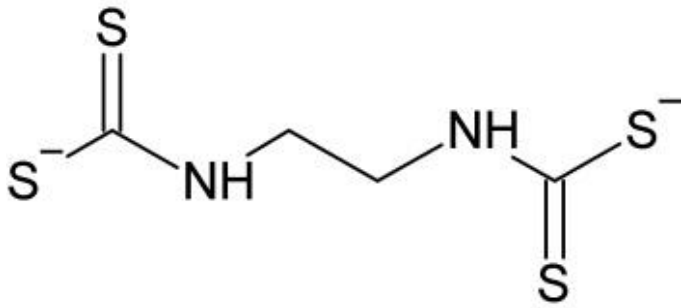
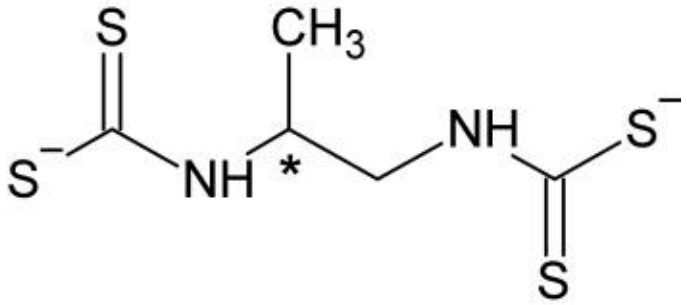
Derivatization of Fungicide Dithiocarbamates
- Current Status -

Dr. Hubert Zipper, EURL-SRM

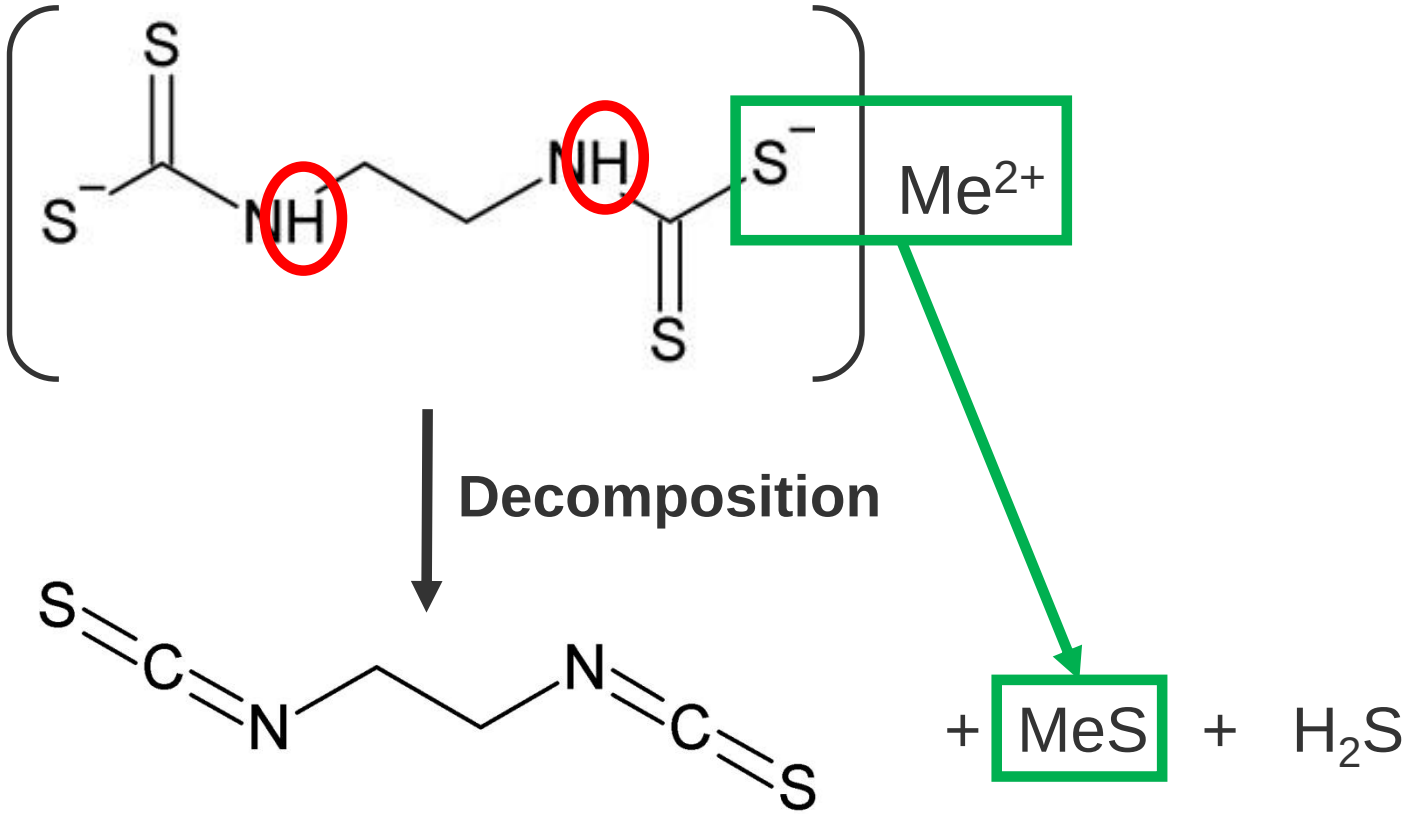
Mono Alkylene-bis-Dithiocarbamates | Metal-based, polymeric complexes

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

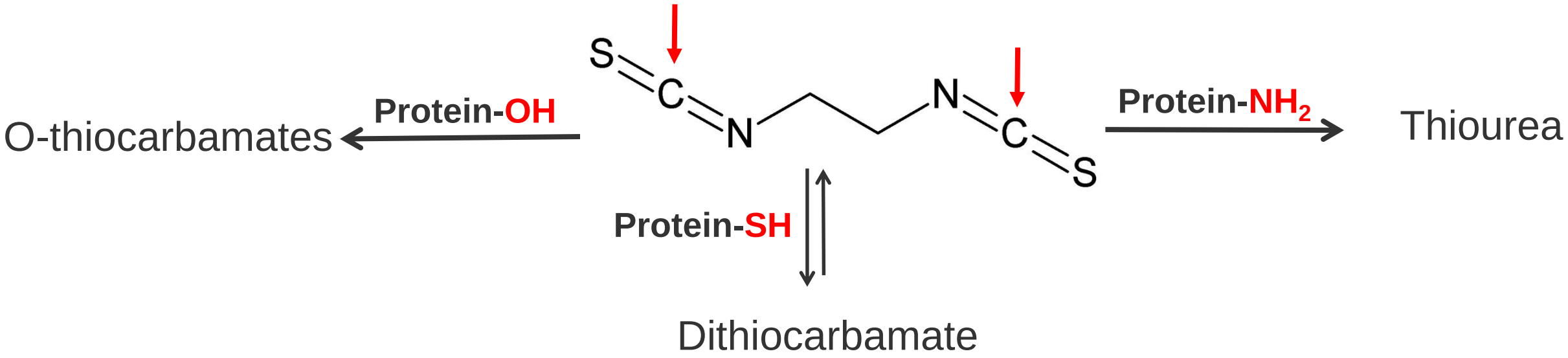


Ethylene-bis-isothiocyanate (eBIC)

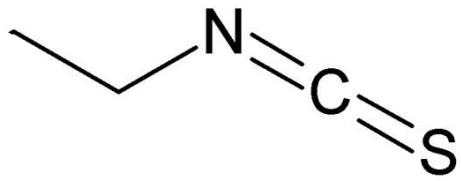
- for other degradation products see literature (e.g. EFSA-reports)

Ethylene-bis-Isothiocyanate (eBIC) | Some Properties

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



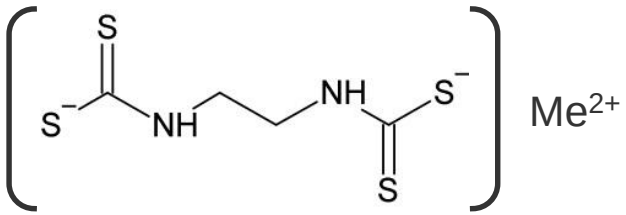
- **Ethyl Isothiocyanat** (degradation prod. (among others) of chloroprene rubber) (**):
  - suspected to be culprit of **allergic contact dermatitis** caused by chloroprene rubber



(*) 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

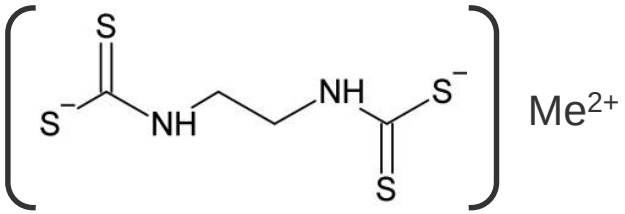


DTC-Analysis - Challenges

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?

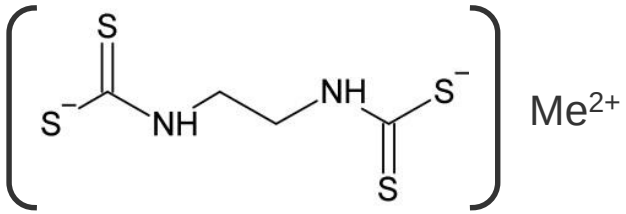
DTC-Analysis - Challenges

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

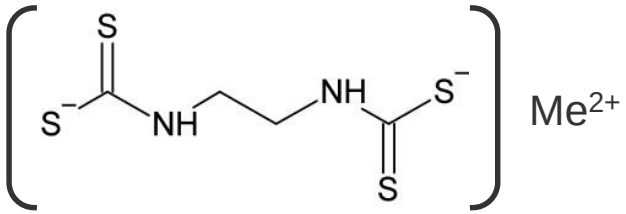
→ QuEChERS amenable

eBIC, pBIC

DTC-Suspension

→ polymeric DTC-structure intact

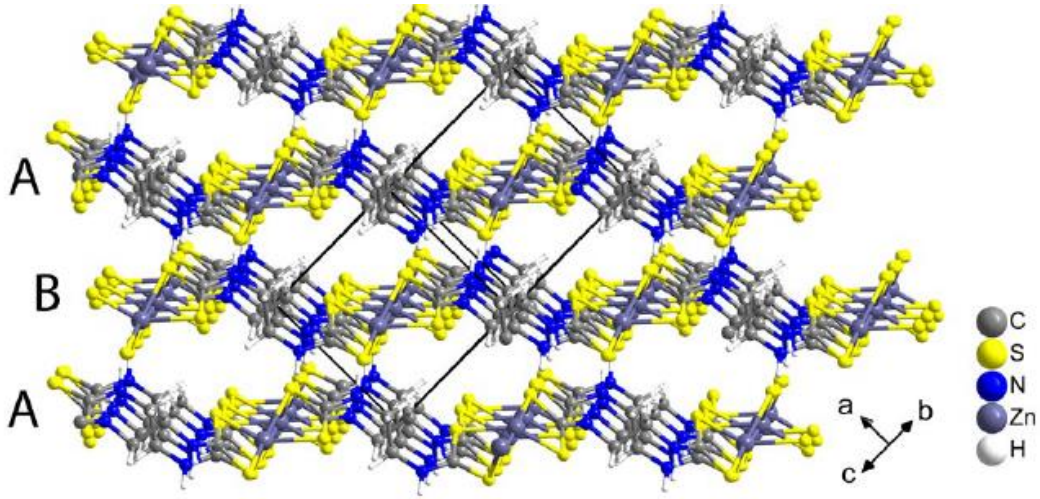
Metal-based, polymeric DTC-complexes



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)

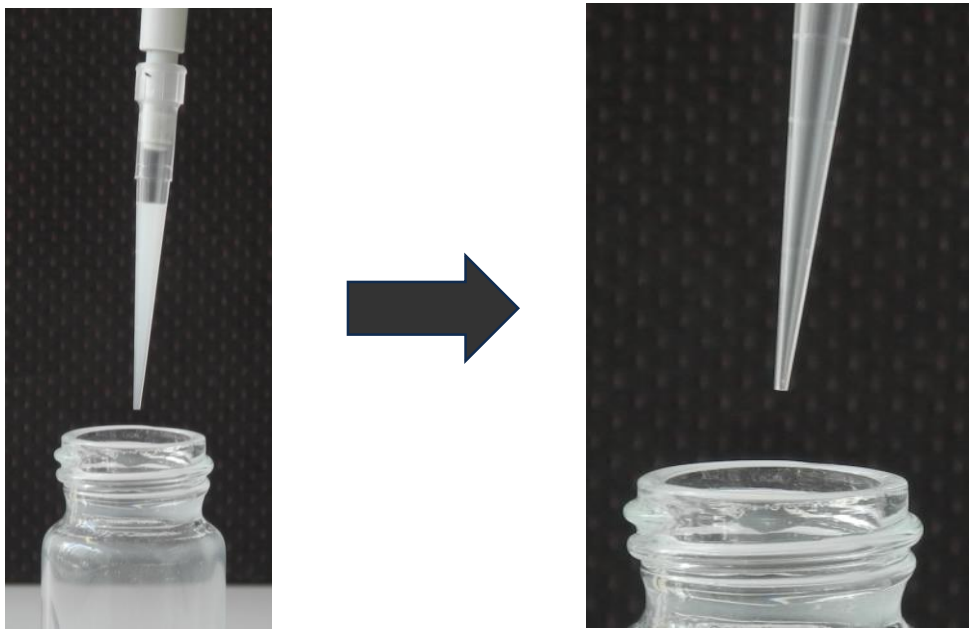
Preparation of DTC stock/working suspensions

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)



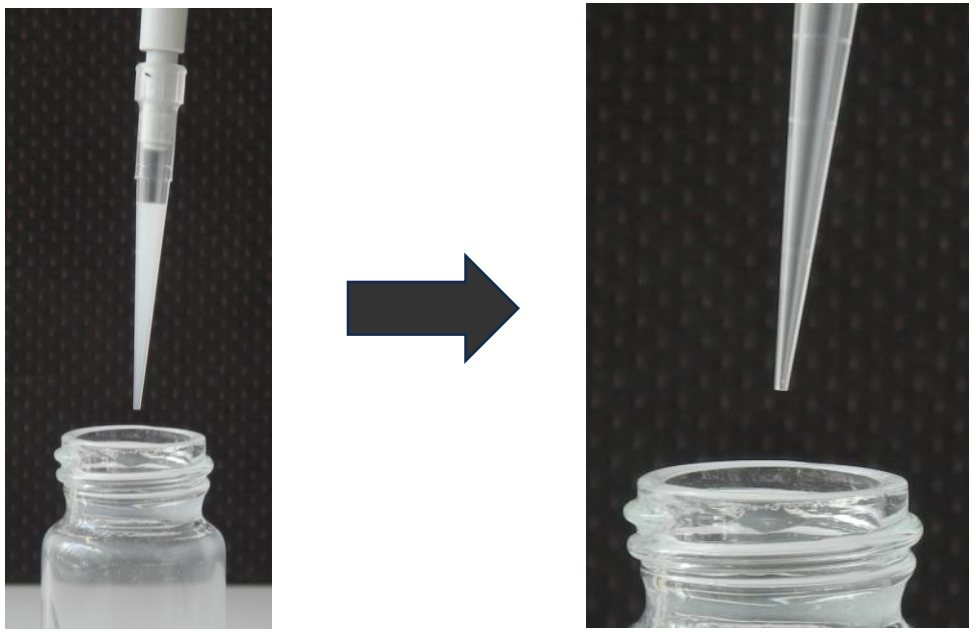
Preparation of DTC stock/working suspensions

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

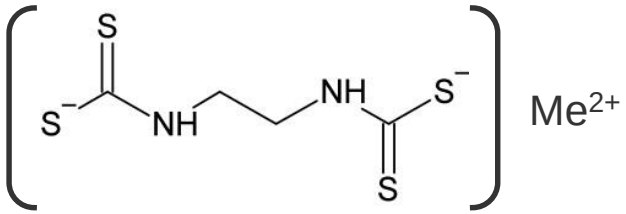
eBIC, pBIC

DTC-Suspension

→ polymeric DTC-structure intact

xanthan-solution

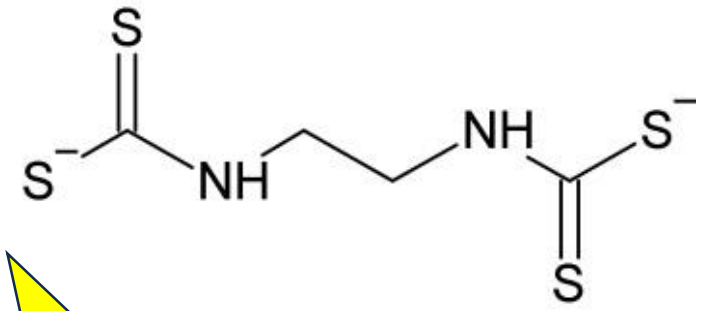
Metal-based, polymeric DTC-complexes



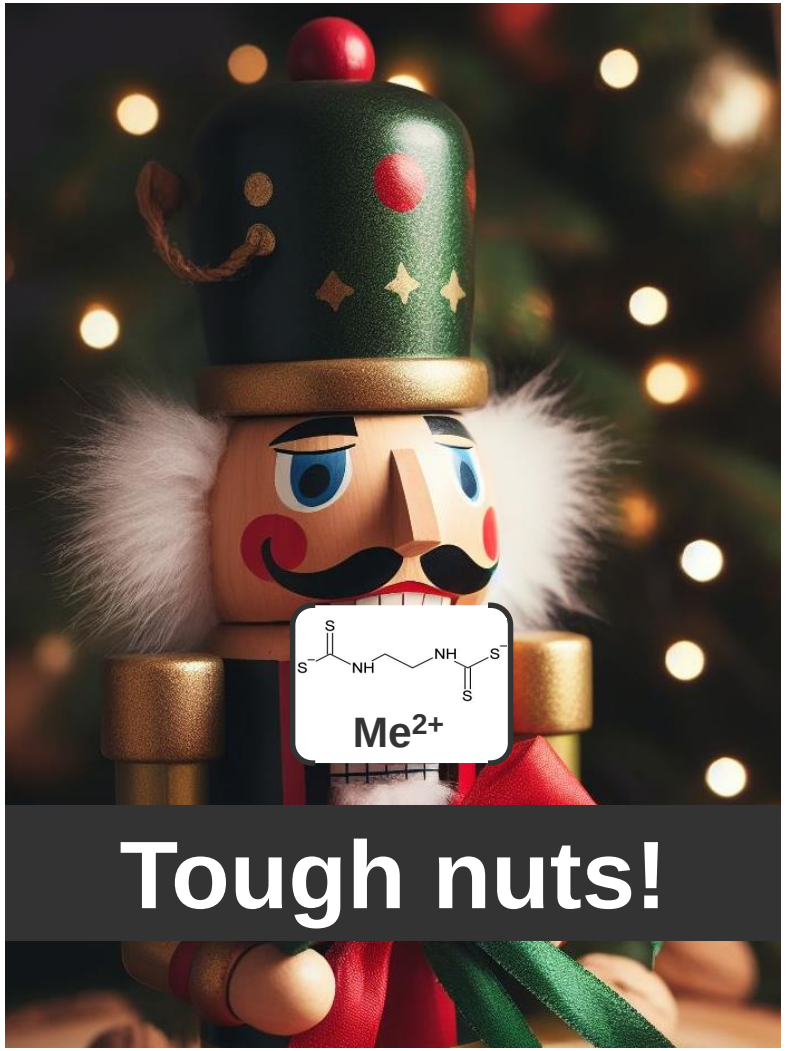
Hydrolysis of DTC complexes

→ 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

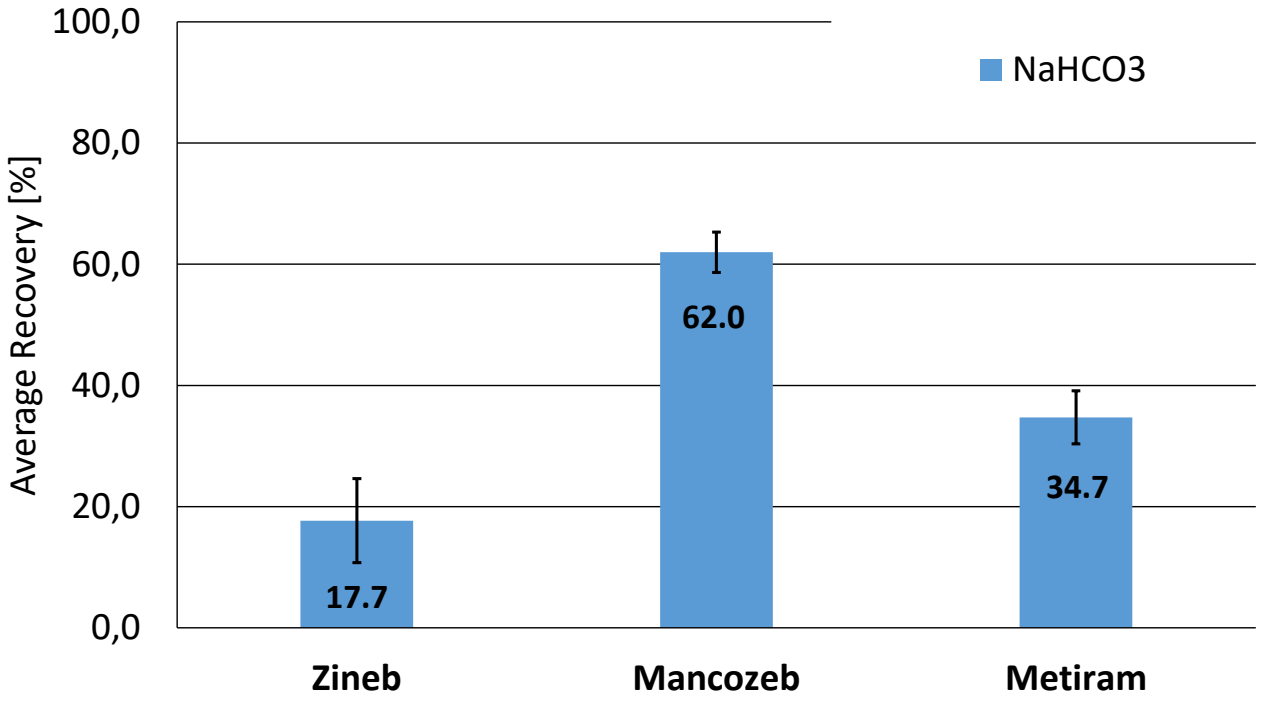
These DTCs have to be hydrolyzed with the same efficiency!



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

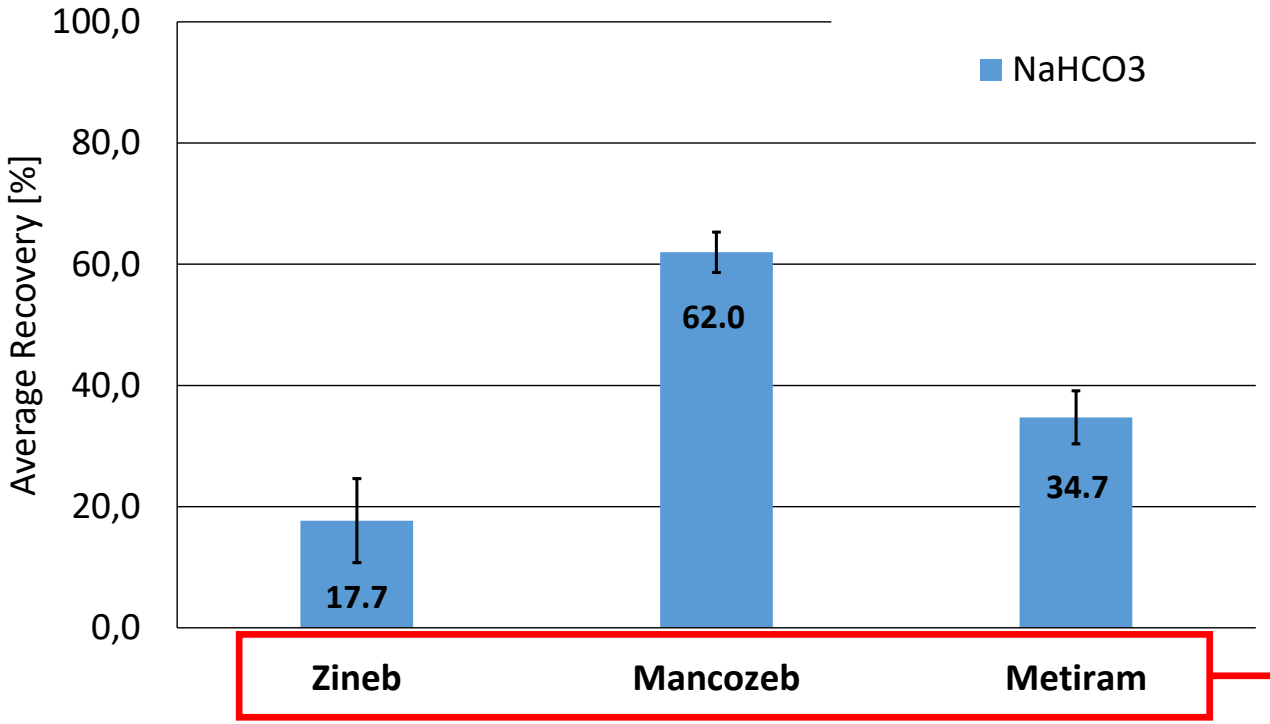


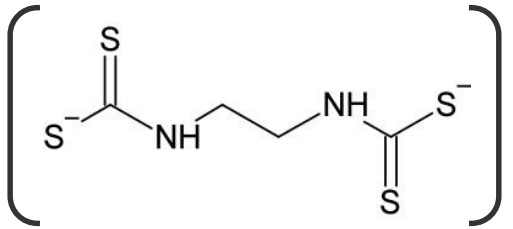
* Kakitani et al., J Pestic Sci. 2017 24;42(4):145-150. doi: 10.1584/jpestics.D17-025

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

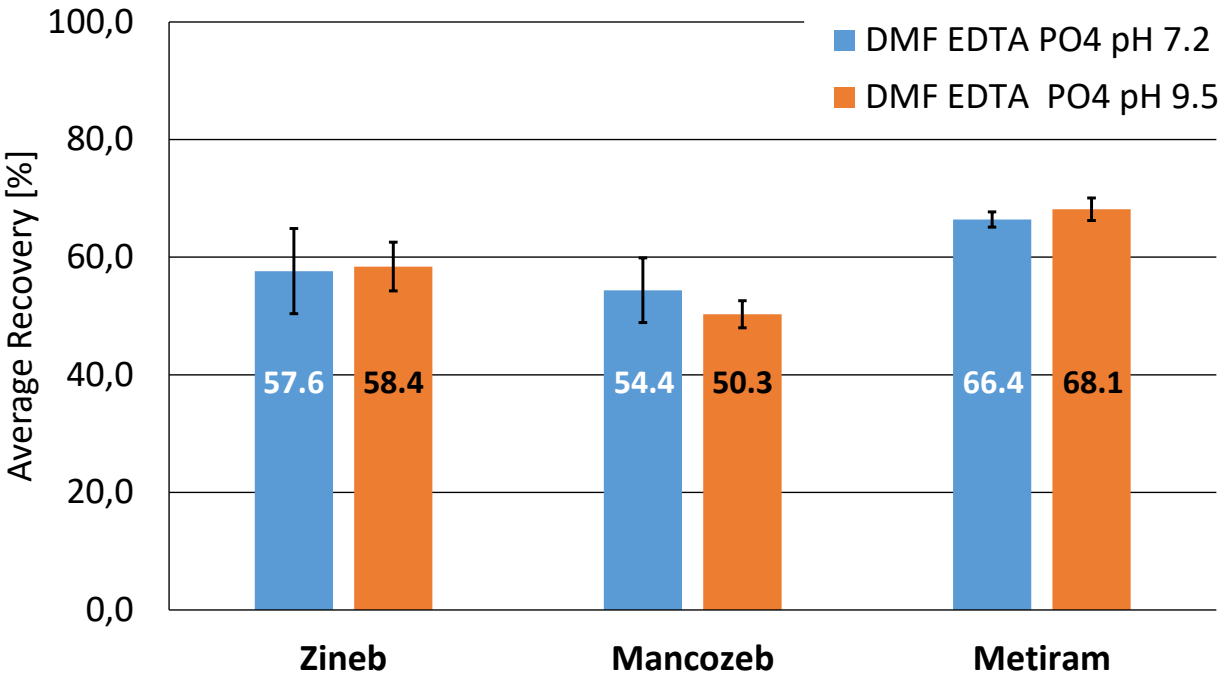
- similar results obtained with phosphate-buffer (pH 9.5, 3 M)/EDTA

Hydrolysis of DTC complexes

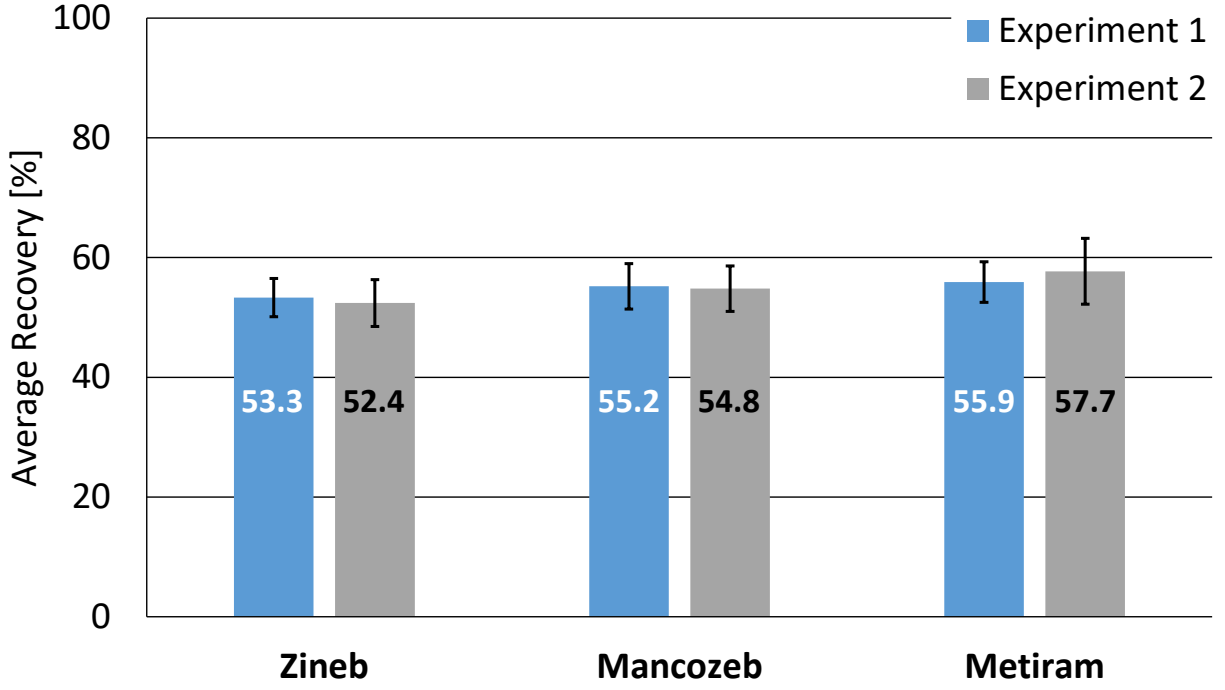
Tomato-homogenate (pH 4.4) as matrix:

- 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

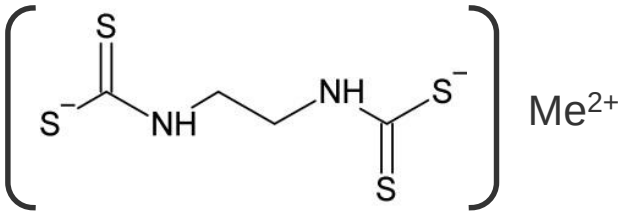
→ QuEChERS amenable

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

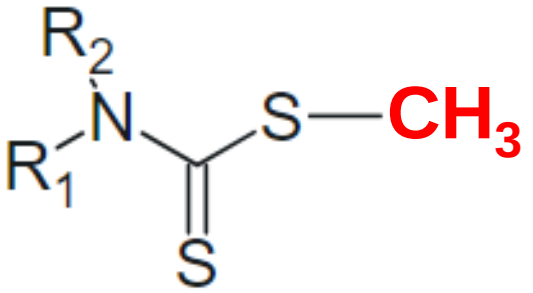
DTC-Derivatization – by Methylation

Zineb, …

Propineb

Ziram, Thiram

Methylation step →



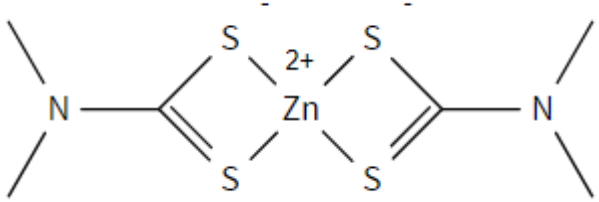
Electrophilic methylation at QuEChERS conditions:

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

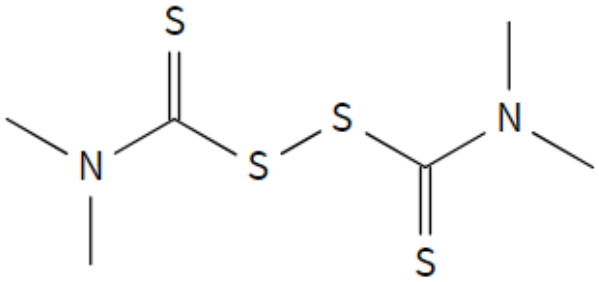
– alternative, less toxic (!) methylating agents tested:
dimethyl dicarbonate(*), dimethyl carbonate, trimesium, trimethylphosphate
=> no methylation products detected

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

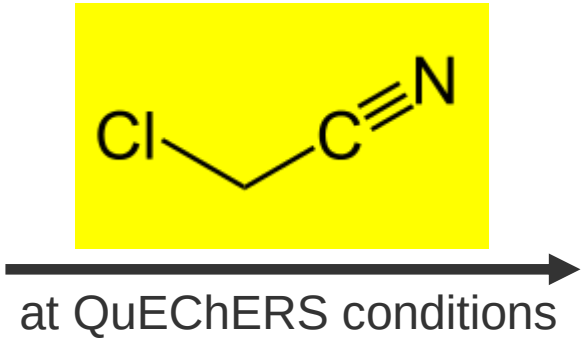
DTC-Derivatization – by Chloroacetonitrile

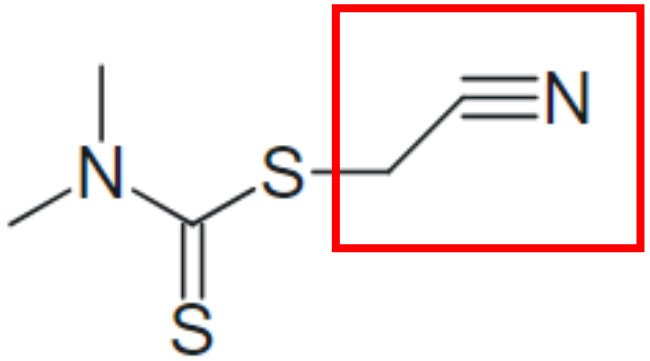


Ziram



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

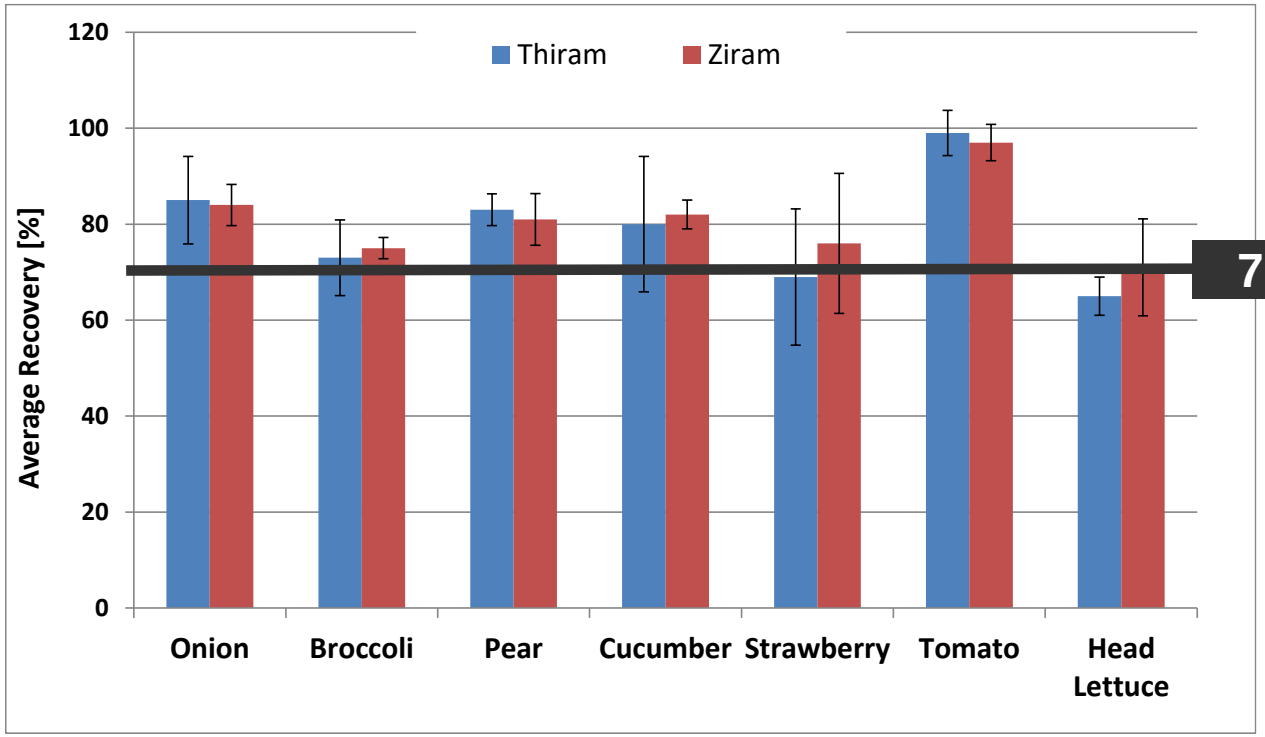
↓

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

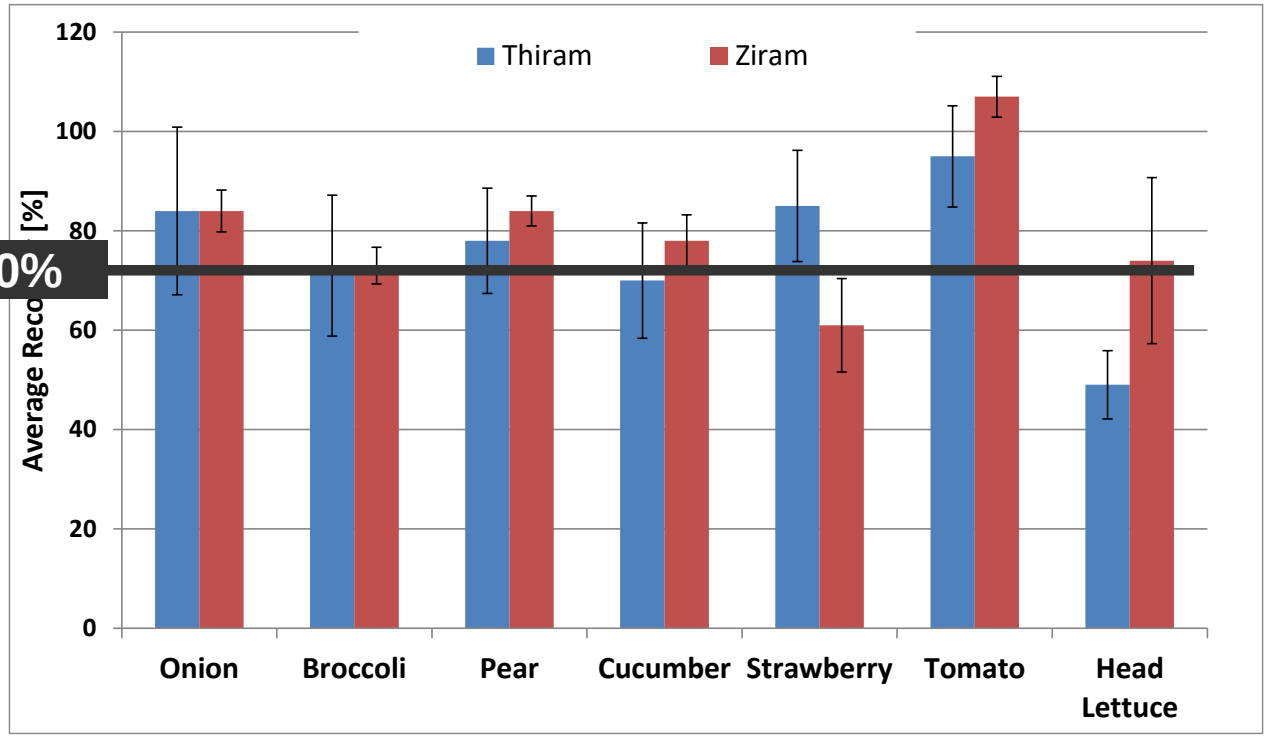
Thiram/Ziram-Derivatization – by Chloroacetonitrile

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

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



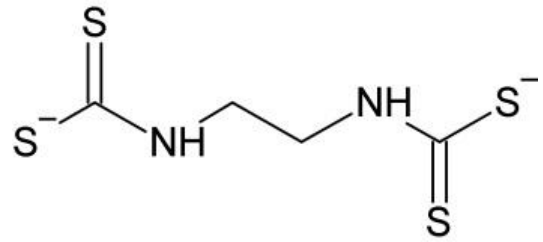
Spiking level: **0,05 mg/kg**

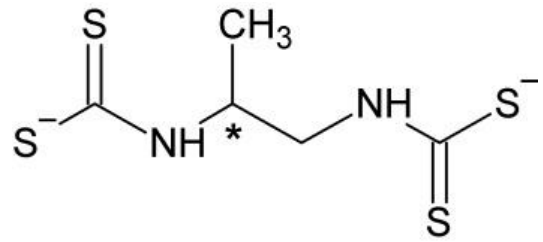


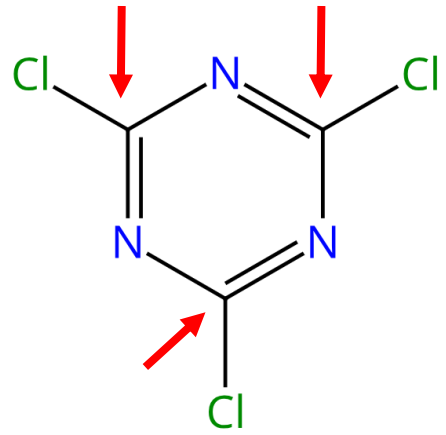
DTC-Derivatization – by Cyanuric chloride (*)

Reaction Scheme (in short):

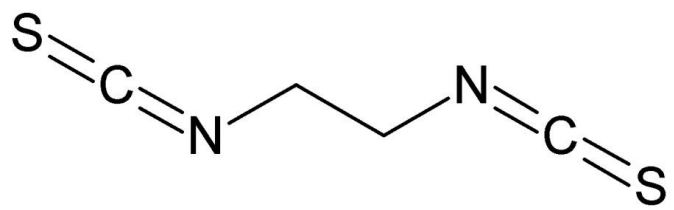
Zineb	Zn^{2+}
Maneb	Mn^{2+}
Mancozeb	Mn^{2+}, Zn^{2+} (94:6)
Metiram	Zn^{2+}, NH_3
Mancopper	13,7% Mn, 4 % Cu
Propineb	Zn^{2+}



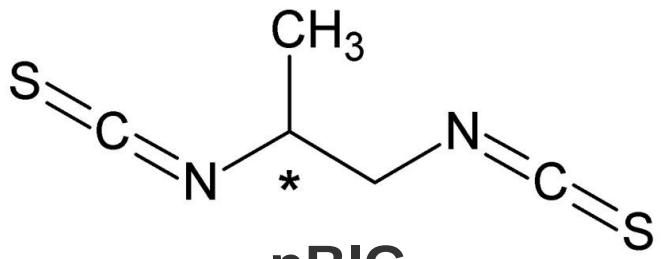




at QuEChERS conditions



eBIC
(ethylene-*bis*-isothiocyanate)

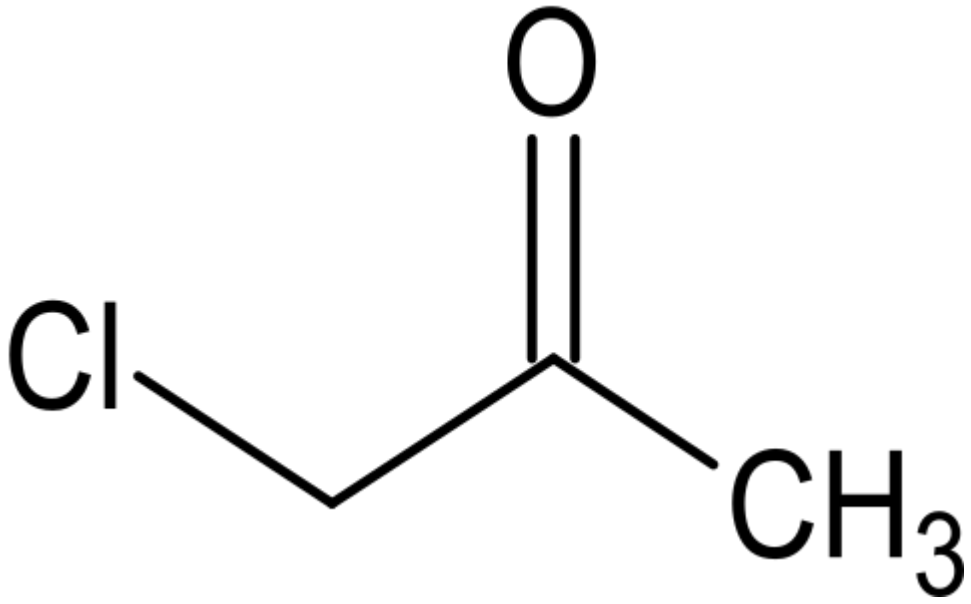


pBIC
(Propylene-*bis*-isothiocyanate)

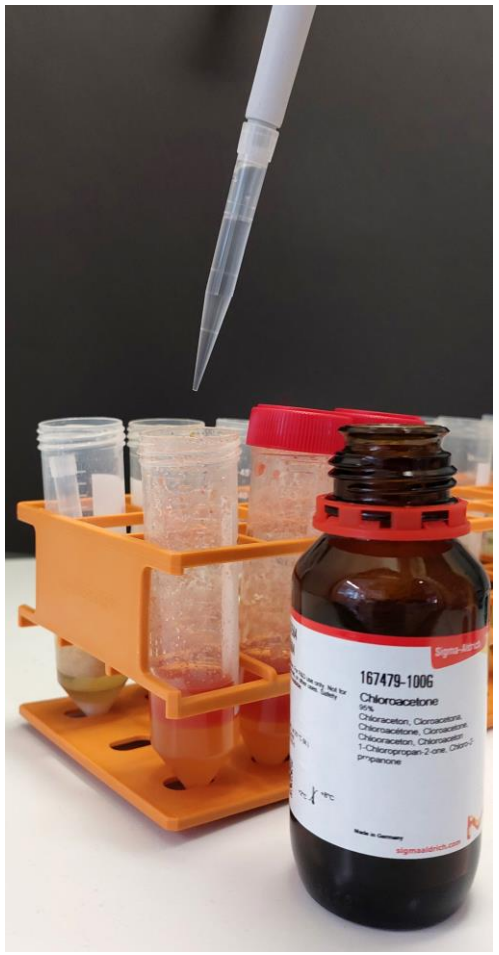
- Validation pending
- no conversion of Thiram and Ziram ☹

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

DTC-Derivatization – by Chloroacetone



Chloroacetone



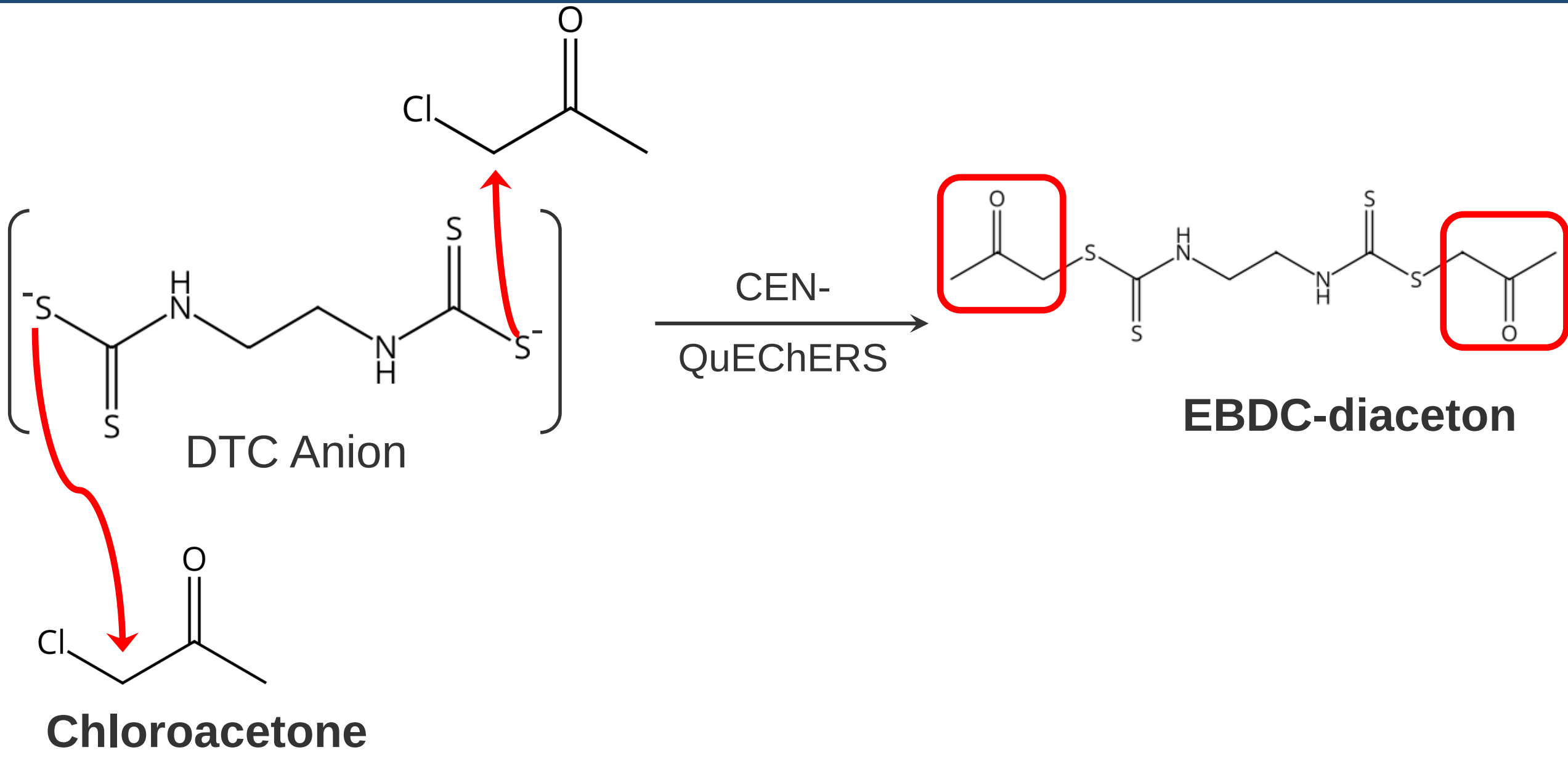
Chloroaceton - Hazards

flammable liquid and vapour;

causes skin burns, eye damage and respiratory irritation

=> working in fume hood is strongly recommended!

DTC-Derivatization – by Chloroacetone

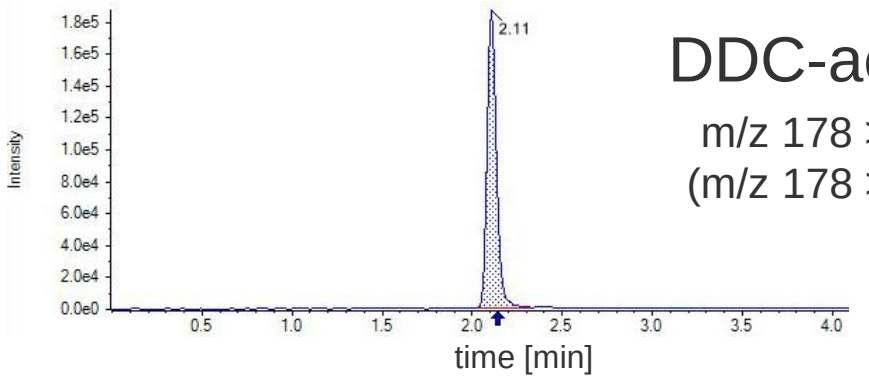


* Tsuboi *et al.*, Bulletin of the Chemical Society of Japan, 61(9), 3205-9; 1988

DTC-Derivatization – by Chloroacetone

Tomato spiked at 0.01 mg/kg with

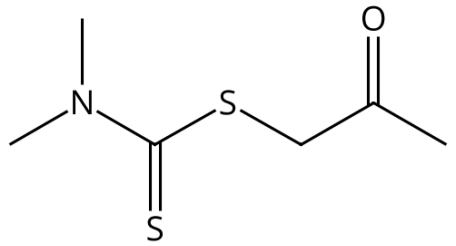
Ziram



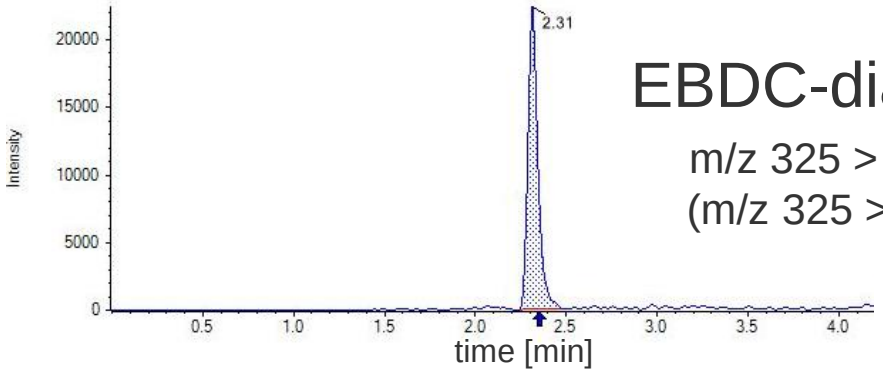
DDC-aceton

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

Chemical Structure

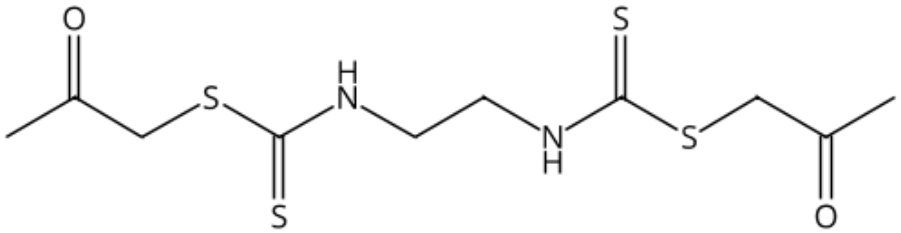


Mancozeb

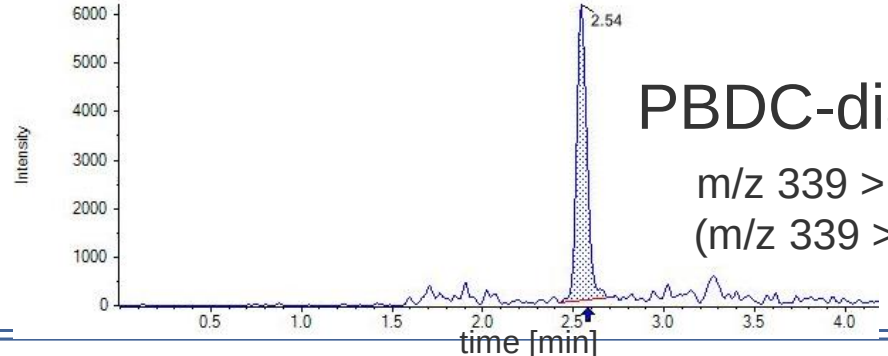


EBDC-diaceton

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

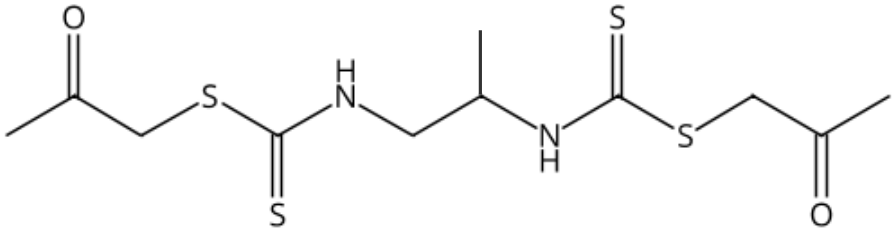


Propineb



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.

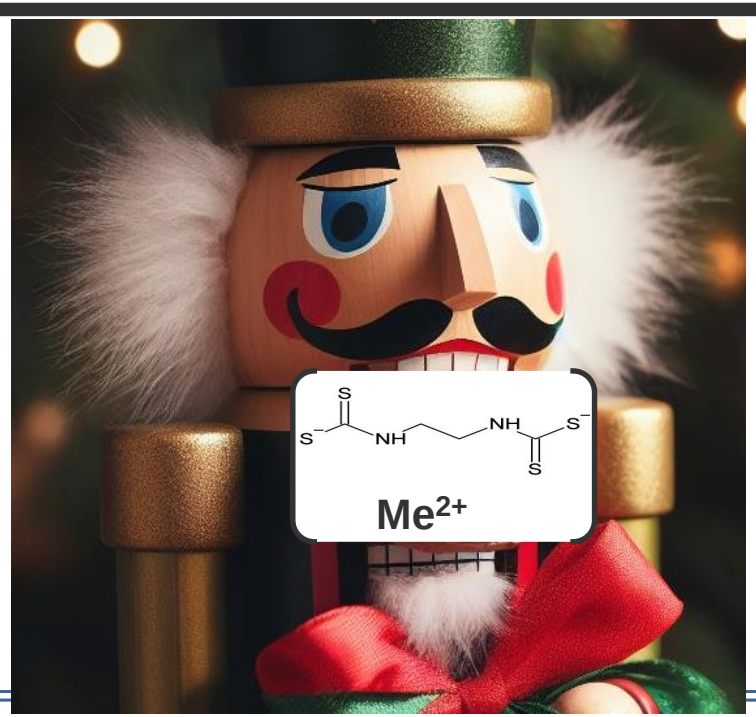
↓

Transfer 1 mL extract to vials

↓

LC-MS/MS

Validation: pending! ☹



Summary

Screening Marker

→ QuEChERS amenable

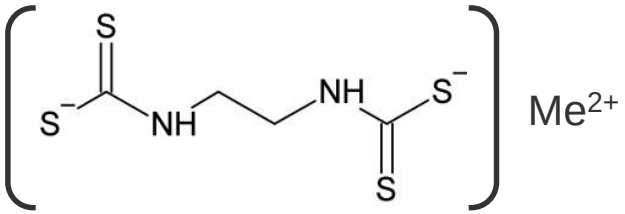
eBIC, pBIC

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

www.eurl-pesticides.eu

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

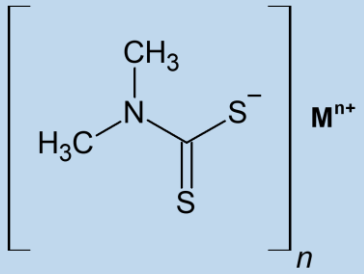
E. Eichhorn, H. Zipper, D. Mack, G. Cerchia, A. Karst, K. Rothenbächer, S. Goerlich, C. Ullrich, I. Sigalov, E. Scherbaum, M. Anastassiades

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

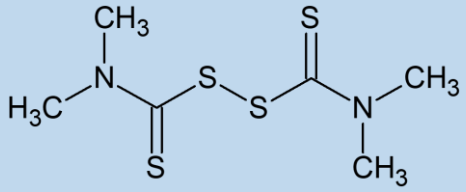
EURL Joint Workshop
18th-20th September 2023, Fellbach, Germany

Dithiocarbamates (DTC) | Introduction

N,N-Dimethyldithiocarbamate group



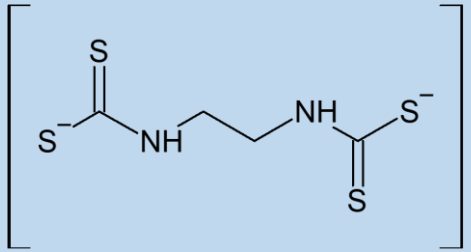
e. g. $M^{n+} = Zn^{2+}$: Ziram
$M^{n+} = As^{3+}$: Asomate
$M^{n+} = Fe^{3+}$: Ferbam



Import tolerances for bananas and mangoes

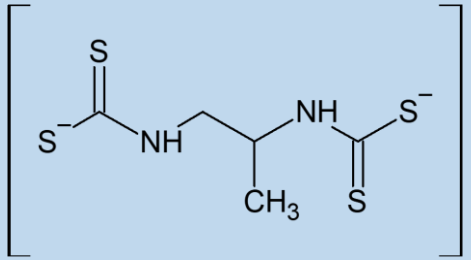
Thiram

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

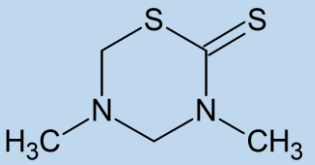
Legal status within the EU

accord. to Reg. (EC) 1107/2009:

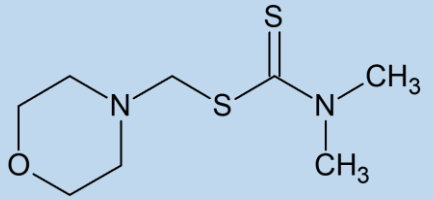
Currently approved active substance

Approval expired in the last years

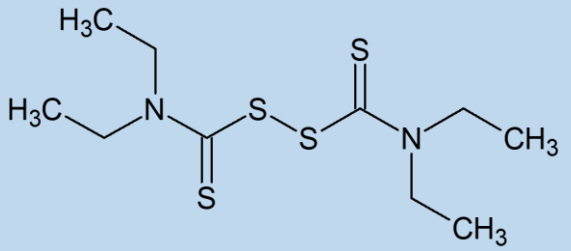
Group of other purely organic dithiocarbamates (selection)



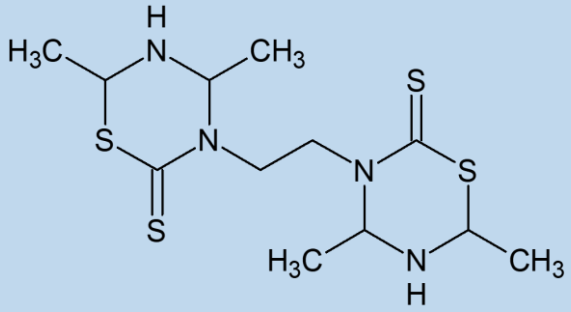
Dazomet



Carbamorph



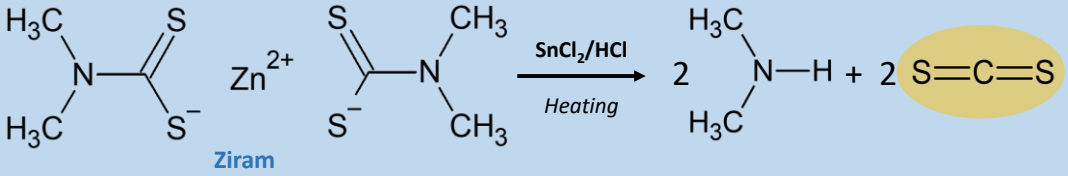
Disulfiram



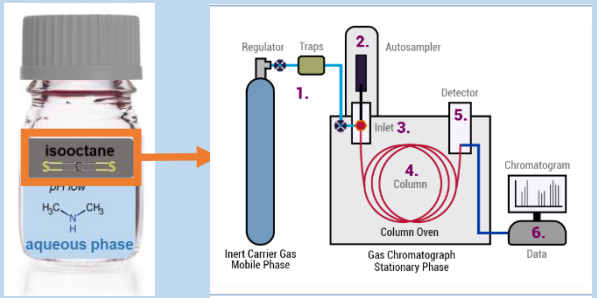
Milneb

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

Reductive cleavage with $SnCl_2$/HCl



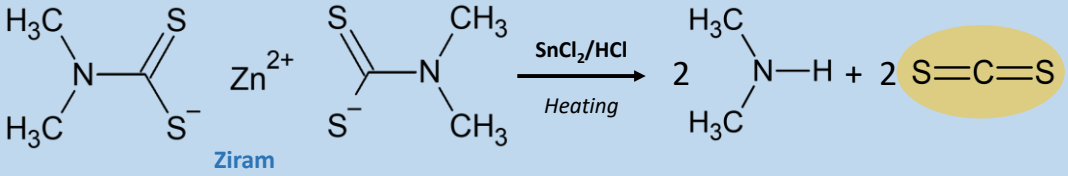
Ziram



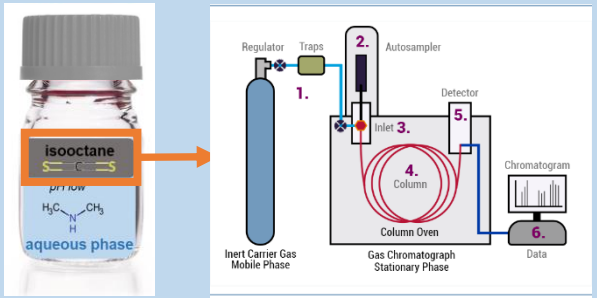
Drawbacks:

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

Reductive cleavage with $SnCl_2$/HCl



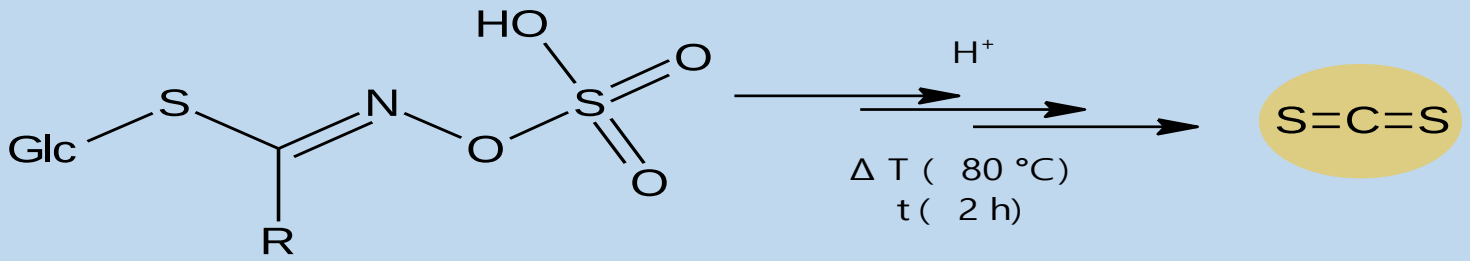
Ziram



Drawbacks:

- **No distinction of CS_2 origin**

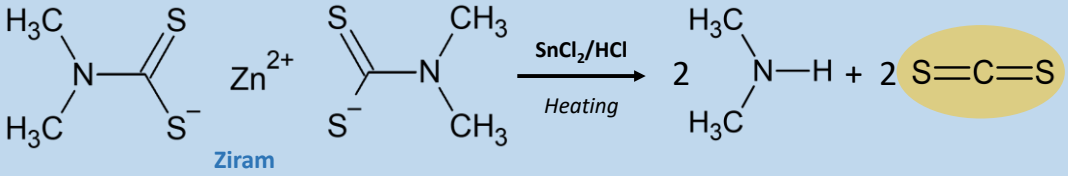
  (i.e. CS_2 from DTC-fungicides versus CS_2 from natural components in matrix, e.g. *Brassicaceae* and *Allium* genus)



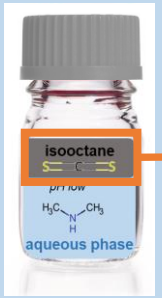
Glucosinalotes
(naturally occuring in commodities
of e.g. *Brassicaceae* and *Allium* genus)

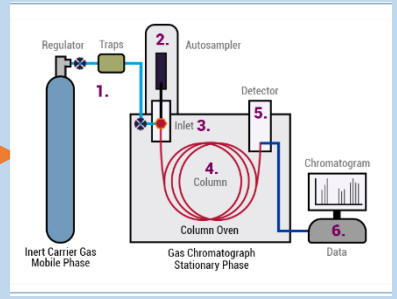
Dithiocarbamates (DTC) | Common moiety method: analysis as CS_2

Reductive cleavage with $SnCl_2$/HCl



Ziram





Drawbacks:

- **No distinction of CS_2 origin**

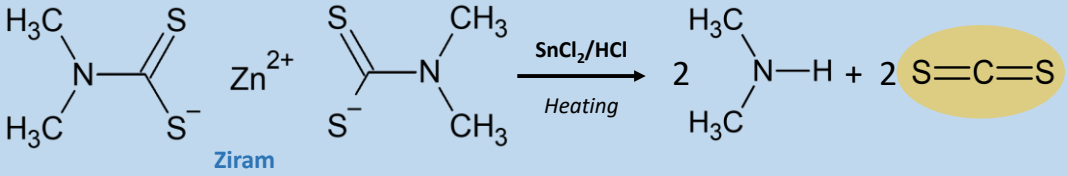
  (i.e. CS_2 from DTC-fungicides versus CS_2 from natural components in matrix, e.g. *Brassicaceae* and *Allium* genus)

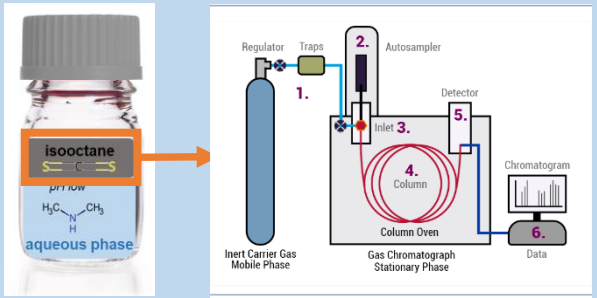
- **No distinction between individual DTC-groups**

  (not to mention distinction between individual active substances)

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

Reductive cleavage with $SnCl_2$/HCl





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)

- **No distinction between individual DTC-groups**

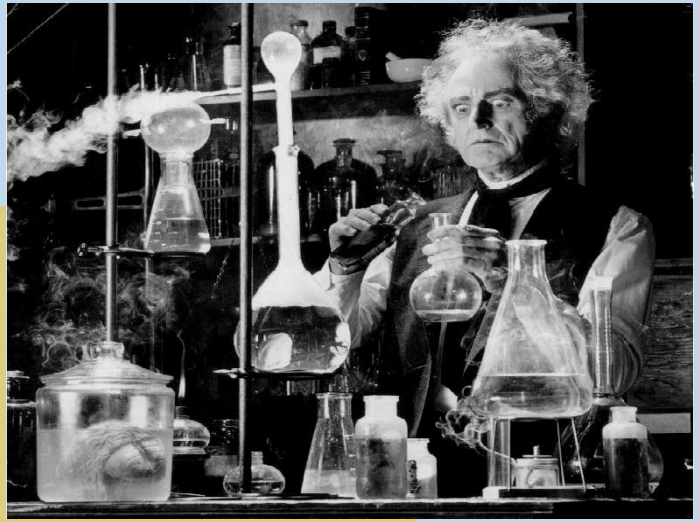
  (not to mention distinction between individual active substances)

- **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

Aim of our study

- 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

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

Aim of our study

- 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

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

- 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]
  - **for DTC-markers** by using
    - CEN-QuEChERS, followed by GC-MS/MS and GC-Orbitrap
    - QuPPe [2], followed by LC-MS/MS

[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]
  - **for DTC-markers** by using
    - CEN-QuEChERS, followed by GC-MS/MS and GC-Orbitrap
    - QuPPe [2], followed by LC-MS/MS
- Each determined marker was evaluated individually regarding its correlation with CS_2 findings

[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]
  - **for DTC-markers** by using
    - CEN-QuEChERS, followed by GC-MS/MS and GC-Orbitrap
    - QuPPe [2], followed by LC-MS/MS
- Each determined marker was evaluated individually regarding its correlation with CS_2 findings
- If an appropriate correlation for a marker was found,
  - an individual threshold level was set (if possible)
  - the marker was considered for a DTC group-specific evaluation

[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]
  - **for DTC-markers** by using
    - CEN-QuEChERS, followed by GC-MS/MS and GC-Orbitrap
    - QuPPe [2], followed by LC-MS/MS
- Each determined marker was evaluated individually regarding its correlation with CS_2 findings
- If an appropriate correlation for a marker was found,
  - an individual threshold level was set (if possible)
  - 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)

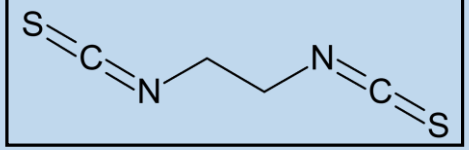
[1] https://www.eurl-pesticides.eu/library/docs/srm/meth_DithiocarbamatesCS2_EurlSrm.PDF
[2] https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/EurlSrm_meth_QuPPe_PO_V12_1.pdf; last update: 17.03.2023

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

➢ Correlation between eBIC and CS_2 concentrations?

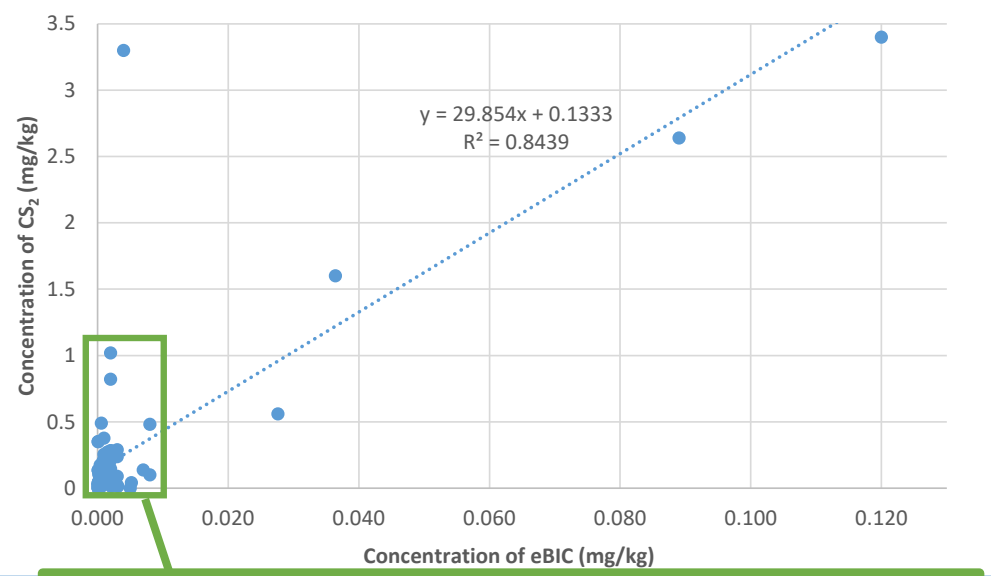
ethylene-*bis*-isothiocyanate (eBIC)

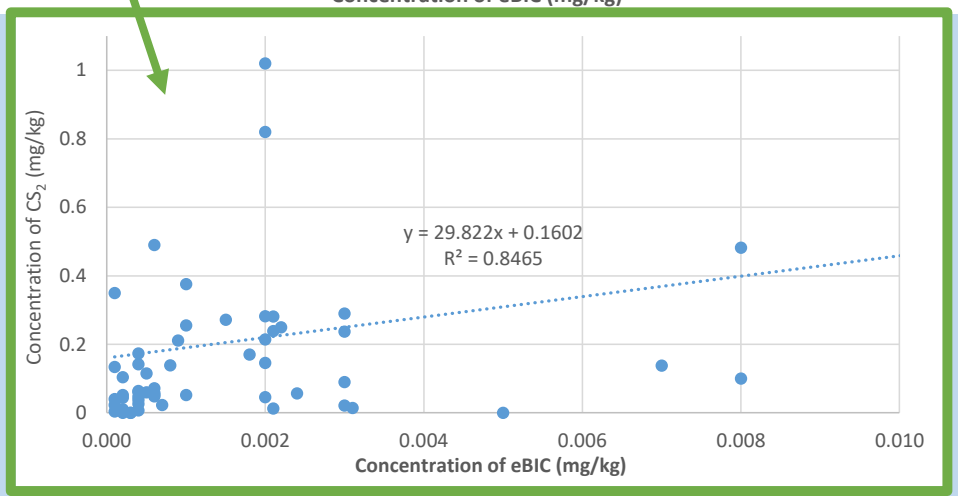
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap

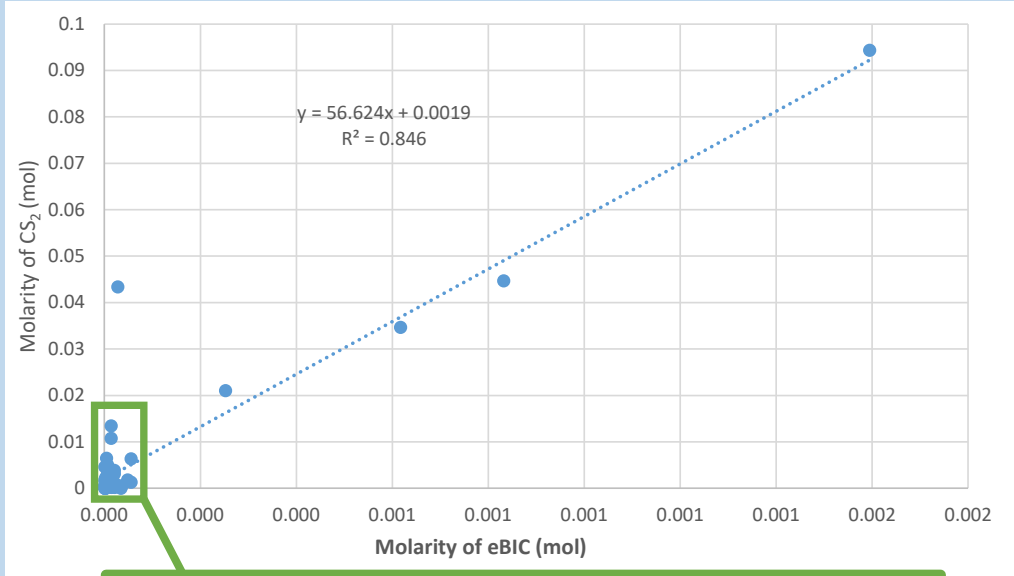


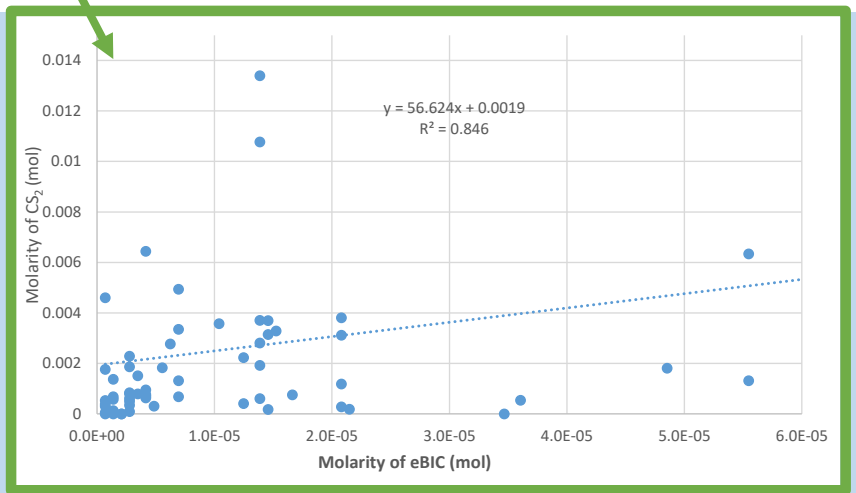
DTC-Markers | Exemplary results for Ethylene-*bis*-isothiocyanate (eBIC)

➢ Correlation between eBIC and CS_2 concentrations?



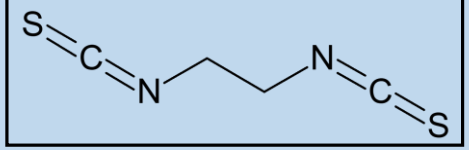






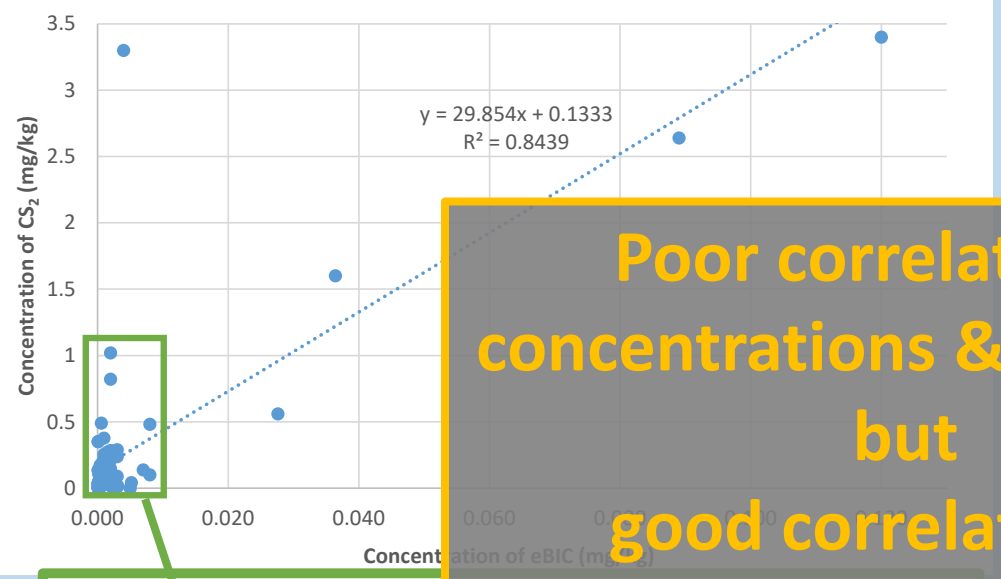
ethylene-*bis*-isothiocyanate (eBIC)

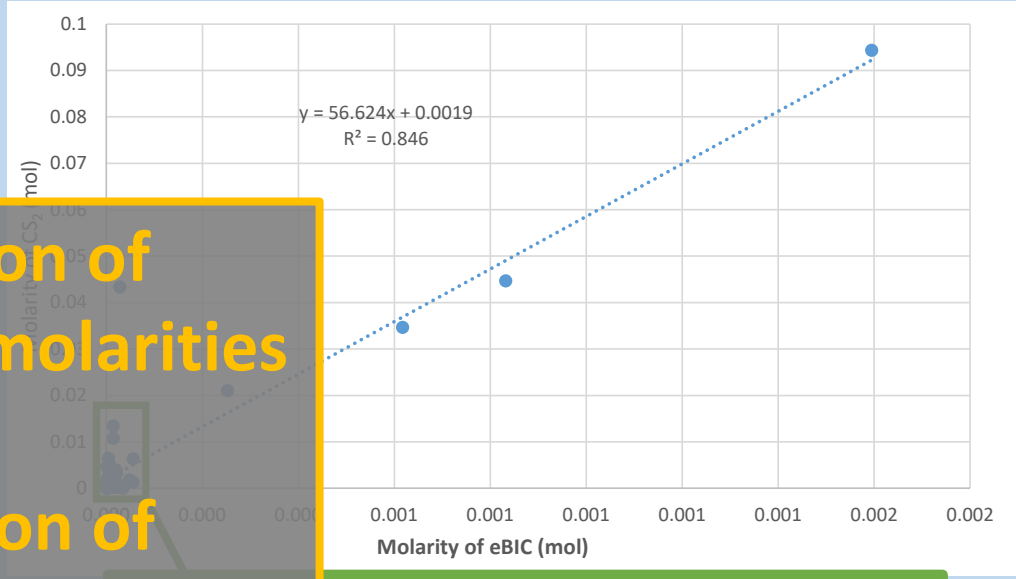
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap



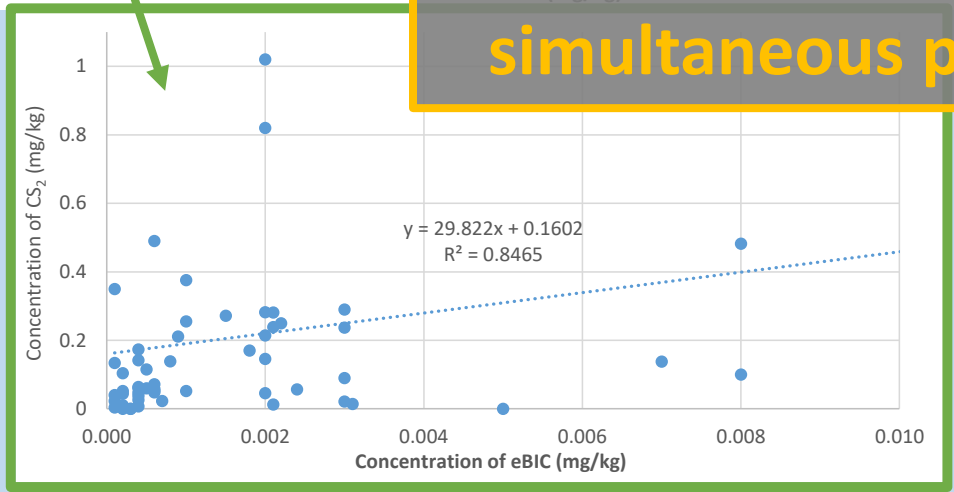
DTC-Markers | Exemplary results for Ethylene-*bis*-isothiocyanate (eBIC)

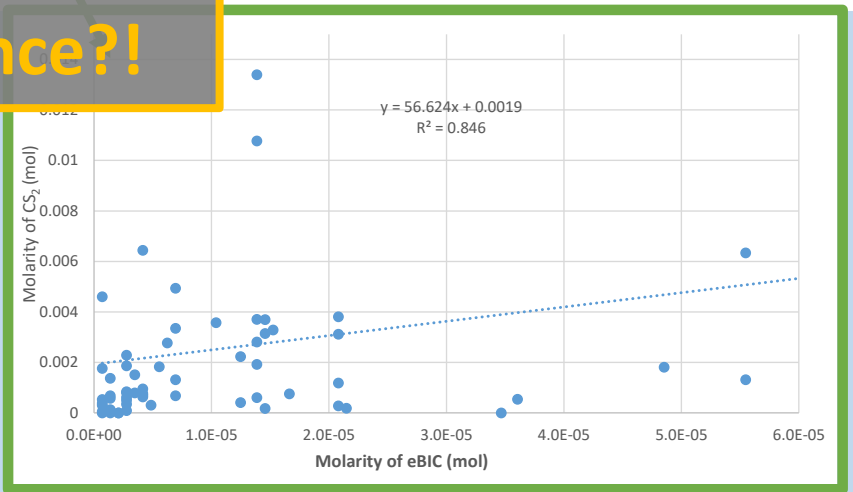
➢ Correlation between eBIC and CS_2 concentrations?





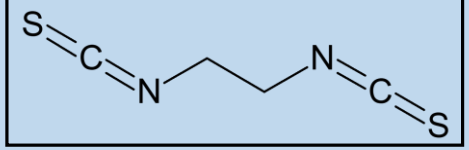
Poor correlation of concentrations & molarities but good correlation of simultaneous presence?!





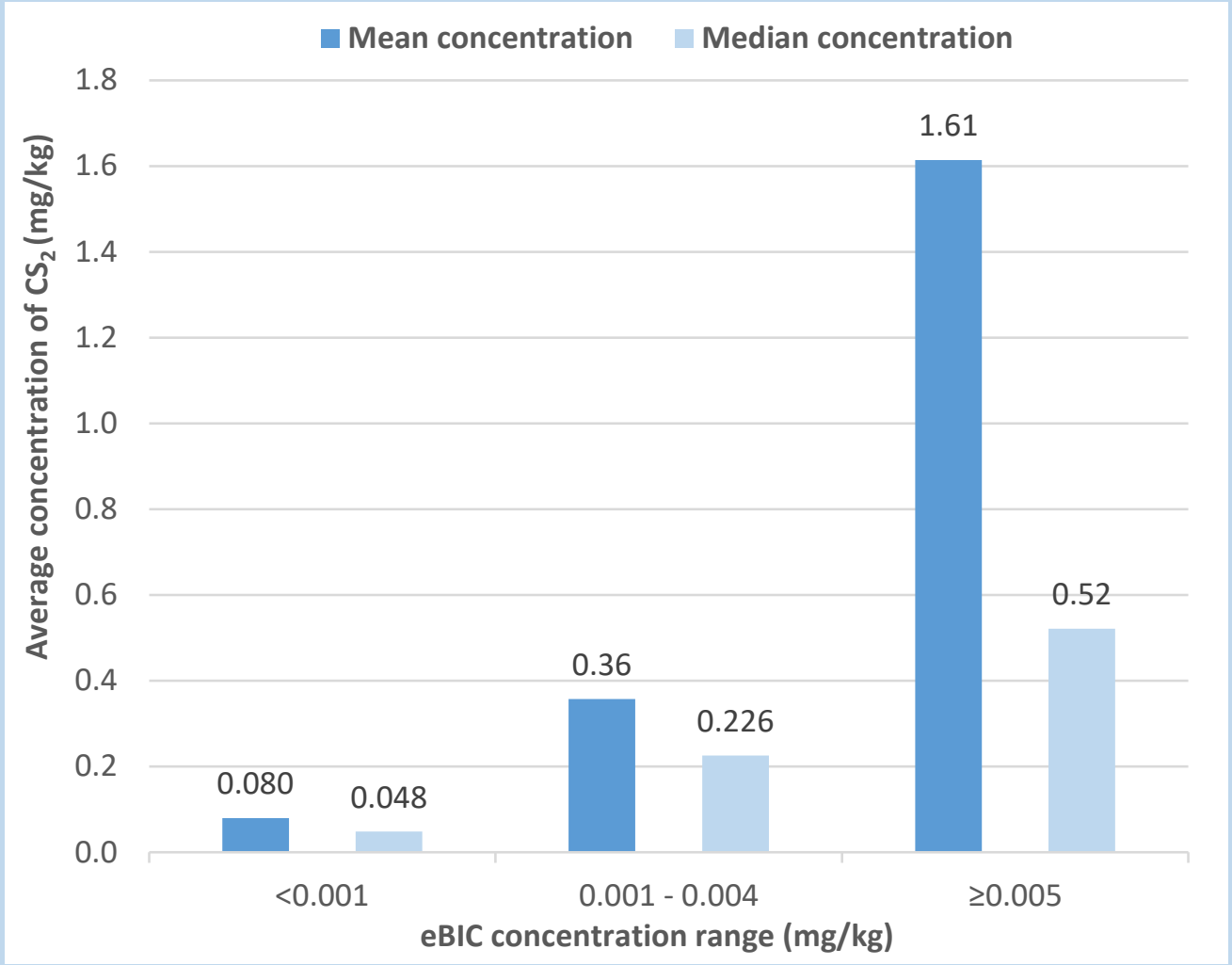
ethylene-*bis*-isothiocyanate (eBIC)

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



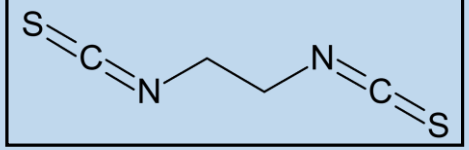
DTC-Markers | Exemplary results for Ethylene-*bis*-isothiocyanate (eBIC)

➢ Correlation between eBIC and CS_2 concentrations?



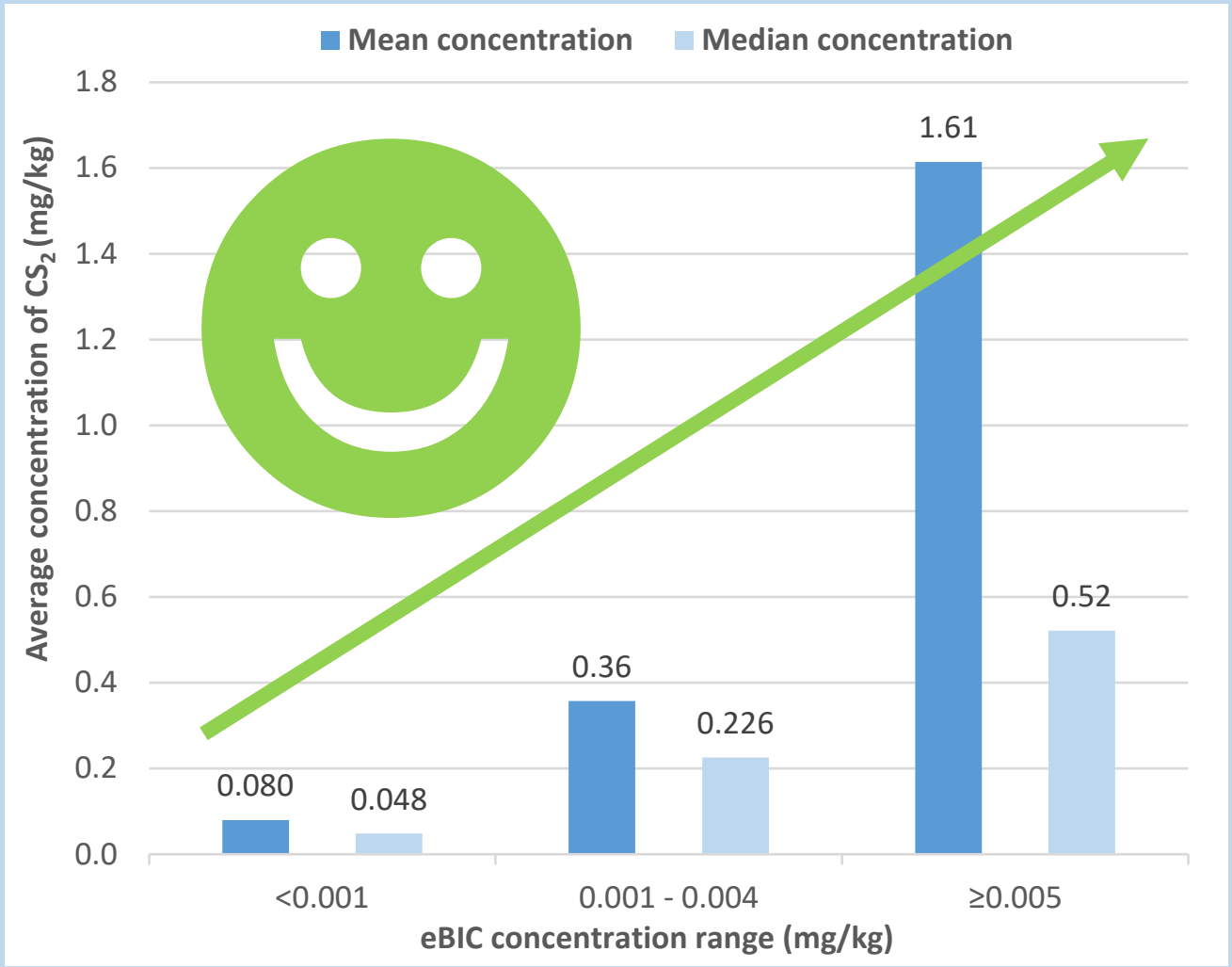
ethylene-*bis*-isothiocyanate (eBIC)

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



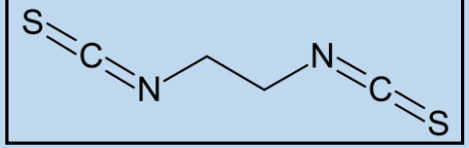
DTC-Markers | Exemplary results for Ethylene-*bis*-isothiocyanate (eBIC)

➢ Correlation between eBIC and CS_2 concentrations?



ethylene-*bis*-isothiocyanate (eBIC)

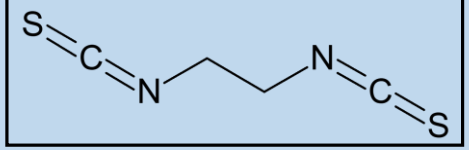
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap

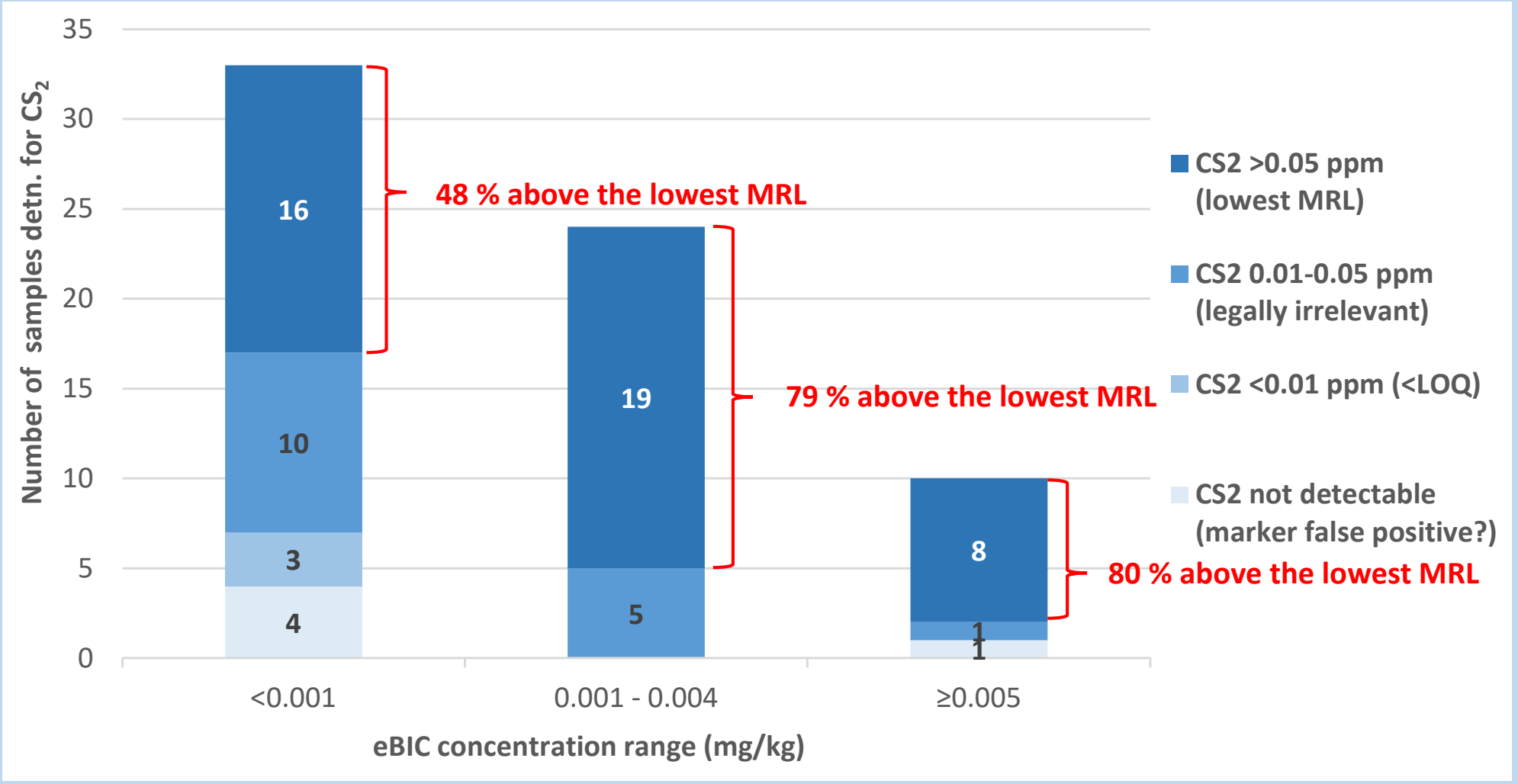


DTC-Markers | Exemplary results for Ethylene-*bis*-isothiocyanate (eBIC)

➢ Correlation between eBIC and CS_2 concentrations?

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

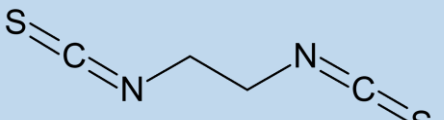


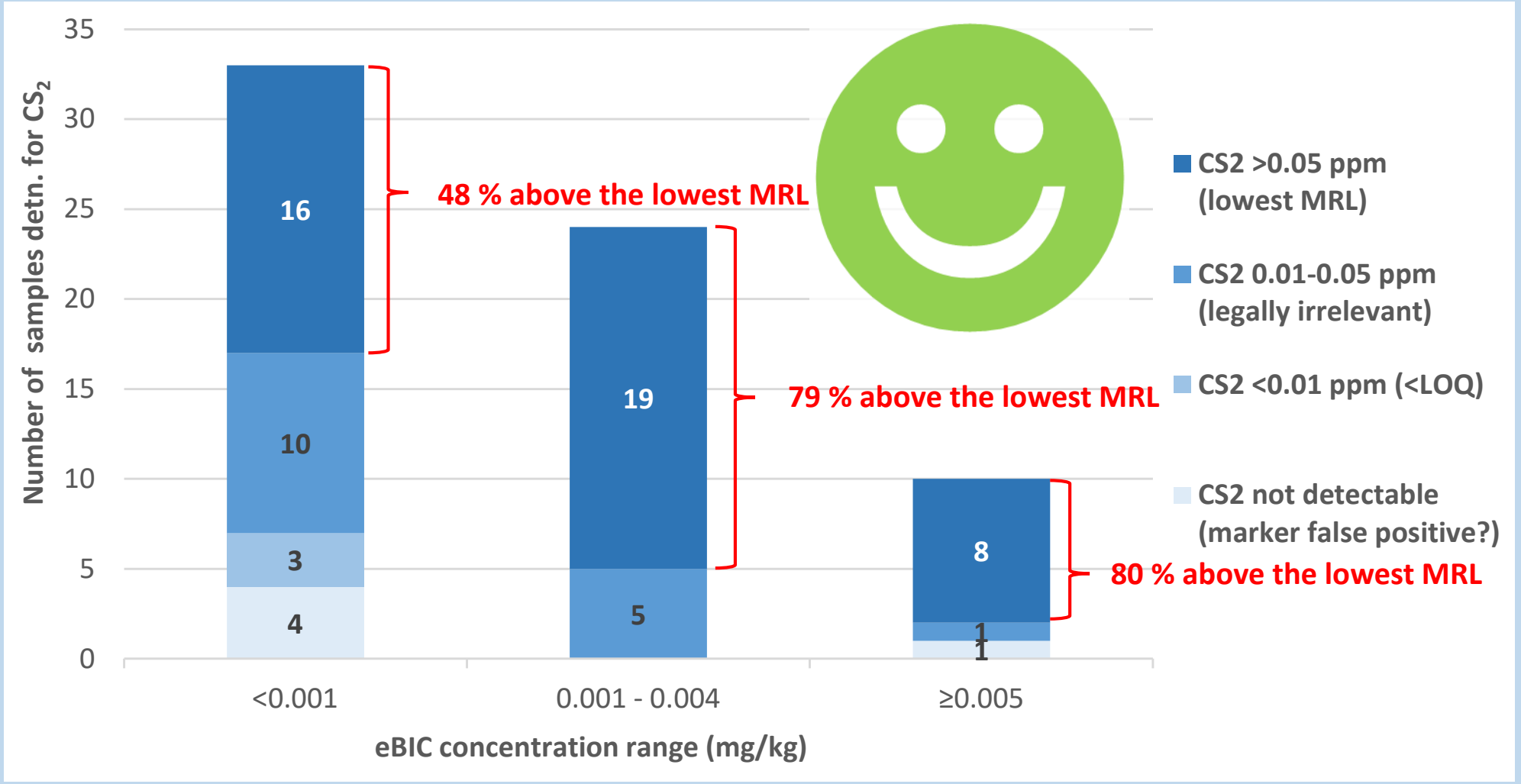


DTC-Markers | Exemplary results for Ethylene-*bis*-isothiocyanate (eBIC)

➢ Correlation between eBIC and CS_2 concentrations?

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

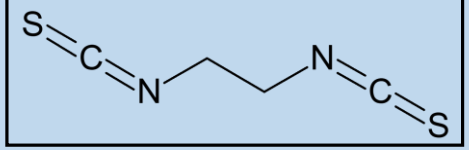


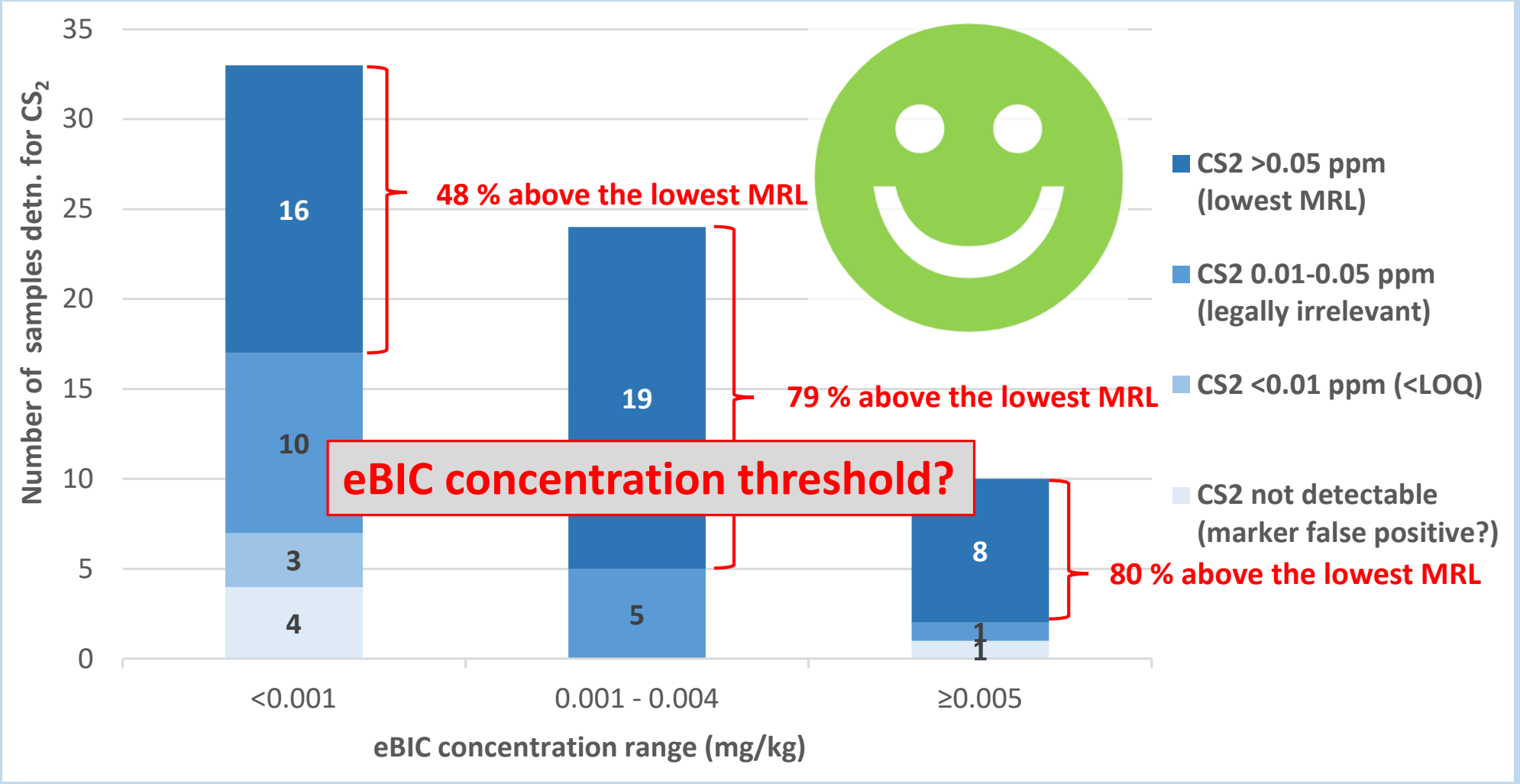


DTC-Markers | Exemplary results for Ethylene-*bis*-isothiocyanate (eBIC)

➢ Correlation between eBIC and CS_2 concentrations?

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

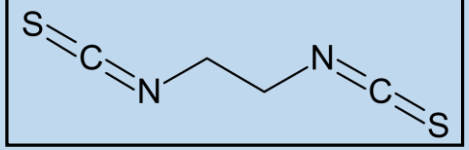


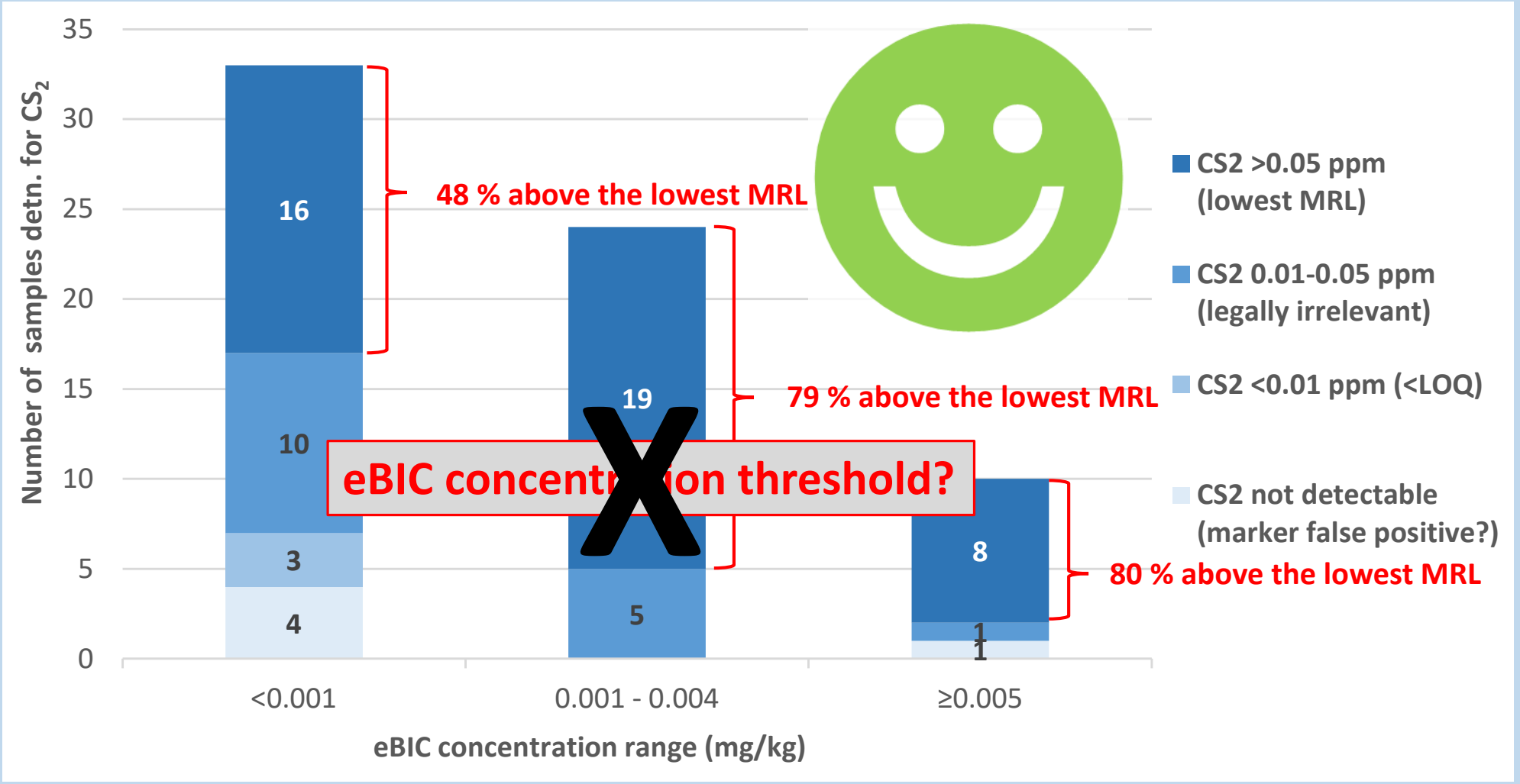


DTC-Markers | Exemplary results for Ethylene-*bis*-isothiocyanate (eBIC)

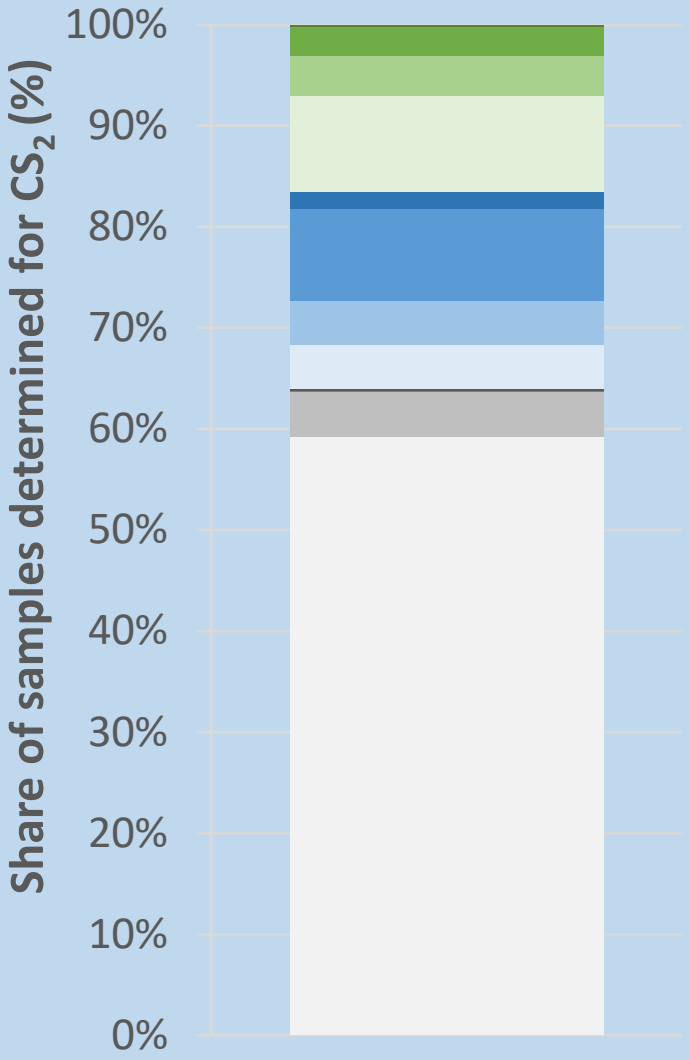
➢ Correlation between eBIC and CS_2 concentrations?

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

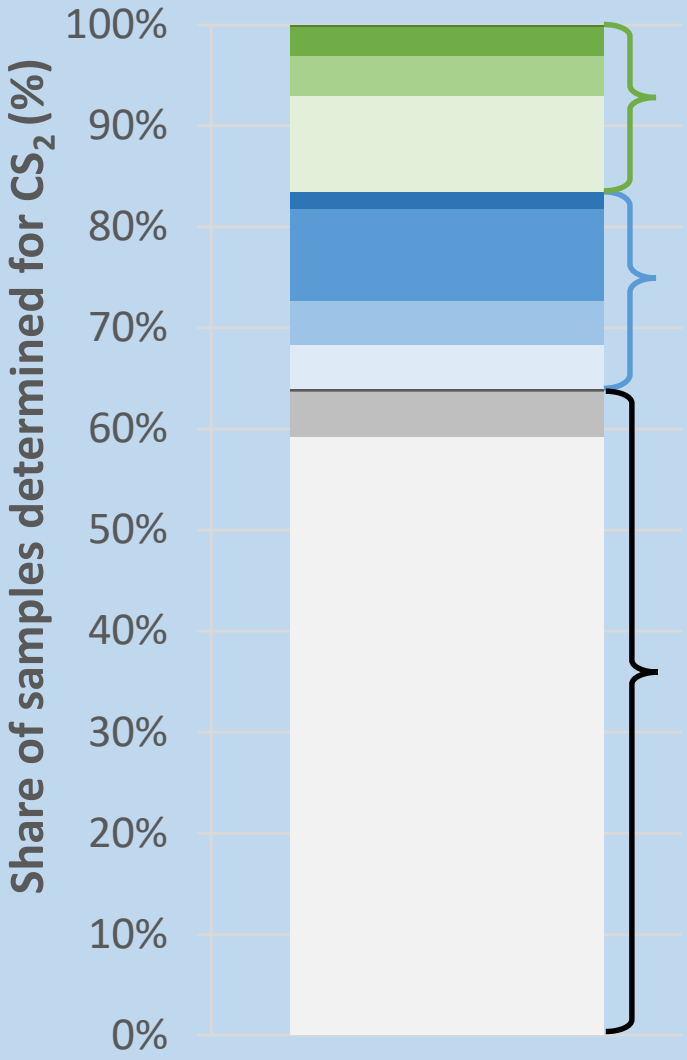




DTC-Markers | Overview of results



DTC-Markers | Overview of results

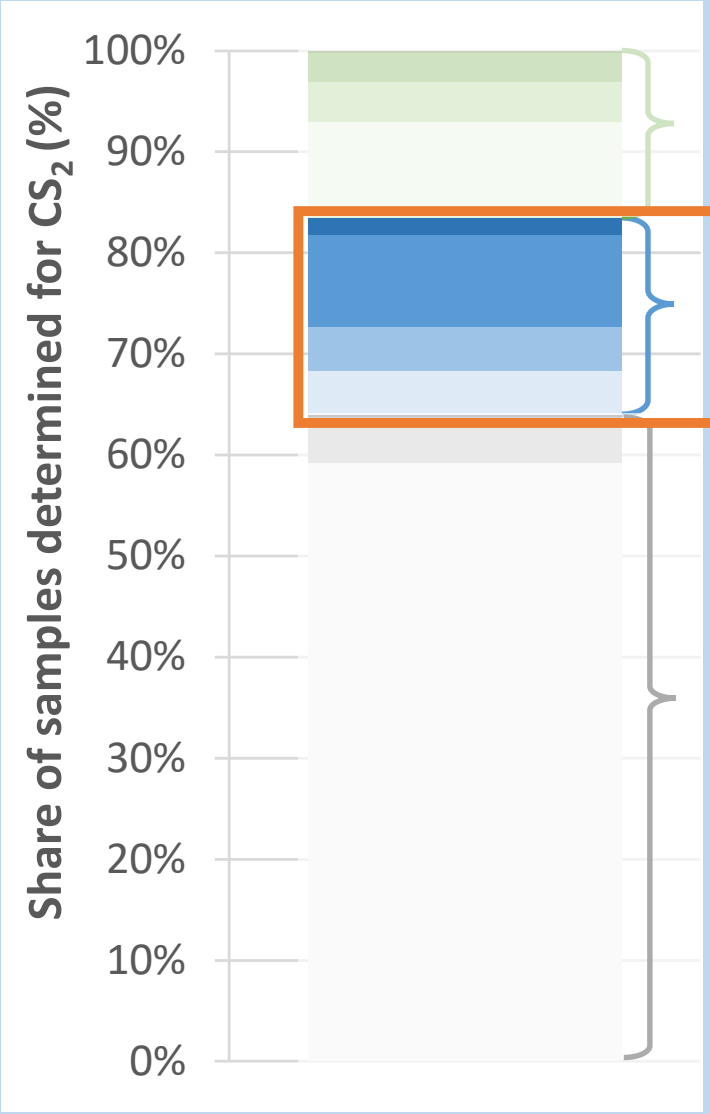


„OTHER MARKERS“: Samples containing exclusively or additionally any of the other DTC-group markers
(propylene-*bis*-DTC- or N,N-dimethyl-DTC markers)

Samples with ethylene-*bis*-DTC markers

Samples w/o detectable DTC-markers

DTC-Markers | Overview of results



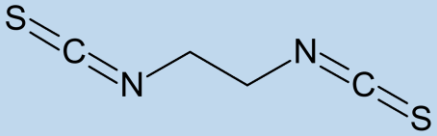
„OTHER MARKERS“: Samples containing exclusively or additionally any of the other DTC-group markers (propylene-*bis*-DTC- or N,N-dimethyl-DTC markers)

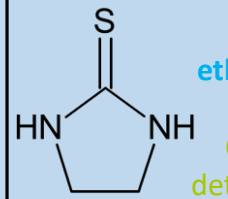
Samples with ethylene-*bis*-DTC markers

Samples w/o detectable DTC-markers

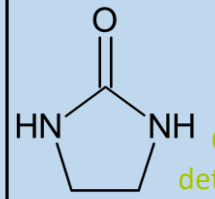
DTC-Markers | Results for the ethylene-*bis*-DTC (EBDTC) group

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



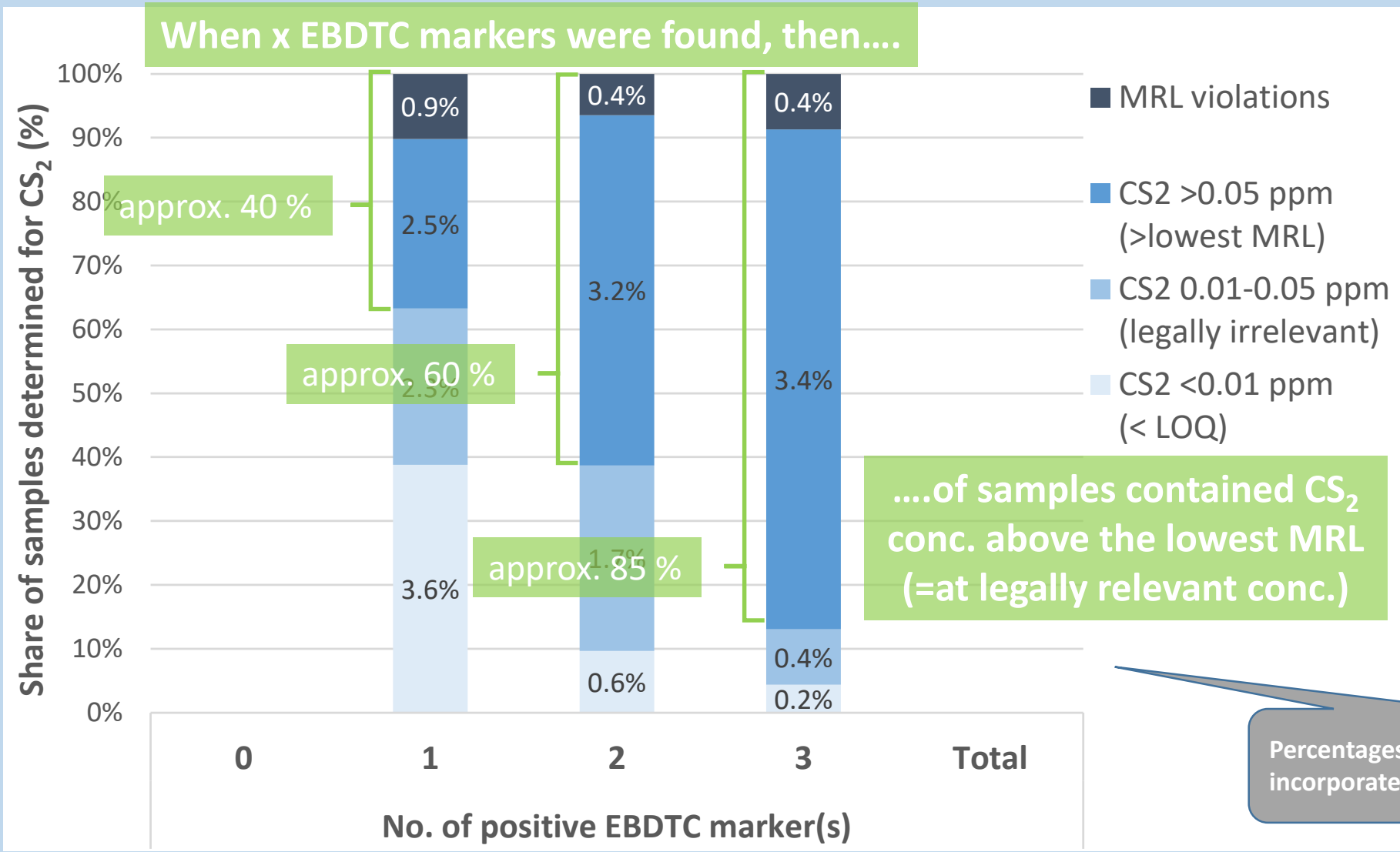


ethylene thiourea (ETU)
QuPPe-amenable
det. with LC-MS/MS

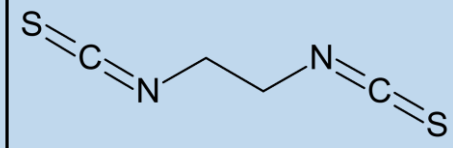


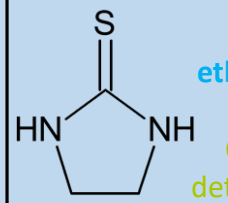
ethylene urea (EU)
QuPPe-amenable
det. with LC-MS/MS

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

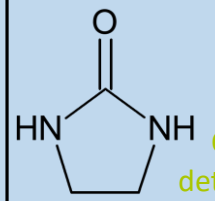


ethylene-*bis*-isothiocyanate (eBIC)
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap





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

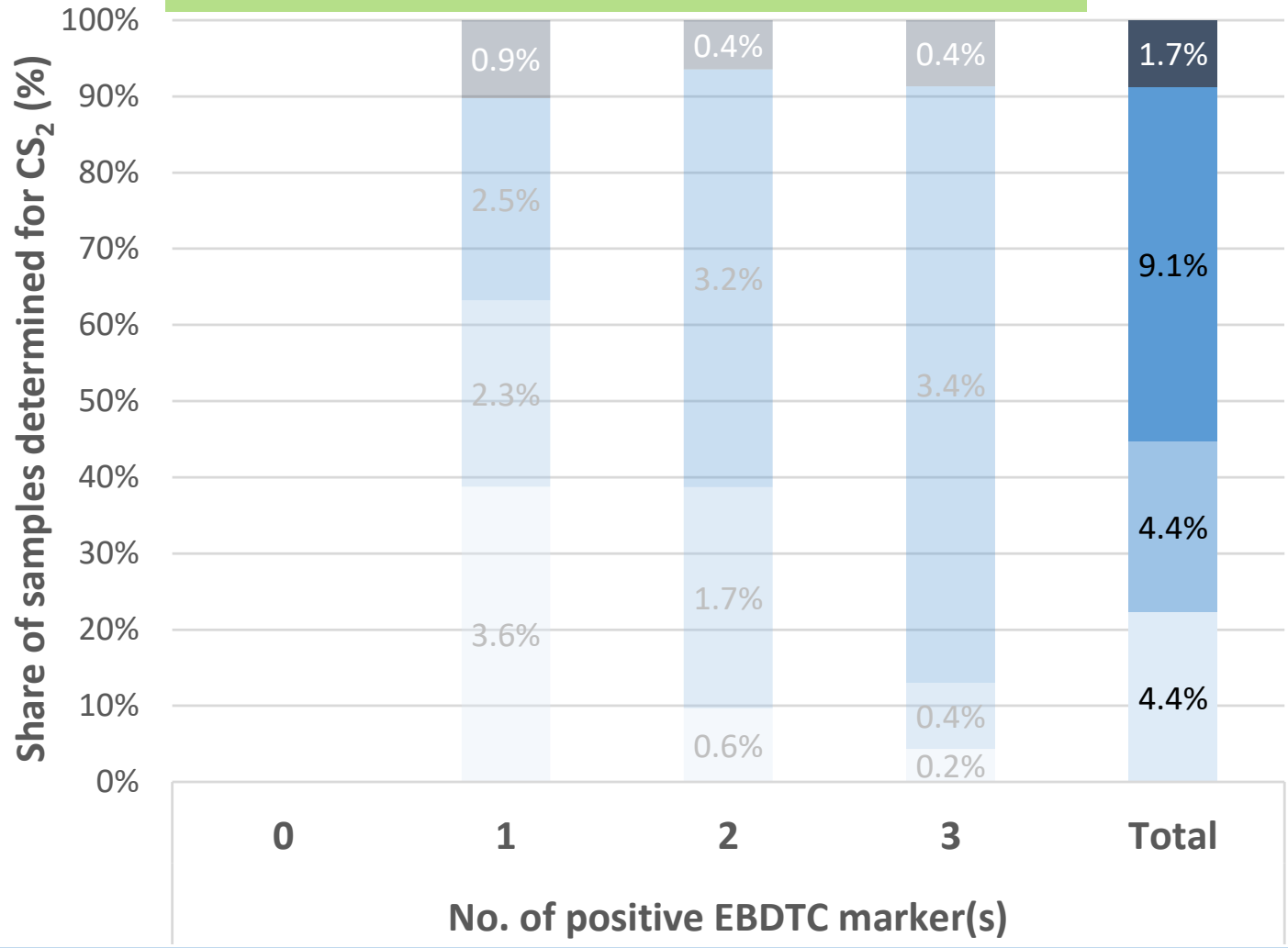


ethylene urea (EU)
QuPPe-amenable
det. with LC-MS/MS

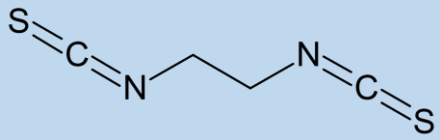
Percentages refer to the total number of incorporated samples (N = 528)

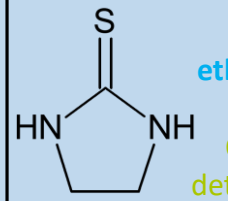
DTC-Markers | Results for the ethylene-*bis*-DTC (EBDTC) group

When x EBDTC markers were found, then….

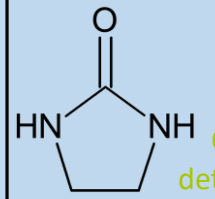


ethylene-*bis*-isothiocyanate (eBIC)
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap





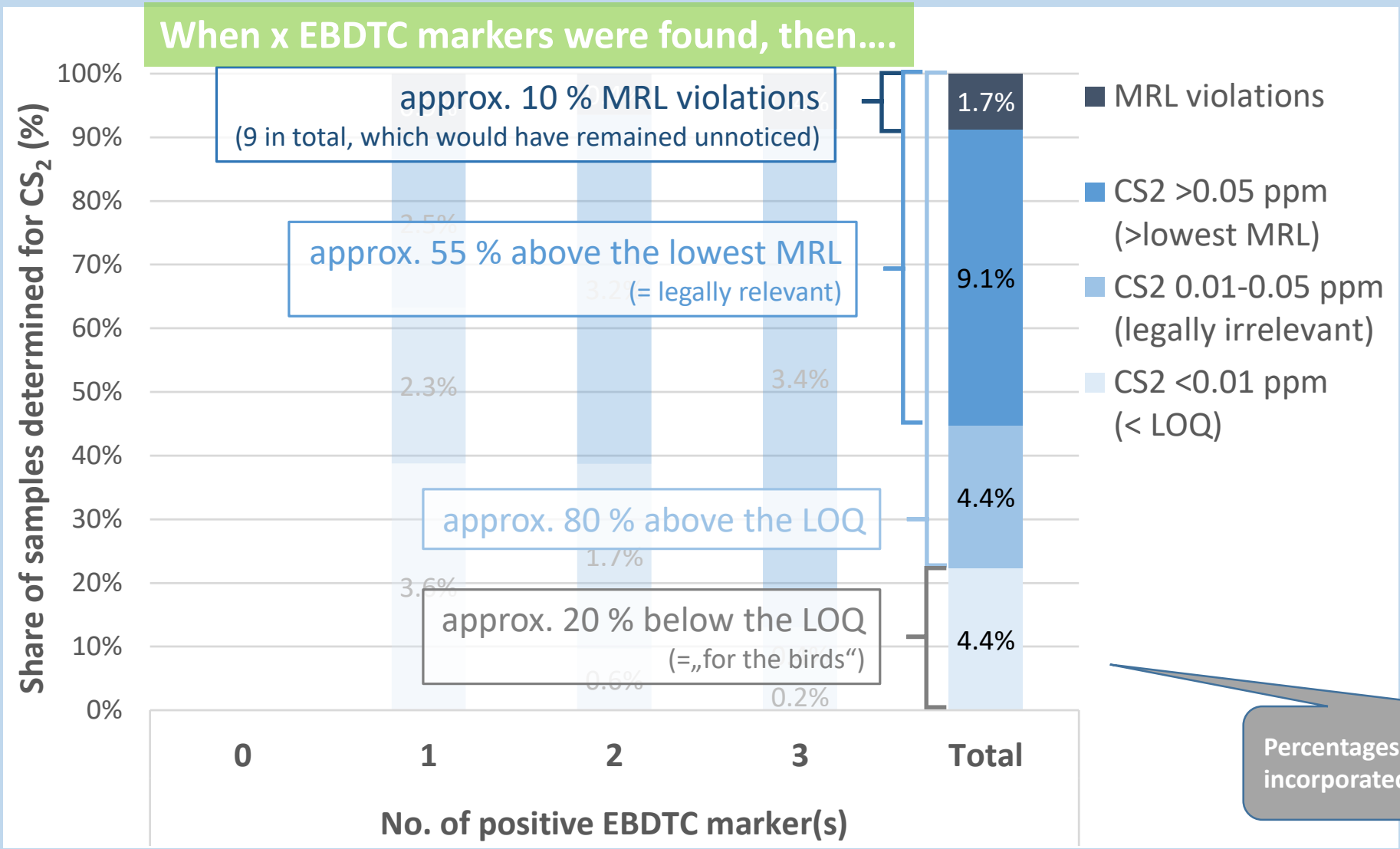
ethylene thiourea (ETU)
QuPPe-amenable
det. with LC-MS/MS



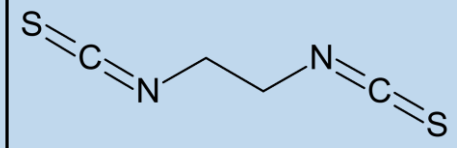
ethylene urea (EU)
QuPPe-amenable
det. with LC-MS/MS

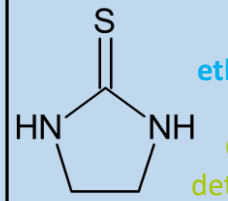
Percentages refer to the total number of incorporated samples (N = 528)

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

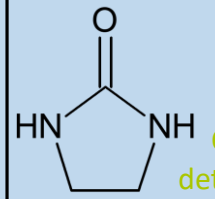


ethylene-*bis*-isothiocyanate (eBIC)
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap





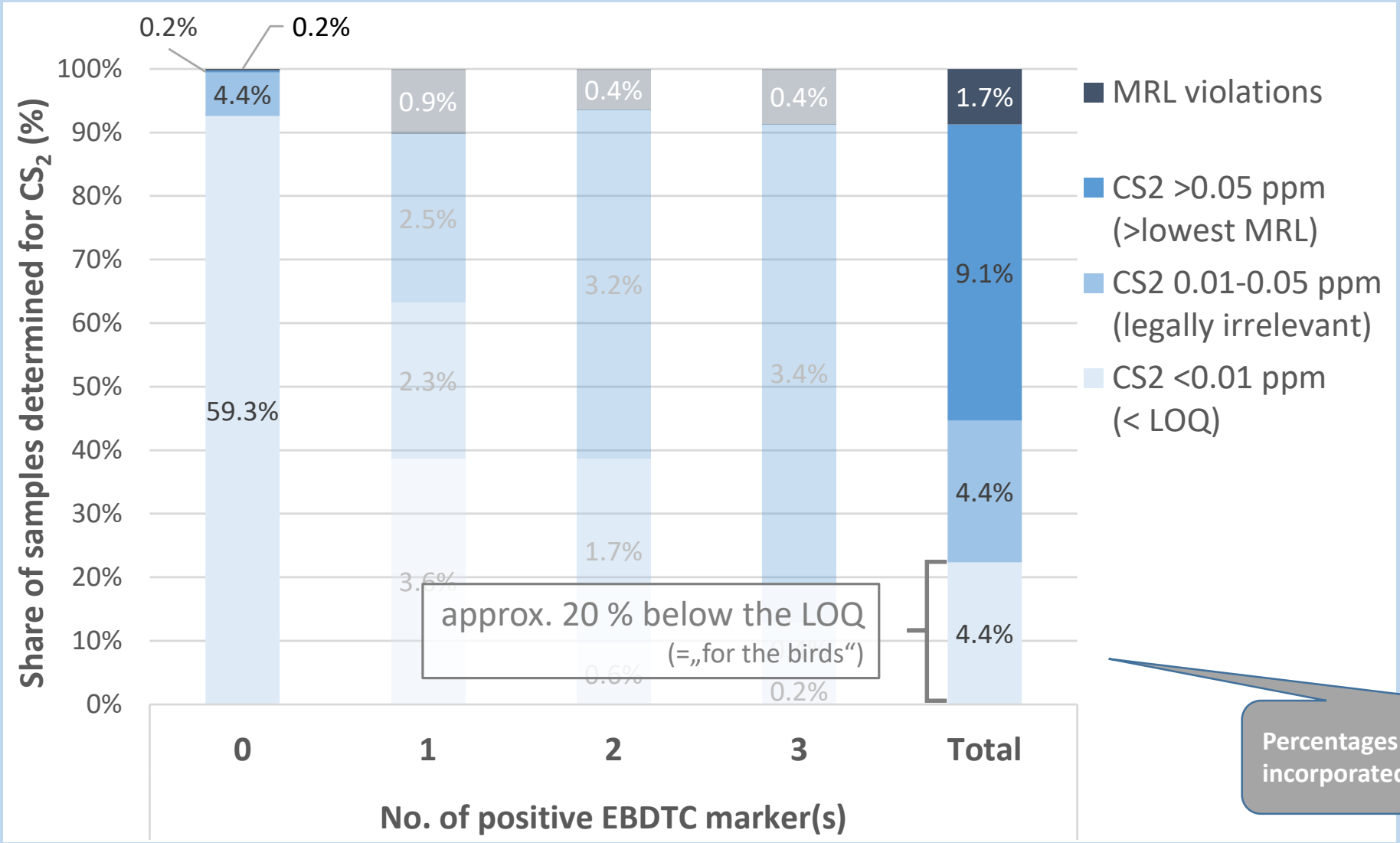
ethylene thiourea (ETU)
QuPPe-amenable
det. with LC-MS/MS



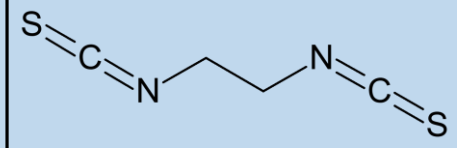
ethylene urea (EU)
QuPPe-amenable
det. with LC-MS/MS

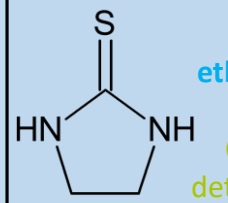
Percentages refer to the total number of incorporated samples (N = 528)

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

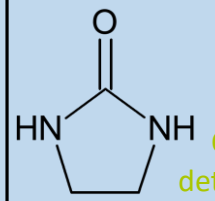


ethylene-*bis*-isothiocyanate (eBIC)
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap





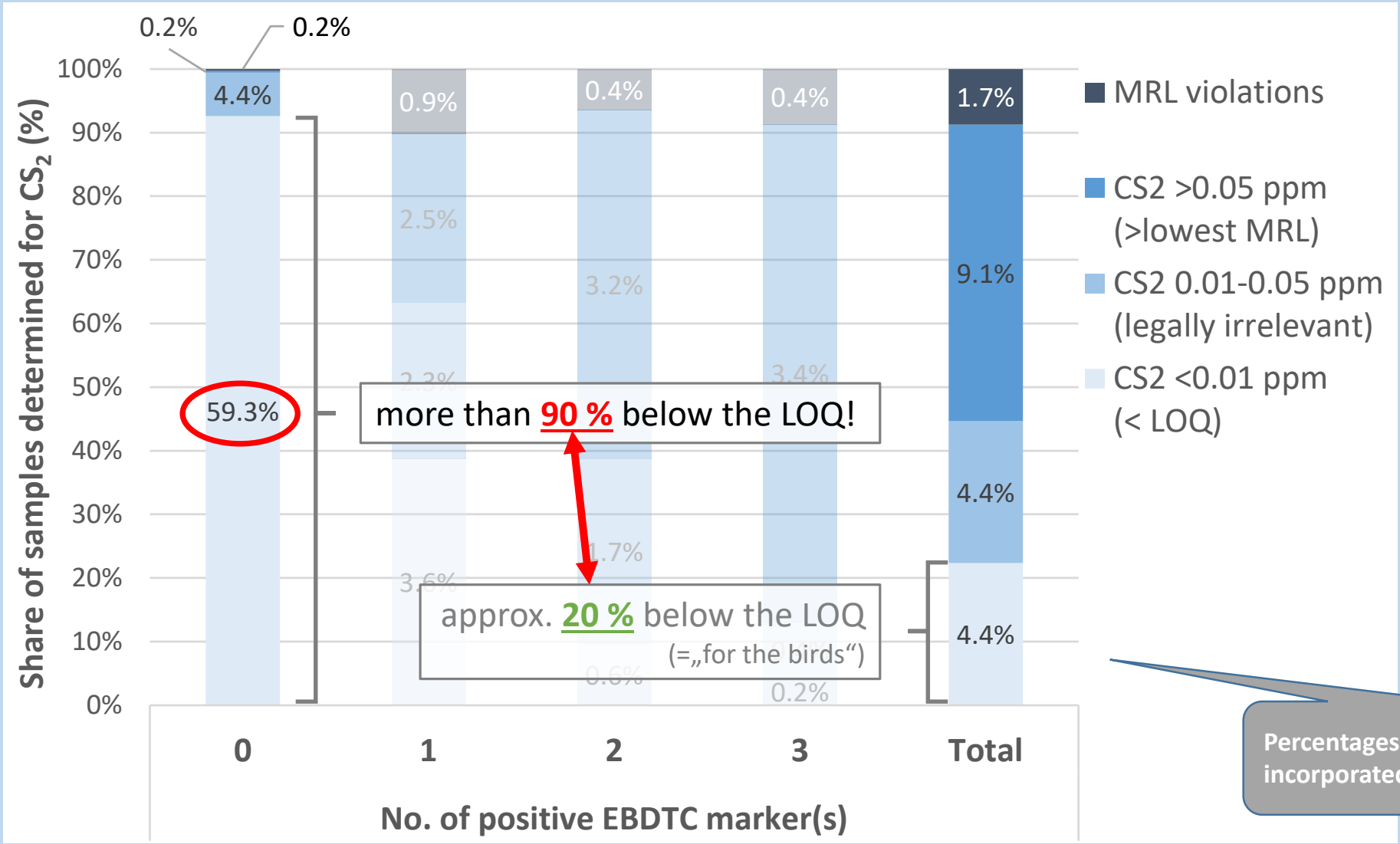
ethylene thiourea (ETU)
QuPPe-amenable
det. with LC-MS/MS



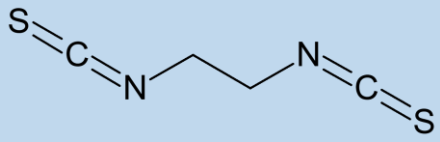
ethylene urea (EU)
QuPPe-amenable
det. with LC-MS/MS

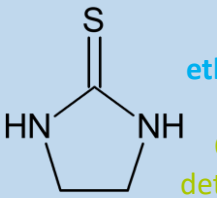
Percentages refer to the total number of incorporated samples (N = 528)

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

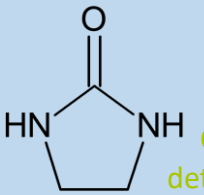


ethylene-*bis*-isothiocyanate (eBIC)
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap





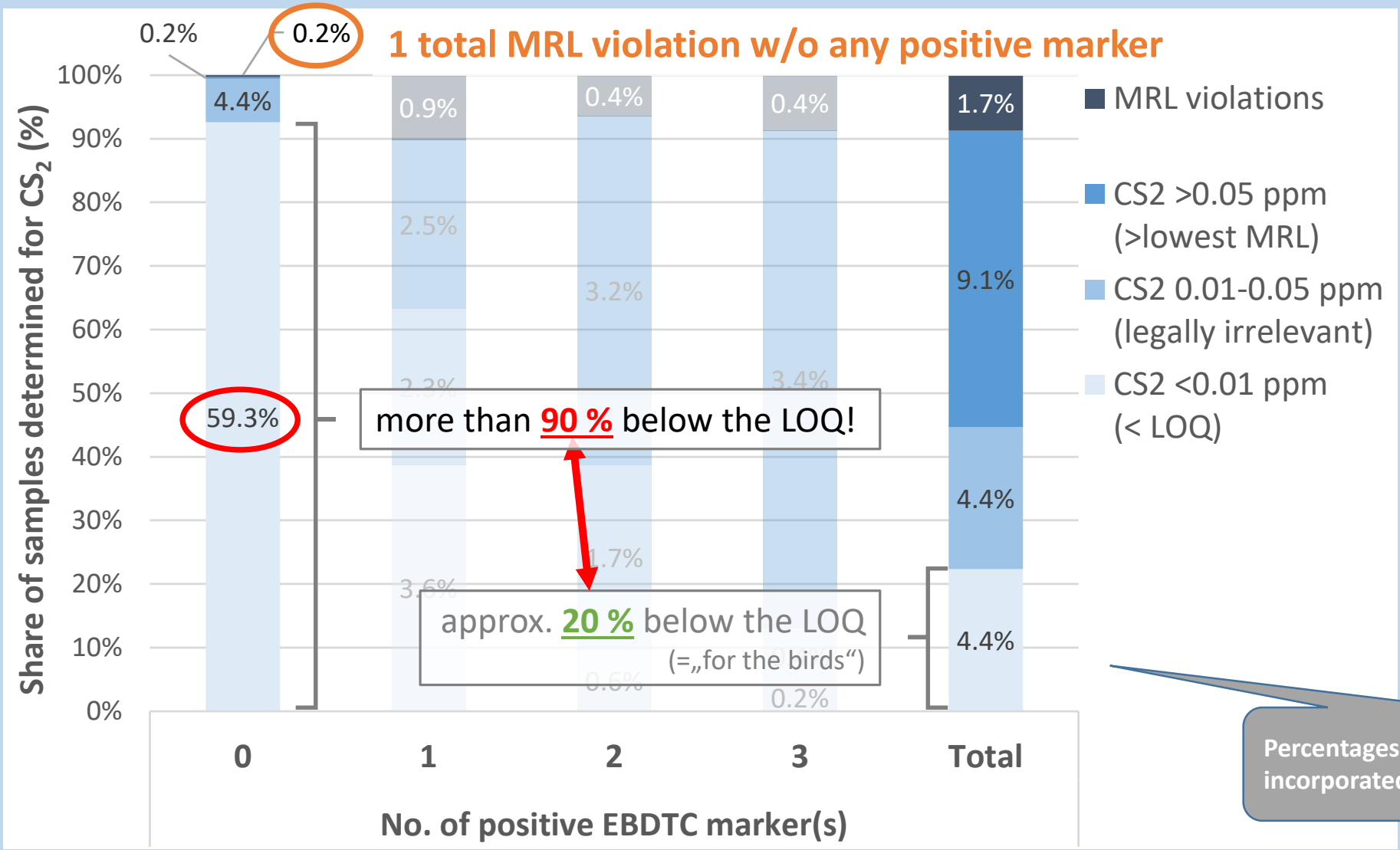
ethylene thiourea (ETU)
QuPPe-amenable
det. with LC-MS/MS



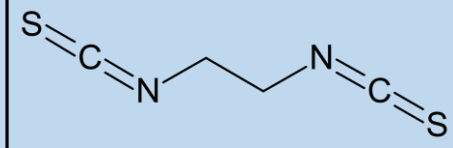
ethylene urea (EU)
QuPPe-amenable
det. with LC-MS/MS

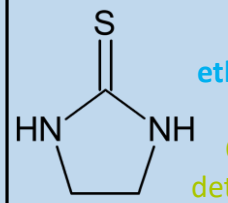
Percentages refer to the total number of incorporated samples (N = 528)

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

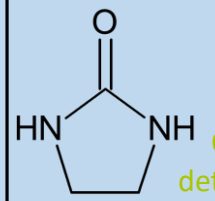


ethylene-*bis*-isothiocyanate (eBIC)
QuEChERS- amenable,
det. with GC-MS/MS or -Orbitrap





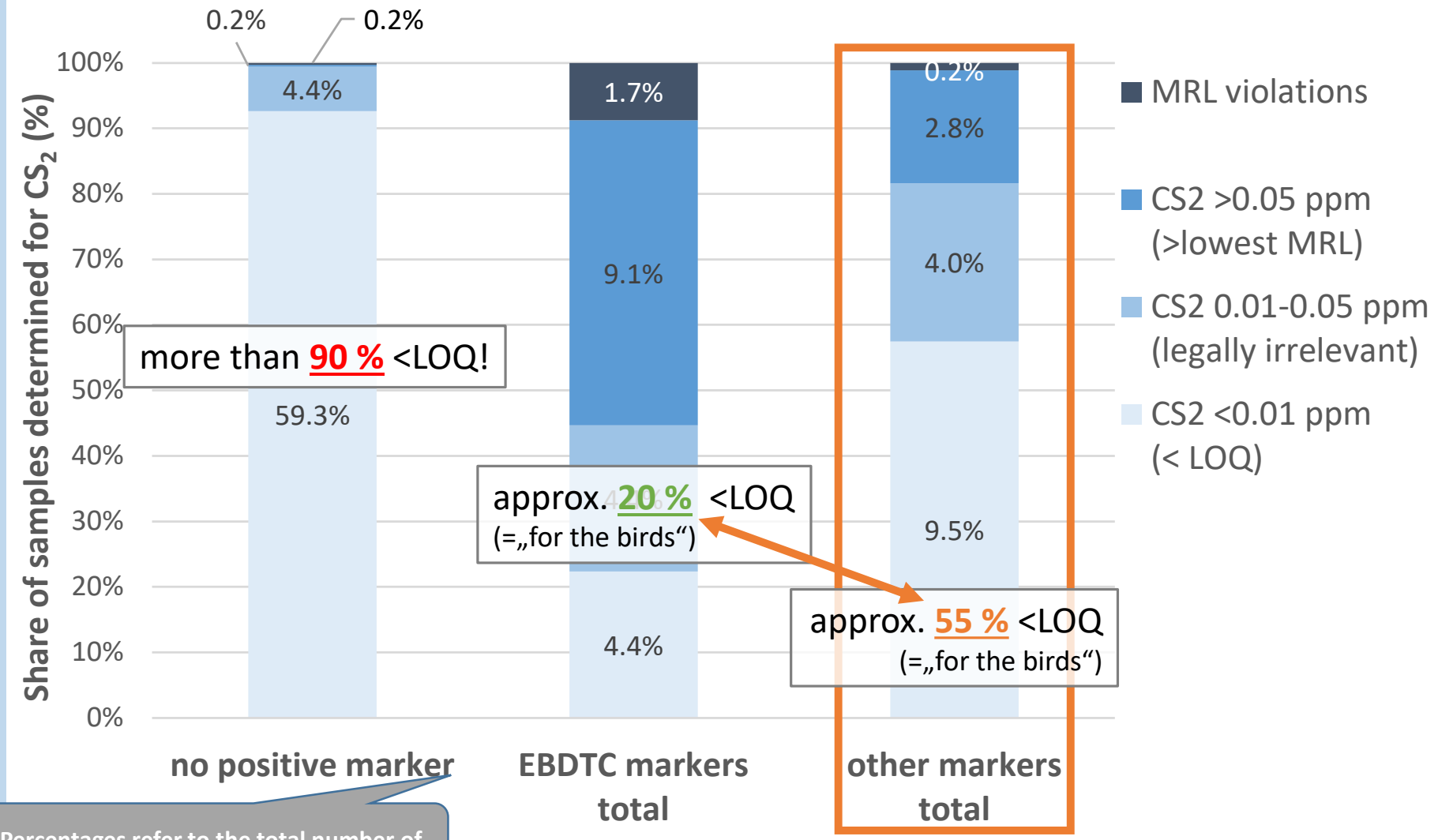
ethylene thiourea (ETU)
QuPPe-amenable
det. with LC-MS/MS



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

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

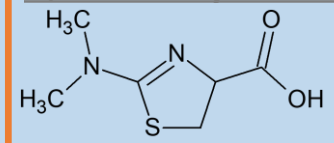
DTC-Markers | Results for the other markers of different DTC-groups



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

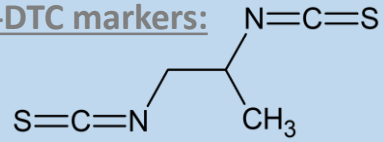
Essential other markers:

N,N-Dimethyl-DTC marker:

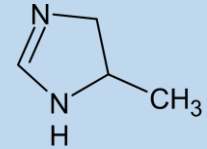


2-(dimethylamino)-4,5-dihydro-1,3-thiazole-4-carboxylic acid (M1)
QuPPe-amenable,
det. with LC-MS/MS

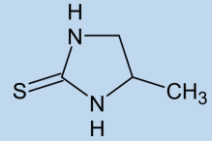
Propylene-bis-DTC markers:



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



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

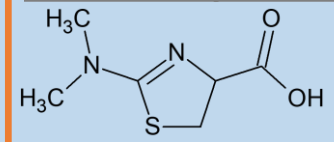


Propylene thiourea (PTU)
QuPPe-amenable
det. with LC-MS/MS

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

Essential other markers:

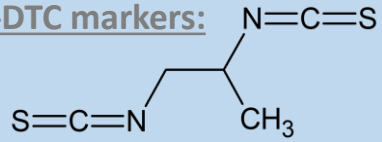
N,N-Dimethyl-DTC marker:



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

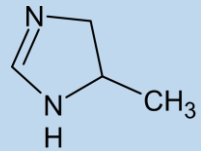
QuPPe-amenable,
det. with LC-MS/MS

Propylene-bis-DTC markers:



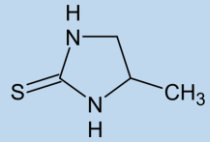
Propylene-bis-isothiocyanate (pBIC)

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



4-Methyl-imidazoline (MIDZ)

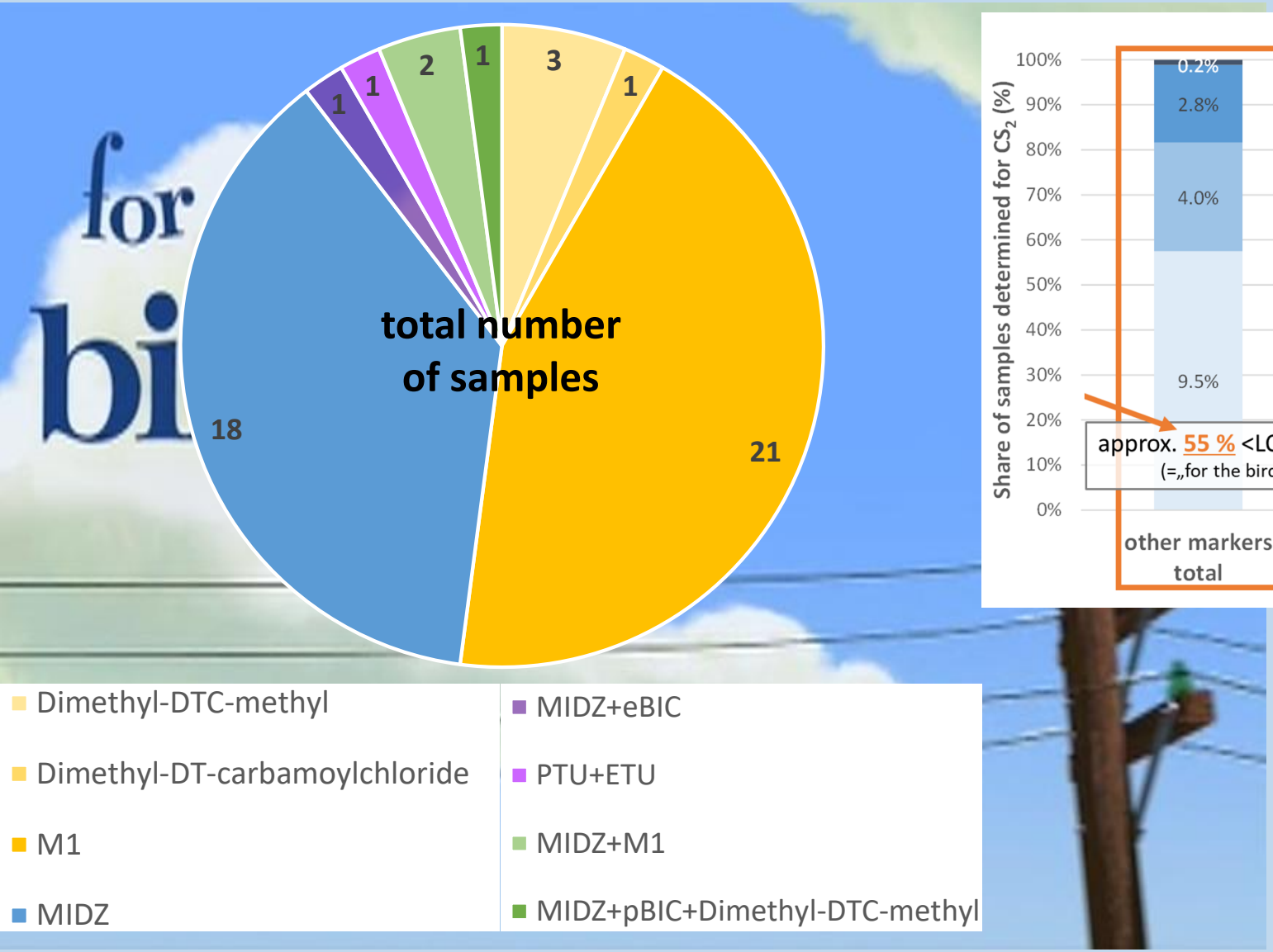
QuPPe-amenable
det. with LC-MS/MS



Propylene thiourea (PTU)

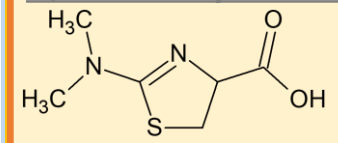
QuPPe-amenable
det. with LC-MS/MS

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



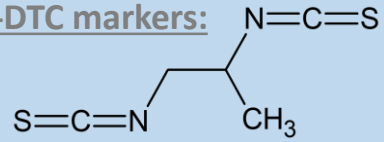
Essential other markers:

N,N-Dimethyl-DTC marker:

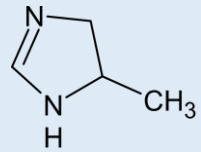


2-(dimethylamino)-4,5-dihydro-1,3-thiazole-4-carboxylic acid (M1)
QuPPe-amenable,
det. with LC-MS/MS

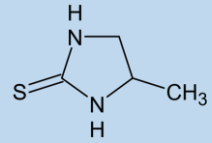
Propylene-bis-DTC markers:



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

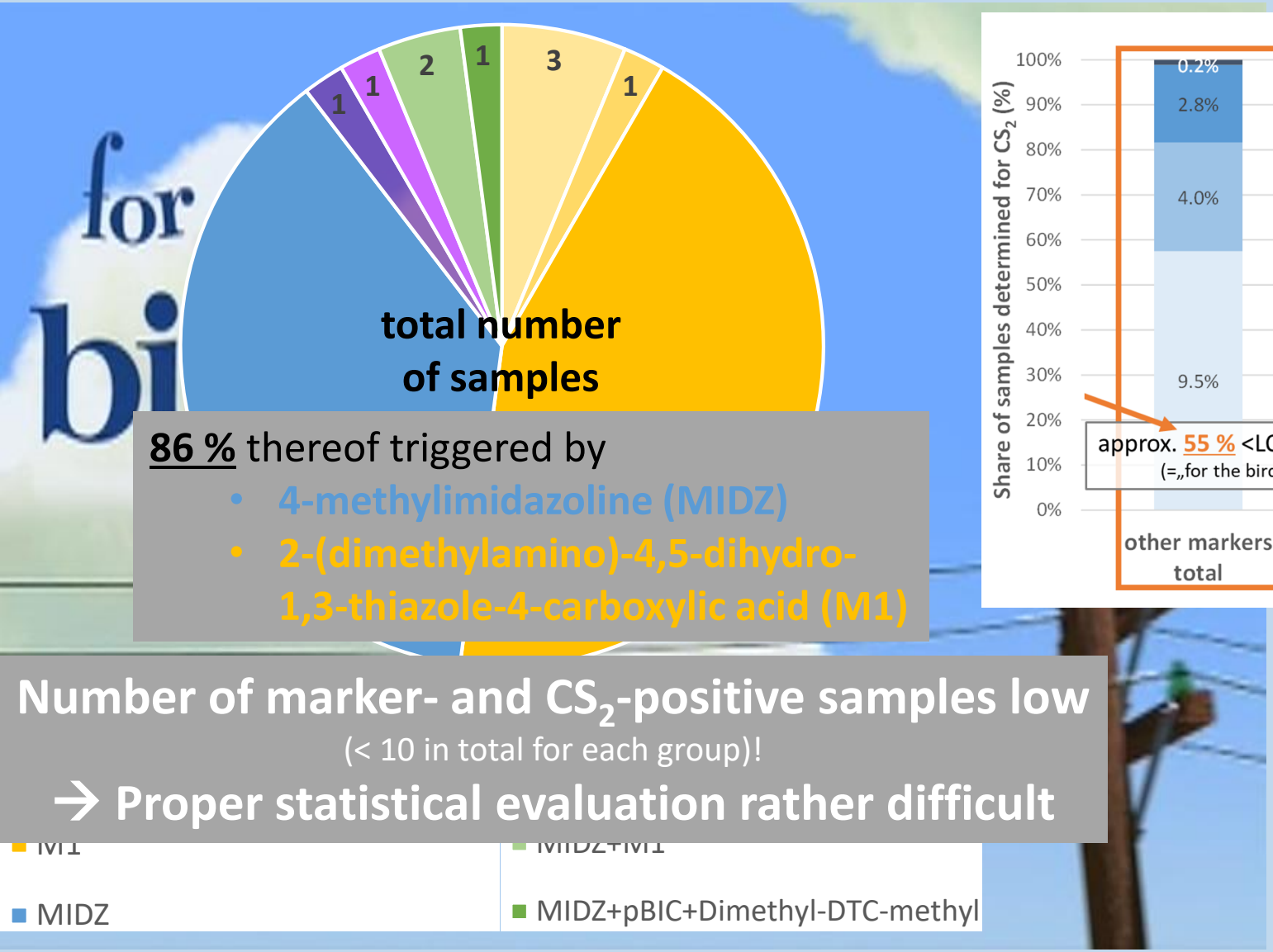


4-Methyl-imidazoline (MIDZ)
QuPPe-amenable
det. with LC-MS/MS



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

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



86 % thereof triggered by

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

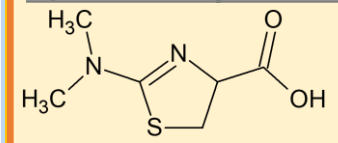
Number of marker- and CS_2-positive samples low

(< 10 in total for each group)!

→ **Proper statistical evaluation rather difficult**

Essential other markers:

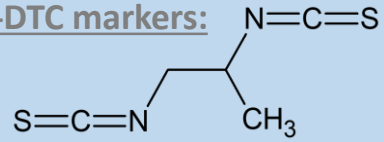
N,N-Dimethyl-DTC marker:



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

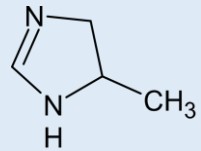
QuPPe-amenable,
det. with LC-MS/MS

Propylene-bis-DTC markers:



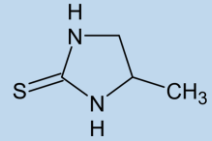
Propylene-bis-isothiocyanate (pBIC)

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



4-Methyl-imidazoline (MIDZ)

QuPPe-amenable
det. with LC-MS/MS

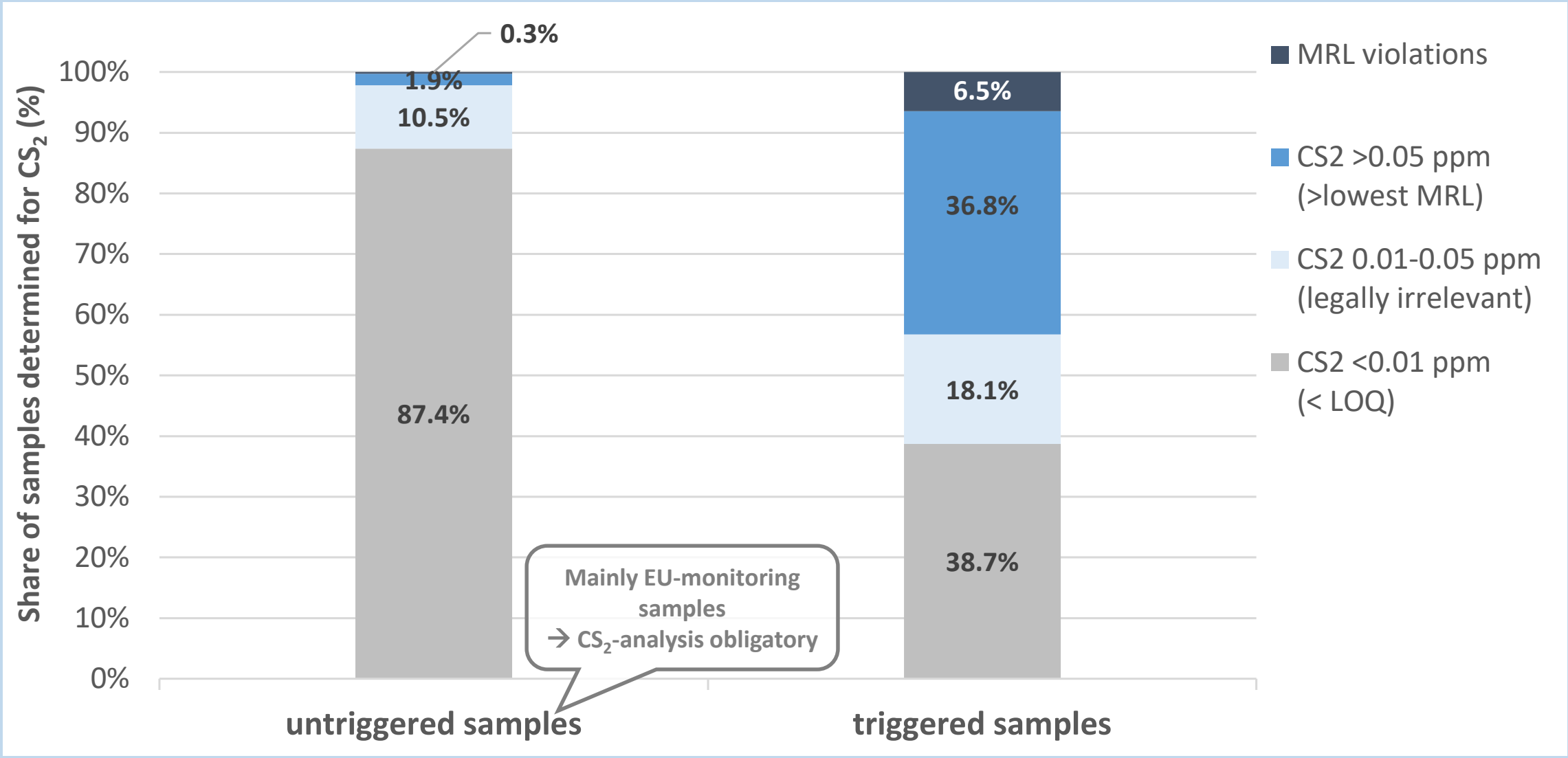


Propylene thiourea (PTU)

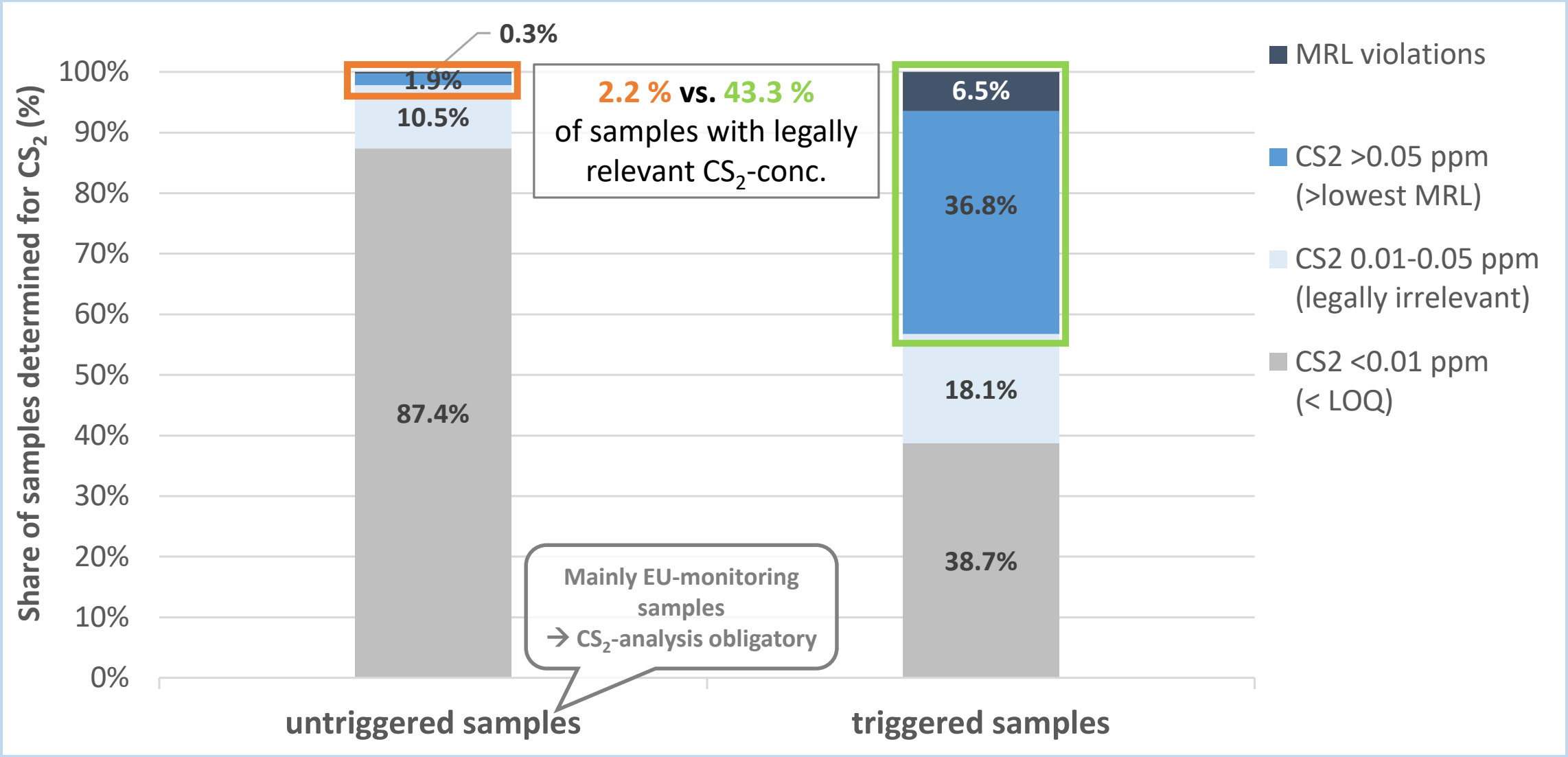
QuPPe-amenable
det. with LC-MS/MS

DTC-Markers | Summarized results

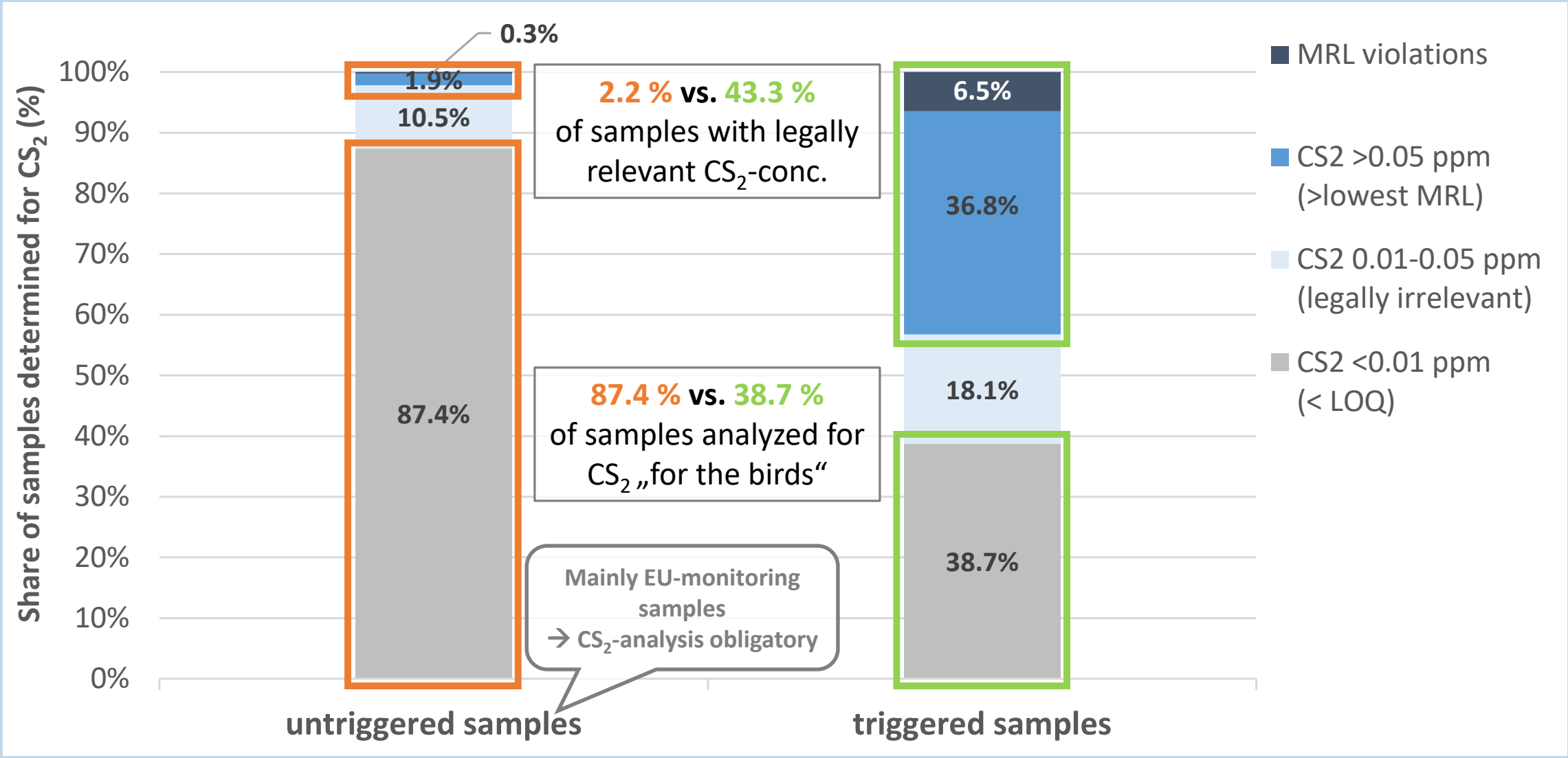
DTC-Markers | Summarized results



DTC-Markers | Summarized results



DTC-Markers | Summarized results

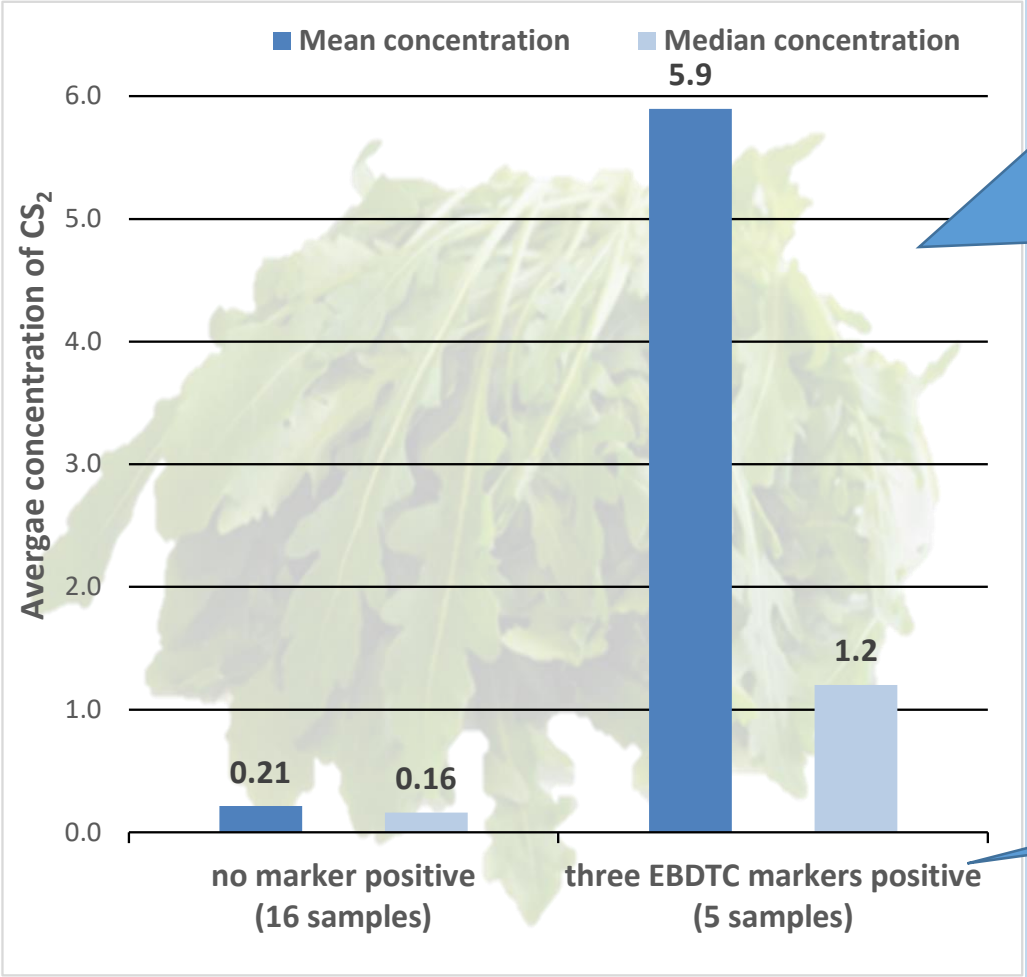


DTC-Markers | Summarized results

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

DTC-Markers | Summary

- ✓ A monitoring program was conducted (in total 528 samples determined)
  - 13 DTC markers analyzed by routine methods (QuEChERS and QuPPe)
  - CS_2 analyzed by traditional common moiety method

DTC-Markers | Summary

- ✓ A monitoring program was conducted (in total 528 samples determined)
  - 13 DTC markers analyzed by routine methods (QuEChERS and QuPPe)
  - CS_2 analyzed by traditional common moiety method
- ✓ Three EBDTC markers (ETU, EU and eBIC) with a correlation to relevant CS_2-findings were identified
  - ➢ propability of relevant CS_2-findings increases with increasing number of encountered markers

DTC-Markers | Summary

- ✓ A monitoring program was conducted (in total 528 samples determined)
  - 13 DTC markers analyzed by routine methods (QuEChERS and QuPPe)
  - CS_2 analyzed by traditional common moiety method
- ✓ Three EBDTC markers (ETU, EU and eBIC) with a correlation to relevant CS_2-findings were identified
  - ➢ propability of relevant CS_2-findings increases with increasing number of encountered markers
- ✓ These **three EBDTC markers** seem to be **suitable for indicating** if a sample had **a DTC-treatment history**
  - ➢ **10 MRL-violations** would have remained unnoticed otherwise!
  - ➢ (Indications of) high specificity in commodities with natural components generating CS_2

DTC-Markers | Summary

- ✓ A monitoring program was conducted (in total 528 samples determined)
  - 13 DTC markers analyzed by routine methods (QuEChERS and QuPPe)
  - CS_2 analyzed by traditional common moiety method
- ✓ Three EBDTC markers (ETU, EU and eBIC) with a correlation to relevant CS_2-findings were identified
  - ➢ propability of relevant CS_2-findings increases with increasing number of encountered markers
- ✓ These **three EBDTC markers** seem to be **suitable for indicating** if a sample had **a DTC-treatment history**
  - ➢ **10 MRL-violations** would have remained unnoticed otherwise!
  - ➢ (Indications of) high specificity in commodities with natural components generating CS_2
- ✓ Enhancement of the lab's effectivity by using these EBDTC markers as a trigger for a subsequent CS_2- analysis

DTC-Markers | Summary

- ✓ A monitoring program was conducted (in total 528 samples determined)
  - 13 DTC markers analyzed by routine methods (QuEChERS and QuPPe)
  - CS_2 analyzed by traditional common moiety method
- ✓ Three EBDTC markers (ETU, EU and eBIC) with a correlation to relevant CS_2-findings were identified
  - ➢ propability of relevant CS_2-findings increases with increasing number of encountered markers
- ✓ These **three EBDTC markers** seem to be **suitable for indicating** if a sample had **a DTC-treatment history**
  - ➢ **10 MRL-violations** would have remained unnoticed otherwise!
  - ➢ (Indications of) high specificity in commodities with natural components generating CS_2
- ✓ 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

DTC-Markers | Summary

- ✓ A monitoring program was conducted (in total 528 samples determined)
  - 13 DTC markers analyzed by routine methods (QuEChERS and QuPPe)
  - CS_2 analyzed by traditional common moiety method
- ✓ Three EBDTC markers (ETU, EU and eBIC) with a correlation to relevant CS_2-findings were identified
  - ➢ propability of relevant CS_2-findings increases with increasing number of encountered markers
- ✓ These **three EBDTC markers** seem to be **suitable for indicating** if a sample had **a DTC-treatment history**
  - ➢ **10 MRL-violations** would have remained unnoticed otherwise!
  - ➢ (Indications of) high specificity in commodities with natural components generating CS_2
- ✓ 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

DTC-Markers | Summary

- ✓ A monitoring program was conducted (in total 528 samples determined)
  - 13 DTC markers analyzed by routine methods (QuEChERS and QuPPe)
  - CS_2 analyzed by traditional common moiety method
- ✓ Three EBDTC markers (ETU, EU and eBIC) with a correlation to relevant CS_2-findings were identified
  - ➢ propability of relevant CS_2-findings increases with increasing number of encountered markers
- ✓ These **three EBDTC markers** seem to be **suitable for indicating** if a sample had **a DTC-treatment history**
  - ➢ **10 MRL-violations** would have remained unnoticed otherwise!
  - ➢ (Indications of) high specificity in commodities with natural components generating CS_2
- ✓ 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:

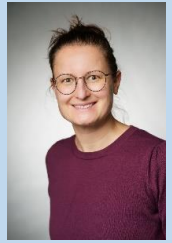
eric.eichhorn@cvuas.bwl.de **or** eurl-srm@cvuas.bwl.de

Ann-Kathrin Schäfer

Andrea Karst

Anja Barth

Sabrina Goerlich



Anne Benkenstein

Bärbel Illg

Bendedikt Hornung

Cristin Wildgrube

Doro Mack

Dorota Stanislawcyk

Eric Eichhorn

Ellen Scherbaum

Heike Welzel

Giovanna Cerchia

Pesticide team of the CVUA Stuttgart

Kathi Hacker

Bruno Sauer

Marc Wieland

Michelangelo Anastassiades

Hubert Zipper

Leonie Moser

Irina Sigalov

Karin Rothenbächer

Sigrid Schüler

Silvia Zechmann

Florian Hägele

Hanna Dias

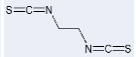
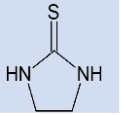
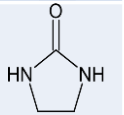
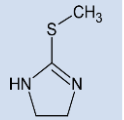
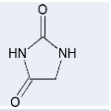
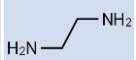
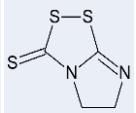
Pat Schreiter

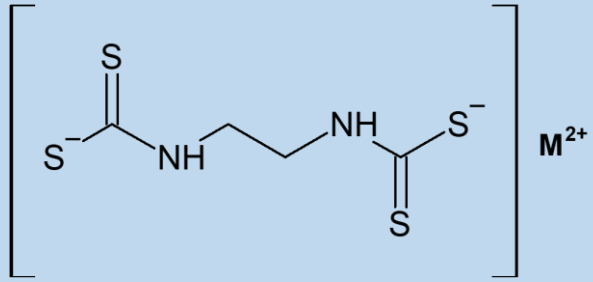
Rebekka Lötterle

Sahra Fieberg

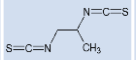
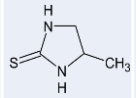
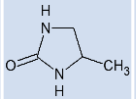
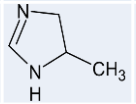
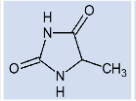
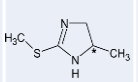
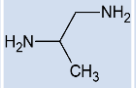
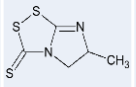
DTC-Markers | ANNEX

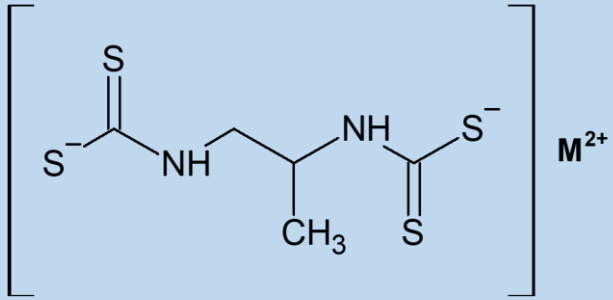
DTC-Markers | Overview of the considered ethylene-*bis*-DTC markers

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

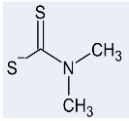
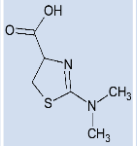
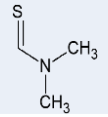
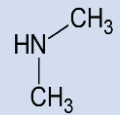
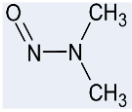
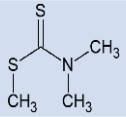


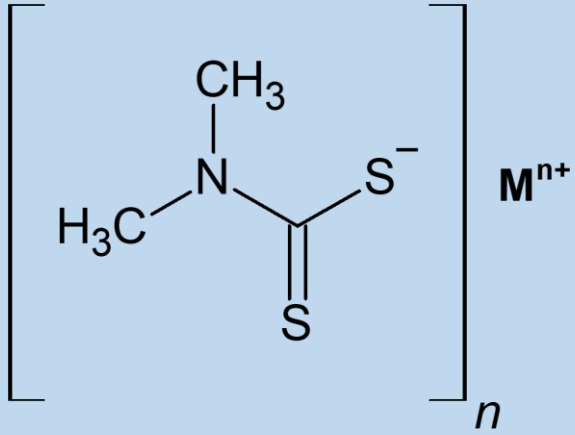
DTC-Markers | Overview of the considered propylene-*bis*-DTC markers

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	-	-

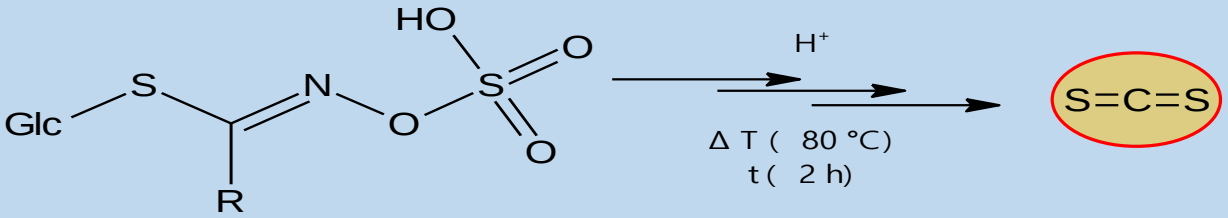


DTC-Markers | Overview of the considered N,N-dimethyl-DTC markers

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)