A one-year experience with pesticide residue analysis in hops using QuEChERS based method: Living up to expectations or blind alley?

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2016 – A look back

Adaptation of the QuEChERS method for Hop matrix

- Composition of SPE sorbent mixtures (PSA, GCB, C18, zirconia based sorbent) ***vs.*** Matrix effects
- Composition of SPE sorbent mixtures ***vs.*** Recovery of pesticides
- Cartridge SPE ***vs.*** Dispersive SPE
- Sample dilution for overcoming or reducing of matrix effects

Sample preparation

- Unbuffered original QuEChERS procedure
- 58 pesticides + 3 x ISTD (TPP, thiamethoxam-d3 and azoxystrobin-d4)
- HR/AM LC-MS/MS (Q Exactive MS), operating in PRM mode: two HR-MRM transitions per analyte

2017 - Final QuEChERS procedure for hops

Weight 1 g of minced hops in 50 mL extraction vessel

↓

Add 10 mL water and wait 30 min

↓

Add 10 mL Acetonitrile + ISTDs

↓ Shake

Add partitioning/Buffer Salts

- 4 g $MgSO_4$
- 1 g NaCl

↓ Shake / Centrifuge (4,500 rpm, 7 min)

d-SPE: 1 mL aliquot and mix with

150 mg $MgSO_4$ + 100 mg PSA + 25 mg C18 + 25 mg Z-Sep

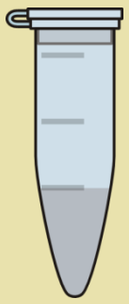
↓ Shake / Centrifuge (13,000 rpm, 2 min)

Re-acidify Extract (10 µL formic acid 5% (v/v) per mL)

↓

LC-MS

Dispersive SPE



- **PSA** – soft and hard resins, polar pigments
- **C18** – fatty acids, essential oils
- **Z-Sep** – pigments, non-polar compounds

Validation of QuEChERS method

- Recoveries obtained for two fortification levels (0.05 and 0.50 mg/kg) were 70-120% with satisfactory precision (RSD<20%) for **46** from **58** pesticides involved in the study.
- Recovery of **Propamocarb** and especially of **Pymetrozin** is about 15%.
- The LOQ was 0.02 or 0.05 mg/kg for most of the pesticides.
- Matrix matched calibration or standard addition were used for routine analysis of hop samples due to remaining strong matrix effects.

Alternative sample preparation procedures

Two suitable sample preparation protocols were published for hops:

1) Hengel M. J. et al.: *J. Am. Soc. Brew. Chem. 69, 121-126 (2011)*

LC: extraction by acetonitrile, SPE clean-up

2) Biendel M. *et al.*: *BrewingScience 67, 108-115 (2014)*

GC: extraction by acetone/dichlormethane, SPE with silica gel and magnesium silicate

LC: extraction by acetone/dichlormethane, SPE clean-up with PSA

eurofins, Germany

GC: DFG S19 – gel permeation chromatography

LC: Klein & Alder method – extraction by methanol and clean-up on ChemElut cartridge

AHA collaborative trial "Pesticides 2017/1"

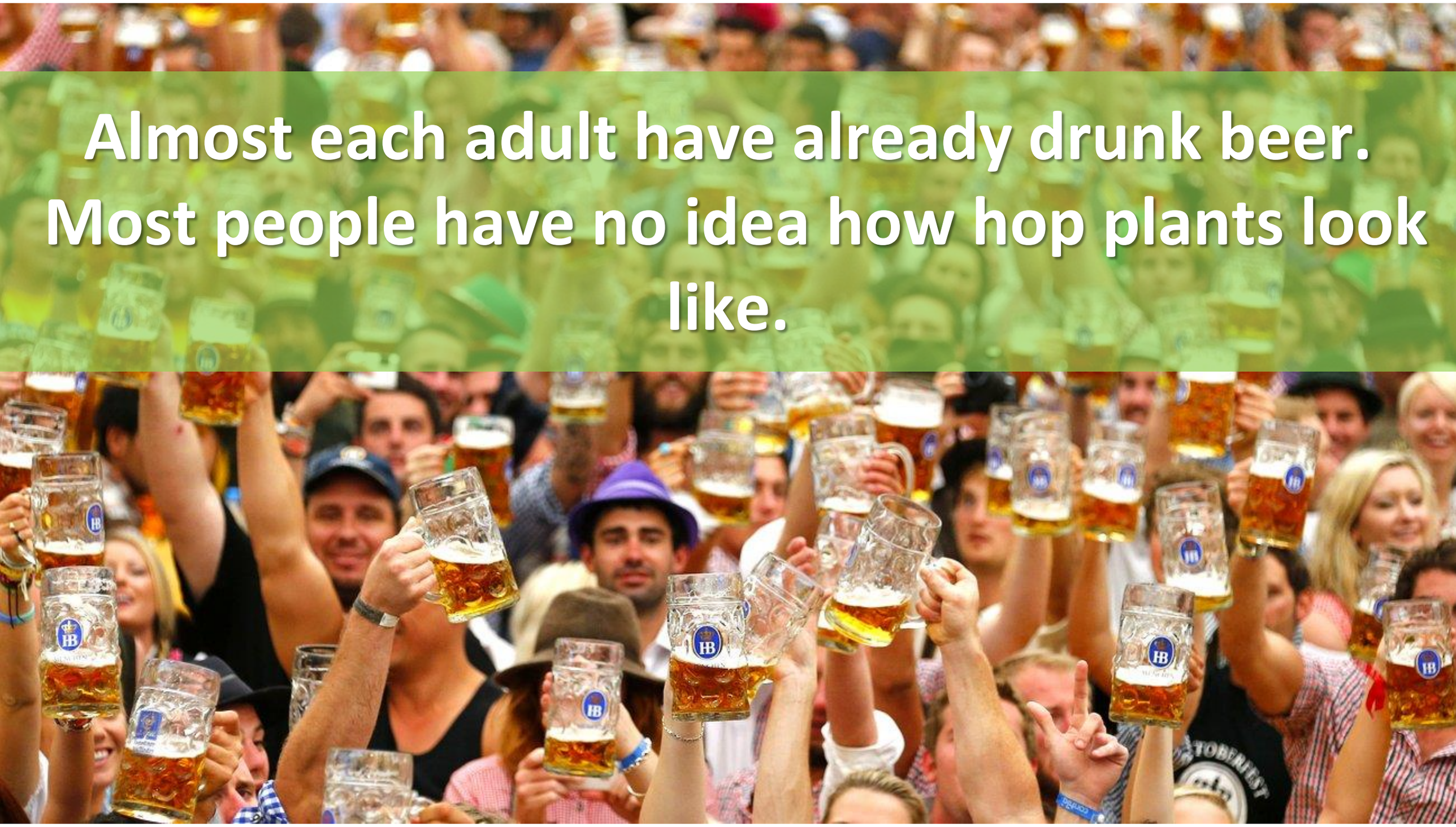
- 11 participants (5x Germany, 2x Czech Republic, 2x Japan, France, USA)
- 3 samples of hop pellets (P1-P3) and 1 spiked hop extract

Pesticide		Mean (mg/kg)	QuEChERS method - RIBM		JASBC (Hengel) method - PAL	
			x (mg/kg)	z-score	x (mg/kg)	z-score
Azoxystrobin	P1	**5.52**	6.33	0.60	**5.12**	**-0.36**
Azoxystrobin	P3	**1.01**	1.27	0.98	**0.81**	**-0.80**
Boscalid	P1	**16.82**	20.02	0.75	**14.50**	**-0.59**
Dimethomorph	P1	**2.51**	**2.76**	**0.36**	1.90	-1.17
Dimethomorph	P2	**15.51**	**16.13**	**0.18**	11.45	-1.49
Flonicamid	P1	**0.54**	**0.54**	**0.03**	0.15	-2.02
Mandipropamid	P3	**11.60**	**13.55**	**0.56**	9.74	-0.69
Metrafenone	P2	**5.42**	6.15	0.36	**5.91**	**0.22**
Myclobutanil	P3	**1.08**	**1.43**	**1.26**	0.63	-1.54
Pyraclostrobin	P1	**1.83**	**1.97**	**0.12**	0.92	-0.89
Spirodiclofen	P2	**2.18**	2.72	0.73	**2.08**	**-0.15**

PROC/CONS

- Unmodified QuEChERS extraction is efficient for all tested pesticides except pymetrozine.
- Dispersive clean-up procedure could be easy optimized by the change of sorbents or the composition of their mixture.
- Use of primary-secondary amine (PSA) significantly reduces recovery pesticides as phenoxy alkanoic acids or flonicamid metabolites (TFNA, TFNG).
- Recoveries some tested pesticides are negatively affected by other dSPE sorbents, for example: C18 and imazalil.
- Matrix effects still remain quite high and in some cases they decrease the signal intensity up to 80%.

Almost each adult have already drunk beer. Most people have no idea how hop plants look like.



Pesticide residue analysis in BEER - Overview

- Beer is a matrix without strong interferences of coextracted compounds
- Very low concentration of pesticides => sensitive instrument and/or clean-up and pre-concentration of sample

Sample preparation options:

1. Direct injection of beer
2. Direct injection of QuEChERS extract – limited volume of injection volume 2 µL
3. Solvent exchange of QuEChERS extract - to acidified mix. MeOH/H2O
4. Cartridge SPE clean-up on PSA column, sample pre-concentration in combination with solvent exchange - to acidified mixture of MeOH/H2O

Pesticide residue analysis in BEER - Screening

- 8 foreign, 11 homeland beers of Pilsner type and 5 craft beers

	Beer Style	Azoxystrobin	Boscalid	Dimethomorph	Mandipropamid	Metrafenone	Propamocarb
1.	Pilsner type	0.6 µg/L	7.4	2.0	2.0	-	-
2.	Pilsner type	< 0.5	2.9	1.0	0.8	-	-
3.	Pilsner type	< 0.5	2.7	1.7	**20.7**	-	**10.7**
4.	Pilsner type	1.0	4.2	-	-	-	-
5.	Pilsner type	0.5	3.8	20.6	0.7	-	-
6.	American Pale Ale	< 0.5	**15.1**	**68.0**	2.7	1.3	0.5
7.	Indian Pale Ale (IPA)	< 0.5	5.5	**18.1**	0.6	< 0.5	< 0.5
8.	Indian Pale Ale	< 0.5	6.0	**19.1**	0.6	< 0.5	< 0.5
9.	Premium Bitter/ESB	0.5	3.2	**-**	-	-	< 0.5
10.	Strong Pale Lager	1.9	3.3	**10.0**	< 0.5	< 0.5	< 0.5

Method: LC-MS/MS, 68 pesticides, SPE clean-up (100 mg PSA/mL), 2:1 pre-concentration, injection vol. 5 µL

Pesticide residue analysis in BEER - Screening

„Beer making is decontamination process.“ Yes, but…





Pesticide	1. (µg/500 ml)	2. (µg/500 ml)	3. (µg/500 ml)	Red princ (µg/apple)	Golden del. (µg/apple)	Gala (µg/apple)
Azoxystrobin	0.25	-	0.5	-	-	-
Boscalid	0.2	0.7	2.4	17.5	12.0	-
Dimethomorph	0.2	0.15	3.3*	-	-	-
Mandipropamid	0.3	0.25	1.3	-	-	-
Pirimicarb	-	-	0.5	12.5	-	13.0
Pyraclostrobin	-	-	-	6.0	-	-
Spiroxamine			0.5	-	-	-

Why widely used pesticides as Ametoctradin, Pyraclostrobin and Quinoxyfen, Spirotetramat were not detected at any sample?

Picobrewery experiment

Why widely used pesticides as Ametoctradin, Pyraclostrobin and Quinoxyfen, Spirotetramat were not detected at any sample?

Experiment: Adopt beer making procedure of "**pilsner**" style for laboratory scale and make a beer from pesticide enriched hops

- 10 g organic hops spiked with mixture of 58 pesticides at the level **10** mg/kg.
- Theoretical concentration in beer should be **20** µg/L.

Distribution of pesticides during beer making

Mashing

Water + **Malt** → **Wort**

Boiling

(2x1.5L) Wort + **3 g of Hops spiked at the concentration 10 ppm**

Boiling wort for 90 min

Sample A

Hopped Wort

Lager yeast

Fermenting

1. Open fermenting vat, **10** °C, **7 days**

Sample B

Green beer (20 µg/L)

2. Fermenting tank, **3** °C, **3 weeks**

Sample C

Beer (10.23% Plato, Alcohol 3.5%)

Distribution of pesticides during beer making

1) Transfer pesticides from hops to wort during boiling

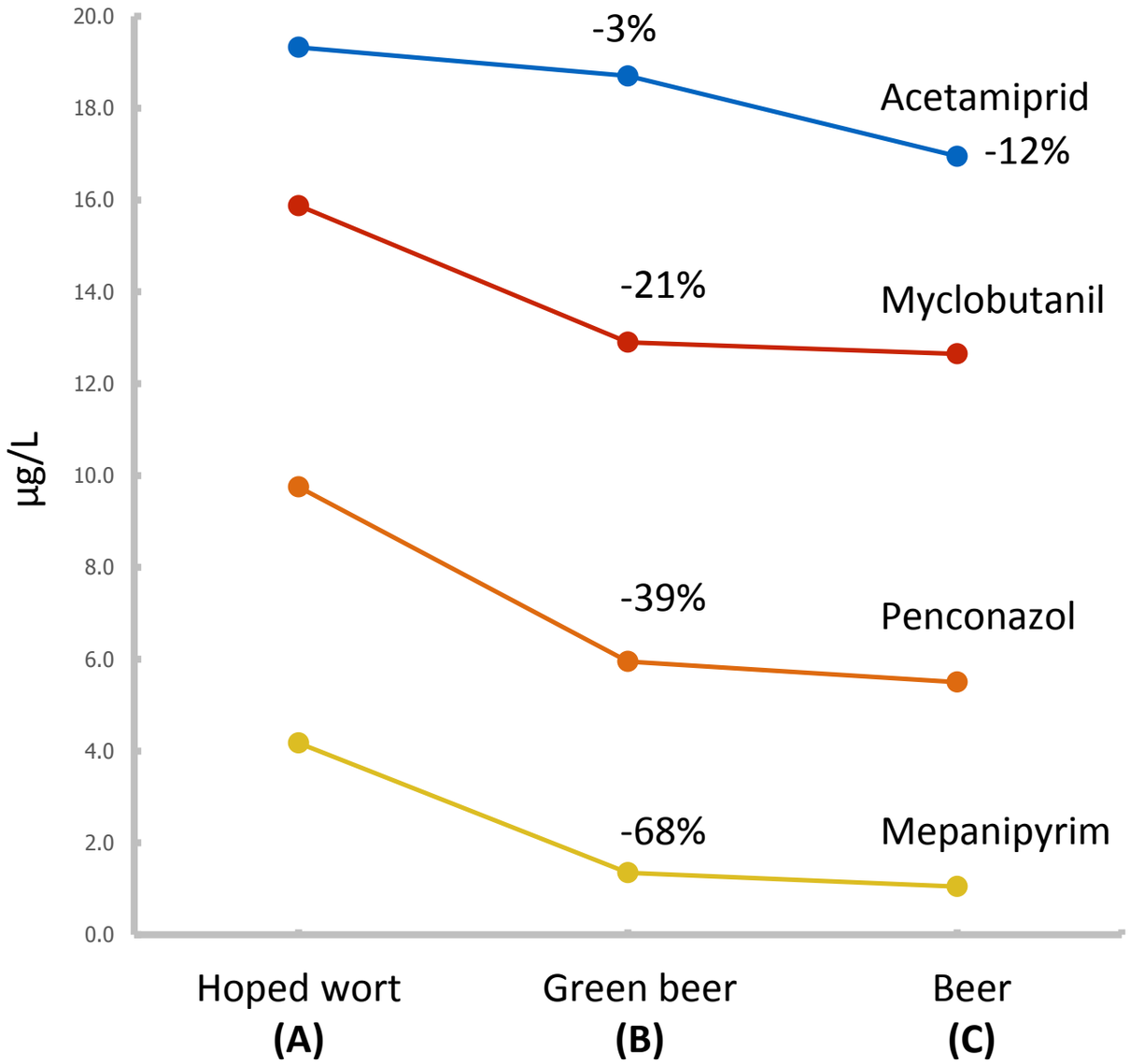
I.	Acetamiprid, Carbendazin, Clothianidin, Dimethomorph, Flonicamid, Imidacloprid, Metalaxyl, Myclobutanil, Pirimicarb, Propamocarb, Propiconazole, Thiabendazole, Triadimenol
II.	Acephate, Azoxystrobin, Boscalid, Fenarimol, Fluopicolide, Fluopyram, Imazalil, Mandipropamid, Penconazole, Spiroxamine, Tebuconazole, Tebufenozide, Thiamethoxam, Triadimefon
III.	Fenpropimorph, Hexythiazox, Indoxacarb, Malaoxon, Malathion, Mepanipyrim, Metrafenone, Oxadiazon, Pendimethalin, Pyridaben, **Quinoxyfen**, Tebufenpyrad, Trifloxystrobin
IV.	**Ametoctradin**, Avamectin B1A, Chlorpyrifos, Cyazofamid, Cymoxanil, Fenpyroximate, Propargite, **Pyraclostrobin**, Spirodiclofen, **Spirotetramat**

I. Pesticide is almost fully extracted during boiling wort.
II. Approximately 50% of pesticide is extracted.
III. At least ¼ of pesticide is extracted or just traces could be observed in hoped wort.
IV. Pesticide is not extracted.

Solubility in water/buffer: Spirotetramat = 34 mg/L; Pyraclostrobin 2 mg/L; Ametoctradin 0.23 mg/L (pH=4); Cyazofamid 0.12 mg/L (pH=5).

Distribution of pesticides during beer making

2) Change of pesticides during fermentation



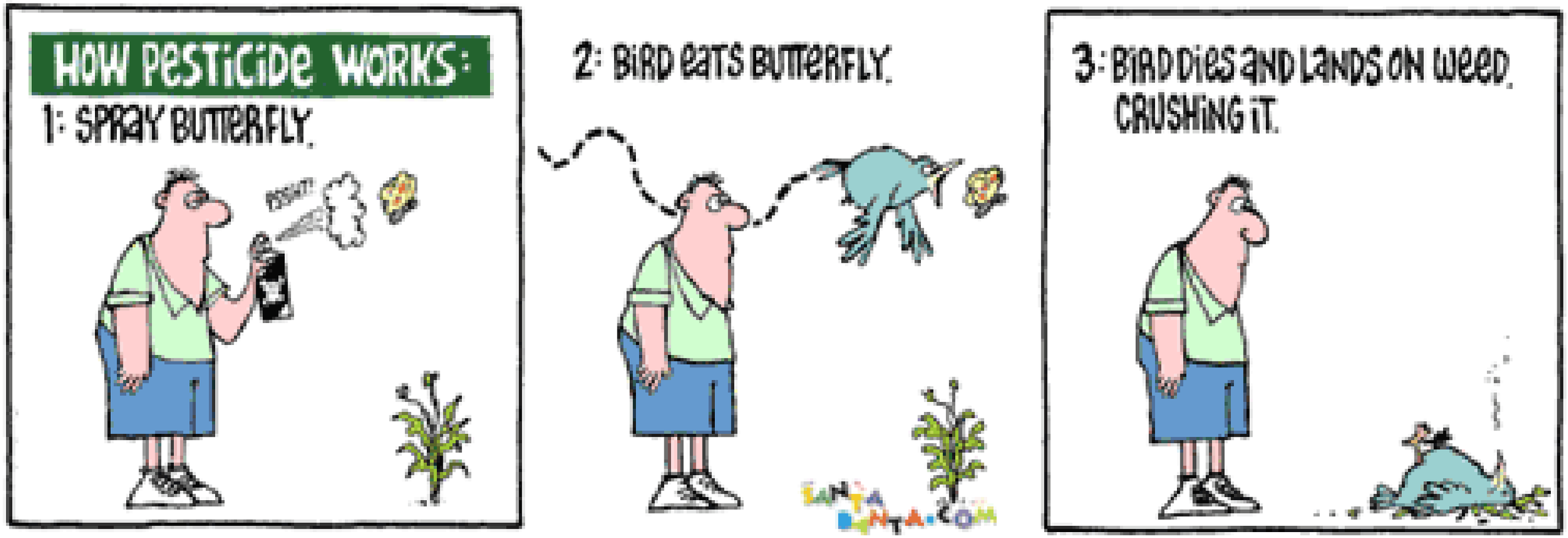
- During fermentation, the concentration of pesticides decreases.
- The intensity of the decline is individual and varies from 5-70%.

Conclusion

- Approach based on QuEChERS approach is comparable to others worldwide used methods.
- Hop is crops with intensive chemical protection and some of these pesticides are fully or particularly transferred into beer during beer making.
- The screening of 19 commercial beers show that the concentration of pesticides are at the level bellow MRL for baby food.
- Highly hoped beers from craft breweries could increase daily intake of pesticides.
- If you drink no more than 6 pints a week you will be at low risk of **alcohol*** and **pesticides** *(coming from hops)* effecting your health.
  **drinkaware.co.uk*

Acknowledgement

This study was supported by the **Ministry of Agriculture of the Czech Republic**, project **RO1917** „Research on quality and processing of malting and brewing raw materials“.



Thank you for your attention