News on Single Residue Methods

1) M. Anastassiades (various aspects), slides 1-93
2) E. Eichhorn (Screening of DTC-markers), slides 94-150
3) H. Zipper (Derivatisation of DTCs), slides 150-179

Contributions by the entire Pesticide Residues Team

European Union Reference Laboratory for Pesticides requiring Single Residue Methods,
located at the Chemical and Veterinary Analysis Agency (CVUA) Stuttgart, Fellbach, Germany

Overview of Current Activities

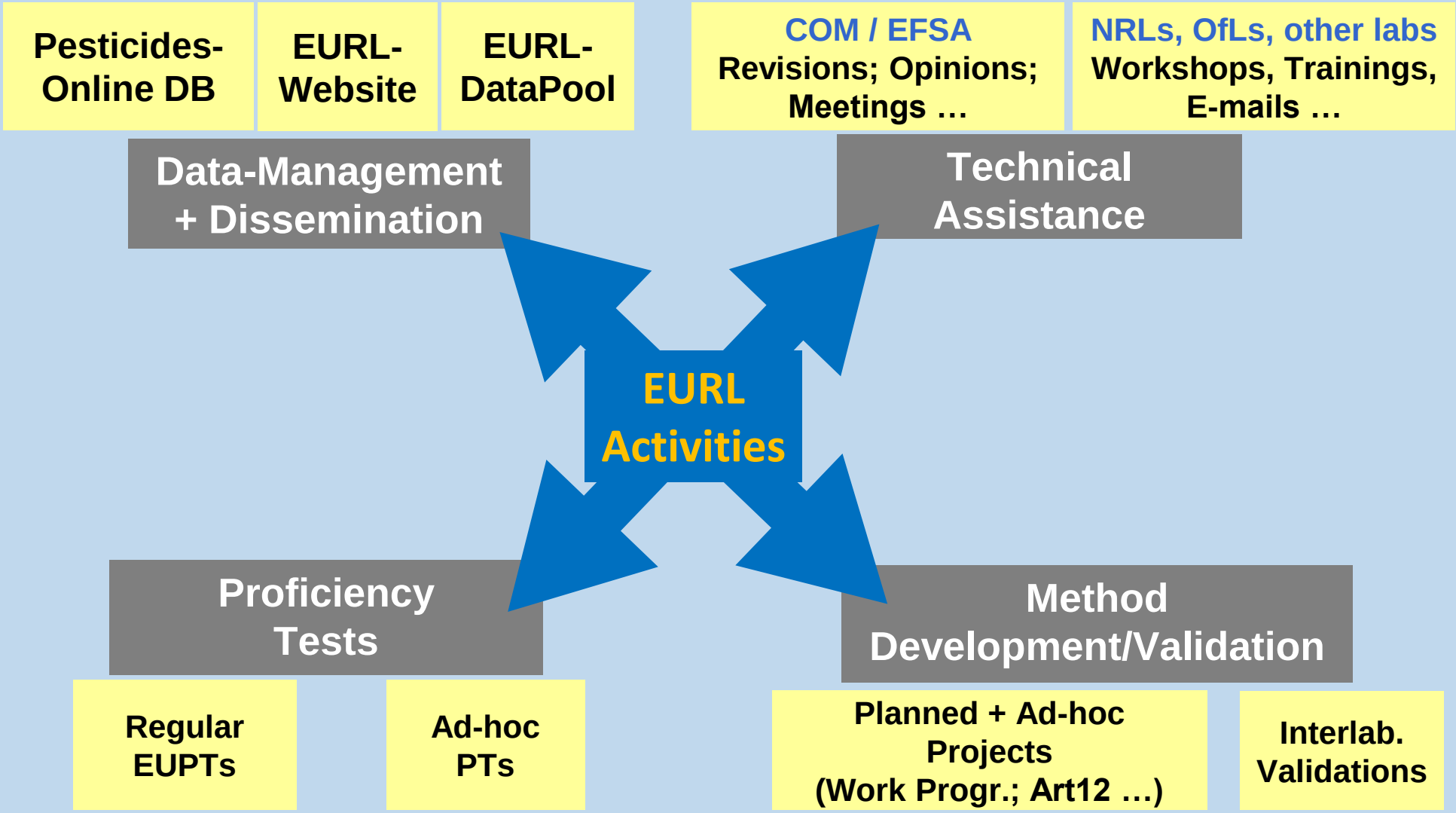
Michelangelo Anastassiades

Enabling a more judicious and efficent analysis of alkylene-bis-DTC fungicides by screening for marker substances

Eric Eichhorn

Facing analytical challenges DTC analysis – step by step

Hubert Zipper


Pesticides-Online DB
EURL-Website
EURL-DataPool
COM / EFSA
Revisions; Opinions; Meetings …
NRLs, OfLs, other labs
Workshops, Trainings, E-mails …
Data-Management + Dissemination
Technical Assistance
EURL Activities
Proficiency Tests
Regular EUPTs
Ad-hoc PTs
Method Development/Validation
Planned + Ad-hoc Projects (Work Progr.; Art12 …)
Interlab. Validations

Technical Assistance to COM/EFSA

Pesticide (re-)evaluations

MRL-Re-evaluations (periodic): Art. 12 of Reg. 396/2005/EC

MRL-Re-evaluations in case of **exposure concerns**: Art. 43 of Reg. 396/2005

Renewal Assessments: Art. 12 of Reg. 1107/2009/EC (Reg. 844/2012/EC)

Other (e.g. Background levels evaluation, Evaluation of substances not requiring MRLs …)

EURL-Input

EFSA	**EFSA**	**COM**	**COM**
Member State-Consultation	Draft Reasoned Opinion	Consultation	Draft Regulation

Technical Assistance to COM/EFSA

Pesticide (re-)evaluations (Art. 12, Art. 43, New Active Substances,..)

- **Check proposed RDs & suggest improvements if needed**
  - **Analytical feasibility** = monitorability (can RD be reasonably covered using typical routine instrumentation, are necessary analytical standards commercially available …),
  - **Specificity** (check if analytes can originate from other sources)
  - **Plausibility** (view residue findings and check whether proposed markers are all relevant and if other markers are more relevant; check lipophilicity for „(F)“ symbol)
- **Propose analytically feasible LOQs** for main commodity groups (used for setting the MRL*s)
- Prepare **Draft Evaluation Report** and submit to EFSA
- Prepare **Comments on Draft Reasoned Opinion** (drRO) and submit to EFSA
- Address open points during preparation of **draft Reg. by COM**
- Comment on draft Regulation (sometimes)

Technical Assistance to COM/EFSA

Pesticide (re-)evaluations

In 2022-23 alone *(until end of August '23)*

→ **136 Requests** by COM / EFSA, concerning **111 Active Substances**

Requests by EFSA

coordinated by

EURL-SRM

Requests by COM

coordination shared between

EURL-SRM & EURL-CF

Technical support to DG-SANTE and EFSA

Evaluation / re-evaluation of pesticides (Context: Art.12 of Reg. 396/2005 and other)

Active Substance	Request						Experiments conducted				
	EFSA				COM		Method developm.	Metabolite(s) to consider	Standard valid.(s)	Hydrolysis valid./exper.	Other
	ER	dRO	DAR	RAR	LOQs	Other					
(Z)-13-Hexadecen-11yn-1-ylacetate					x						
(Z,Z,Z,Z)-7,13,16,19-Docosatetraen-1-ylisoburyrate					x						
1-Methylcyclopropene (1-MCP)				x							
2-Phenylphenol				x						x	
8-Hydroxyquinoline				x							x
Acetolachlor		x				x					
Acrinathrin					x						
Azimsulfuron					x						
Azocyclotin	x	x									x
Bacillus paralicheniformis strain FMCH001			x								
Bensulfuron-methyl				x							
Benzobicyclon			x								
Bifenazate					x						
Bifenthrin	x	x									
Bispyrac					x						
Bixlozone			x				x		x		
Buprofezin				x							
Calcium phosphide					x						
Capric acid (decanoic acid)				x							
Caprylic acid (octanoic acid)				x							
Carbetamide					x						
Carboxin plus 2 metabolites					x						
Chlorfenapyr	x	x									
Chlorsulfuron					x						
Clove oil				x							
Cyhexatin	x	x									x
Cypermethrins		x			x						
Cyproconazole					x						
Cyromazine					x						
Deltamethrin					x						
Desmedipham					x			x	x		
Diazinon	x	x									
Dicofol	x	x					x	x	x		
Diethofencarb					x						
Difenacoum					x						
Difenoconazole	x	x									
Dimetholachlor				x							
Dithianon		x			x						
Dithiocarbamates		x					x		x		
Diuron					x						
Elemental iron			x								
Endosulfan	x	x									
Epoxiconazole					x						
Etridiazole					x						
Eugenol				x							
Famoxadone					x						
fat distillation rersidues				x							
Fenamiphos					x		x		x		
Fenarimol	x	x									
Fenbuconazole					x						
Fenoxycarb					x						
Fenpropathrin	x	x									
Fluazaindolizine			x						x		
Flurochloridone				x							

Active Substance	Request						Experiments conducted				
	EFSA				COM		Method developm.	Metabolite(s) to consider	Standard valid.(s)	Hydrolysis valid./exper.	Other
	ER	dRO	DAR	RAR	LOQs	Other					
Flutriafol					x						
Fosthiazate				x							
gamma-Cyhalothrin	x							x	x		
Glufosinate					x			x	x		
Halosulfuron-methyl				x							x
Haloxyfop-P	x	x								x	
Indolylbutyric acid				x							
Indoxacarb	x	x							x		x
Isopyrazam					x						
Isoxaben					x	x			x		
Lemon essential oil					x						
Lufenuron					x						
Maltodextrin				x							
MCPA				x						x	
MCPA-Thioethyl				x						x	x
MCPB				x						x	
Mecoprop-P				x							
Metalaxyl					x			x	x	x	
Methyl-nonylketone					x						
Metosulam					x						
Metyltetraprole			x								
Myclobutanil					x			x	x	x	
Nicotine					x	x			x		x
Novaluron					x	x					
Oryzalin					x						
Oxamyl					x	x					
Oxasulfuron					x						
Paraffin oil				x							
Penconazole				x							
Pencycuron					x						
Penoxsulam					x						
Penthiopyrad+PAM					x						
Phosmet	x	x			x						
Phosphane/phosphine				x					x		
Potassium permanganate					x						
Prochloraz					x						
Profenofos	x	x									
Profoxydim					x						
Proquinazid				x							
Pyrethrins				x							
Pyriproxyfen		x			x			x	x		
Quinoxyfen					x						
Sodium aluminium silicate					x						
Sodium hypochlorite					x						
Sodium silver thiosulfate					x						
Spirodiclofen					x			x	x		x
Tee tree oil				x							
Teflubenzuron					x						
Tetraconazole					x	x					
Thiabendazol					x			x	x		
Thymol				x			x		x		
Topramezone					x				x		
Triazoxide					x						
Trifloxystrobin					x			x	x		
Triflumizole					x						
Triflumuron					x						
Zoxamide	x	x					x	x	x		

Evaluation Report

Prepared under Article 12 of Regulation (EC) No 396/2005

6 February 2023

Example Zoxamide

Analytical validations by the EURLs and capability of official laboratories to be considered for the review of the existing MRLs for zoxamide

efsa JOURNAL

REASONED OPINION

Draft-RO

Circulated for Consultation

EU-MSs and EURLs

APPROVED: dd Month 20YY

doi:10.2903/j.efsa.20YY.NNNN

Review of the existing maximum residue levels for zoxamide according to Article 12 of Regulation (EC) No 396/2005 and setting of an import tolerance for onions, garlic and shallots

European Food Safety Authority (EFSA)

Authors' list

-DRAFT-

Example Zoxamide

Report on the MS Consultation for EFSA-Q-2008-00649, EFSA-Q-2019-00404

EFSA Question Number:	EFSA-Q-2008-00649, EFSA-Q-2019-00404
Type of assessment:	MRL Review (Reg. 396/2005 – Art. 12.2, Art 10)
Active Substance:	zoxamide
Subject:	Review of the existing MRLs for zoxamide and setting of an import tolerance for onions, garlic and shallots
Commenting period started on:	24 July 2023
Commenting period ended on:	
Comments received from:	
Comments evaluated by EFSA on:	
Pesticides MRL Expert Meeting on:	
Document finalised on:	

No.	*Reference*	*MS Comment*	*EFSA Co*
1.	Summary and Section 2 and Conclusions	EURLs Please take notice of the **updated ER** by the EURLs, introducing new validation data for zoxamide in **honey**. Also, validation data for the metabolite RH-141452 in the four main matrix groups of plant origin as well as in liver and milk as well as validation data for RH-141455 in high water content commodities is provided.	

Example Zoxamide

Evaluation Report

Prepared under Article 12 of Regulation (EC) No 396/2005

6 February 2023
Update: 31 August 2023

Analytical validations by the EURLs and capability of official laboratories to be considered for the review of the existing MRLs for zoxamide

Considering Metabolites RH-141452 RH-141452

Analysis of Fluoride

FLUORIDE

Anthropogenic sources

- Coal burning, steel production, brick manufacuring, fertilizer production
- Contained in a variety of products, e.g. tooth paste, table salt (to prevent caries)

Natural Presence

- Minerals (apatite, fluorite, fluorapatite, cryolith …)
- Water (fluoride ion, hexafluorosilicate complex …), higher levels in calcium deficient waters

Uptake by all living organisms.

Background levels in food
need to be considered when setting MRLs !!

Pesticide Use

Degradation product of **sulfuryl fluoride**

- Approved A.S. within the EU
- Fumigant for disinfestation of dry products, e.g. before transportation, storage, packing, custom clearance
- Separate MRLs for sulfuryl fluoride & fluoride anion [recently revised by Reg. (EU) 2022/1321],

Several MRLs were lowered!

	Old MRL (mg/kg)	**New MRL** (mg/kg)
Coconuts	30	15
Cocoa beans	10	5
Animal tissues	1	0.3
Fruits and vegetables	2	0.2

Determination of fluoride ions in food

➢ Protocol for Fresh Fruits and Vegetables:
„DIRECT“ measurement approach

Measurement by Ion-Selective Electrode (ISE)

SevenCompact pH/ion meter & PerfectION™ comb F electrode; Mettler Toledo

Weigh sample homogenate into a 50 mL centrifuge tube
Fruit and vegetables: 10 g ± 0.1 g;
Dry commodities: 5 g ± 0.05 g

ADJUST WATER CONTENT of sample to 10 mL (Mandatory as no IL-IS can be used)
e.g. +0.5 mL for matrices with 90% water content; +2 mL water to 10 g potato; + 3.5 mL water to 10 g garlic; +10 mL to dry commodities

ADD EXTRACTION SOLVENT (10 mL methanol containing 1 % formic acid)

SHAKE thoroughly for 1 min

Preferably FREEZE-OUT extract until completely frozen

Standard QuPPe protocol

preferably cryogenic centrifugation (e.g. at -10 °C)
(if centrifuge is not refrigerated, swiftly proceed with centrifugation and the following step to avoid redissolvation of matrix)

dSPE to Remove Lipids for High Oil Content samples (e.g. avocado):
(this step may be skipped if sample was centrifuged frozen at ≤ -10 °C and ≥ 20 min)

TRANSFER 4 mL raw extract into a tube containing 200 mg **C_{18}-sorbent,**
SHAKE for 1 min and **CENTRIFUGE** (>3,000 g for 5 min)

WITHDRAW SUPERNATANT AND FILTER it into a plastic vessel suitable for measurement
(use syringe filter of 0.2 µm pore size; e.g. H-PTFE)
(withdraw cold supernatant quickly after centrifugation to avoid that matrix components redissolve)

Take an aliquot, e.g. 2 mL, and add 2 mL TISAB II

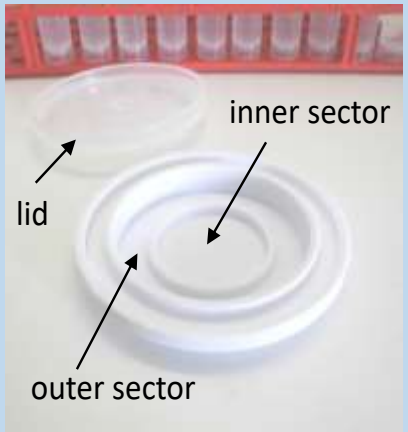
Measurement by ISE

Determination of fluoride ions in food

➢ Protocol for Dry Commodities

(also applicable to wet commod. dried within the cell)

Procedure using Microdiffusion Cells:



Measurement using an Ion-Selective Electrode (ISE) after microdiffusion

SevenCompact pH/ion meter & PerfectION™ comb F electrode; Mettler Toledo

Weigh sample homogenate into the outer sector of the microdiffusion cell
Dry commodities: 0.5 g ± 0.005 g

↓

Fill the inner sector of the microdiffusion cell with 1 mL 0.1 M NaOH (Fluoride trapping solution)

↓

Add 4 mL HMDS-saturated 5 M $HClO_4$ solution to the sample homogenate in the outer sector of the microdiffusion cell (Diffusion solution)

↓

Close the cell gas tight by using Vaseline on the lid

↓

Fluoride diffusion:
a) 48 h at room temperature
OR b) 5 h at 50 °C

↓

Add 1 mL TISAB II solution to the trap solution in the inner sector

↓

Transfer the solution into a vessel suitable for ISE measurement, e.g. tapered 10 mL or 50 mL-centrifugal tube

↓

Measurement by ISE

Determination of fluoride ions in food

Impact of Temperature to Speed up Microdiffusion

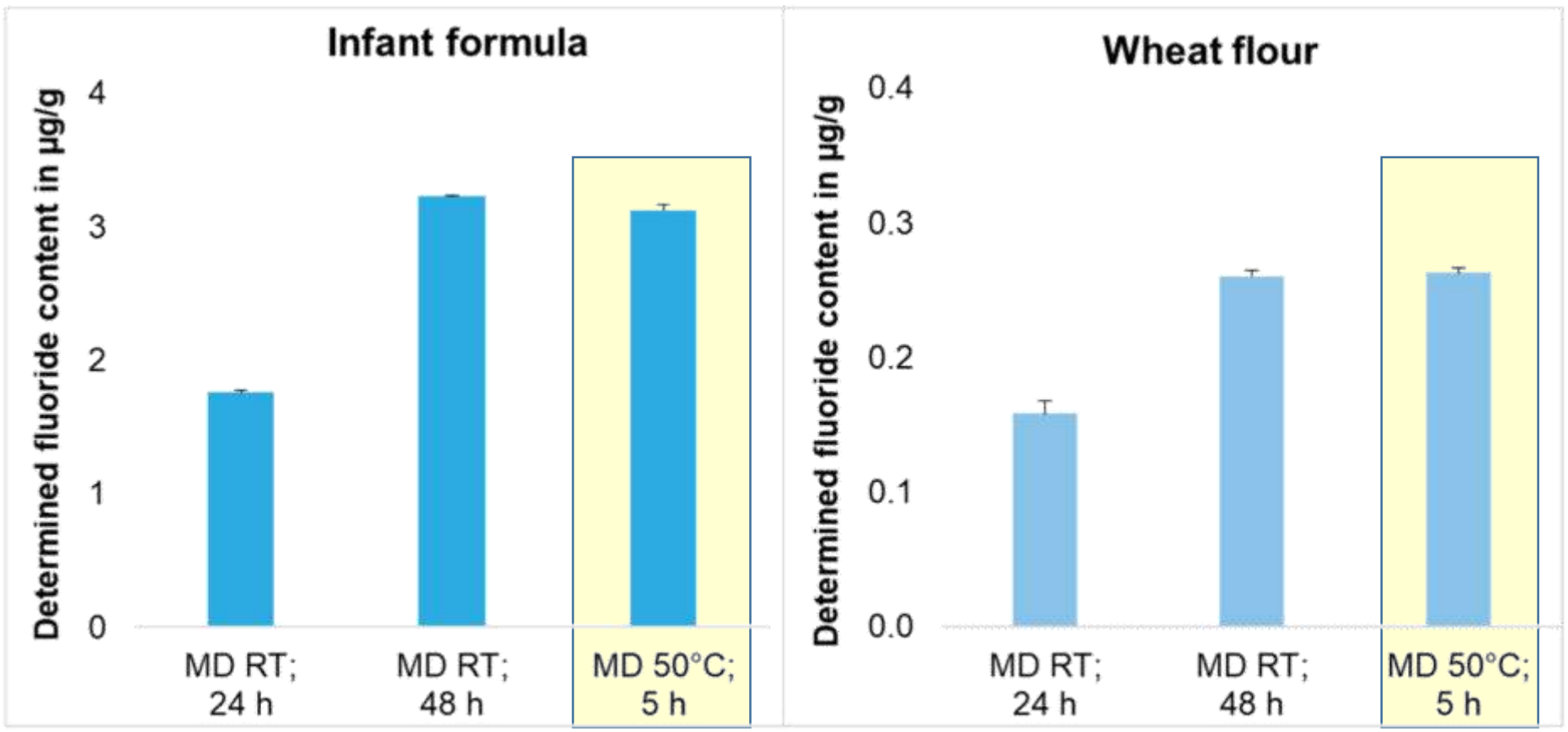
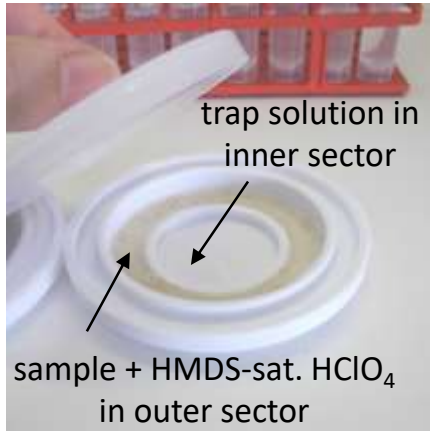
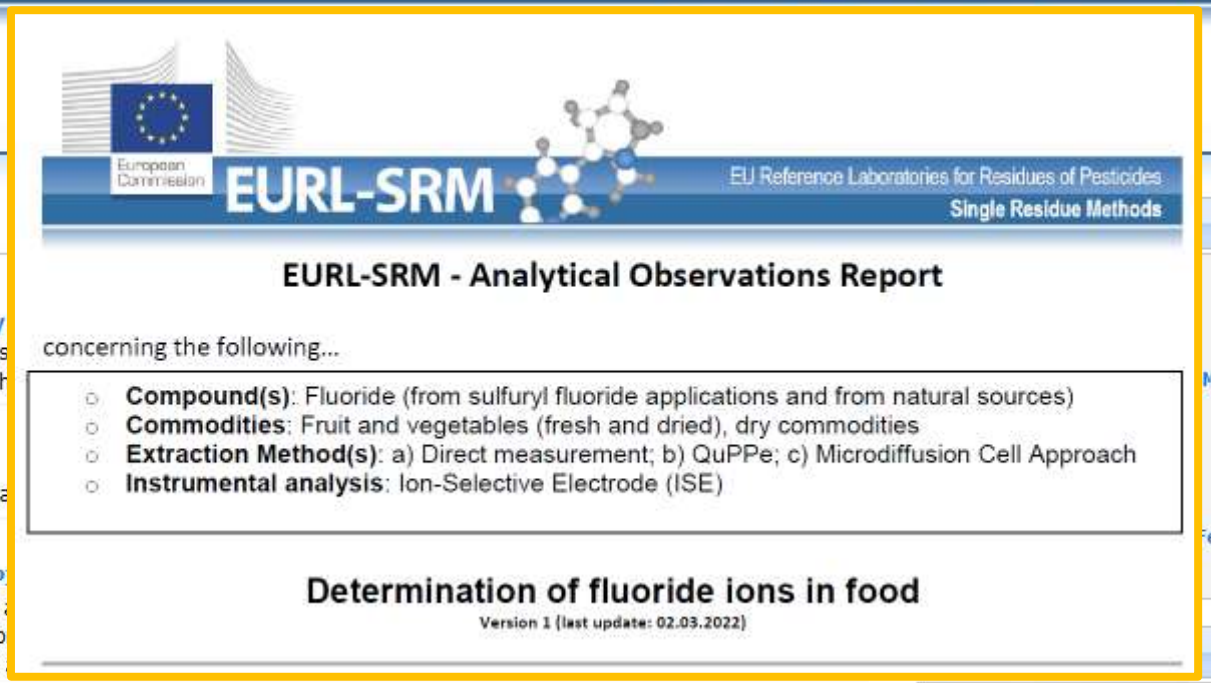


Figure 6: *Comparison of measurement and microdiffusion conditions for infant formula powder and wheat flour. MD = microdiffusion; MeOH-FA = methanol containing 1% formic acid.*





Topics

EURL-SRM Network
NRL-SRM Network

Proficiency Tests
EUPT-SRM Overview
EUPT-SRM18 (Honey)
EUPT-SRM17 (Tomatoes)
EUPT-SRM16 (Sesame)
EUPT-SRM15 (Rice)

Workshops
Workshop Overview
Joint Workshop 2023
Joint EURL/NRLs (SRM-FV) 2022

Services
ILISs Distribution
CheckYourScope
SRM-PinBoard
EURL-SRM Methods
Analytical Observations
Residue Observations
Downloads
Sources of Standards

Internet
EURL DataPool
QuEChERS - Website
QuPPe - Website
PestiPedia

Data Submission

Latest News

19-04-2023 | EURL-SRM
Risk of False Positives of Chloridazon-Desphenyl in Honey by
A new EURL-SRM Analytical Observations Report concerning the ris
Various chromatographic separation methods for chloridazon-desph

17-03-2023 | EURL-SRM
QuPPe-PO-Method Version 12.1
The QuPPe-PO-Method has been updated (now includes more deta

16-03-2023 | EURL-SRM
Analysis of the Folpet and Captan degradants Phthalimide (P
The Analytical Observation Report (SRM-49) on the analysis of PI
update also includes results of experiments concerning the transfo
Phthalimide (PI), during various steps of the QuEChERS procedure

03-03-2023 | EURL-SRM
Determination of fluoride ion in food
Two approaches for the determination of Fluoride Ion via selective electrodes (ISE) are described: a) direct measurement in QuPPe extracts and b) measurement in diffusates derived by microdiffusion.

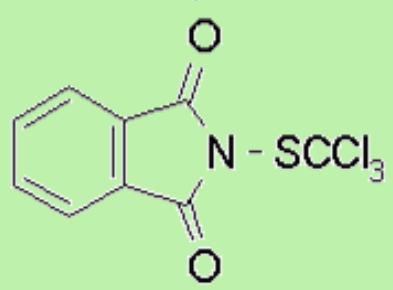
27-02-2023 | EURL-SRM
Compilation of Residue Observations Reports of QuPPe Compounds
A new compilation of residue findings of QuPPe compounds in food products, analysed in 2022, was uploaded. The report additionally encompasses findings of ethylene oxide / 2-chloroethanol. Aim of these annual compilations of residue findings is to help OfLs to localize analyte/matrix combinations that are worthwhile monitoring.

24-02-2023 | EURL-SRM
New Analytical Observations Report on QACs analysis
The EURL report on QACs analysis in food via QuEChERS and LC-MS/MS, was updated by introducing a simple and practical approach for separating background contaminations of QACs during LC-MS/MS analysis involving the use of a trap-column.

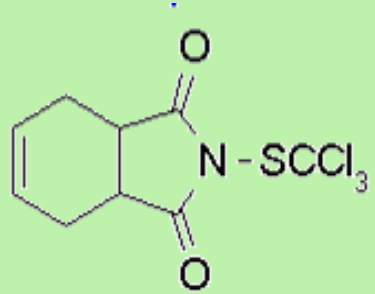
10-02-2023 | EURL-SRM
Joint EURLs/NRLs Workshop | 18–20 October 2023 in Stuttgart (Fellbach)
The Joint EURLs/NRLs Workshop for Pesticide Residues will be held from 18 to 20 October 2023 in Stuttgart.

Analysis of Captan/THPI & Folpet/PI

Determination of phthalimide and tetrahydrophthalimide using LC-MS/MS



FOLPET



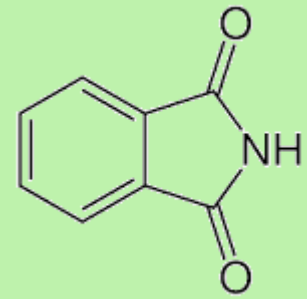
CAPTAN

Degradation especially at

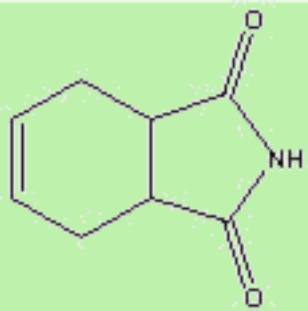
- ***High pH***
- ***High temperature***

e.g. during:

- ***Homogenization***
- ***Storage of defrosted Homogenates***
- ***Extraction/Cleanup***
- ***Storage of Extracts***
- ***GC-injectiion***



Phthalimide



Tetrahydrophthalimide

Analysis of Captan (sum) and Folpet (sum)

New EU Residue Definitions since 2016:

Captan including tetrahydrophthalimid (THPI), calculated as captan

Folpet including phthalimid (PI), calculated as folpet

Analysis of Captan (sum) and Folpet (sum)

DILEMMA 1: Convert Parents to THPI/PI or Not?

a) Transform Parents into THPI/PI prior to Measurement

- ✓ **Circumvent Problems in GC (analysis by LC-MS/MS or by GC in absence of parents not problematic)**
- ✓ **One analyte per RD** quantified (➔ LOQ clear)
- × **In case of preliminary screening LOQs/SDLs not clear** (LOQs important for negative findings)
  - **Both parents and THPI/PI** initially measured/screened (➔ summed LOQ (by consensus) or summed SDL (?))
  - **Only parents** initially measured/screened (➔ LOQ/SDL does‘t cover full RD)
  - **Only THPI/PI** initially measured/screened (➔ LOQ/SDL does‘t cover full RD)
- × **No info about presence of parents (unless screened separately)** (presence provide usefull evidence about use of parents, e.g. PI has multiple sources)
- × **Risk asessment compromized** (no info about residue levels of individual components)

b) Measure all Components Individually (Captan/Folpet and THPI/PI)

- ✓ **Full information about residue situation and LOQs** of all components (accurate risk asessement possible)
- ✓ **Difficulties in GC-analysis** (need to compensate ME of parents, avoid overestimation of THPI/PI)

Analysis of Captan (sum) and Folpet (sum)

DILEMMA 2: How to Transform Captan/Folpet into THPI/PI

Transformation in wet homogenates:

- Passive transformation
- Active transformation (at higher pH)

Typically **good conversion yields**,
but … not suitable for a multiresidue setup
(other analytes get lost)

Transformation QuEChERS extracts:

- Passive: non-re-acidified extracts after PSA
- Active: adding base to extract)

Conversion in non-acidified extracts too slow.
At more harsh conditions (pH ▲, Temp. ▲),
conversion yields drop ▼ (further degradation of THPI/PI ?)

Determination of phthalimide and tetrahydrophthalimide using LC-MS/MS

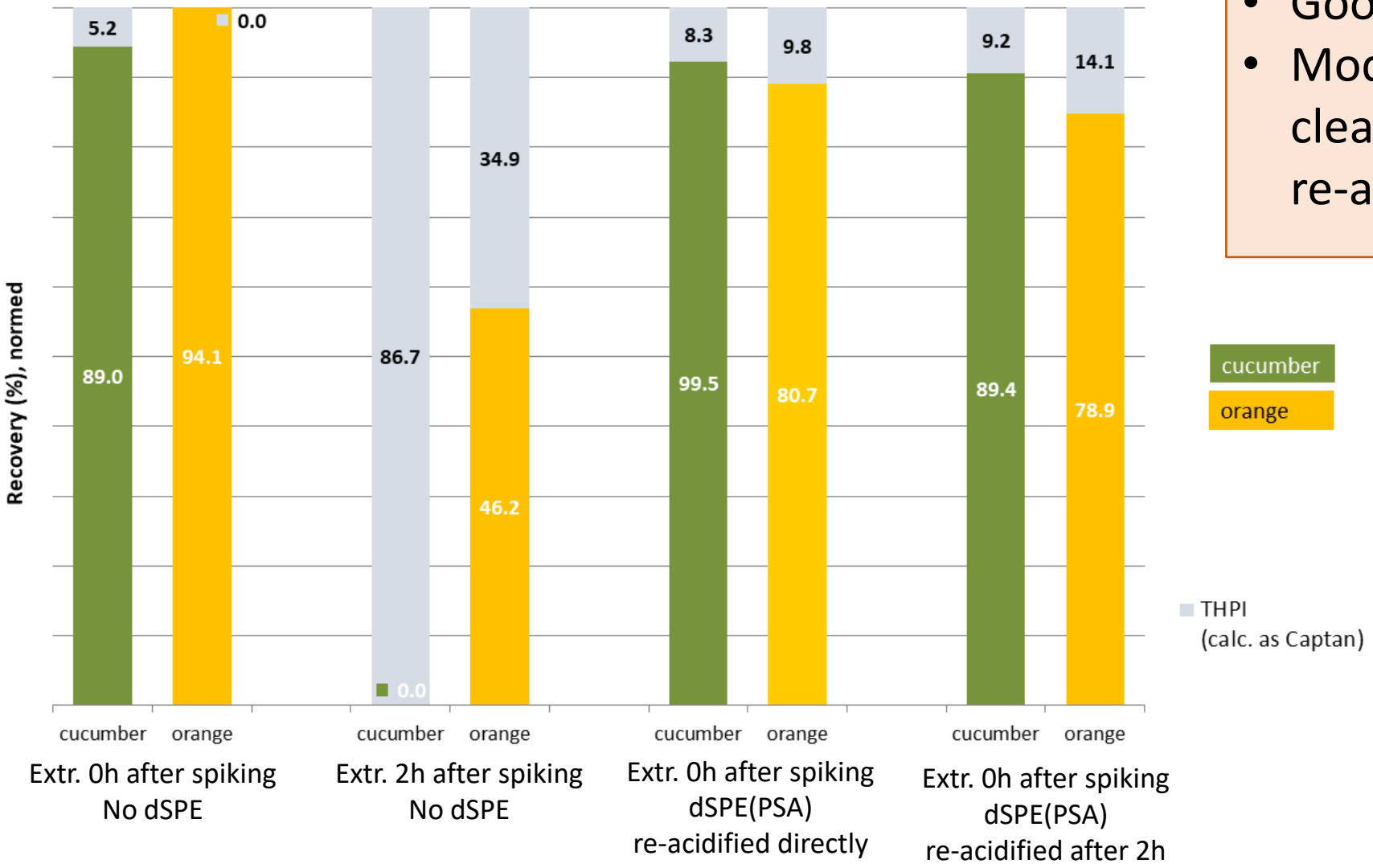
Hydrolysis in Homogenates Prior/During Extraction (at increased pH)

Matrix: Grapes

pH adjusted	Delay between pH-adjustment and extraction	Extraction method	Shaking time	THPI	Captan	SUM Captan		PI	Folpet	SUM Folpet
pH 7	No delay	QuEChERS	15 min	4	106	110		2	113	115
pH 10	No delay			47	9	56		42	1	43
pH 7	4h at RT			78	5	83		73	13	86
pH 10	4h at RT			55	0.0	55		2	0.0	2

→ Conversion Yields not quantitave

Stability of Captan during QuEChERS-Extraction:



Avoid prolongued standing of thawed homogenates !!

- Good Rec. w. QuEChERS
- Moderate losses during PSA-cleanup and if extracts are not re-acidified

Similar results for Folpet, Captafol

Both parents and degradants determined by LC-MS/MS in this experiment

DILEMMA 3: Which Techniques to use for Measurement

Sample Contains
only THPI/PI
(absence of parents difficult to judge, if extensively degrading during procedure)

Sample Contains
Parents & THPI/PI
(often the case)

Passive or Active Transformation of parents to THPI/PI in Homogenate or Extract
(Conversion not always quantitative, No data on parent)

THPI/PI via GC-MS(/MS)

THPI/PI via LC-MS(/MS)

Approach 1:
Parents & THPI/PI via GC-MS/MS

Tricky, risk to overestimate degradants
special procedure
+ Excel sheet (SRM-07)

Approach 2:
Parents via GC-MS/MS
THPI/PI via LC-MS/MS

CURRENTLY PREFERRED
but make sure to address MEs during measurement (e.g. ILIS, APs)

Approach 3:
Parents via LC-MS/MS
THPI/PI via LC-MS/MS

Parent analysis via LC-MS/MS lacks sensitivity

GC-ANALYSIS: OVERESTIMATION OF PI AND THPI IN PRESENCE OF PARENTS

FOLPET	PI	PI measured (calibrated w. PI)	overestimation of PI
spiked in one vial [ppm]		[ppm]	error [%]
0,1	0,1	0,11	27%
0,2	0,1	0,11	22%
0,3	0,1	0,14	53%
0,6	0,1	0,19	116%
1	0,1	0,24	178%

CAPTAN	THPI	THPI measured (calibrated w. THPI)	overestimation of THPI
spiked in one vial [ppm]		[ppm]	error [%]
0,1	0,1	0,13	31%
0,2	0,1	0,12	20%
0,3	0,1	0,15	50%
0,6	0,1	0,19	102%
1	0,1	0,25	159%

Tomato blank extract (QuEChERS, d-SPE, AP)
Spiked w. Folpet/ Captan and PI/THPI at different levels
Simultaneous measurement by GC-MS/MS

Situation in PT-material

		Captan/ THPI	Folpet/ PI
Parent (mg/kg)		0,172	0,249
Degradant (mg/kg)		0,59	0,10
Ratio Parent/ Degradant	Conc.	1 : 3.5	2.5 : 1
	Mols	1 : 7	1.3 : 1

Proportionally More parent

PROBLEM IN GC-ANALYSIS

THE HIGHER THE PARENT: DEGRADANT RATIO
THE MORE PRONOUNCED THE OVERESTIMATION
OF THE RESPECTIVE DEGRADANT !!!

Direct Analysis of PI/THPI using GC or LC-MS/MS - OVERVIEW

GC (see SRM-07)

Captan/Folpet (quant)

Need to Compensate MEs (e.g. using AP+ ILIS) ✓

THPI/PI (quant)

Risk of overestimation & FPs!

Formed in inlet from parents + other potential sources, e.g.

Phtalanhydride ► **PI,** ✗

Captafol ► **THPI,**

Special GC-Quantif. involving corr. of PI/THPI levels via calc. (Excel file linked in SRM-07) ✓

THPI/PI (qual)

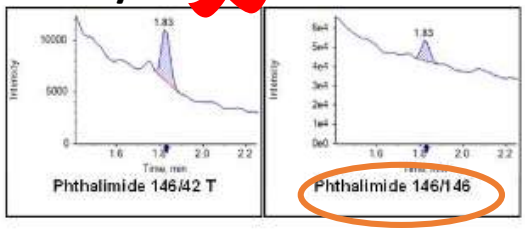
Useful for routine screening ! ✓

LC-MS/MS (see SRM-42 and SRM-49)

ESI-Mode

Neg. mode (SRM-42)

THPI/PI: ✗

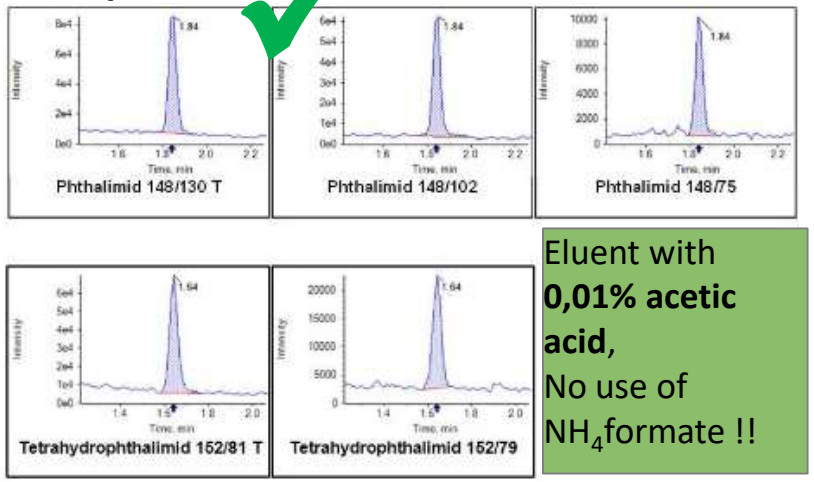


Lack of sensitivity depending on gradient and instrument, Only one useful MRM for PI

Parents: ✗

Pos. mode (SRM-49)

THPI/PI: ✓



Eluent with **0,01% acetic acid**, No use of NH_4formate !!

Parents: ✗

$[M+H]^+$ or $[M+NH_4]$ adducts sensitivity not bad but variable

APCI-Mode

Neg. mode (SRM-42)

THPI/PI and Parents

*Possible but **tricky**!! (insource effects), extra requirements, cross-interferences. e.g. Folpet D4 and PI-D4 interfere with Captan (analyzed as THPI) and THPI respectively*

✗

Topics

EURL-SRM Network
NRL-SRM Network

Proficiency Tests
EUPT-SRM Overview
EUPT-SRM18 (Honey)
EUPT-SRM17 (Tomatoes)
EUPT-SRM16 (Sesame)
EUPT-SRM15 (Rice)

Workshops
Workshop Overview
Joint Workshop 2023
Joint EURL/NRLs (SRM-FV) 2022

Services
ILISs Distribution
CheckYourScope
SRM-PinBoard
EURL-SRM Methods
Analytical Observations
Residue Observations
Downloads
Sources of Standards

Internet
EURL DataPool
QuEChERS - Website
QuPPe - Website
PestiPedia

Data Submission

Latest News

19-04-2023 | EURL-SRM
Risk of False Positives of Chloridazon-Desphenyl in Honey by LC-MS/MS
A new EURL-SRM Analytical Observations Report concerning the risk of false posit
Various chromatographic separation methods for chloridazon-desphenyl were test

17-03-2023 | EURL-SRM
QuPPe-PO-Method Version 12.1
The QuPPe-PO-Method has been updated (now includes more detailed information on Honey analysis).

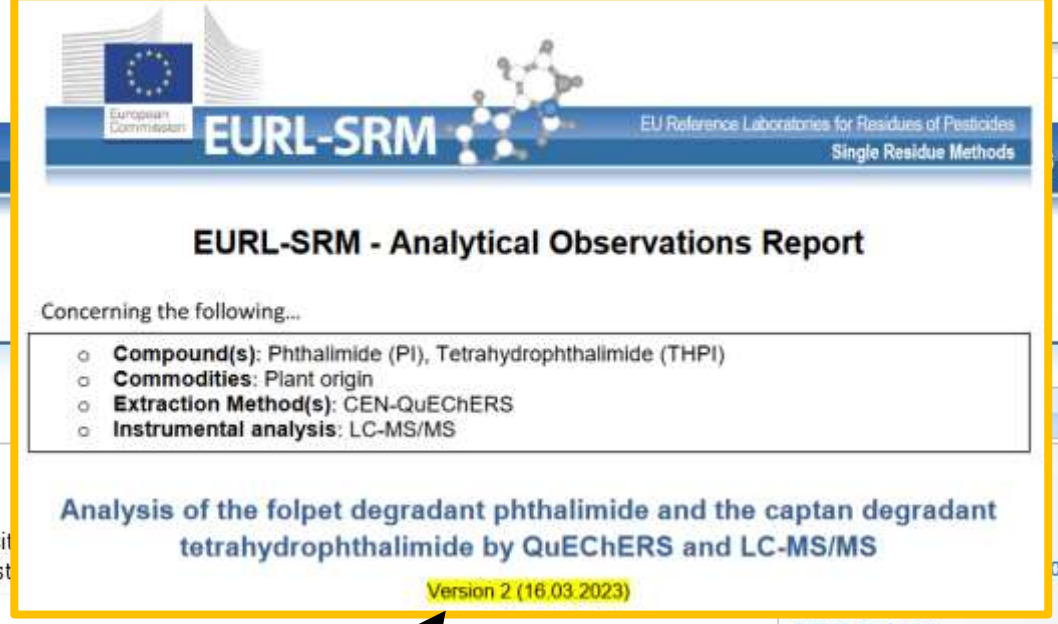
16-03-2023 | EURL-SRM
Analysis of the Folpet and Captan degradants Phthalimide (PI) and Tetrahydrophthalimide (THPI) by QuEChERS and LC-MS/MS
The Analytical Observation Report (SRM-49) on the analysis of PI and THPI via LC-MS/MS was updated by introducing additional validation data. This update also includes results of experiments concerning the transformation of Captan and Captafol to Tetrahydrophthalimide (THPI) and of Folpet to Phthalimide (PI), during various steps of the QuEChERS procedure and especially in thawed sample homogenates prior to extraction.

03-03-2023 | EURL-SRM
Determination of fluoride ion in food
Two approaches for the determination of Fluoride Ion via selective electrodes (ISE) are described: a) direct measurement in QuPPe extracts and b) measurement in diffusates derived by microdiffusion.

27-02-2023 | EURL-SRM
Compilation of Residue Observations Reports of QuPPe Compounds
A new compilation of residue findings of QuPPe compounds in food products, analysed in 2022, was uploaded. The report additionally encompasses findings of ethylene oxide / 2-chloroethanol. Aim of these annual compilations of residue findings is to help OfLs to localize analyte/matrix combinations that are worthwhile monitoring.

24-02-2023 | EURL-SRM
New Analytical Observations Report on QACs analysis
The EURL report on QACs analysis in food via QuEChERS and LC-MS/MS, was updated by introducing a simple and practical approach for separating background contaminations of QACs during LC-MS/MS analysis involving the use of a trap-column.

10-02-2023 | EURL-SRM
Joint EURLs/NRLs Workshop | 18–20 October 2023 in Stuttgart (Fellbach)
The Joint EURLs/NRLs Workshop for Pesticide Residues will be held from 18 to 20 October 2023 in Stuttgart.



CIRCA BC Login
RASFF Portal DB (COM)
How to Use CIRCA BC
InfoNote: Processed Food/Feed (COM)
EUPT Registration Website

Pinboard

Show more Pinboard Messages...

Compilation of Analytical Observations Reports

The table below compiles various observations made during the analysis of pesticide residues.

Compound(s)	No. of Method Finder List/Version/Date of Update	Link
Captan & Tetrahydrophthalimid, Folpet & Phthalimid	**SRM-07/(V3)/06.04.2017** and **SRM-42/(V1)/30.06.2019** **SRM-49/(V1)/16.03.2023**	Report SRM-07 (GC) Report SRM-42 (LC) | Report SRM-49 (LC)

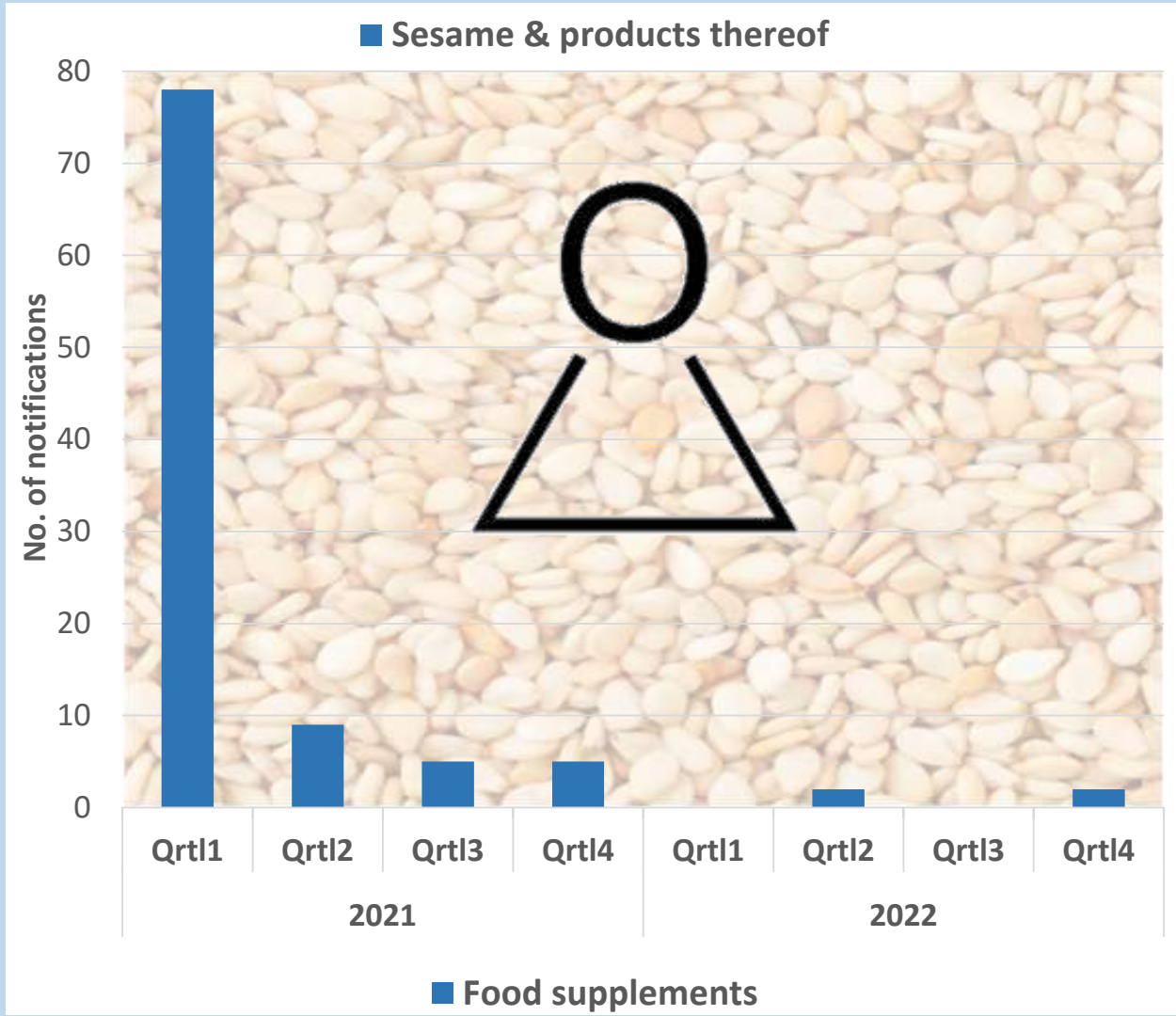
Short Description of SRM-07: This document describes approaches for the analysis of Captan and Folpet in QuEChERS extracts via GC-MS or GC-MS/MS. Different approaches for correcting the results of the parent molecules for matrix effects during GC analysis or for losses during the entire procedure are presented and discussed. In addition two approaches for analyzing Captan and Folpet next to their legally relevant metabolites Tetrahydrophthalimide (THPI) and Phthalimide are presented and discussed.
Short Description of SRM-42: Various possibilities for the LC-MS/MS analysis of Captan/THPI and Folpet/Phthalimide were studied employing APCI and ESI interfaces. LC-MS/MS analysis circumvents problems related to GC-analysis but further efforts to improve sensitivity are required. The active hydrolysis of Captan and Folpet to their respective degradants (THPI and PI), was also studied aiming to reduce the number of analytes to be measured. Unfortunately, conversion yields were often not satisfacory and further studies are needed.

Short Description of SRM-49: This report describes a simple and sensitive method for the analysis of PI and THPI. The analysis is based on QuEChERS extraction and LC-MS/MS determination in the ESI-pos. mode using a C18 column for separation. Validations of THPI and PI were conducted on various commodities at 0.005 and 0.010 mg/kg, which correspond to ~0.01 and ~0.02 mg/kg when expressed as Folpet and Captan respectively.
PI-validation at these levels was successful for three mass-transitions (m/z 148/130 and 148/102 and 148/75) in cucumber, grapes, wheat flour and peanut butter. Validation of THPI was successful for both measured mass-transitions (m/z 152/81 and 152/79) in cucumber and grapes at both tested levels (0.005 mg/kg and 0.010 mg/kg). However, in wheat flour and peanut butter, THPI validation at 0.005 mg/kg and 0.010 mg/kg was only successful at one single mass-transition (m/z 152/81). The second mass transition (m/z 152/79) was interferred thus affecting identification of THPI at very low low levels. The parent compounds Captan and Folpet show a rather poor sensitivity in LC-MS/MS not allowing accurate analyses at low levels. For such analyses the well-established GC methodology is thus recommended (see SRM-07). As Captan and Folpet may degrade to THPI and PI at various stages of the procedure, it is important to analyse THPI/PI and Captan/Folpet from the same extract and within a reasonably short time distance.
Overall conclusion: It could be shown, that THPI and PI can be potentially incorporated into the multiresidue scheme of labs unsing the procedure SRM-49. If the LC-gradient of SRM-49 does not fit to the routine approach it may also run as a standalone procedure to be employed following detection of a marker compound during routine GC analysis (e.g. detection of Captan and/or THPI by a GC-based method, see SRM-07).
Based on validation experiments on Captan and Folpet using GC (see SRM-07), and on THPI and PI via LC-MS/MS in the ESI-pos. mode (see SRM-49), the lowest MRLs for Captan (Sum) and Folpet (Sum) in acidic and non-acidic commodities of high water content (at 0.02* and 0.03* mg/kg- expressed as parent - respectively), are considered well enforceable. In dry commodities of low or high fat content the MRLs at 0.07* seem well achievable for PI whereas more experiment are required for THPI.

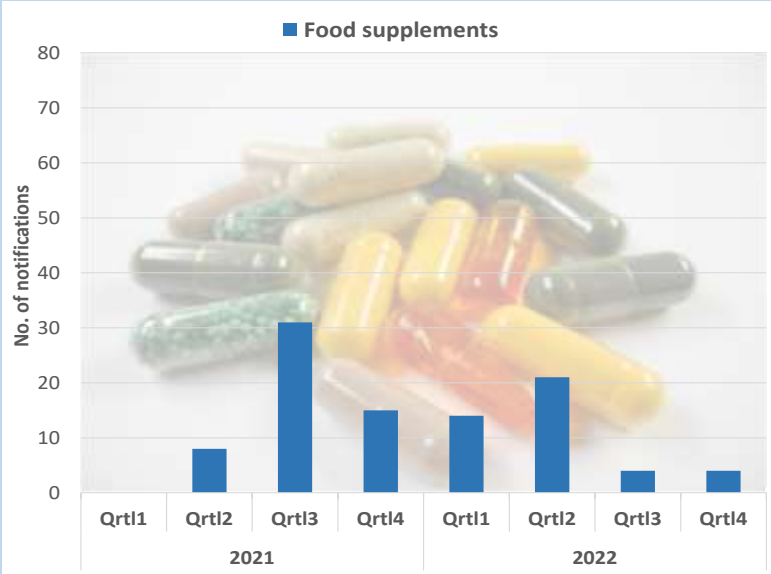
Analysis of Ethylene Oxide

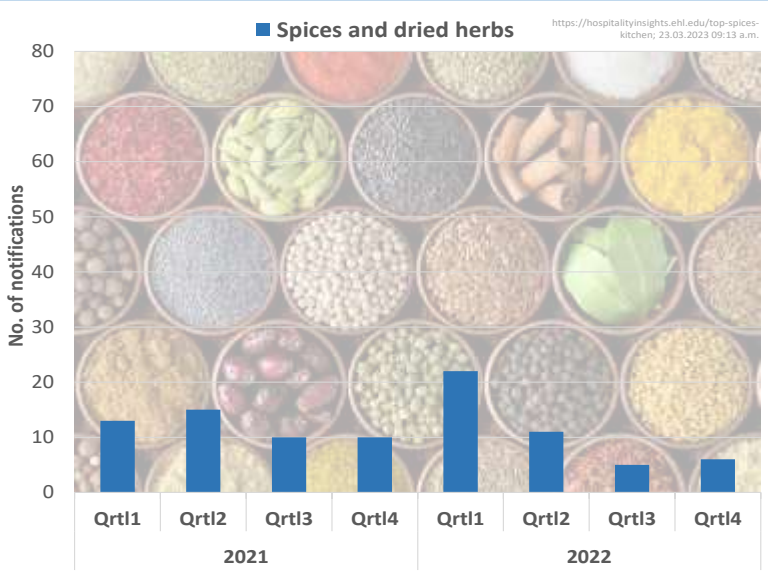
Ethylene Oxide Crisis – RASFF Notifications

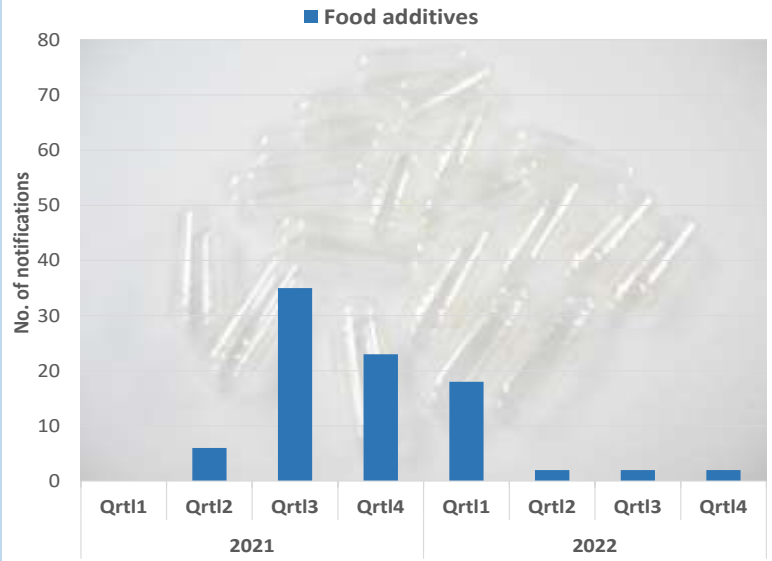
It all began in the autumn of 2020…

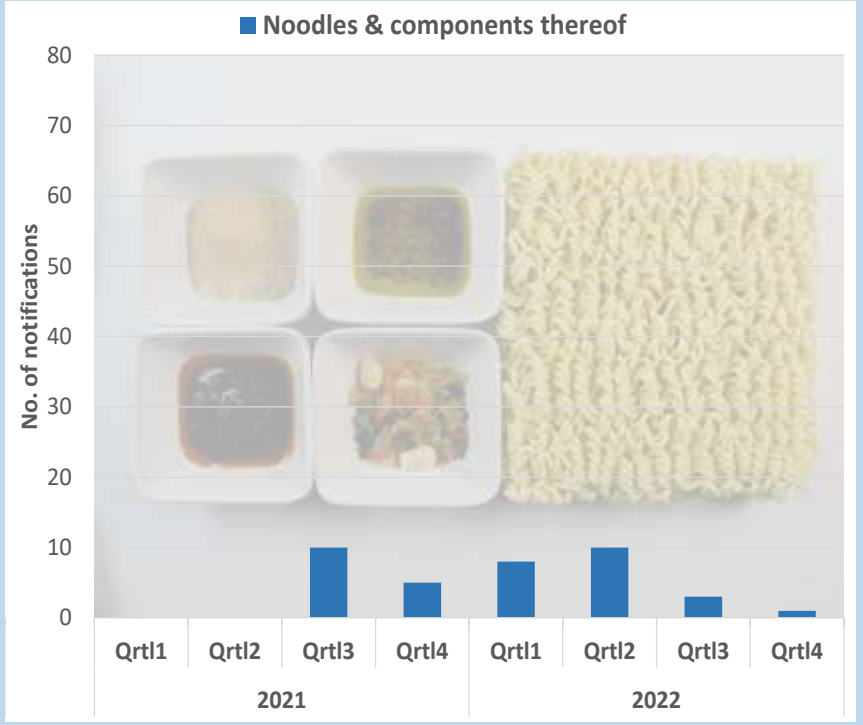


Ethylene Oxide Crisis – RASFF Notifications





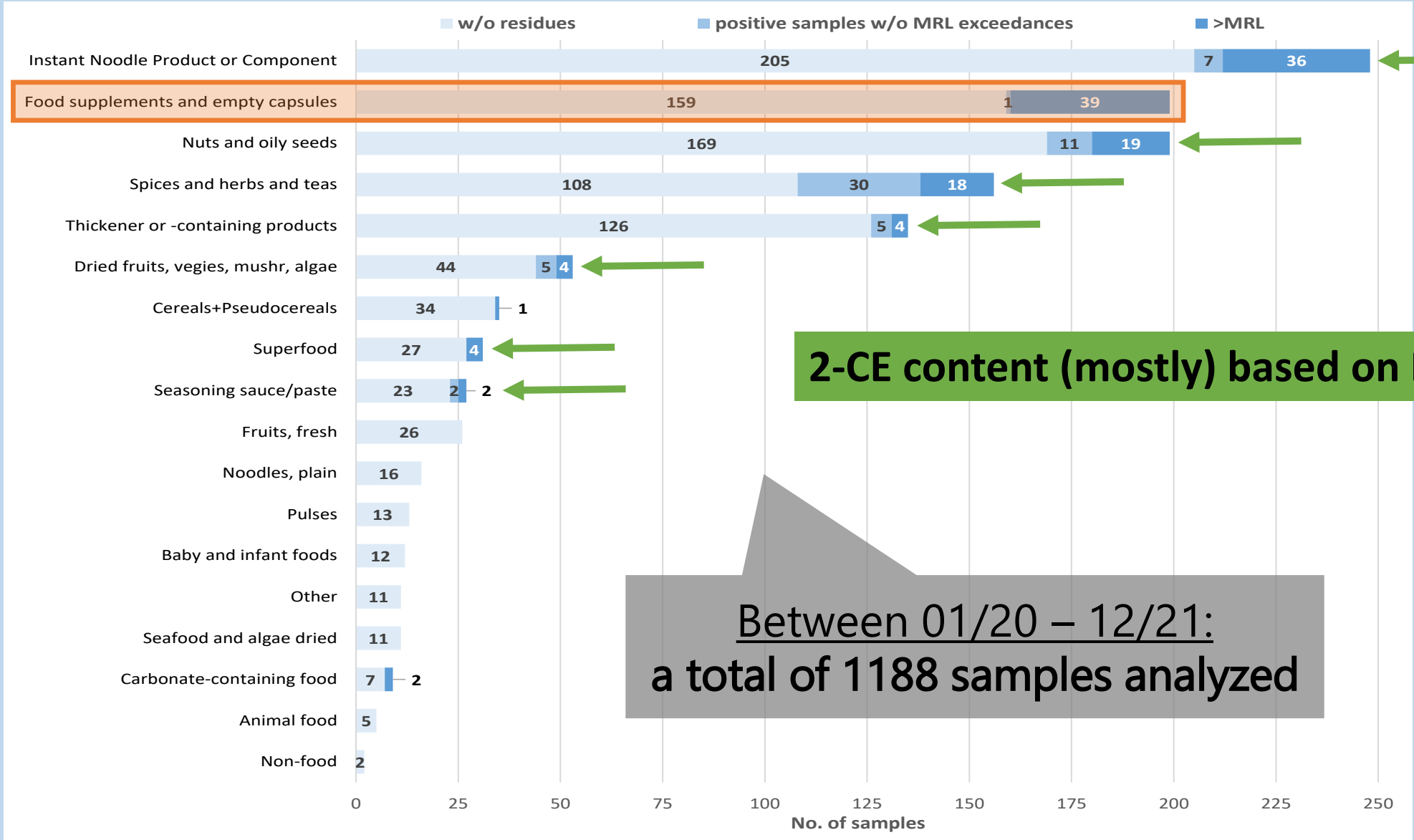




2023 (Q1-Q3): 52 Notifications

- **Spices + Herbs:** 30 (mainly IN)
- **Food Supplements:** 16
- **Other:** 6 (2x beans, 1x sesame, 1x guar gum, 2x salsa)

Ethylene Oxide Crisis – Comprehensive Pilot Monitoring EURL-SRM / CVUAS



2-CE content (mostly) based on EO-fumigations

Between 01/20 – 12/21:
a total of 1188 samples analyzed

Ethylene Oxide Crisis – Comprehensive Pilot Monitoring EURL-SRM / CVUAS

Example: 2-CE as a Processing Contaminant:

- **Capsules**: consisting of modified celluloses

  → **EO-fumigated cellulose raw material**

- **Carbonate-containing Products**:

– Added as micro-nutrient (Ca, Mg salts) and for pH adjustment

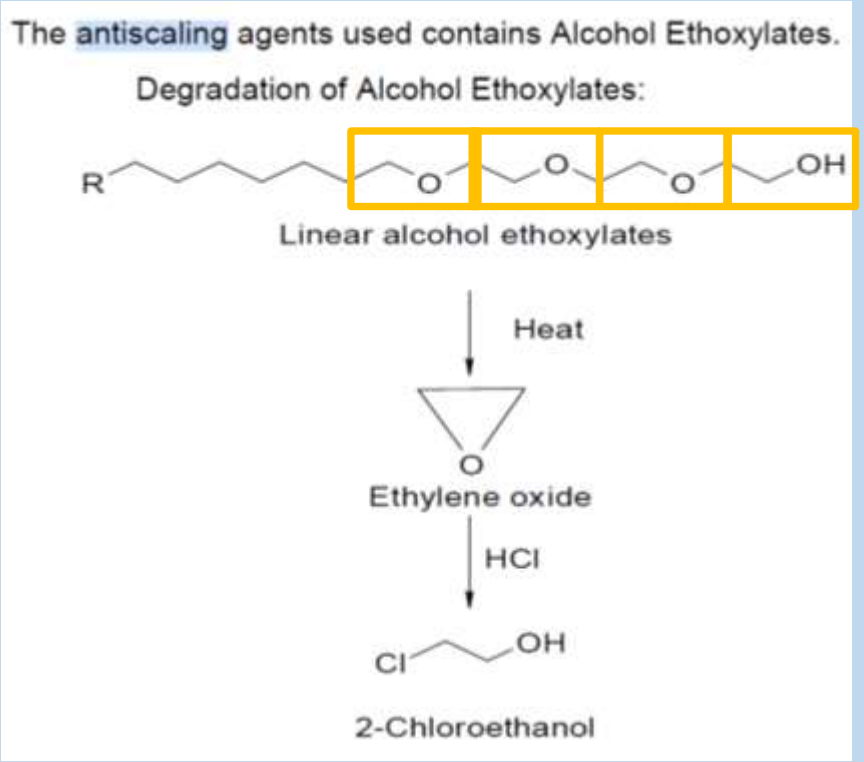
→Fumigation of carbonate salts unlikely (microbiological stable)!

BUT…..

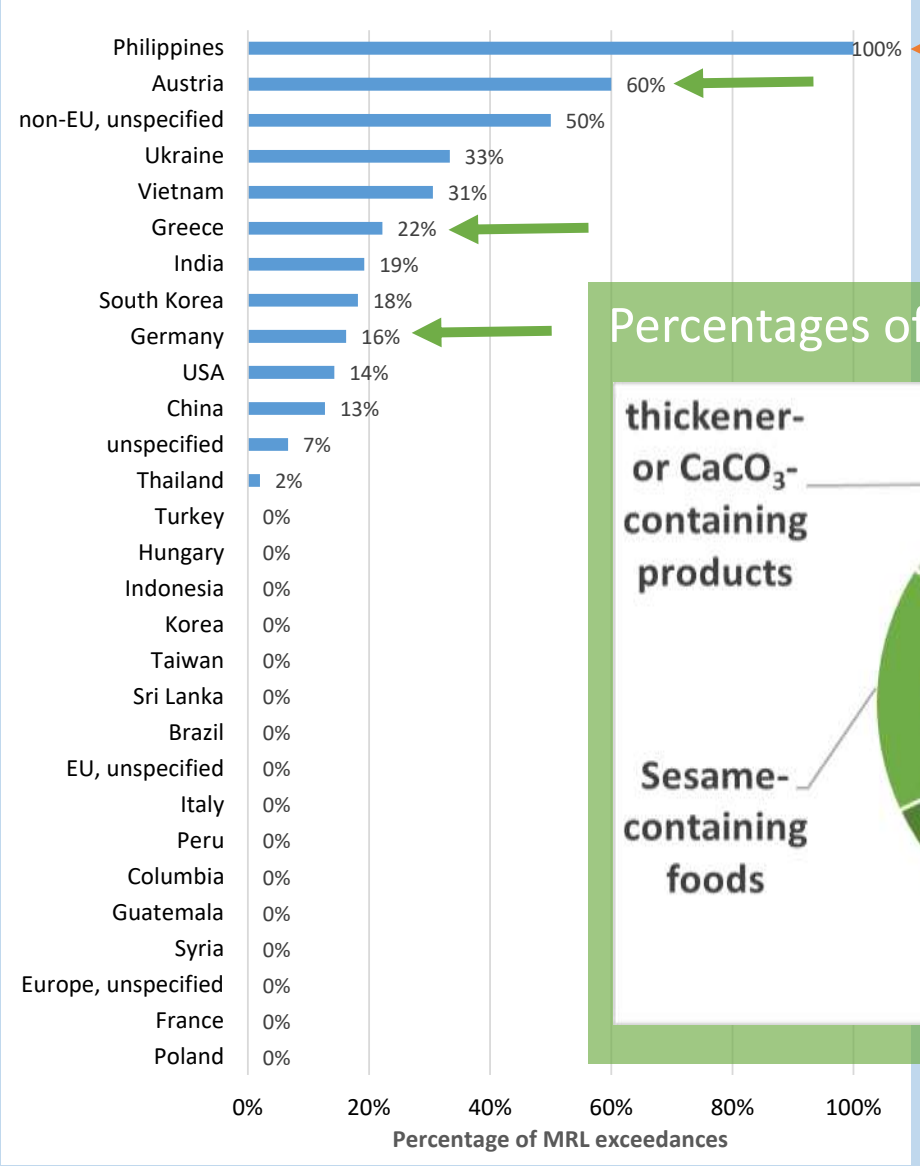
– Carbonate salts are industrially washed, dried and powdered

– Containers cleaned w. **polyethoxylate**-containing cleansing agents

→ **Degradation resulted in a 2-CE (processing contamination)**



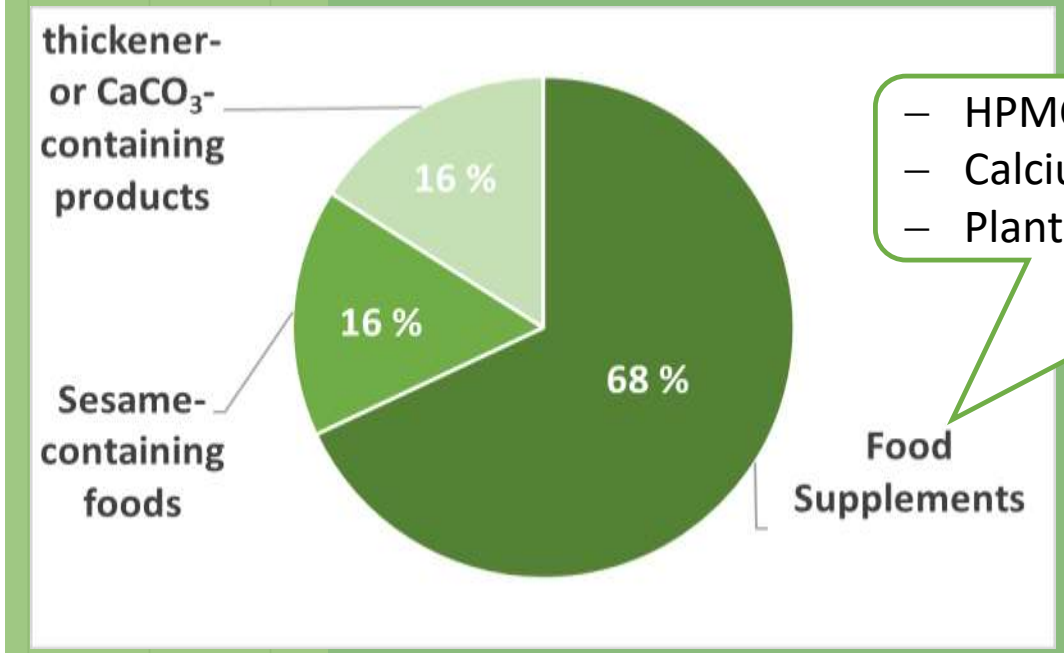
Ethylene Oxide Crisis – Results of a comprehensive monitoring program



7 out of 9 samples: Noodle products and/or seasoning sauces

EO-fumigation is prohibited within the EU since the 1980s

Percentages of MRL-exceedances for European countries were remarkably high....



- HPMC-capsules
- Calcium carbonate-containing products
- Plant powders from non-EU countries

Analysis of Esters and Conjugates of acidic pesticides

Intro

Many residue definitions (RDs) entail **Esters and/or Conjugates.**

Examples:

- *„Sum of 2,4-D, its salts, its esters and its conjugates, expr. as 2,4-D"*
- *"Sum of thiabendazole, 5-hydroxythiabendazole and its sulfate conjugate, expressed as thiabendazole*

Some RDs entail conjugates w/o explicitly mentioning "conjugates":

Example:

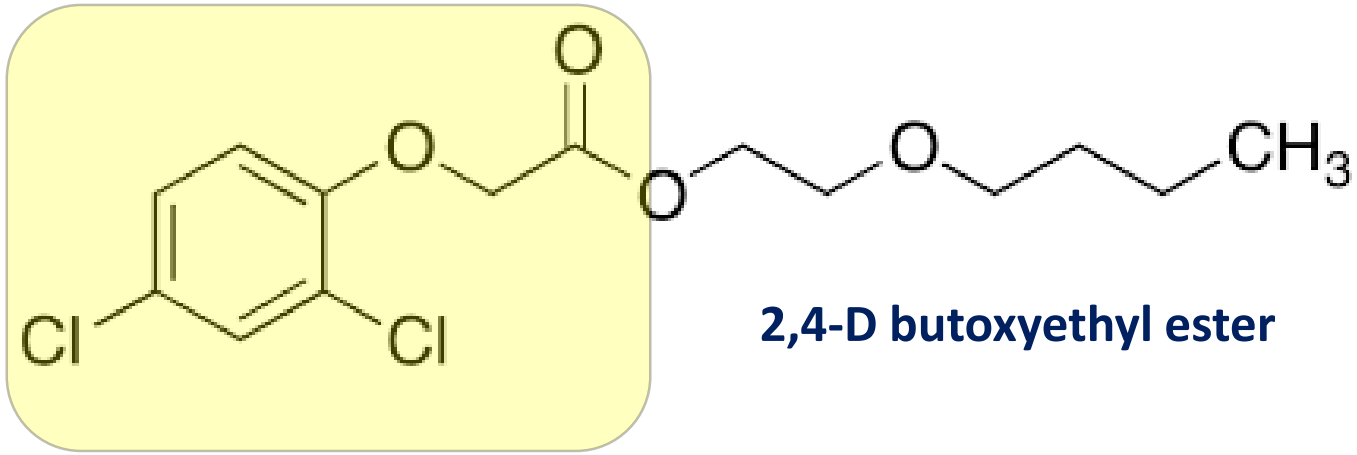
„Sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as flufenacet equivalent"

What is understood under „**Esters**“ within RDs?

Acidic herbicides are approved as active substances and may be used in different **variants, within PPP-formulations.**

Example:

Active Subst.: 2,4-D



2,4-D butoxyethyl ester

Variants:

- **Free acids**
- **Esters:** -ethylhexyl; -isopropyl; -octyl; butoxyethyl ….
- **Salts:** -dimethylamine ~; -diethanolamine ~; sodium ~

Important to know!

Acids or **Salts** → free acid/anion in equilibrium ► taken up **via roots** (high polarity)

Esters taken up via leafs (low to intermediate polarity)

Esters reported to be quickly enzymatically hydrolysed in plants

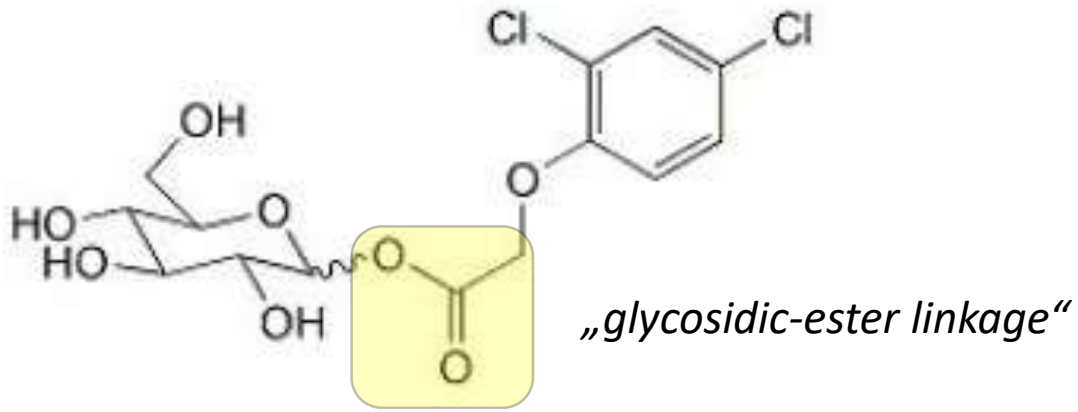
→ Residues in food are rare (e.g. fluazifop-butyl sometimes found)

Still **esters need to be analytically targeted (formally)**

- There can be **late applications**, where esters are not yet fully metabolized.
- They are explicitly **mentioned in the residue definitions**

What is understood under „**Esters**“ within RDs?

Any esters of acids that are **secondarily** formed within the plant/animal through **reversible covalent bonding to matrix components** are considered **conjugates**. E.g. glucosides



„glycosidic-ester linkage“

Glucoside Conjugate of 2,4-D

→ Word “Esters” in RDs is not to be understood in the broad chemical sense!

How/Why are conjugates formed – Conj. Phases

Organisms have developed **metabolic mechanisms** to **reduce harmfulness** and/or **facilitate elimination of xenobiotics***

Specialized enzymes are often involved in these processes.

Xenobiotics-Detoxification often divided into **3 Phases**:

- **Modification** (bioactivation); e.g hydrolysis, introduction –OH group
- **Conjugation**
- **Transport/Excretion** (animals) or **compartmentation/segragation** (plants)

* ***Xenobiotics*** *[Greek = foreign to living organism] = compounds* ***foreign to living organisms*** *(plants/animals)*

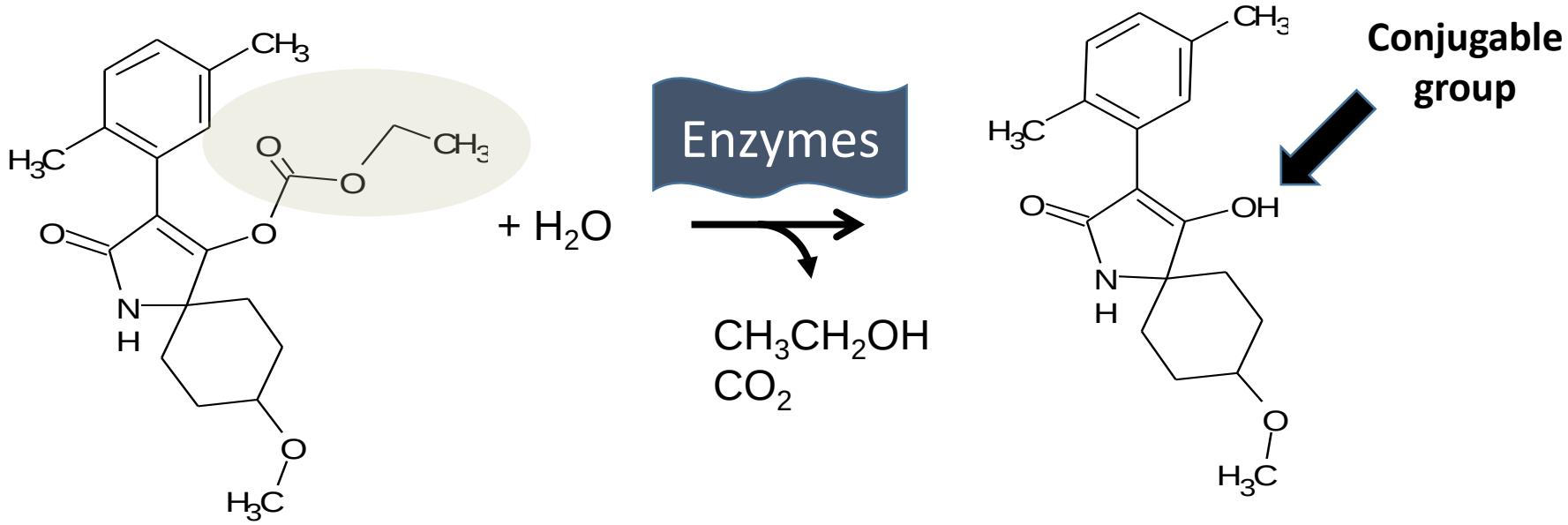
Detoxification Phases

Phase I: Introduction of a reactive chemical group

(e.g. via hydrolysis, oxidation (e.g. hydroxylation), reduction)

(may be skipped if xenobiotic already entails a reactive group)

Example:



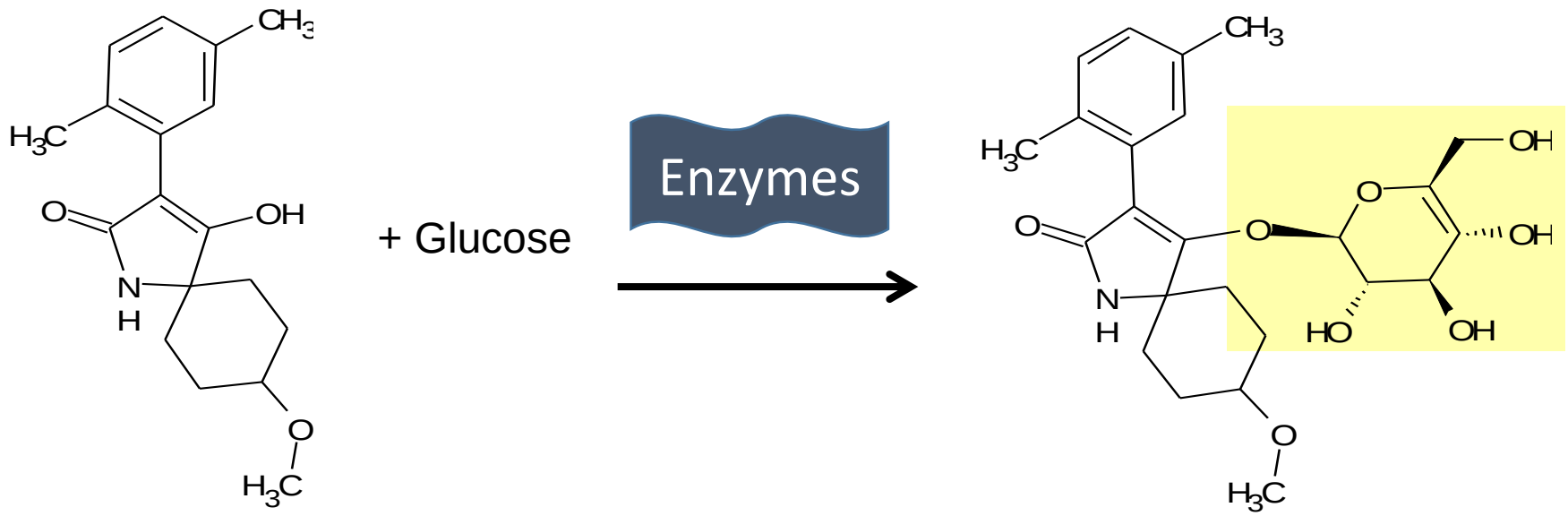
Spirotetramate

Spirotetramate enol

Phase II: Covalent bonding to natural compound

(e.g. sugar, amino acid) (➔ **Conjugation**)

Example:

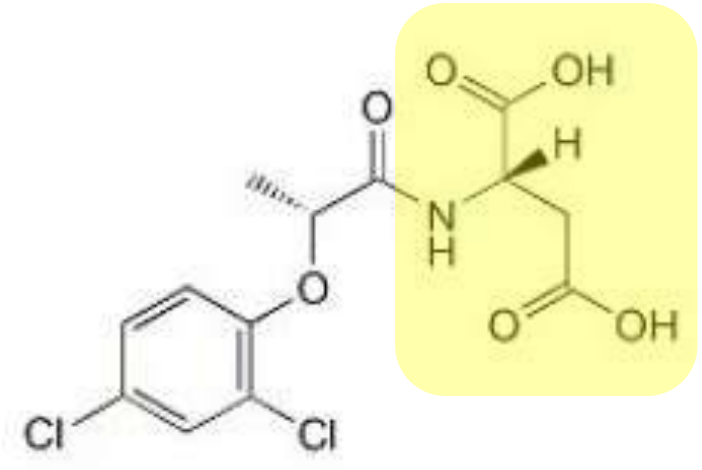


Spirotetramate enol

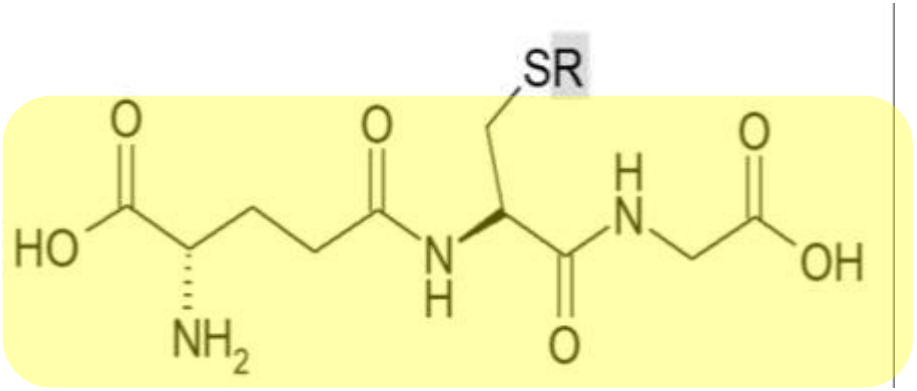
Spirotetramate enol glucoside

Examples of conjugates

- **Amino acid conjugates**



Aspartate

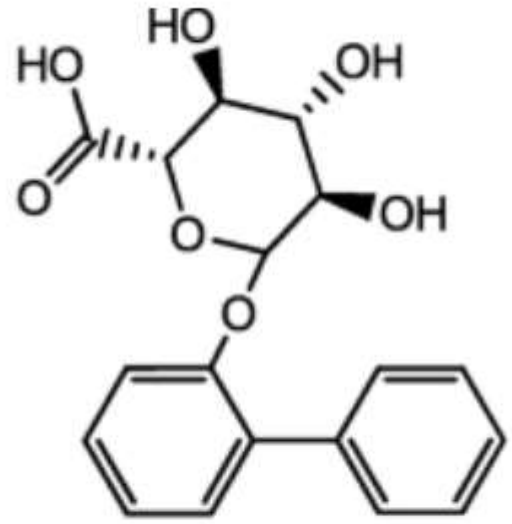


Glutathion-Conjugate (R-SG)

Glutathion is a tripeptide composed of Glutamin (linked through γ-carboxy group), Cyctein, Glycin

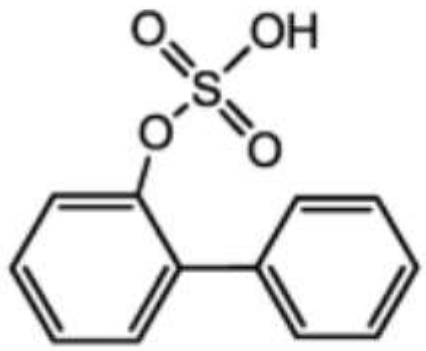
Examples of conjugates

Glucuronides and Sulfate conjugates (in animals)



Ortho-phenylphenol glucuronide (OPP-G)

Glucuronide



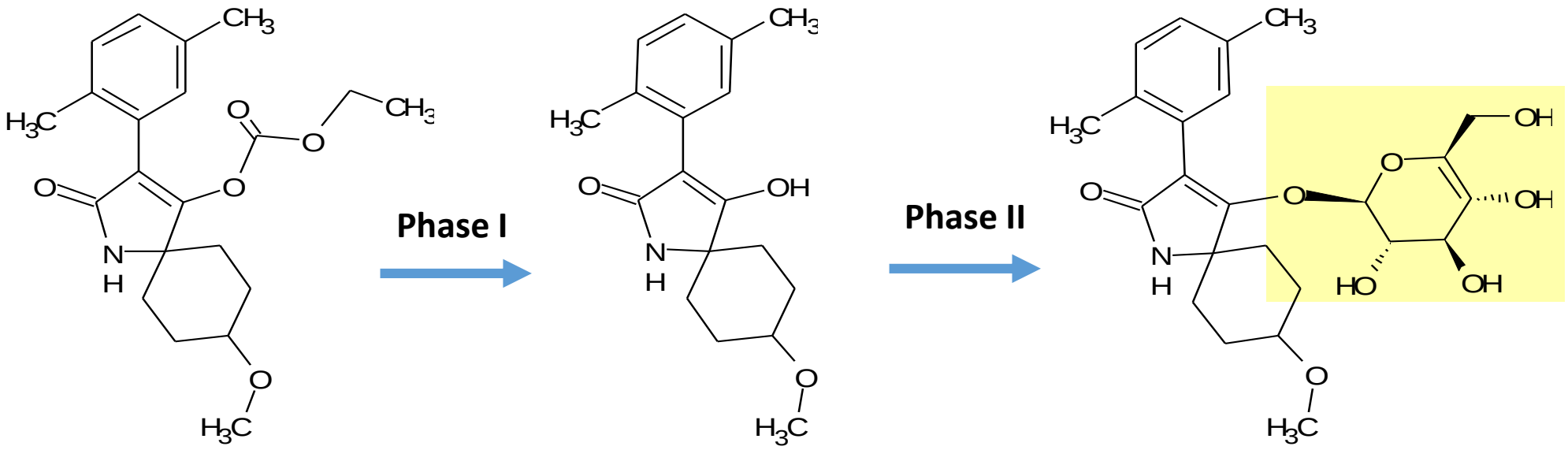
Ortho-phenylphenol sulfate (OPP-S)

Sulfate

R. Bérubé et al.: Analytical and Bioanalytical Chemistry volume 410, pages 7275–7284 (2018)

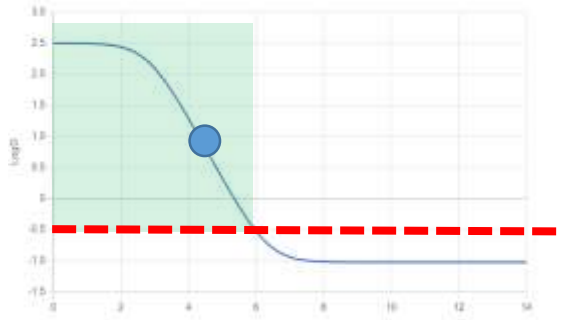
How does polarity shift through glucosylation?

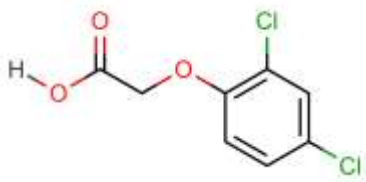
Spirotetramat Enol glucoside



- **Parent**
  logKow **3.5**
  at any pH<10
  (computed)
- **Enol**
  logKow **2.4**
  at pH 4.5
  (computed)
- **Enol glucoside**
  logKow **0.3**
  (at any pH<10)
  (computed)

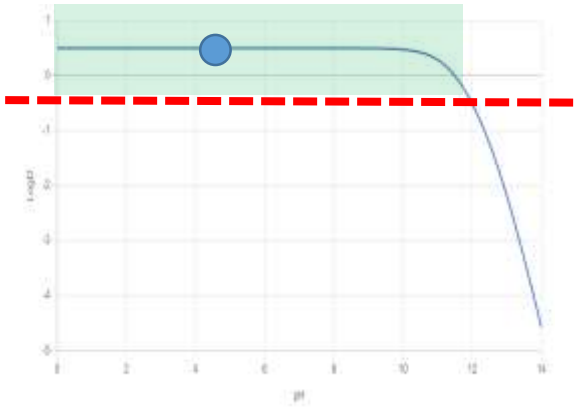
2,4-D

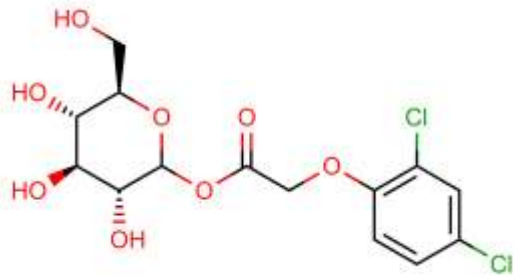




logKow **0.82** (at pH 4.5)
logKow **-0.52** (at pH 6)

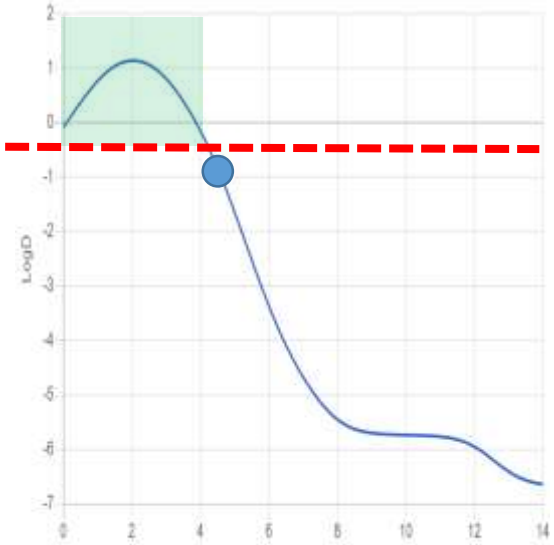
2,4-D glucoside

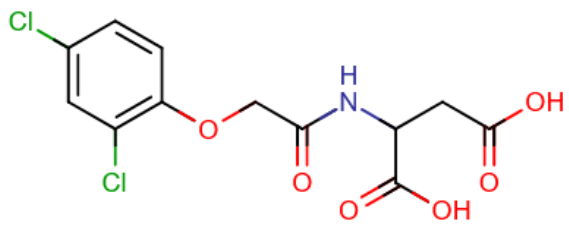




logKow **0.50**
(at any pH<10)

2,4-D aspartate

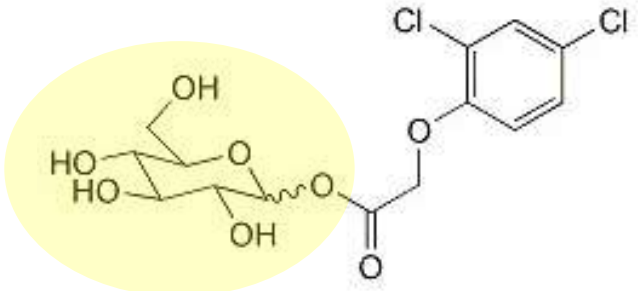


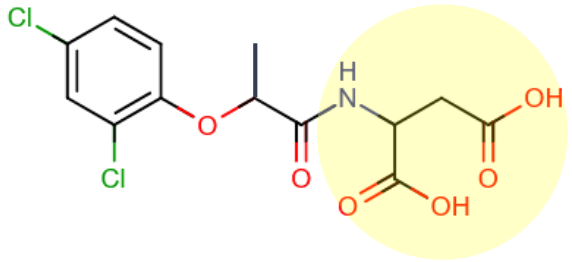


logKow **-0.8** (at pH 4.5)
logKow **-3.3** (at pH 6)

Analysis of Phenoxy acid Conjugates as such (via QuEChERS)

	QuEChERS-Recoveries of glucoside-conjugates of phenoxy acids (extracted and measured as such)
2,4-DP-Glucoside	102
2,4-D-Glucoside	100
Haloxyfop-Glucoside	95
MCPA-Glucoside	87
2,4-DP-Aspartate	95





Problem: Too many possible esters for some acids

2,4-D, 2-butoxyisopropyl ester
2,4-D, 2-ethylhexyl ester
2,4-D, 2-octyl ester
2,4-D, butoxy ethoxy propanol ester
2,4-D, butoxypolyethoxypropyl ester
2,4-D, butoxypropyl ester
2,4-D, butyl ester
2,4-D, chlorocrotyl ester
2,4-D, ethoxyethoxyethyl ester
2,4-D, ethyl ester
2,4-D, isobutyl ester
2,4-D, isopropyl ester
2,4-D, methyl ester
2,4-D, nonyl ester
2,4-D, octyl ester
2,4-D, polypropoxybutyl ester
2,4-D, polypropylene glycol ester
2,4-D, propyl ester
2,4-D, propylene glycol butyl ether ester
2,4-D, propylene glycol isobutyl ether ester
2,4-D, tetrahydrofurfuryl ester
2,4-C, tripropylene glycol isobutyl ether ester

Example 2,4-D-Esters

Problem: Too many possible esters for some acids

Analysis of all Esters individually ✗

- Many analytes to validate / analyse (most unlikely to be found)
- Many LOQs
- Huge summed-LOQ

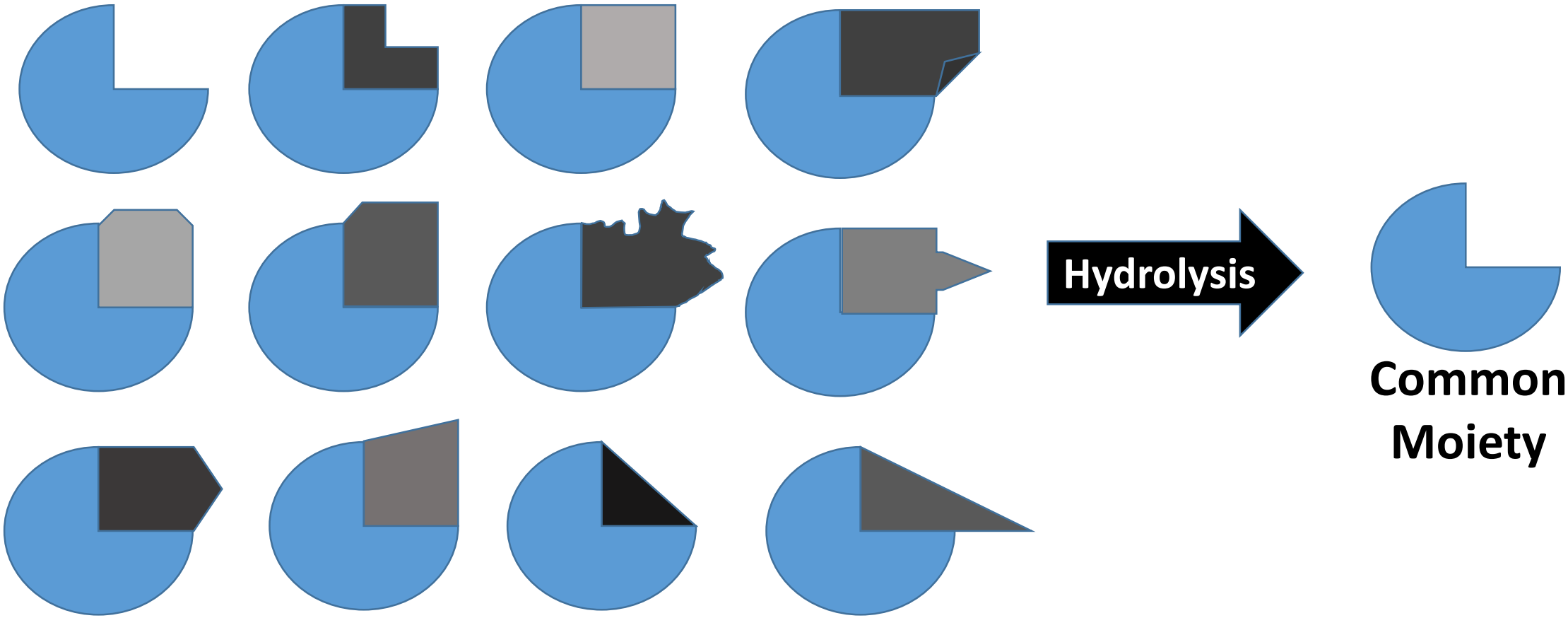
Common moiety analysis (involving hydrolysis of Esters) ✓

- Single analyte to determine
- One LOQ
- Approach not MRM compatible
- More work-intensive than normal MRM (pre-screening for selective use)
- Screening LOQs/SDLs different than actual LOQs of hydrolysis approach.

NOTE: Non-selective **HYDROLYSIS** will typically cleave both **esters and conjugates** (at least partly)

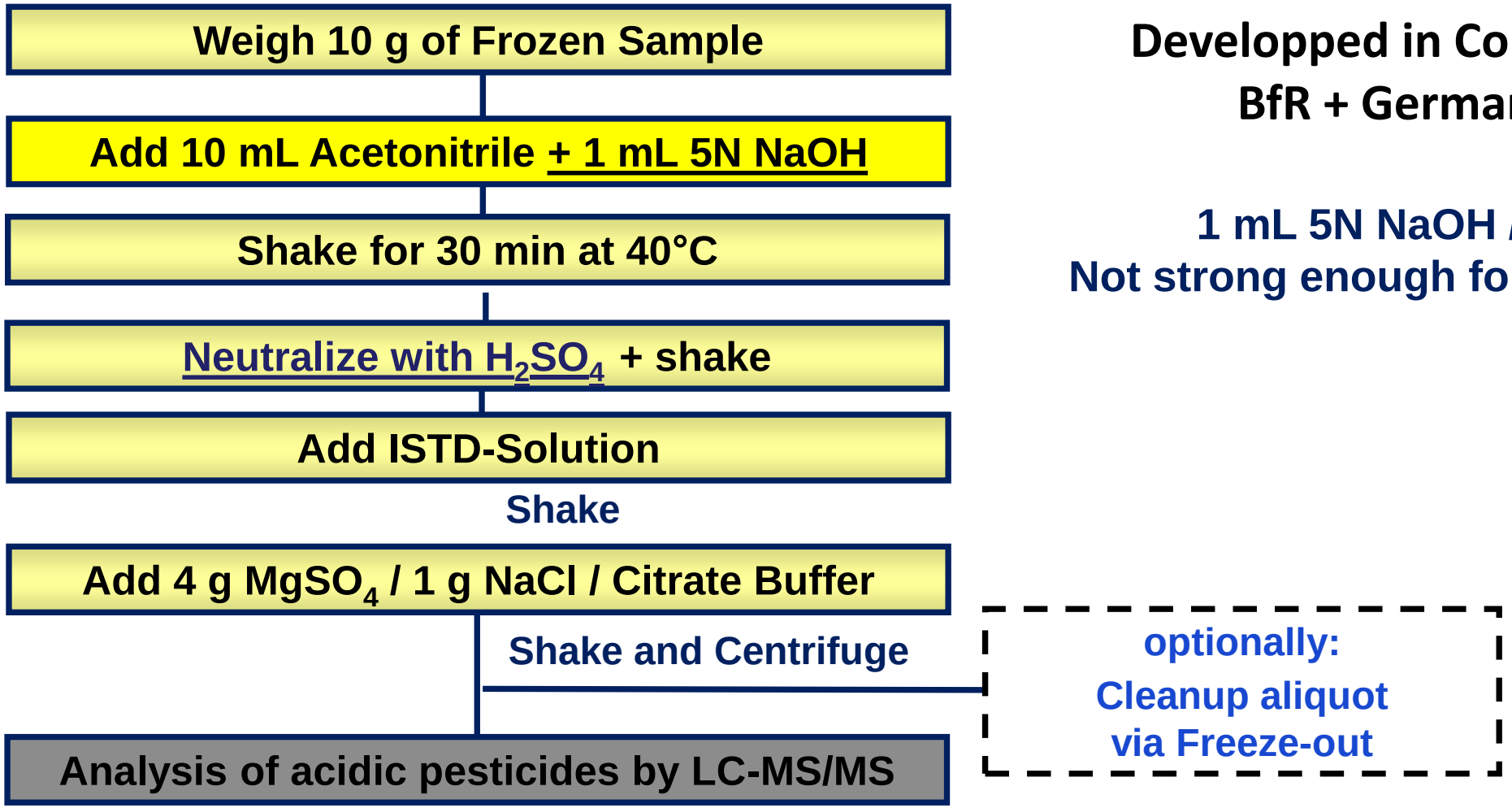
➔ **Esters & Conjugates typically go as a package in RDs**

Common Moiety Approach



QuEChERS combined with Alkaline Hydrolysis

- **Previous Method**: Base added directly to sample homogenate
- **Current Method**: Hydrolysis after ACN-addition (1st extraction step)
  → Efficient Hydrolysis in Dry Matrices and of Resistant Esters



Developped in Collaboration with
BfR + German NRL-SRM

1 mL 5N NaOH / 30 min / 40°C
Not strong enough for complex matrices !

Deconjugation Strategy

Current Approaches (CEN-QuEChERS-based):

- EN 15662: **Fixed hydrolysis conditions (1 mL NaOH/40°C/30 min), roughly representing those of applicants.**
- SRM-43: **with 2 additional hydrolysis conditions introduces to cover resistant esters in difficult matrices (= 3 conditions in total, depending on matrix)**

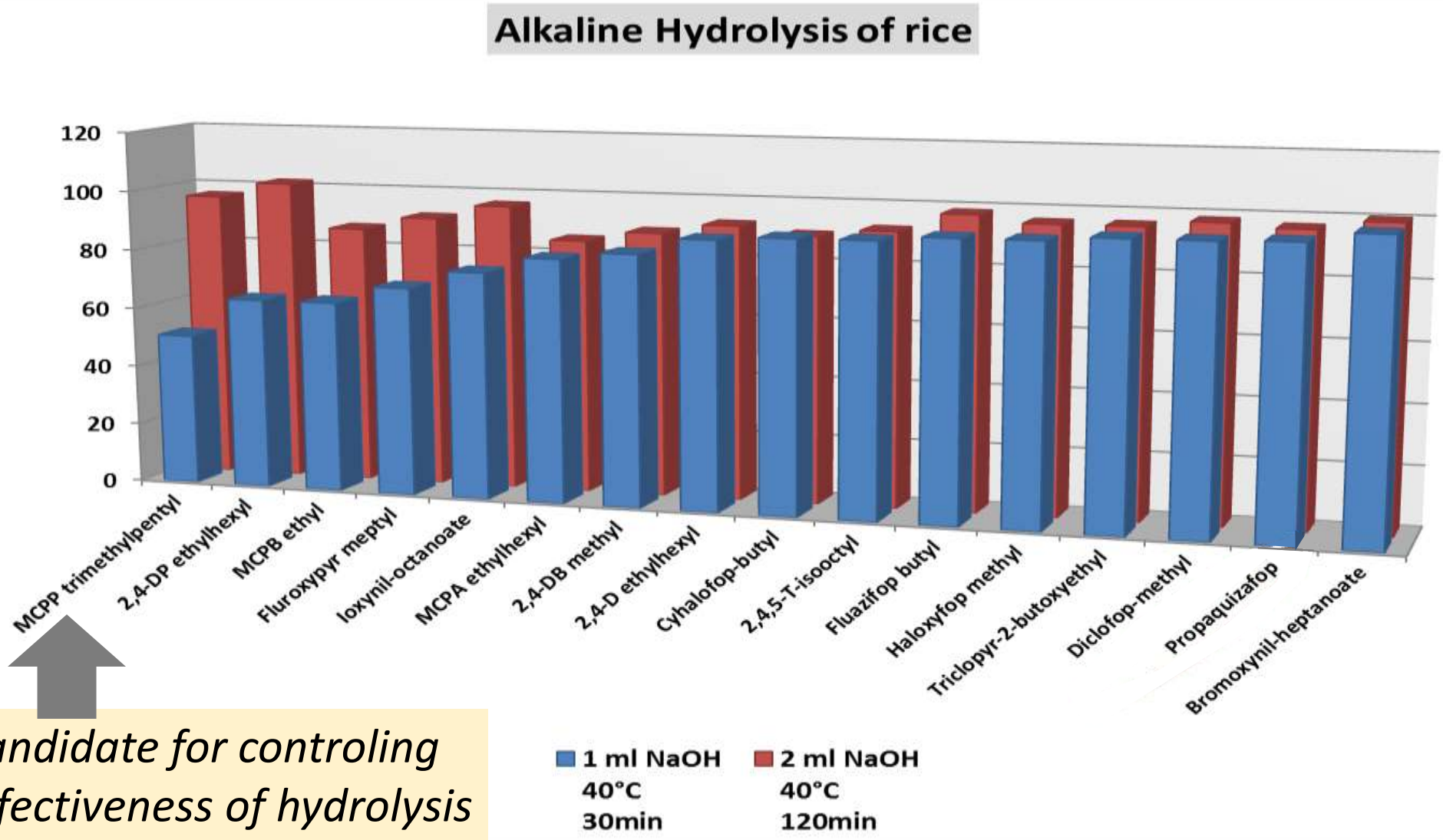
Envisaged New Approach (Performance-based and method independent):

Successful hydrolysis judged by the hydrolysis yield of marker compounds
(i.e. judiciously selected hydrolysis-resistant esters and conjugates)

What needs to be done?

- ► Check hydrolysis conditions required for saponifying a selected range of esters in a variety of matrices
- ► Check impact of hydrolysis conditions on matrix effects
- ► Decide if procedure can be simplified to one single (strong) hydrolysis condition for all types of matrices
- ► Select suitable marker compound(s) for checking hydrolysis success

Various Esters show different resistance to hydrolysis



Candidate for controling effectiveness of hydrolysis

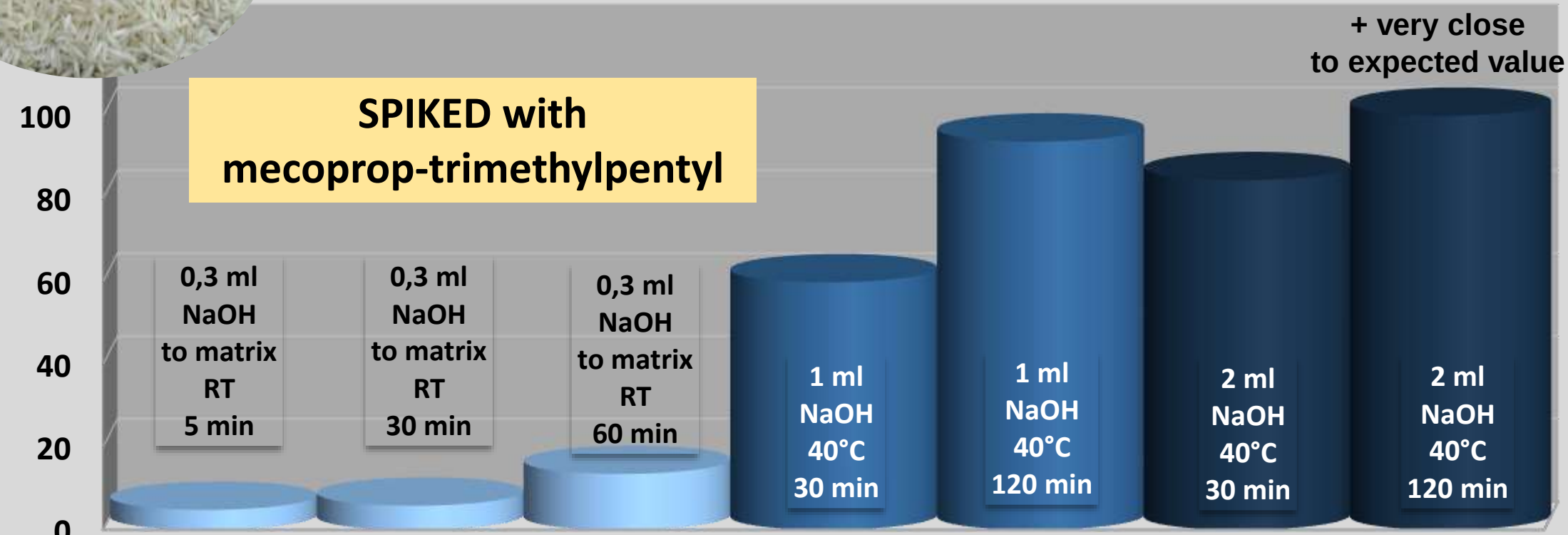
Mecoprop (sum) in EUPT-SRM15 material (Rice Flour)

MCPP free acid

at different conditions of alkaline Hydrolysis

SPIKED with mecoprop-trimethylpentyl

Set at 100%
+ very close to expected value



Checking suitability of MCPP-TMP Ester for performance check
Extraction of samples with INCURRED residues

	QuEChERS	40°C/30 min 1 mL NaOH	40°C/120 min 2 mL NaOH	60°C/60 min 2 mL NaOH
CHIA				
Incurred Haloxyfop	0,071	0,101	0,107	0,105
MCPP-TMP (Hydrolysis Control)	-	68	95	101
PEPPER				
Incurred 2,4-D	0,098	0,124	0,114	0,130
MCPP-TMP (Hydrolysis Control)	-	94	102	99
PAPRIKA SPICE				
Incurred Fluazifop	0,008	0,066	0,074	0,078
Incurred 2,4-D	0,004	0,009	0,010	0,011
MCPP-TMP (Hydrolysis Control)	-	24	99	100

No hydrolysis | EN15662 | SRM-43 | SRM-43

HYDROLYSIS FACTOR

HF=1.5

HF=1.3

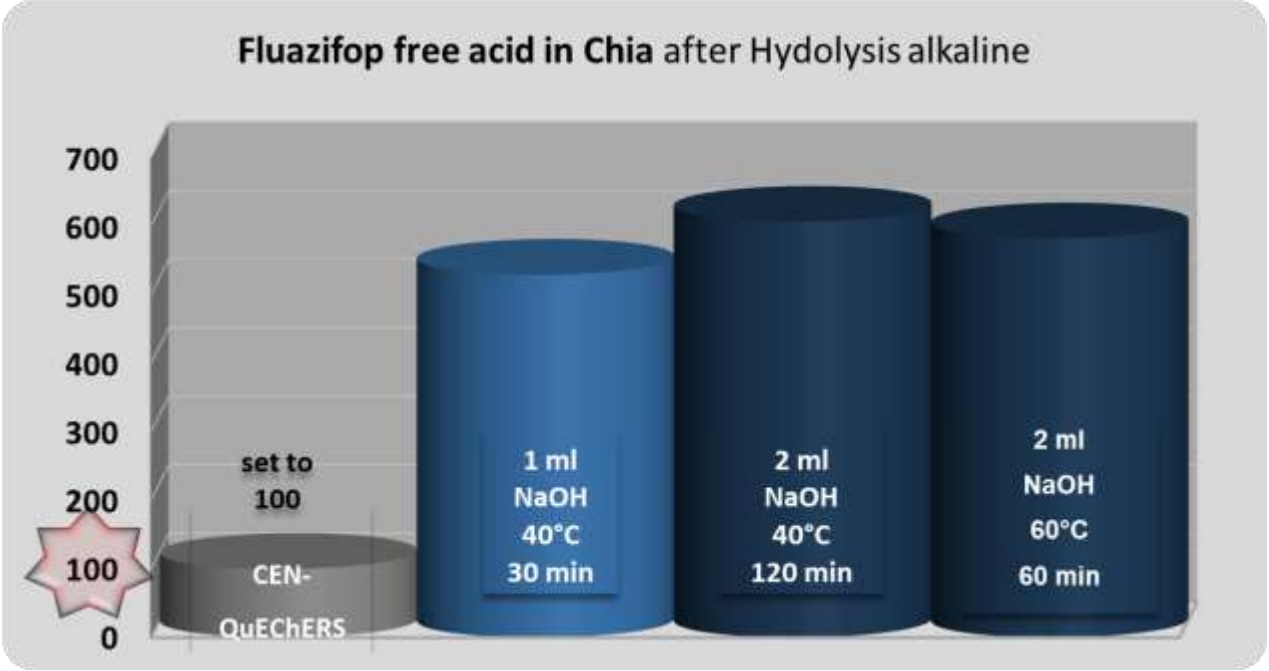
HF=9.8

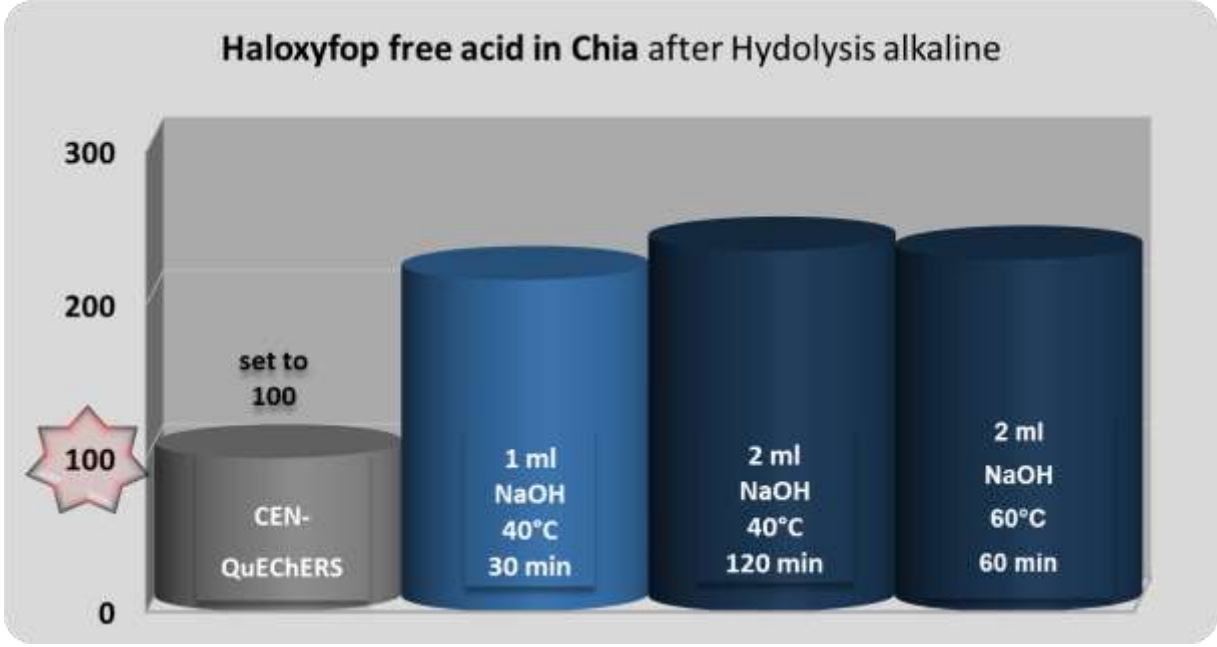
HF= 2.8

Note: EN15662 conditions are typically sufficient for breaking up conjugates!

Hydrolysis of Conjugates

INCURRED residues of **Fluazifop** hydrolyzed at various conditions

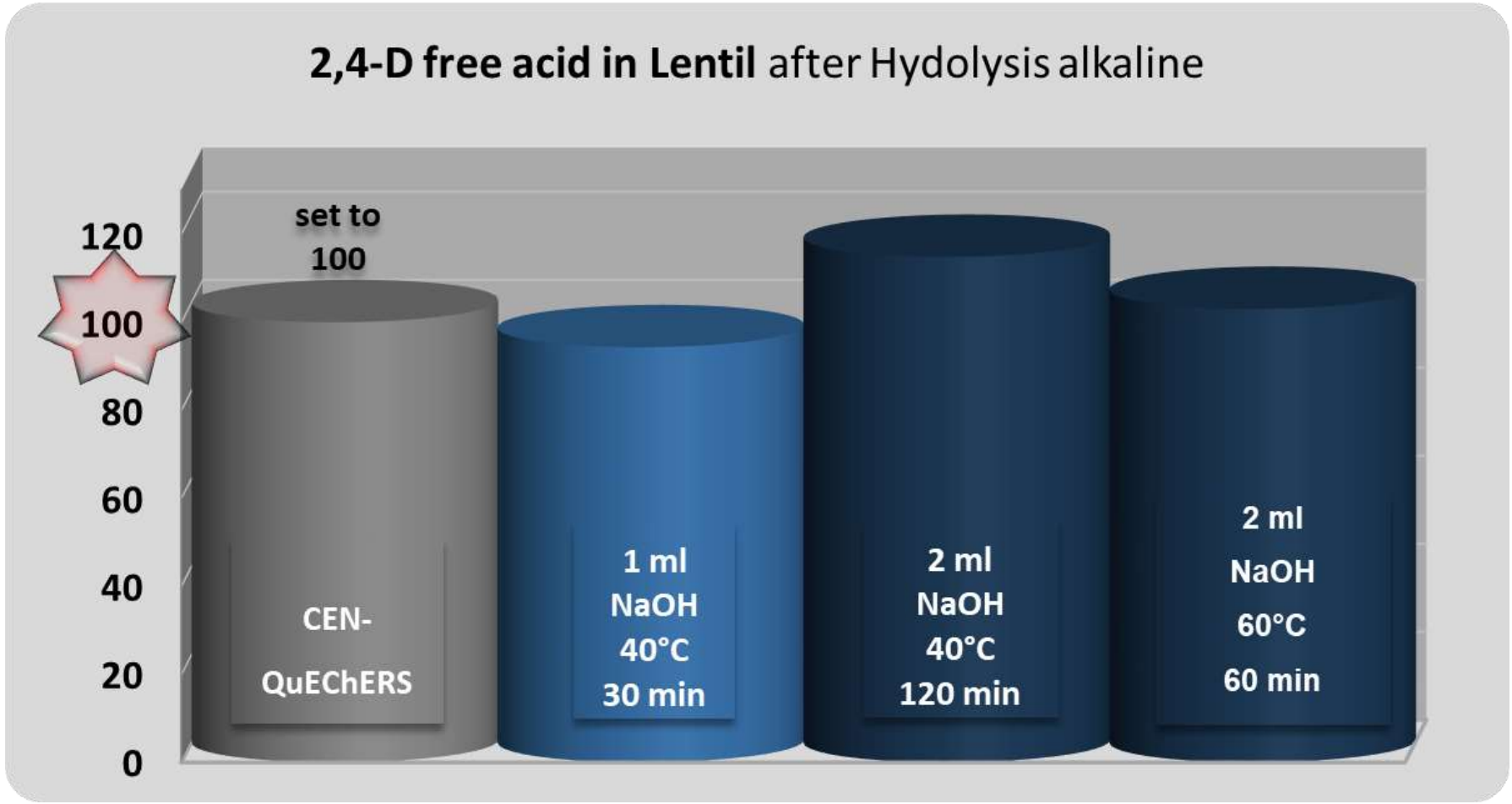




HF=6 **HYDROLYSIS FACTORS** **HF=2.2**

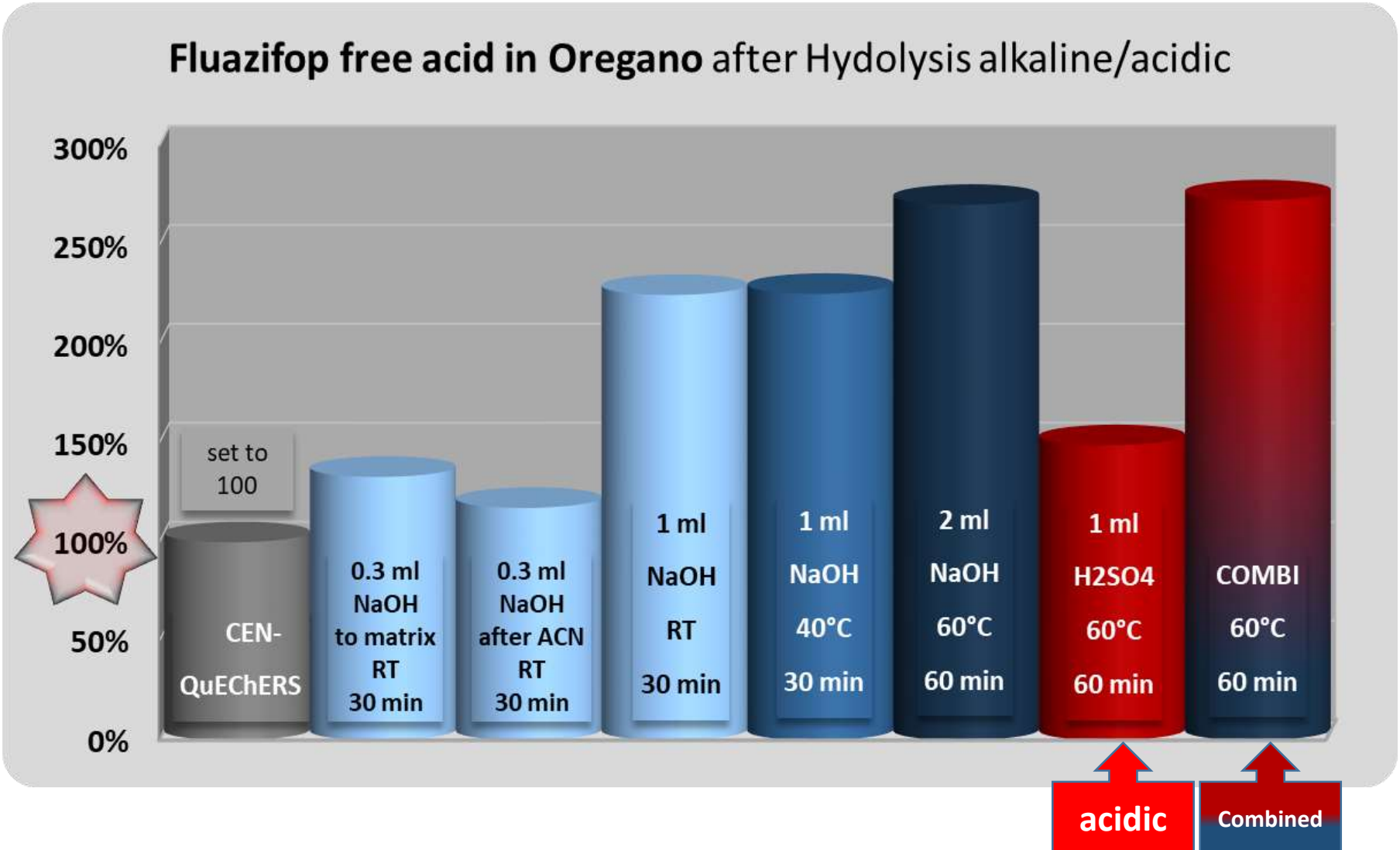
Hydrolysis of **Conjugates**

Incurred residues of **2,4-D in lentils**



HYDROLYSIS FACTOR
VERY SMALL
HF close to 1

Incurred residues of **Fluazifop** hydrolyzed at various conditions



CEN-QuEChERS
mild alkaline hydrolysis conditions
medium alkaline hydrolysis conditions
strong alkaline hydrolysis conditions
strong acidic hydrolysis conditions

HYDROLYSIS FACTOR (HF) = 2.7

How can a method(s) involving hydrolysis be used efficiently in routine ?

Several questions come up:

Q1: Which commodities are relevant in terms of residues of acidic herbicides?

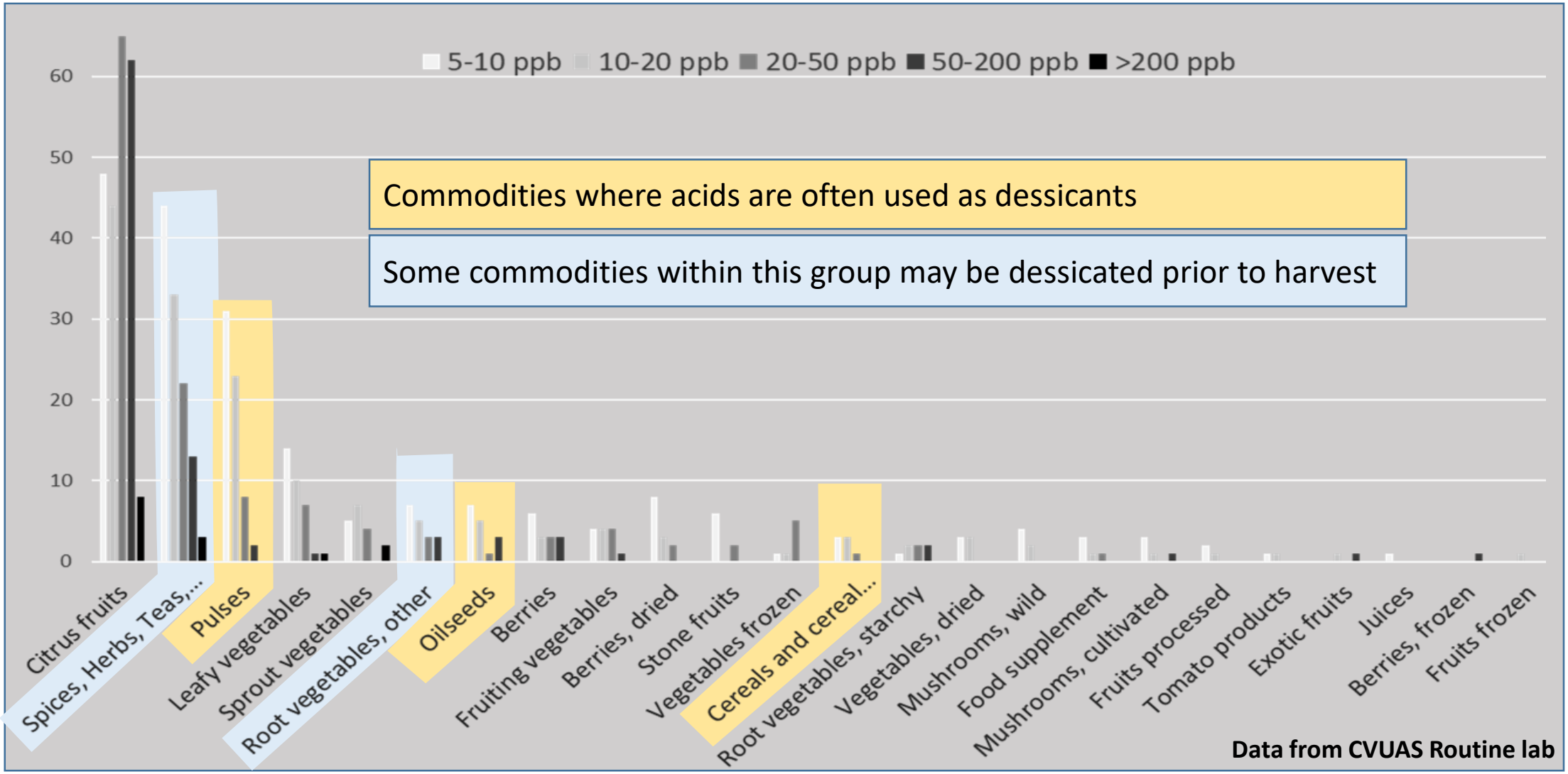
Q2: What is the share of conjugated acids in samples with incurred residues?

Q3: Is it always worthwhile hydrolyzing samples w. residues of acidic pesticides ?

Q4: Down to which level should free acids be analyzed in the pre-screening, to ensure that re-analysis involving hydrolysis is triggered (if levels for the sum exceed LOQ)?

Q1: Which commodities are relevant in terms of residues of acidic herbicides?

Commodity Types in which acidic herbicides have been encountered (2010 – 2022)



How can the method(s) involving hydrolysis be used efficiently in routine ?

What needs to be done?

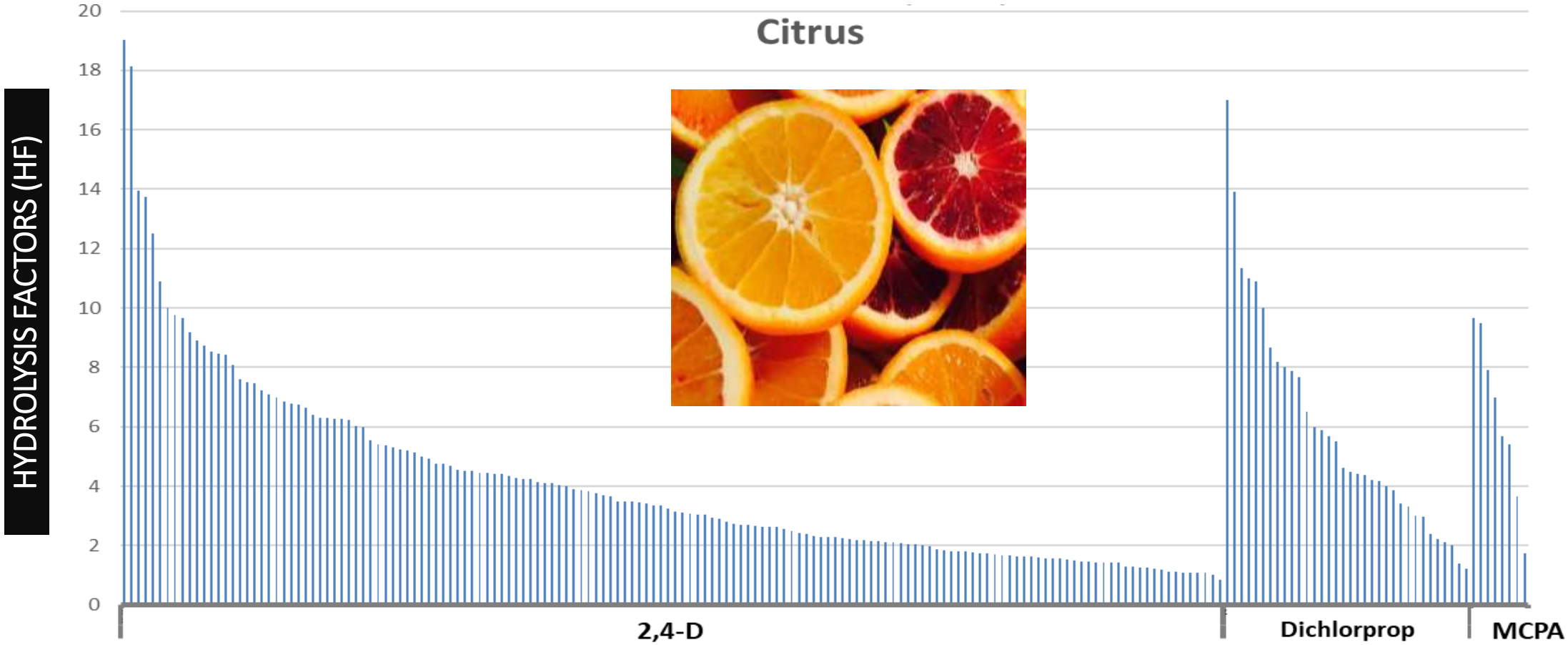
1. **Analyze samples by two approaches and collect the data:**
   a. *by a method **NOT** entailing de-conjugation/hydrolysis*
   b. *by a method **entailing de-conjugation/hydrolysis***

2. **Calculate Hydrolysis Factors**

$$\text{Hydrolysis Factor (HF)} = \frac{\text{Conc. of total acid determined after Hydrolysis}}{\text{Conc. of free acid determined w/o Hydrolysis}}$$

Q2: What is the share of conjugated acids in samples with incurred residues?

Example: Citrus fruits



How can the method(s) involving hydrolysis be used efficiently in routine ?

What needs to be done?

3. **Evaluate the HF-collection and set a reasonable/desired "quasi-worst-case" HF (QWC-HF)**

4. **Considering various factors** *(achievable LOQ/SDL of pre-analysis, achievable LOQ for the sum, lowest desired RL for the sum, MRLs …)* **labs can set reasonable Trigger Levels (TrL) for the pre-analysis and if needed adjust RLs for the (sum)**

Trigger Level (TrL) * QWC-HF should be ≤ RL (for sum)

Importance of Collecting Hydrolysis Factors (HFs)

HFs facilitate establishment of reasonable trigger levels for re-analysis entailing de-conjugation.

If HF is high, the trigger level should be low, e.g. :

MRL=0.02 mg/kg
Quasi worst case HF = 5

Trigger level = 0.004 mg/kg

MRL=0.02 mg/kg
Quasi worst case HF = 1.3

Trigger level = 0.01 mg/kg

NOTE: There is some uncertainty in the collected data

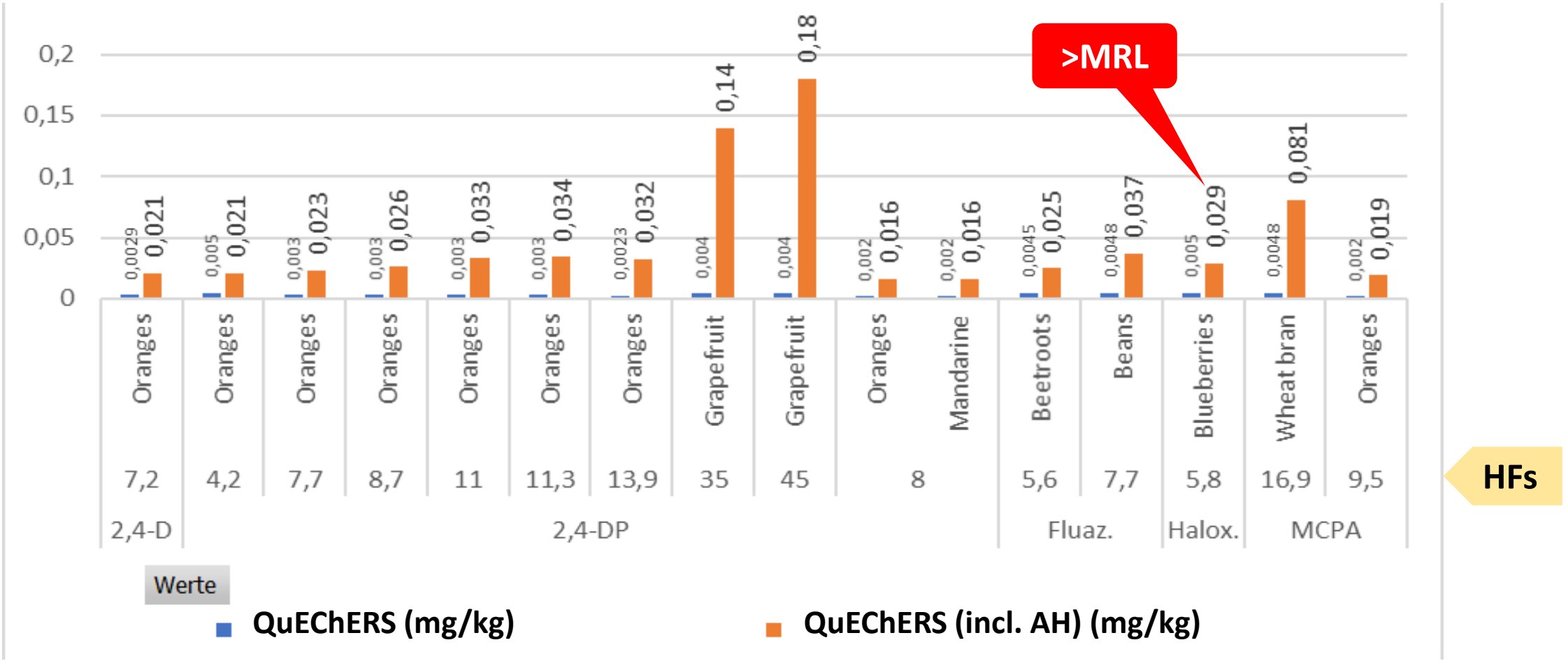
- Hydrolysis conditions might have been to weak for the case
- Matrix-effects may have not been properly corrected for MEs

Q2: What is the share of conjugated acids in samples with incurred residues?

Example: Citrus fruits

QWC-HF = 10
could be a reasonable value for 2,4-D, 2,4-DP and MCPA (sensitivity allowing)

Examples of samples where pre-analysis values were ≤ 0.005 mg/kg and summed result was >0.01 mg/kg



Q2: What is the share of conjugated acids in samples with incurred residues?

Example: Spices

Possible QWC-HF = 10
Differentiation between individual matrices would be reasonable

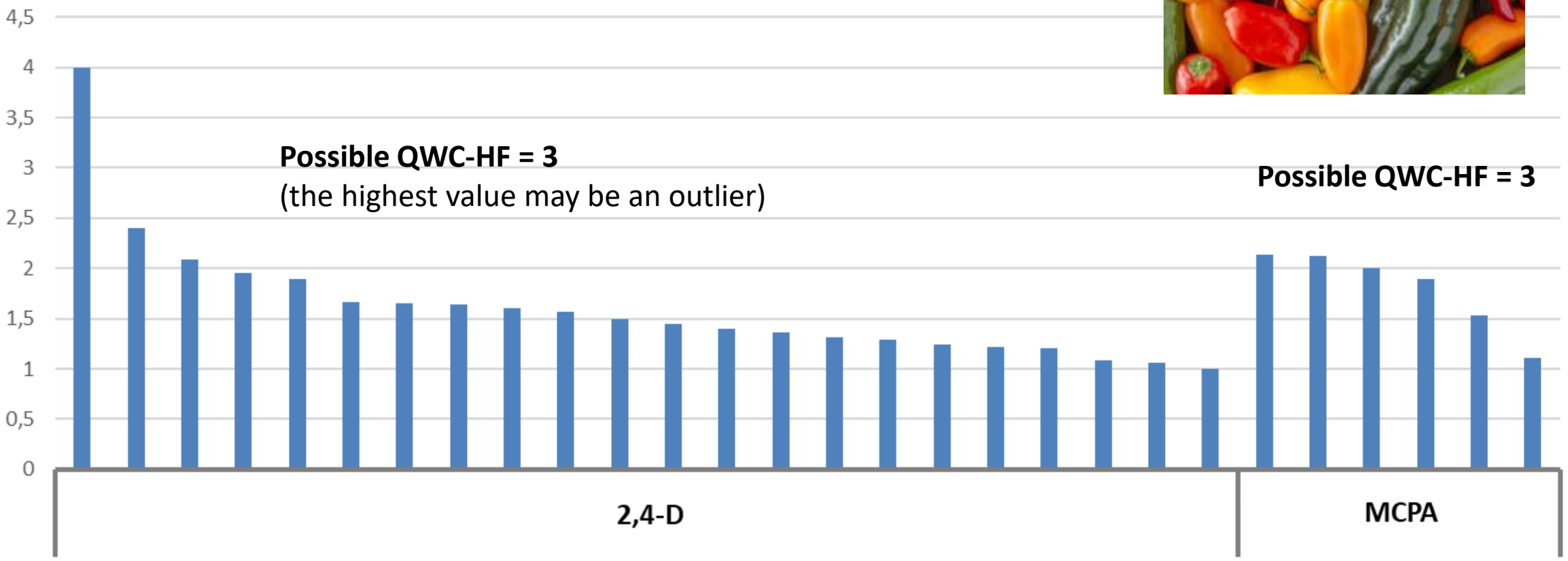
Possible QWC-HF = 4
(considering small population of data)

Possible QWC-HF = 4
(considering the small population of data)

Source: CVUA Stuttgart

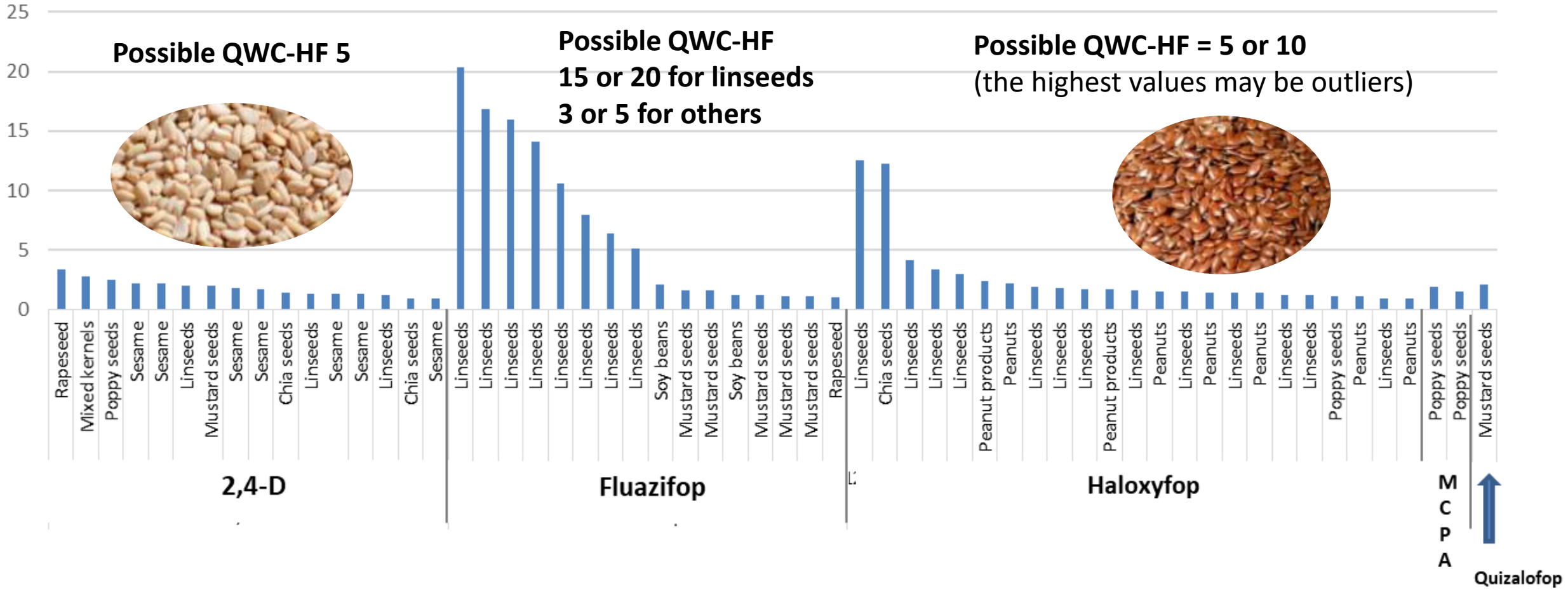
Q2: What is the share of conjugated acids in samples with incurred residues?

Example: Solanaceae



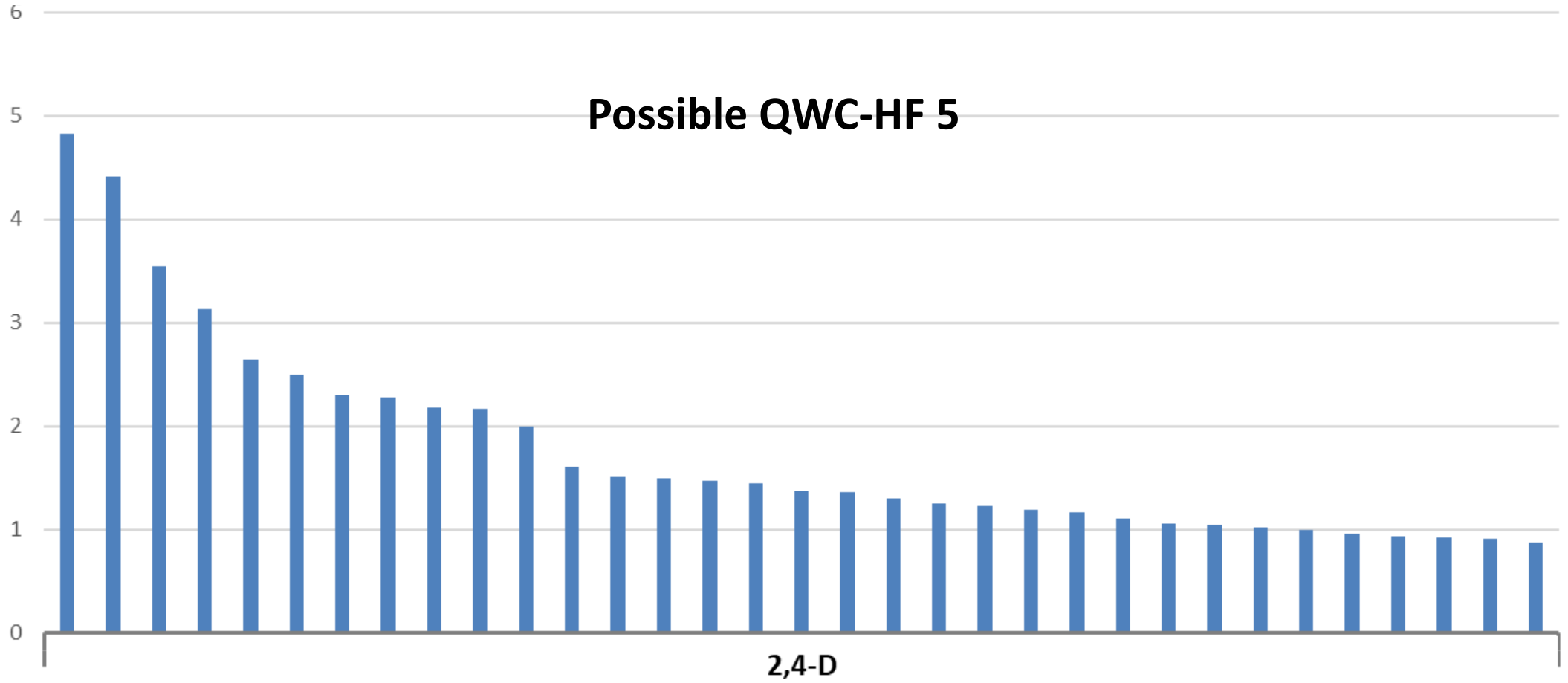
Q2: What is the share of conjugated acids in samples with incurred residues?

Example: Oilseeds



Q2: What is the share of conjugated acids in samples with incurred residues?

Example: Cocoa



Hydrolysis of **Conjugates**

Enzyme Group	Enzyme	Note	working pH optimum
xxxNONExxx	Acid-H (60°C/60 min)	2 mL 5N H2SO4	~0
Amidase (protease)	Pepsin	Porcine Gastric Mucosa	~2
Protease + Lipase	Rabbit Gastric Extract	first optimum	~2
Glucosidase	alpha glucosidase	Rice	~4,5
Glucosidase	beta Glucosidase	almonds	~4,5
Glucuronidase	beta Glucuronidase	Patella Vulgata	~6
Amidase (protease)	Papain	Papaya	~6
Amidase (protease)	Protease	B. licheniformis	~7,5
Esterase	Esterase	Porcine liver	~7,5
Protease + Lipase	Rabbit Gastric Extract	second optimum	~8
xxxNONExxx	Alk-H (60°C/60 min)	2 mL 5N NaOH	~14

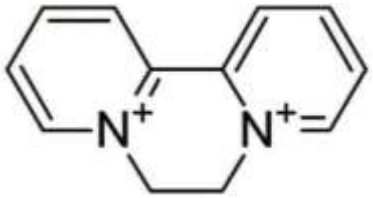
Hydrolysis of **Conjugates**

		Ester	Glycoside-Conjugates				Amino acid Conj.
			Glycosyl-Esters		Acetals		Amide
					Aliphatic	Phenolic	
Enzyme/Hydrolysis	pH	MCPP-TMP	MCPA-Glucoside	Haloxyfop-Glucoside	Pinoxaden Metabolit M5	2.4-D-O-Glucoside	Dichlorprop-Aspartat
Acid-H (60°C/60 min)	~0	✓✓✓	✓✓✓	✓✓	✓	✓	✓
Pepsin	~2	✓	✓	✓	x	x	x
Rabbit Gastric Extract	~2	✓	✓	✓	x	x	x
alpha glucosidase	~4,5	✓✓	✓✓✓	✓	x	✓	x
beta Glucosidase	~4,5	✓✓	✓✓✓	✓	✓✓✓	✓✓✓	x
beta Glucuronidase	~6	✓✓	✓✓✓	✓✓	x	x	x
Papain	~6	✓✓	✓✓✓	✓✓	x	x	x
Protease	~7,5	✓✓	✓✓✓	✓✓✓	x	x	x
Esterase	~7,5	✓✓✓	✓✓✓	✓✓✓	x	x	x
Rabbit Gastric Extract	~8	✓✓	✓✓✓	✓✓	x	x	x
Alk-H (60°C/60 min)	~14	✓✓✓	✓✓✓	✓✓✓	x	✓✓	x

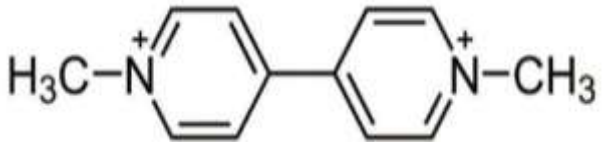
Analysis of Paraquat and Diquat

Analysis of Diquat (DQ) and Paraquat (PQ)

- Both are non-selektive herbicides
- Both are banned in the EU … but are still widely used elswhere (e.g. as crop-desiccants (e.g. on potatoes, oilseeds, cereals)



Diquat (DQ)



Paraquat (PQ)

Analysis of Diquat (DQ) and Paraquat (PQ) – Critical Points

- EU-MRLs mostly at LOQ with some exeptions

e.g.:

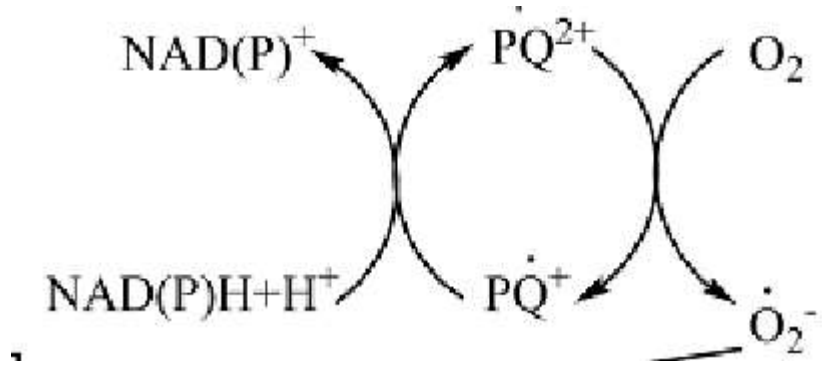
- **PARAQUAT**
  - **Rice**: 0.05 ppm

- **DIQUAT**
  - **Oats**: 2 ppm
  - **Potatoes**: 0.1 ppm
  - **Oil seeds:** Linseed 5 ppm, Rapeseed: 1.5 ppm, Sunflower seed 0.9 ppm, Soy 0.3 ppm, Oat 2 ppm
  - **Pulses:** 0.2 ppm (Peas 0.3 ppm),
  - **Tree nuts:** 0.2 ppm
  - **Tree fruits:** Citrus, Pome fruit, Stone fruit …: 0.02 ppm
  - **Other fruits:** Strawberries: 0.05 ppm; Bananas 0.02 ppm

Vázquez C (2015)

Background of herbicidal and toxicological activity

- PQ / DQ get oxidized by O_2 forming PQ / DQ radicals and **Oxigen Radicals**
- PQ/DQ radicals react w. NADPH and **PQ/DQ are re-generated**
- Organism looses all ist reduction power and dies



Check ladungsbalance
sollte PQ^{3+} heißen ?
Oder geht $2xH^+$ weg?

→ $HO^{\bullet}$ (Hydroxy radicals)
H_2O_2

NADPH (reduction cofactor in cells)

CHALLENGES IN THE ANALYSIS OF PQ and DQ:

MRM-Ion-Ratios Variable Depending on Matrix

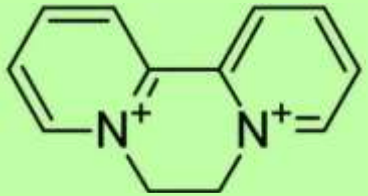
DQ and PQ form **various precursor ions**:

- **Dications $[M]^{2+}$**
- **Radical cations $[M]^{+*}$**
- **Deprotonated cations $[M\text{-}H^{+}]^{+}$**

Relative generation rate of precursor ions depends on:

- Composition of mobile phase during elution **(incl. co-eluting matrix)**
- Design and condition of the LC-MS/MS interface

Paraquat (PQ)



Diquat (DQ)

CHALLENGES IN THE ANALYSIS OF PQ and DQ:

TAKE HOME MESSAGE:

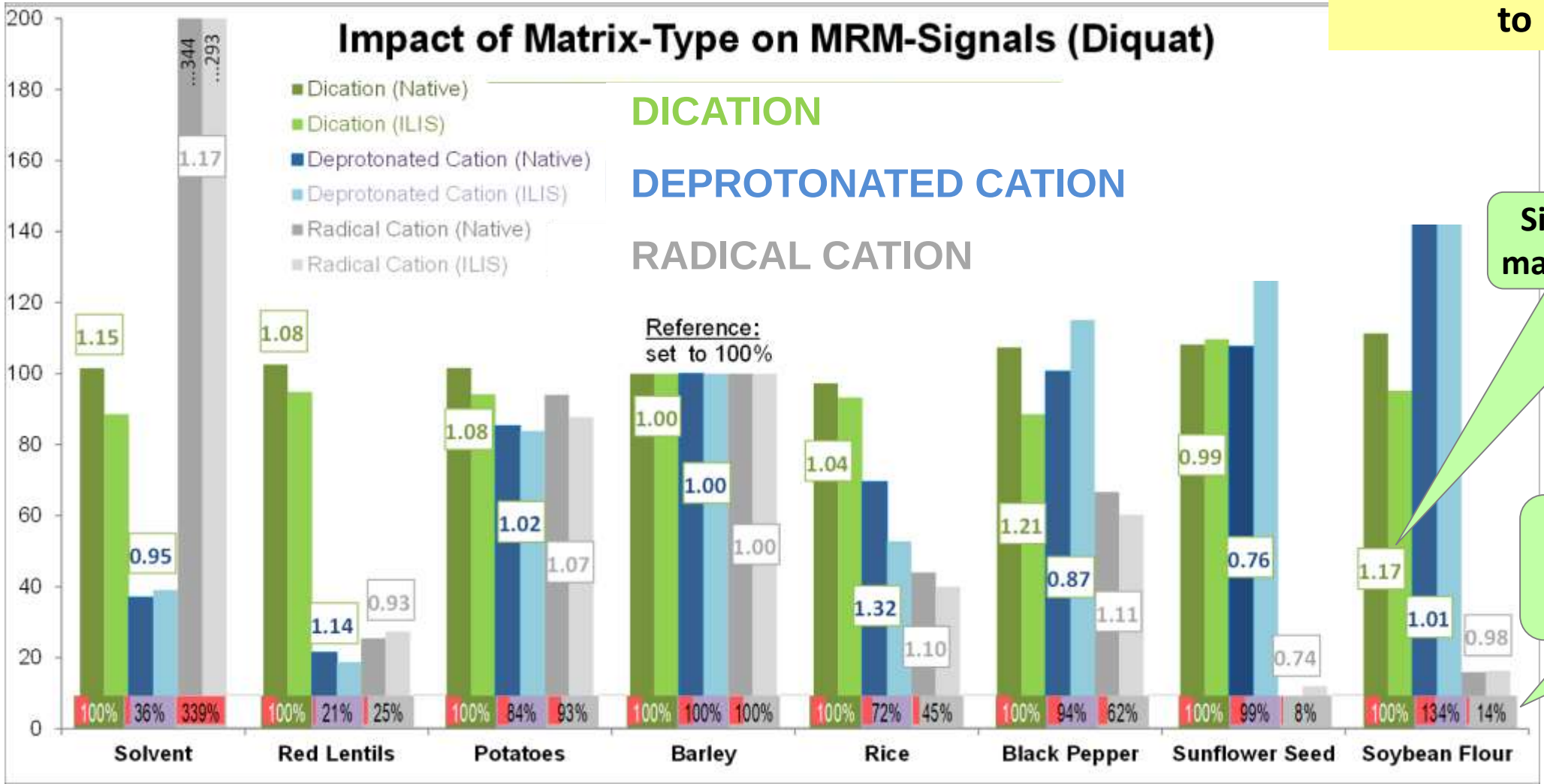
For AQC-based identifications use MRMs of same precursor!

If from diff. precursors, use signal-ratios against ILIS for calc.

… but use matching MRMs of native compound and ILIS !!

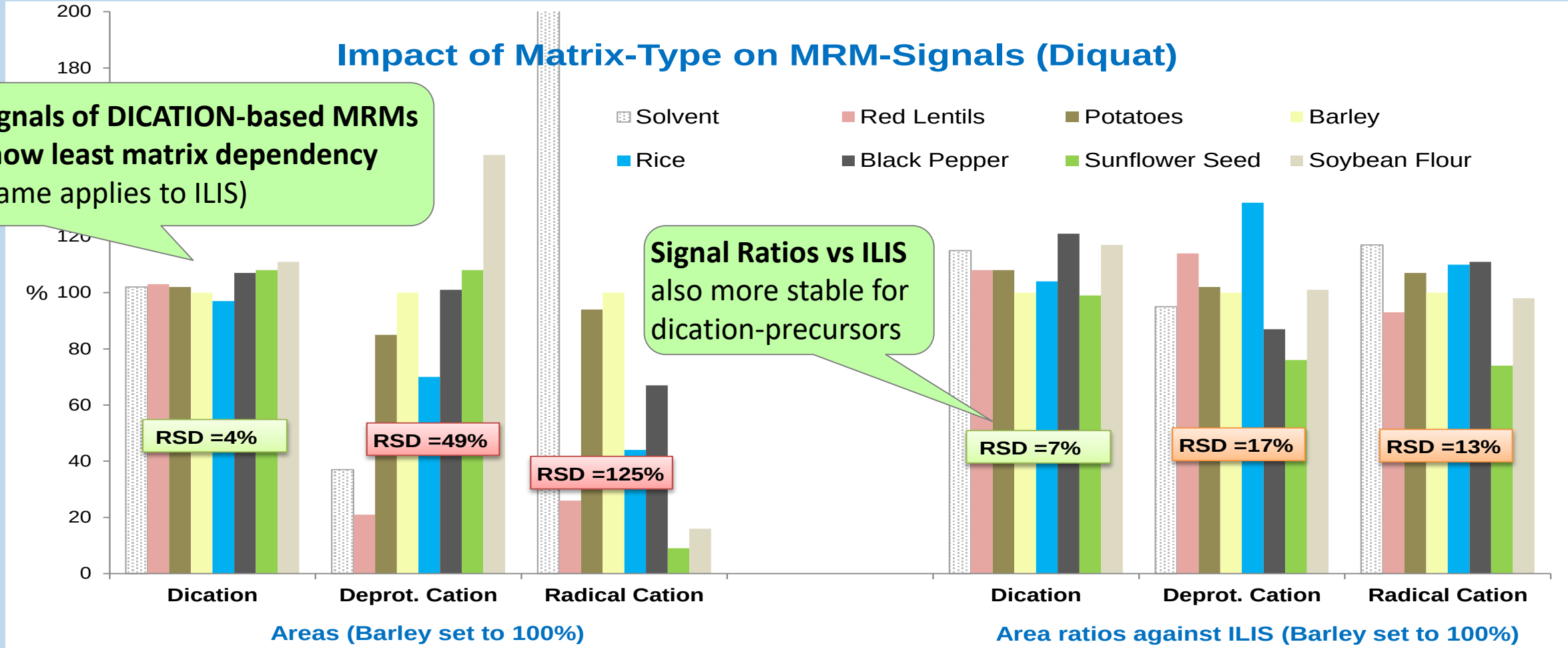
MRM-Ion-Ratios Variable Depending on Matrix

Signals in Barley normalized to 100%!!



Signal Ratio against matching MRM of ILIS

Rel. signal strengths of MRMs (dication signal set at 100%)



Signals of DICATION-based MRMs show least matrix dependency (same applies to ILIS)

Signal Ratios vs ILIS also more stable for dication-precursors

Deprotonated Cation & Radical Cation seem to be „**competing**“

Dication precursor preferable **!!!**
(limited rel. matrix effects)

CHALLENGES IN THE ANALYSIS OF PQ and DQ:

Extractability Issues

ILIS-based correction works well, even at poor abs. recoveries

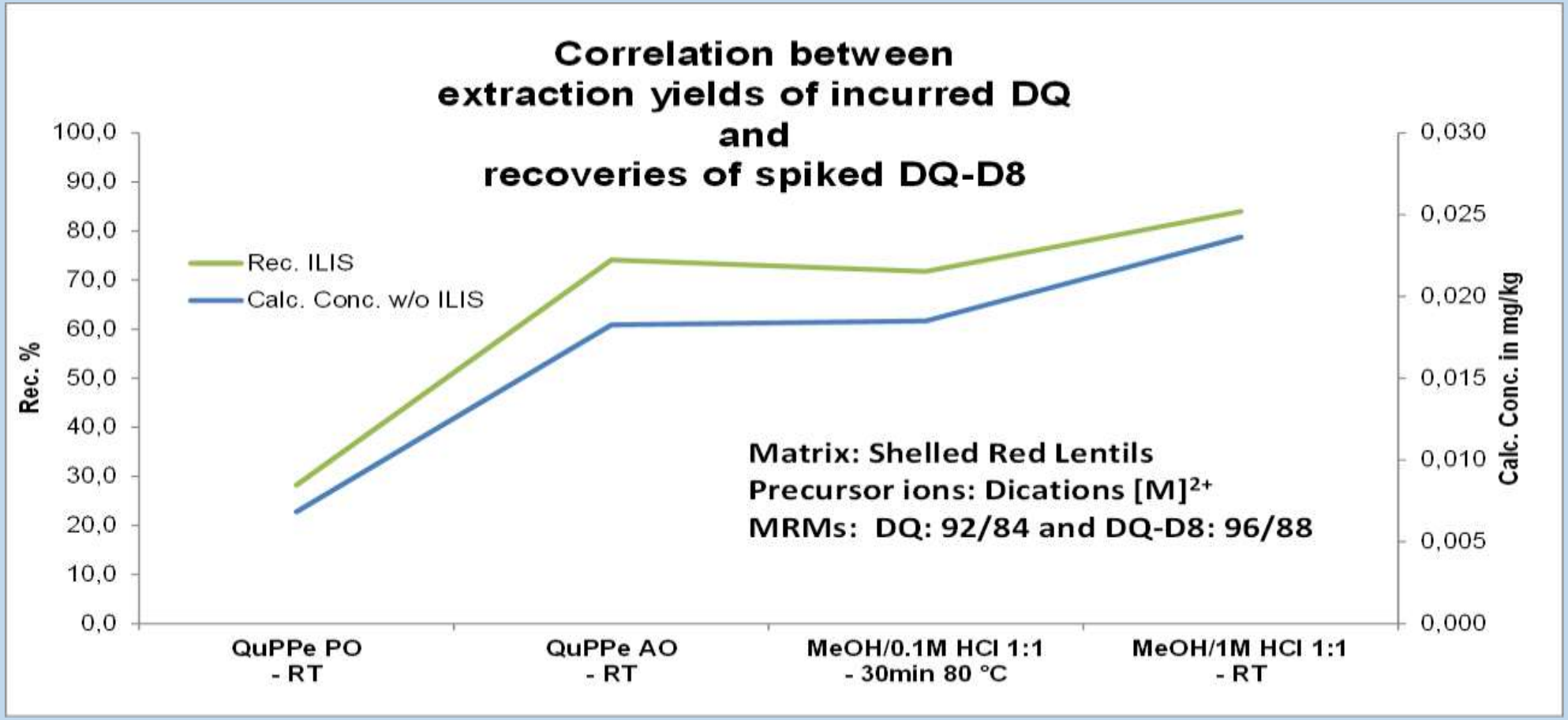
- DQ / PQ tend to **interact with matrix** during extraction
- **Poor recoveries especially for oily seeds** (e.g. flax, chia) and **pulses**

Absolute and ILIS-corrected recovery rates of spiked DQ and PQ (level 0.2 mg/kg)		QuPPe AO (EDTA /1% FA / RT)	QuPPE PQ/DQ **1M** HCl / RT	QuPPE PQ/DQ 0,1M HCl / **80 °C**	QuPPE PQ/DQ **1M** HCl / **80 °C**	QuPPe AO (EDTA /1% FA / RT)	QuPPE PQ/DQ **1M** HCl / RT	QuPPE PQ/DQ 0,1M HCl / **80 °C**	QuPPE PQ/DQ **1M** HCl / **80 °C**
		no ILIS				with ILIS			
Rice	**DQ**	95	87	81	95	109	105	108	99
	PQ	89	88	85	91	105	97	109	102
Chia	**DQ**	15	74	3	99	108	102	81	99
	PQ	12	60	9	88	107	102	102	96
Lentils (brown, unshelled)	**DQ**	12	50	28	74	110	104	113	100
	PQ	10	15	15	41	114	99	109	97

Stronger acidification (0.1N HCl → 1 N HCl)
improves extraction yields for difficult matrices (also helps to avoid FNs)

QuPPe: Diquat and Paraquat – Extractability

- Yields of incurred residues correlate well with the recoveries of spiked DQ/PQ
  → incurred residues and spiked residues are subject to the same equilibria



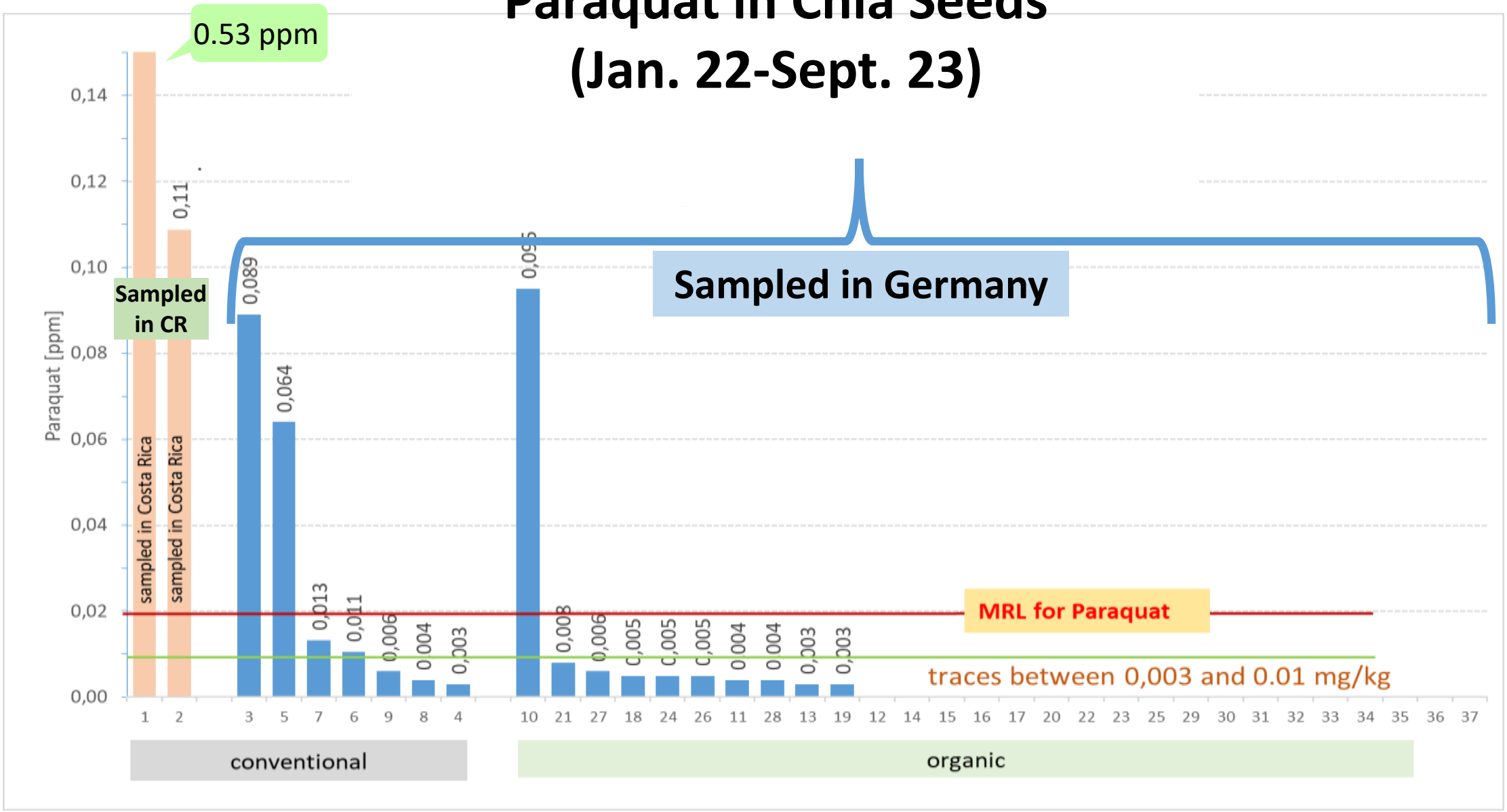
Analysis of Diquat and Paraquat in Market Samples

- Various market samples analyzed for DQ and PQ

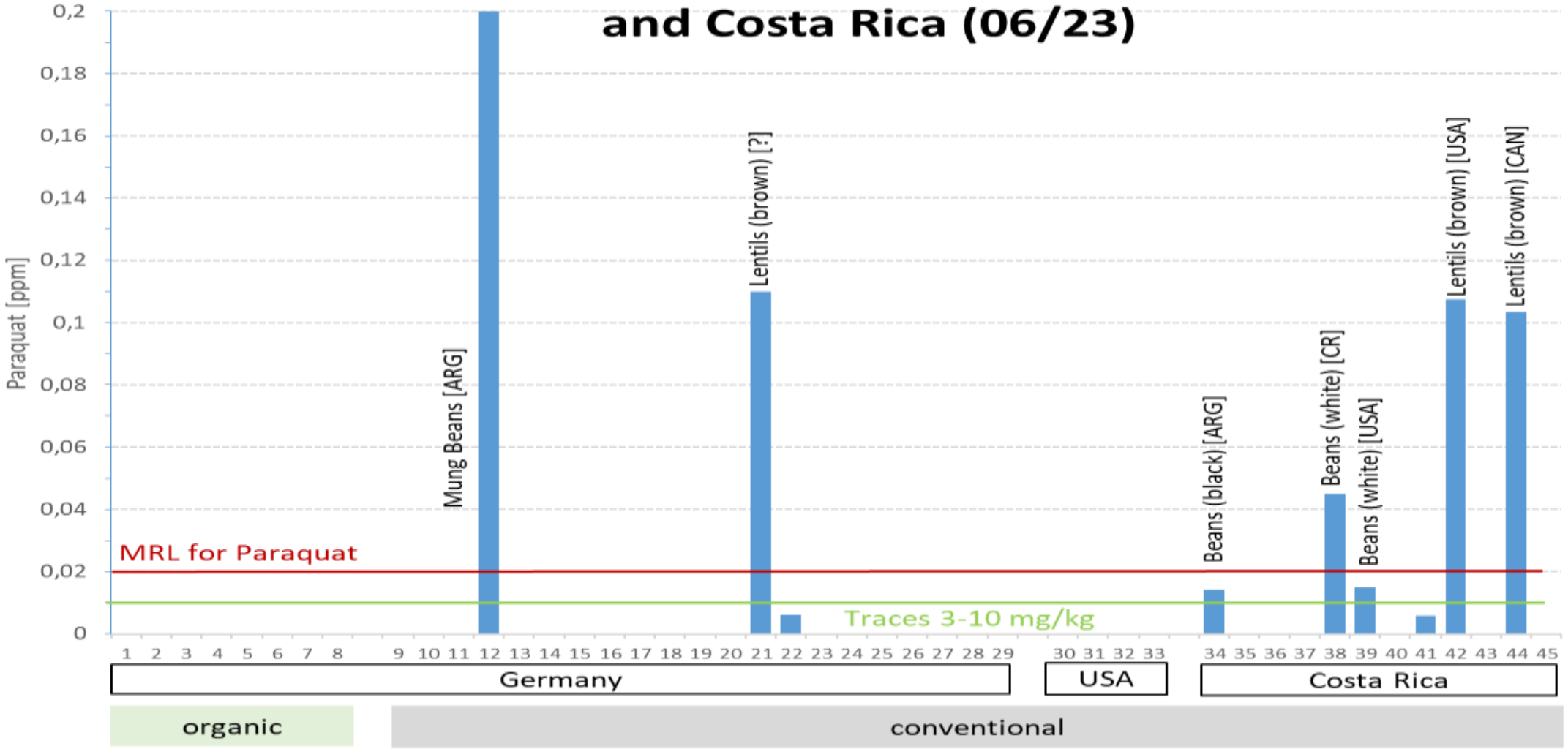
Results of analyzed market samples 04/2022 - 04/2023 (QuPPe 1M HCl)	Analyzed	DQ		PQ	
		> LOQ - < MRL	> MRL	> LOQ - < MRL	> MRL
	Number of Samples **CONVENTIONAL / ORGANIC**				
Linseeds (flax)	**2 / 9**	**1 / 1**	**-**	**-**	**-**
Chia seeds	**5 / 18**	**-**	**-**	**2 / 0**	**3 / 1**
Lentils	**9 / 6**	**-**	**-**	**-**	**1 / 0**
Beans (dried)	**4 / 0**	**-**	**-**	**-**	**1 / 0**
Pepper (black)	**4 / 0**	**-**	**-**	**1 / 0**	**1 / 0**

→ Residues also found in **ORGANIC** Chia (1x >MRL) and Linseed (1x <MRL)

Paraquat in Chia Seeds (Jan. 22-Sept. 23)



Paraquat in Pulses collected from the markets in Germany (01/23 - 09/23), USA (08/23) and Costa Rica (06/23)



Analysis of Dithiocarbamates as CS_2

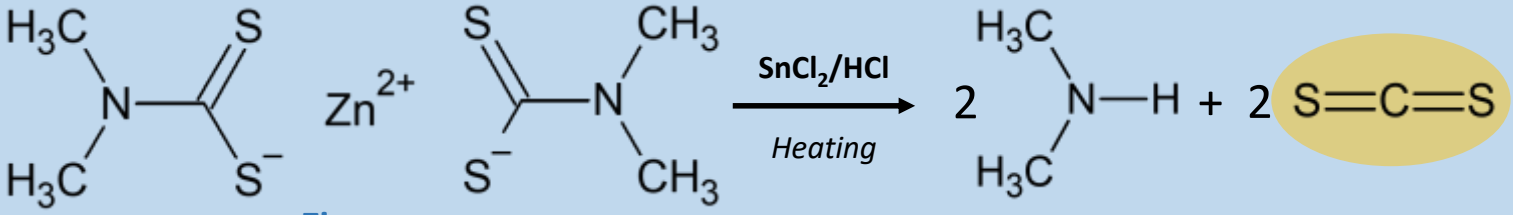
Dithiocarbamates – Status within the EU

Article 12-review initiated for….

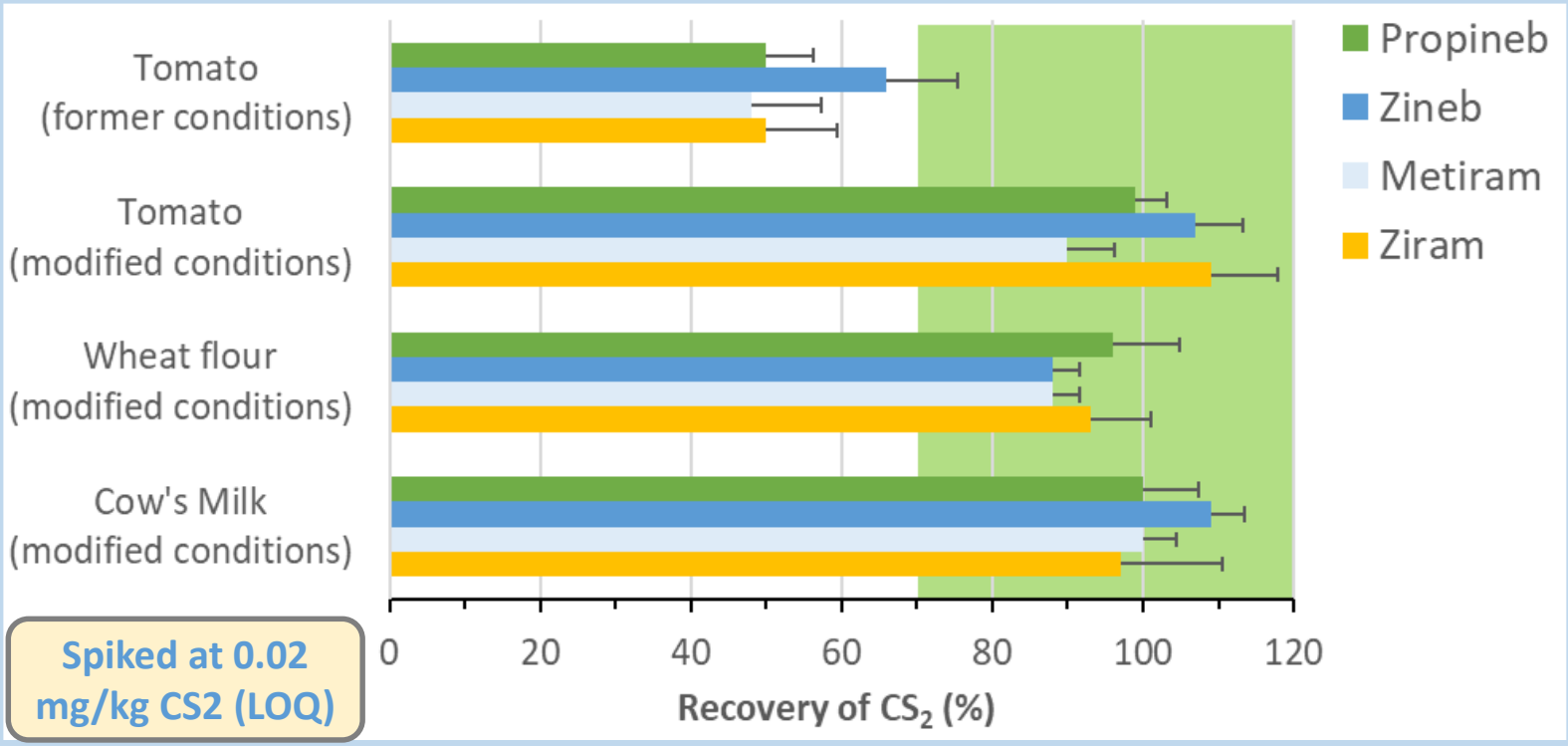
Active substance	Aproval	*Requested by EFSA*		*Requested by COM*
		Evaluation Report	Draft Reasoned Opinion	LOQs
Propineb	**Expired 03/18**	-	20 Apr 2020	10 Nov 2020 & 06 Nov 2023
Thiram	**Expired 10/18**	20 Feb 2020	27 Nov 2020	17 May 2021 & 06 Nov 2023
Ziram	**till 03/25**	16 Mar 2021	09 Nov 2022	06 Nov 2023
Maneb	**Expired 01/17**	06 Apr 2021	09 Nov 2022	*Pending…*
Metiram	**till 01/24**	06 Apr 2021	09 Nov 2022	06 Nov 2023
Mancozeb	**Expired 04/21**	06 Apr 2021	09 Nov 2022	06 Nov 2023

Dithiocarbamates – Modification of the traditional reductive cleavage method

Reductive cleavage with $SnCl_2$/HCl



Ziram



Spiked at 0.02 mg/kg CS2 (LOQ)

Former conditions:

50 g of sample homogenate
+**150 mL** hydrolysis agent
(Agent:Sample-Ratio: 3:1)
+ **25 mL** isooctane (2g sample/mL)
→**2 h @ 80 °C** in a water bath

New conditions:

10 g of sample homogenate
+**75 mL** hydrolysis agent
(Agent:Sample-Ratio: 7.5:1)
+ **10 mL** isooctane (1g sample/mL)
→**3 h @ 85 °C** in a water bath

Hydrolysis agent (0.1 M $SnCl_2$+4 M HCl)

QuPPe-Compounds Residue Situation

Topics

EURL-SRM Network
NRL-SRM Network

Proficiency Tests
EUPT-SRM Overview
EUPT-SRM18 (Honey)
EUPT-SRM17 (Tomatoes)
EUPT-SRM16 (Sesame)
EUPT-SRM15 (Rice)

Workshops
Workshop Overview
Joint Workshop 2023
Joint EURL/NRLs (SRM-FV) 2022

Services
ILISs Distribution
CheckYourScope
SRM-PinBoard
EURL-SRM Methods
Analytical Observations
Residue Observations
Downloads
Sources of Standards

Latest News

19-04-2023 | EURL-SRM
Risk of False Positives of Chloridazon-Desphenyl in Honey by LC-MS/MS
A new EURL-SRM Analytical Observations Report concerning the risk of false positive findings of chloridazon-desphenyl in honey by LC-MS/MS is available. Various chromatographic separation methods for chloridazon-desphenyl were tested to clarify the situation.

17-03-2023 | EURL-SRM
QuPPe-PO-Method Version 12.1
The QuPPe-PO-Method has been updated (now includes more detailed information on Honey analysis).

16-03-2023 | EURL-SRM
Analysis of the Folpet and Captan degradants Phthalimide (PI) and Tetrahydrophthalimide (THPI) by QuEChERS and LC-MS/MS
The Analytical Observation Report (SRM-49) on the analysis of PI and THPI via LC-MS/MS was updated by introducing additional validation data. This update also includes results of experiments concerning the transformation of Captan and Captafol to Tetrahydrophthalimide (THPI) and of Folpet to Phthalimide (PI), during various steps of the QuEChERS procedure and especially in thawed sample homogenates prior to extraction.

03-03-2023 | EURL-SRM
Determination of fluoride ion in food
Two approaches for the determination of Fluoride Ion via selective electrodes (ISE) are described: a) direct measurement in QuPPe extracts and b) measurement in diffusates derived by microdiffusion.

27-02-2023 | EURL-SRM
Compilation of Residue Observations Reports of QuPPe Compounds
A new compilation of residue findings of QuPPe compounds in food products, analysed in 2022, was uploaded. The report additionally encompasses findings of ethylene oxide / 2-chloroethanol. Aim of these

Quicklinks

EURL-DataPool
EURL Method-Finder List
EU MRLs-Database (COM)
EU Pesticides-DB - Main (COM)
EU-Legisl. on PPPs (COM)
CIRCA BC Login
RASFF Portal DB (COM)
How to Use CIRCA BC
InfoNote: Processed Food/Feed (COM)
EUPT Registration Website

Pinboard

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Topics

EURL-SRM Network
NRL-SRM Network

Proficiency Tests
EUPT-SRM Overview
EUPT-SRM18 (Honey)
EUPT-SRM17 (Tomatoes)
EUPT-SRM16 (Sesame)
EUPT-SRM15 (Rice)

Workshops
Workshop Overview
Joint Workshop 2023
Joint EURL/NRLs (SRM-FV) 2022

Services
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CheckYourScope
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EURL-SRM Methods
Analytical Observations
Residue Observations
Downloads
Sources of Standards

Compilation of Residue Observations Reports

The table below compiles various observation notes and reports related to residue findings of SRM-pesticides in food. Laboratories within the NRL/OL-Network are encouraged to submit their own notes and reports on residue findings. The idea is to gradually build up a large collection of with the aim to conserve knowledge and to offer laboratories a useful and practical source of information. A link to the **EURL-DataPool** is planned.

Compounds covered / Title of Study	Date of reports / version	Link
QuPPe-Compounds as well as Ethylene oxide / 2-Chloroethanol (2022 data)	01.03.2023 / V1	View Document
QuPPe-Compounds (2021 data)	01.04.2022 / V1	View Document
QuPPe-Compounds (2020 data)	30.03.2021 / V1	View Document
QuPPe-Compounds (2019 data)	30.01.2020 / V1	View Document
QuPPe-Compounds (2018 data)	30.04.2019 / V1	View Document
TFA (Trifluoroacetic acid) and DFA (Difluoroacetic acid)	19.06.2017 / V1	View Document
Determination of Triazole Derivative Metabolites (TDMs) in Fruit and Vegetables using the QuPPe Method and Differential Mobility Spectrometry (DMS) and Survey of the Residue Situation in Organic and Conventional Produce	CVUA Stuttgart eJournal, May 2016	View Document
Triazole derivative metabolites (TDMs) - Poster	EPRW 2014 (Dublin)	View Document

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EURL-SRM

EU Reference Laboratories for Residues of Pesticides

Single Residue Methods

Residue Findings of QuPPe-Compounds in Samples of Plant Origin from the German Market in 2022

Reported by: EURL-SRM

Version 1 (last update: 01.03.2023)

The aim of this compilation is to give an overview as to which highly polar (QuPPe-) compounds are currently encountered in food products of plant origin. This should help other institutions when it comes to taking decisions on how to expand the scope of analytes, on how to plan sampling, on which QuPPe compounds are worthwhile checking in the various samples. Ultimately, this contributes to a more targeted and efficient use of lab resources.

At CVUA Stuttgart, 44 QuPPe-compounds were routinely monitored in 2022 (see Table 1). Some of these compounds are not legally relevant, as they are not part of the legal residue definition.

Despite not being a QuPPe compound, a brief overview of the residue findings of 2-chloroethanol (formed from the reaction of the fumigant ethylene oxide with chloride) in 2022 is given at the end of this document. 2-CE findings in various products have been causing severe turbulences in the EU-food market since autumn 2020.

Table 1: Scope of QuPPe-compounds that were routinely monitored by the CVUA Stuttgart in 2022

Compound	Notes on legal limits	General notes
Ammelide	Non-regulated metabolite and contaminant	Ammelide can originate from various sources, similar to ammeline. Ammelide (and ammeline) are formed as intermediates during the gradual transformation of melamine (a compound with multiple sources, see below) to cyanuric acid. Ammelide is reported as a metabolite of various triazine pesticides incl.: cyromazine (insecticide), anilazine (fungicide) and the herbicides terbuthylazine, prometryn, simazine, atrazine, ametrin, cy-

The QuPPe website launched this year

www.quppe.eu

Quick Polar Pesticides Method

QuPPe [kjup] stands for

Quick Polar Pesticides

and is the acronym for a simple analytical approach, that covers multiple residues of highly polar pesticides and metabolites in fruits, vegetables, cereals and commodities of animal origin.

Discover more ...

The QuPPe method is in the process of becoming an EN standard.
Supplementary information regarding the standard will be accessible on this website as an on-line supplement.

Download

Method	Matrix Scope	Latest Version
QuPPe-PO	Products of Plant Origin and Honey	V12.1 Download Full Paper Last update of method: 17.03.2023
QuPPe-AO	Products of Animal Origin excluding Honey	V3.2 Download Full Paper Last update of method: 14.05.2019

Further Information

List of obsolete versions

Using routine methods to screen for marker substances of alkylene-*bis*-dithiocarbamate fungicides to enable a more judicious and efficient further analysis of this pesticide group

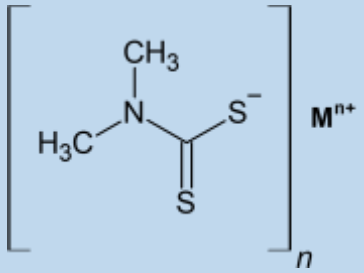
E. Eichhorn, H. Zipper, D. Mack, G. Cerchia, A. Karst, K. Rothenbächer, S. Goerlich, C. Ullrich, I. Sigalov, E. Scherbaum, M. Anastassiades

European Union Reference Laboratory for Pesticides requiring Single Residue Methods, located at the Chemical and Veterinary Analysis Agency (CVUA) Stuttgart, Fellbach, Germany

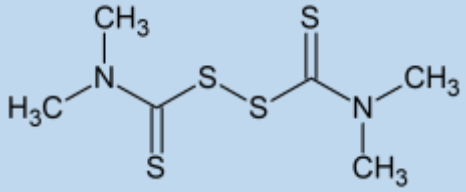
EURL Joint Workshop
18th-20th September 2023, Fellbach, Germany

Dithiocarbamates (DTC) | Introduction

N,N-Dimethyldithiocarbamate group



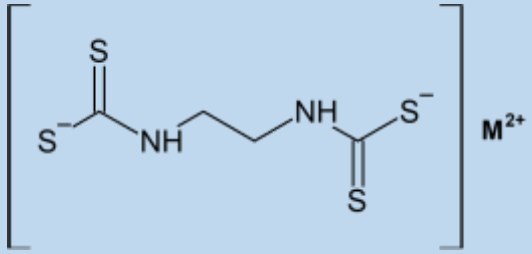
e. g. $M^{n+} = Zn^{2+}$: Ziram
$M^{n+} = As^{3+}$: Asomate
$M^{n+} = Fe^{3+}$: Ferbam



Thiram

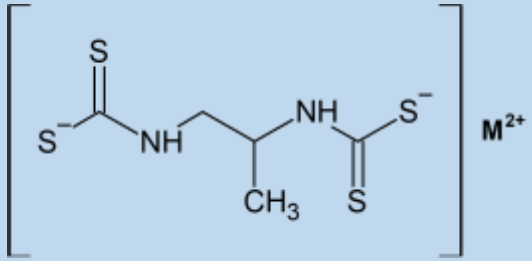
Import tolerances for bananas and mangoes

Ethylene-*bis*-dithiocarbamate group



e. g. $M^{2+} = Zn^{2+}$: Zineb
$M^{2+} = Mn^{2+}$: Maneb
$M^{2+} = Mn^{2+}/Zn^{2+}$ (94/6): Mancozeb
$M^{2+} = Zn^{2+}, NH_3$: Metiram
$M^{2+} = 2\ Na^{+}$: Nabam

Propylene-*bis*-dithiocarbamate group



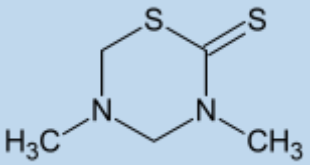
e. g. $M^{2+} = Zn^{2+}$: Propineb

Legal status within the EU
accord. to Reg. (EC) 1107/2009:

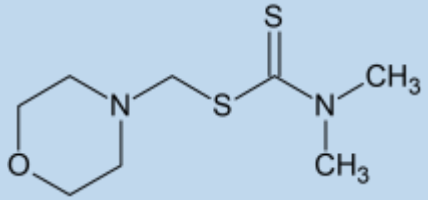
Currently approved active substance

Approval expired in the last years

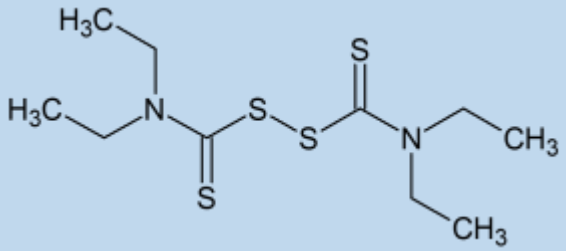
Group of other purely organic dithiocarbamates (selection)



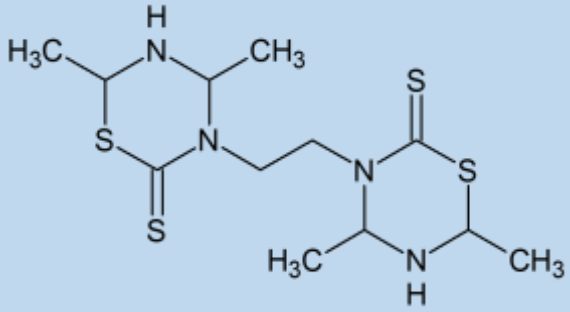
Dazomet



Carbamorph



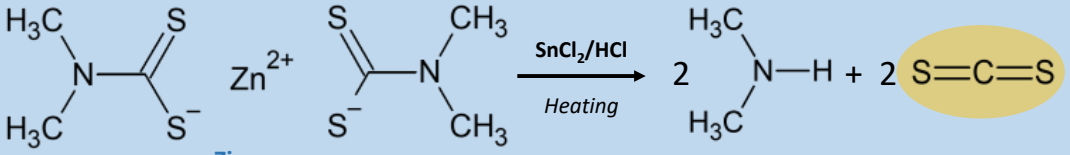
Disulfiram



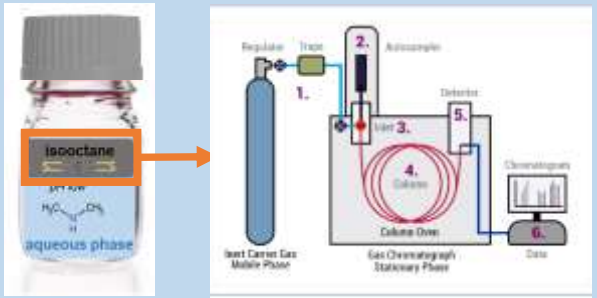
Milneb

Dithiocarbamates (DTC) | Common moiety method: analysis as CS_2

Reductive cleavage with $SnCl_2$/HCl



Ziram



Drawbacks:

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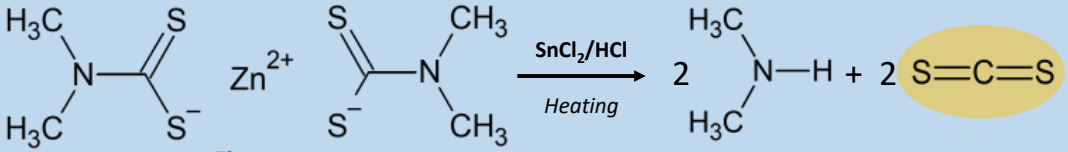
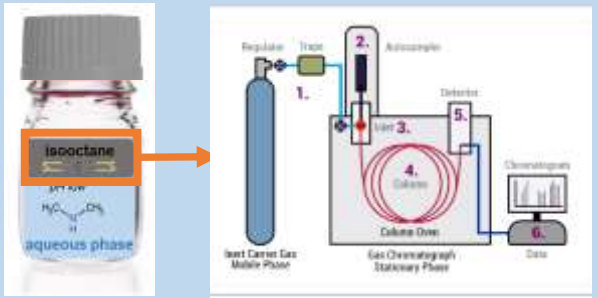
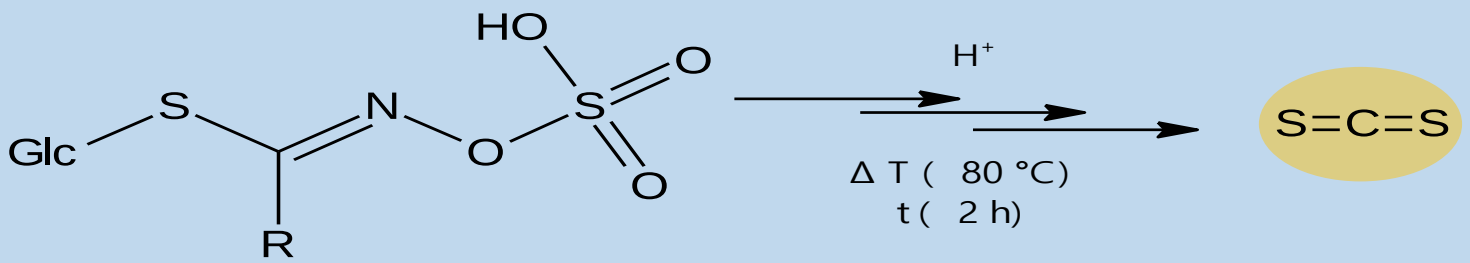




Drawbacks:

- **No distinction of CS_2 origin**

  (i.e. CS_2 from DTC-fungicides versus CS_2 from natural components in matrix, e.g. *Brassicaceae* and *Allium* genus)



Glucosinalotes
(naturally occuring in commodities
of e.g. *Brassicaceae* and *Allium* genus)

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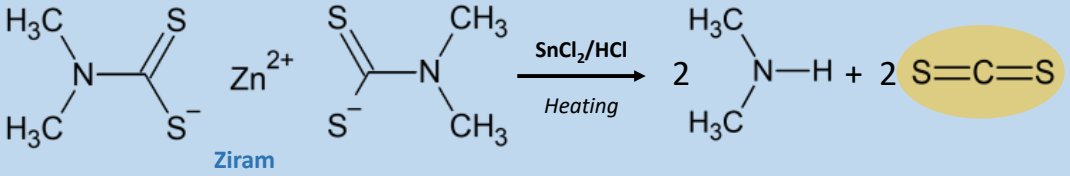

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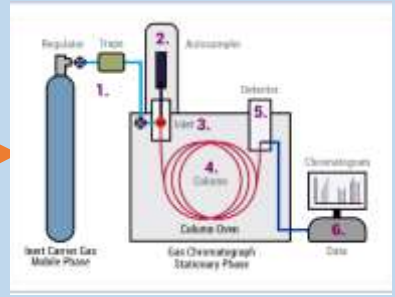

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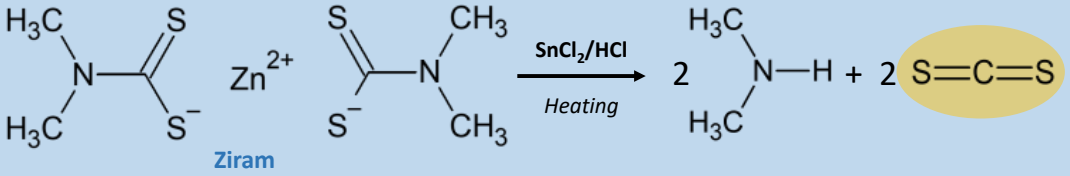

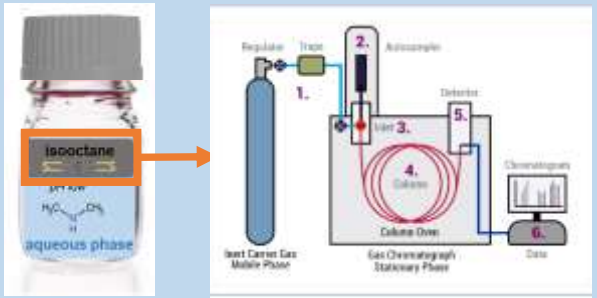

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  (not to mention distinction between individual active substances)

- **Wasteful method**

  (high consumption of HCl and $SnCl_2$)

- **Troublesome method**

  (as the cleavage of the DTCs is usually conducted at elevated temperatures for several hours)

http://die-mikrowelle.de/anwendungen-problemloesungen/page/2/; 28.09.2022 16:08

Aim of our study

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- Identify possible DTC metabolites and/or reaction products ("marker substances")
- Marker substances should be ideally:
  a. suitable as a trigger for any subsequent DTC-analyses (e.g. CS_2-analysis)
  b. specific for a DTC-treatment
  c. amenable to established multi-residue methods such as QuEChERS and QuPPe
  d. analyzable by standard LC/MS and GC/MS equipment
  e. commercially available
- Improve the cost/benefit ratio by preventing the unnecessary use of the common moiety method

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- Improve the cost/benefit ratio by preventing the unnecessary use of the common moiety method

21 markers in total initially considered, MONITORING in routine samples startet with

- 5 Ethylene-*bis*-DTC markers
- 4 Propylene-*bis*-DTC markers
- 4 N,N-Dimethyl-DTC markers

DTC-Markers | Results

[1] https://www.eurl-pesticides.eu/library/docs/srm/meth_DithiocarbamatesCS2_EurlSrm.PDF
[2] https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/EurlSrm_meth_QuPPe_PO_V12_1.pdf; last update: 17.03.2023

DTC-Markers | Results

- A total of **528 samples** were analyzed
  - **for CS_2** using the traditional method involving reductive cleavage with HCl/$SnCl_2$ [1]
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    - CEN-QuEChERS, followed by GC-MS/MS and GC-Orbitrap
    - QuPPe [2], followed by LC-MS/MS

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  - the marker was considered for a DTC group-specific evaluation
- Commodities naturally generating CS_2 were not considered for the evaluation (n = 37; evaluated individually)

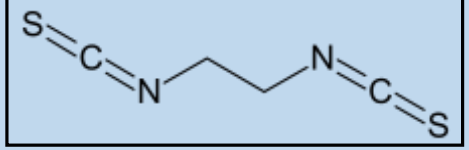
[1] https://www.eurl-pesticides.eu/library/docs/srm/meth_DithiocarbamatesCS2_EurlSrm.PDF
[2] https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/EurlSrm_meth_QuPPe_PO_V12_1.pdf; last update: 17.03.2023

DTC-Markers | Exemplary results for ethylene-*bis*-isothiocyanate (eBIC)

➢ Correlation between eBIC and CS_2 concentrations?

ethylene-*bis*-isothiocyanate (eBIC)

QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap



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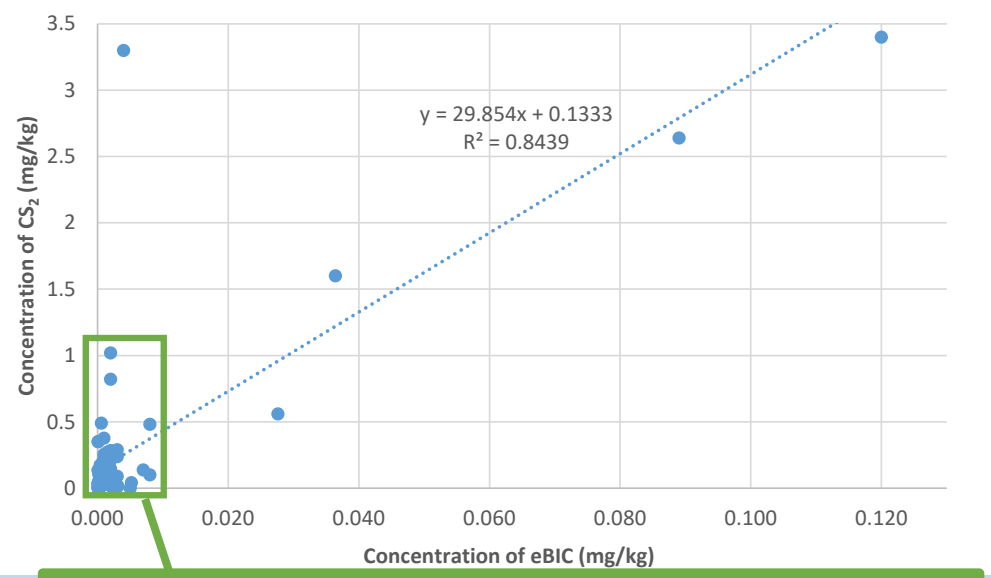
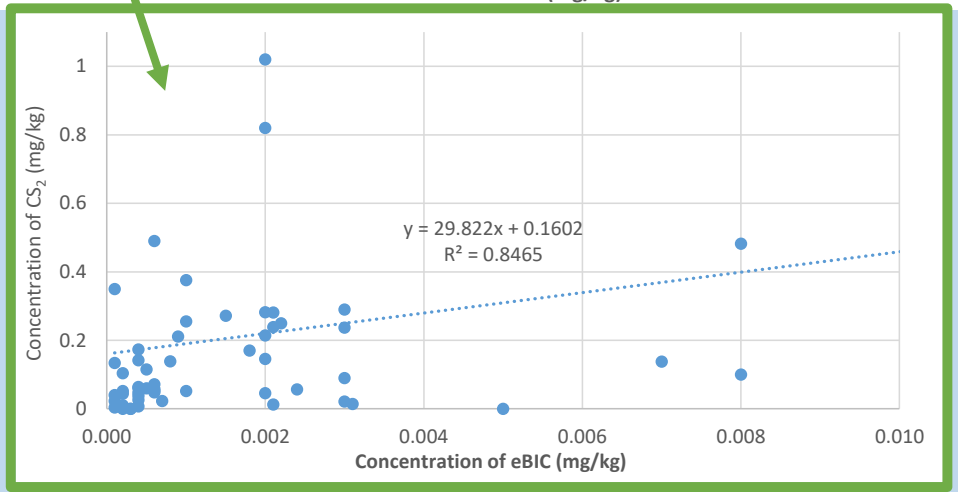
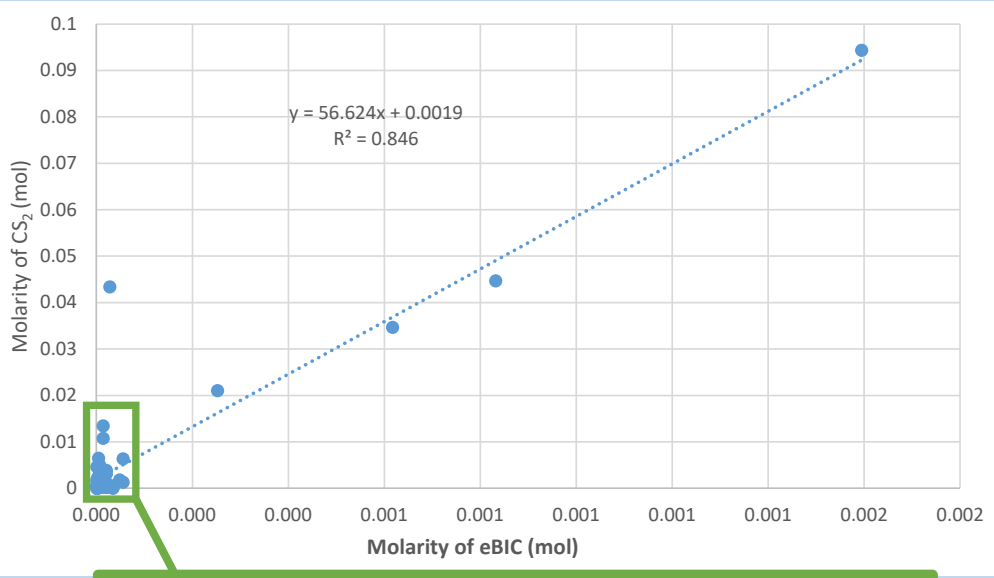
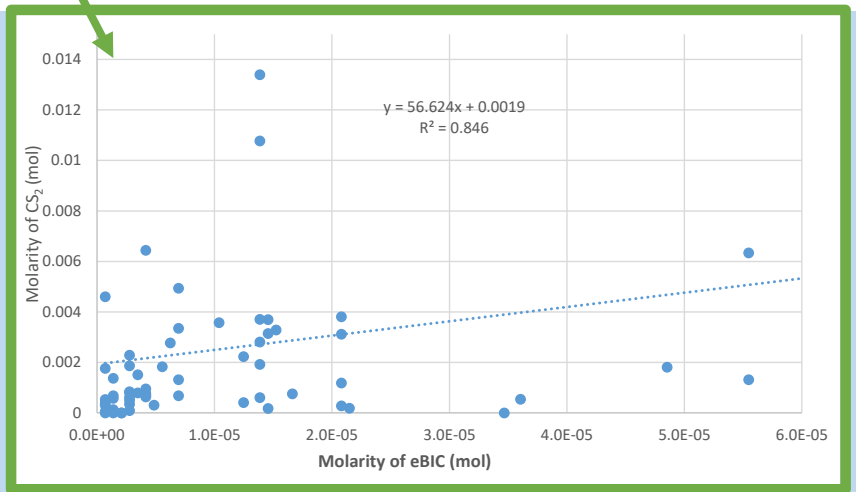


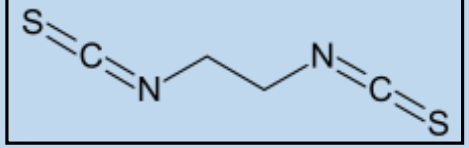






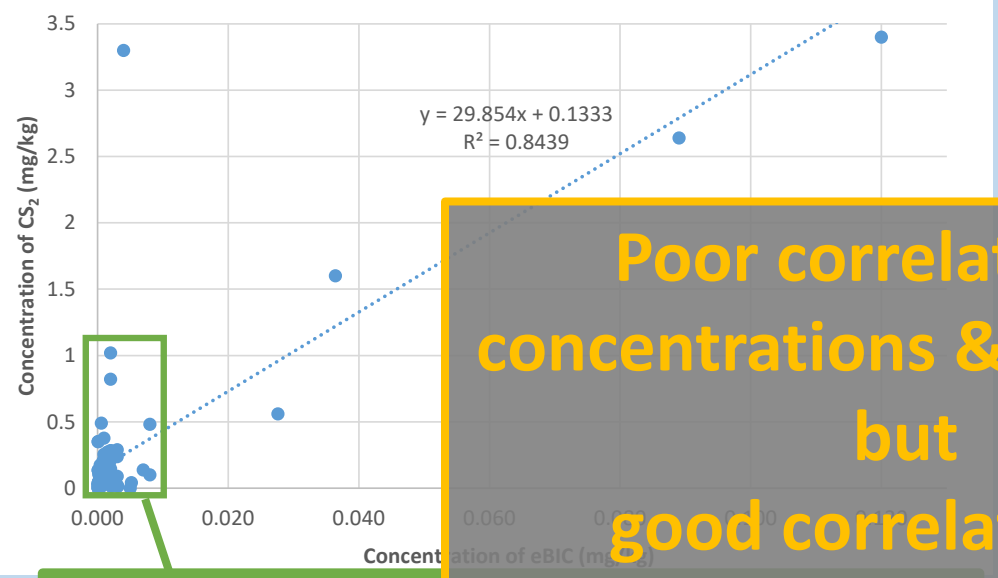
ethylene-*bis*-isothiocyanate (eBIC)

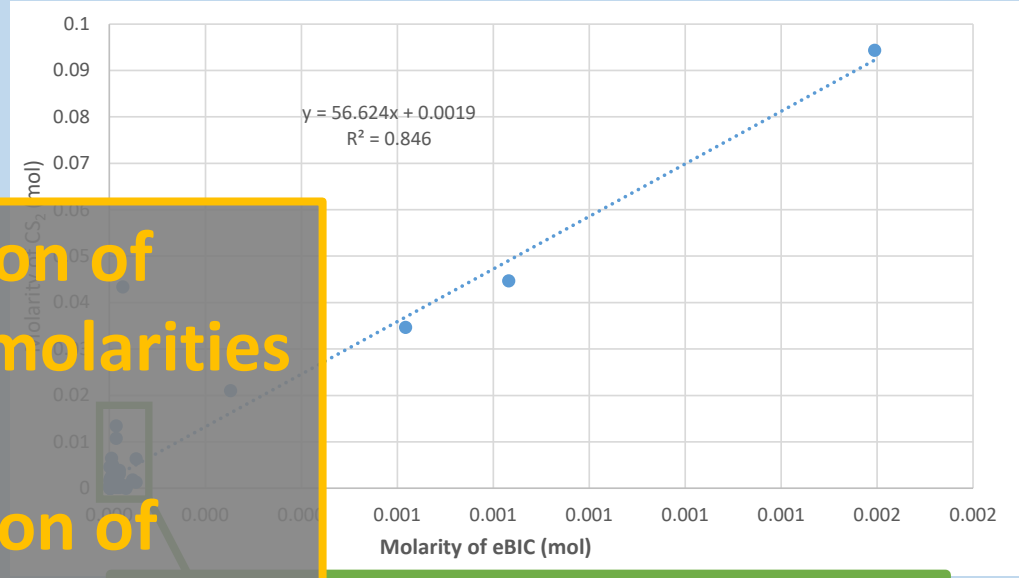
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap



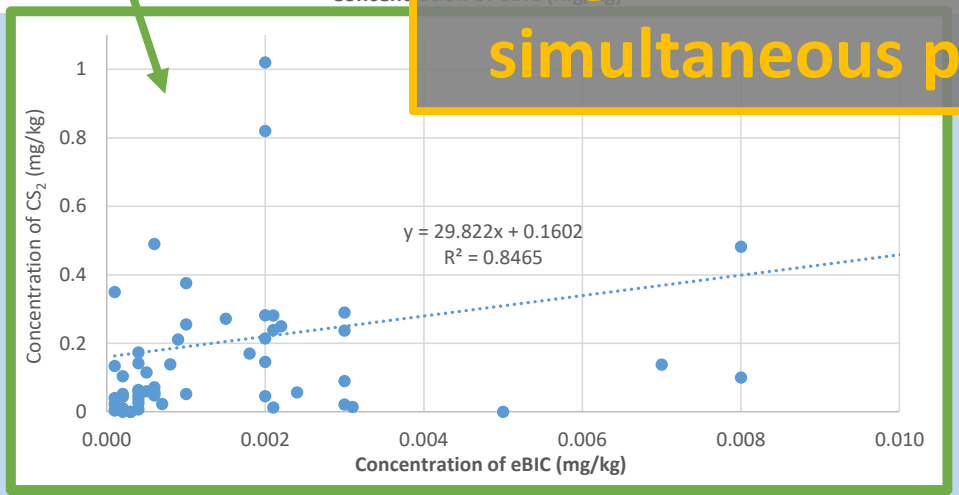
DTC-Markers | Exemplary results for Ethylene-*bis*-isothiocyanate (eBIC)

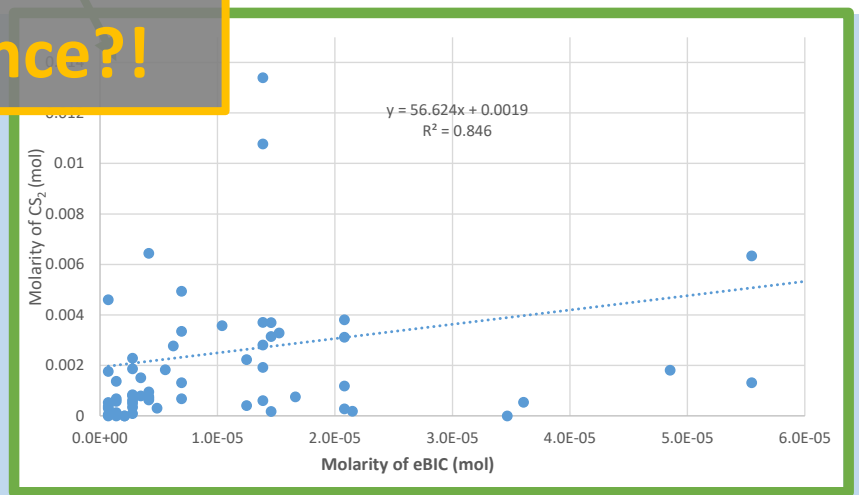
➢ Correlation between eBIC and CS_2 concentrations?





Poor correlation of concentrations & molarities but good correlation of simultaneous presence?!





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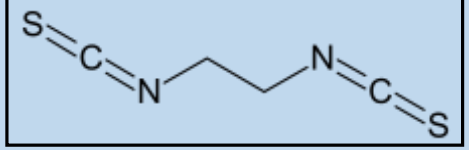
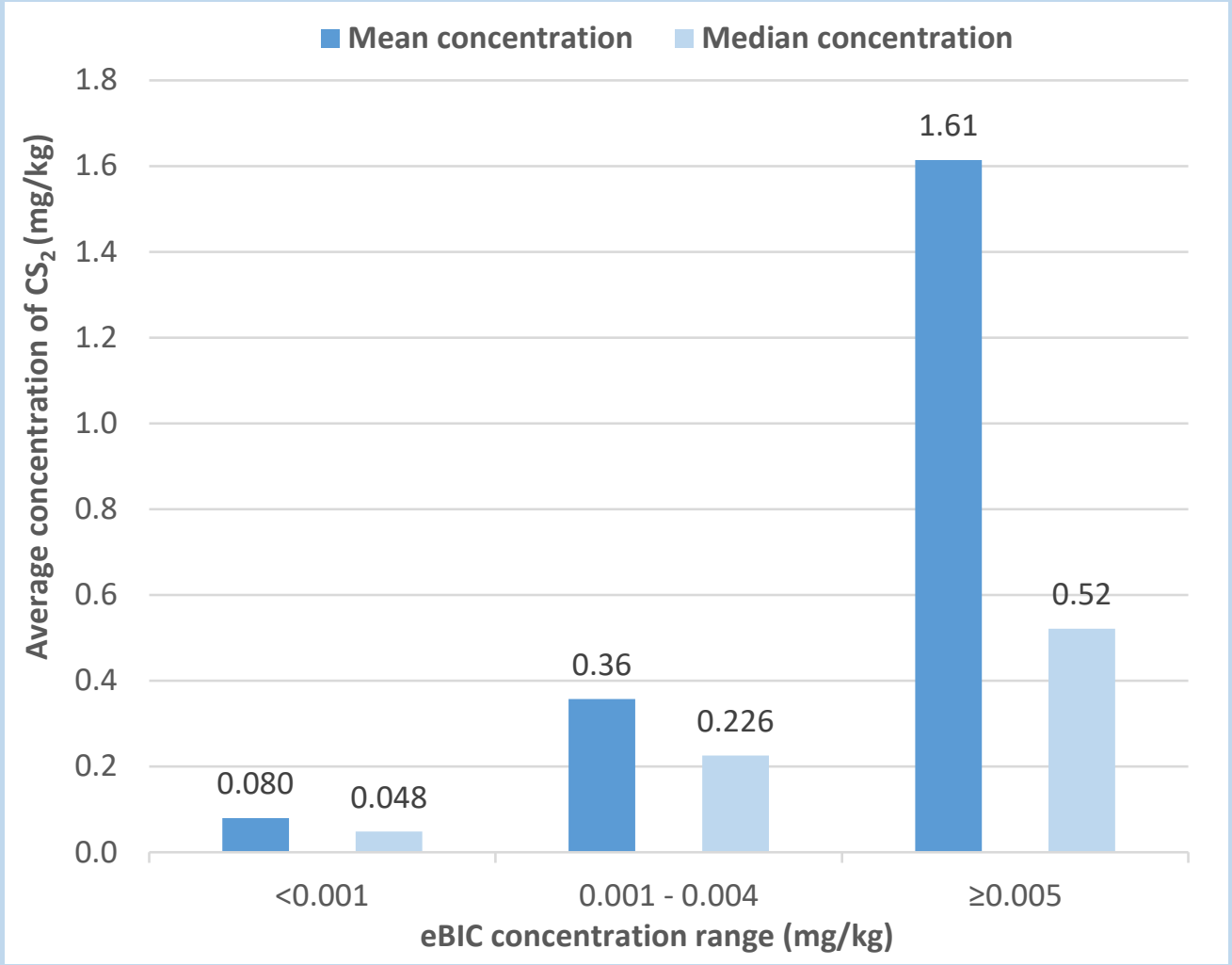
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap



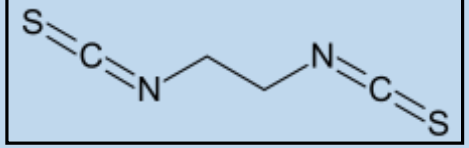
DTC-Markers | Exemplary results for Ethylene-*bis*-isothiocyanate (eBIC)

➢ Correlation between eBIC and CS_2 concentrations?



ethylene-*bis*-isothiocyanate (eBIC)

QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap



DTC-Markers | Exemplary results for Ethylene-*bis*-isothiocyanate (eBIC)

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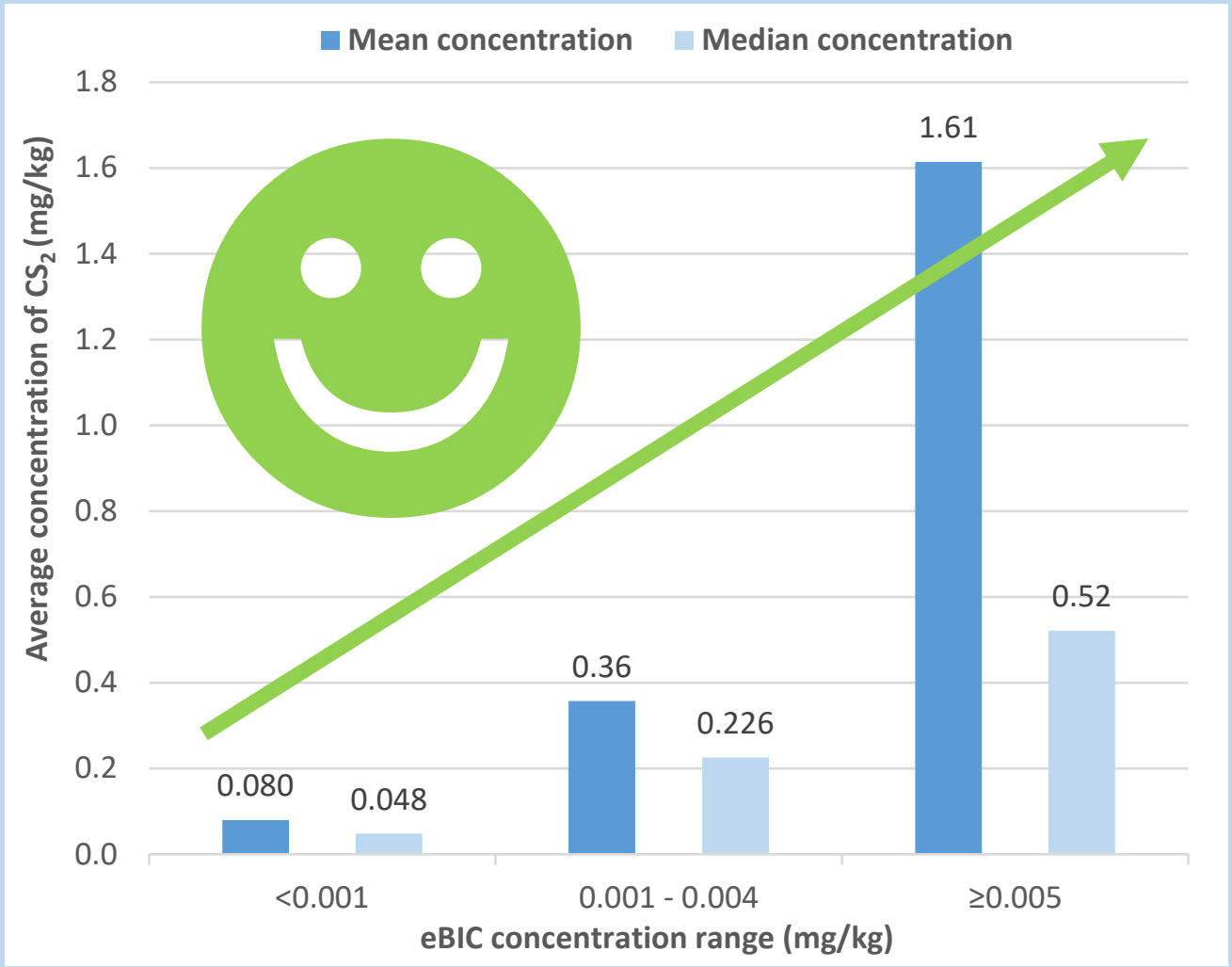
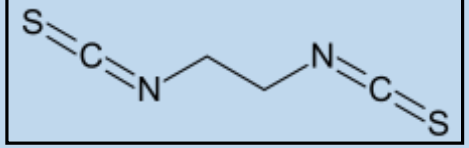


ethylene-*bis*-isothiocyanate (eBIC)

QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap



DTC-Markers | Exemplary results for Ethylene-*bis*-isothiocyanate (eBIC)

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ethylene-*bis*-isothiocyanate (eBIC)
QuEChERS- amenable,
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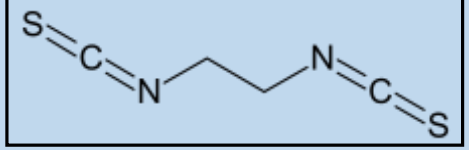

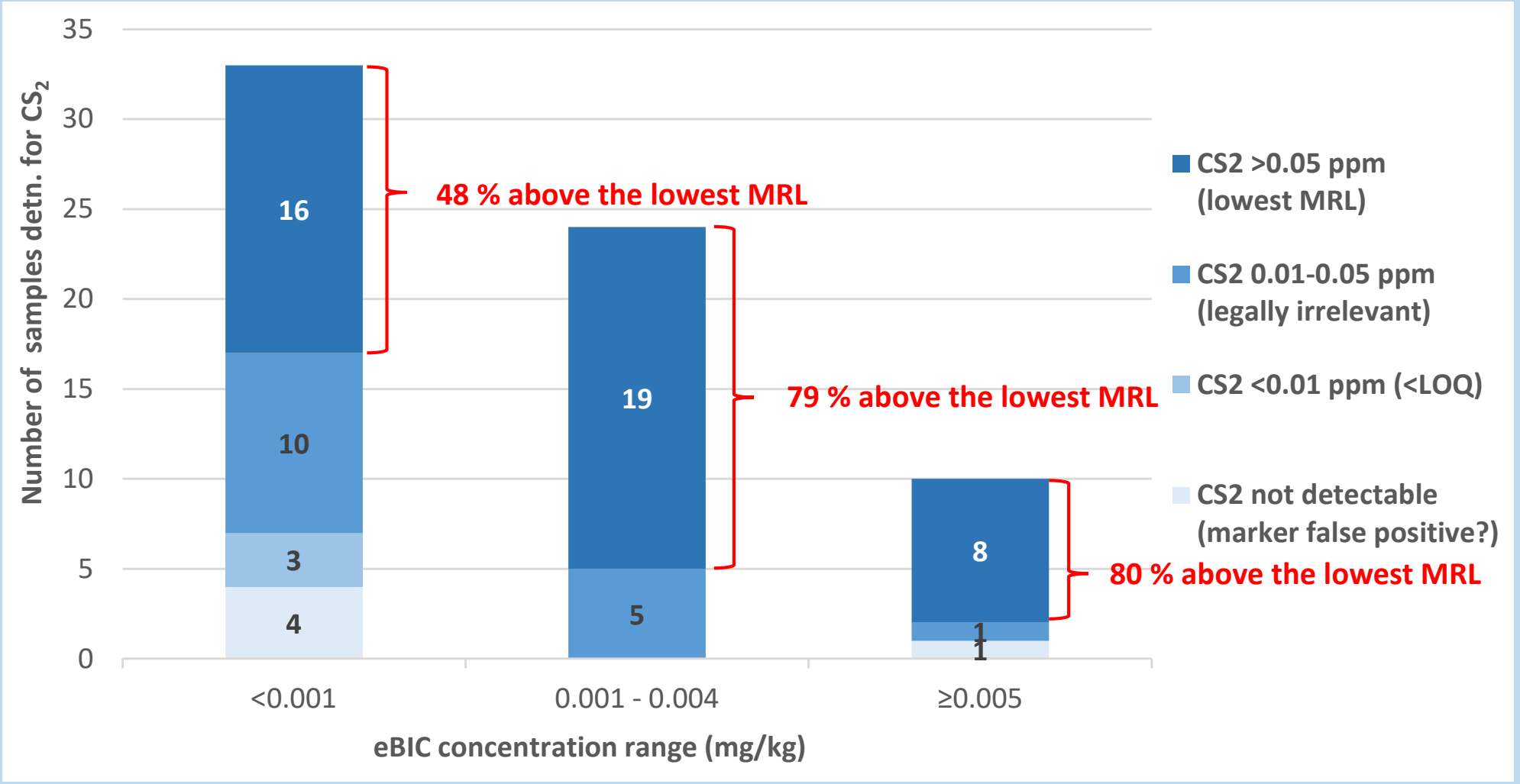

DTC-Markers | Exemplary results for Ethylene-*bis*-isothiocyanate (eBIC)

➢ Correlation between eBIC and CS_2 concentrations?

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QuEChERS- amenable,
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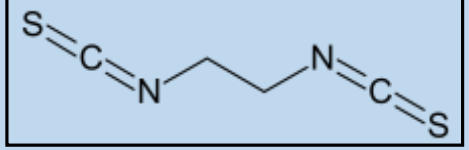

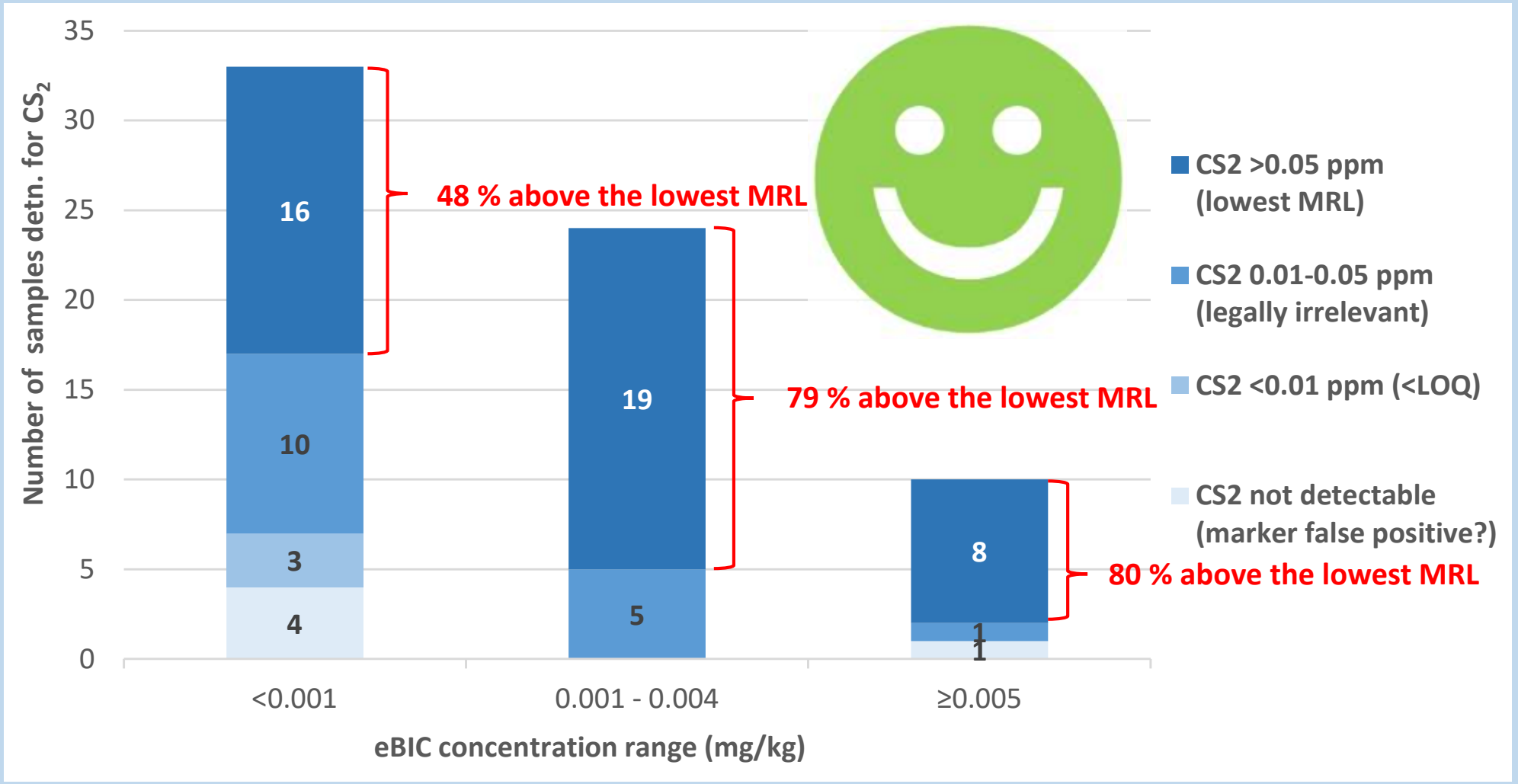

DTC-Markers | Exemplary results for Ethylene-*bis*-isothiocyanate (eBIC)

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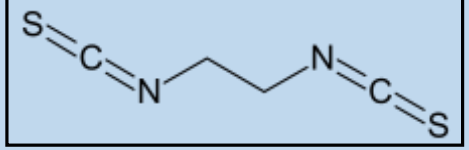

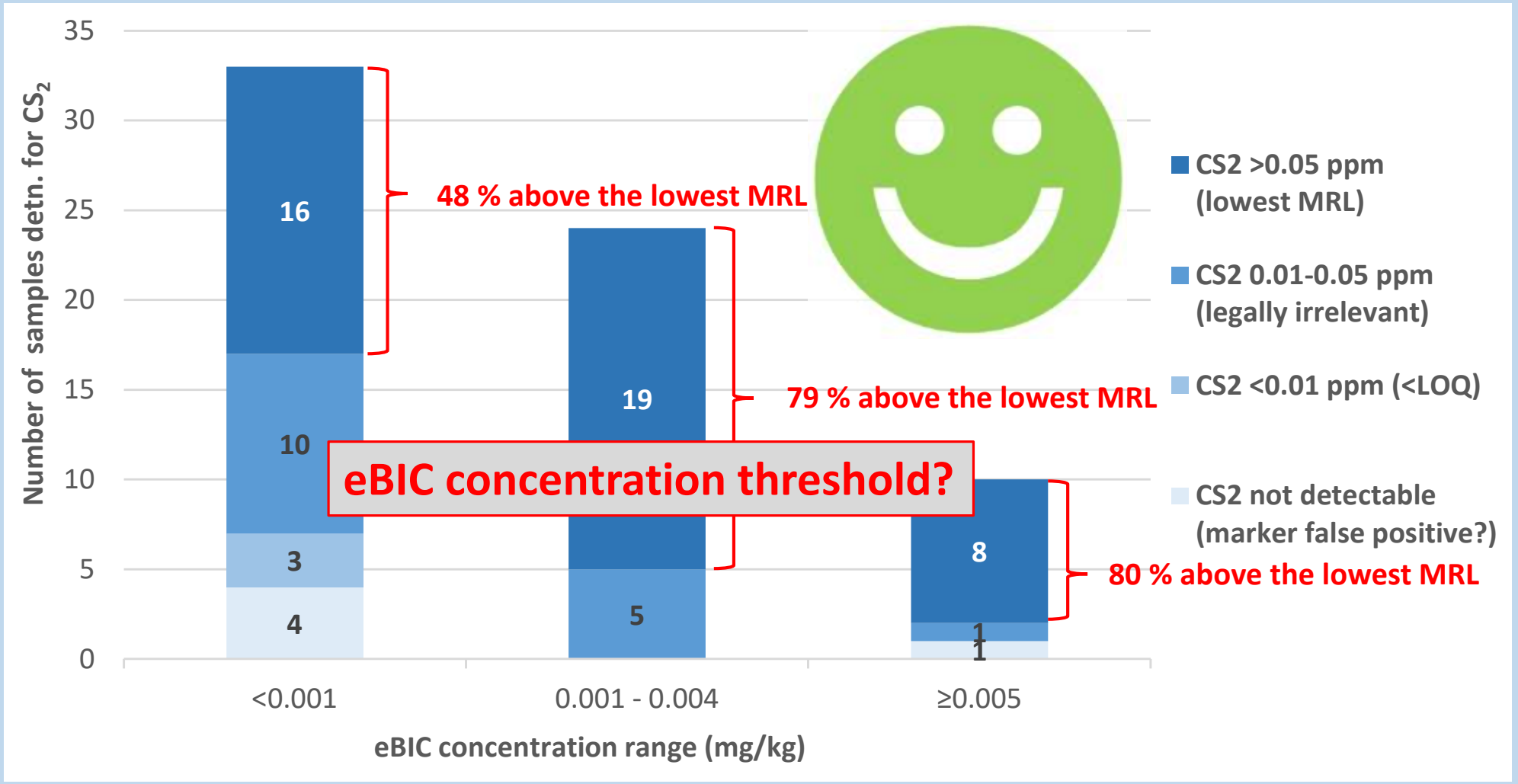

DTC-Markers | Exemplary results for Ethylene-*bis*-isothiocyanate (eBIC)

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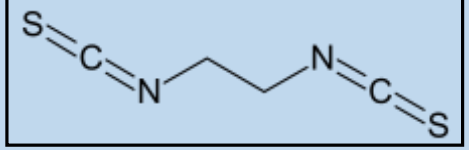
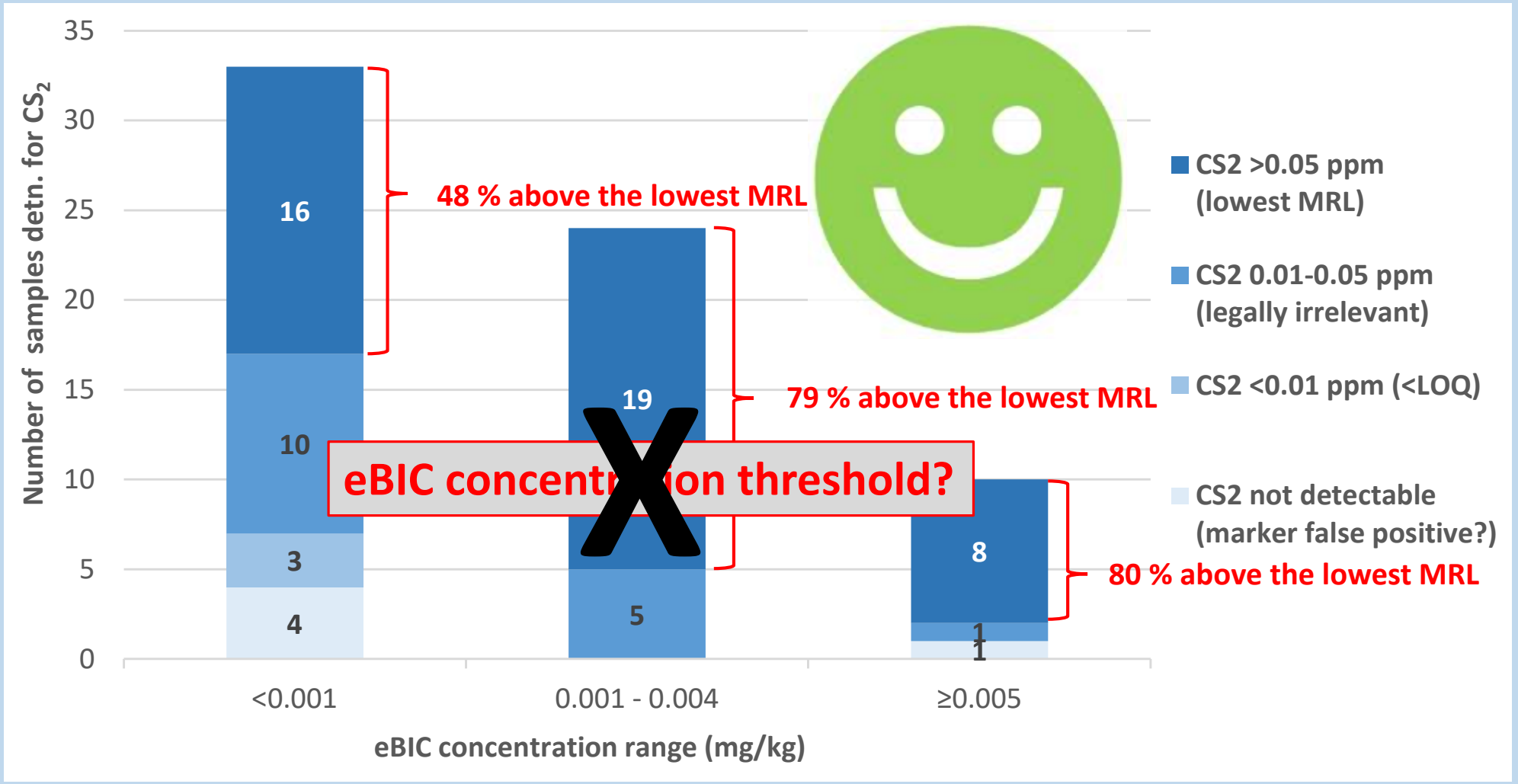
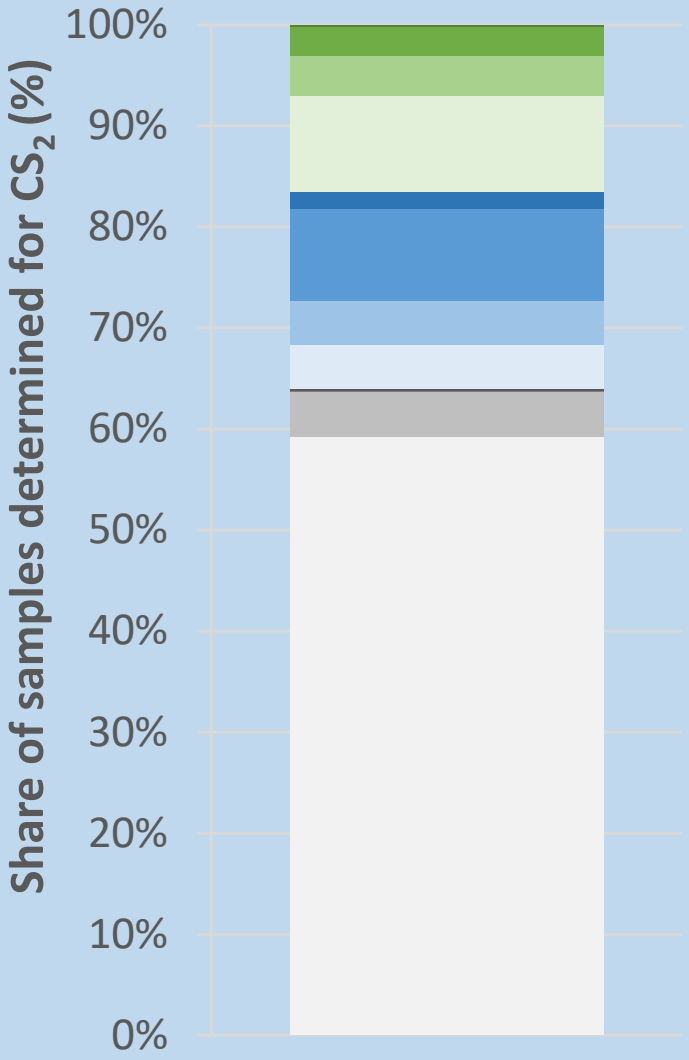
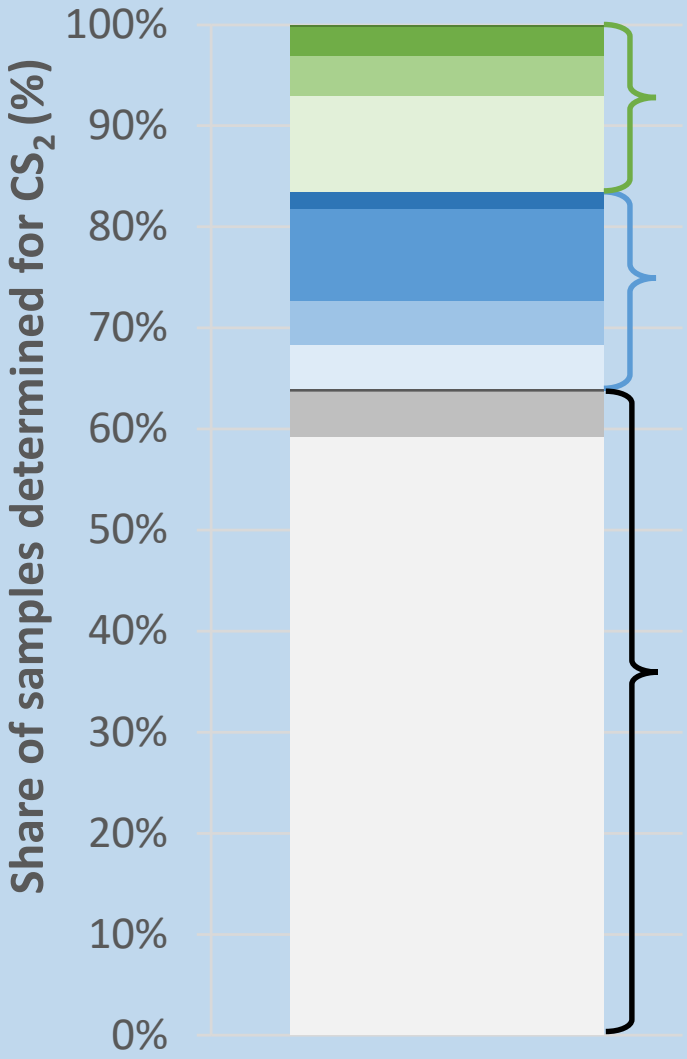




DTC-Markers | Overview of results



DTC-Markers | Overview of results

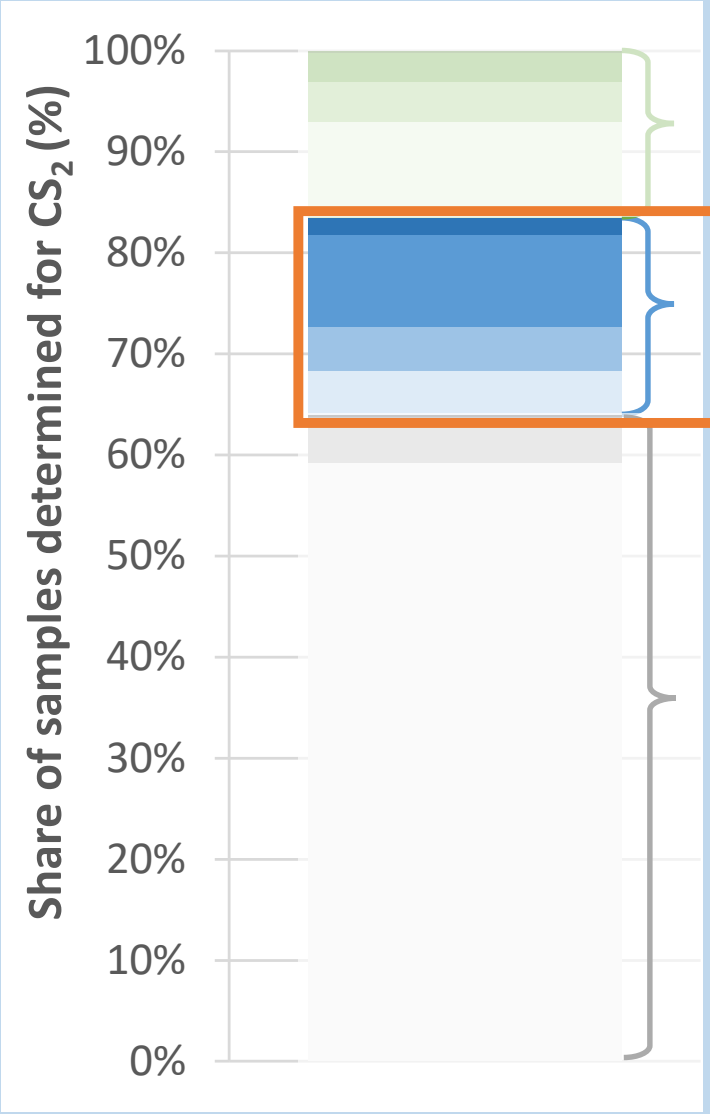


„OTHER MARKERS“: Samples containing exclusively or additionally any of the other DTC-group markers (propylene-*bis*-DTC- or N,N-dimethyl-DTC markers)

Samples with ethylene-*bis*-DTC markers

Samples w/o detectable DTC-markers

DTC-Markers | Overview of results



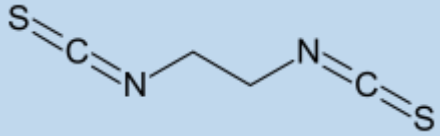
„OTHER MARKERS“: Samples containing exclusively or additionally any of the other DTC-group markers (propylene-*bis*-DTC- or N,N-dimethyl-DTC markers)

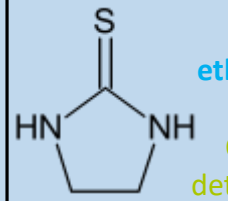
Samples with ethylene-*bis*-DTC markers

Samples w/o detectable DTC-markers

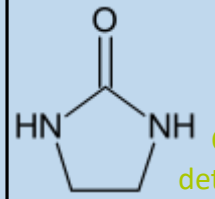
DTC-Markers | Results for the ethylene-*bis*-DTC (EBDTC) group

ethylene-*bis*-isothiocyanate (eBIC)
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap



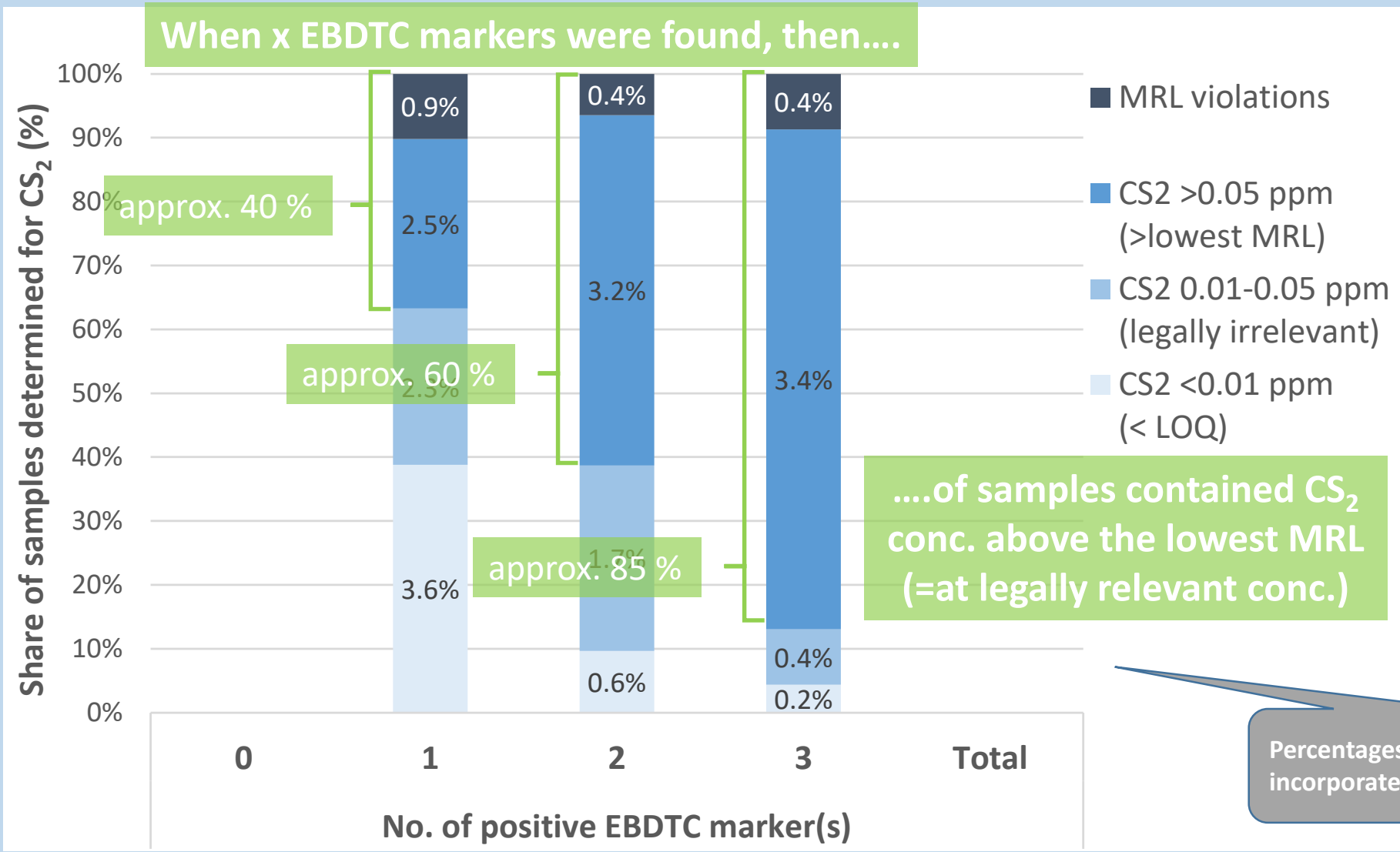


ethylene thiourea (ETU)
QuPPe-amenable
det. with LC-MS/MS

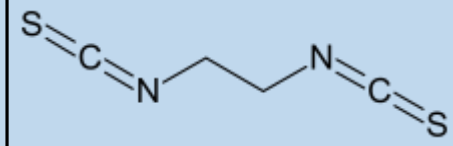


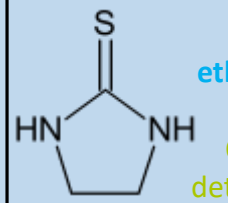
ethylene urea (EU)
QuPPe-amenable
det. with LC-MS/MS

DTC-Markers | Results for the ethylene-*bis*-DTC (EBDTC) group

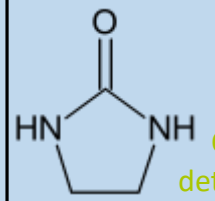


ethylene-*bis*-isothiocyanate (eBIC)
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap





ethylene thiourea (ETU)
QuPPe-amenable
det. with LC-MS/MS

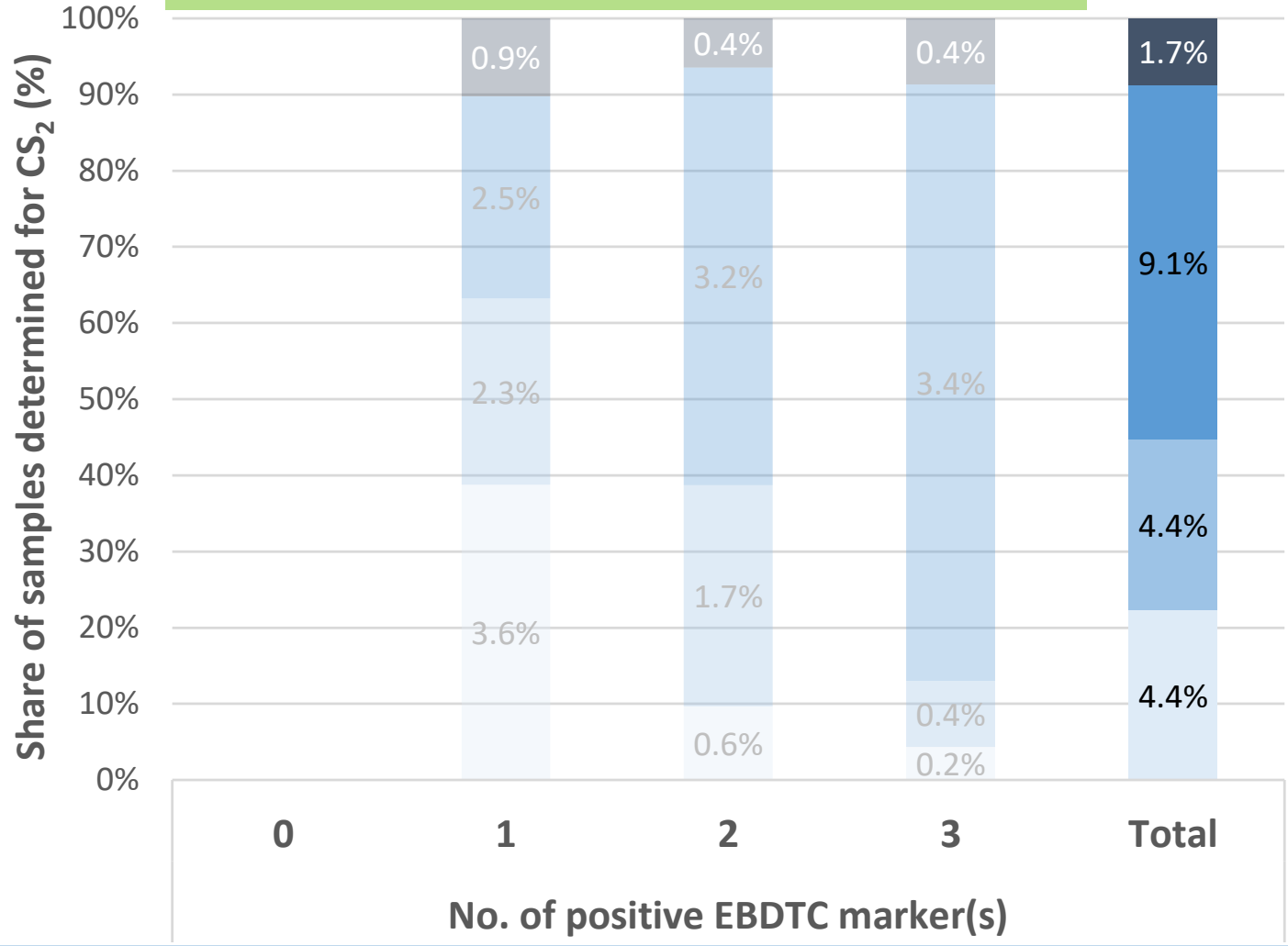


ethylene urea (EU)
QuPPe-amenable
det. with LC-MS/MS

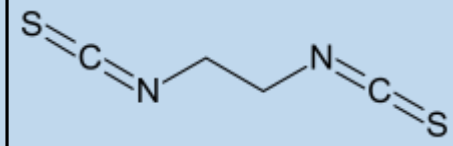
Percentages refer to the total number of incorporated samples (N = 528)

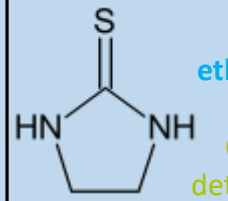
DTC-Markers | Results for the ethylene-*bis*-DTC (EBDTC) group

When x EBDTC markers were found, then….

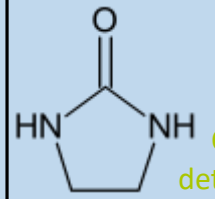


ethylene-*bis*-isothiocyanate (eBIC)
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap





ethylene thiourea (ETU)
QuPPe-amenable
det. with LC-MS/MS

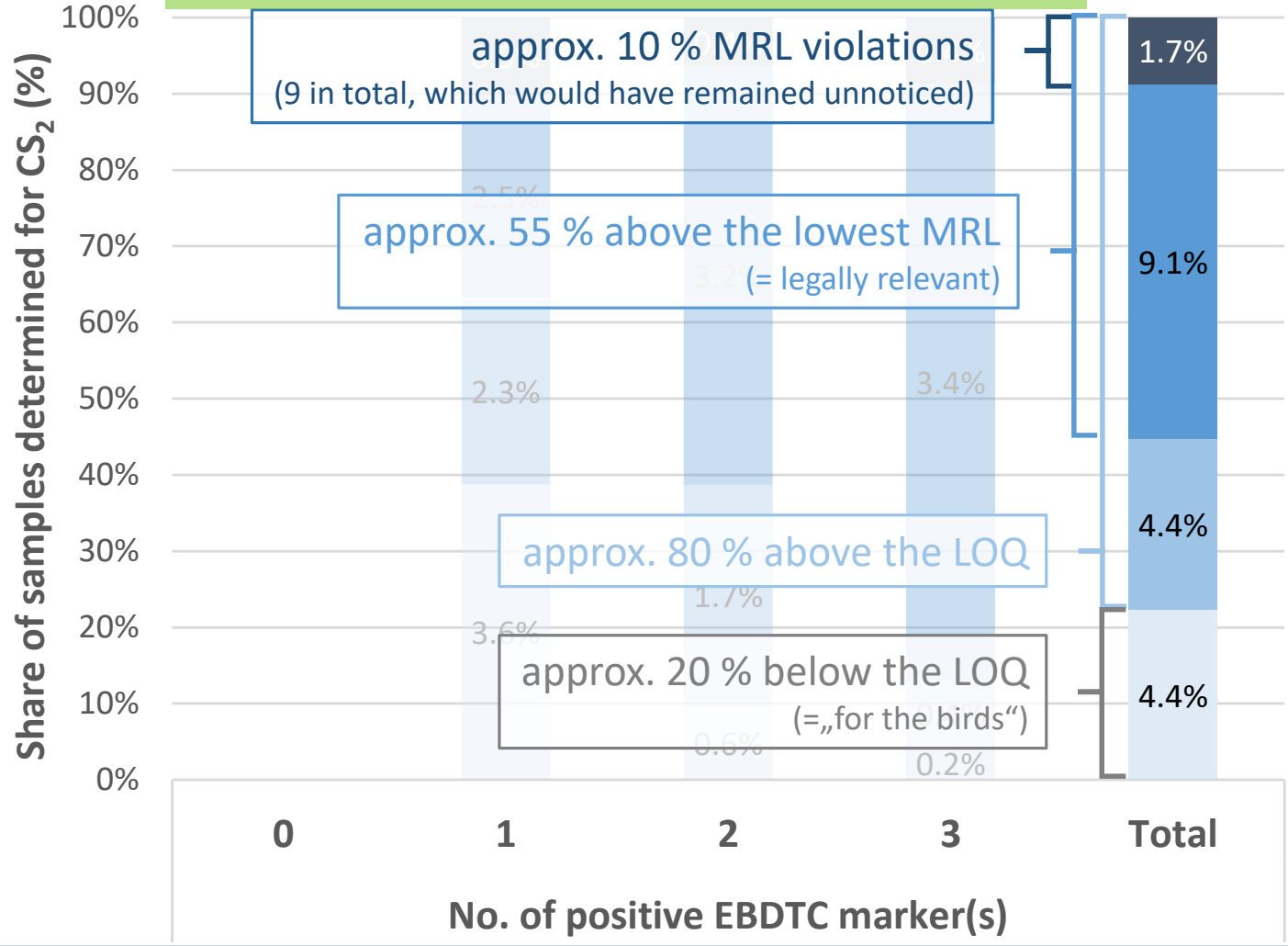


ethylene urea (EU)
QuPPe-amenable
det. with LC-MS/MS

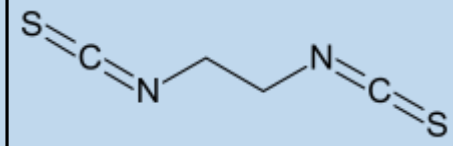
Percentages refer to the total number of incorporated samples (N = 528)

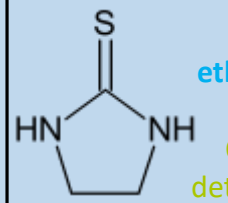
DTC-Markers | Results for the ethylene-*bis*-DTC (EBDTC) group

When x EBDTC markers were found, then….

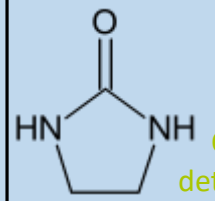


ethylene-*bis*-isothiocyanate (eBIC)
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap





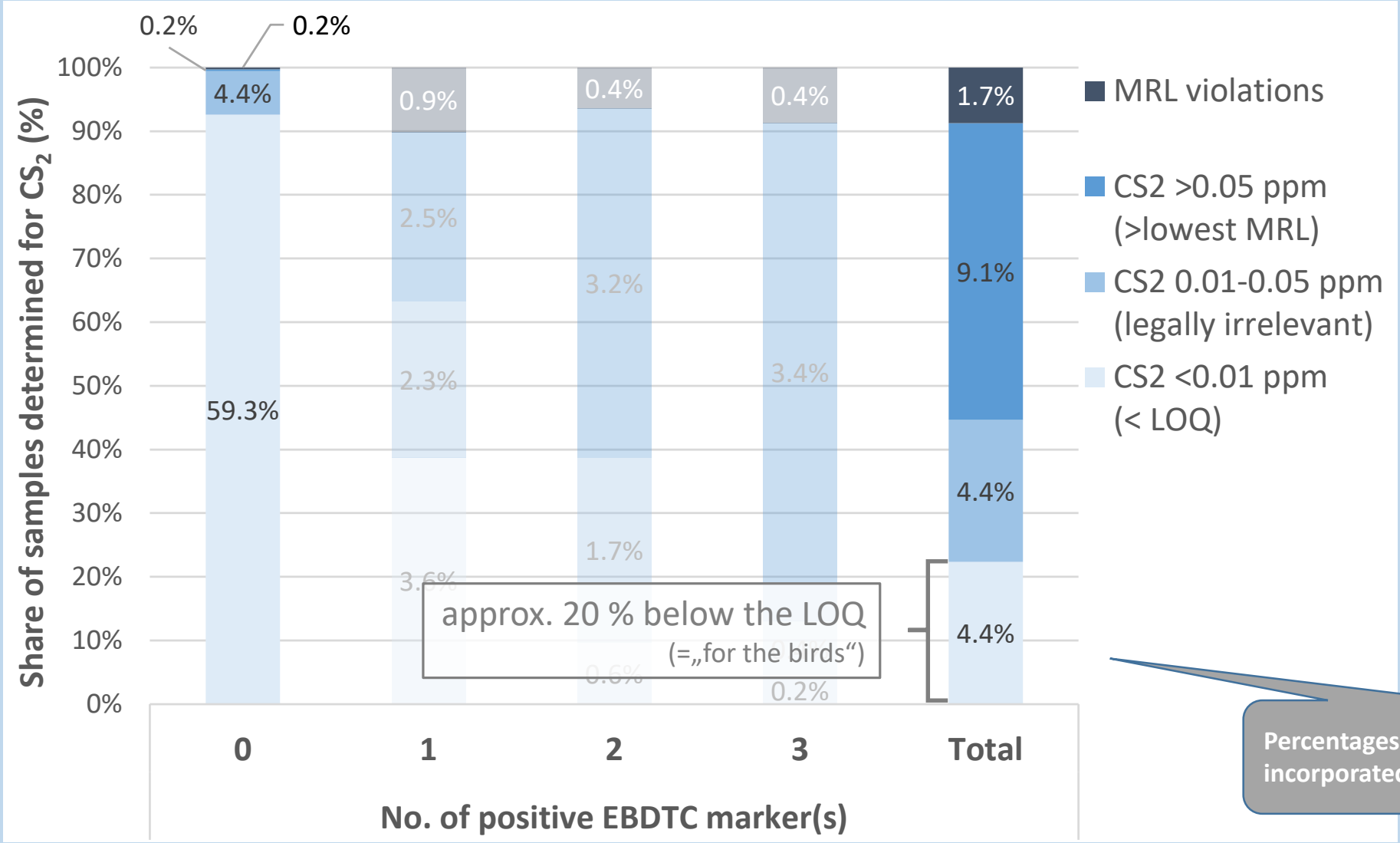
ethylene thiourea (ETU)
QuPPe-amenable
det. with LC-MS/MS



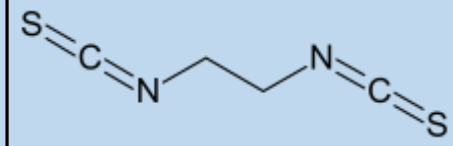
ethylene urea (EU)
QuPPe-amenable
det. with LC-MS/MS

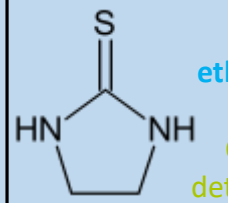
Percentages refer to the total number of incorporated samples (N = 528)

DTC-Markers | Results for the ethylene-*bis*-DTC (EBDTC) group

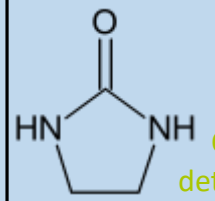


ethylene-*bis*-isothiocyanate (eBIC)
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap





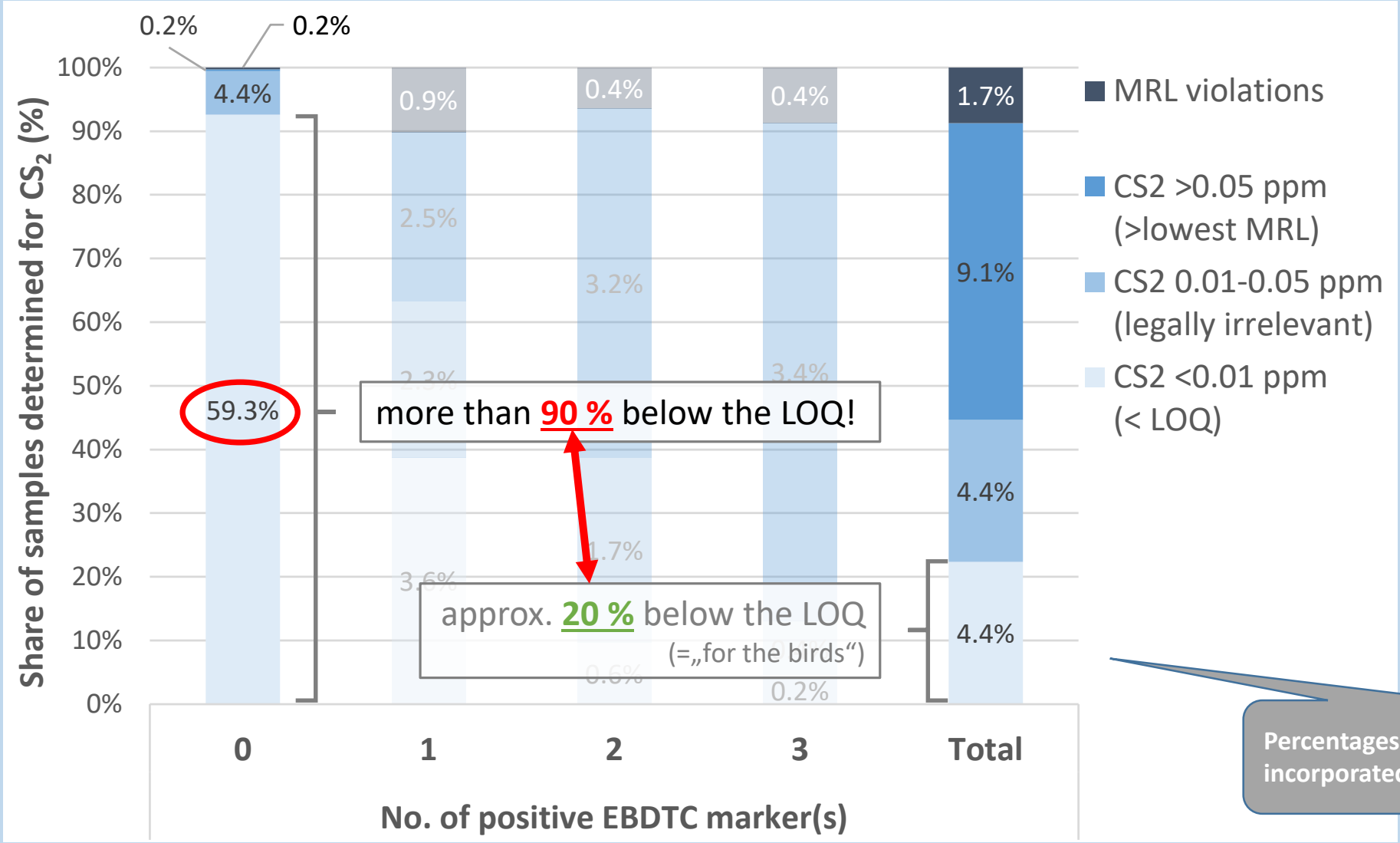
ethylene thiourea (ETU)
QuPPe-amenable
det. with LC-MS/MS



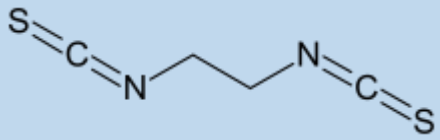
ethylene urea (EU)
QuPPe-amenable
det. with LC-MS/MS

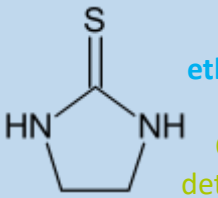
Percentages refer to the total number of incorporated samples (N = 528)

DTC-Markers | Results for the ethylene-*bis*-DTC (EBDTC) group

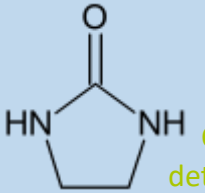


ethylene-*bis*-isothiocyanate (eBIC)
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap





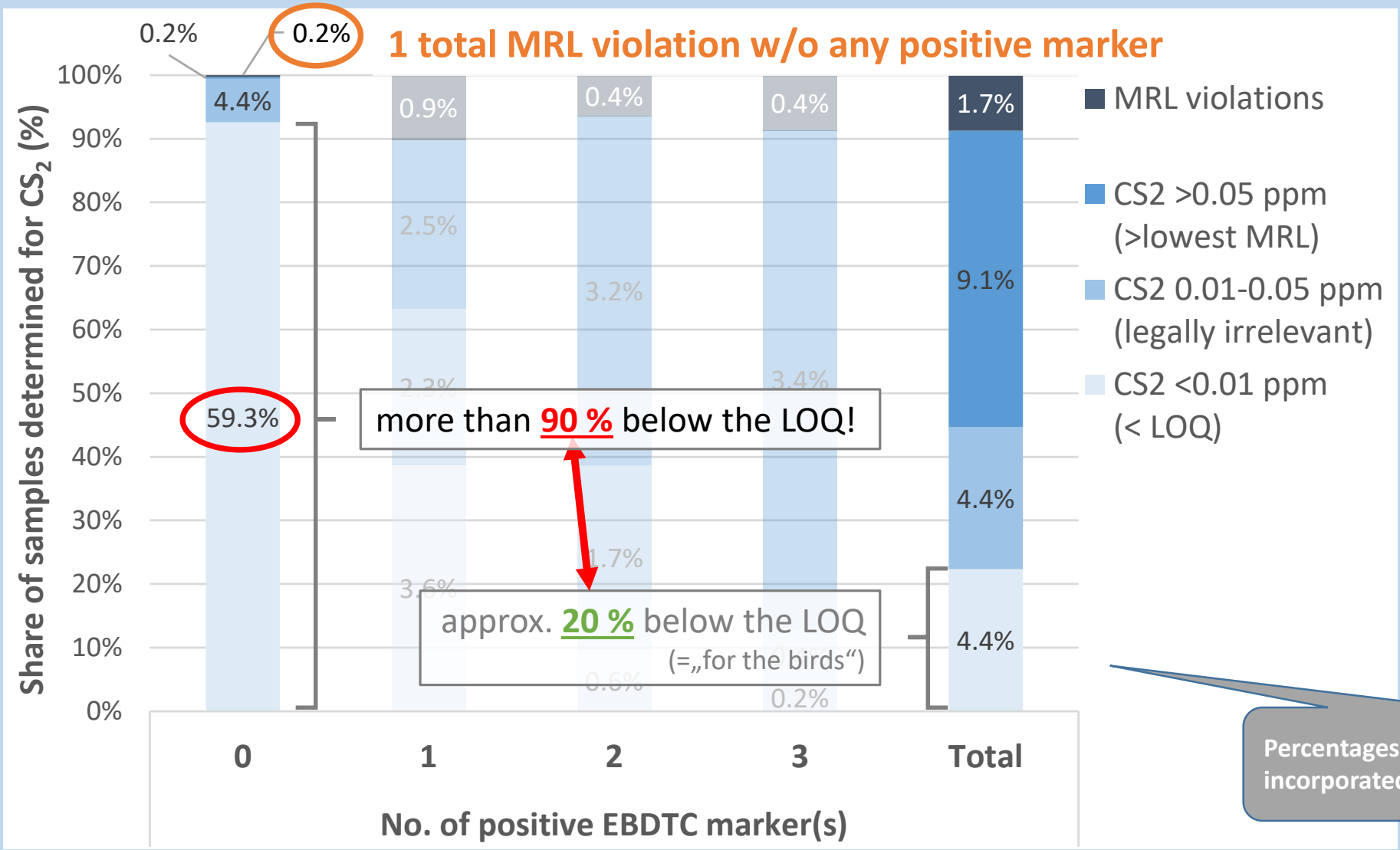
ethylene thiourea (ETU)
QuPPe-amenable
det. with LC-MS/MS



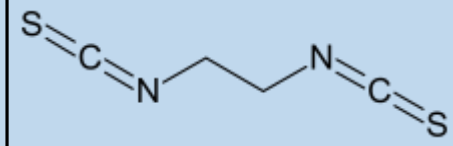
ethylene urea (EU)
QuPPe-amenable
det. with LC-MS/MS

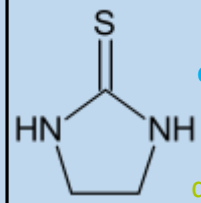
Percentages refer to the total number of incorporated samples (N = 528)

DTC-Markers | Results for the ethylene-*bis*-DTC (EBDTC) group

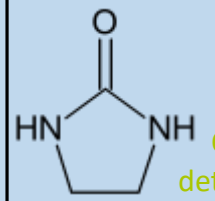


ethylene-*bis*-isothiocyanate (eBIC)
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap





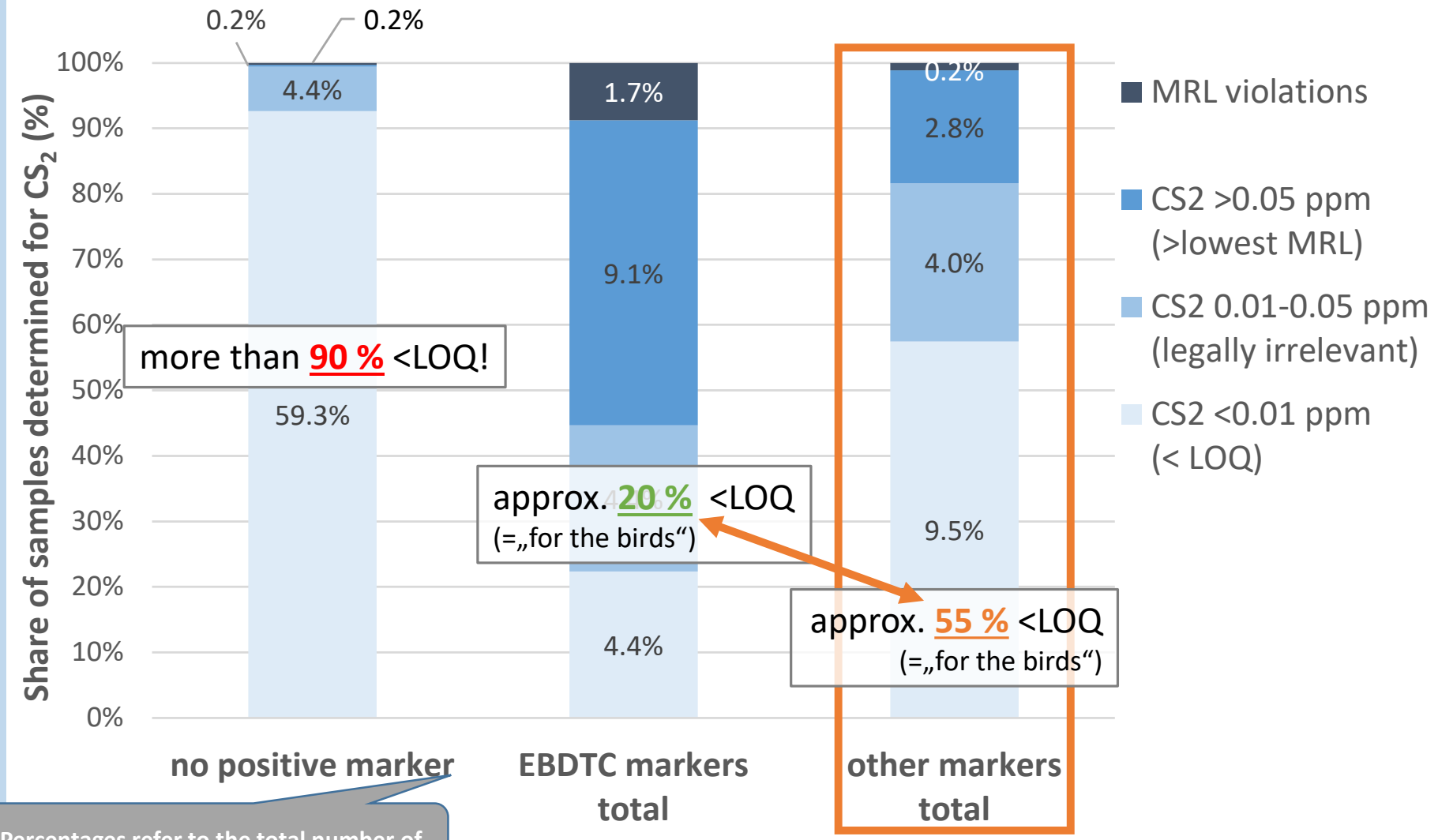
ethylene thiourea (ETU)
QuPPe-amenable
det. with LC-MS/MS



ethylene urea (EU)
QuPPe-amenable
det. with LC-MS/MS

Percentages refer to the total number of incorporated samples (N = 528)

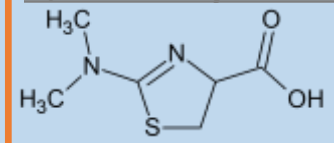
DTC-Markers | Results for the other markers of different DTC-groups



Percentages refer to the total number of incorporated samples (N = 528)

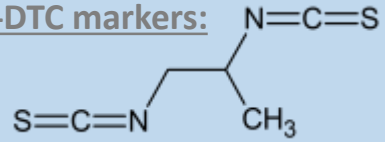
Essential other markers:

N,N-Dimethyl-DTC marker:

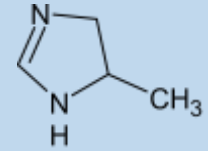


2-(dimethylamino)-4,5-dihydro-1,3-thiazole-4-carboxylic acid (M1)
QuPPe-amenable,
det. with LC-MS/MS

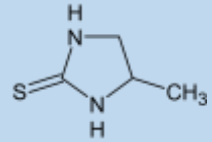
Propylene-bis-DTC markers:



Propylene-bis-isothiocyanate (pBIC)
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap



4-Methyl-imidazoline (MIDZ)
QuPPe-amenable
det. with LC-MS/MS

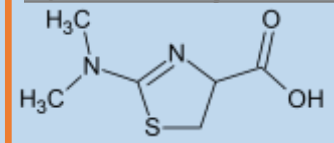


Propylene thiourea (PTU)
QuPPe-amenable
det. with LC-MS/MS

DTC-Markers | Results for the other markers of different DTC-groups

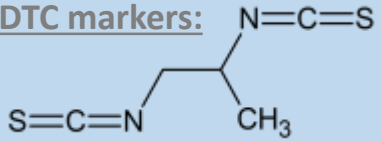
Essential other markers:

N,N-Dimethyl-DTC marker:

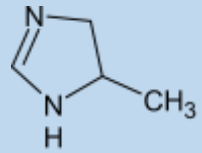


2-(dimethylamino)-4,5-dihydro-1,3-thiazole-4-carboxylic acid (M1)
QuPPe-amenable,
det. with LC-MS/MS

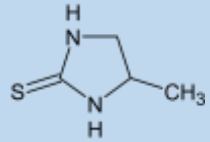
Propylene-bis-DTC markers:



Propylene-bis-isothiocyanate (pBIC)
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap

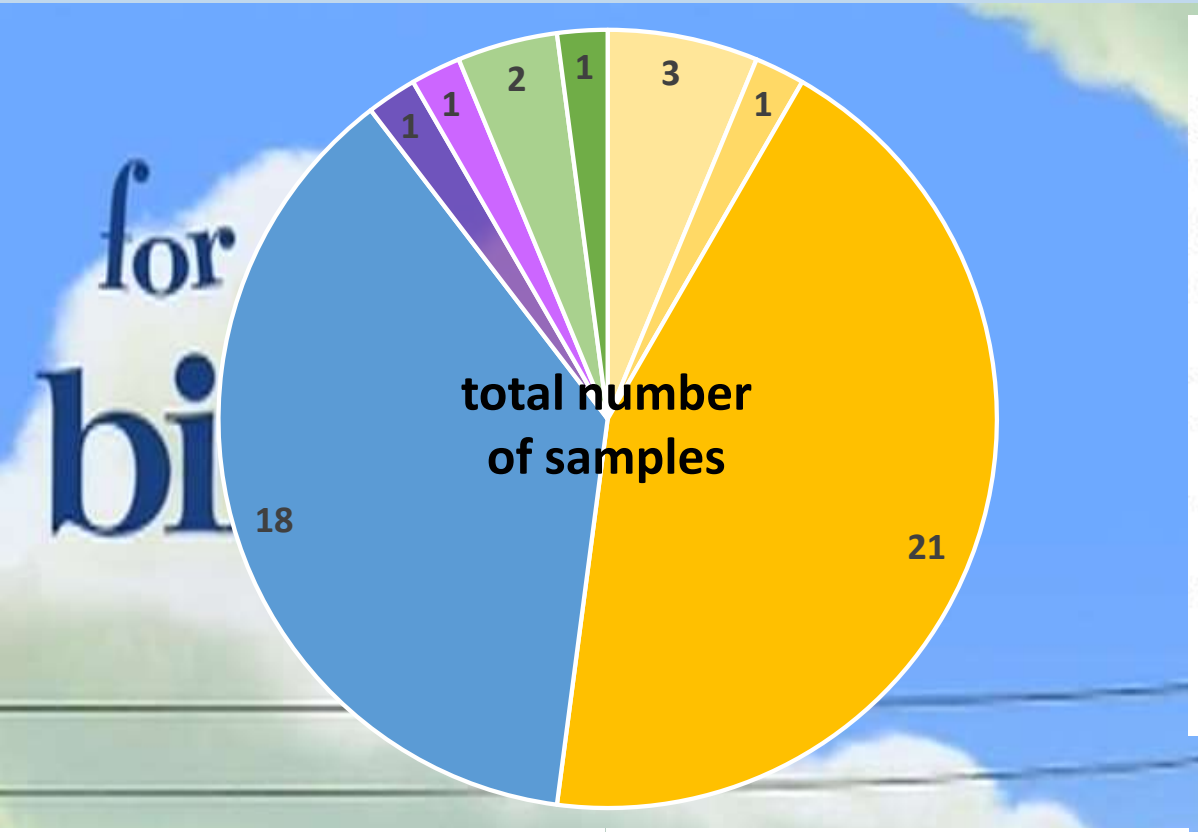


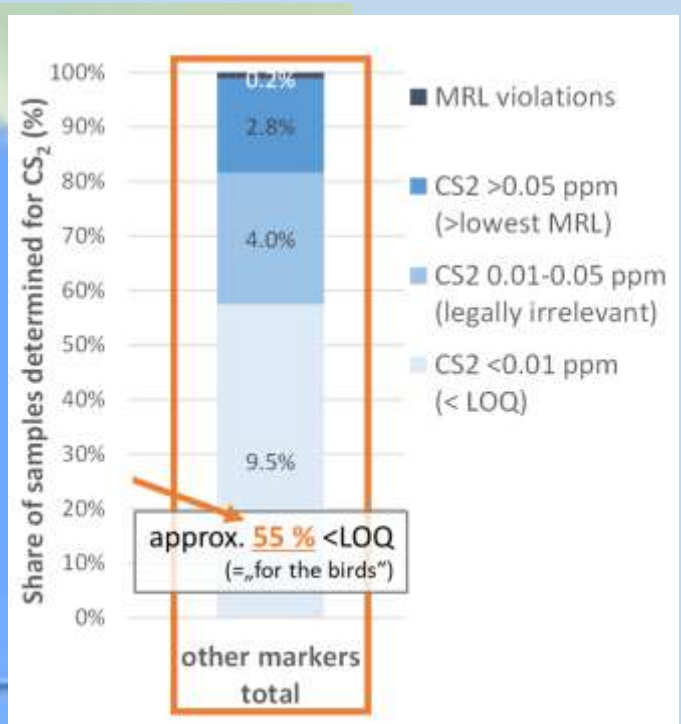
4-Methyl-imidazoline (MIDZ)
QuPPe-amenable
det. with LC-MS/MS

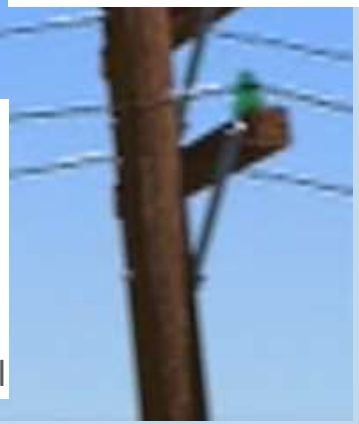


Propylene thiourea (PTU)
QuPPe-amenable
det. with LC-MS/MS

DTC-Markers | Results for the other markers of different DTC-groups

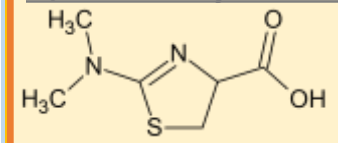






Essential other markers:

N,N-Dimethyl-DTC marker:



2-(dimethylamino)-4,5-dihydro-1,3-thiazole-4-carboxylic acid (M1)
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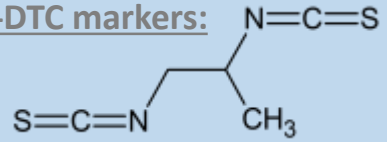
Propylene-bis-DTC markers:



Propylene-bis-isothiocyanate (pBIC)
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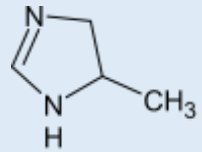
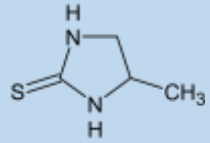


4-Methyl-imidazoline (MIDZ)
QuPPe-amenable
det. with LC-MS/MS



Propylene thiourea (PTU)
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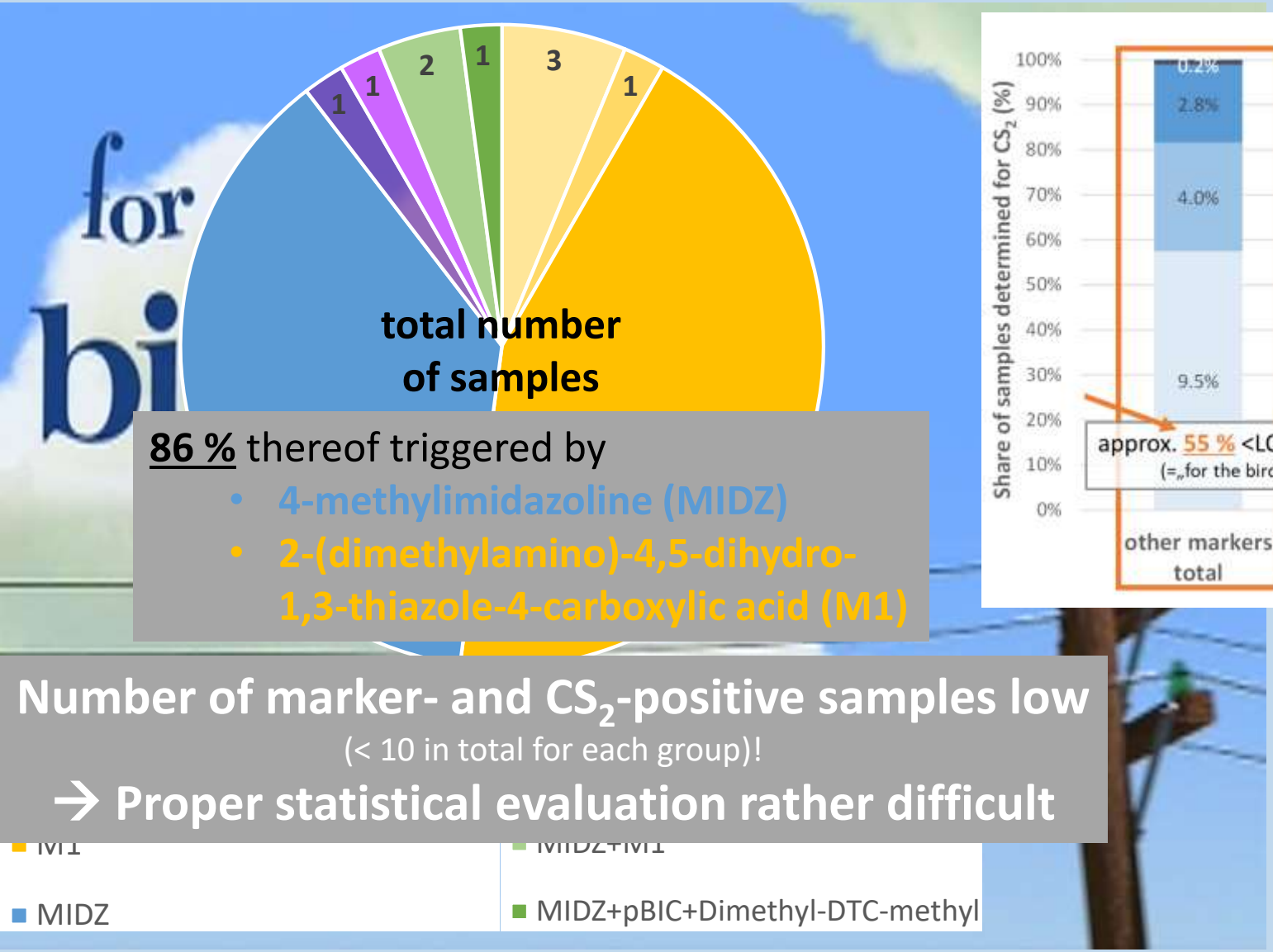
DTC-Markers | Results for the other markers of different DTC-groups



86 % thereof triggered by

- 4-methylimidazoline (MIDZ)
- 2-(dimethylamino)-4,5-dihydro-1,3-thiazole-4-carboxylic acid (M1)

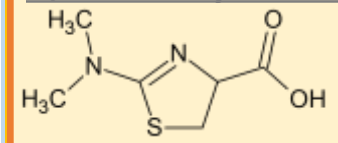
Number of marker- and CS_2-positive samples low

(< 10 in total for each group)!

→ **Proper statistical evaluation rather difficult**

Essential other markers:

N,N-Dimethyl-DTC marker:



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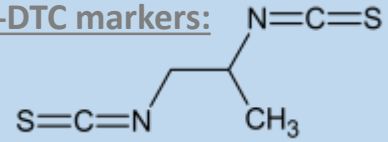

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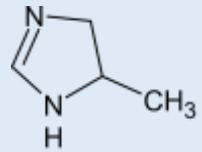

4-Methyl-imidazoline (MIDZ)

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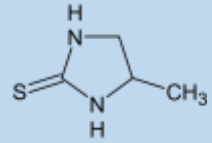
det. with LC-MS/MS



Propylene thiourea (PTU)

QuPPe-amenable
det. with LC-MS/MS

M1
MIDZ
MIDZ+M1
MIDZ+pBIC+Dimethyl-DTC-methyl

DTC-Markers | Summarized results

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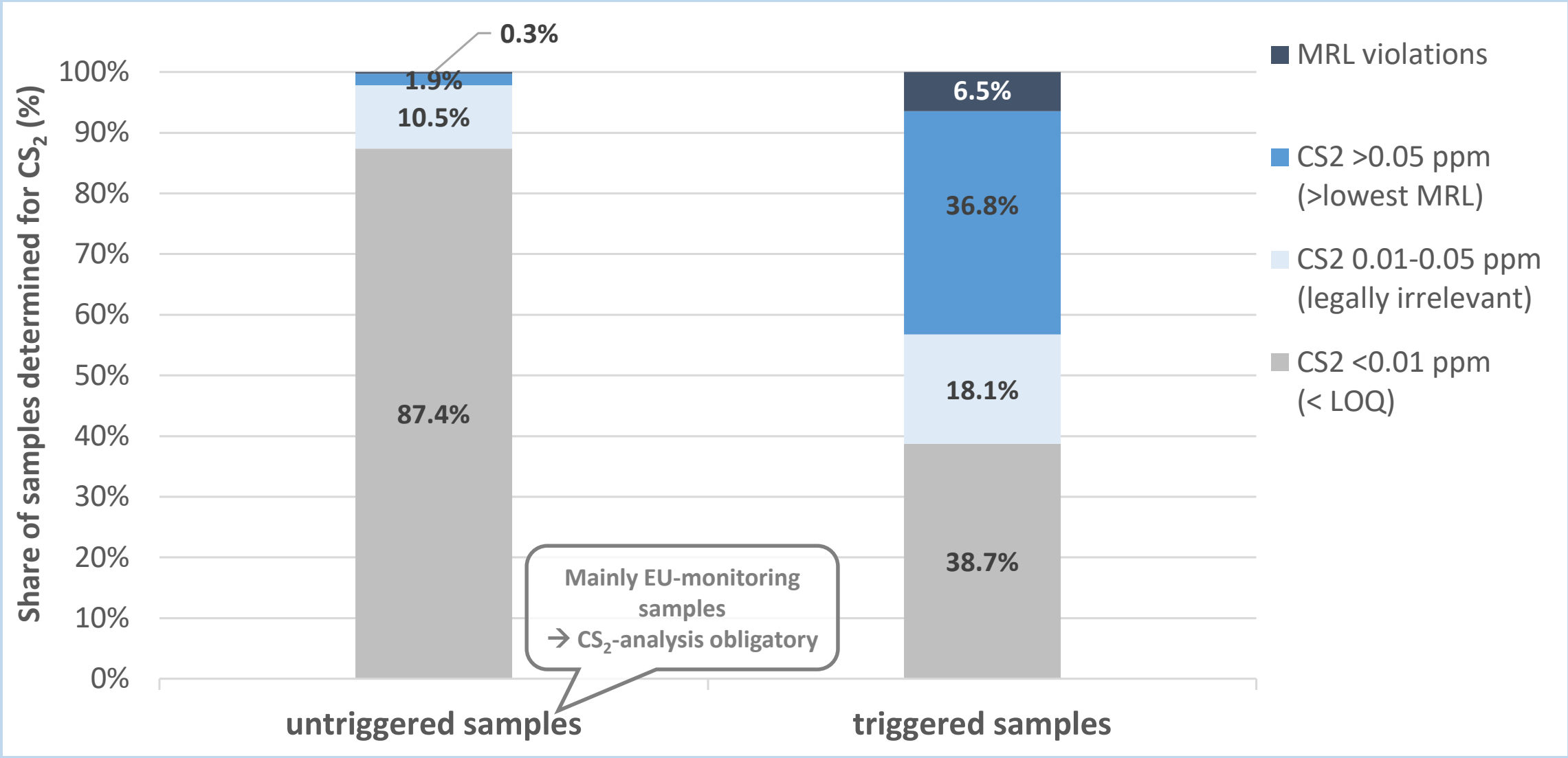

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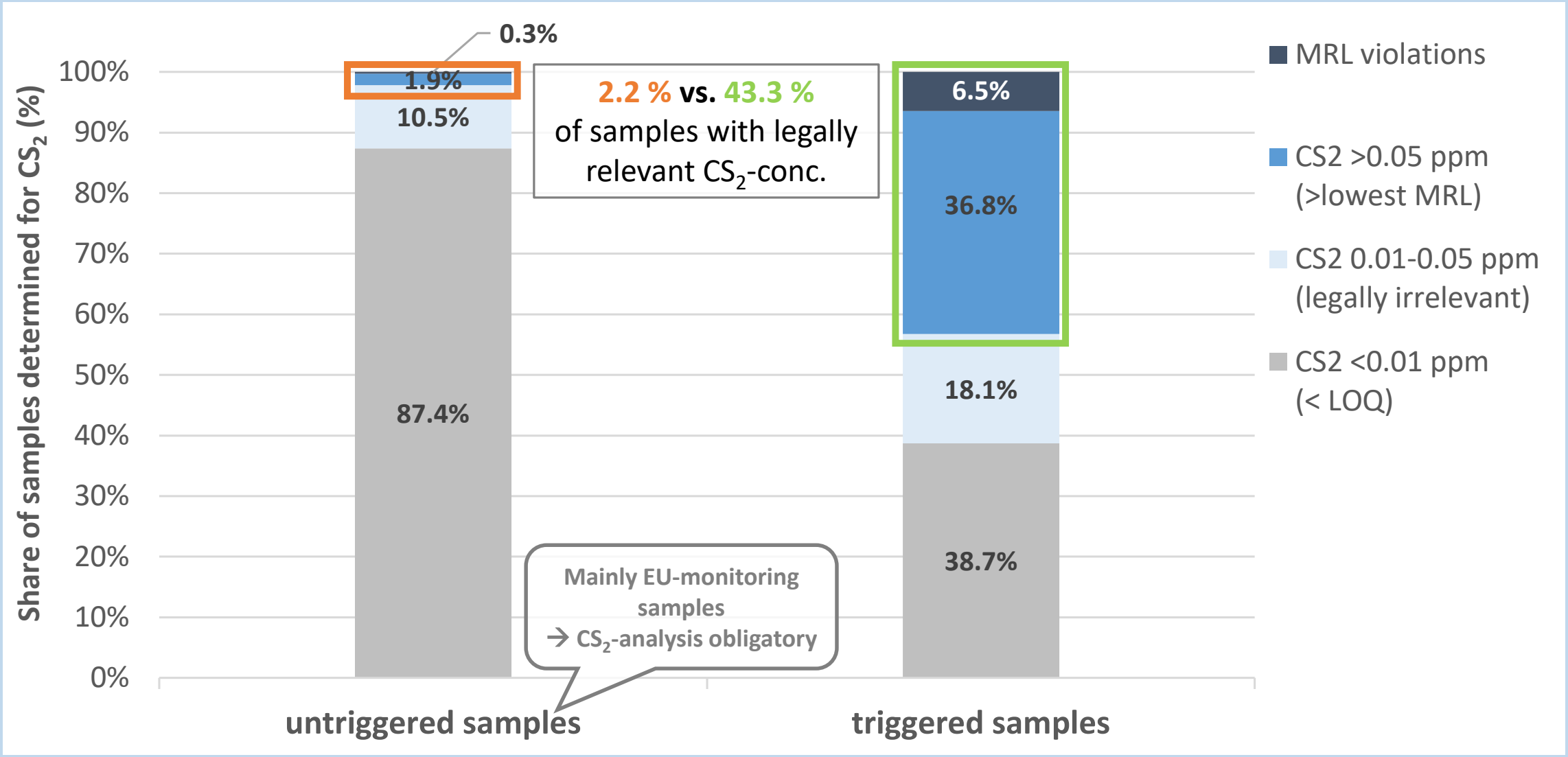

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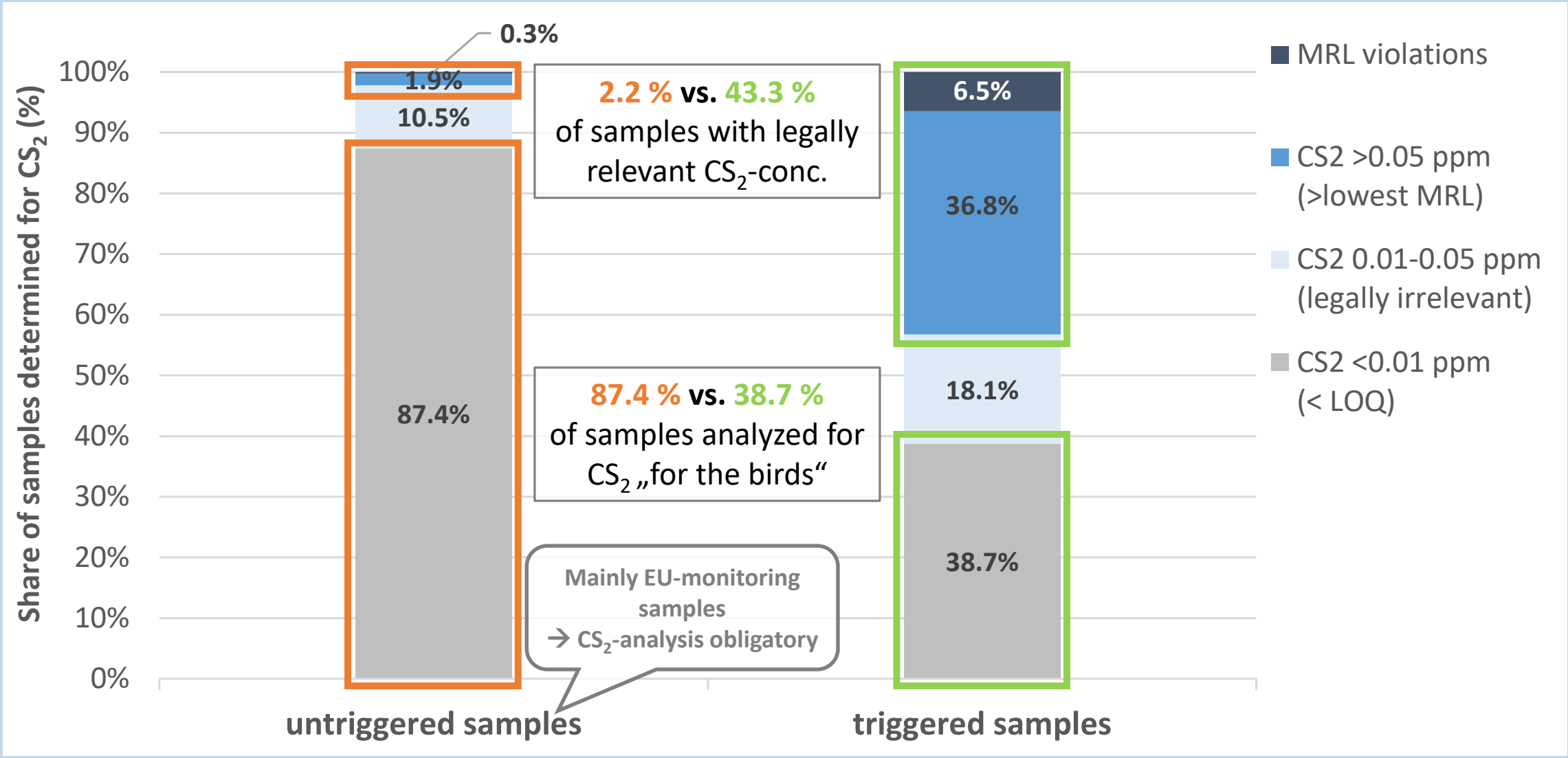
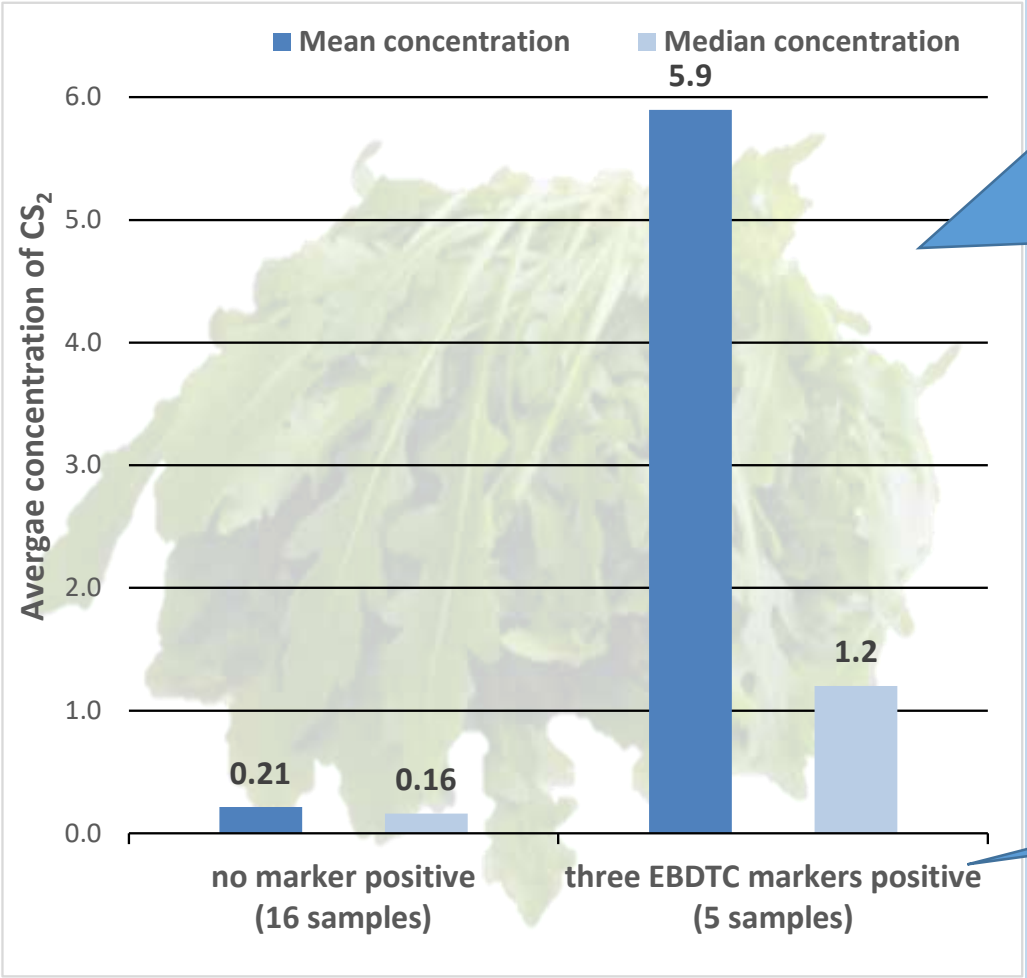


DTC-Markers | Summarized results

But how specific are these markers in case of commodities which naturally contain CS_2-generating components?

DTC-Markers | Samples with background levels

EXAMPLE: Rucola – 21 samples analyzed



	CS_2 (mg/kg)	ETU (mg/kg)	EU (mg/kg)	eBIC (mg/kg)
Sample 1	0.39	0.004	0.021	0.003
Sample 2	0.73	0.006	0.020	0.003
Sample 3	1.5	0.21	0.11	0.013
Sample 4	1.2	0.16	0.039	0.012
Sample 5	**25.7**	**0.99**	**0.38**	**0.84**

MRL violation! (MRL 5 mg/kg)

No cases for rucola, where just one or two EBDTC marker(s) were positive

DTC-Markers | Summary

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- ✓ A monitoring program was conducted (in total 528 samples determined)
  - 13 DTC markers analyzed by routine methods (QuEChERS and QuPPe)
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- ✓ Enhancement of the lab's effectivity by using these EBDTC markers as a trigger for a subsequent CS_2- analysis
- ✓ **Improvement of the cost/benefit ratio** by preventing the unnecessary use of the common moiety method
- ✓ Proper statistical evaluation for the markers of other DTC groups rather difficult at this stage

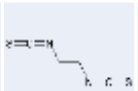
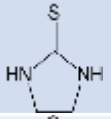
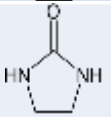
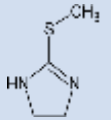
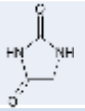
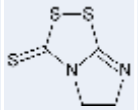
Thank you for your attention!

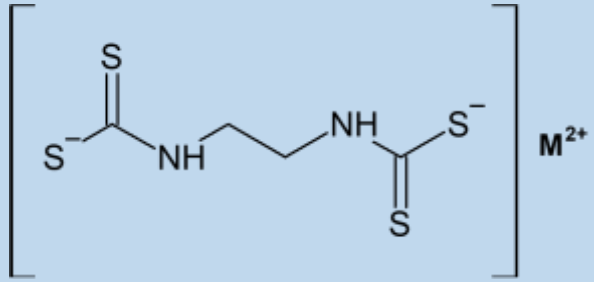
Questions to:

eric.eichhorn@cvuas.bwl.de **or** eurl-srm@cvuas.bwl.de

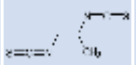
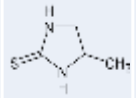
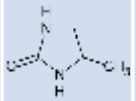
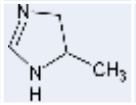
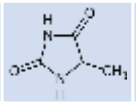
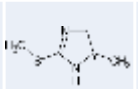
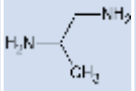
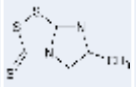
DTC-Markers | Supplementary Info

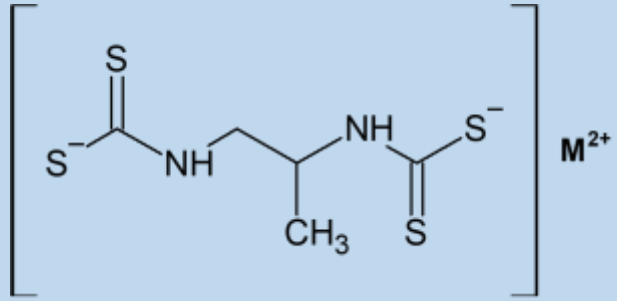
DTC-Markers | Overview of the considered ethylene-*bis*-DTC markers

Marker substance	Chemical structure	Chroma-tography	MS-ionization	Remarks	Incorporated in study?	Usefulness as a trigger
Ethylene-*bis*-dithiocarbamate markers						
Ethylene-*bis*-isothiocyanate („eBIC“) CAS 3688-08-2		GC	EI neg.		yes	High
Ethylene thiourea („ETU“) CAS 96-45-7		LC (HILIC) [1)]	ESI pos.		yes	High
Ethylene urea („EU“) CAS 120-93-4		LC (HILIC) [1)]	ESI pos.		yes	High
S-methyl-ethylene thiourea („S-Me-ETU“) CAS 20112-79-2		LC (HILIC) [1)]	ESI pos.	• Often found at very low levels (<1 ppb); of low specificty as there was no significant difference regarding its findings (and levels) in the group of CS_2-containing and the group of non-CS_2-contaning samples • In relevant samples: always accompanied by eBIC and/or ETU, EU	yes	Low
Hydantoin CAS 461-72-3		LC (HILIC) [1)]	ESI pos. & ESI neg.	• ESI pos.: poor sensitivity • ESI neg.: just one useful MRM available	-	-
Ethylene diamine („EDA“) CAS 107-15-3		LC (HILIC) [1)]	ESI pos.	• Just one useful MRM available • Poor sensitivity	-	-
3H,5H,6H-imidazo[2,1-c]-[1,2,4]dithiazole-3-thione („Etem“) CAS 33813-20-6		LC (HILIC[1)]/ RP)	ESI pos.	• Just two findings out of 540 total samples, together with at least 2 other EBDTC markers • Limited standard stability • Stability issues in matrix extracts	yes	Low



DTC-Markers | Overview of the considered propylene-*bis*-DTC markers

Marker substance	Chemical structure	Chroma-tography	MS-ionization	Remarks	Incorporated in study?	Usefulness as a trigger
Propylene-*bis*-dithiocarbamate markers						
Propylene-*bis*-isothiocyanate („pBIC“) CAS 109704-32-7		GC	EI neg.		yes	**Tentatively high**
Propylene thiourea („PTU“) CAS 2122-19-2		LC (HILIC) [1]	ESI pos.		yes	**Tentatively high**
Propylene urea CAS 6531-31-3		LC (HILIC) [1]	ESI pos.	• Poor sensitivity • High matrix suppression	-	-
4-Methyl-imidazoline CAS 1615-03-8		LC (HILIC) [1]	ESI pos.	• Often found at low levels • Relevance enhanced at a threshold of 5 µg/kg	yes	**Tentatively high**
5-methyl-hydantoin CAS 616-03-5		LC (HILIC) [1]	ESI pos.	• ESI pos.: poor sensitivity • ESI neg.: just one useful MRM available	-	-
S-methyl-propylene thiourea („S-Me-PTU“) CAS 55536-61-3		-	-	• Analytical standard not available	-	-
Propylene diamine („PDA“) CAS 78-90-0		LC (HILIC) [1]	ESI pos.	• Poor sensitivity • Determination via ion-pair LC after traditional acidic hydrolysis for CS_2 as it is legally regulated according to Reg. (EC) No. 396/2005 [2]	-	-
6-Methyl-5,6-dihydroimidazo-[2,1-C][1,2,4]dithiazole-3-thione („Propineb-DIDT“) CAS N/A		LC (HILIC[1]/ RP)	ESI pos.	• Limited standard stability • Stability issues in matrix extracts	-	-



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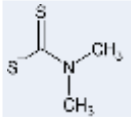
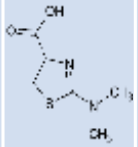
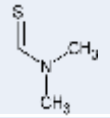
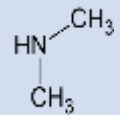
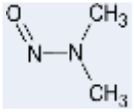
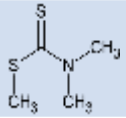
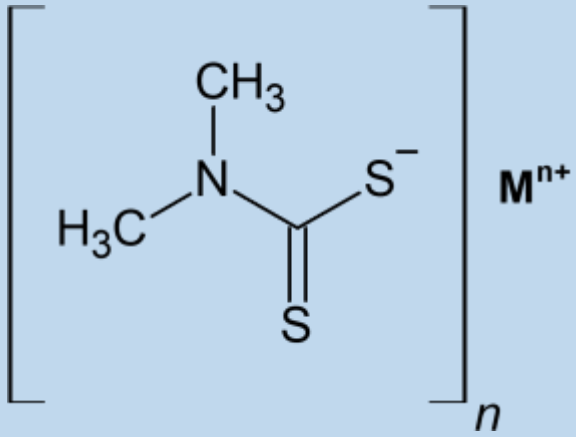
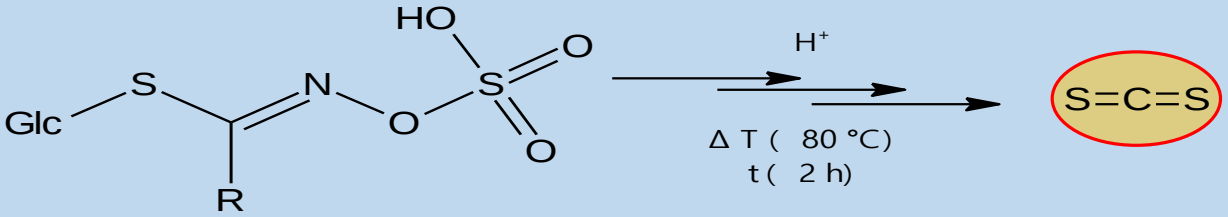
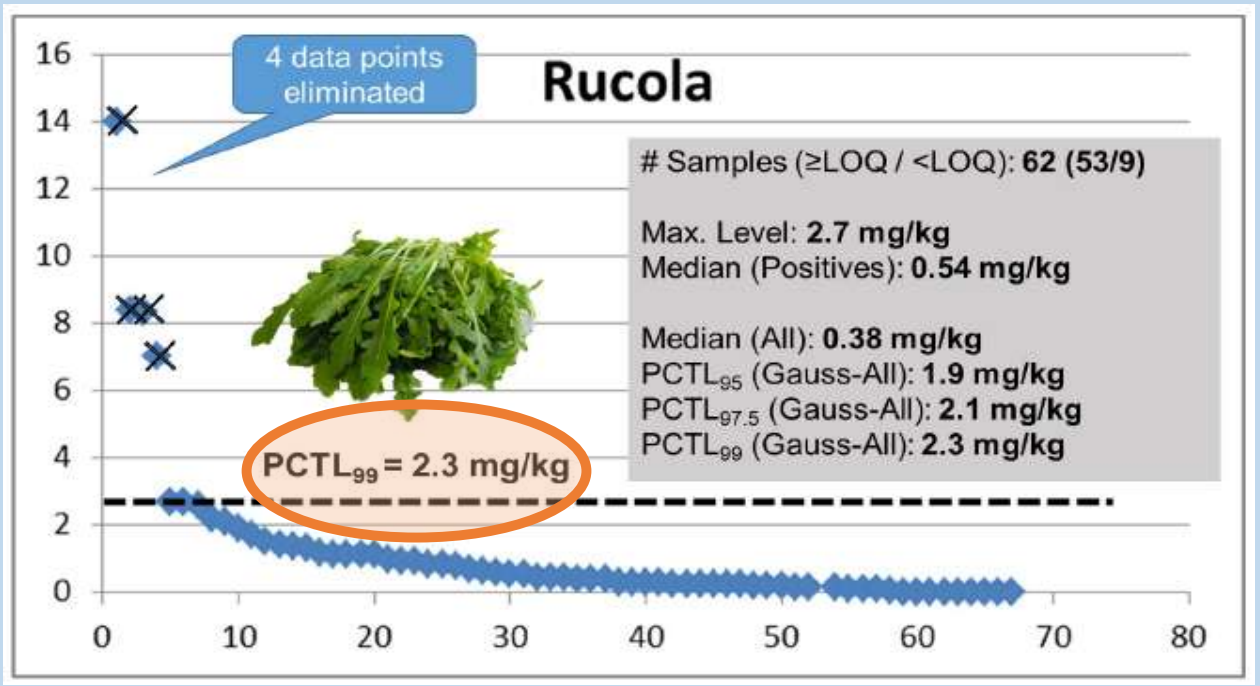
Marker substance	Chemical structure	Chroma-tography	MS-ionization	Remarks	Incorporated in study?	Usefulness as a trigger
Dimethyl-dithiocarbamate markers						
N,N-dimethyldithiocarbamate („Dibam“) CAS 128-04-1		LC (?)	ESI neg.	• Limited standard stability (highly reactive) • Chromatography difficult	-	-
2-(dimethylamino)-4,5-dihydro-1,3-thiazole-4-carboxylic acid („M1“) CAS 1417542-99-4		LC (HILIC) [1)]	ESI pos. / (ESI neg.?)	• Standard not commercially available yet	yes	**TBD**
Dimethylthioformamide (DMTF) CAS 758-16-7		GC / LC (HILIC) [1)]	EI neg. / ESI pos.		yes	**TBD**
Dimethylamine („DMA“) CAS 124-40-3		LC (HILIC) [1)]	ESI pos.	• Ubiquitous up to amounts of approx. 1 mg/kg	yes	**Very low**
N-Nitrosodimethylamine CAS 62-75-9		LC (HILIC) [1)]	ESI pos.	• Reported formation during water treatment • Poor sensitivity	-	-
N,N-dimethyldithiocarbamate-methyl CAS 3735-92-0		GC	EI neg.		yes	**TBD**

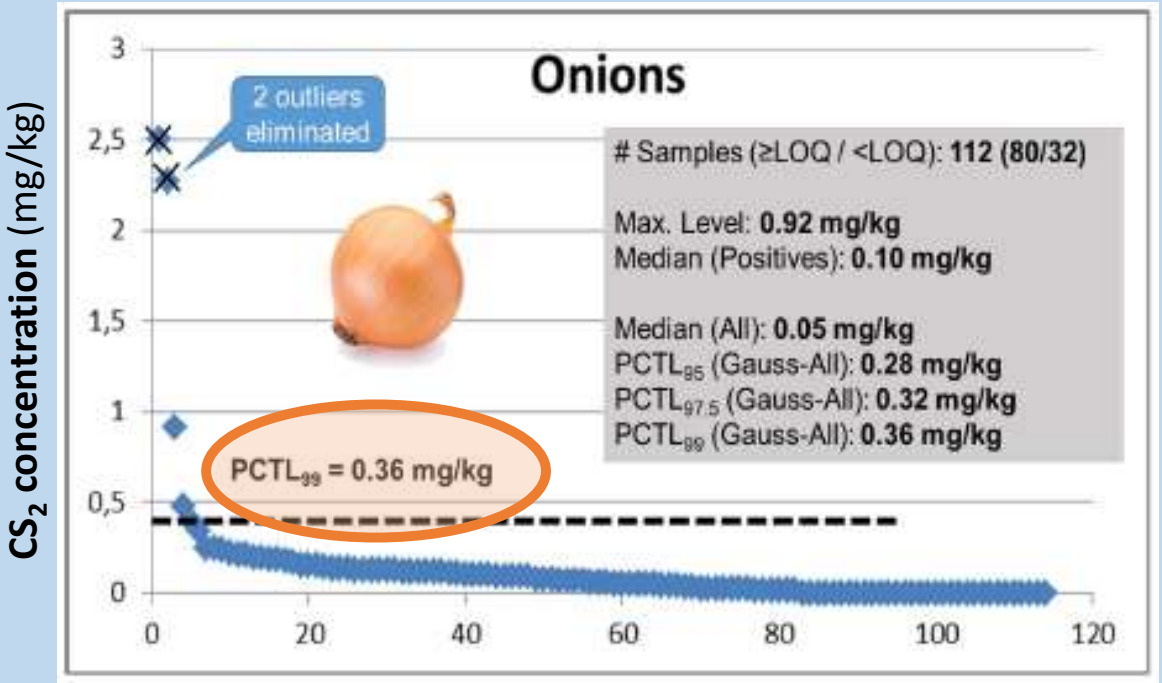


Dithiocarbamates (DTC) | Excursus: CS_2 background levels



Glucosinalotes
(naturally occuring in commodities
of e.g. Brassicaceae and Allium genus)

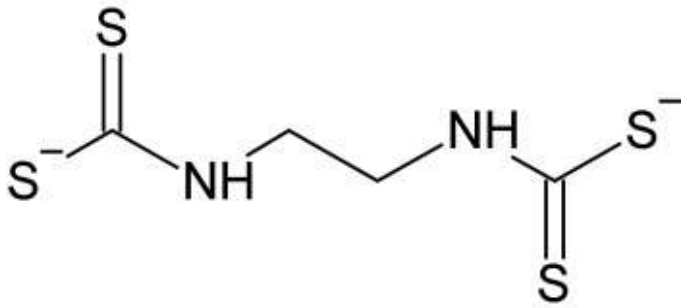
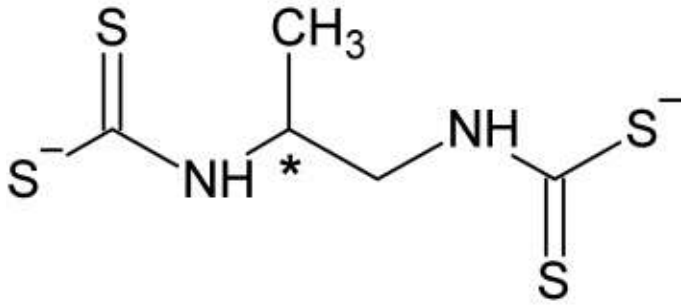




Derivatization of Fungicide Dithiocarbamates - Current Status -

Dr. Hubert Zipper, EURL-SRM

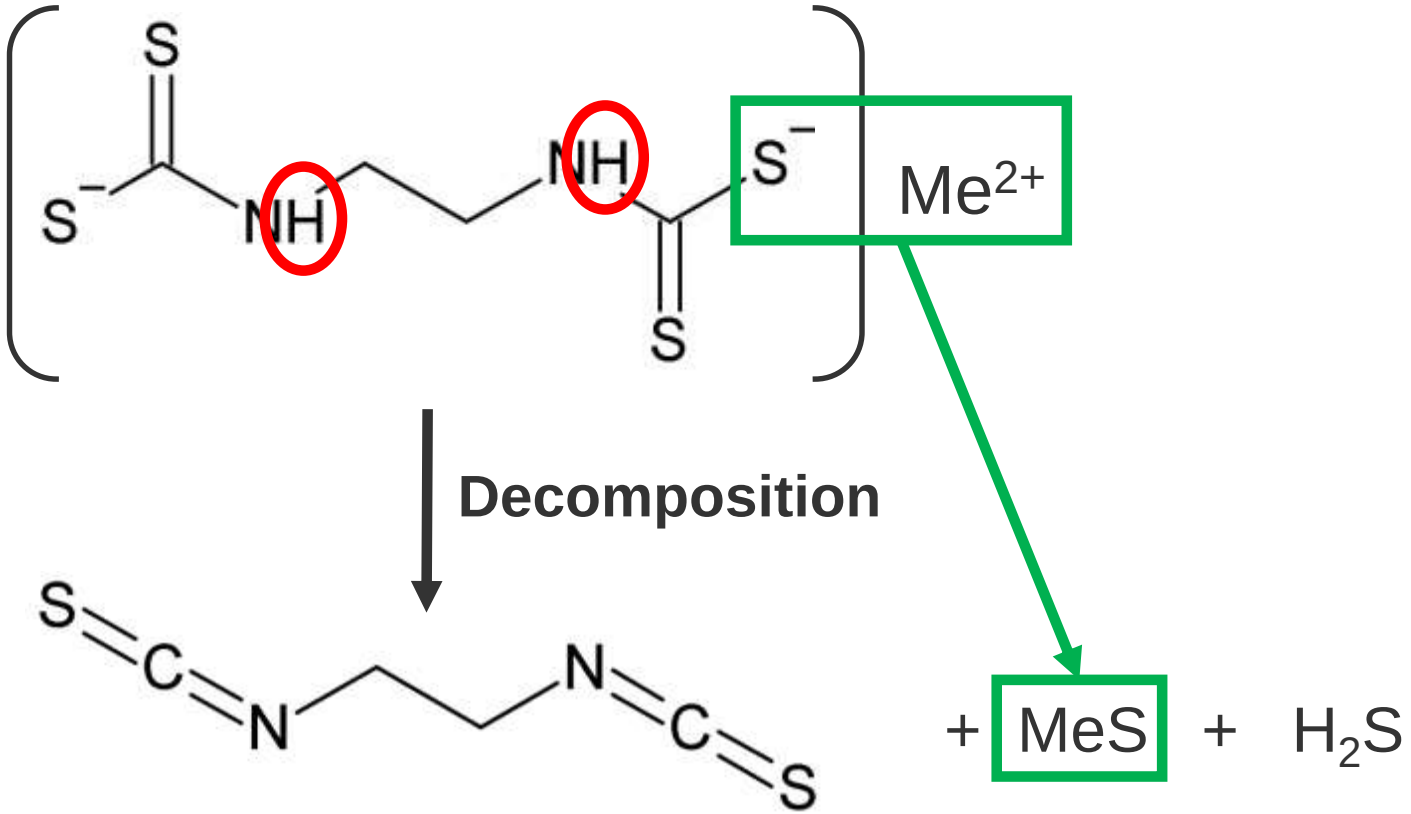
Mono Alkylene-bis-Dithiocarbamates | Metal-based, polymeric complexes

DTC-Fungicide*	Structure: common organosulphur skeleton	Structure: counter ion(s)
Zineb		Zn^{2+}
Maneb		Mn^{2+}
Mancozeb		Mn^{2+}, Zn^{2+} (94:6)
Metiram		Zn^{2+}, NH_3
Mancopper		13,7% Mn, 4 % Cu
Propineb		Zn^{2+}

- **poor/no solubility** in water & organic solvents
- DTC-anions react as **nucleophiles**
- DTCs of **primary amine origin are not stable**

* Other fungicide DTCs not shown

Decomposition of Mono Ethylene-bis-Dithiocarbamates to Ethylene-bis-Isothiocyanate

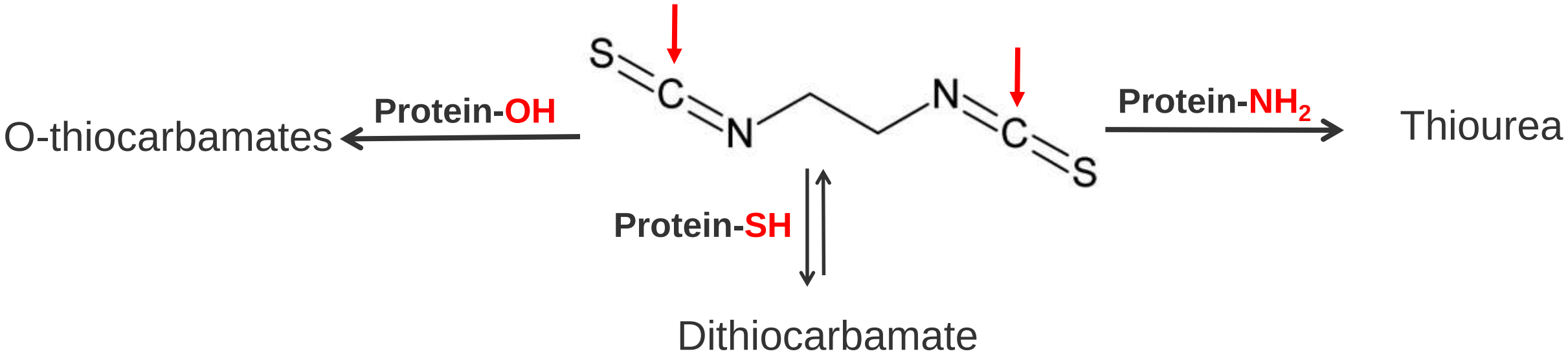


Ethylene-bis-isothiocyanate (eBIC)

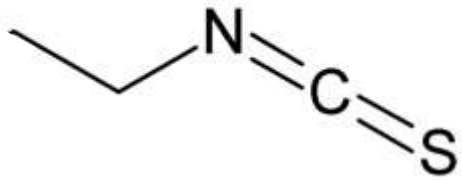
- for other degradation products see literature (e.g. EFSA-reports)

Ethylene-bis-Isothiocyanate (eBIC) | Some Properties

- only few studies on toxicology of eBIC in literature (*)
- Potential modifications of a protein target:



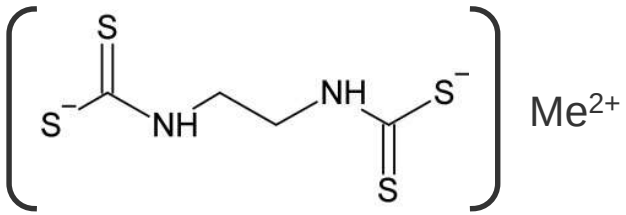
- **Ethyl Isothiocyanat** (degradation prod. (among others) of chloroprene rubber) (**):
  - suspected to be culprit of **allergic contact dermatitis** caused by chloroprene rubber



(*) Chernoff et al., Effects of chemically induced maternal toxicity on prenatal development in the rat, 1990, Teratology, vol. 42
(**) Ramzy et al., Investigation of diethylthiourea and ethyl isothiocyanate as potent skin allergens in chloroprene rubber, 2014, Contact Dermatitis, 72, 139–146

DTC-Analysis - Challenges

Metal-based, polymeric DTC-complexes

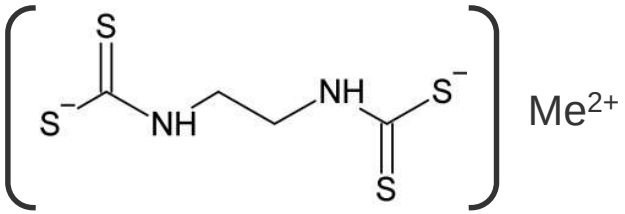


DTC-Analysis - Challenges

Which samples to analyze for DTC?

How to prepare stock/working solutions?

Metal-based, polymeric DTC-complexes



How to efficiently hydrolyse the DTC complexes?

Which derivatization reagent to use?

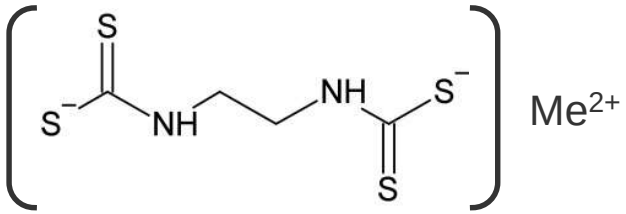
DTC-Analysis - Challenges

DTC-Screening Marker

→ QuEChERS amenable

eBIC, pBIC

Metal-based, polymeric DTC-complexes



Implemented in our routine lab for two years!

(see Eric‘s presentation for results.)

DTC-Analysis - Challenges

DTC-Screening Marker

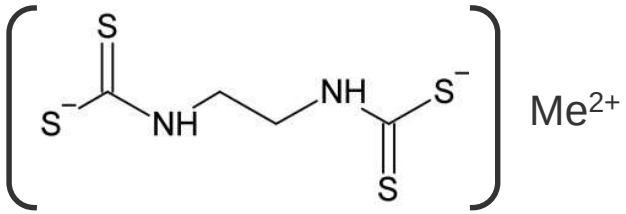
→ QuEChERS amenable

eBIC, pBIC

DTC-Suspension

→ polymeric DTC-structure intact

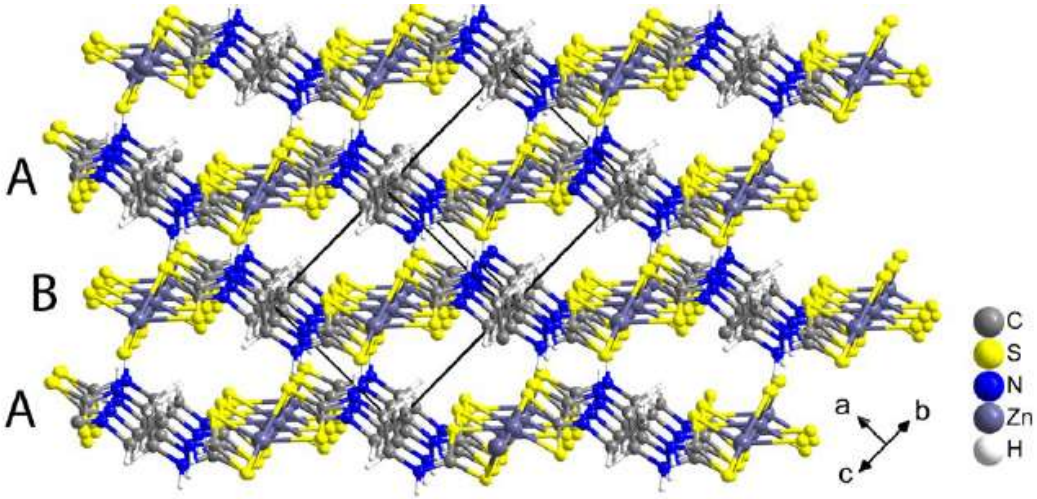
Metal-based, polymeric DTC-complexes



Preparation of DTC stock/working suspensions

Solvent: **0.2 % xanthan gum in H_2O/acetonitrile-solution 95/5 (V/V)**

- polymeric DTC structure remains intact



Lefton *et al.*, The Crystal Structure of Zineb, 75 years later. ChemRxiv. Cambridge Open Engage; 2019

Preparation of DTC stock/working suspensions

Solvent: **0.2 % xanthan gum in H_2O/acetonitrile-solution 95/5 (V/V)**

- polymeric DTC structure remains intact
- low rate of sedimentation

Zineb-stock-suspension
(1 mg/ml)

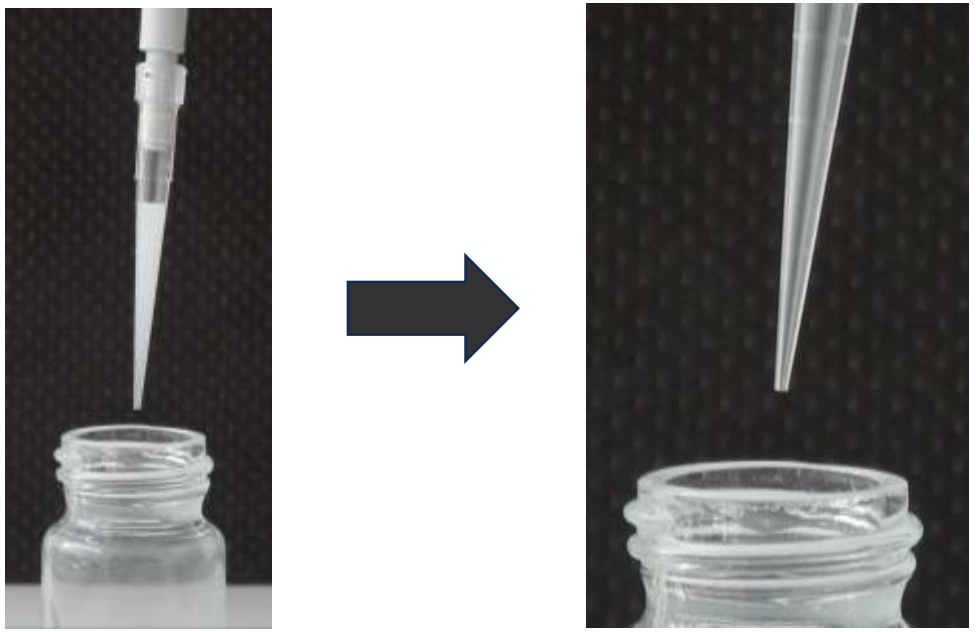
Preparation of DTC stock/working suspensions

Solvent: **0.2 % xanthan gum in H_2O/acetonitrile-solution 95/5 (V/V)**

- polymeric DTC structure remains intact
- low rate of sedimentation
- good flow properties

  ⇒ „classic“ pipett tips can be used

  ⇒ correct amount of pesticide-standard (e.g. in spiking experiments)



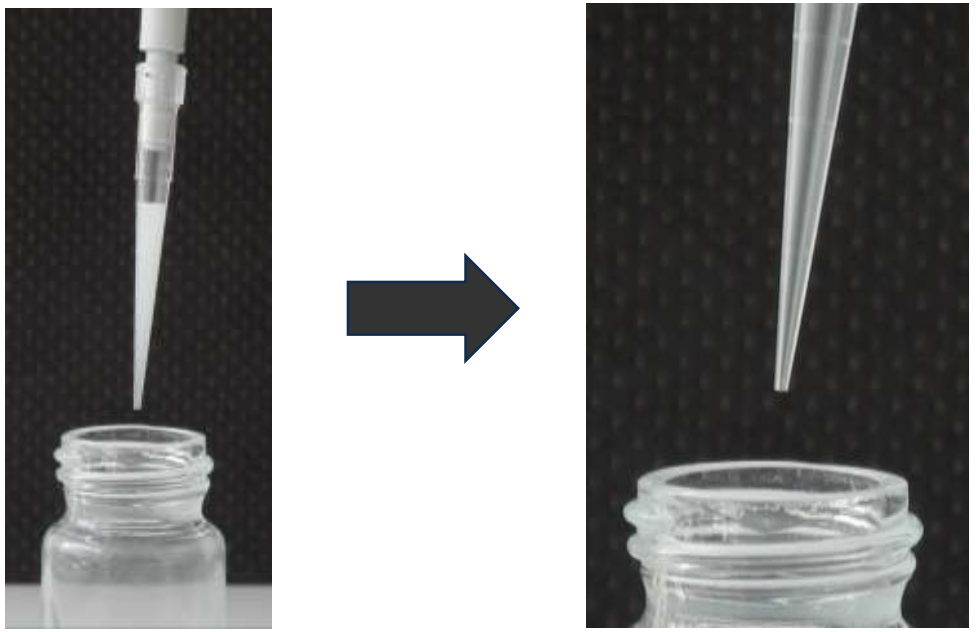
Preparation of DTC stock/working suspensions

Solvent: **0.2 % xanthan gum in H_2O/acetonitrile-solution 95/5 (V/V)**

- polymeric DTC structure remains intact
- low rate of sedimentation
- good flow properties

  ⇒ „classic“ pipett tips can be used

  ⇒ correct amount of pesticide-standard (e.g. in spiking experiments)



Applications

- Method optimization
- Validation of CS_2-Method ($SnCl_2$/HCl-cleavage) with metiram, zineb, propineb, …
- Method development

DTC Survey 2022 (106 participating EU-labs):
only few labs have validation-data for metiram, propineb, …

DTC-Analysis - Challenges

Screening Marker

→ QuEChERS amenable

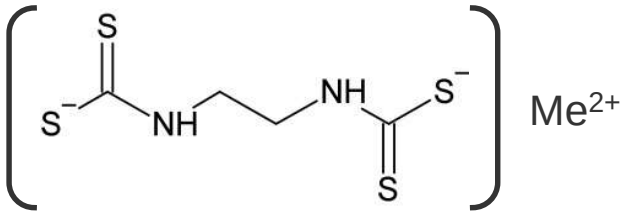
eBIC, pBIC

DTC-Suspension

→ polymeric DTC-structure intact

xanthan-solution

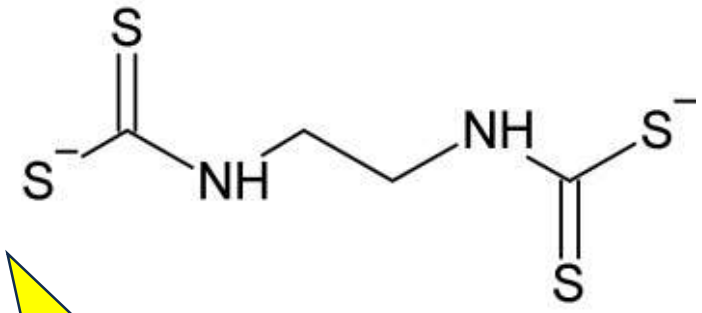
Metal-based, polymeric DTC-complexes



Hydrolysis of DTC complexes

→ same efficiency for EBDC

Hydrolysis of EDBC complexes

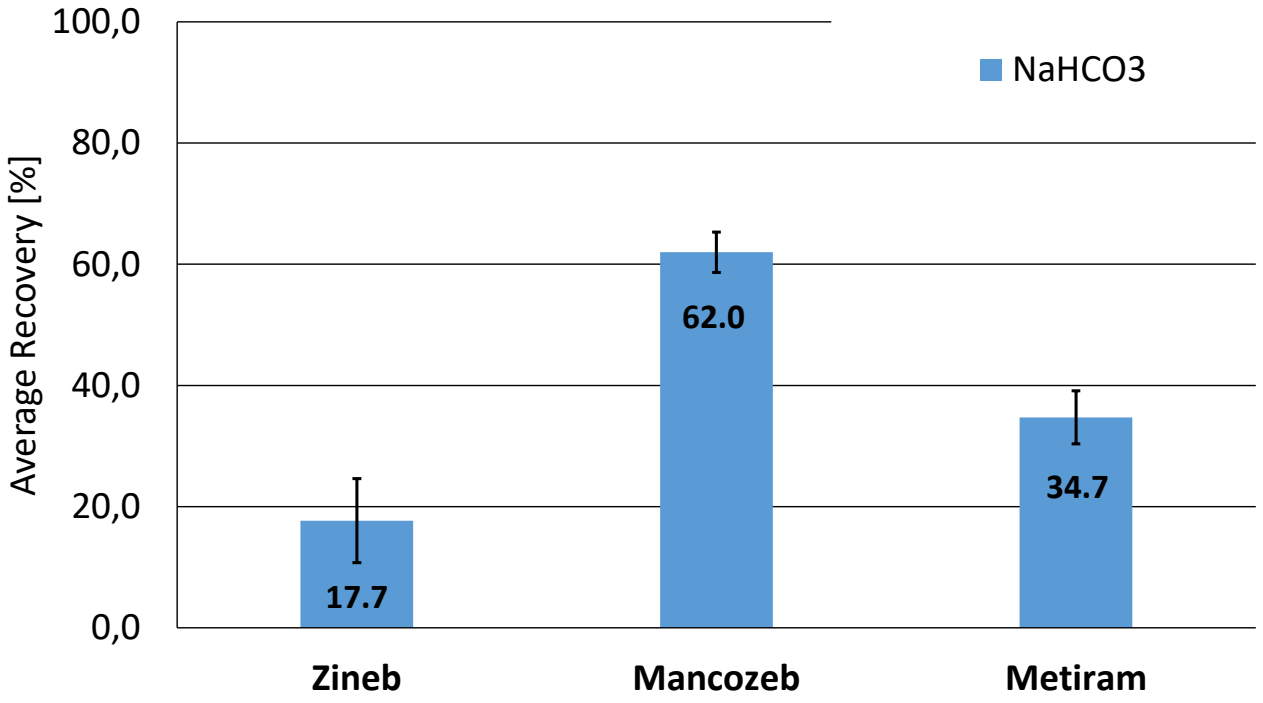
DTC-Fungicide	Structure: common organosulphur skeleton	Structure: counter ion(s)
Zineb		Zn^{2+}
Maneb		Mn^{2+}
Mancozeb		Mn^{2+}, Zn^{2+} (94:6)
Metiram		Zn^{2+}, NH_3
Mancopper		13,7% Mn, 4 % Cu

These DTCs have to be hydrolyzed with the same efficiency!

Hydrolysis of DTC complexes | $NaHCO_3$-Solution (*)

Tomato-homogenate (pH 4.4) as matrix:

- spiking level: 0.1 mg/kg zineb/mancozeb/metiram (n = 3)
- + chloroaceton, + acetonitrile, **+ 1 ml $NaHCO_3$ (1 M)**
- incubation time: 30 min
- solvent calibration

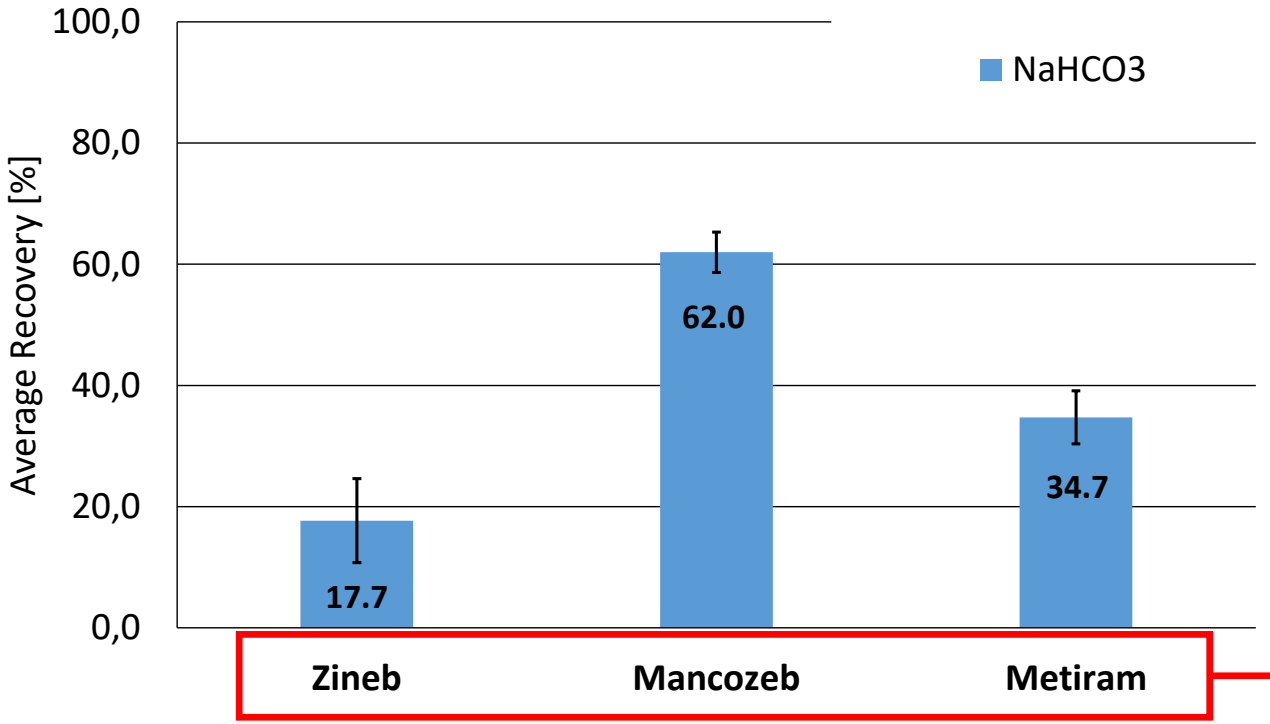


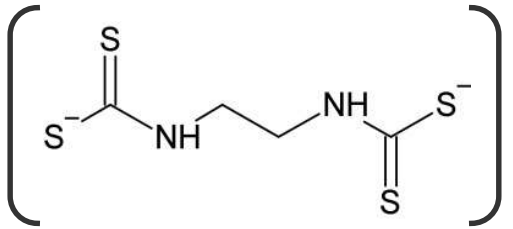
* Kakitani et al., J Pestic Sci. 2017 24;42(4):145-150. doi: 10.1584/jpestics.D17-025

Hydrolysis of DTC complexes | $NaHCO_3$-Solution

Tomato-homogenate (pH 4.4) as matrix:

- spiking level: 0.1 mg/kg zineb/mancozeb/metiram (n = 3)
- + chloroaceton, + acetonitrile, **+ 1 ml $NaHCO_3$ (1 M)**
- incubation time: 30 min
- solvent calibration



	Structure	
	common organosulphur skeleton	counter ion(s)
Zineb		Zn^{2+}
Mancozeb		Mn^{2+}, Zn^{2+} (94:6)
Metiram		Zn^{2+}, NH_3

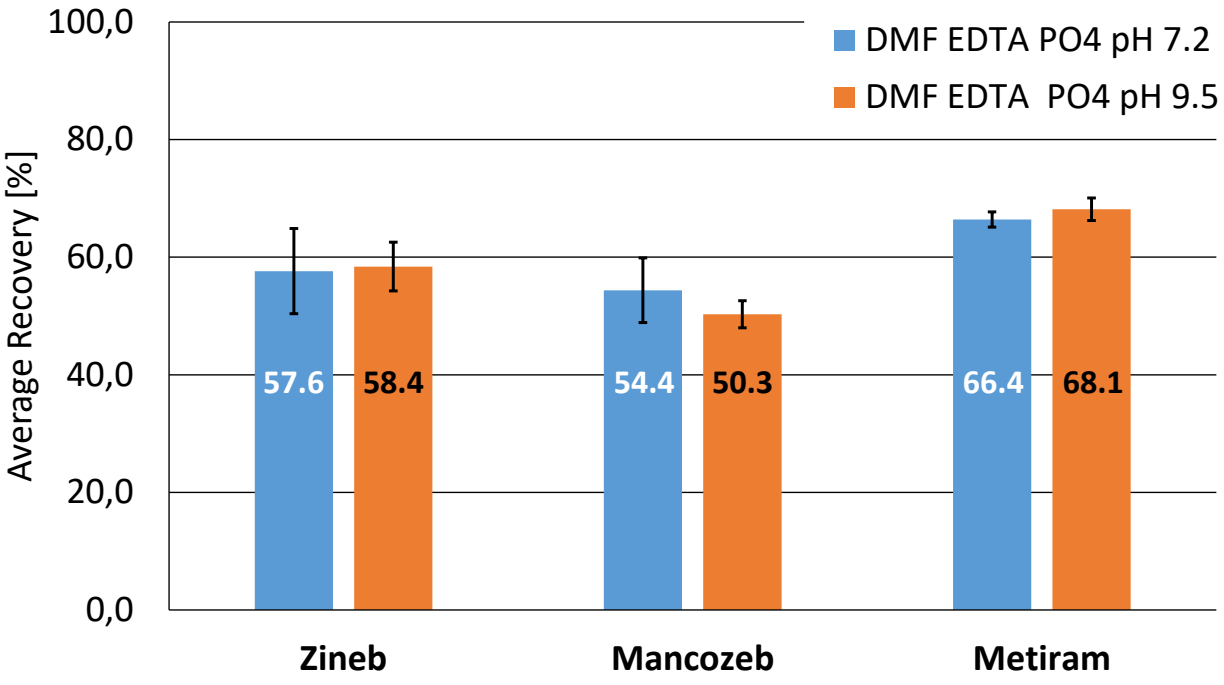
- similar results obtained with phosphate-buffer (pH 9.5, 3 M)/EDTA

Hydrolysis of DTC complexes

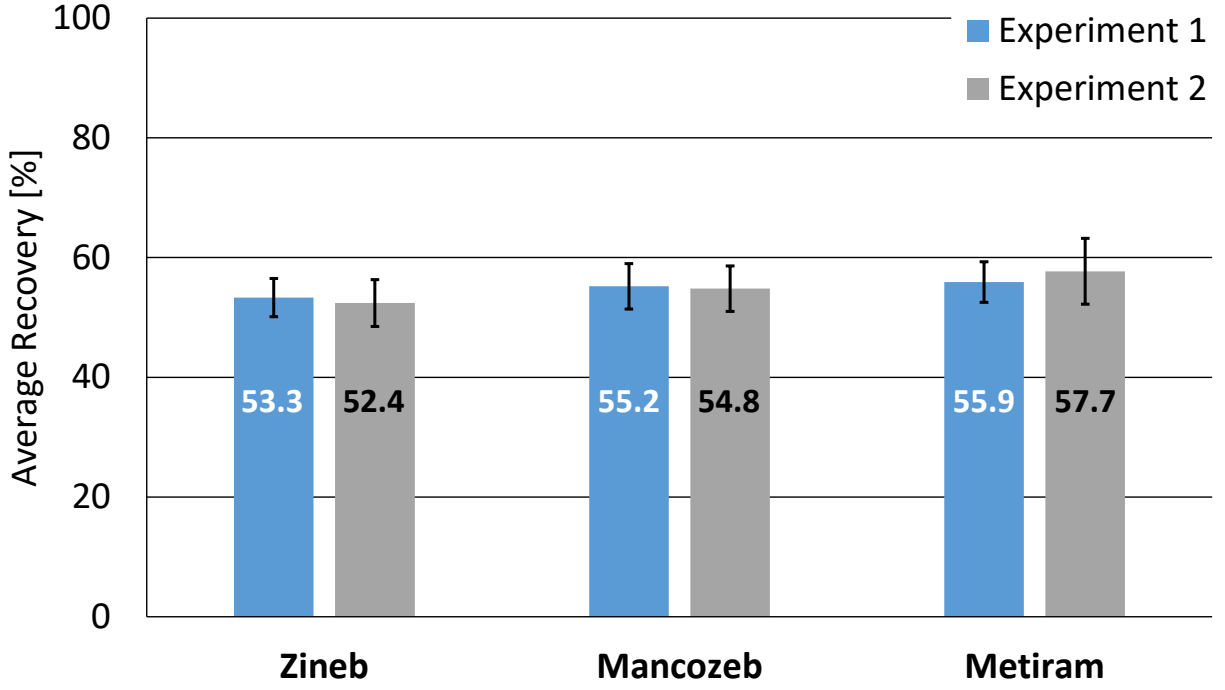
Tomato-homogenate (pH 4.4) as matrix:

- 0.1 mg/kg zineb/mancozeb/metiram (n = 3); derivatization reagent: chloroaceton
- solvent calibration

DMF / EDTA / PO_4-Buffer



DMSO (25%) / EDTA (20%) (w/w) sln.



DTC-Analysis - Challenges

Screening Marker

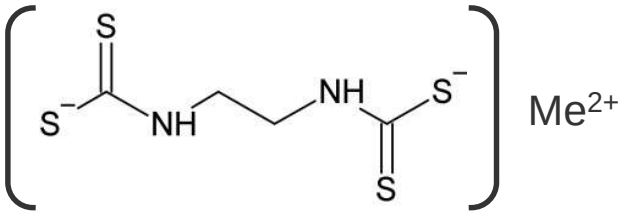
→ QuEChERS amenable

eBIC, pBIC

DTC-Suspension

→ polymeric DTC-structure intact

Metal-based, polymeric DTC-complexes



Hydrolysis of DTC complexes

based on DMSO/EDTA-sln.

DTC-Derivatization

→ non-carcinogenic reagent

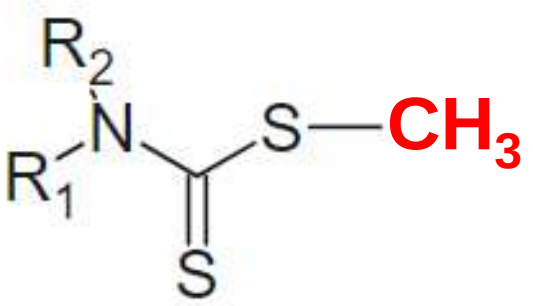
DTC-Derivatization – by Methylation

Zineb, …

Propineb

Ziram, Thiram

Methylation step →



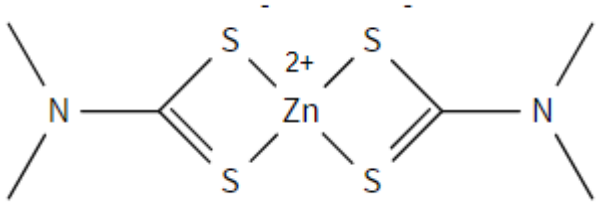
Electrophilic methylation at QuEChERS conditions:

✓ **dimethyl sulfate, methyl iodid** (see literature) (carcinogenic)

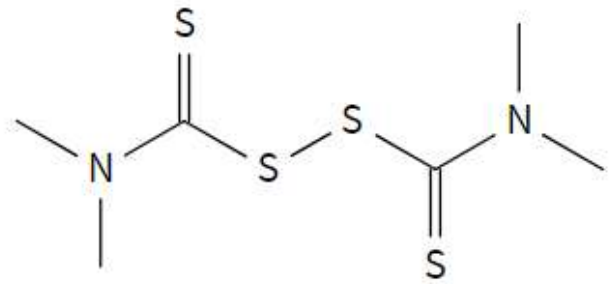
– alternative, less toxic (!) methylating agents tested:
dimethyl dicarbonate(*), dimethyl carbonate, trimesium, trimethylphosphate
=> no methylation products detected

(*) EU Scientific Committee on Food, FDA in the United States and JECFA of WHO have confirmed the safe use in beverages.

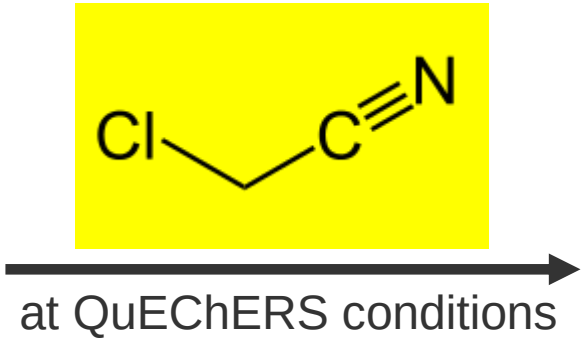
DTC-Derivatization – by Chloroacetonitrile

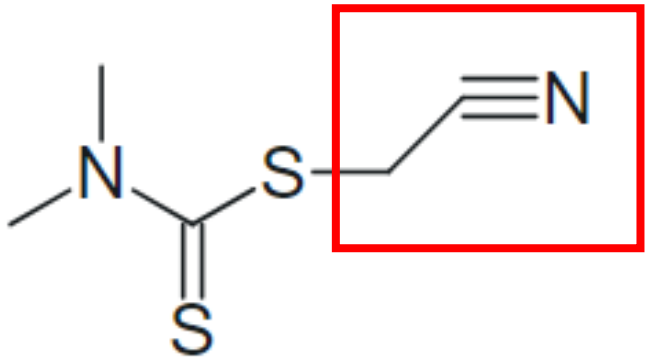


Ziram



Thiram





Cyanomethyl N,N-dimethyldithiocarbamate
CAS 61540-35-0
LC-MS amenable

- no conversion of propineb and EBDC-group (zineb, metiram, mancozeb, …) ☹

Thiram/Ziram-Derivatization – by Chloroacetonitrile

Workflow - Sample Preparation (in short)**:**

Weigh 10.0 g **sample homogenate** in 50 mL centrifuge tube

↓

Add 100 µL **internal standard** solution & 100 µL **DTT-solution** (1 M, water as solvent)

↓

Add 1 mL **phosphatebuffer** (3 M Potassium dihydrogenphosphate, **pH=9,5**) & 10 mL **ACN**

↓

Add 1,5 mL **EDTA/DMSO** (20% (g/g) EDTA, 25% (g/g) DMSO)

↓

Add 100 µL **Chloroacetonitrile**

↓

Shake thouroughly for 15 min & centrifuge

↓

Add QuEChERS salts and shake for 1 min

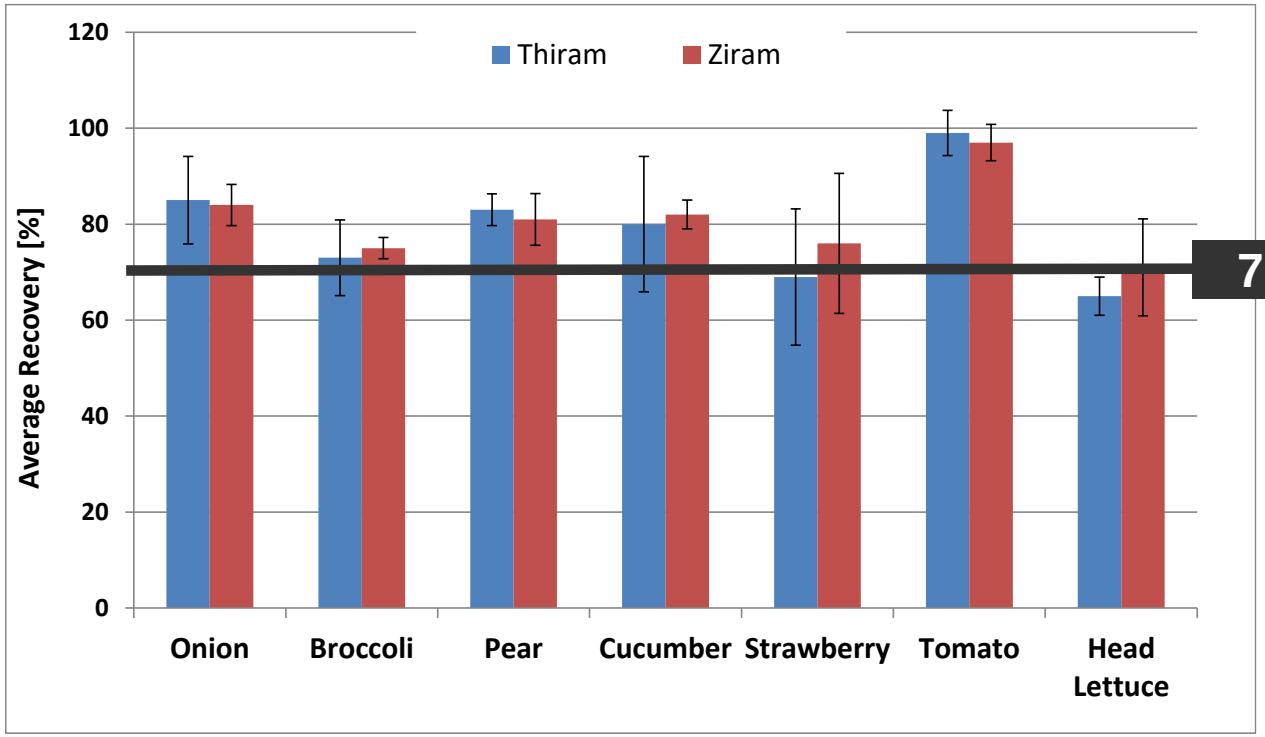
↓

Centrifuge e. g. at 4000 rpm for 5 min & **LC-MS/MS analysis**

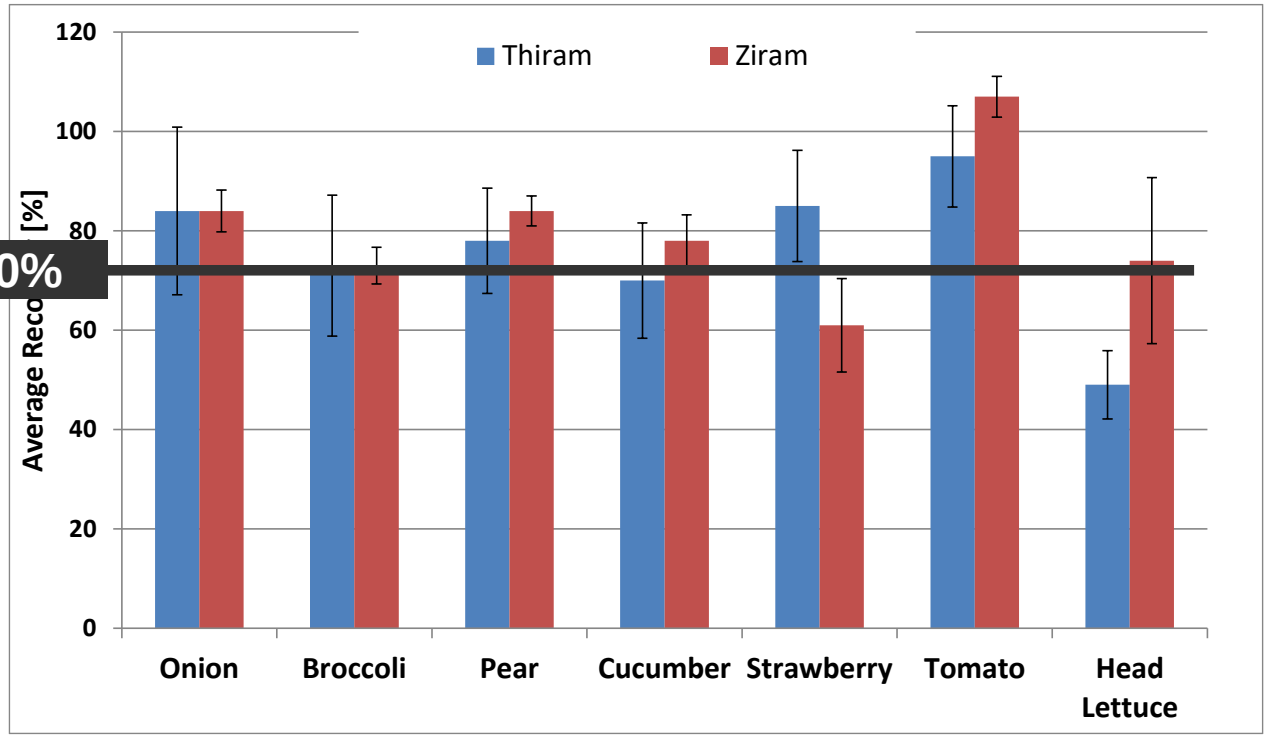
Thiram/Ziram-Derivatization – by Chloroacetonitrile

- Validation data for Thiram and Ziram (n=5):

Spiking level: **0,1 mg/kg**



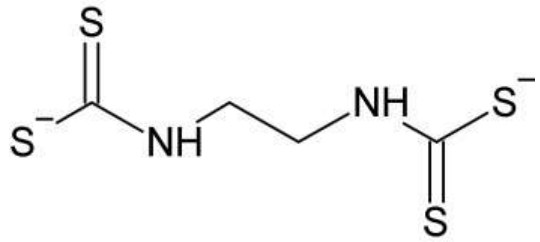
Spiking level: **0,05 mg/kg**

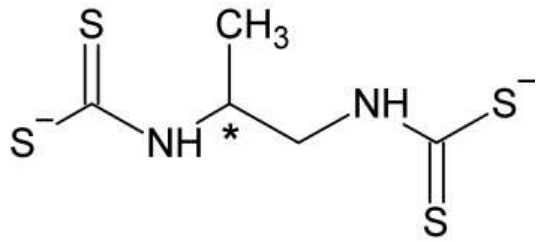


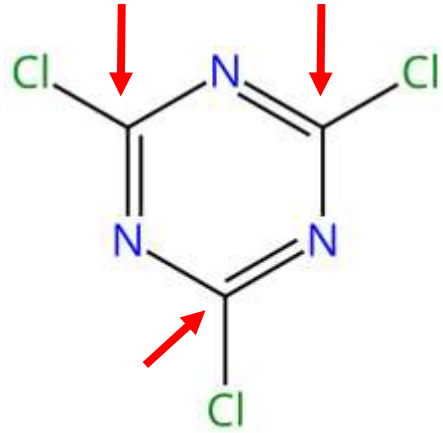
DTC-Derivatization – by Cyanuric chloride (*)

Reaction Scheme (in short):

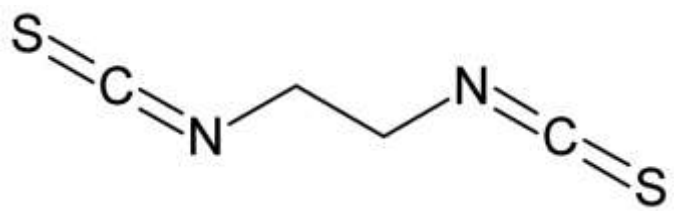
Zineb	Zn^{2+}
Maneb	Mn^{2+}
Mancozeb	Mn^{2+}, Zn^{2+} (94:6)
Metiram	Zn^{2+}, NH_3
Mancopper	13,7% Mn, 4 % Cu
Propineb	Zn^{2+}







at QuEChERS conditions



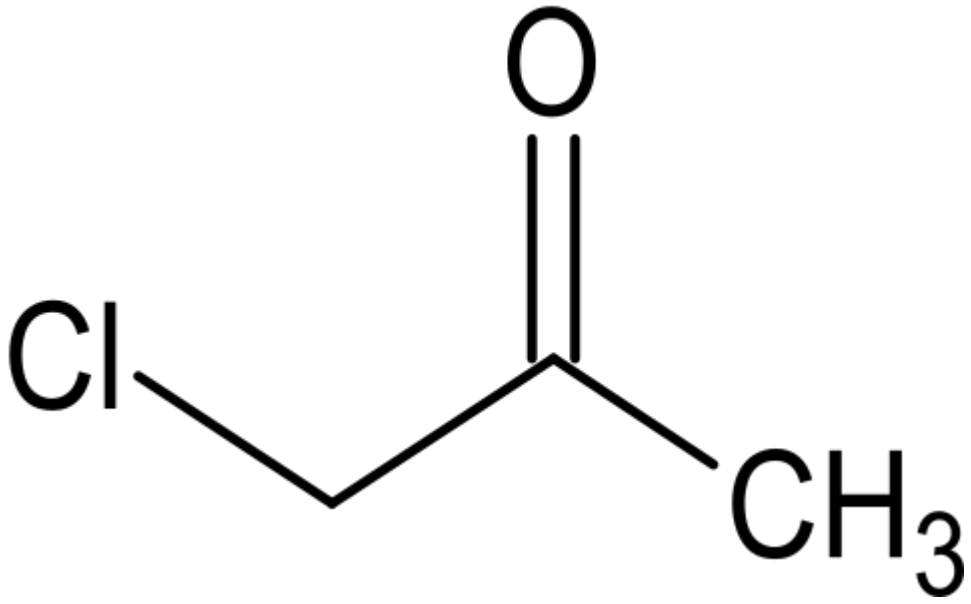
eBIC
(ethylene-*bis*-isothiocyanate)

pBIC
(Propylene-*bis*-isothiocyanate)

- Validation pending
- no conversion of Thiram and Ziram ☹

(*) Sun et al., Beilstein J Org Chem. 2012; 8: 61–70

DTC-Derivatization – by Chloroacetone



Chloroacetone

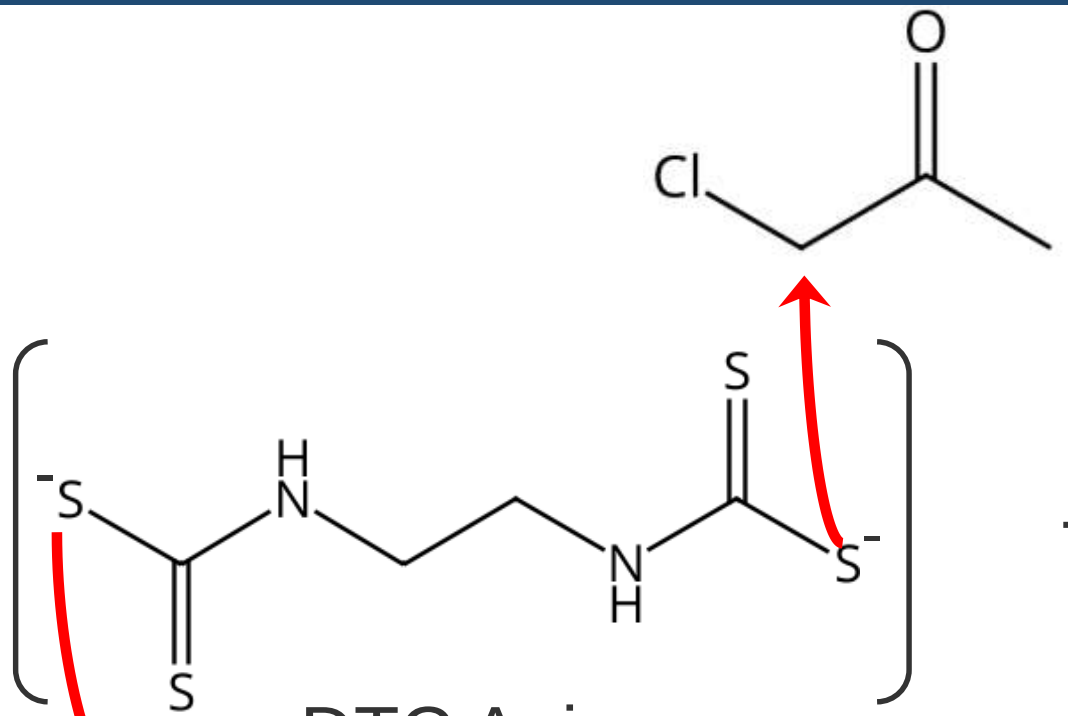
Chloroaceton - Hazards

flammable liquid and vapour;

causes skin burns, eye damage and respiratory irritation

=> working in fume hood is strongly recommended!

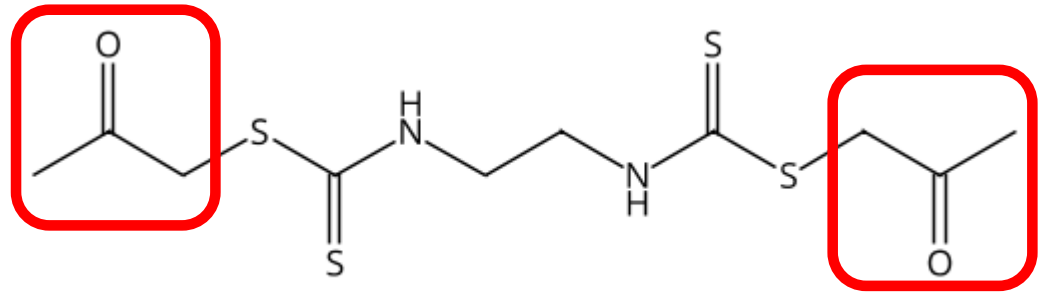
DTC-Derivatization – by Chloroacetone



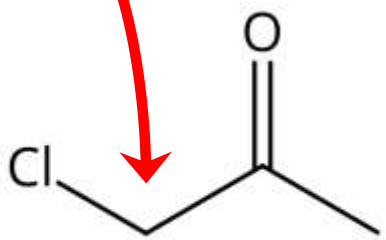
DTC Anion

CEN-

QuEChERS



EBDC-diaceton



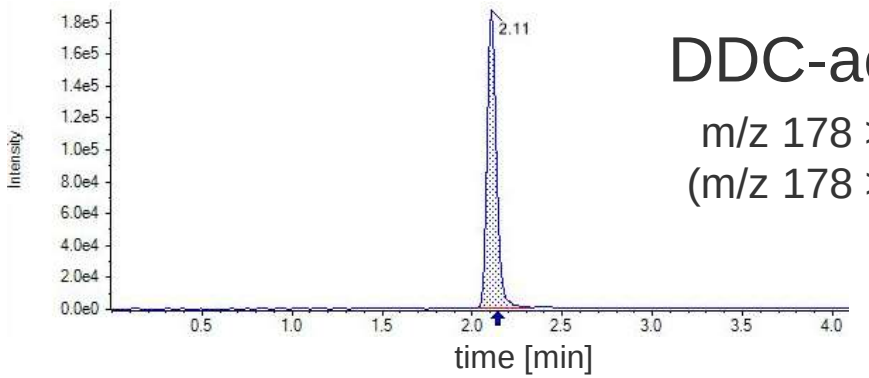
Chloroacetone

* Tsuboi *et al.*, Bulletin of the Chemical Society of Japan, 61(9), 3205-9; 1988

DTC-Derivatization – by Chloroacetone

Tomato spiked at 0.01 mg/kg with

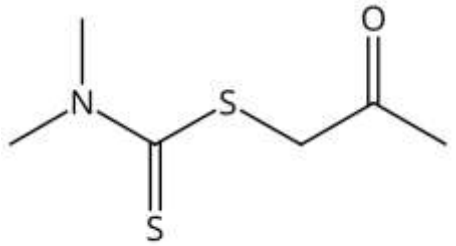
Ziram



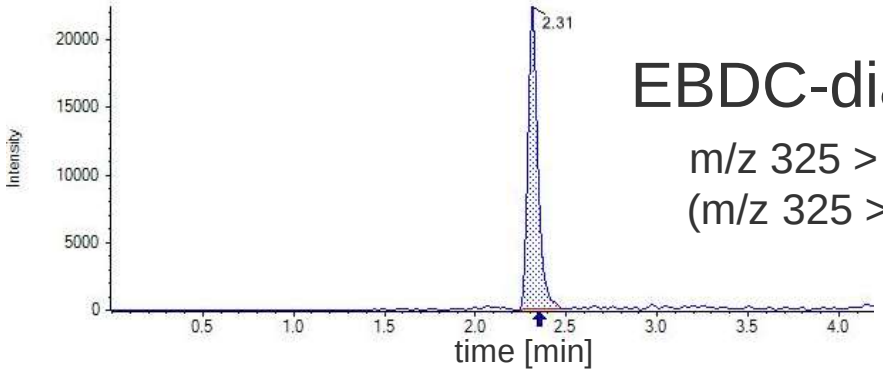
DDC-aceton

m/z 178 > 88
(m/z 178 > 73)

Chemical Structure

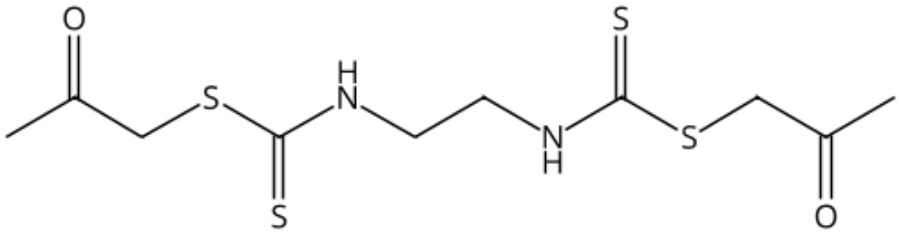


Mancozeb

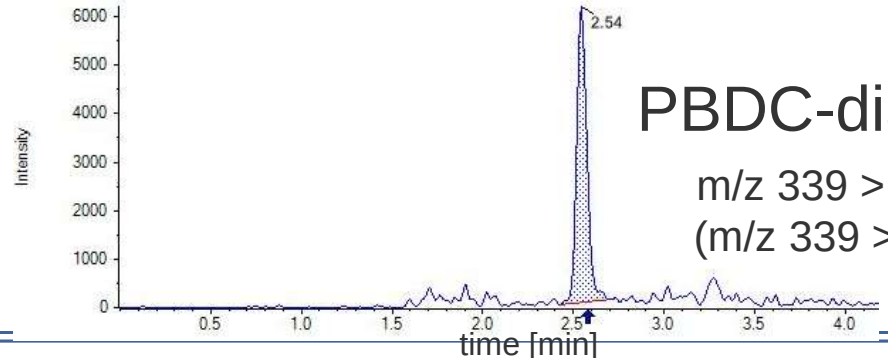


EBDC-diaceton

m/z 325 > 158
(m/z 325 > 88)

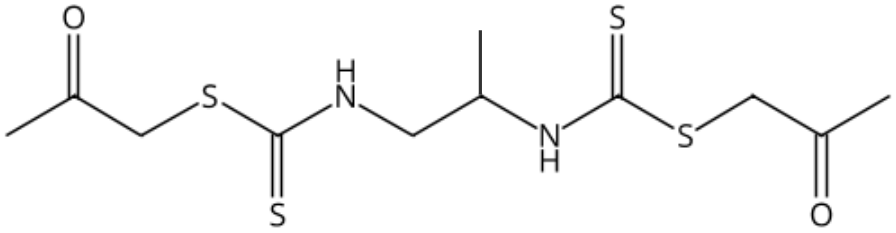


Propineb



PBDC-diaceton

m/z 339 > 231
(m/z 339 > 75)



Group-specific Quantification of DTC | Workflow

Weigh 10.0 g frozen sample homogenate
in 50 mL centrifuge tube

↓

Add 100 µL internal standard solution

↓

Add 10 mL ACN

- Add 0.5 ml chloroacetone
- Add 1 ml DMSO (25%)/EDTA (20%) (w/w) solution

↓

Shake thoroughly for 15 min.

↓

Add QuEChERS salts and shake for 1 min.

↓

Centrifuge e. g. at 4000 rpm for 5 min.

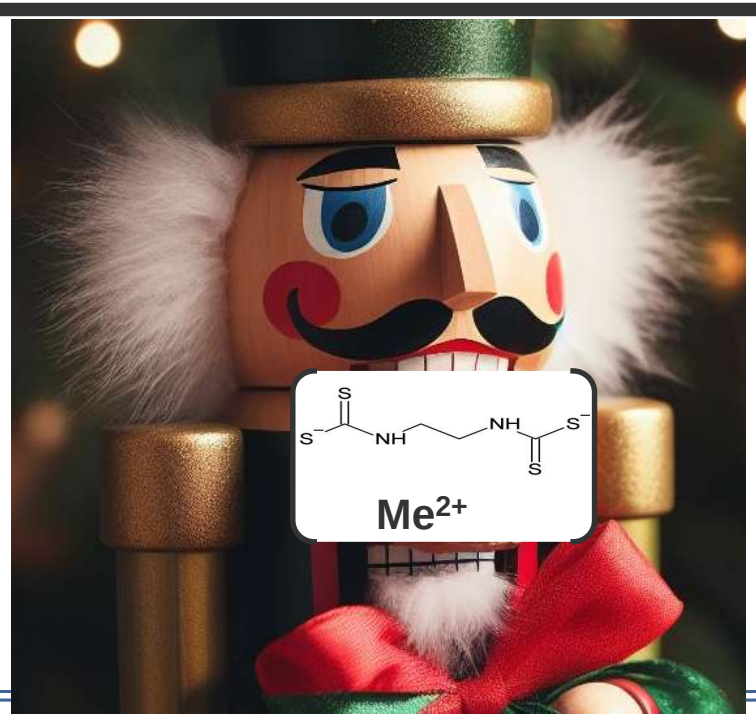
↓

Transfer 1 mL extract to vials

↓

LC-MS/MS

Validation: pending! ☹



Summary

Screening Marker

→ QuEChERS amenable

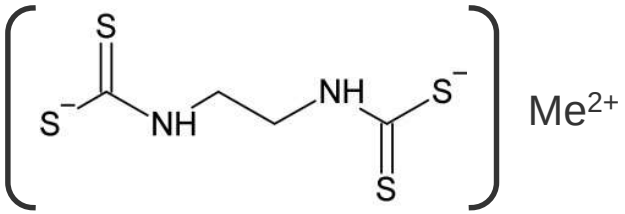
eBIC, pBIC

DTC-Suspension

→ polymeric DTC-structure intact

xanthan-solution

Metal-based, polymeric DTC-complexes



Hydrolysis of DTC complexes

based on DMSO/EDTA-sln.
(optimization needed)

DTC-Derivatization

→ non-carcinogenic reagent!

Chloroacetone = good candidate
testing of other substances

Thank you for your attention!

Questions to:

eurl-srm@cvuas.bwl.de