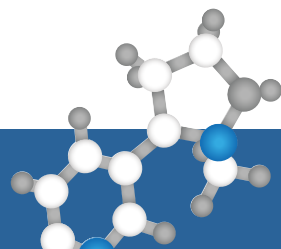




European
Commission

EURL-SRM



EURL-SRM Workshop July 2024

*EURLs for Residues of Pesticides
Single Residue Methods*

Comparative study of four HILIC type columns for the analysis of highly polar pesticides

Ann-Kathrin Schäfer¹⁾, Sedef Torun²⁾, Eric Eichhorn¹⁾ and Michelangelo Anastassiades¹⁾

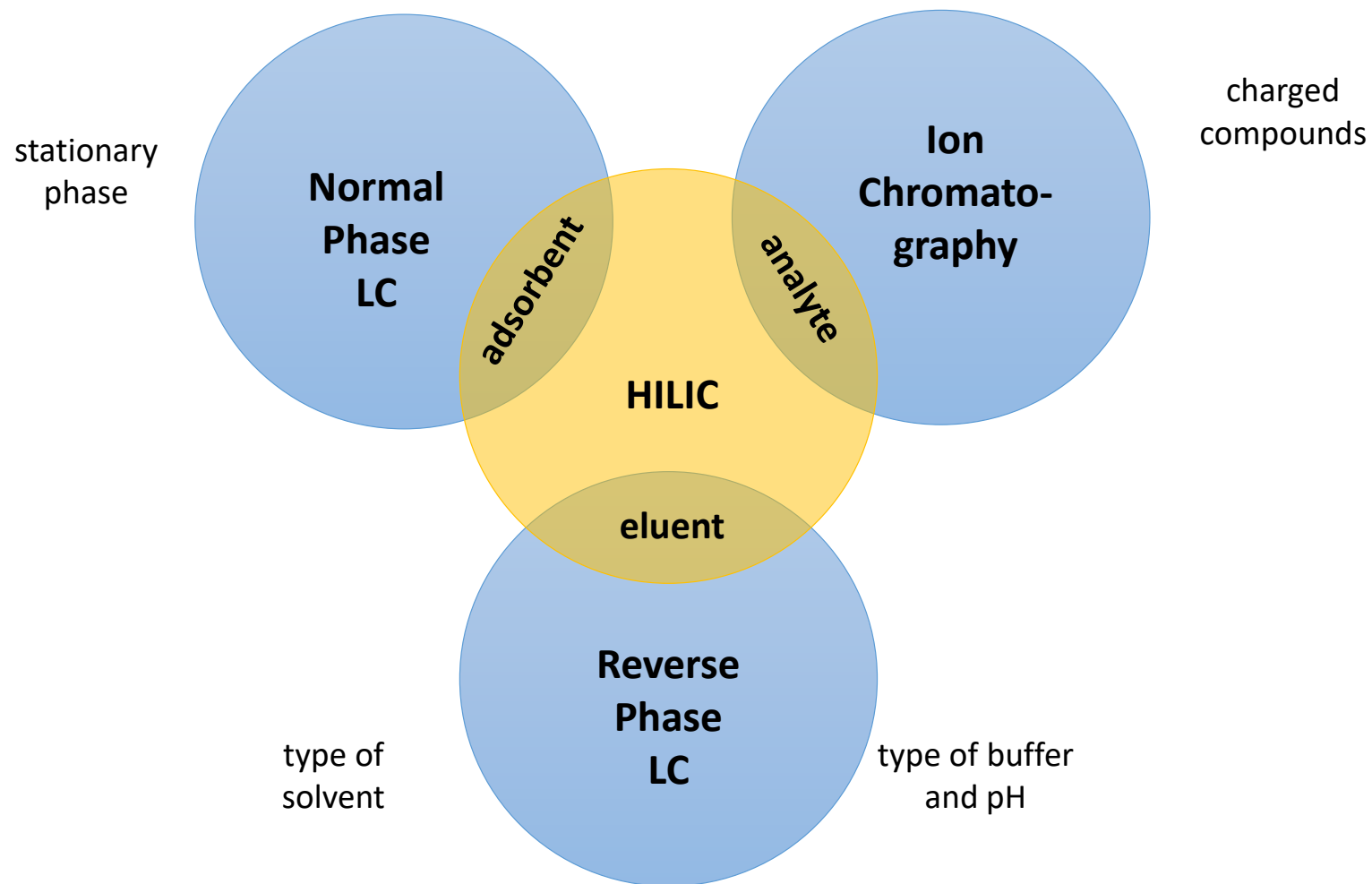
1) EURL-SRM, located at the CVUA Stuttgart, Fellbach, Germany

2) Institute for Food Chemistry, University of Hohenheim, Stuttgart, Germany

**EURL-SRM Workshop
1st and 2nd July 2024, Fellbach, Germany**

Hydrophilic Interaction Liquid Chromatography

- HILIC combines characteristics of the major LC techniques
- for highly polar analytes with $\log P < -1$

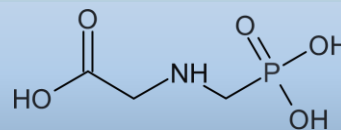


Hydrophilic Interaction Liquid Chromatography

- Polar stationary phase
- mobile phase:
 - starts with high organic content
 - Increase of the polar solvent leads to elution of polar analytes

Mostly Organic Mobile Phase

Aqueous Sublayer



Retention of Polar Analytes

Polar Stationary Phase

Hydrophilic Interaction Liquid Chromatography

Anionic Polar Pesticides Column

- Waters
- 130 Å, 5 µm, 2.1 x 100 mm

‘APPC’

Luna Polar Pesticides

- Phenomenex
- 100 Å, 3 µm, 2.1 x 100 mm

‘LunaPP’

Raptor Polar X

- Restek
- 90 Å, 2.7 µm, 2.1 x 30 mm

Obelisc N

- Sielc
- 100 Å, 5 µm, 2.1 x 150 mm

Hydrophilic Interaction Liquid Chromatography

Anionic Polar Pesticides Column

- Waters
- 130 Å, 5 µm, 2.1 x 100 mm

‘APPC’

Also reversed mode

Luna Polar Pesticides

- Phenomenex
- 100 Å, 3 µm, 2.1 x 100 mm

‘LunaPP’

with ion exchange

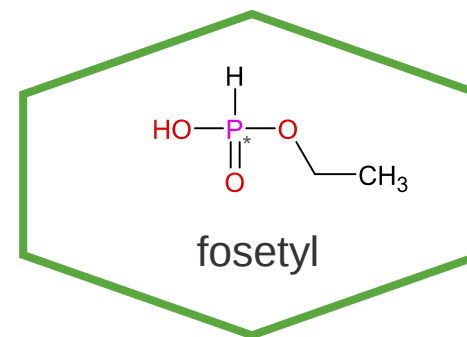
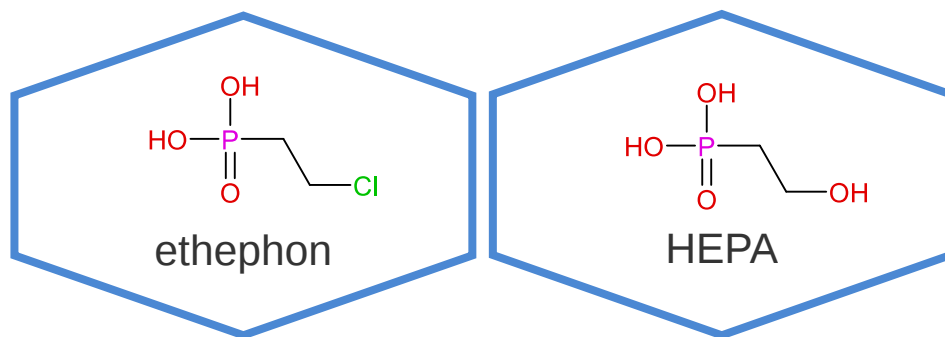
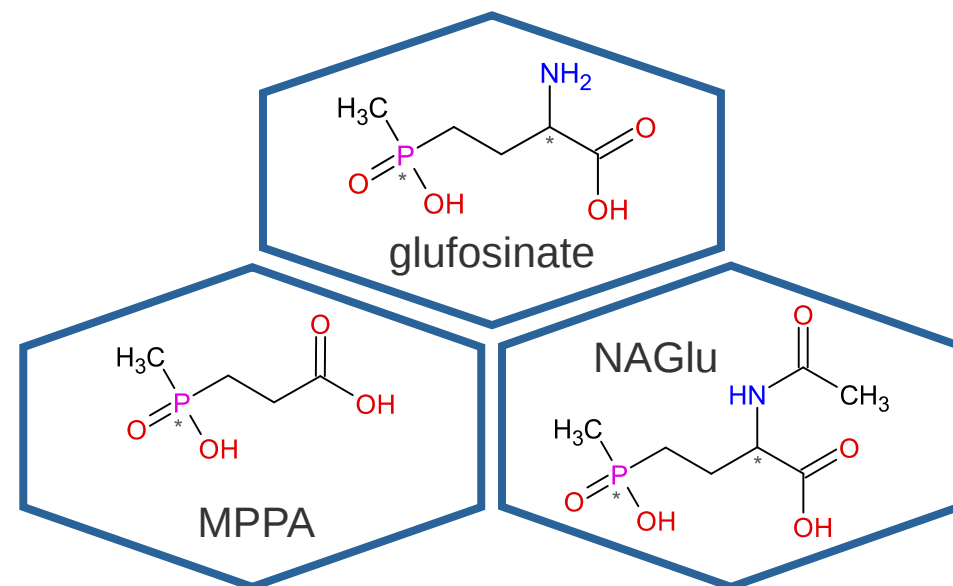
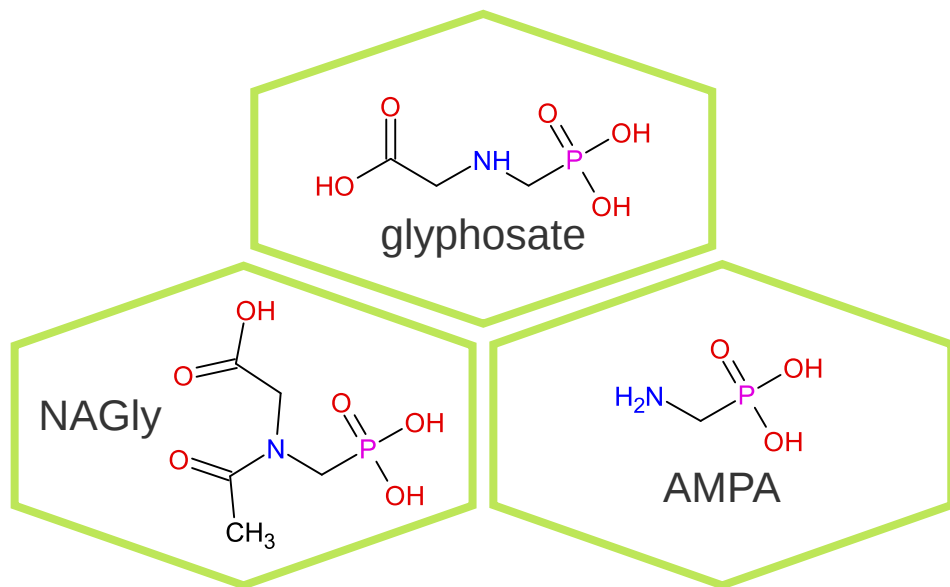
Raptor Polar X

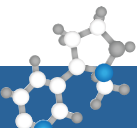
- Restek
- 90 Å, 2.7 µm, 2.1 x 30 mm

Obelisc N

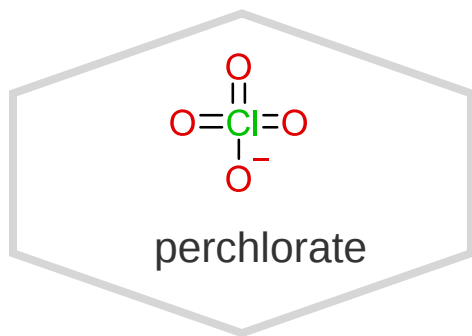
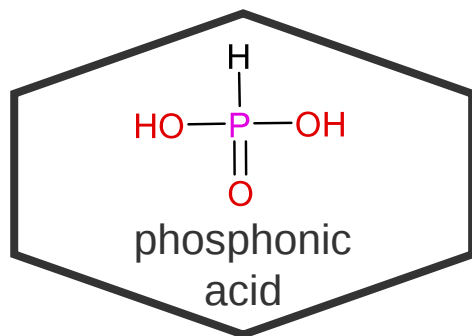
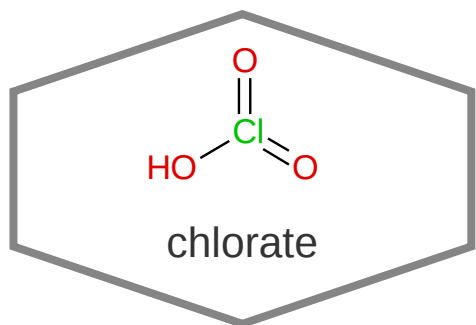
- Sielc
- 100 Å, 5 µm, 2.1 x 150 mm

Anionic Polar Pesticides

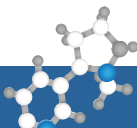




Anionic Polar Pesticides



- not included in project because
- separate methods,
need to separate standard mixtures
containing fosetyl and phosphonic acid
- **issues with finding residue free
matrix for validation**



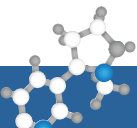
Instrumentation

LC: Acquity UPLC i-Class (Waters):

- glyphosate analysis well established on the instrument; no conditioning/passivation of the LC was required, but of the columns
- for each column, the same oven position was used!

MS: API 5500 QTrap (Sciex):

- duration of the measurements approx. 5-6 weeks

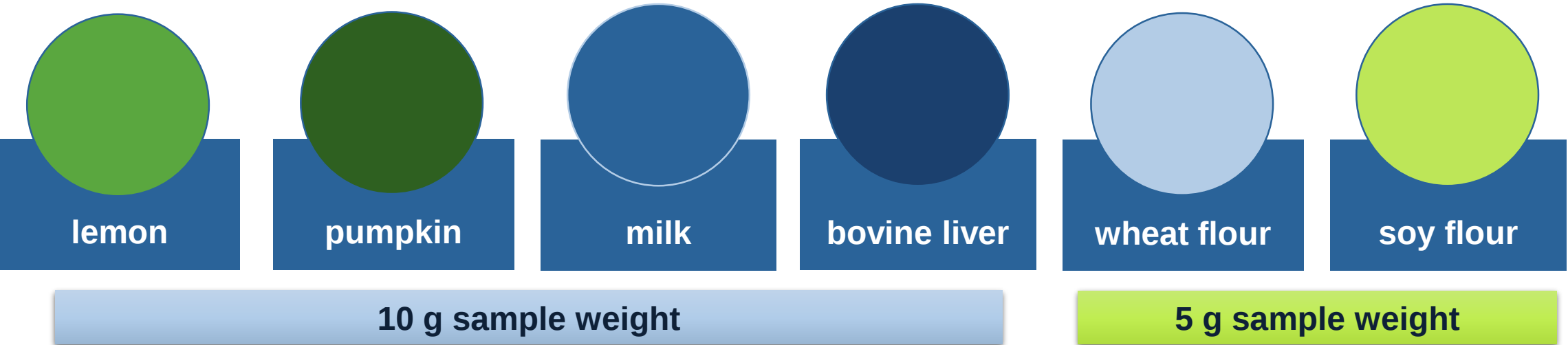


LC-Methods

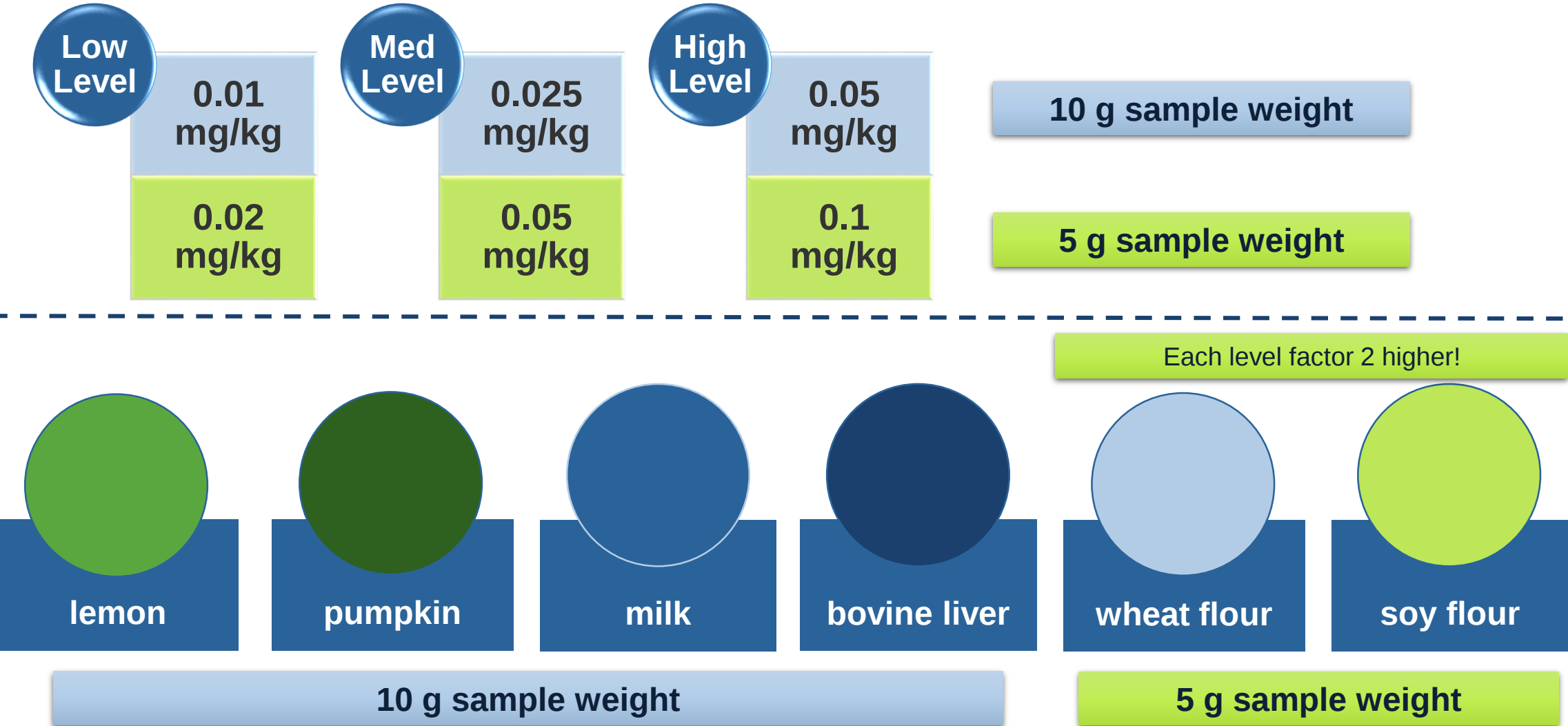
For details on LC methods, the used eluents and gradients,
see in the QuPPe document:

[QuPPe Doc](#)

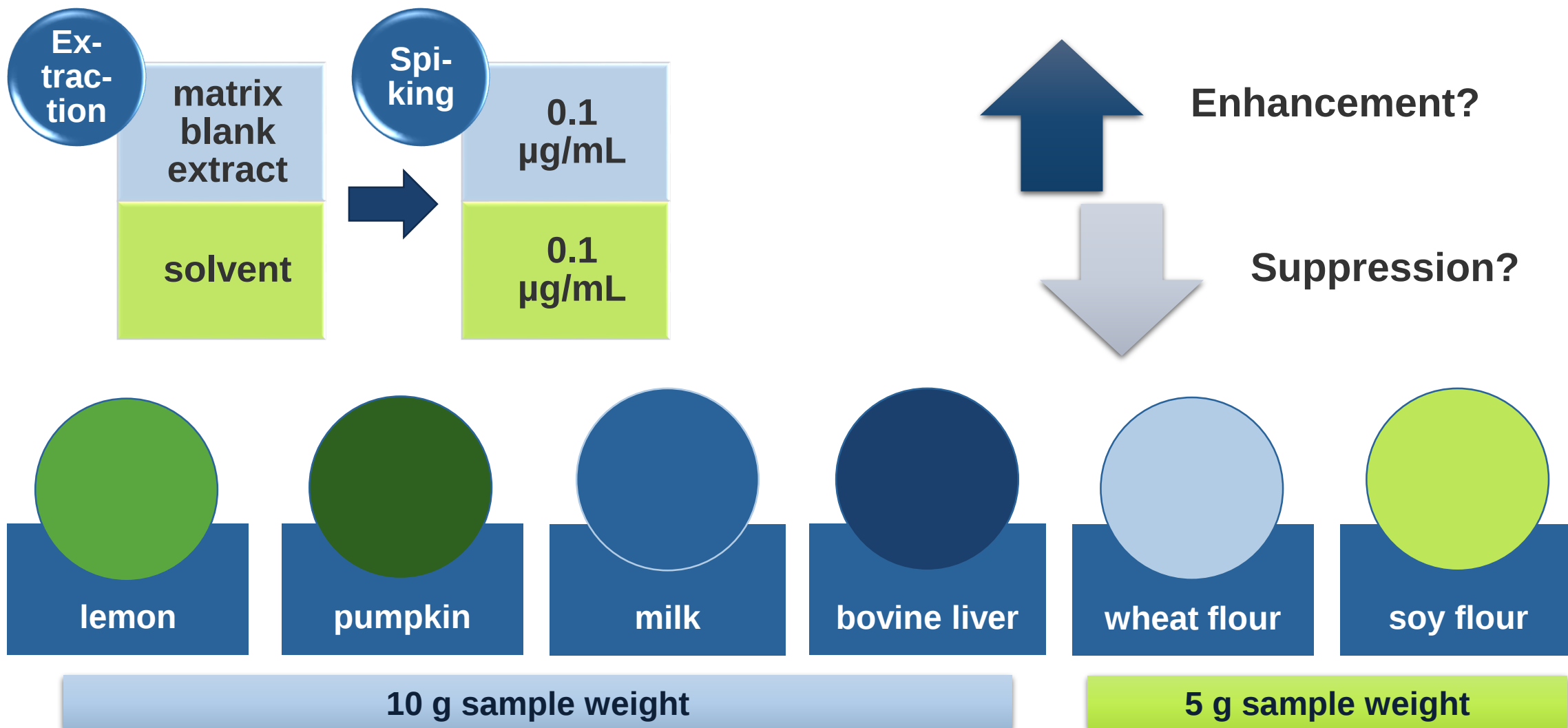
Validation Study



Validation Study

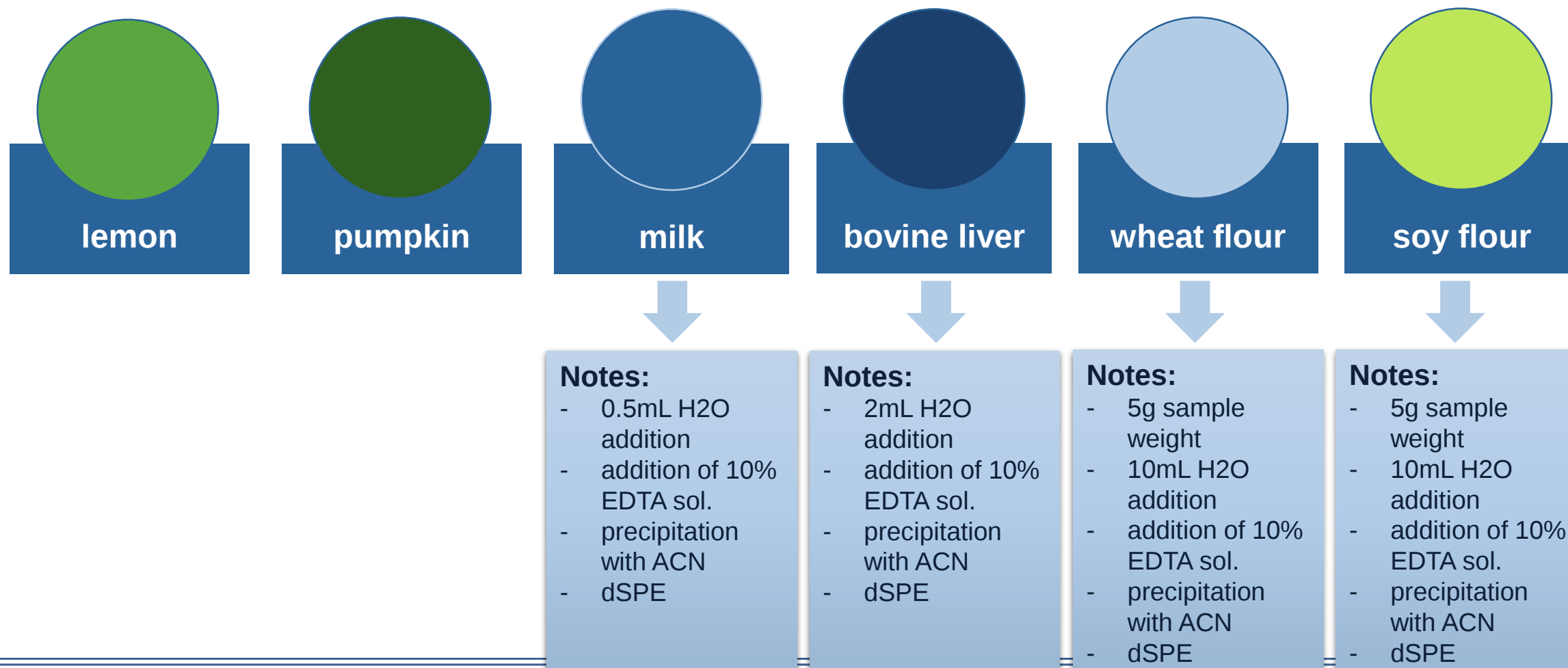


Study of matrix effects

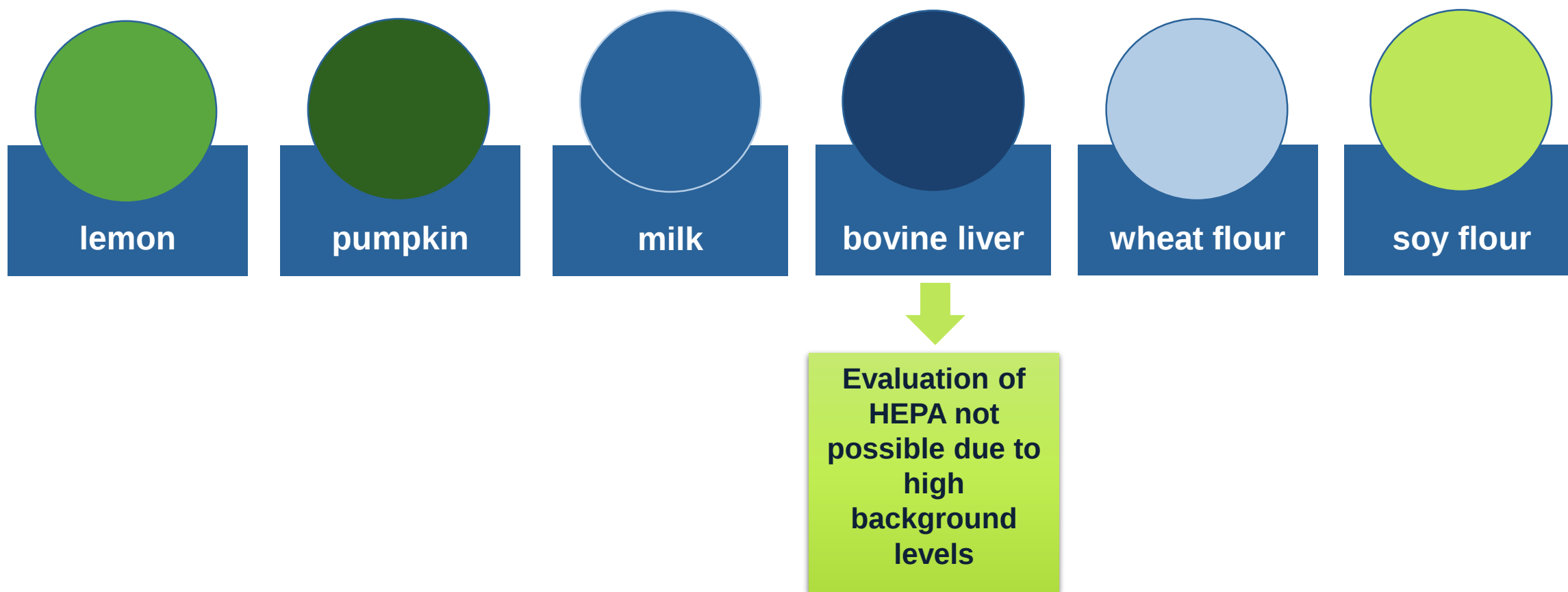


QuPPe extraction procedure

[QuPPe Doc](#)



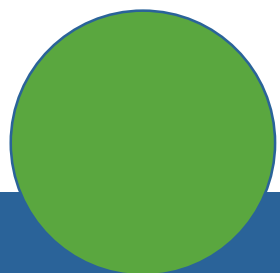
QuPPe extraction procedure



APPC – Successful Low Level Validation of...

Low Level = 0.01 mg/kg

Low Level = 0.02 mg/kg

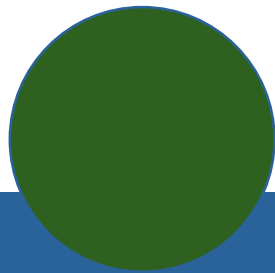


lemon

Compounds:

- ✓ glyphosate
- ✓ AMPA
- ✓ NA-glyphosate
- ✓ fosetyl
- ✓ ethephon
- ✓ HEPA

6/9

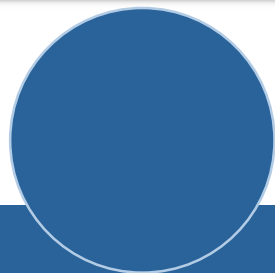


pumpkin

Compounds:

- ✓ glyphosate
- ✓ NA-glyphosate
- ✓ MPPA
- ✓ NA-glufosinate
- ✓ fosetyl
- ✓ HEPA

6/9

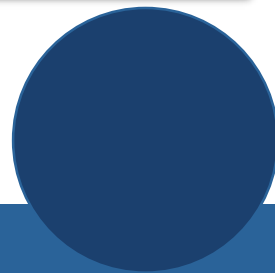


milk

Compounds:

- ✓ glyphosate
- ✓ AMPA
- ✓ MPPA
- ✓ NA-glufosinate
- ✓ fosetyl
- ✓ ethephon
- ✓ HEPA

7/9

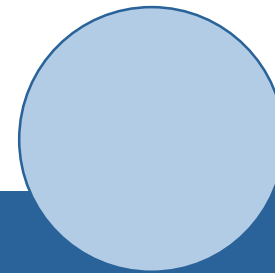


bovine liver

Compounds:

- ✓ AMPA
- ✓ MPPA
- ✓ NA-glufosinate
- ✓ fosetyl
- ✓ ethephon

5/8

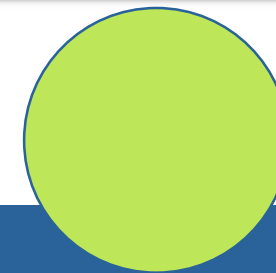


wheat flour

Compounds:

- ✓ MPPA
- ✓ NA-glufosinate
- ✓ fosetyl
- ✓ HEPA

4/9



soy flour

Compounds:

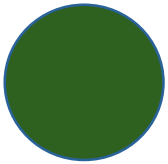
- ✓ MPPA
- ✓ fosetyl
- ✓ HEPA

3/9

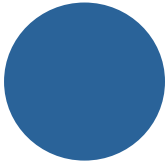
APPC – Matrix effects



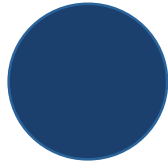
lemon



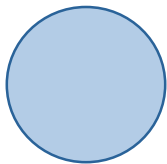
pumpkin



milk



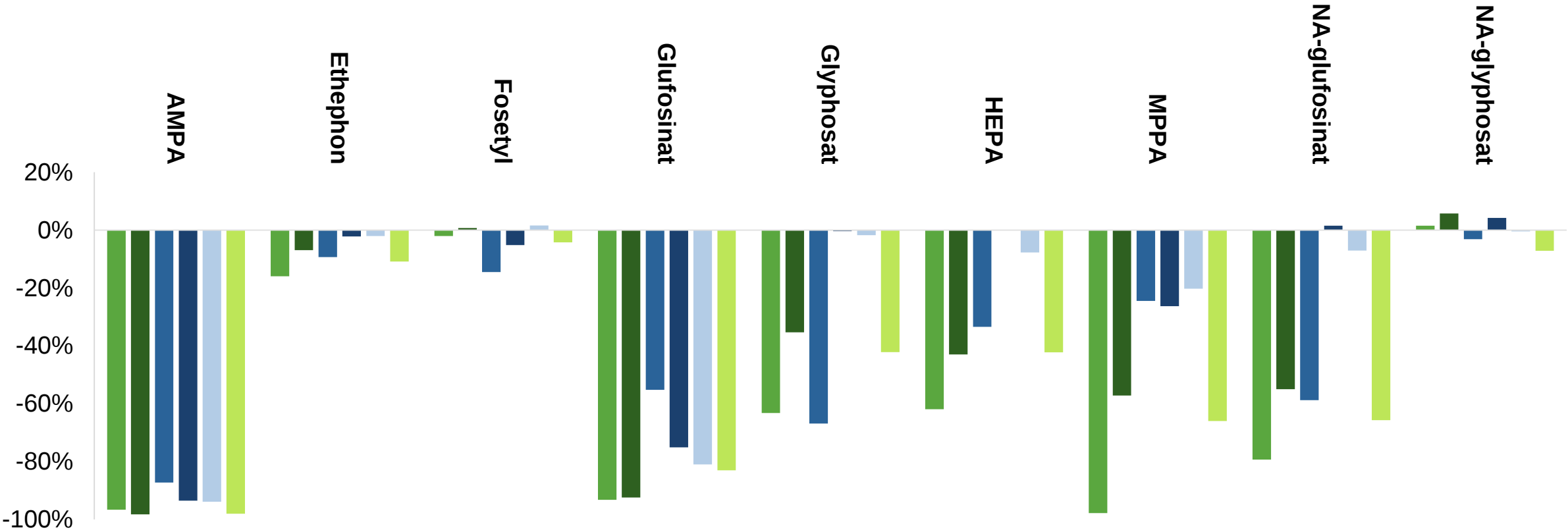
bovine liver

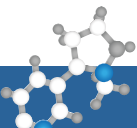


wheat



soy flour





APPC – Notes, Pros and Cons

Pros

good longtime performance:
>2000 samples per column

no issues when not used for a while

Initial conditioning of column optional;
usually not required

Cons

odd peakshape of NA-glyphosate

needs long column equilibration (>5min) after
each run

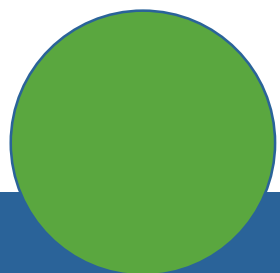
Further Notes

- most experience with this column

LunaPP – Successful Low Level Validation of...

Low Level = 0.01 mg/kg

Low Level = 0.02 mg/kg

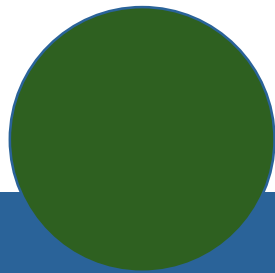


lemon

Compounds:

- ✓ glyphosate
- ✓ AMPA
- ✓ NA-glyphosate
- ✓ glufosinate
- ✓ MPPA
- ✓ NA-glufosinate
- ✓ fosetyl
- ✓ ethephon
- ✓ HEPA

9/9

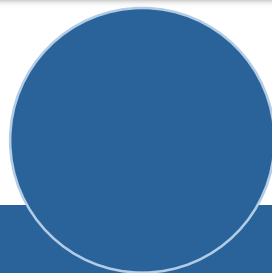


pumpkin

Compounds:

- ✓ glyphosate
- ✓ glufosinate
- ✓ NA-glufosinate
- ✓ fosetyl
- ✓ ethephon

5/9

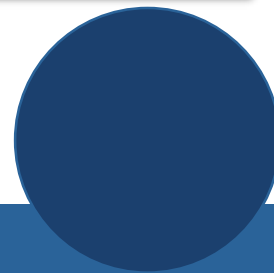


milk

Compounds:

- ✓ AMPA
- ✓ NA-glyphosate
- ✓ MPPA
- ✓ fosetyl
- ✓ ethephon
- ✓ HEPA

6/9

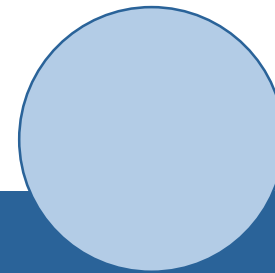


bovine liver

Compounds:

- ✓ glyphosate
- ✓ NA-glyphosate
- ✓ MPPA
- ✓ NA-glufosinate
- ✓ fosetyl
- ✓ ethephon

6/8

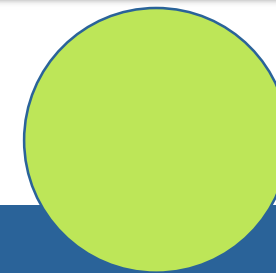


wheat flour

Compounds:

- ✓ glyphosate
- ✓ MPPA
- ✓ NA-glufosinate
- ✓ fosetyl

4/9



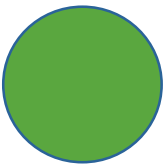
soy flour

Compounds:

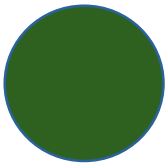
- ✓ NA-glufosinate
- ✓ fosetyl
- ✓ HEPA

3/9

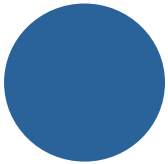
LunaPP – Matrix effects



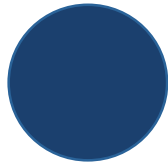
lemon



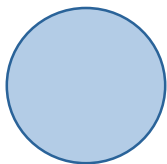
pumpkin



milk



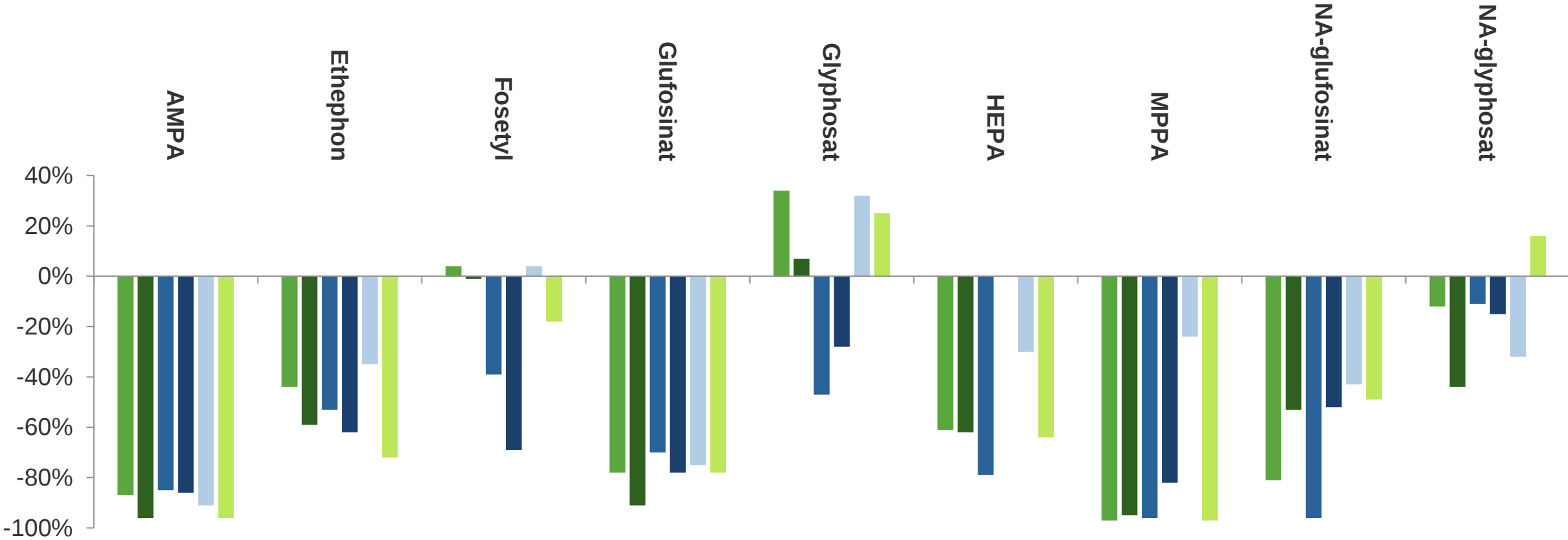
bovine liver

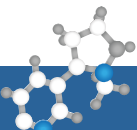


wheat



soy flour





LunaPP – Notes, Pros and Cons

Pros

same eluents for ,Gly&Co‘ and ,PerChloPhos‘

,flexible‘: can be used in reversed mode
and for cationic pesticides
(not tested thoroughly in our lab)

Cons

needed conditioning/passivation

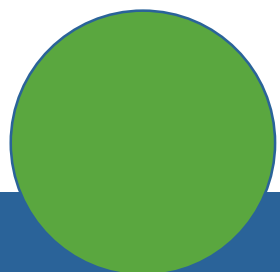
Further Notes

- newest column, least experience

Restek Polar X – Successful Low Level Validation of...

Low Level = 0.01 mg/kg

Low Level = 0.02 mg/kg

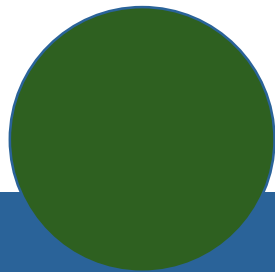


lemon

Compounds:

- ✓ glyphosate
- ✓ fosetyl
- ✓ ethephon

3/9

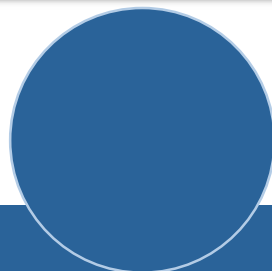


pumpkin

Compounds:

- ✓ glufosinate
- ✓ MPPA
- ✓ NA-glufosinate
- ✓ fosetyl
- ✓ ethephon
- ✓ HEPA

6/9

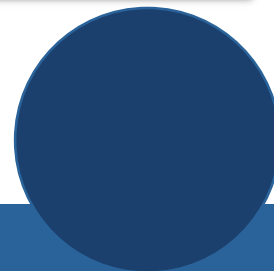


milk

Compounds:

- ✓ AMPA
- ✓ glufosinate
- ✓ NA-glufosinate
- ✓ fosetyl
- ✓ ethephon
- ✓ HEPA

6/9

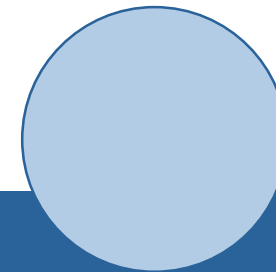


bovine liver

Compounds:

- ✓ AMPA
- ✓ glufosinate
- ✓ fosetyl
- ✓ ethephon

4/8

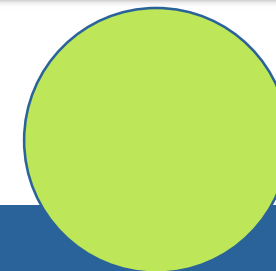


wheat flour

Compounds:

- ✓ glufosinate
- ✓ MPPA
- ✓ NA-glufosinate
- ✓ fosetyl
- ✓ ethephon
- ✓ HEPA

6/9



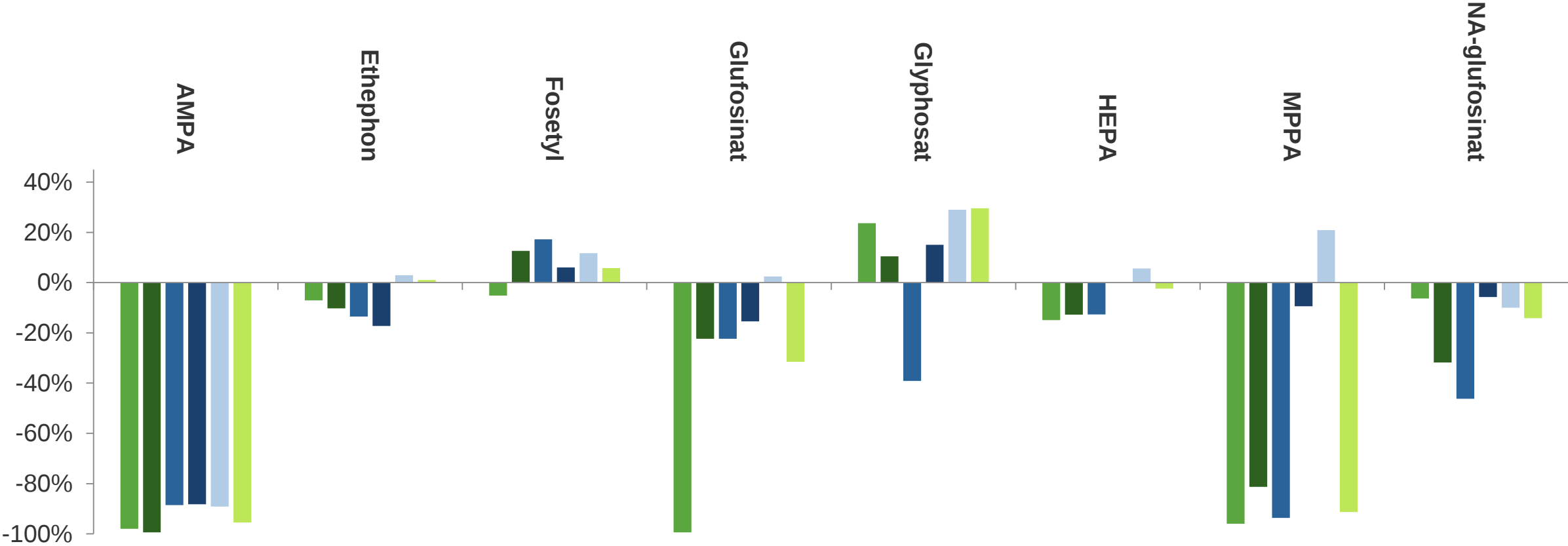
soy flour

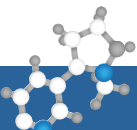
Compounds:

- ✓ AMPA
- ✓ glufosinate
- ✓ MPPA
- ✓ fosetyl
- ✓ ethephon
- ✓ HEPA

6/9

Restek Polar X – Matrix effects





Restek Polar X – Notes, Pros and Cons

Pros

low or moderate suppression, except for
AMPA, MPPA

same eluents for ,Gly&Co‘ and ,PerChloPhos‘

Cons

no retention or elution (?) of NA-glyphosate

needs conditioning with medronic acid!

poor performance in matrices with high acidic
or ionic (?) content (lemon),
due to ion exchange groups?

issues when not used for a while

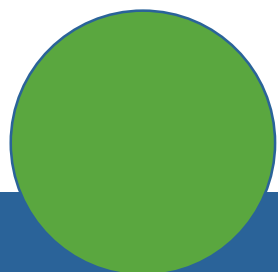
Further Notes

- issues during measurement with the stability of retention times
- new phase material expected next year

Obelisc N – Successful Low Level Validation of...

Low Level = 0.01 mg/kg

Low Level = 0.02 mg/kg

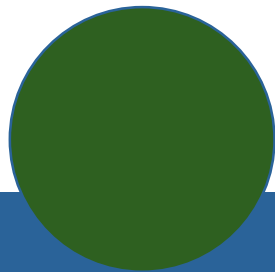


lemon

Compounds:

- ✓ glyphosate
- ✓ NA-glyphosate
- ✓ glufosinate
- ✓ NA-glufosinate
- ✓ ethephon
- ✓ HEPA

6/9

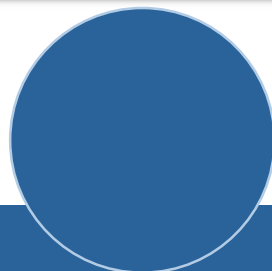


pumpkin

Compounds:

- ✓ glyphosate
- ✓ MPPA
- ✓ NA-glufosinate

3/9

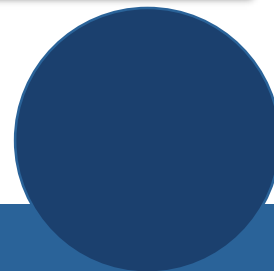


milk

Compounds:

- ✓ glyphosate
- ✓ NA-glyphosate
- ✓ MPPA
- ✓ NA-glufosinate
- ✓ ethephon

5/9

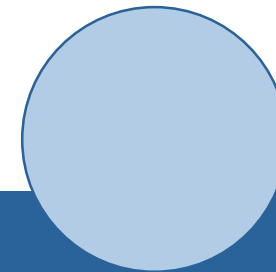


bovine liver

Compounds:

- ✓ MPPA
- ✓ NA-glufosinate

2/8

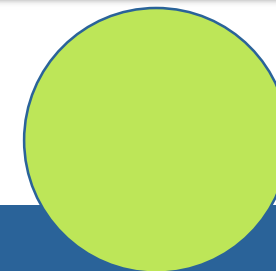


wheat flour

Compounds:

- ✓ AMPA
- ✓ NA-glyphosate
- ✓ MPPA
- ✓ NA-glufosinate
- ✓ ethephon
- ✓ HEPA

6/9

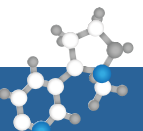


soy flour

Compounds:

- ✓ glyphosate
- ✓ NA-glufosinate
- ✓ ethephon

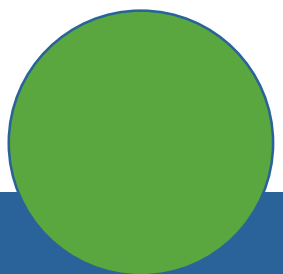
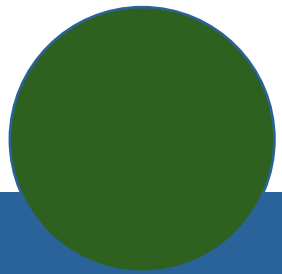
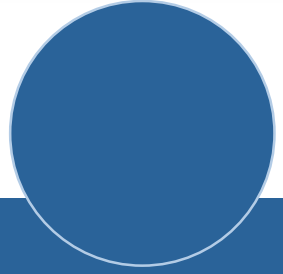
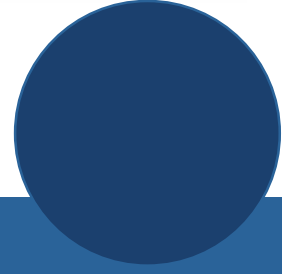
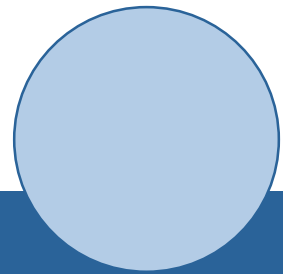
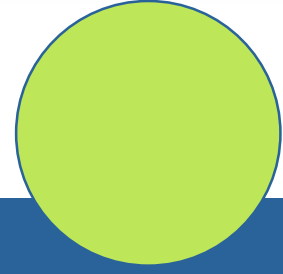
3/9



Obelisc N – Successful Low Level Validation of...

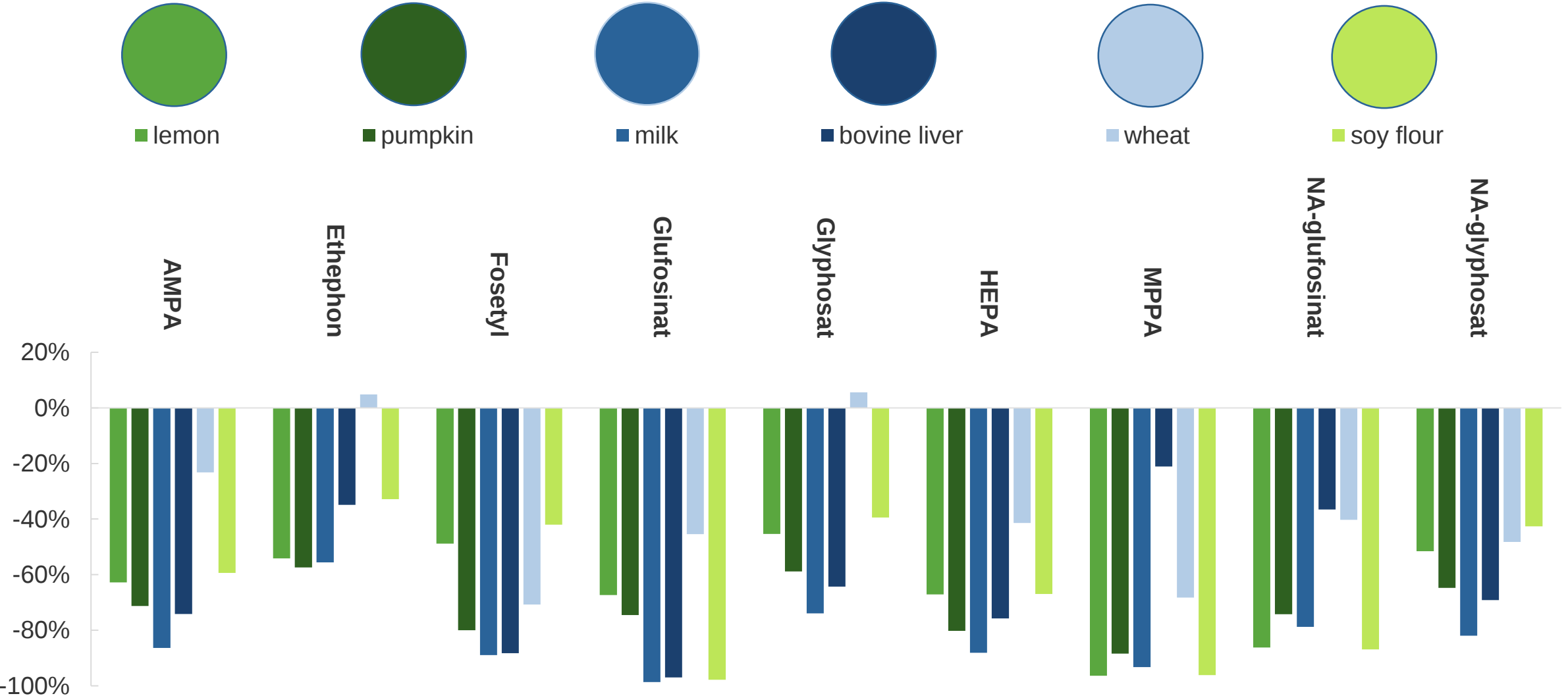
Low Level = 0.01 mg/kg

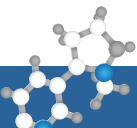
Low Level = 0.02 mg/kg

					
lemon	pumpkin	milk	bovine liver	wheat flour	soy flour
Compounds: ✓ glyphosate ✓ NA-glyphosate ✓ glufosinate ✓ NA-glufosinate ✓ ethephon ✓ HEPA	Compounds: ✓ glyphosate ✓ MPPA ✓ NA-glufosinate	Compounds: ✓ glyphosate ✓ NA-glyphosate ✓ MPPA ✓ NA-glufosinate ✓ ethephon	Compounds: ✓ MPPA ✓ NA-glufosinate	Compounds: ✓ AMPA ✓ NA-glyphosate ✓ MPPA ✓ NA-glufosinate ✓ ethephon ✓ HEPA	Compounds: ✓ glyphosate ✓ NA-glufosinate ✓ ethephon

6 issues with the measurement of fosetyl (background)

Obelisc N – Matrix effects





Obelisc N – Notes, Pros and Cons

Pros

no conditioning of the column required

Best retention of AMPA

same eluents for ,Gly&Co‘ and ,PerChloPhos‘

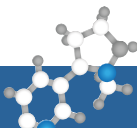
Cons

no signal of glufosinate in matrix detected at all in this study

strong suppression for all compounds

Further Notes

- issues with the measurement of fosetyl (background)



Summary, outlook and perspective

- each column has benefits and drawbacks; there is still not one for everything

Outlook

- study of matrix effects in different dilution steps (current internship)
- validation study on chlorate, perchlorate, phosphonic acid ...
(still looking for suitable sample material)
- new column phase of Raptor Polar X probably next year
- a new column?
- a more sensitive instrument???

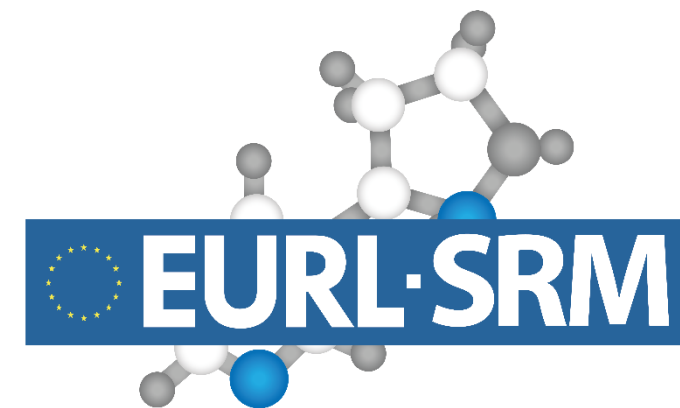
Acknowledgement

Scientific internship of Sedef Torun



Acknowledgement

The Pesticides Team



Chemisches und
Veterinäruntersuchungsamt
Stuttgart

Thank You for Your Attention



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