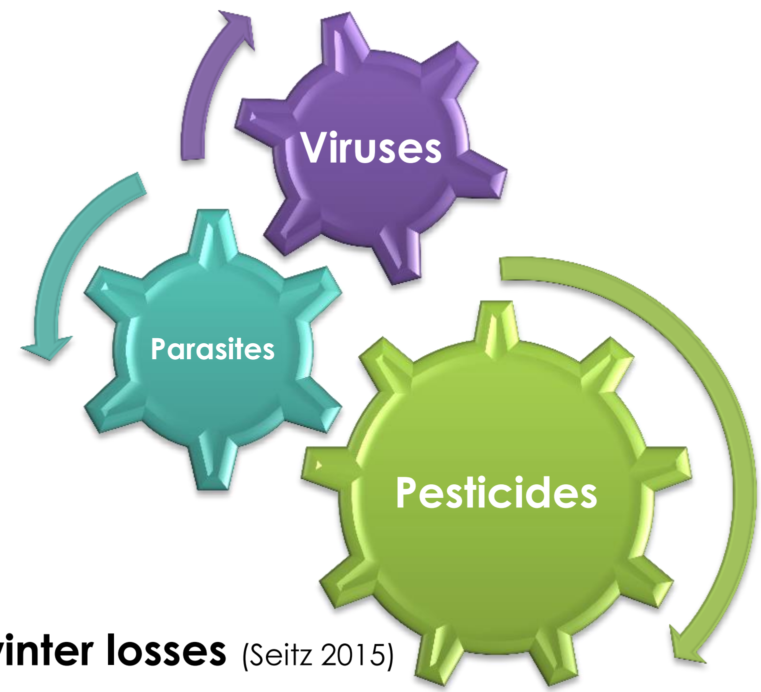
Multiple residues of pesticides and pesticide metabolites in honeybees: mass-spectrometry based method, exposure assessment of honeybee colonies and evaluation of risks

Tomasz Kiljanek, Alicja Niewiadowska, Marta Gaweł, Stanisław Semeniuk, Milena Borzęcka, Andrzej Posyniak

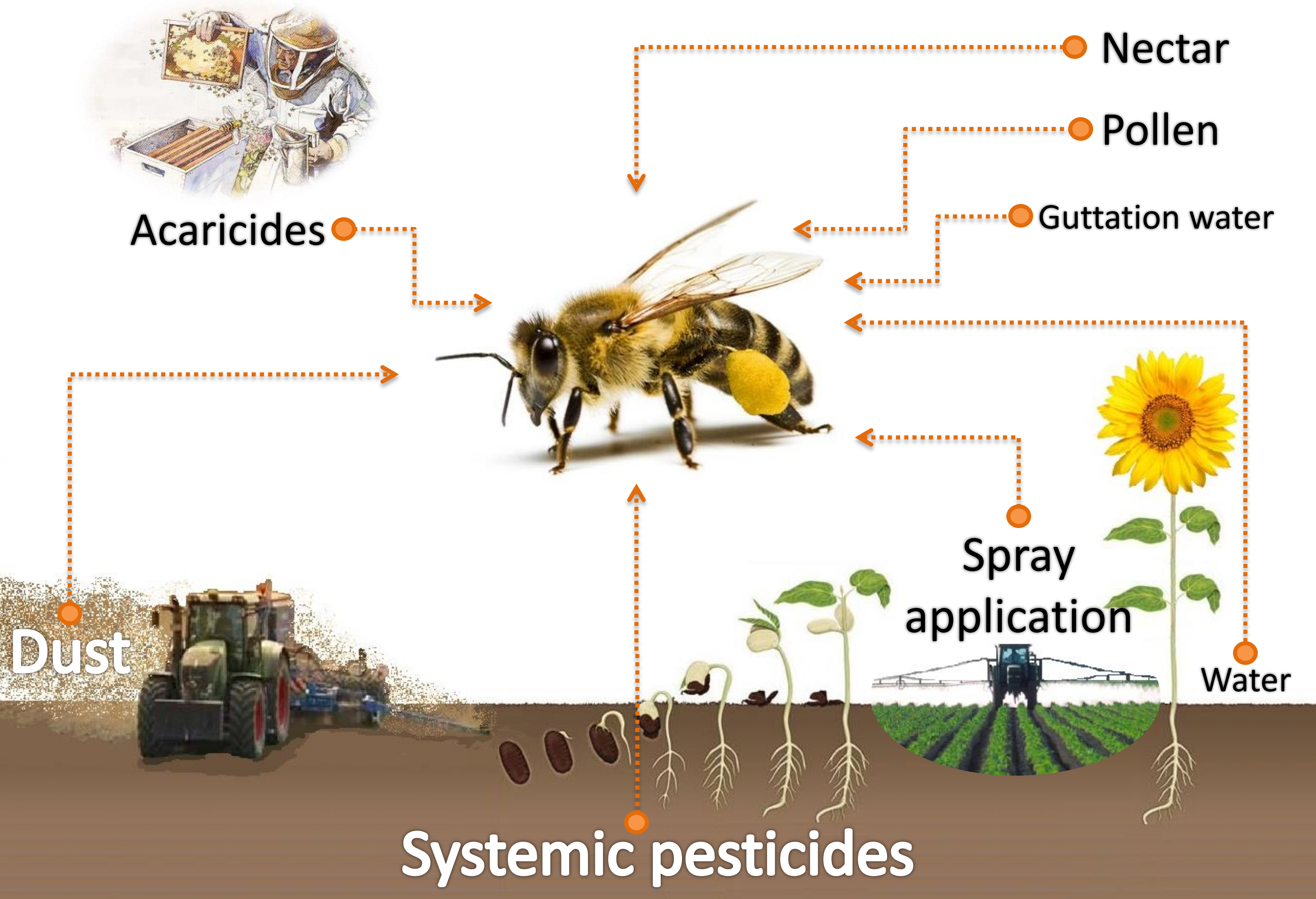
National Veterinary Research Institute, Poland

Bee Health

- In Europe production for over 80% of crops depends at least to some extent upon animal pollination (Klein 2007)
- Pollinators contribute at least 22 billion EUR each year to the European agriculture industry (EC)



- CCD in North America and Europe
- Losses of honey bee colonies:
  - up to 36 % in Europe (Laurent 2014)
  - up to 45% in the USA (Seitz 2015)
- For the first time summer losses greater than winter losses (Seitz 2015)


Nectar
Pollen
Guttation water
Acaricides
Dust
Spray application
Water
Systemic pesticides

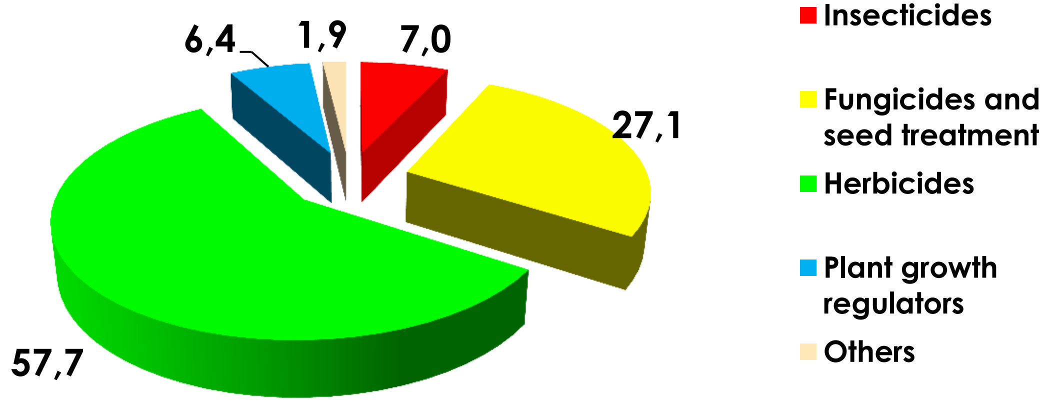
Plant Protection Products (PPPs)

- **There are more than <u>1800</u> different PPPs authorized to use in Poland**
- **Level of PPPs sale in Poland in 2015 reached <u>67 298 tons</u>**

  and by active ingredients 22 204 tons

  (Central Statistical Office of Poland , Statistical Yearbook of Agriculture 2016)

Sale of individual categories of PPPs (%)



Determination of pesticides <u>currently approved to use in EU</u>

- **Method for the determination of 200 pesticides and pesticide metabolites in honeybee samples**
- **195 compounds – substances approved to use within EU as PPPs or varroacides**

Kiljanek, T., Niewiadowska, A., Semeniuk, S., Gaweł, M., Borzęcka, M., Posyniak, A., Multi-residue method for the determination of pesticides and pesticide metabolites in honeybees by liquid and gas chromatography coupled with tandem mass spectrometry – honeybee poisoning incidents, Journal of Chromatography A 1435 (2016)100–114.
DOI: 10.1016/j.chroma.2016.01.045

Analytical Method

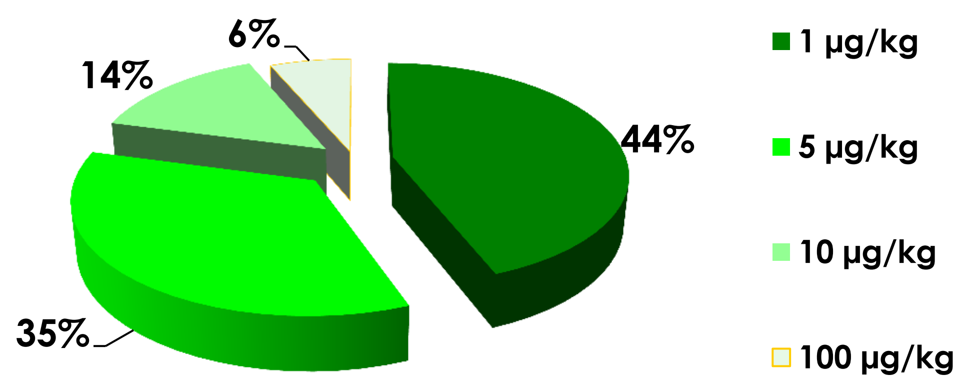
200 Pesticides + matrix → QuEChERS Z-Sep+ PSA → LC-MS/MS GC-MS/MS → Reliable data

LC-MS/MS QTrap 6500

GC-MS/MS Agilent 7000

Analytical Capabilities

- **Validation according to the SANCO/12571/2013 guidance document**
- **EUPT AO-10 (honey) organised by EURL for Residues of Pesticides in Food of Animal Origin**
- **AZ^2=0.15 (the Average of the Squared z-Score)**



LOQ

Analysis of 417 samples of honeybees

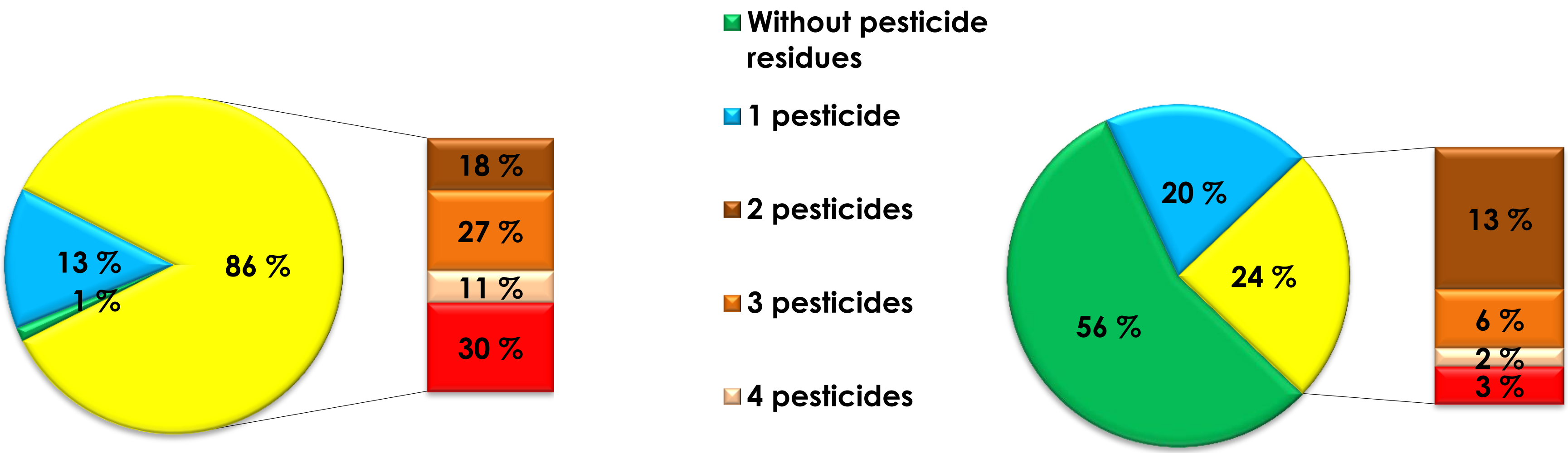
Diagnosis of bee poisoning incidents

Monitoring of pesticides in apiaries

74 samples of poisoned honeybees

343 samples of live honeybees

Number of pesticide residues



Samples of poisoned honeybees contained as average four times more pesticide residues than samples of live honeybees

81 pesticide residues

- **34 insecticides, acaricides, varroacides and metabolites**
- **32 fungicides**
- **15 herbicides**
- **30 various modes of action (MoA)**

Kiljanek T., Niewiadowska A., Gaweł M., Semeniuk S., Borzęcka M., Posyniak A., Pohorecka K., Multiple pesticide residues in live and poisoned honeybees – preliminary exposure assessment
Chemosphere 175 (2017) 36–44.
DOI: 10.1016/j.chemosphere.2017.02.028

Insecticides, Acaricides & Varroacides

MoA	Compound
Acetylcholinesterase inhibitors	**Chlorpyrifos**
	Chlorpyrifos-methyl
	Coumaphos
	Dimethoate
GABA-gated chloride channel blockers	Fipronil
	Fipronil-carboxamide
	Fipronil-desulfinyl
	Fipronil-sulfide
	Fipronil-sulfone
Sodium channel modulators	alpha-Cypermethrin
	Beta-Cyfluthrin
	Deltamethrin
	Etofenprox
	lambda-Cyhalothrin
	tau-Fluwalinate
	zeta-Cypermethrin

MoA	Compound
Nicotinic acetylcholine receptor competitive modulators	Acetamiprid
	Clothianidin
	Imidacloprid
	Imidacloprid-urea
	Thiacloprid
	Thiacloprid-amide
	Thiamethoxam
nAChR allosteric modulators	Spinosyn A
Glutamate-gated chloride channel allosteric modulators	Abamectin
Mite growth inhibitors	Etoxazole
Octopamine receptor agonists	DMA
	DMF
	DMPF
Mitochondrial complex III electron transport inhibitors	Bifenazate
	Fenazaquin
	Fenpyroximate
Inhibitors of acetyl coa carboxylase	Spirodiclofen
UN	Bromopropylate

Fungicides

MoA	Compound
Nucleic acids synthesis	Bupirimate
	Metalaxyl-M/Metalaxyl
Cytoskeleton and motor proteins	Carbendazim
	Thiophanate-methyl
Respiration	Azoxystrobin
	Boscalid
	Dimoxystrobin
	Kresoxim-methyl
	Trifloxystrobin
Amino acids and protein synthesis	**Cyprodinil**
	Mepanipyrim
	Pyrimethanil
Signal transduction	Fludioxonil
	Iprodione
	Quinoxyfen
Lipid synthesis and membrane integrity	Propamocarb

MoA	Compound
Sterol biosynthesis inhibition (SBI)	Difenoconazole
	Epoxiconazole
	Fenhexamid
	Fenpropidin
	Flusilazole
	Metconazole
	Myclobutanil
	Prochloraz
	Propiconazole
	Spiroxamine
	Tebuconazole
	Tetraconazole
Cell wall biosynthesis	Dimethomorph
Multi-site contact activity	Chlorothalonil
	Dithianon
UN	Cyflufenamid

Herbicides

MoA	Compound
Inhibition of acetyl CoA carboxylase	Propaquizafop
Inhibition of photosynthesis at photosystem II	Desmedipham
	Lenacil
	Phenmedipham
	Terbuthylazine
	Bentazone
Inhibition of carotenoid biosynthesis at the phytoene desaturase step	Flurochloridone

MoA	Compound
Inhibition of lycopene cyclase	Clomazone
Microtubule assembly inhibition	Pendimethalin
Inhibition of cell division	Metazachlor
	S-Metolachlor
Inhibition of lipid synthesis	Prosulfocarb
Action like indole acetic acid	2.4-D
	MCPA
Photosynthesis inhibitor	Quinoclamine

Hazard Quotient (HQ)

- Correlation of predicted environmental concentration (PEC) of pesticides with LD50
- Honeybees illustrate PEC in the best way – connection of all possible sources of exposure
- Comparison of multiple pesticide related risk for typical live honeybee colony and for poisoned colony – the worst case scenario

$$HQ = \frac{Concentration\ of\ pesticide\ in\ honeybee\ sample}{LD_{50}} \frac{[ng/g]}{[\mu g/bee]}$$

Overview of calculated HQ scores

	Live honeybee samples	**Poisoned honeybee samples**
Analysed samples	343	74
Samples without residues	56,0% (192)	1,4% (1)
HQ < 50	37,6% (129)	6,8% (5)
HQ 50+	**6,4% (22)**	**91,9% (68)**
HQ 1000+	0	59,5% (44)
Min HQ	0,01	0,1
Mean HQ ± SE	19,2 ± 35,3	15984 ± 36902
Median HQ	0,8	1504
Max HQ	181,5	164501

If the resulting HQ is 50 or less, then the risk reflects acceptable background mortality 5.3% suggested by EFSA (EFSA, 2013)

HQ 50+ as a limit value that confirms pesticide poisoning

Thank you for your attention