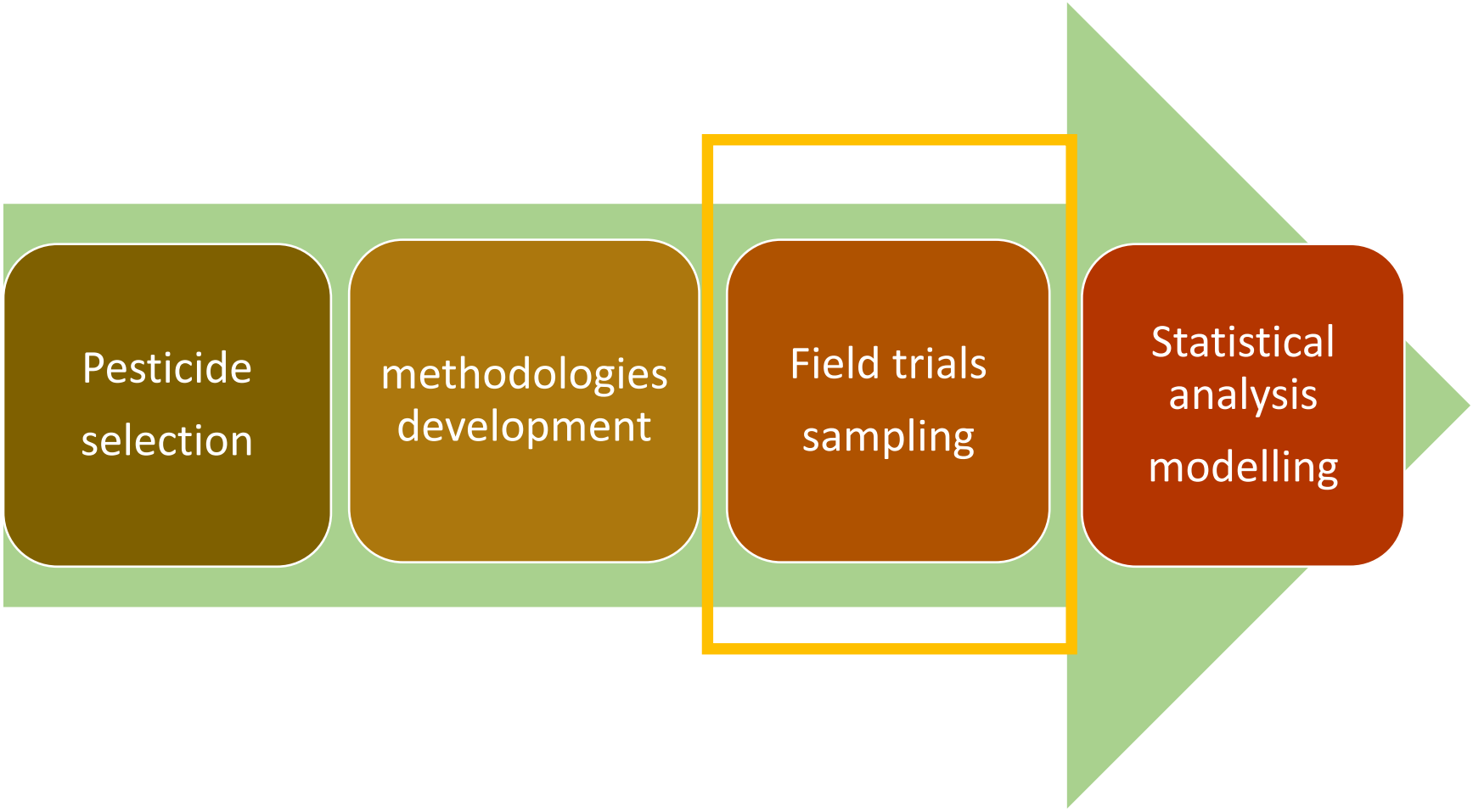
PESTICIDA	0,01 mg/kg		0,05 mg/kg		LOQ (mg/kg)	LOD (mg/kg)
	% Rec	% RSD	% Rec	% RSD		
Azoxistrobin	98	5	84	5	0,01	0,003
Boscalid	82	19	89	7	0,01	0,01
Carbendazim	86	5	80	2	0,01	0,01
Metsulfuron metil	84	3	79	3	0,01	0,003
Iprodione	104	6	92	7	0,01	0,01
Tebuconazole	89	6	86	4	0,01	0,003
Metomilo	83	6	87	3	0,01	0,003
Dimetoato	87	5	84	3	0,01	0,003
Carbarilo	87	7	87	4	0,01	0,003
Haloxifop metil	95	4	87	2	0,01	0,003
Hexitiazox	116	4	98	11	0,01	0,003
Imazalil	72	12	87	5	0,01	0,003
Piraclostrobin	95	1	86	11	0,01	0,003
Tiacloprid	91	3	87	2	0,01	0,003
Cumafos	96	3	94	3	0,01	0,003
Imidacloprid	73	7	84	6	0,01	0,003
Acetamiprid	88	4	86	3	0,01	0,003
Clotianidin	82	6	90	5	0,01	0,003
Tiametoxam	90	7	96	4	0,01	0,01



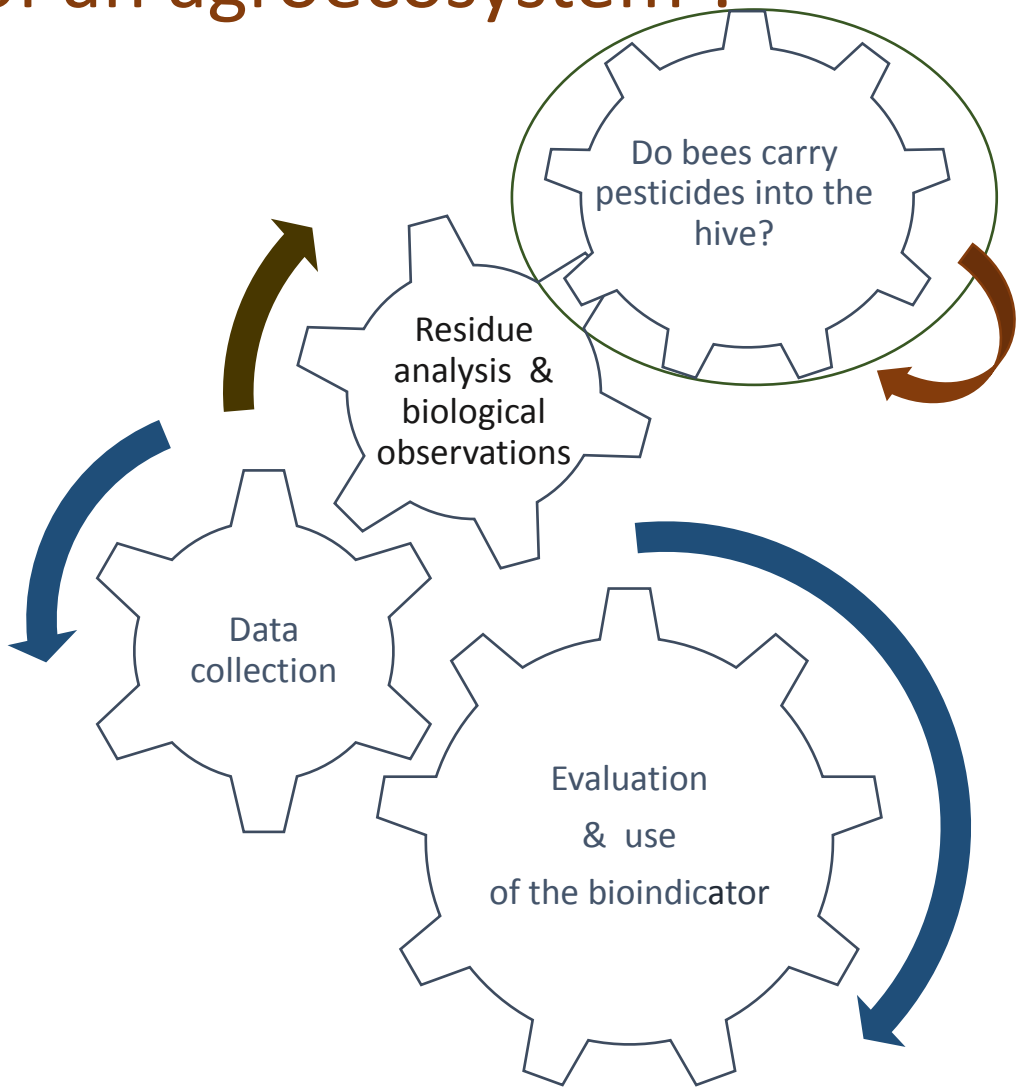


Let´s put them to work!!!!

strategy

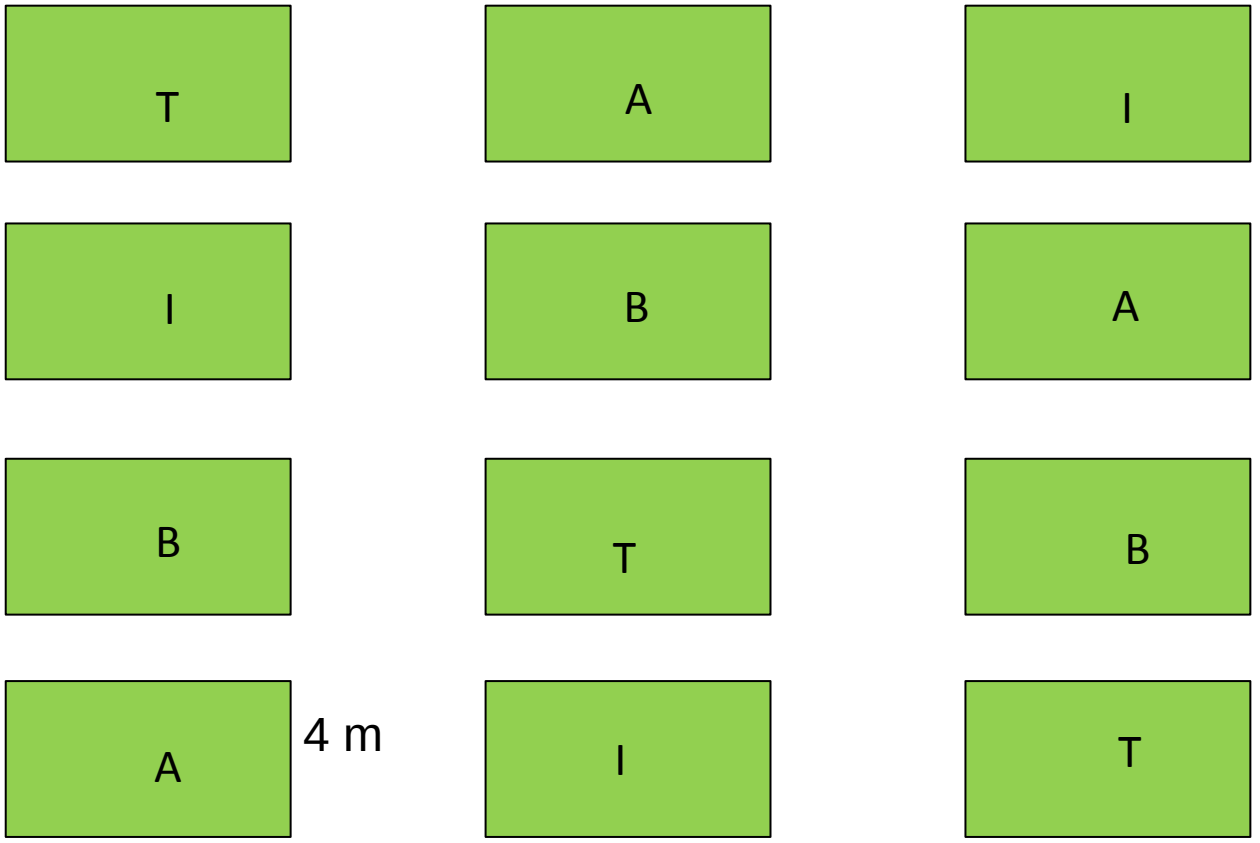

Pesticide selection
methodologies development
Field trials sampling
Statistical analysis modelling

Is the beehive an indicator of the status of an agroecosystem ?



Controlled semi-field studies

Tents over flowering soybean crop:



I=Imidacloprid
A=Acetamprid
T=Thiametoxam
B=Blank

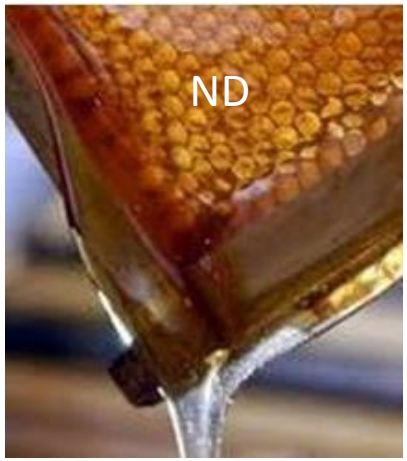
Niell et al Sci Tot Env submitted

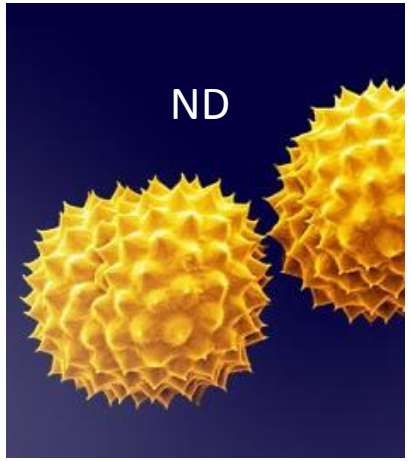
The experiment

24hs after application

Niell et al Sc Tot Env submitted

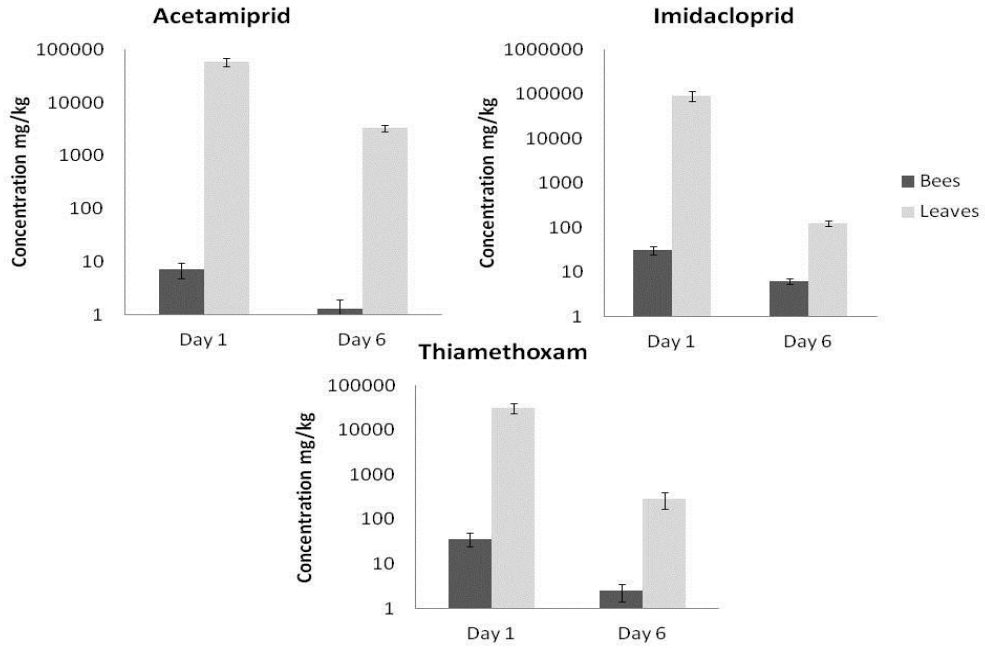
The analysis

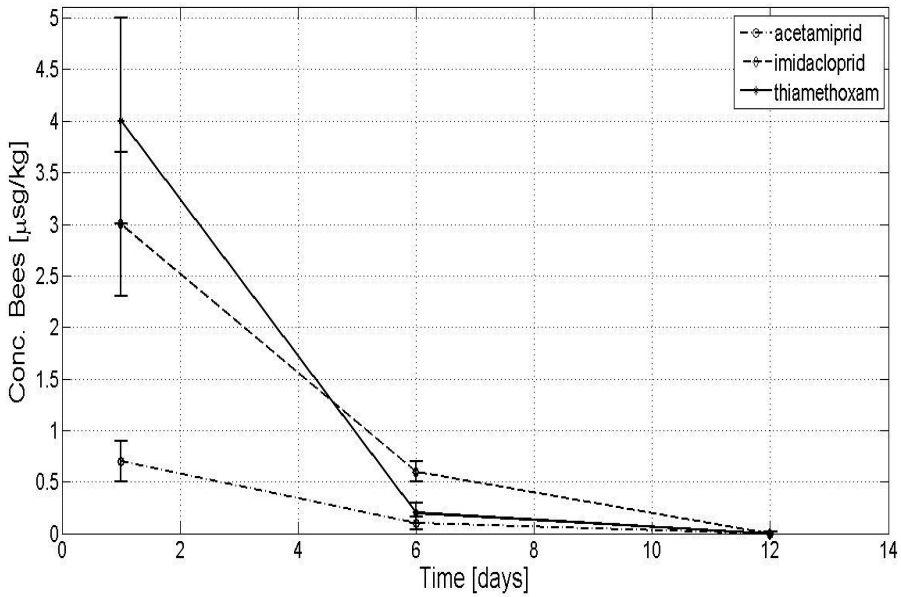




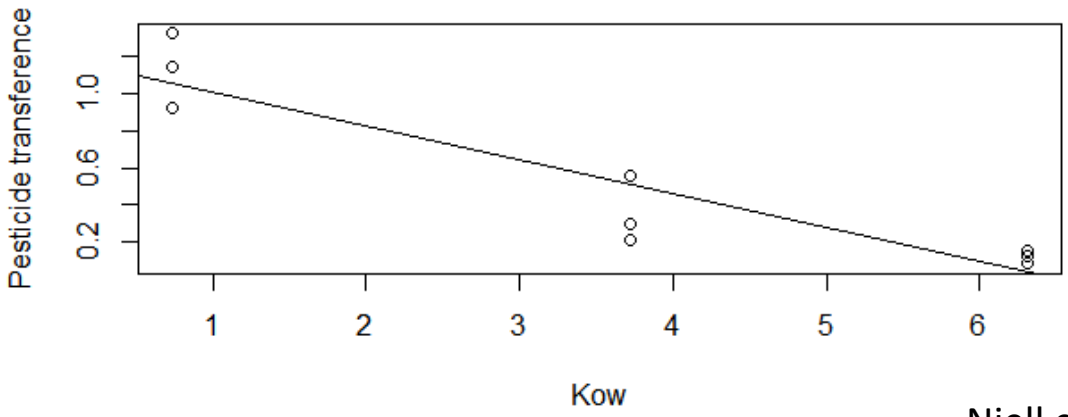
PESTICIDE	thiamethoxam	imidacloprid	acetamiprid
Day 1 (µg/kg)	4	3	0.7
Day 6 (µg/kg)	0.3	1	0.1

Niell et al Sci Tot Env submitted



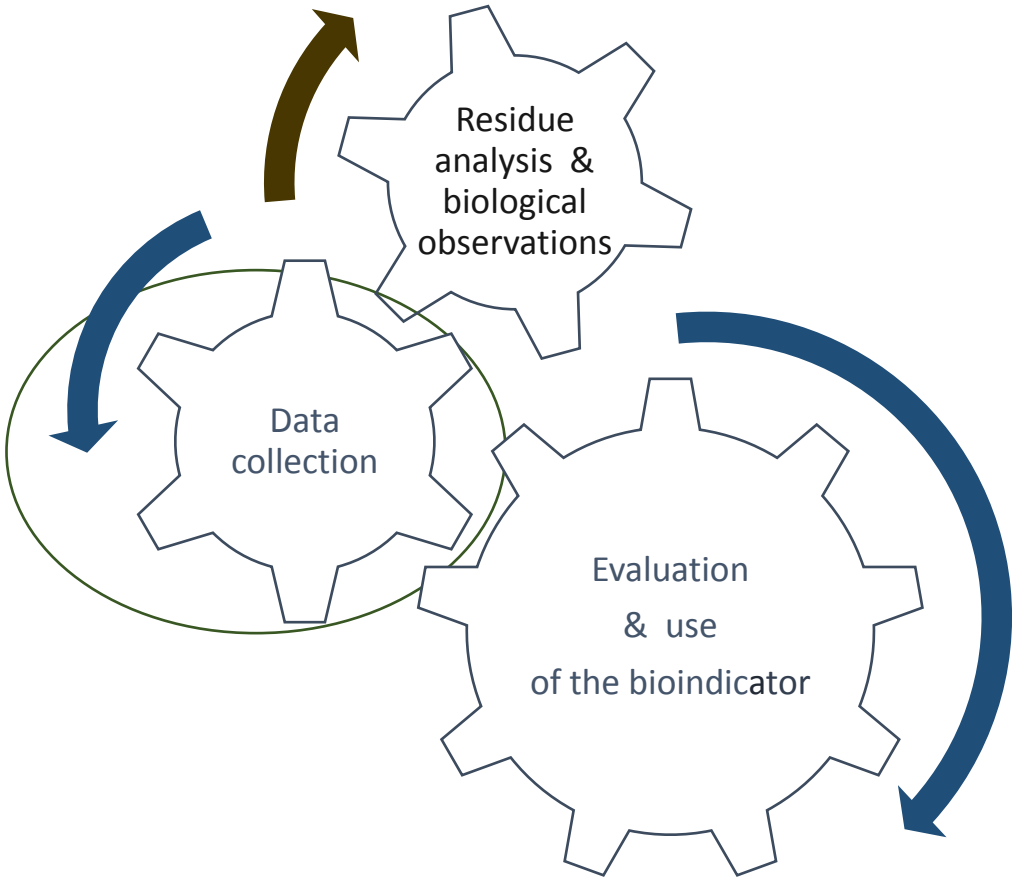


	[leaves]mgkg^{-1}	transf	Vp	MW	Kow	K Henry
Thiamethoxam	3.23	1.13E-03	6.60E-06	291	0.74	4.70E-10
Imidacloprid	9.44	0.00035992	0.0000004	255	3.72	1.70E-10
Acetamiprid	6.01	0.00012087	0,000173	222	6.31	5.30E-08



Niell et al Sc Tot Env submitted

Is the beehive an indicator of the status of an agroecosystem ?

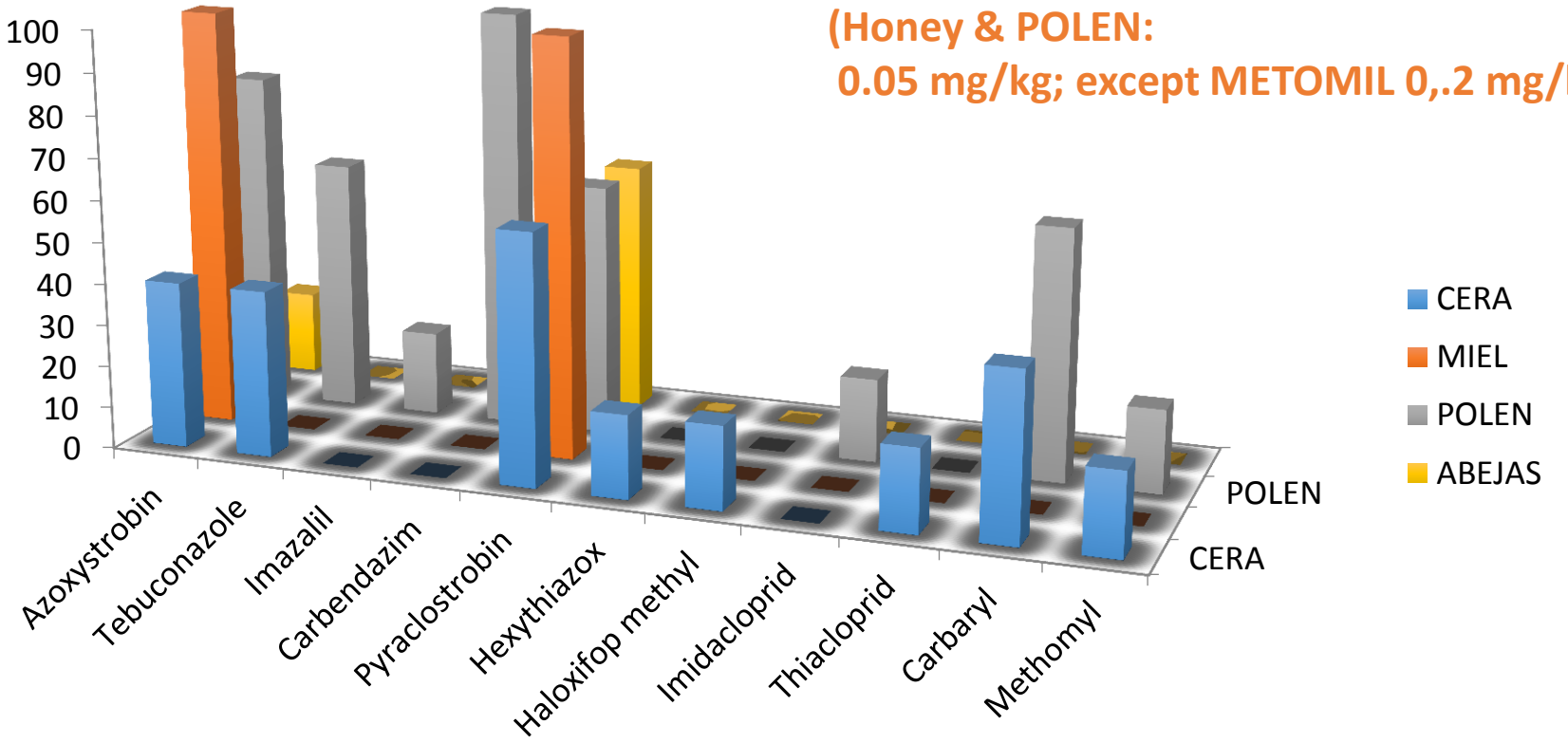


Environments selected for evaluation

POSITIVES FRECUENCY 2012-2013

below MRL

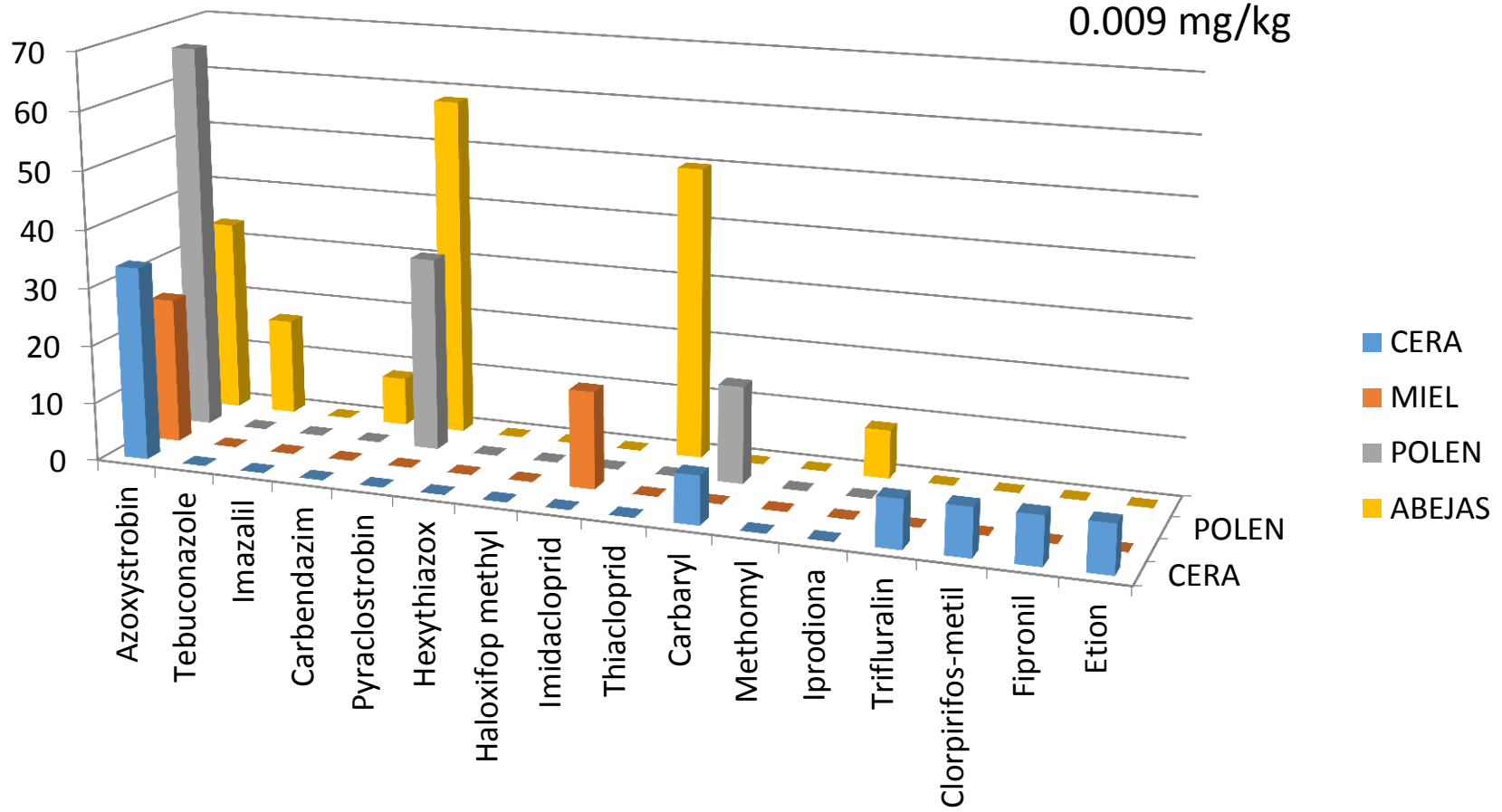
(Honey & POLEN:
0.05 mg/kg; except METOMIL 0,.2 mg/kg)



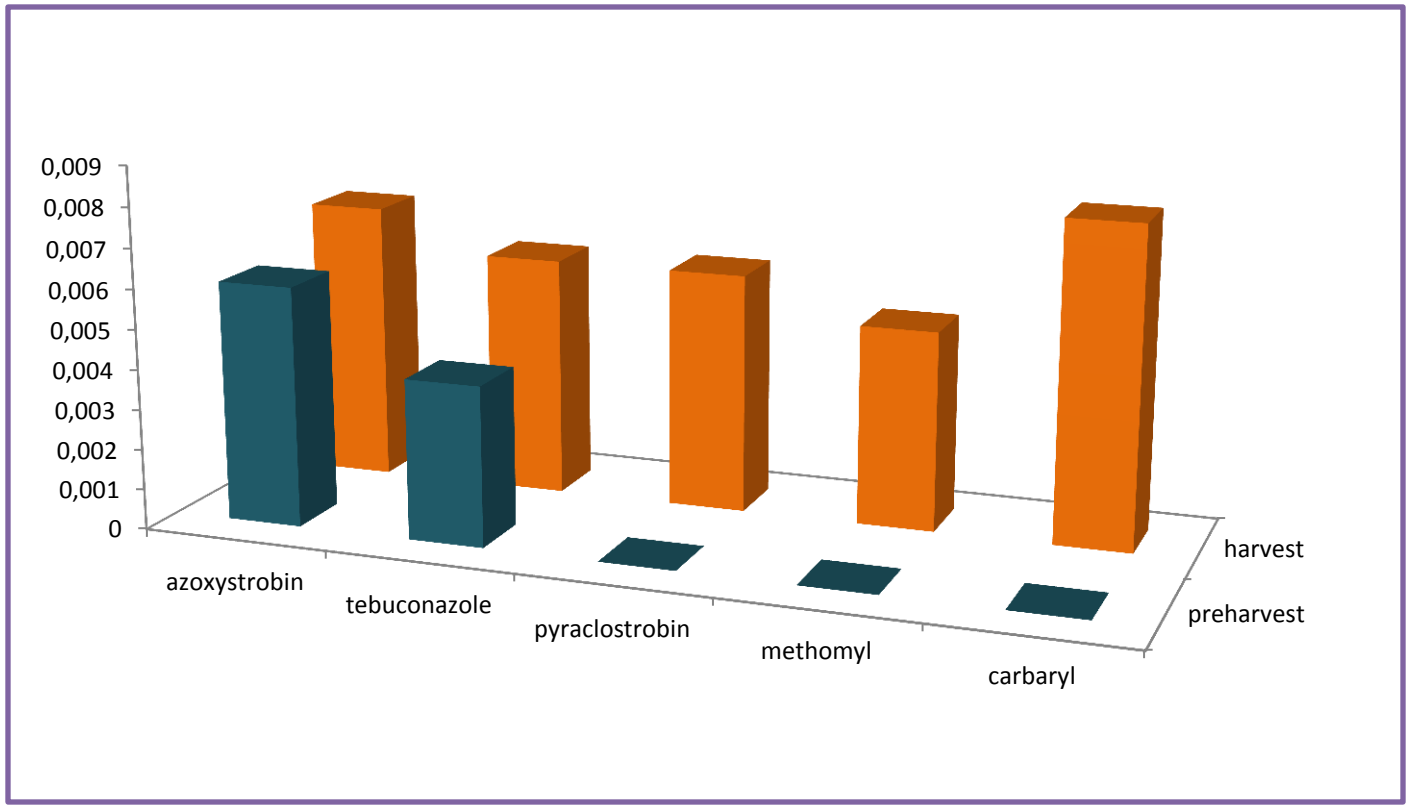
Niell *et al.* (2015) Journal of Agricultural and Food Chemistry (jafc) 63 (18):4484-4492.

POSITIVES FRECUENCY 2014-2015

MAX CONCENTRATION :
0.009 mg/kg

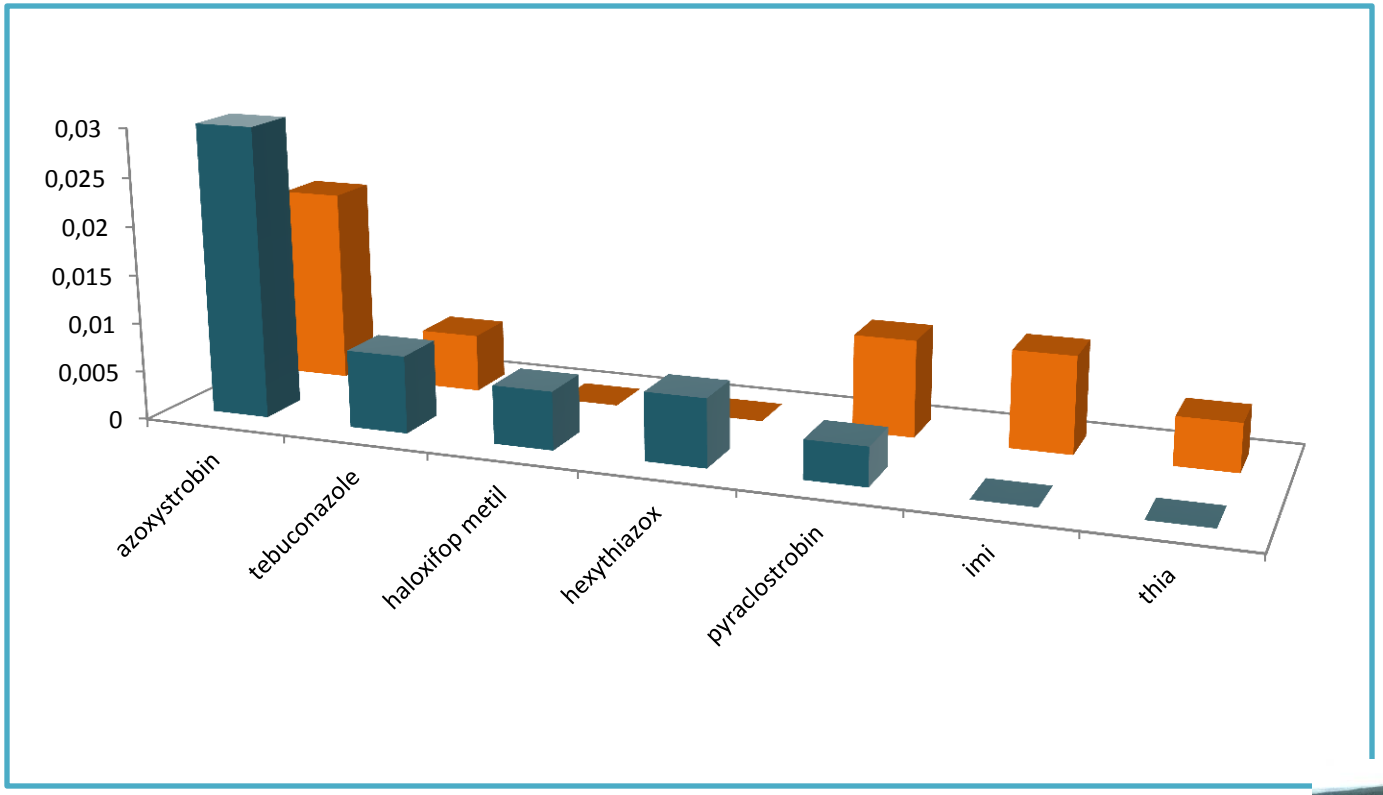


Pesticide findings in wax & bees



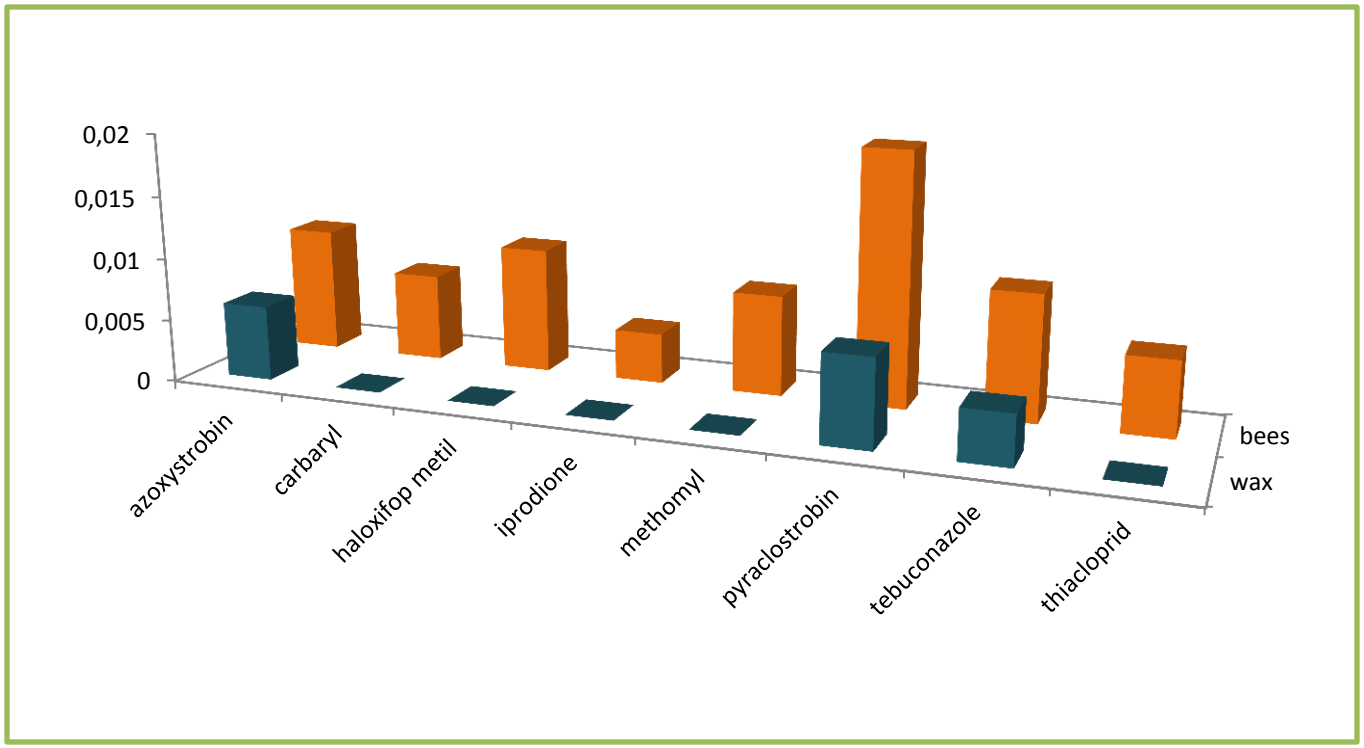
Near soybeans plantations

Pesticide findings in wax & bees



Near soybean plantations

Pesticide findings in wax & bees



Near *Lotus sp.* plantations

Pyraclostrobin
Azoxystrobin
Axoxystrobin
Coumaphos
Tebuconazole
Pyraclostrobin

Azoxystrobin
Pyraclostrobin
Azoxystrobin
Pyraclostrobin
Carbaryl
Methomyl
Haloxifop methyl

Coumaphos
Carbaryl

Azoxystrobin
Pyraclostrobin
Tebuconazole
Azoxystrobin
Coumaphos
Pyraclostrobin

Pyraclostrobin
Coumaphos
Pyraclostrobin
Hexythiazox

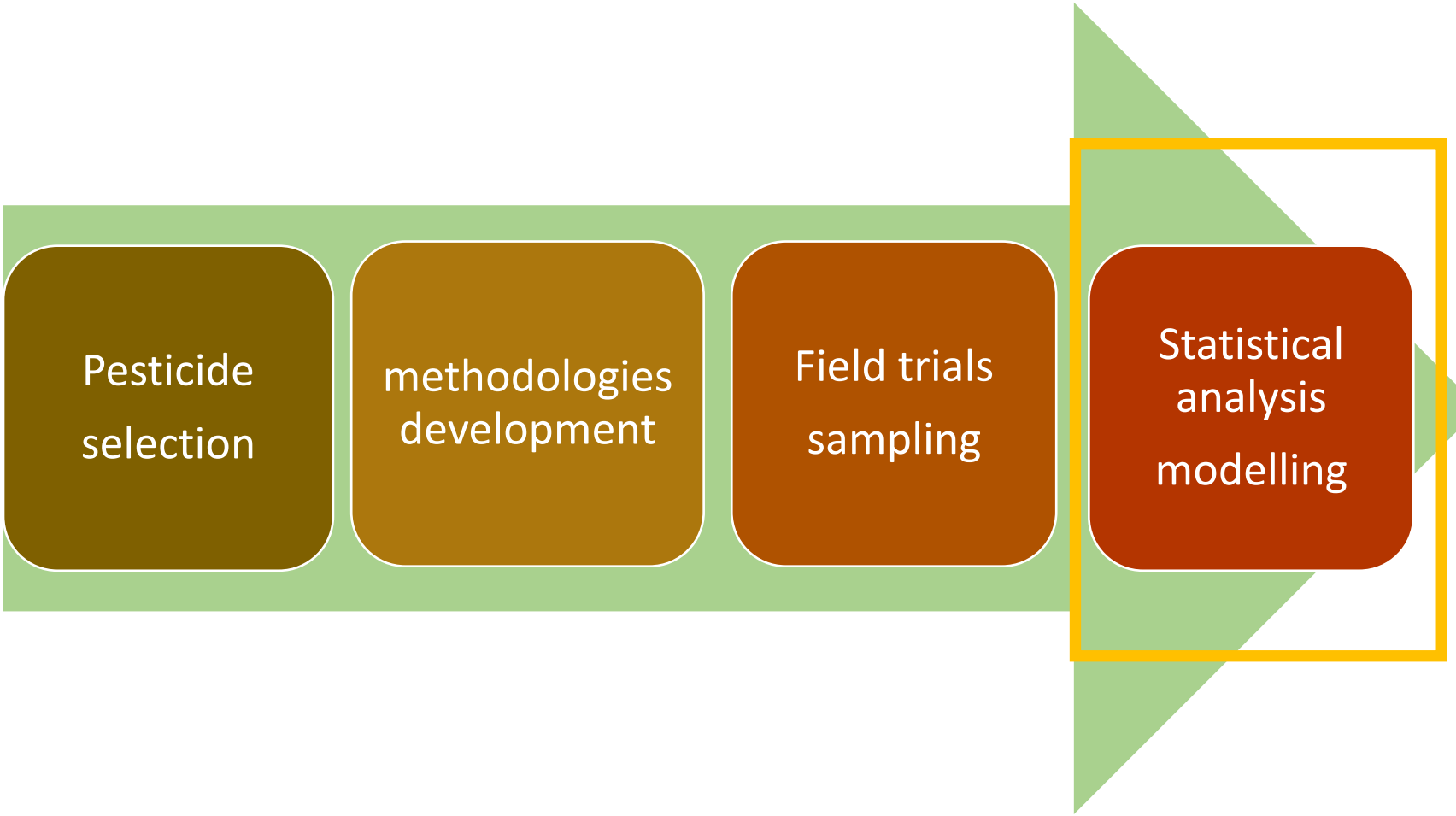
Pyraclostrobin
Azoxystrobin
Coumaphos
Pyraclostrobin
Coumaphos

Azoxystrobin
Azoxystrobin
Coumaphos

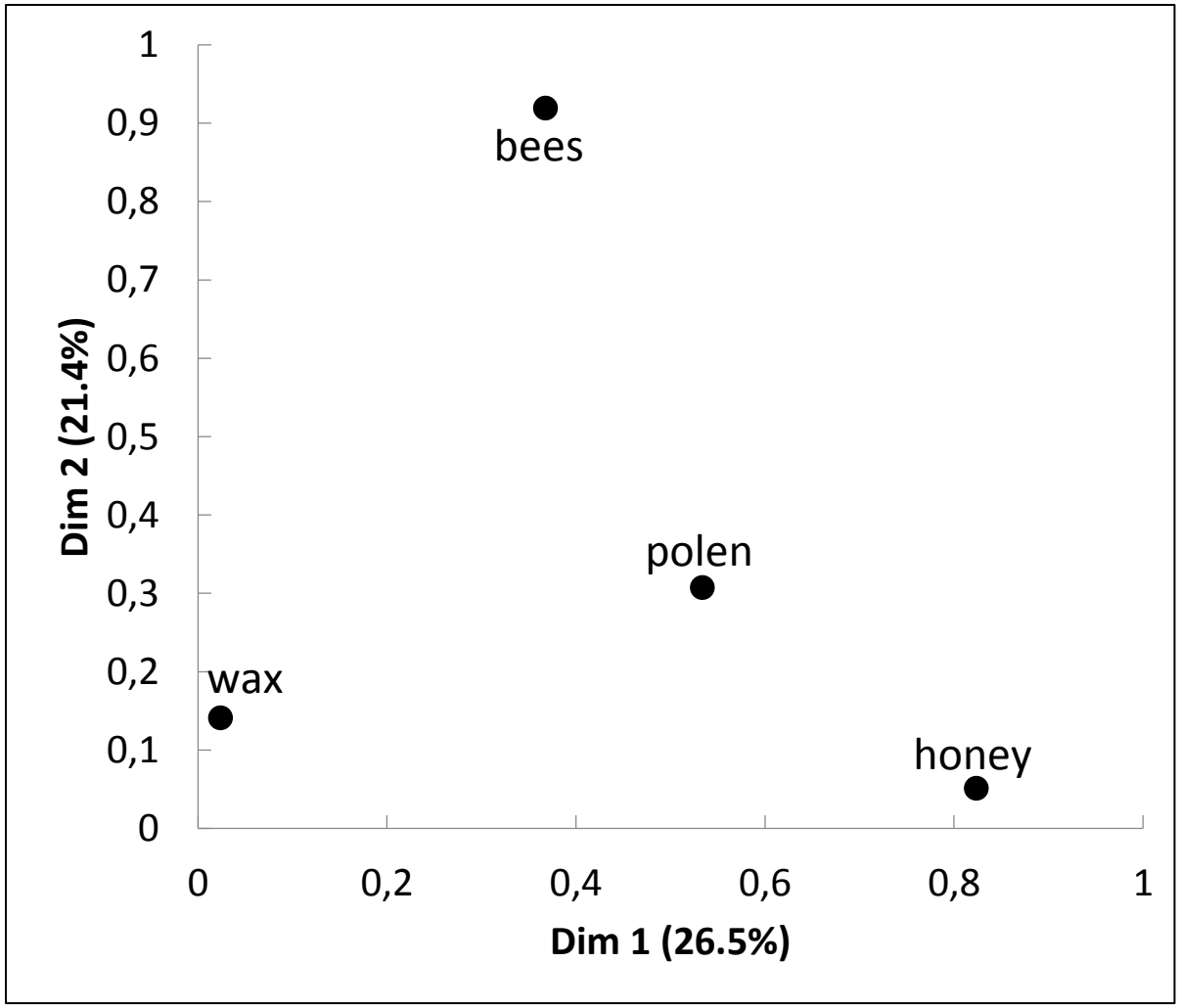
Coumaphos
Pyraclostrobin
Coumaphos
Carbaryl

Coumaphos
Tebuconazole
Pyraclostrobin
Carbaryl
Methomyl
Dimethoate
Haloxifop methyl
Thiacloprid

strategy


Pesticide selection
methodologies development
Field trials sampling
Statistical analysis modelling

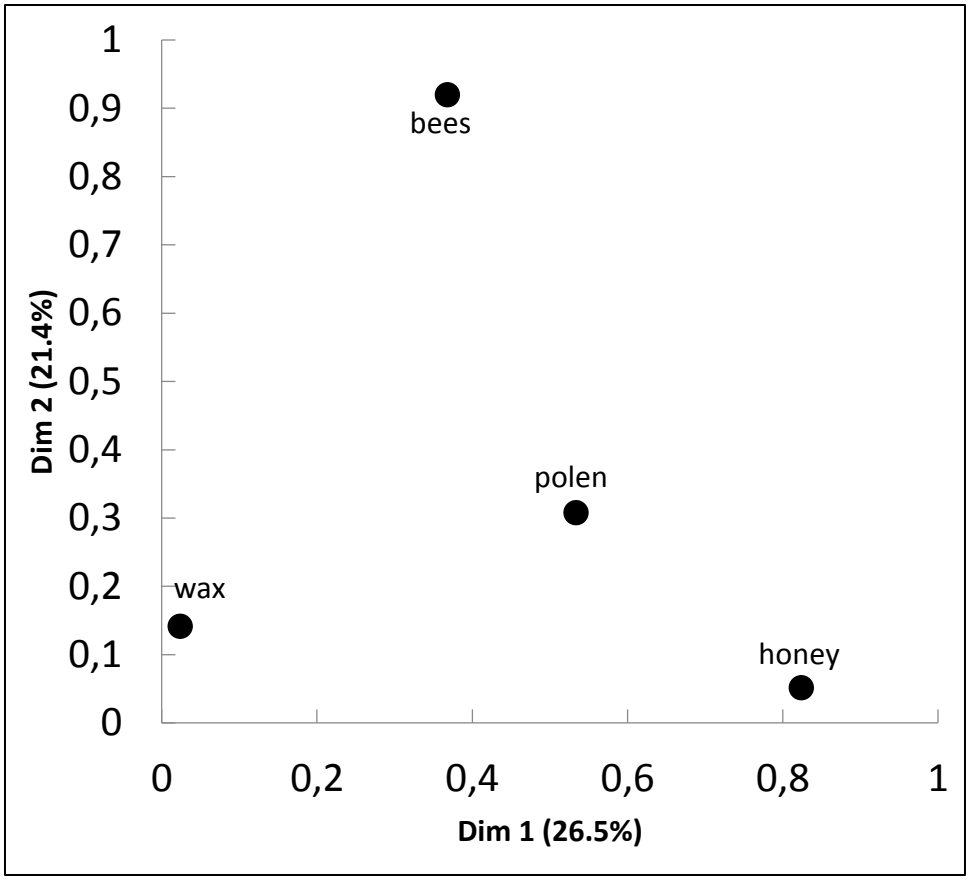
Multiple factor analysis



Niell *et al.* (2015) Journal of Agricultural and Food Chemistry 63 (18):4484-4492

The environmental meaning of the pesticide residues detected in different beehive matrices

- **Bees:** they contain the pesticides that are being currently applied
- **Polen** samples contain mostly the systemic pesticides that were applied in the different agroecosystems
- **Wax** is the historical reservoir of the pestices applied, and where the most lipophilic pesticdes are kept
- **Honey** is the matrix where hydrophillic pesticides are found, mainly herbicides



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Gower distances, a tool to compare seasonal variations in the same region

Apiary	Seasons compare	Productive enviironment	G-distance
A	Autumn -spring	Dairy production	0,24
B	Autumn -spring	Forestry	0,24
C	Autumn -winter	Cattle-rice cropping	0,35
D	Winter-spring	Dairy production natural prairies	0,18
E	Autumn -spring	Forestry, native hood	0,15

The lower of the G values, the higher the similarity in the pesticide residue levels for the two seasons of a given region

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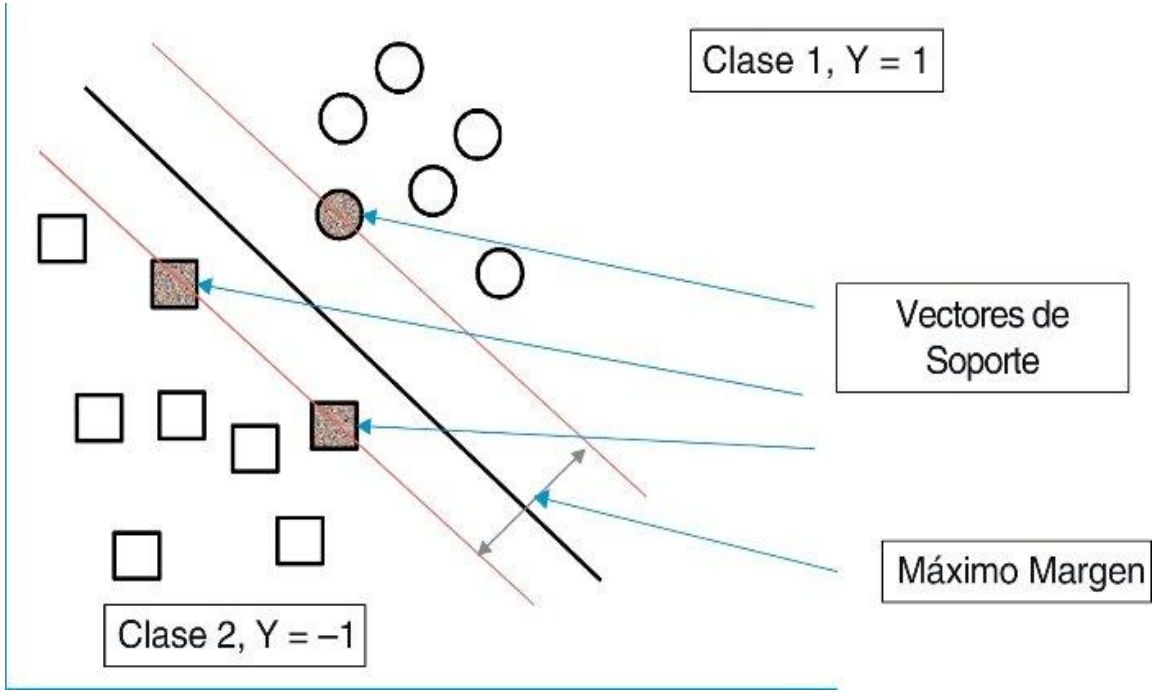
Building up the biomonitor: modelling

Supporting Vector Machines (SVM) in collaboration with Prof. Dr. H. Cancela



- ✓ Algorithms implemented using R software
- ✓ Supervised learnnig on the gathered data
- ✓ The performance of different chemical & bological data subsets as environment indicators was explored.

Niell et al Env. Res. submitted

Parameters selected

chemical

- The simple count of pesticide findings per environment per season (counting each pesticide found in each beehive product: bee, honey, pollen and wax) indicated as ***pestnum***.

- To combine meaningfully multiple pesticide findings per environment per season the **Toxic Units *TUi*** (Sprague, 1970) approach was used; they are defined as the ratio of the measured concentration c_i of a given compound *i* to its LD_{50} acute toxicity:

$$TU_i = \frac{c_i}{LD_{50i}}$$

for each detected compound (honeybees contact acute 48 hour LD_{50} (µg bee^{-1}) for bees and wax findings, and honeybees oral acute 48 hour LD_{50} (µg bee^{-1}) for honey and pollen)

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Parameters selected

Chemical/toxicological

- Under the assumption of the **Concentration Addition model (Loewe and Muischnek, 1926)**, toxic units were aggregated through simple addition of the different pesticide residues occurring in each environment per season. The total toxic units TU for the whole sample mixture are thus:

$$\bullet\ TUtot = \sum_i^n TU_i$$

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Parameters selected

biological

- ❖ Population
- ❖ Brood

Experimental data set

- ✓ 265 colony strength estimations were included
- ✓ 312 samples could be subjected to pesticide multiresidues analysis.

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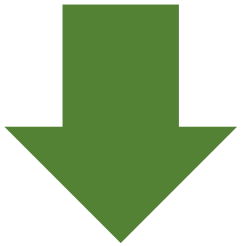
Supervised training

- Environments classification was performed according to field observations and pesticide residues history distinguishing among seasons over the four years.
- the available dataset, is small from an automatic learning point of view



- the validation method chosen was 10-fold cross validation with 41 cross

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Data set

Case	Class	µpop	µbrood	Summer	Fall	Winter	Spring	Pest num	Hive Tutot (bee)	Hive Tutot (mam)
1	Yes	5.2	1.0	0	0	1	0	15	4.13	9.16E-02
2	No	7.8	4.6	0	0	1	0	5	2.60	2.29E-02
3	No	2.8	0.8	0	0	1	0	9	0.24	3.42E-02
4	Yes	6.8	0.7	0	0	1	0	16	165.42	1.26E+00

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Preliminary results

Variables	Best parameters		Best performance	Number of support vectors
	gamma (γ)	Cost (C)		
Seasons&Biological	0.125	1024	0.33	26
Seasons & Chemical	0.0625	256	0.18	22
Seasons & Pestnum	0.0625	32	0.175	27

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Some outputs

- The number of supporting vectors is approximately half of the samples, which (in a context with a small number of samples) is an indicator of lack of overfitting.
- The biological indicators could be as useful as the chemical indicators, leading to classifications of similar accuracy.
- SVM model application using biological indicators can point to risky seasons and ecosystems where further chemical indicators should be studied.

Summary

- Quechers based methodology was developped and applied to determine pestide residues in beecomb matrices
- Bees carry neonicotinoid residues to the hive
- Pesticide residues in beehive compartments give wealthy environmental information
- Each compartment gives specific information on the pesticdes applied
- SVM was applied for the first time to analyze environmental information
- SVM is a powerful tool for developing a model to predict the status of an agroecosystem using either biological parameters or chemical information

DO YOU THINK THE BEEHIVE COULD ACT AS BIOMONITOR OF AGROECOSYSTEMS?

Bees make blue honey after eating M&Ms

SCIENCEALERT STAFF
SATURDAY, 24 MAY 2014

We couldn't make this stuff up if we tried.

Image: Science Dump

French beekeepers were recently shocked when their bees started **producing thick, blue and**


GACT

Acknowledgements


TEATRO SOLIS


MUCHAS GRACIAS!!!!
THANK YOU!!!!