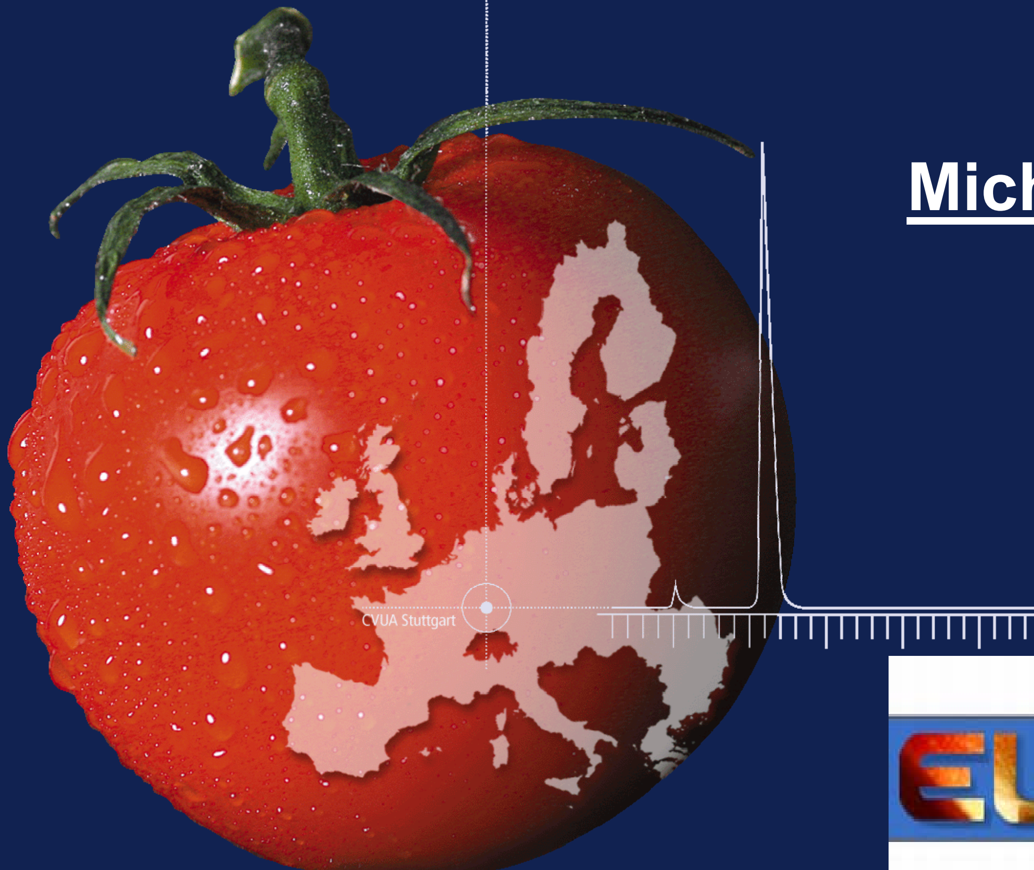
ADVANCES IN THE ANALYSIS OF PESTICIDES TYPICALLY ANALYSED BY SINGLE-RESIDUE METHODS



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SRM-Phobia...

SRM-compounds are not very popular...

SRM-Phobia...

SRMs often require...

❖ **tedious or unfamiliar sample preparation steps e.g.:**
- *pH adjustments*
- *Derivatization*
- *Cleavage*
- *Head-space sampling*

❖ **separation/detection techniques different from those typically used in MRMs and <u>often not available</u> in labs, e.g.:**
- *"Exotic" columns for LC- or GC-separation*
- *"Exotic" mobile phases (LC)*
- *"Exotic" Instrumental-configurations*

SRM-Phobia...

SRMs are thus often Costly …

… and Work-Intensive…

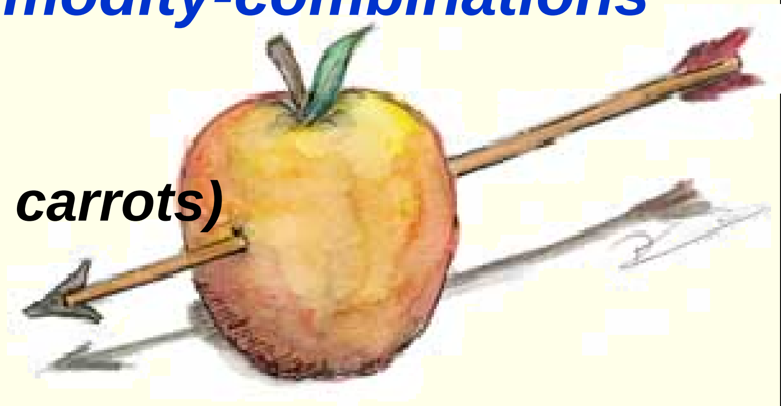
- **Effort : Benefit-Ratio is less favourable than that of MRMs**

As labs are typically overloaded with work…

..most try to avoid SRMs wherever possible using them only when there no other way out ;-)

SRM-Challenge...

If at all, labs employ SRMs very judiciously…
targeting only specific pesticide/commodity-combinations



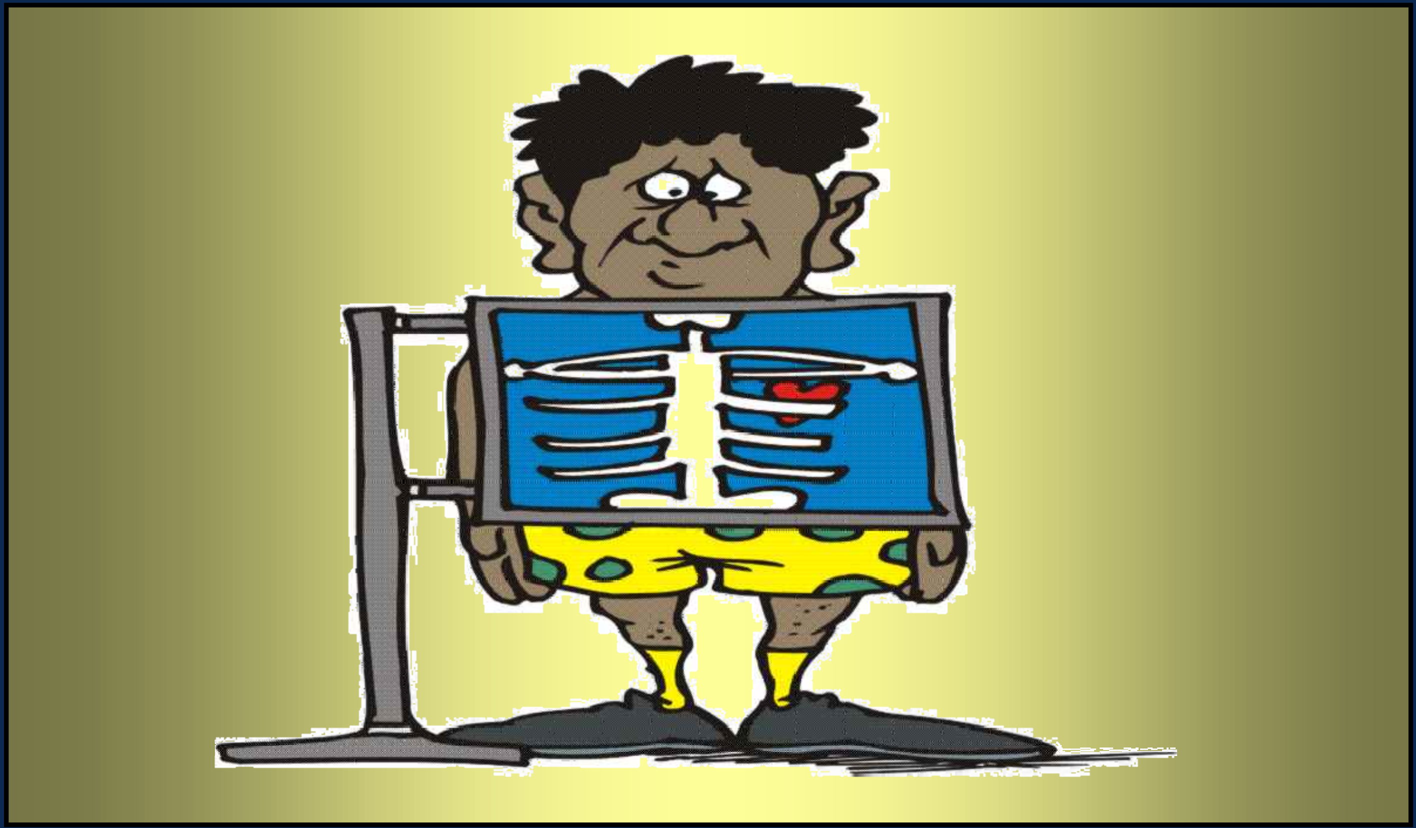
Examples:
Chlormequat (pears, cereals, mushrooms, carrots)
Glyphosate (cereals)
Maleic hydrazide (onions, potatoes)

☛ ***This targeted approach Improves the Effort to Benefit Ratio***

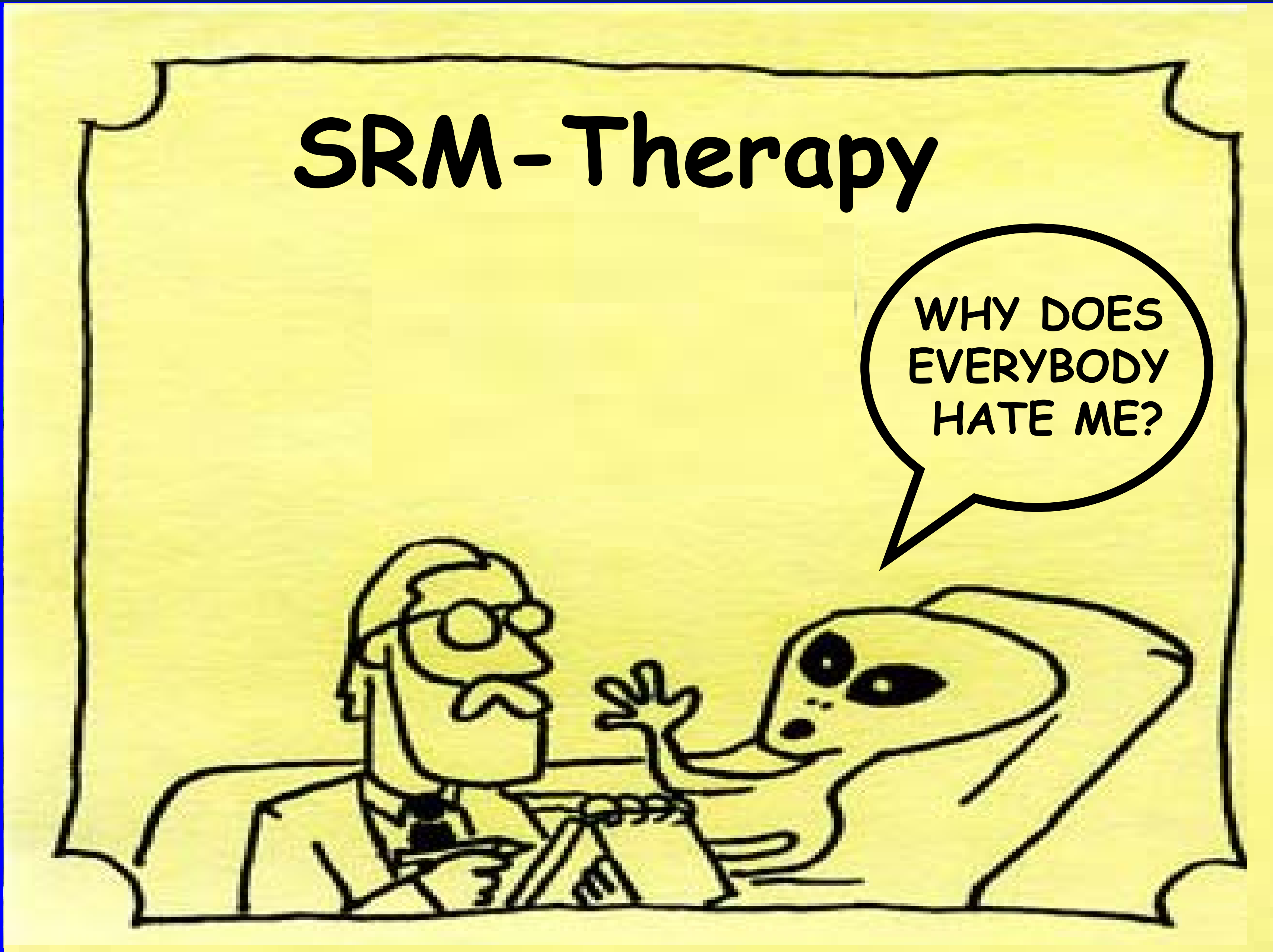
However: Our knowledge about the residue situation of SRM-Pesticides is still very limited…

Question to be answered:
Which SRM-Pesticide/Commodity-Combinations are worth-while monitoring ?

Let’s take a closer look at the SRM pesticides



What makes a compound an SRM-compound?



What makes a compound an SRM-compound?

OBSERVATIONS / REASONS		**EXAMPLES**	**POSSIBLE SRM-APPROACHES**
I. **Poor Chr/phy and/or Detection**	- Bad peak-shape - Poor detector response - Degradation (mostly GC)	- Organotins - Abamectin - Carbamates, dicofol	- Diff. Separation cond. - Diff. Detection cond. - Deriv., APs, Temp. adj.
II. **Degradation - Losses**	- Compound **Degrades** - Hydrolysis - Oxidation - Reactions with matrix	- Captan, Folpet - Ethiofencarb - Chlorothalonil	- Control pH, work fast, keep temp. low
III. **Adsorption / Partitioning -Losses**	- Comp. **interacts w. surfaces**	- Organotins, Paraquat	- Modify solvent (e.g. H^+)
	- Compound too **Polar** - Compound **Acidic/Basic** *+ unfavourable pH*	- Quats, Glyphosate - Phenoxy acids	- One-phase extractions - pH-adjustments
	- Compound too **Lipophilic** *+ high fat commodity* *+ polar extraction solvent*	- DDT, HCB	- Use non-polar solvents for total fat extraction
IV. **Evaporation - Losses**	- Compound too **Volatile**	- Phosphine - Ethylene oxide - CS_2	- Cryo-milling, Special GC-conditions (Headspace sampling…)
V. **Residues not in the form needed**	- Legislative requirements: Residue def. includes bound residues or requires release of common moiety	- Phenoxy-acids - Dithiocarbamates, Amitraz, Prochloraz, Vinclozolin, Diuron	- Cleavage steps to release bound residues or common moieties

Classification SRM or MRM ?

Classification SRM/MRM varies…

- **from lab to lab**
- **over the course of time (as technology is progressing)**

It depends on…

- Sample Preparation Technique Employed:
  - Trend for methods better covering polar compounds, and being LC/MS amenable (QuEChERS, ChemElut, modif. S19/Luke)
- Instrumental Analysis Technique Available:
  - Detection technology
    Sensitivity/selectivity improves over time,
  - Chromatographic separation technology
    LC: UPLC, U-HPLC, new stationary phases e.g. HILIC
    GC: fast GC, GCxGC…

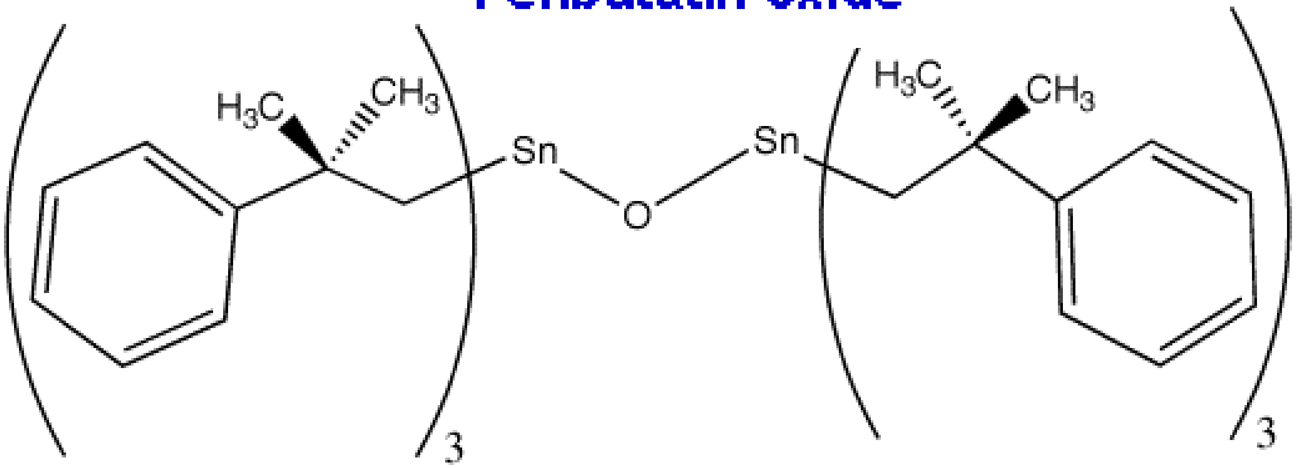
Many believe:
In future virtually all pesticides will be MRM-amenable
("Dilute & Shoot" or "Quick & Dirty")?

I.
Poor Chromatography and/or Detection

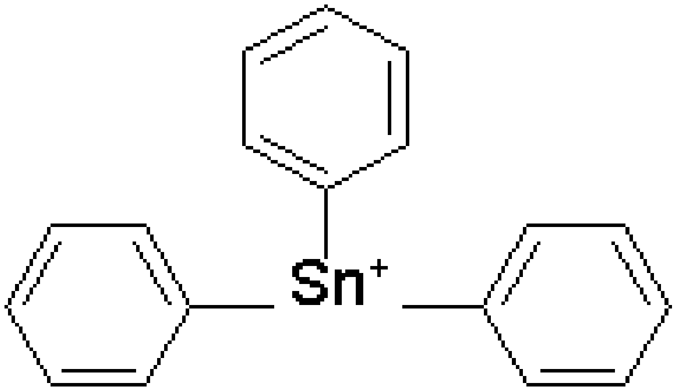
I. Poor Chromatography and/or Detection

Example: Organotins

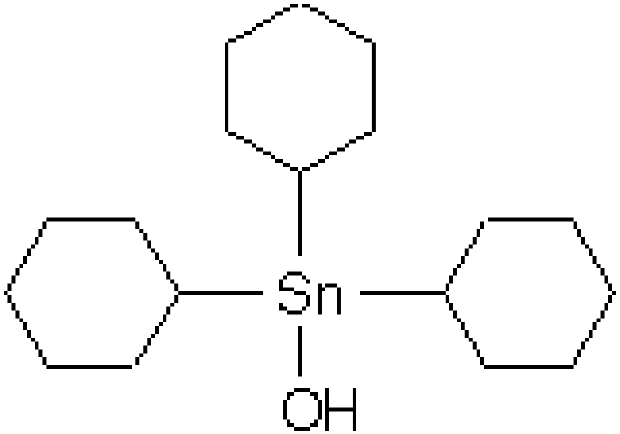
Fenbutatin oxide



Fentin



Cyhexatin



I. Poor Chromatography and/or Detection
Example: Organotins

☑ QuEChERS-Recoveries 70 - 110 %
☒ Very poor peak shapes using standard LC-MS/MS-conditions

➢ Acidic conditions essential to obtain sharp peaks !!

➢ H^+ interrupts unwanted interactions of O-Tins with surfaces

Column:
Zorbax 3,5 µm; Eclipse XDB-C18; 2,1x 50 mm

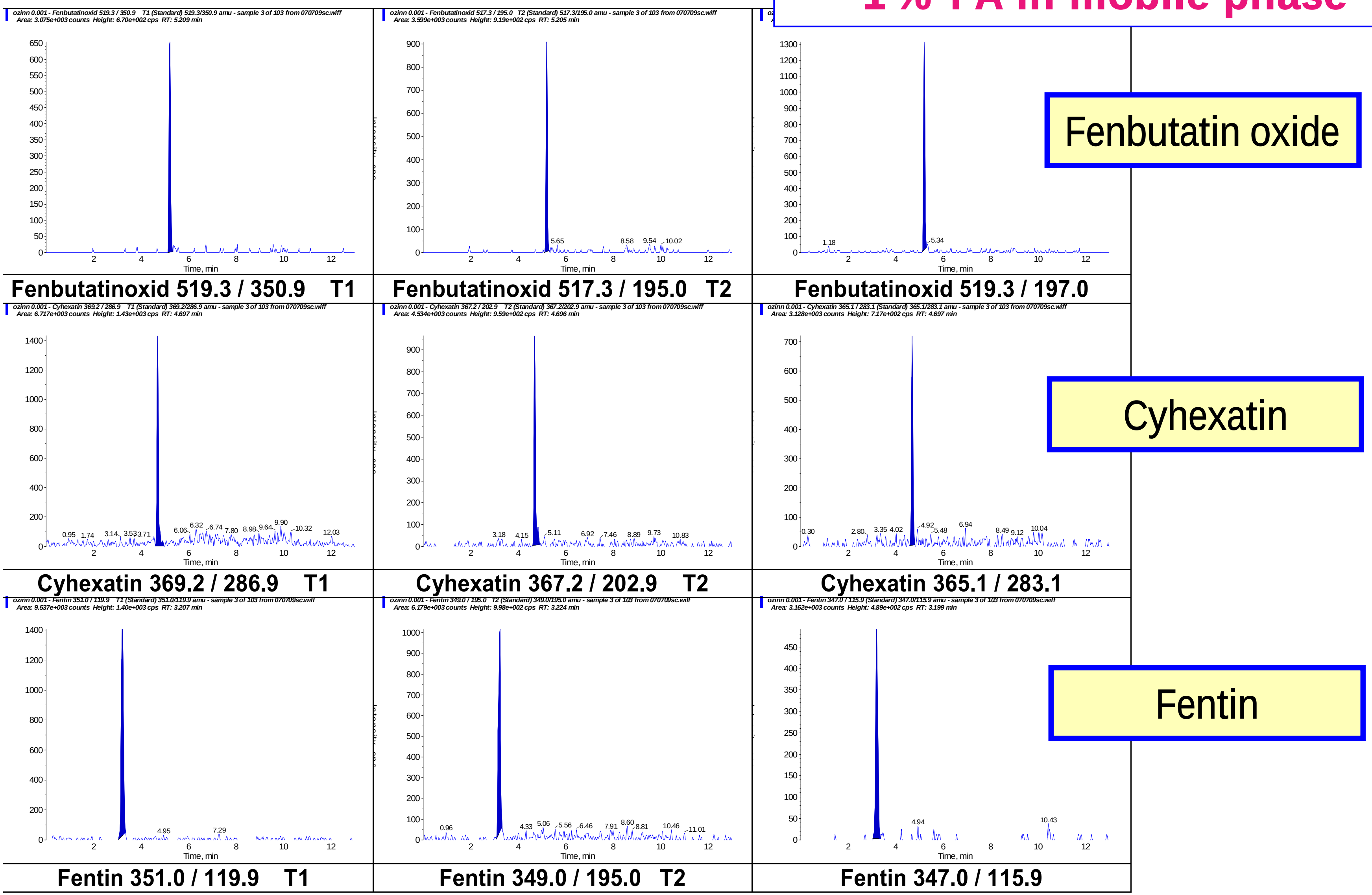
Mobile phase:
A: NH_4formate (5 mmol) in water +**1% formic acid**

B: NH_4formate (5 mmol) in methanol +**1% formic acid**

I. Poor Chromatography and/or Detection

Example: Organotins

Organotins at 0.001 mg / mL
1 % FA in mobile phase



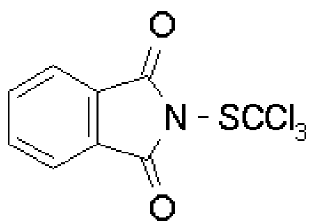
I.
Poor Chromatography and/or Detection

and

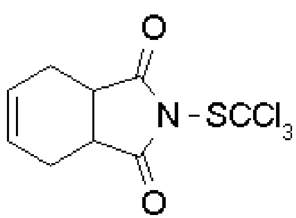
II.
Degradation - Losses

I./II. Poor Chromatography and Degradation losses

Example: Base-Labile Pesticides



FOLPET



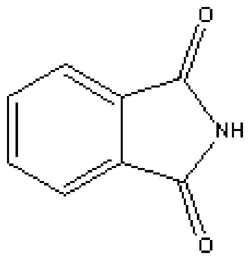
CAPTAN

Degradation at

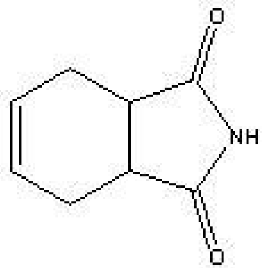
- ***High pH***
- ***High temperatures***

e.g.:

- ***During sample prep.***
- ***In final extract***
- ***In GC-inlet***



Phthalimide



Tetrahydrophthalimide

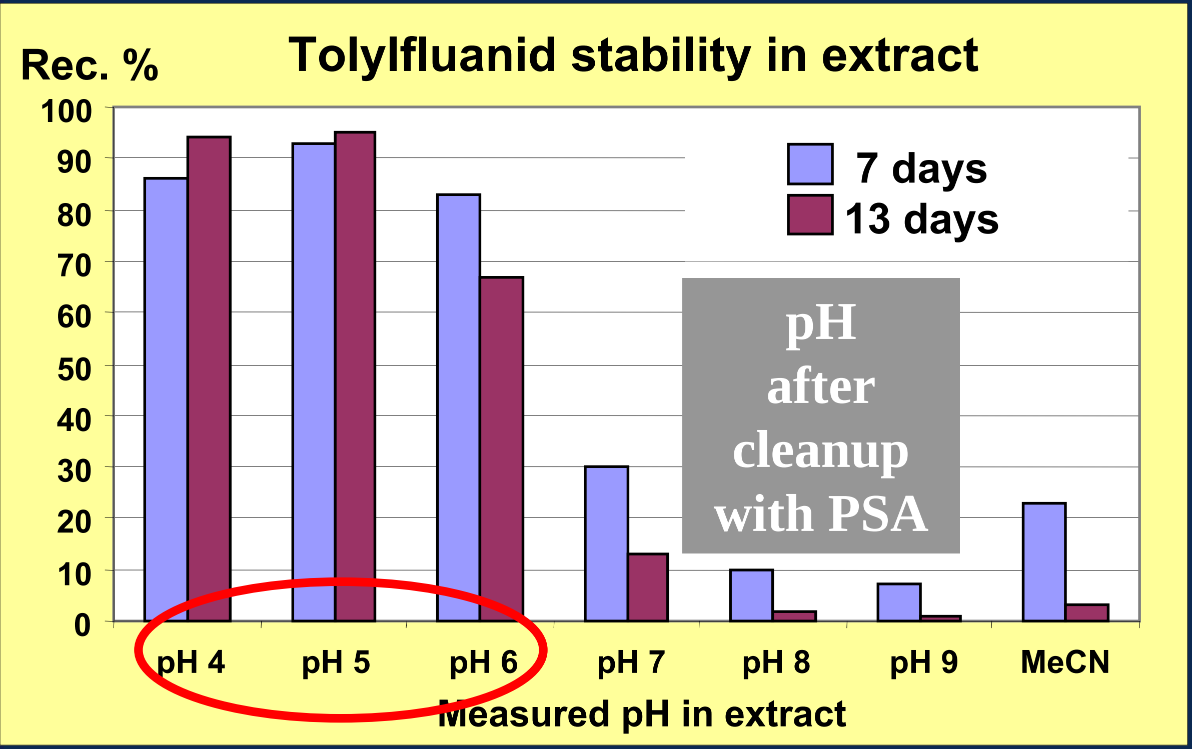
Similar behaviour:

➢ Dicofol, Captafol, Tolylfluanid, Dichlofluanid, Pyridate

Example: Base-Labile Pesticides
Reducing losses in extracts…

Problem:

Degradation in extracts of original QuEChERS following PSA-cleanup



Solution:

Addition of acid
e.g.: Formic acid (5% in ACN)
10 µL per mL extract.

- Brings "pH" to ~5

- **QuEChERS-amenable!**

OK, but what about instrumental analysis?

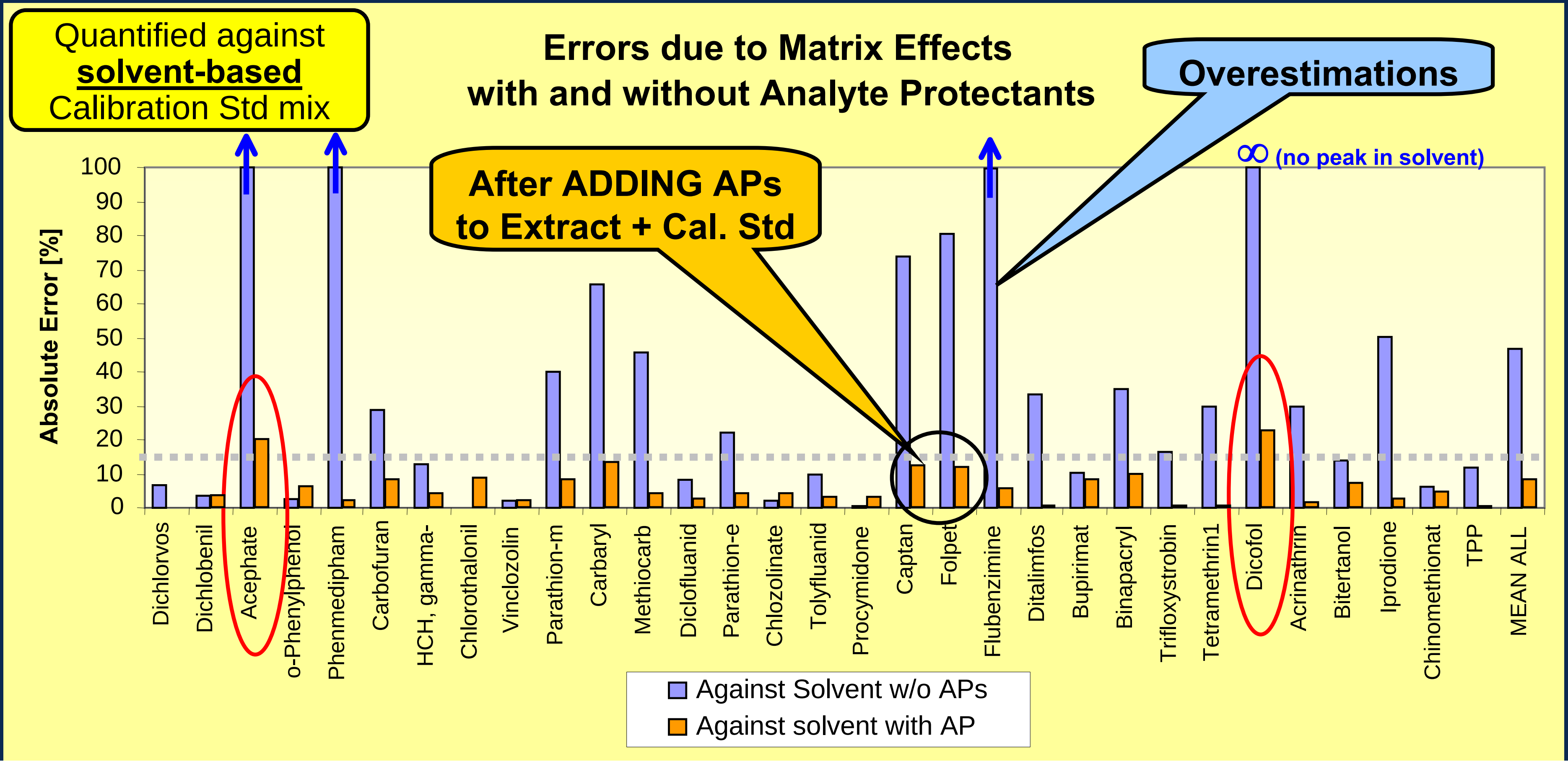
- **Tolylfluanid, Dichlofluanid, Pyridate (amenable to LC-MS/MS)** ☑
- **Captan, Folpet, Dicofol, Captafol (NOT amenable to LC-MS/MS)** ☒

I. Poor Chromatography and/or Detection

Use of Analyte Protectants to improve GC-Analysis

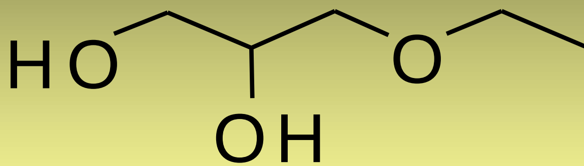
Extract acidification also improves GC-behaviour of Folpet, Captan and Dicofol

- But better results by addition of ANALYTE PROTECTANTS-MIX to extracts
  - Folpet, Captan work perfectly with APs ☑
  - Dicofol still not fully satisfactory ☒

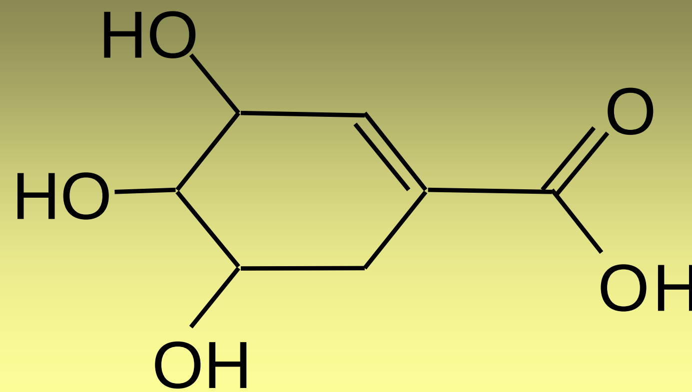


Analyte Protectants Mixture

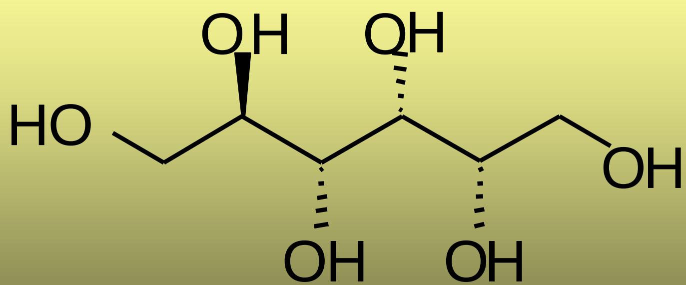
Ethylglycerol



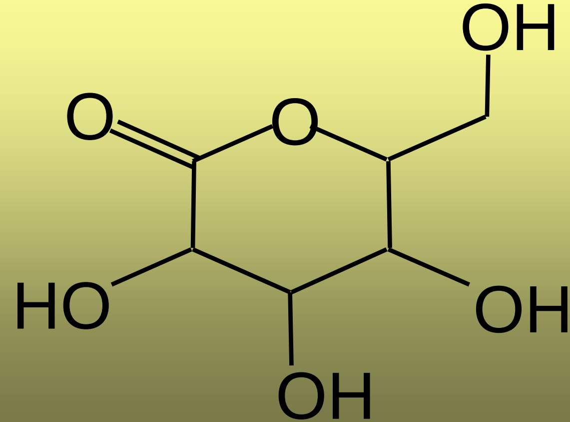
Shikimic acid



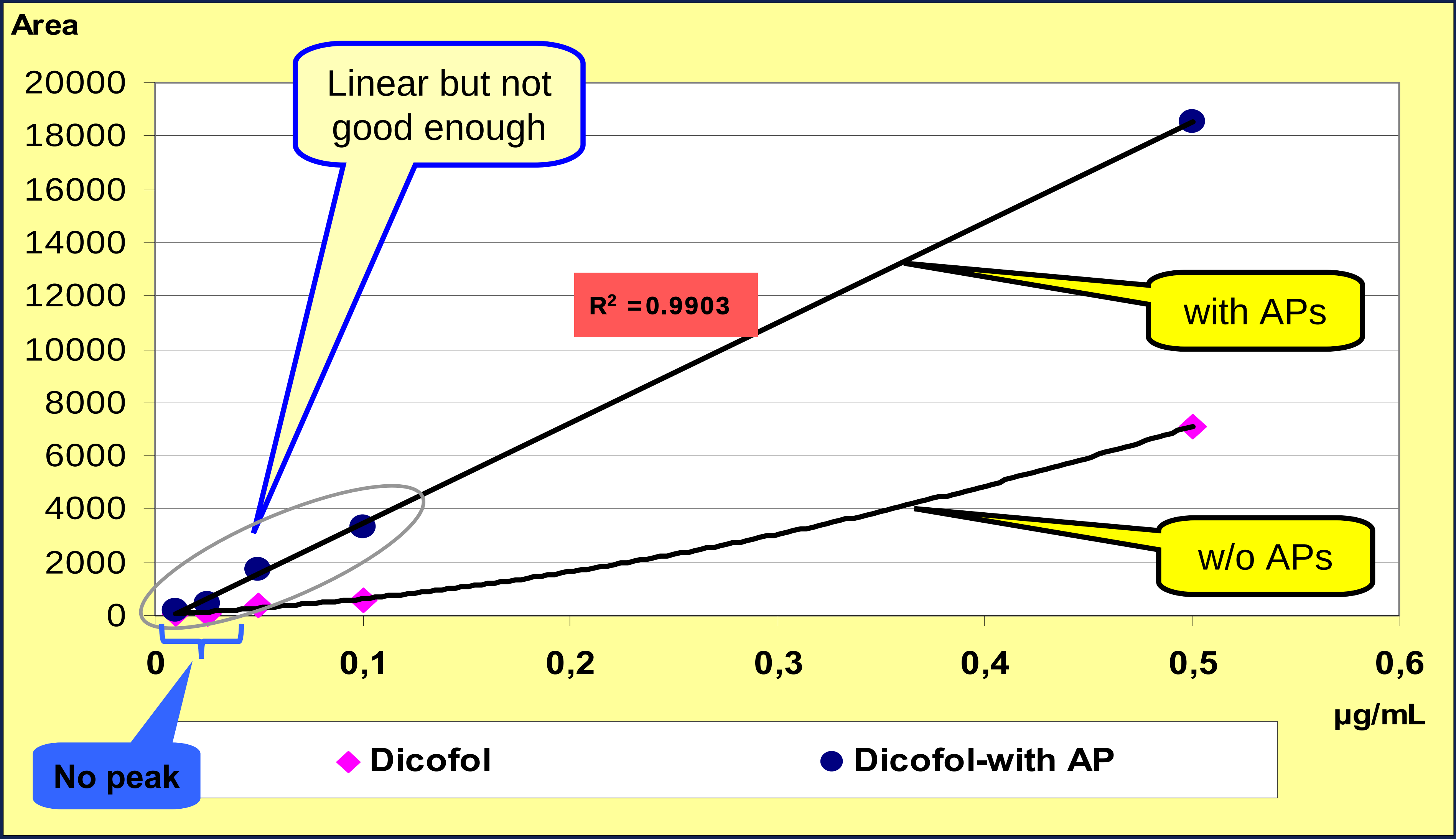
Sorbitol



δ-Gluconolactone

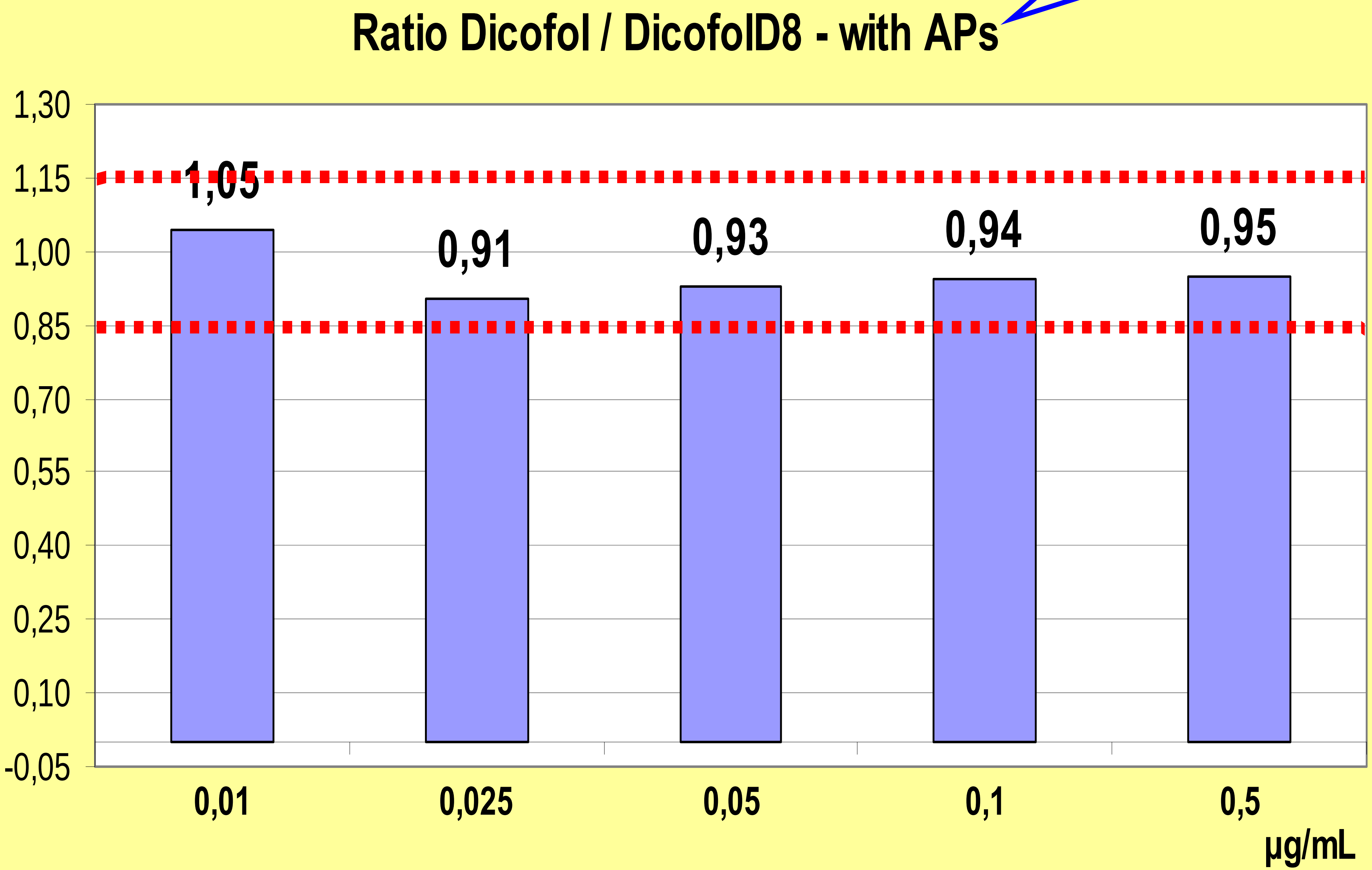


Dicofol calibration curves with and w/o APs

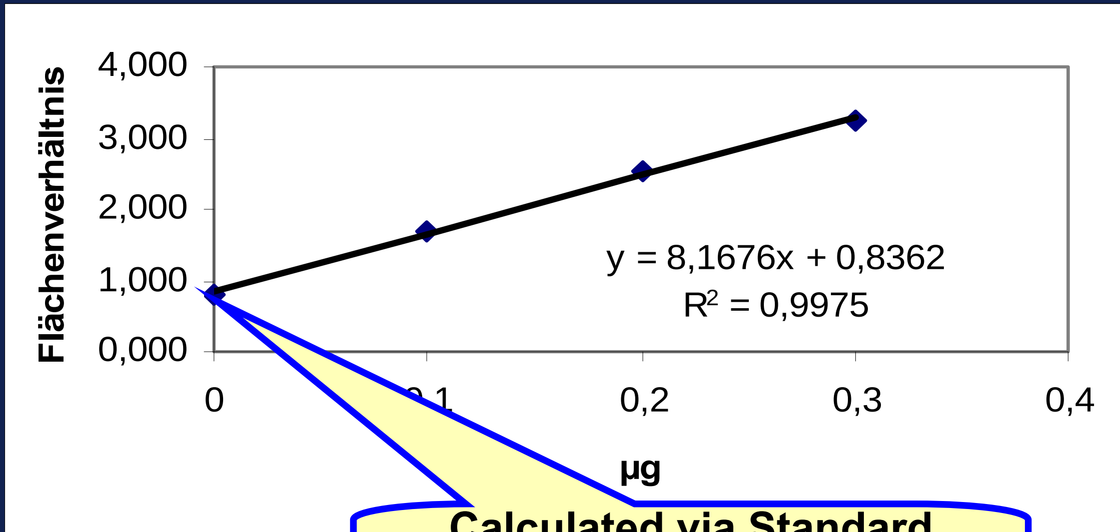


Use of isotopically labelled ISTDs

Better with APs because w/o APs often no peaks

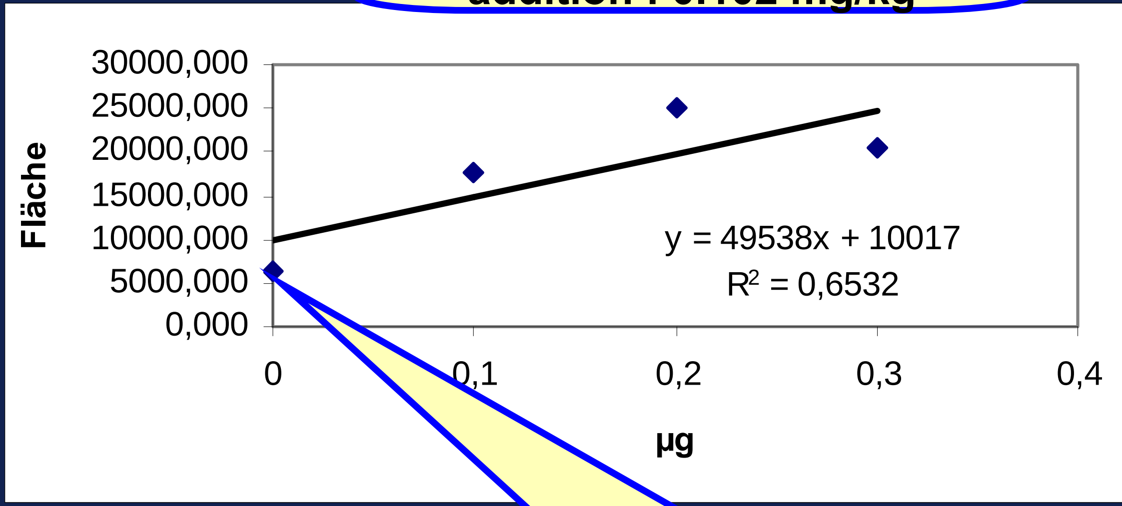


Use of isotopically labelled ISTDs



Calculated via Standard addition : 0.102 mg/kg

Dicofol using IL-ISTD-ratio



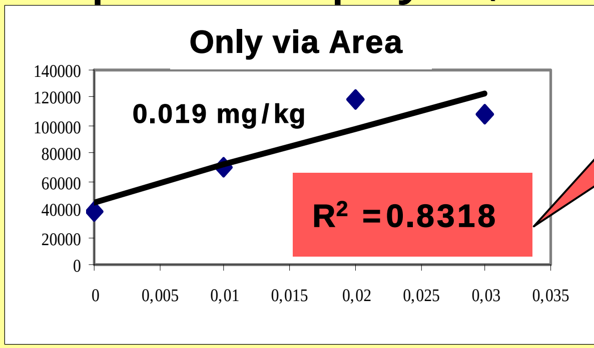
Calculated via Standard addition : 0.201 mg/kg

Dicofol using Area

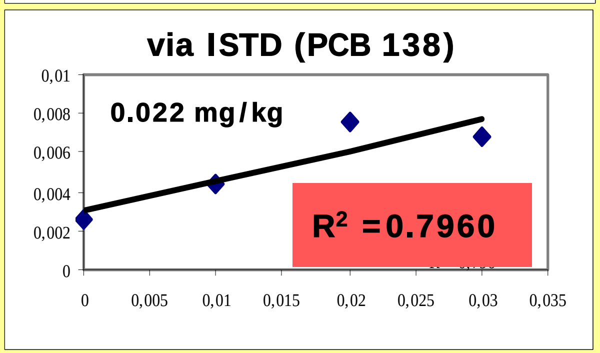
I. Poor Chromatography and/or Detection

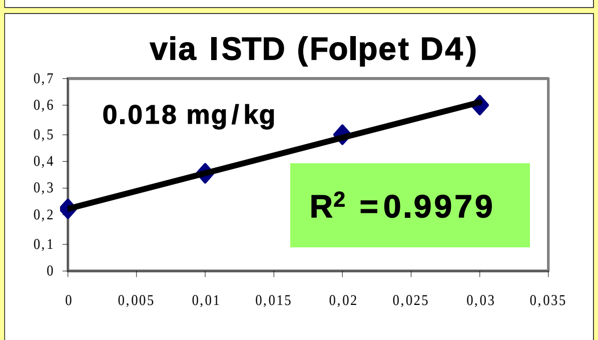
Isotopically Labelled ISTDs

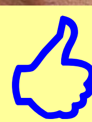
Folpet in Papaya (MRL=0.01 mg/kg)



Unacceptable R^2 Should be at least 0.995

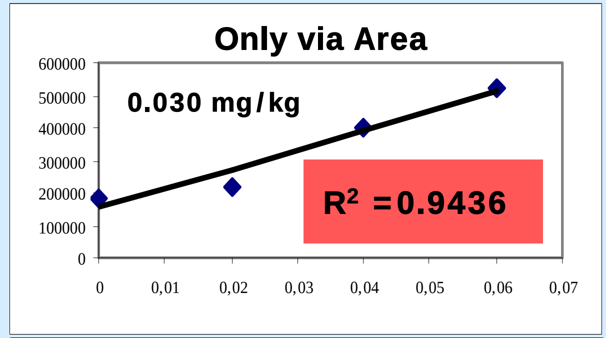


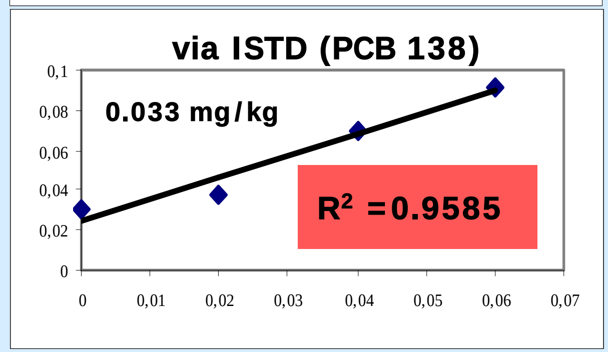


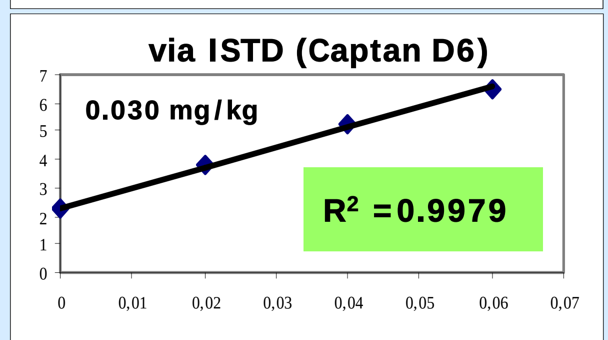


No violation

Captan in Blueberries (MRL=0.01 mg/kg)





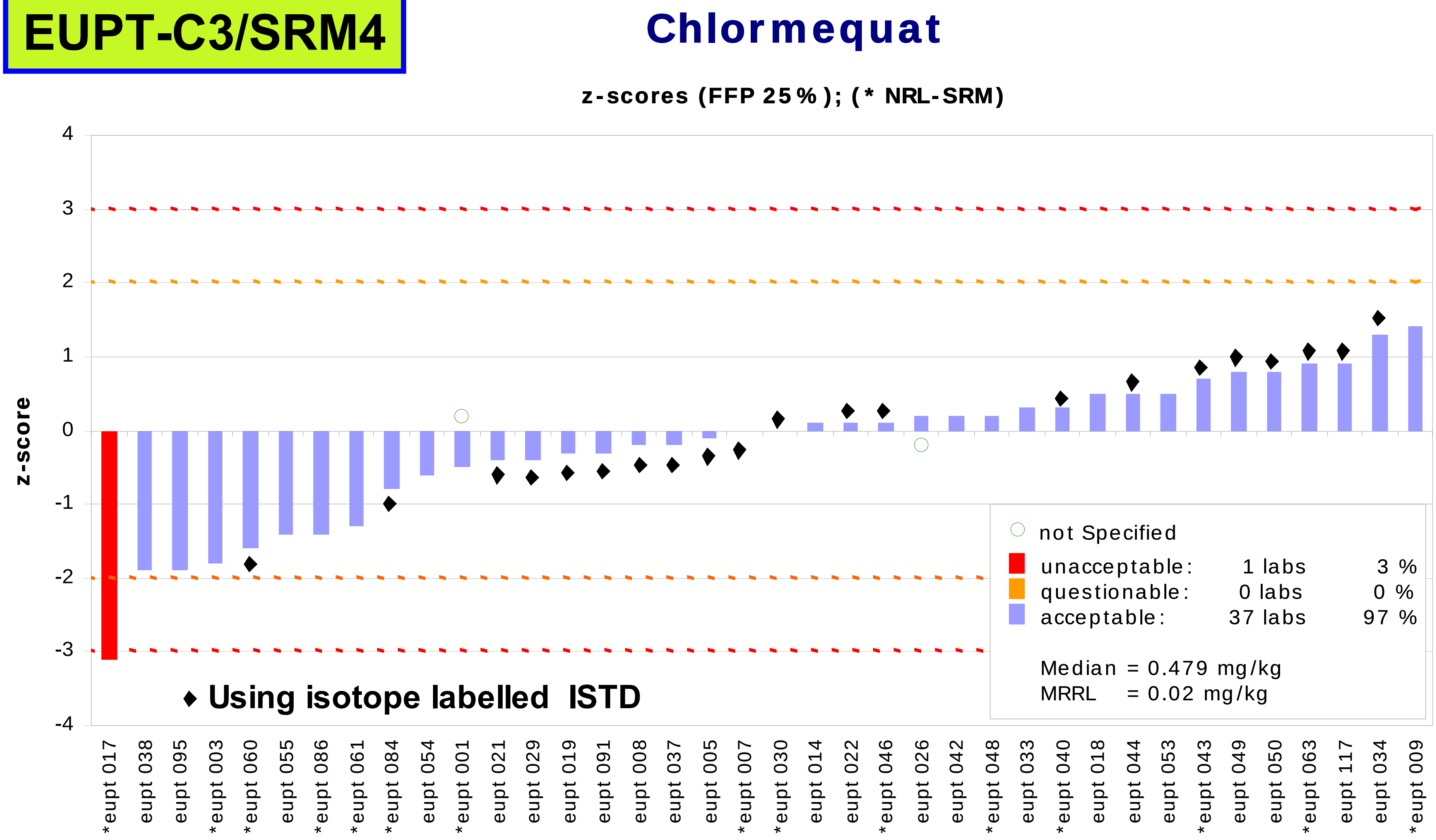


ISTDs added to extract

Violation

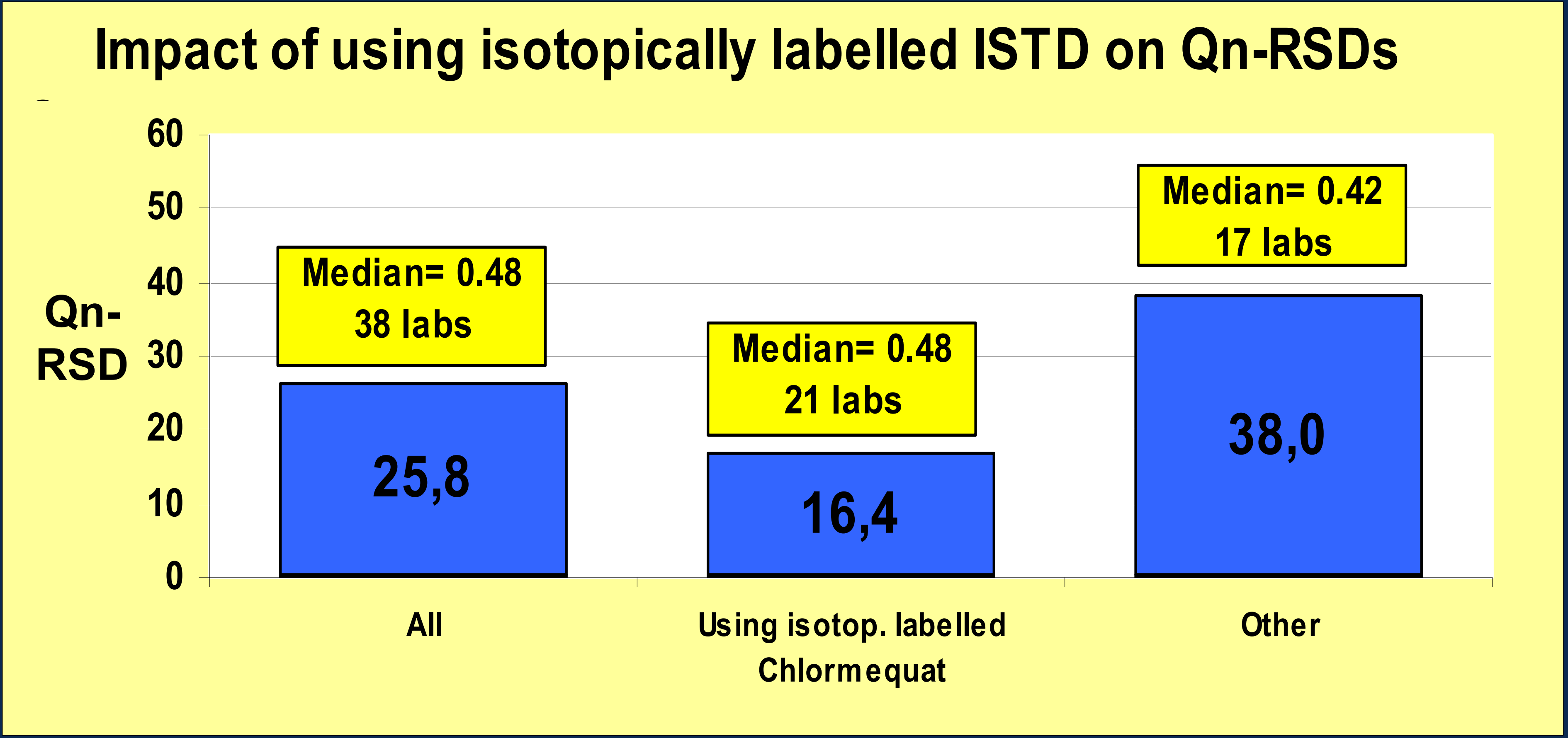
Impact of isotopically labelled ISTDs

EUPT-C3/SRM4



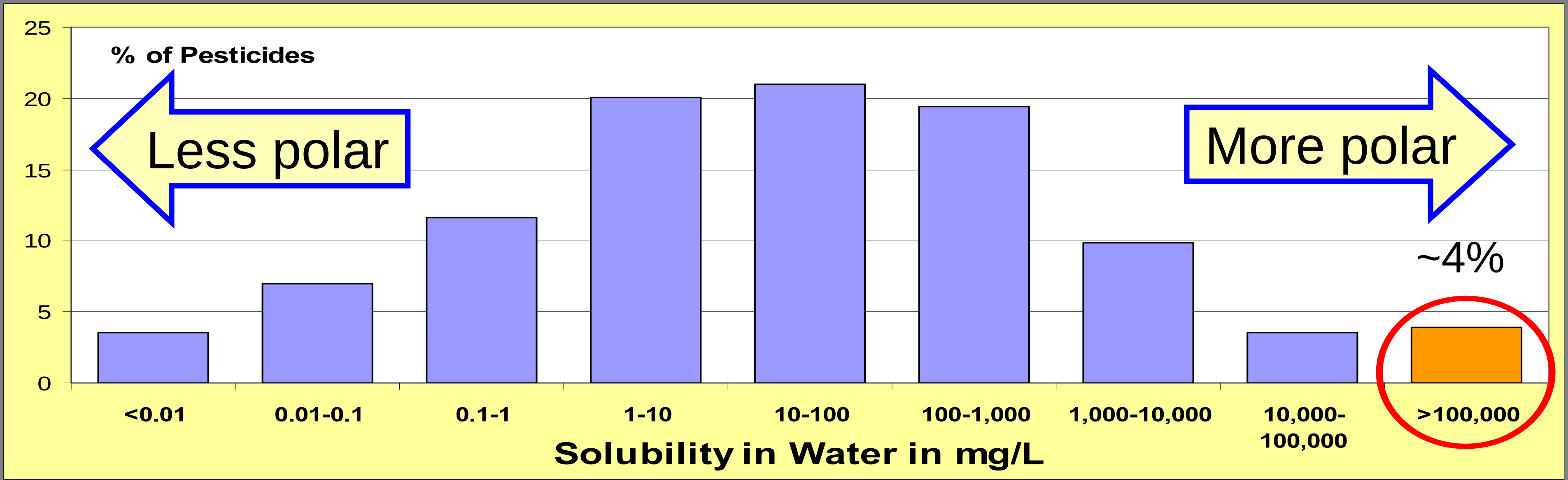
Impact of isotopically labelled ISTDs

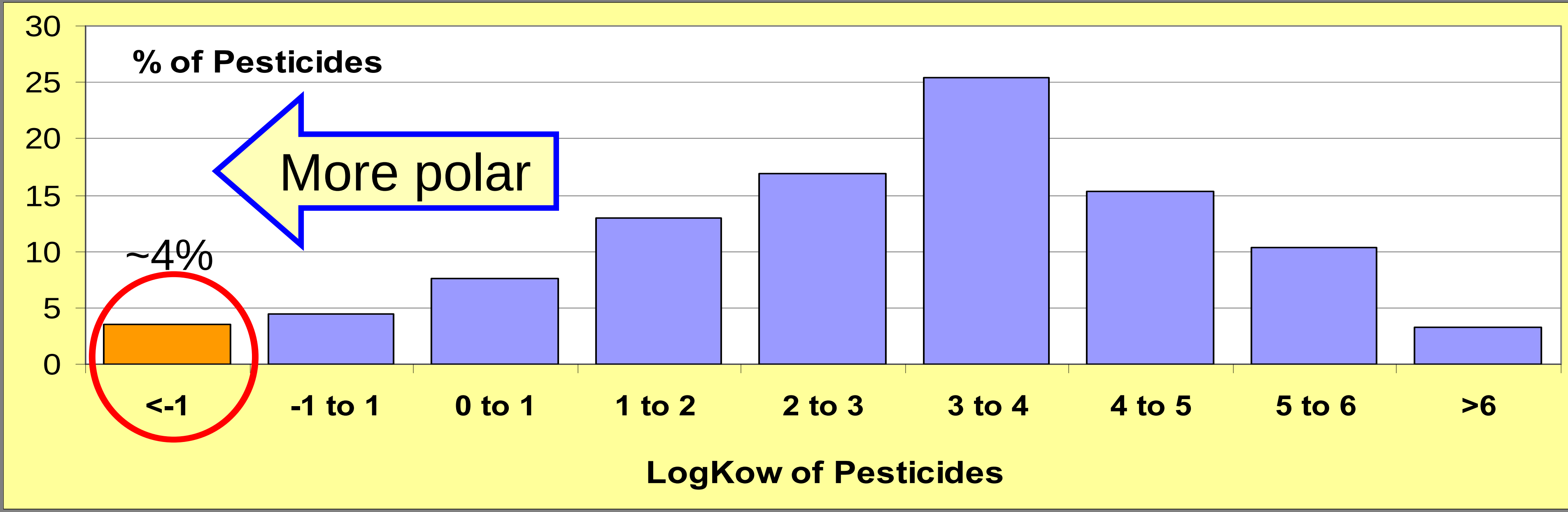
EUPT-C3/SRM4

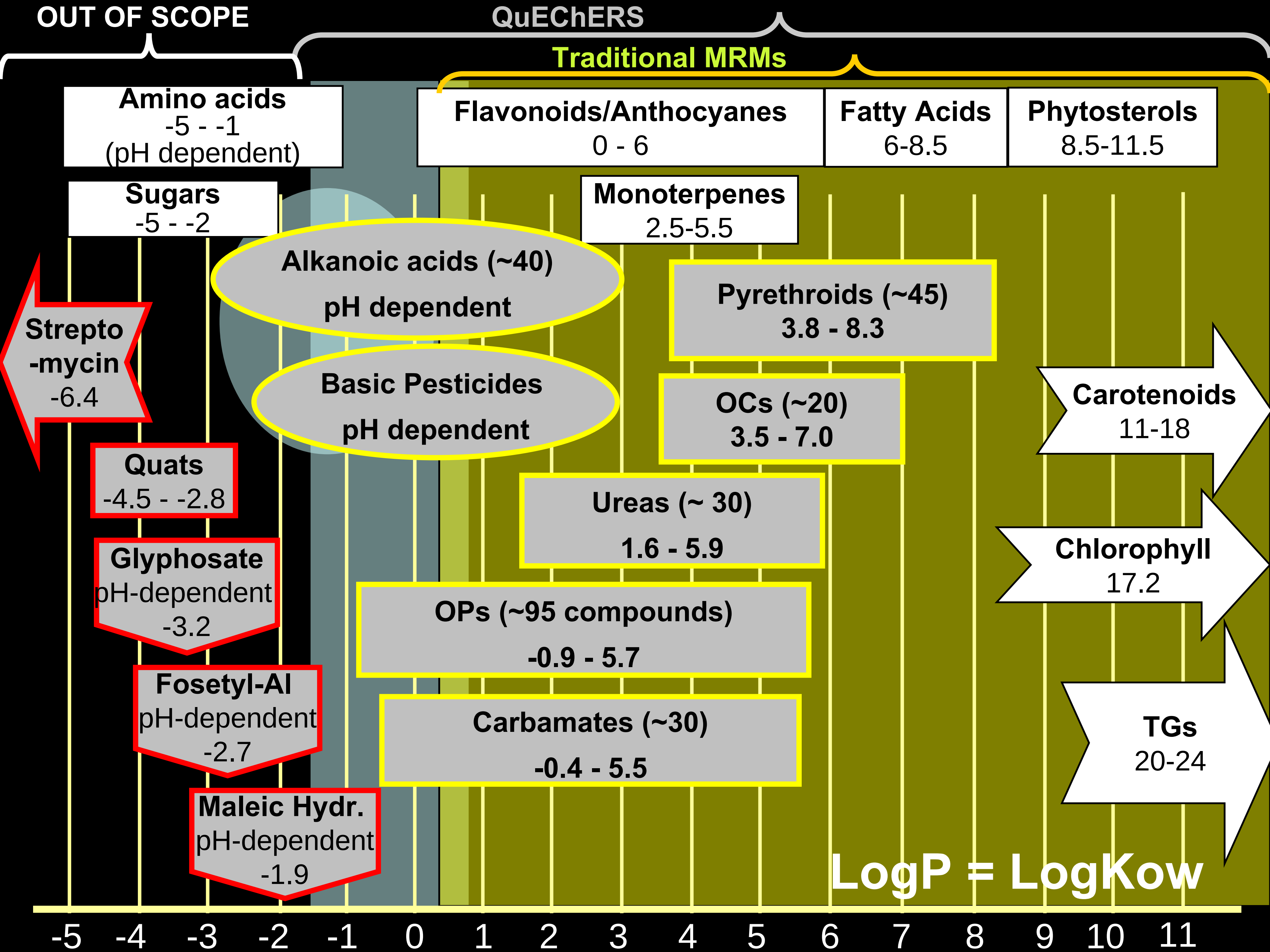


III. Partitioning Losses

Pesticide overview...

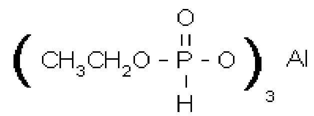
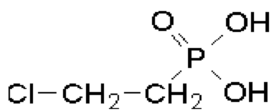
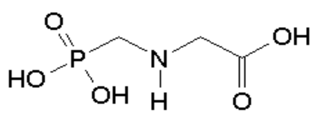
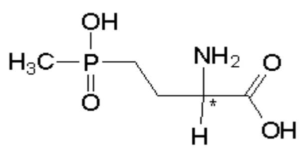
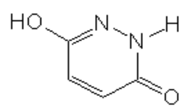
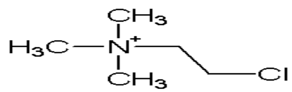
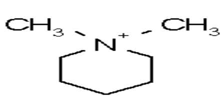
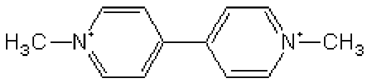
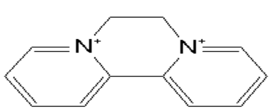





OUT OF SCOPE
QuEChERS
Traditional MRMs
Amino acids
-5 - -1
(pH dependent)
Sugars
-5 - -2
Flavonoids/Anthocyanes
0 - 6
Fatty Acids
6-8.5
Phytosterols
8.5-11.5
Monoterpenes
2.5-5.5
Alkanoic acids (~40)
pH dependent
Basic Pesticides
pH dependent
Strepto
-mycin
-6.4
Pyrethroids (~45)
3.8 - 8.3
OCs (~20)
3.5 - 7.0
Carotenoids
11-18
Quats
-4.5 - -2.8
Ureas (~ 30)
1.6 - 5.9
Glyphosate
pH-dependent
-3.2
Chlorophyll
17.2
OPs (~95 compounds)
-0.9 - 5.7
Fosetyl-Al
pH-dependent
-2.7
Carbamates (~30)
-0.4 - 5.5
TGs
20-24
Maleic Hydr.
pH-dependent
-1.9
LogP = LogKow
-5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11

EURL-PROJECT

METHOD
FOR THE ANALYSIS OF
HIGHLY POLAR PESTICIDES

Name	Formulas	LogKow	H_2O sol. g /L	pka	ADI/ARfD mg/ kg bw	91/414 Status
Fosetyl Al		-2,7 (pH4)	111 (pH6)	4.7	3 / -	IN
Ethephon		-1.89 (pH7)	800 (pH4)	2.82 7.21	0.03 / 0.03	IN
Glyphosate		-3.2 (pH5)	900 (pH?)	0.78 2.29 5.96 11	0.3 / -	IN
Glufosinate		-3.77 (pH5)	500 (pH7)	0.8 2.9 9.8	0.02 / 0.02	IN
Maleic Hydrazide		-1.96 (pH7)	144 (pH7)	5.62	0.25 / -	IN
Chlormequat		-3.47	886	-	0.05 / 0.05	IN
Mepiquat		-2.82	500	-	0.3 / 0.3	IN
Paraquat		-4.5	620	-	0.004 / 0.005	(OUT)
Diquat		-4.6	700	-	0.002 / 0.01	IN

Recoveries with QuEChERS...

Name	QuEChERS Recoveries from Cuccumber		LogP
	ORGANIC phase	WATER phase	
Maleic Hydrazide	30 %	74 %	-1.96 (pH7)
Ethephon	2 %	87 %	-1.89 (pH7)
Daminozide	6 %	87 %	-1.5 (pH7)
Fosetyl Al	< 1 %	83 %	-2,7 (pH4)
Amitrol	5 %	105 %	-0.97 (pH7)

➢ **Method to be developed**

… should not involve liquid-liquid-partitioning

Goals of Project...

- Develop an **Attractive Method** that labs can readily implement

❖ Select relevant pesticides

❖ Keep procedure simple and efficient

- Avoid troublesome steps (derivatisation, SPE-enrichm. etc.)

*- Use **isotopically labelled ISTDs**!*

❖ Use instr. available or shortly available to most labs **(LC-MS/MS)**

❖ **Where possible, simultaneous sample prep. & chromatogr.**

...**LIMITATIONS!**

❖ Test real samples

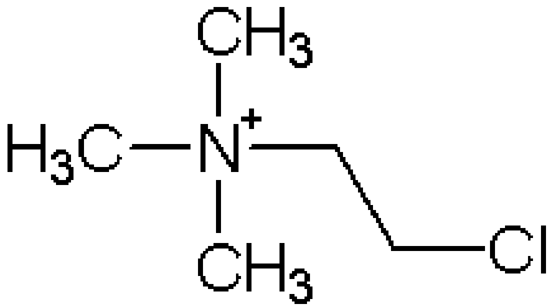
...to localize relevant Pesticide/Commodity Combinations

Overview LC-Conditions / ISTD-availability

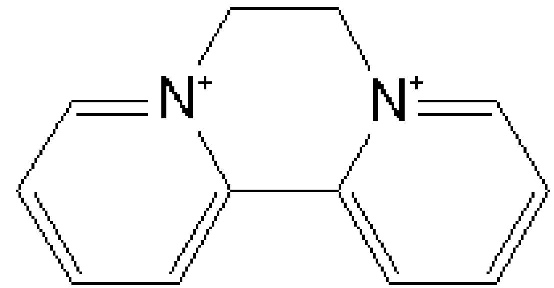
Name	LC-MS/MS ESI (+)	LC-MS/MS ESI (-)	Isotopically Labelled ISTDs
Ethephon	-	++	NEW (D4)
Glyphosate	+	++	Existed
Glufosinate	++	++	NEW (D3)
Chlormequat	++	-	Existed
Daminozide	++	-	Existed
HEPA	-	++	Donation
ETU	++	-	Existed
N-Acetylglufosinate	-	++	NEW (D3)
Streptomycin	++	-	dyhidrostreptomycin
Cyromazine	++	-	Existed
MPPA	-	++	NEW (D3)
AMPA	-	++	Existed
Mepiquat	++	-	Existed
Paraquat	++	-	Existed
Diquat	++	-	Existed
Amitrol	++	-	ordered
Fosetyl Al	+	++	NEW (D15)
Maleic Hydrazide	+	++	NEW (D2)

Polar Pesticides
-
Short Introduction

Chlormequat

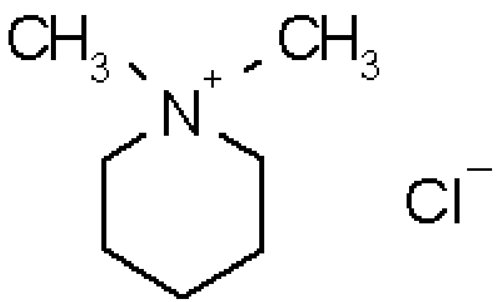


Diquat

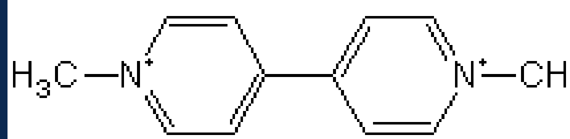


Quats

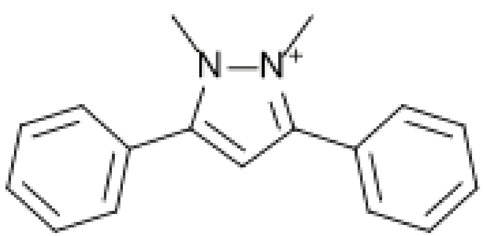
Mepiquat chloride



Paraquat



Difenzoquat



Amenable to QuEChERS

Chlormequat and Mepiquat

❖ **Uses:**

- Growth regulators in many crops (cereals, grapes, pears…)

-In cereals as stem shorteners / strengtheners

-Illegal use of chlormequat in carrots, pears

❖ **Mode of Action:**

- Systemic

❖ **Toxicity to mammals:**

- Comparably low

Paraquat and Diquat

❖ **Uses:**

- Pre- and post-emergency herbicides
- Used on potatoes, canola for defoliation to facilitate mechanical harvest
- Used in banana, coffee, palm, citrus plantations to kill weeds incl. for snake precaution
- Pre-season weed control for many cultivations

❖ **Mode of Action:**

- Non-Systemic (contact-Herbicides)
- Non-selective (kill everything)

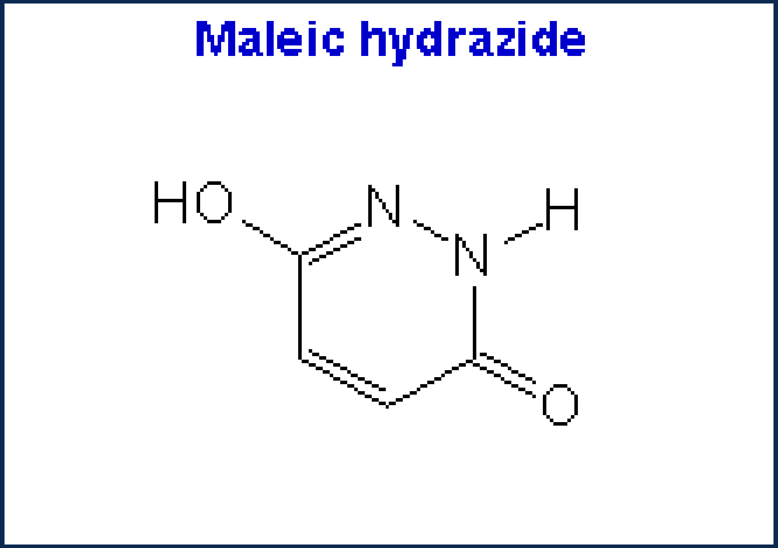
❖ **Dissipation:**

- Quite stable, strongly bind on clay thus low residues expected on crops

❖ **Toxicity:**

- Paraquat (high), Diquat (intermediate)
- High Acute Toxicity to farmers upon inhalation (irreversible lung damage)

Maleic hydrazide



Maleic Hydrazide

- **Use:**

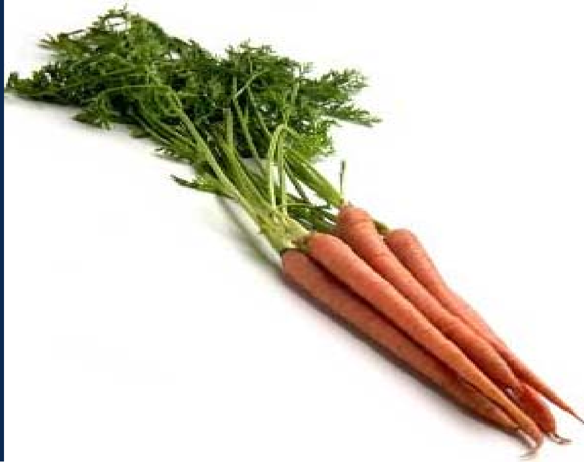
Plant growth regulator, herbicide
- Inhibits sprouting (potatoes, onions, carrots)
- Induces dormancy in citrus fruits
- Used as herbicide in combination with 2,4-D

- **Mode of Action:**

- Systemic, absorbed by leaves and roots, inhibits cell division

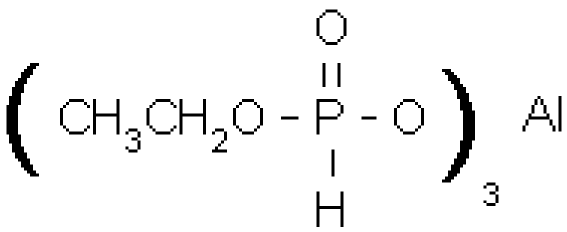
- **Toxicity:**

- Comparably low toxicity to mammals.

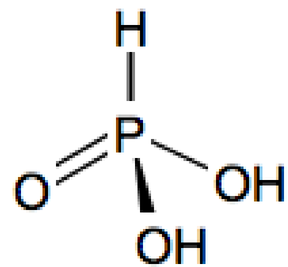


Fosetyl Aluminium

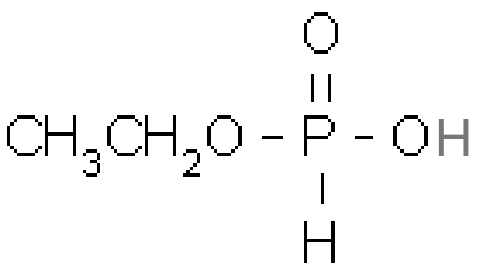
Fosetyl-aluminium



Phoshorous acid (Phosphonic acid)



Fosetyl (Ethylphosphonic acid)



Current Residue Definition:
Fosetyl-Al (sum of fosetyl, phosphorous acid and their salts, expressed as fosetyl)

Fosetyl-Aluminium

❖ **Use:**

- Fungicide
- Control of diseases caused by fungi (e.g. *Phythophthora*)
- On a variety of crops (e.g. berries, fruiting vegetables, vine)

Note: *Fosetyl-Al not the only source of phosphonic acid*

- *Phosphonic acid + salts used as plant strengtheners (incl. organic farming).*
- *Additional source: foliar P fertilizers*.

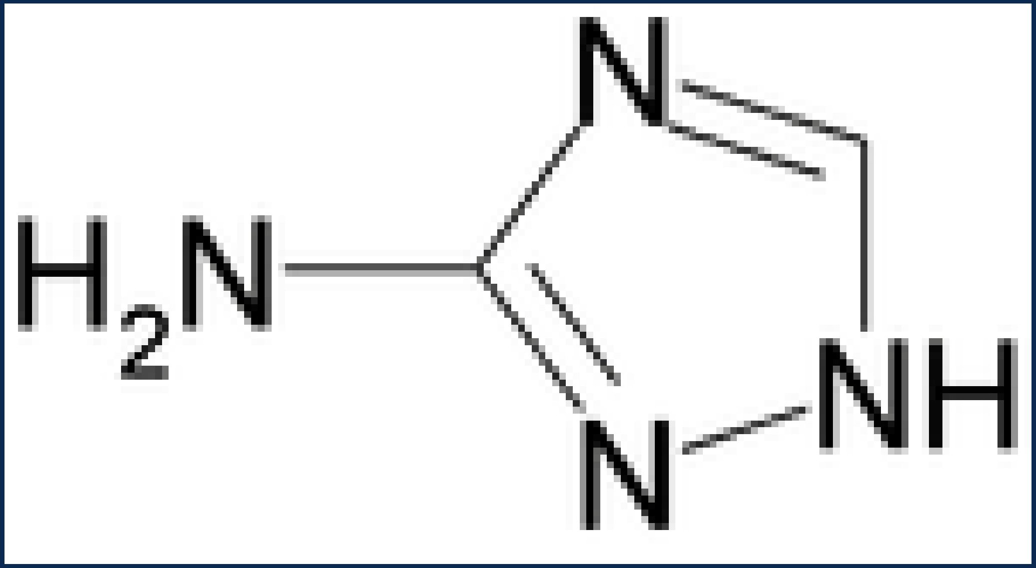
❖ **Mode of Action:**

- Systemic, absorbed by leaves and roots,
Phosphonic acid degradant activates plant's defence system against fungal infections, e.g. peronospora in vine cultures

❖ **Toxicity:**

- Comparably low toxicity to mammals.

Amitrol = 3AT



Amitrol

❖ **Use:**

- Non-Selective Herbicide (unlike all other triazoles ➔ Fungicides)
- Control annual weeds mostly in non-crop lands
- Also in food cultivations e.g. Kiwi (NZ), Soy, Cereals (pre-planting)

❖ **Mode of Action:**

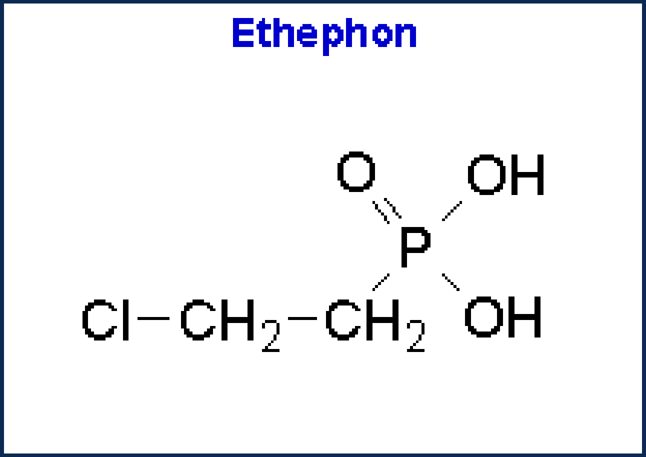
- Systemic, absorbed by leaves and roots,

❖ **Toxicity:**

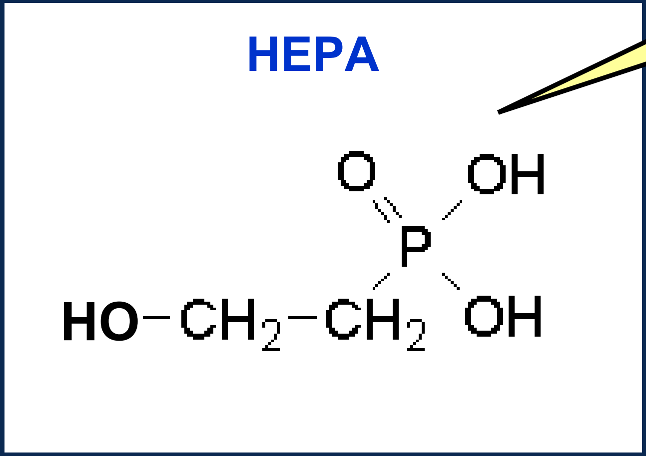
- Proven endocrine disruption activity,
- Strongly suspected to induce thyroid cancer

Included in a biocide ban
approved by European Parliament in Jan. 2009

Ethephon / HEPA



Currently, no legal relevance in food but toxicological concerns



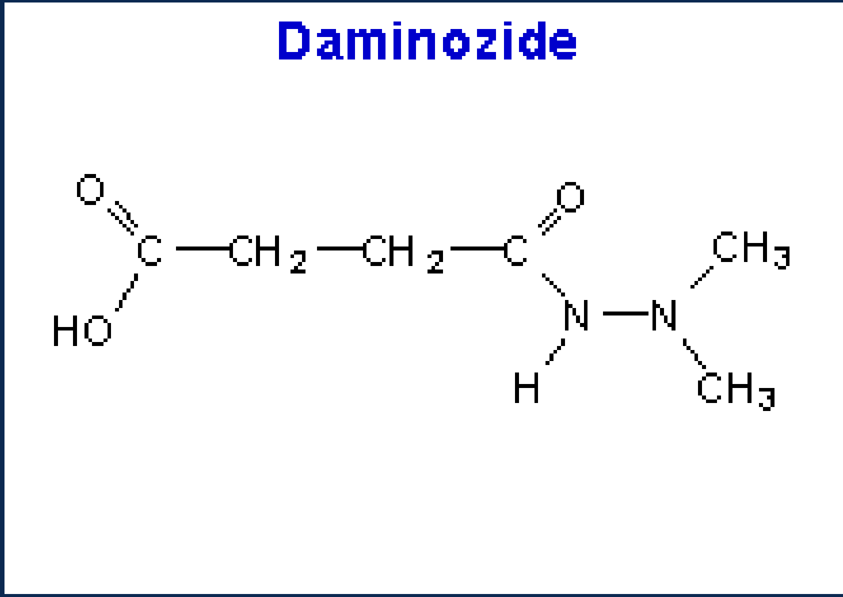
Ethephon

❖ **Use:**
- Plant growth regulator
- promotes pre-harvest ripening in apples, berries, citrus etc.
- facilitates harvesting by loosening the fruits
- shortens and strengthens the stem in cereals, maize… etc.
- accelerates post-harvest ripening in mango, pine apples etc.

❖ **Mode of Action:**
- Systemic, decomposes to ethylene, affects growth and ripening

❖ **Toxicity:**
Comparably low toxicity to mammals.

Daminozide



Daminozide

❖ Use:

- Plant growth regulator
- Inhibition of internodial elongation. More compact plants.
- Formerly used on apples and peanuts
- Not used in Europe any more

❖ Mode of Action:

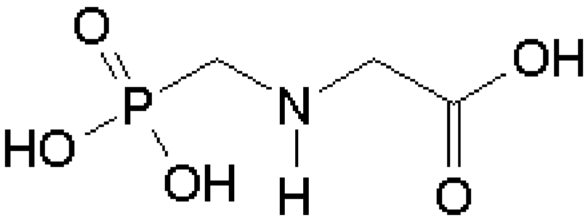
- Systemic, absorbed by the leaves, translocated throughout plant

❖ Toxicity:

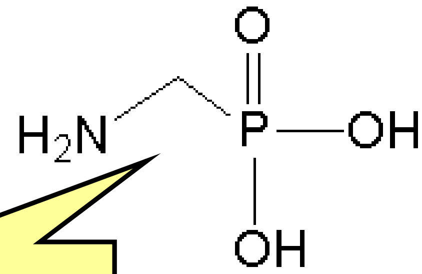
- Comparably low toxicity to mammals.

Glyphosate, Glufosinate

Glyphosate

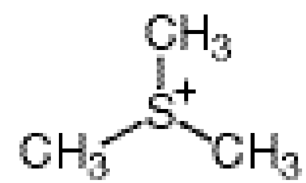


AMPA



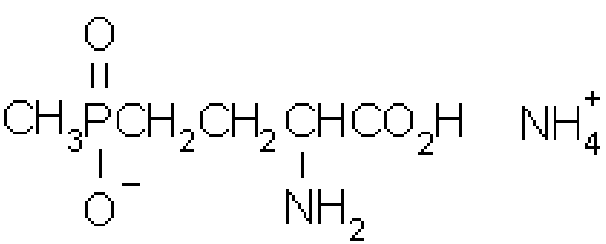
No legal relevance in food

Trimethylsulfonium Trimesium

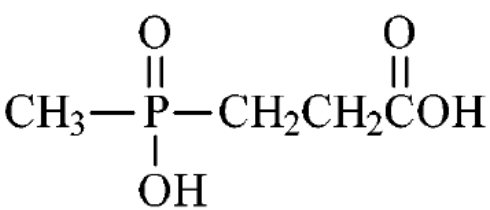


Counter-ion of Glyphosate with proper MRL

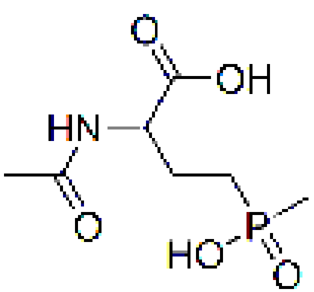
Glufosinate-ammonium



3-MPPA = MPP



NAG = N-acetyl-glufosinate



NAG is formed in transgenic crops as metabolite, next to check…

Glyphosate and Glufosinate

❖ **Use:**

- Broad spectrum Herbicides
  - ➢ Against weeds, grasses,
  - ➢ As desiccants to dry off crops before harvest (potato, canola…)
  - ➢ Transgenic crops resistant to glyphosate / glufosinate (Corn, Soy, Canola…)
  - ➢ Combination with other herbicides (often double resistant GM-crops)

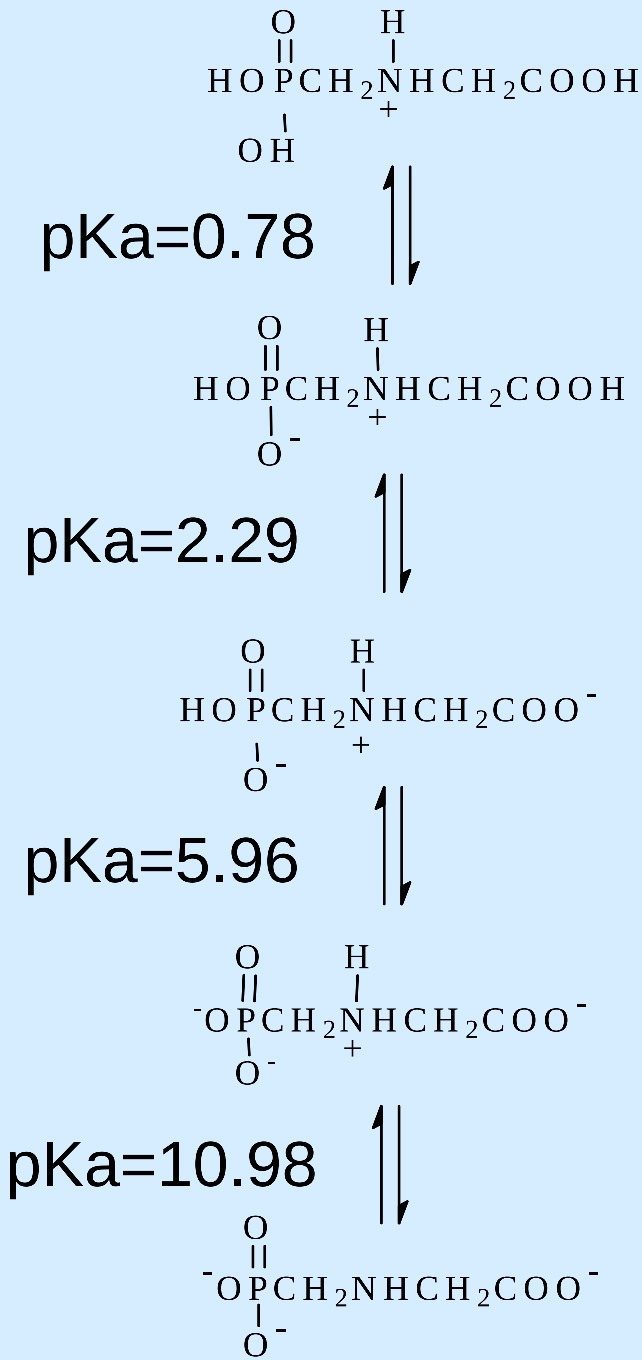
  - ➢ Glyphosate makes 60% of worldwide herbicide sales!!!

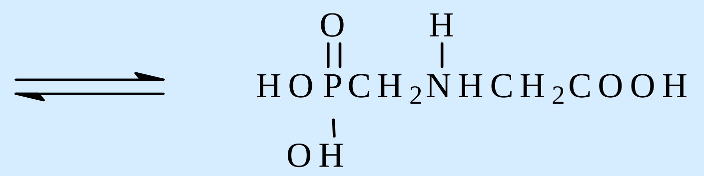
❖ **Mode of Action:**

- Systemic
- Inhibit production of amino acids essential for plant growth

❖ **Toxicity:**

- Comparably low toxicity to mammals and insects.





Minor tautomer

Glyphosate, pH dependency

Ref: Chamberlain et al. (1996). Pestic. Sci., **47.**

Multiresidue Method for „SRM Analytes“

Weigh in 10g of frozen sample
(5 g for dry material)

Add ISTD-solution

Add 10 mL water (for dry material)

Add 10ml of Extraction solvent
(MeOH w. 1% Formic Acid)

Shake vigorously 1 min

Centrifuge 5min, 4000RPM

Filter Aliquot
(through 0,45µm syringe filter)

Fill in vial

Perform LC-MS/MS analysis

Multiresidue Method for „Paraquat Diquat“

Weigh in 10g of frozen sample
(5 g for dry material)

Add ISTD-solution

Add 10 mL water (for dry material)

Add 10ml of Extraction solvent
MeOH + 0.1 M HCl in water (1:1)

Shake 1 min, heat 15 min at 80°C and re-shake 1 min

Centrifuge 5min, 4000RPM

Filter Aliquot
(through 0,45µm syringe filter)

Fill in vial

Perform LC-MS/MS analysis

Variation for Paraquat + Diquat

Polar Pesticides

- Measurement
- Validation
- Analysis of real samples

Pesticide Name	Methods				Isotopically labelled ISTDs		ESI neg	ESI pos
	"Amitrol & Co."	„Quats & Co."	"Glyphosate & Co."	"Fosetyl /MH"				
Amitrol	x				Ordered			
ETU	x				NEW	D4	-	++
PTU	x				NEW	D6	-	++
Chlormequat	x	x			Existed	D4	-	++
Mepiquat	x	x			Existed	D3	-	++
Daminozide	x	x			Existed	D6		
Cyromazine	x	x			NEW	D6	+	++
Paraquat		x			Existed	D4	-	++
Diquat		x			Existed	D6	-	++
Glufosinate/MPPA/NAG			x		3x NEW	D3/D3/D3	++	++/++/-
Glyphosate/AMPA			x		Existed	**G:** $^{13}C2$ ^{15}N **AMPA:** ^{13}C ^{15}N	++	(+)
Ethephon/HEPA			x		2x NEW	D4/D4	++	-
N-Acetylglufosinate			x		NEW	D3	++	-
Fosetyl-Al			Screening	x	NEW	D15 (=3x5)	++	(+)
Maleic hydrazid				x	NEW	D2	++	(+)

	COLUMN: ObeliscR (SIELC) 150x2mm 5µ;
Quats & Co.	**Eluent**: A: 20 mmol NH_4-formate in H2O (**pH 3** w. FA); B: ACN (**Gradient:** 20%A to 80%A)
Amitrol & Co.	**Eluent**: A: 50 mM NH_4formate in H2O; B: ACN (**Gradient:** 3%A to 70%A)
Glyphosate& Co.	**COLUMN: AS11 (Dionex) 250x2mm;** **Eluent**: A: H2O; B: 1mM citric acid adj. to pH11 w. DMA (**Gradient:** 0%B to **50%B**)
Fosetyl / MH	**COLUMN: ObeliscR (SIELC) 150x2mm 5µ;** **Eluent**: A: 50 mM NH4formate + 0,1% FA ; B: ACN (**Gradient:** 3%A to 70%A)

"Quats & co."

- Chlormequat
- Mepiquat
- Diquat
- Paraquat

Require stronger extraction conditions than other compounds

Generic conditions OK for screening

Problems with simultaneous analysis of this pair

- Daminozide
- Trimesium cation
- Amitrol

LC-MS / MS Method: "Quats & co."

Paraquat/Diquat and Amitrol could NOT be included in same LC-method

- Paraquat/Diquat require acidic eluent:

A: 20 mMol NH_4formate in water (pH3 with FA)
B: ACN
Gradient: 20% A to 90% A

- Amitrol "hates" acidity

A: 50 mMol NH_4formate in H2O;
B: ACN
Gradient: 3% A to 70% A

Chlormequat
Mepiquat
Daminozide
Trimesium

} work well under both conditions

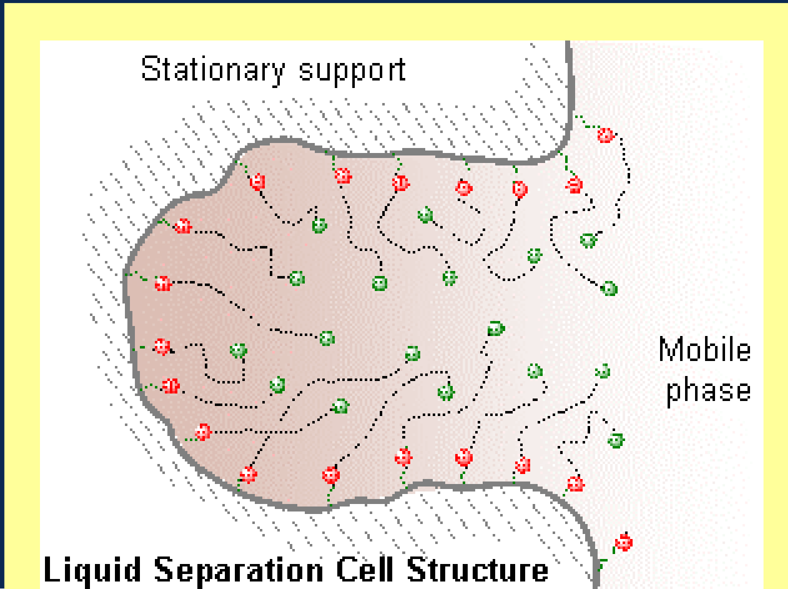
LC-Column used...

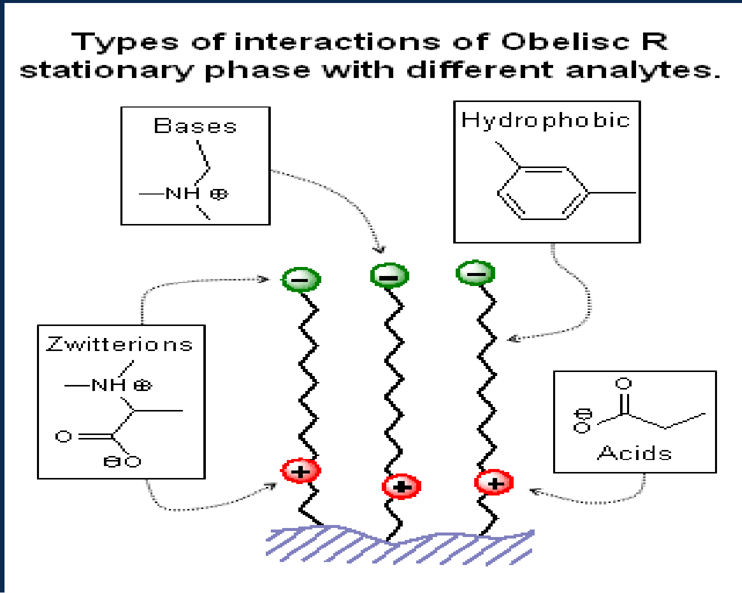
Column used for „Quats“: **ObeliscR** by Sielc

R stands for RP-Charakter, but we have **used it in NP-mode!**

LiSC (Liquid Separation Cell)

- Zwitterionic Ligands
- „Small pores act like living cells„
- „Pore-openings act as a membrane“
- „Different environment inside and outside the cells“

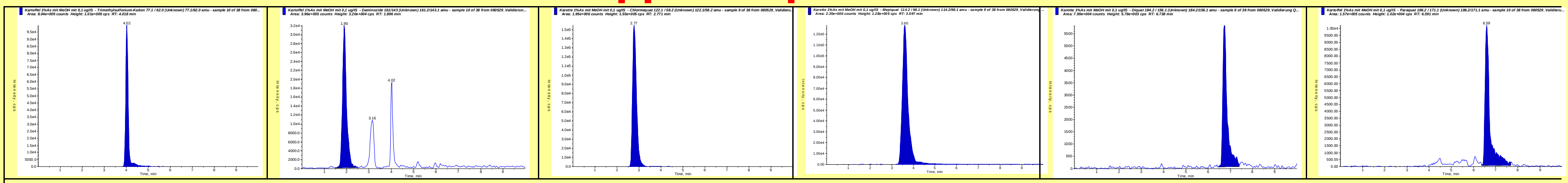




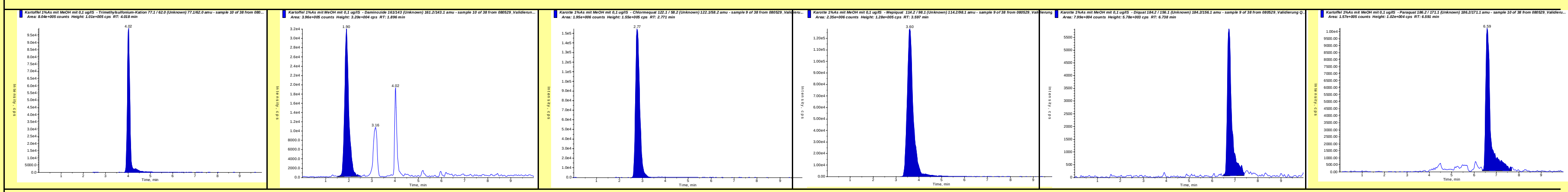
LC-MS/MS Method: “Quats-Co“ with ObeliscR

Trimesium	Daminozide	Chlormequat	Mepiquat	Diquat	Paraquat
77/62	161/143	122/58	114/98	184/156	186/171

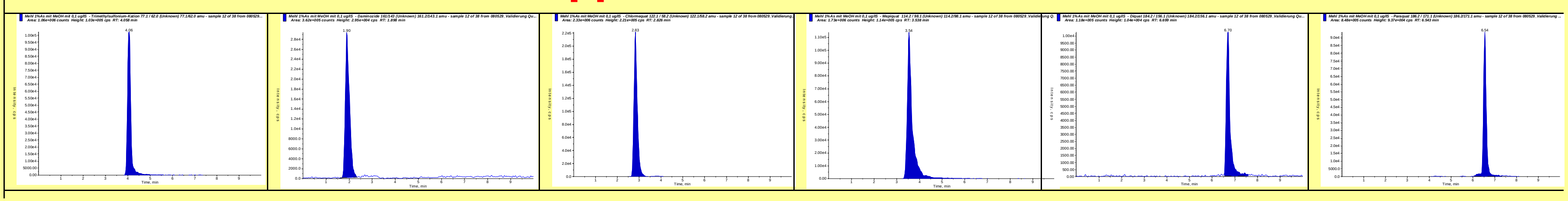
0.1 ppm on potato



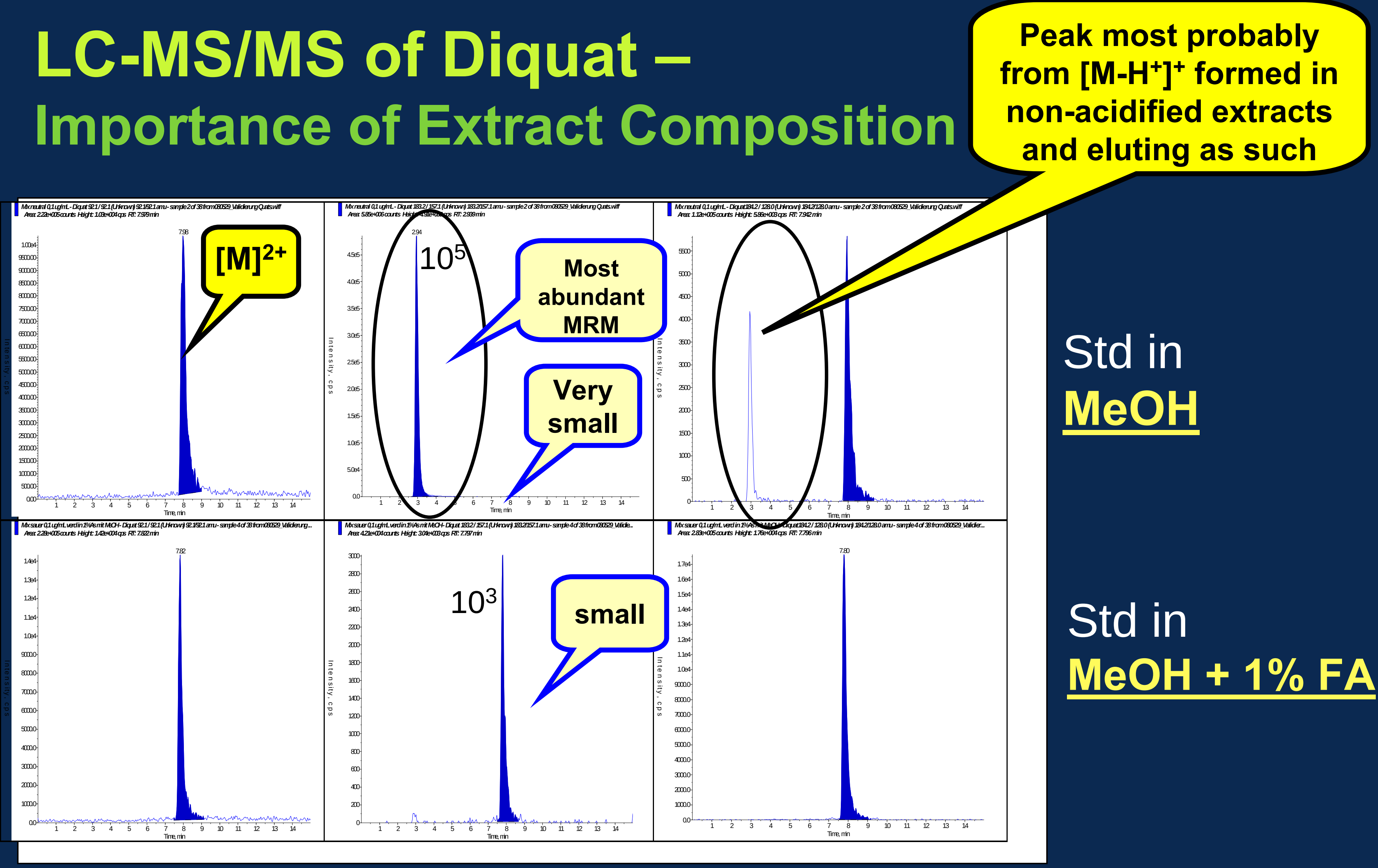
0.1 ppm on pineapple



0.2 ppm on wheat flour



LC-MS/MS of Diquat –
Importance of Extract Composition



Std in **MeOH**

Std in **MeOH + 1% FA**

m/z 92/92
M^{2+}

m/z 183/157
$[M-H^+]^+$
Deprotonated D.

m/z 184/128
$M^{+\bullet}$
Reduced D.
(Radical)

Recoveries - „Quats & Co.“-method

Matrix: Wheat flour 0.1 mg/kg

Name	Matrix mached cal. (with ISTD)		matrix mached cal.) (w/o ISTD)		Matrix Effects cal. in solv.= 100%	
	Rec.	RSD	Rec.	RSD	Compound	ISTD
Chlormequat	106	5.5	106	5.5	82	80
Mepiquat	106	1.9	108	3.1	77	70
Diquat	103	6.2	69	4.4	72	60
Paraquat	114	6.5	75	9.4	37	34
Daminozide	101	2.8	113	4.5	95	99
Trimesium	no ISTD	no ISTD	104	1.9	85	-

Recoveries using the generic extraction method

Column: ObeliscR (SIELC) 150x2mm 5µ;
Eluent: A: 20 mMol NH4formate at pH 3.0; B: acetonitrile (gradient: 20% A to 80% A)

Recoveries - „Amitrol & Co.“-method

Matrix: Cucumber 0.01 mg/kg

Name	Matrix-Mached cal. (with ISTD)		Solvent-Based Cal. (with ISTD)		Matrix Effects cal. in solv.= 100%
	Rec.	RSD	Rec.	RSD	Compound
Amitrol	107 (no ISTD)	4.6	90 (no ISTD)	3.6	93
Mepiquat	106	5.7	105	5.3	93
Chlormequat	107	1.6	103	1.6	100
ETU	95	2.2	93	2.8	96
PTU	99 (no ISTD)	3.1	91 (no ISTD)	3.7	93
Cyromazine	97	4.3	106	3.2	94
Daminozide	107	3.5	86	3.1	80

Recoveries

Matrix: Orange 0.01 mg/kg

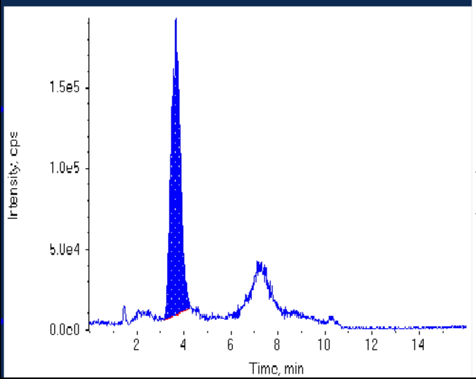
Name	Matrix-Mached cal. (with ISTD)		Solvent-Based Cal. (with ISTD)		Matrix Effects cal. in solv.= 100%
	Rec.	RSD	Rec.	RSD	Compound
Amitrol	109 (no ISTD)	6.0	56 (no ISTD)	5.5	46
Mepiquat	107	8.2	101	9.0	38
Chlormequat	102	3.7	101	3.5	42
Daminozide	120	7.7	72	5.9	50

Column: ObeliscR (SIELC) 150x2mm 5µ;
Eluent: A: 50 mMol NH4formate; B: acetonitrile

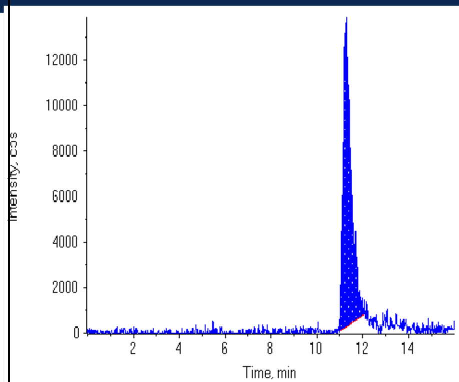
Recoveries - „Amitrol & Co.“-method

Matrix: Cucumber 0.01 mg/kg

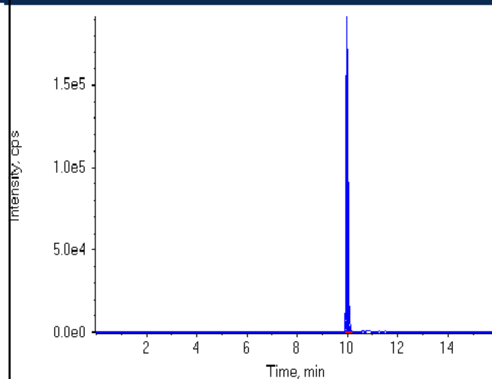
Amitrol 85 /43



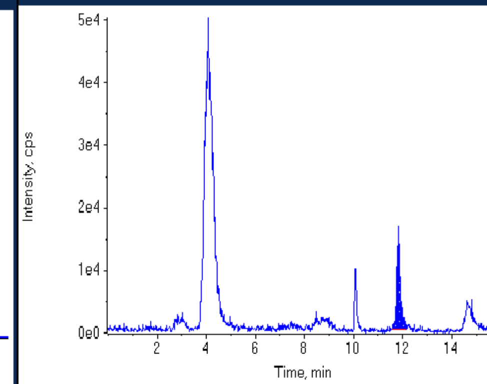
Mepiquat 114/98



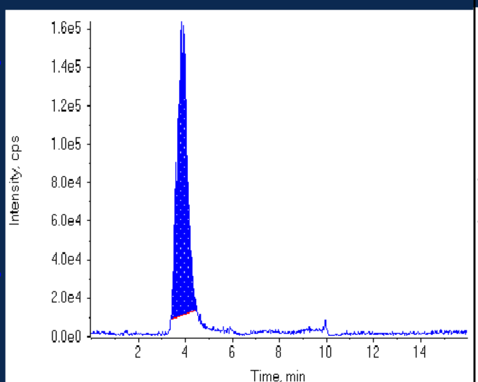
Chlormequat 122/58



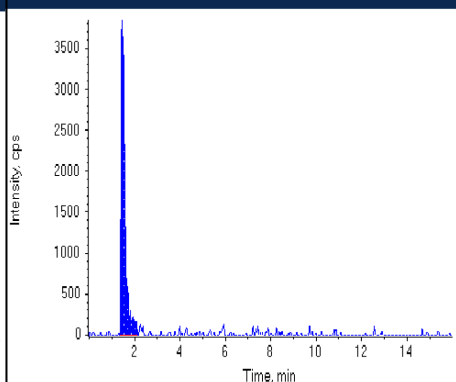
Daminozide 161 /143



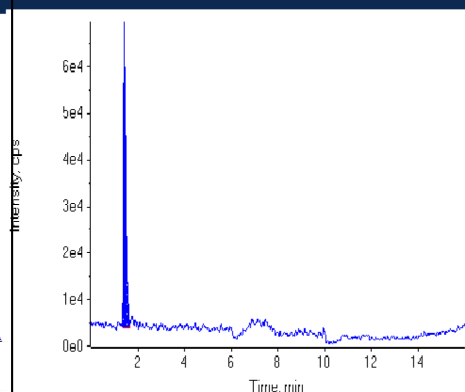
Cyromazine 167/68



ETU 103/44



PTU 117/100



“Glyphosate & Co.”

- Glyphosate
- Glufosinate
- AMPA
- 3-MPPA

- Ethephon
- HEPA

LC-Column used

Anion-Exchange Column

Active group: e.g. quarternary amine (permanently cationic)

$$-N(CH_3)_3^+ \ OH^-$$

Column used for Glyphosate & Co.:

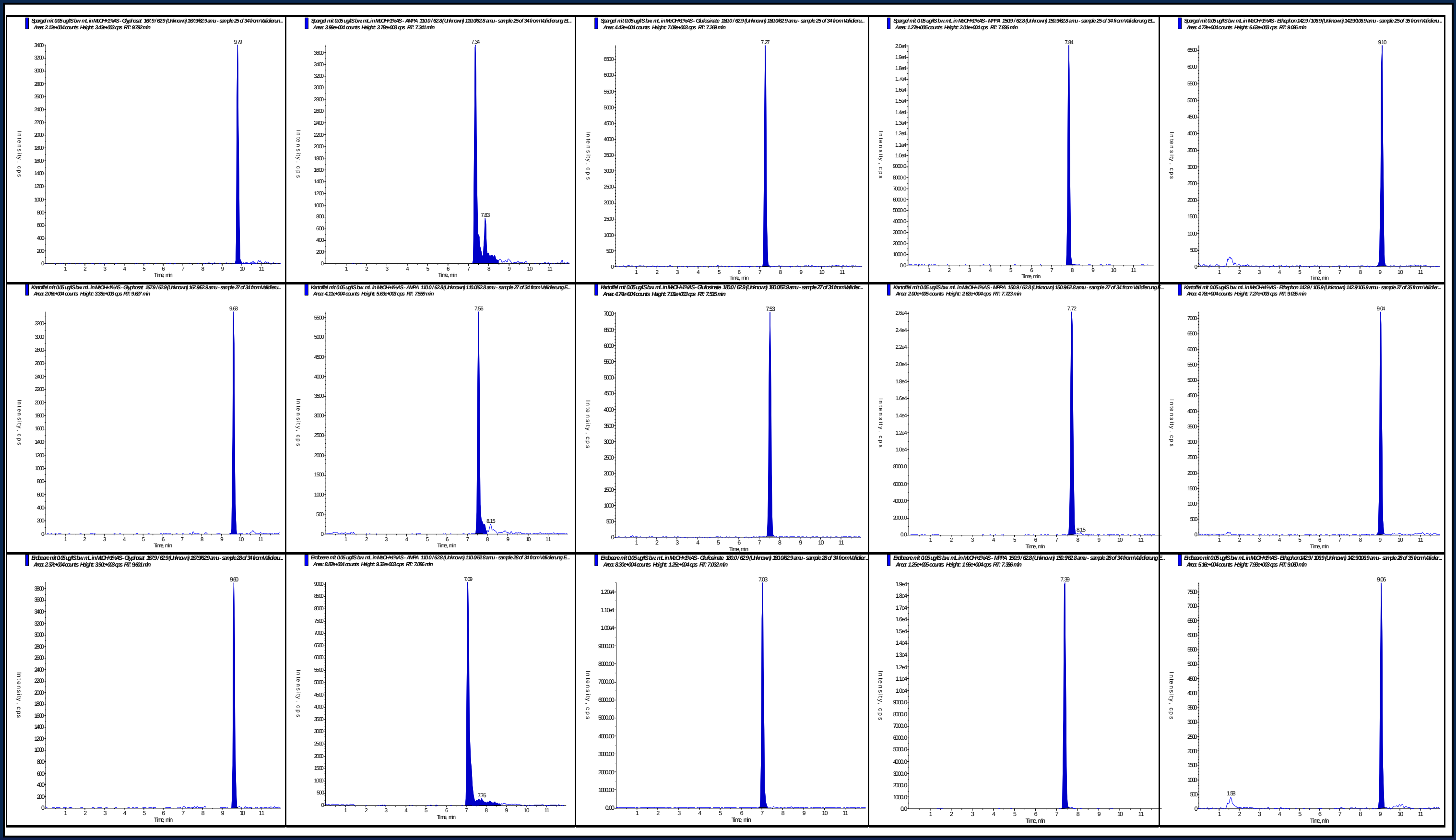
IonPac AS11 by Dionex

Quarternary Ammonium bound on a DVB/Ethyl-DVB co-polymer

LC-MS/MS

problems with RT-shift / peak shape can occur depending on matrix!
(ISTD fortunately matches for this)

Glyphosate	AMPA	Glufosinate	MPPA	Ethephon
168/63	110/63	180/63	150/63	143/107



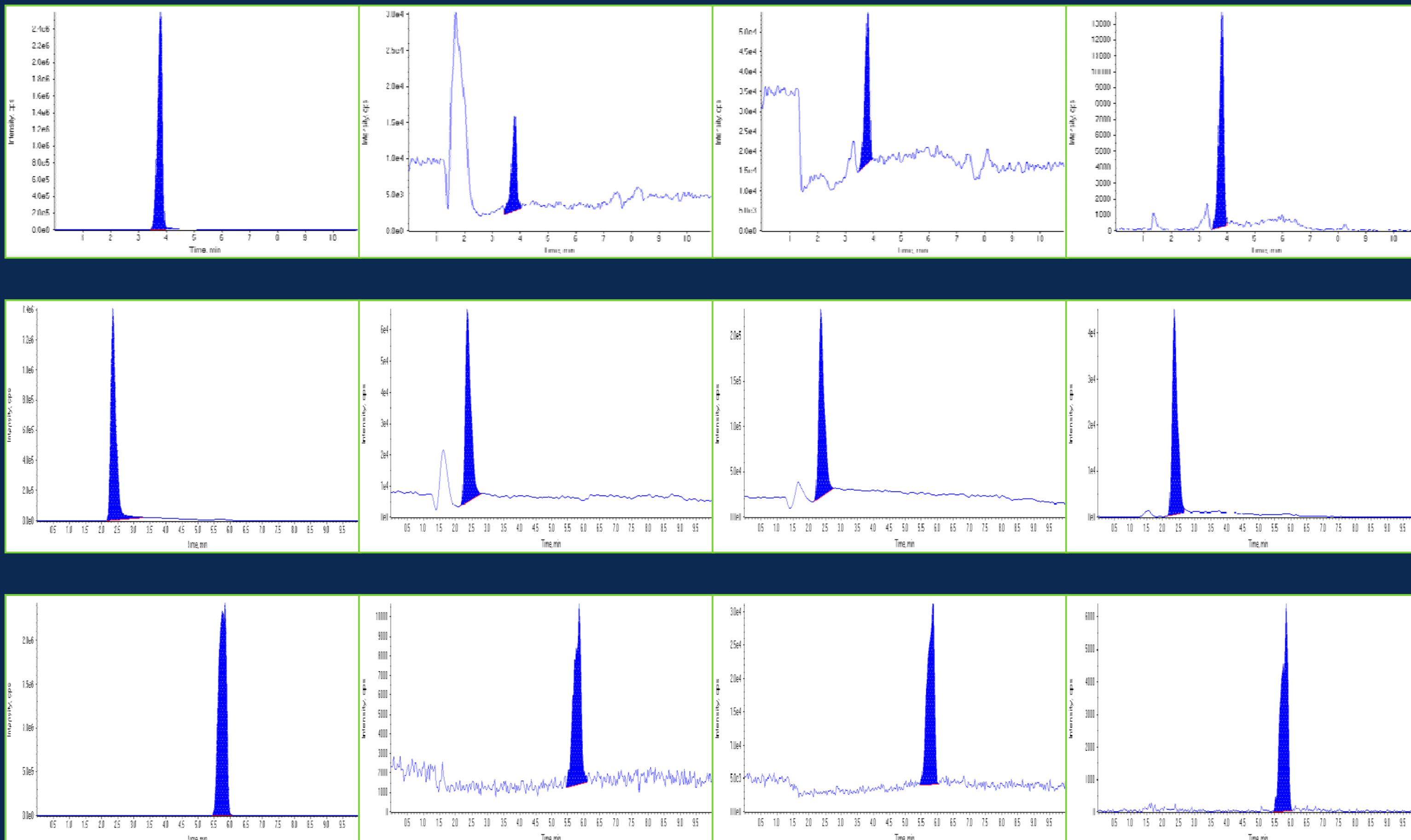
0,1 ppm asparagus

0,1 ppm potato

0,1 ppm strawberry

HEPA in real samples

Note RT shift, but ISTD behaves the same…



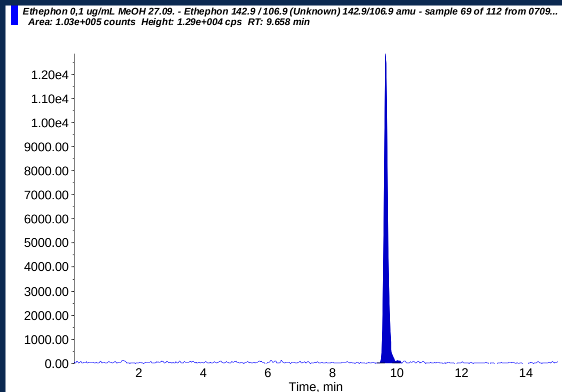
Oats
0.034 mg/kg

Figs
0.12 mg/kg

Pineapple
0.009 mg/kg

HEPA D4 (IS)	HEPA	HEPA	HEPA
129/78.8	125/94.8	125/78.9 T	125/62.8

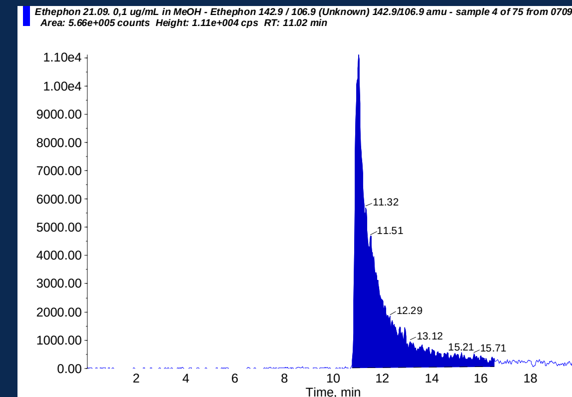
Chromatographic experiments - Ethephon

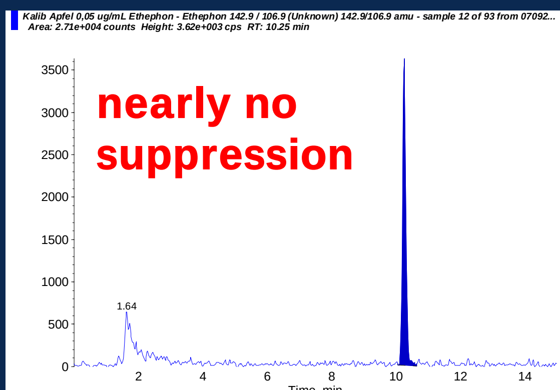


Ethephon

0,1 µg/mL

In SOLVENT

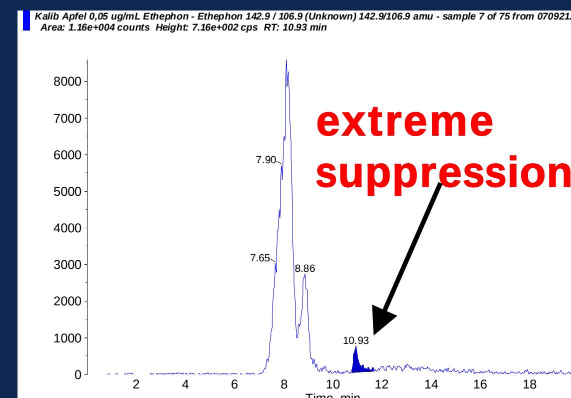




Ethephon

0,05 µg/mL

in APPLE



Dionex AS11 250x2mm

A: water

B: 1 mMol citric acid in water, adjusted to pH 11 with Dimethylamin

Atlantis HILIC 100x2mm

A: 100 mMol NH4formate in water

B: acetonitrile

Recoveries - „Glyphosate & Co.“- Method

Matrix: Wheat flour 0.1 mg/kg

Name	Matrix mached cal. (with ISTD)		Matrix mached cal. (w/o ISTD)		Matrix Effects cal. in solv.= 100%	
	Rec.	RSD	Rec.	RSD	Compound	ISTD
Glyphosate	118	5.8	83	5.9	277	289
Glufosinate	no ISTD	no ISTD	85	4.6	194	-
AMPA	119	5.7	83	7.7	247	236
3-MPPA	no ISTD	no ISTD	85	1.3	161	-
Ethephon	109	5.9	91	7.1	143	141

HEPA-Recoveries

Matrix: Orange 0.02 mg/kg

Name	Matrix mached cal. (with ISTD)	
	Rec.	RSD (n=5)
HEPA	119	3.5

Isotope-labelled ISTD provided by Bayer CropScience

“Fosetyl, MH” - Method

- Fosetyl-Aluminium
- Maleic Hydrazide

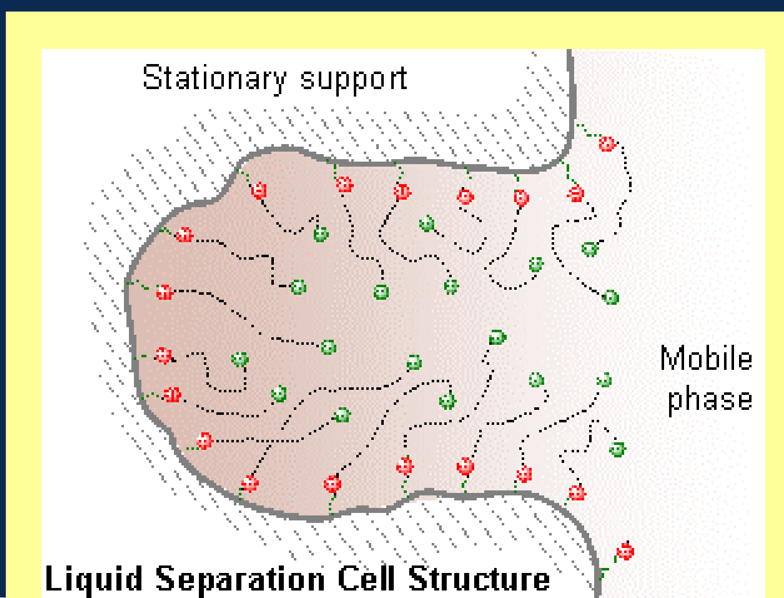
LC-Column used...

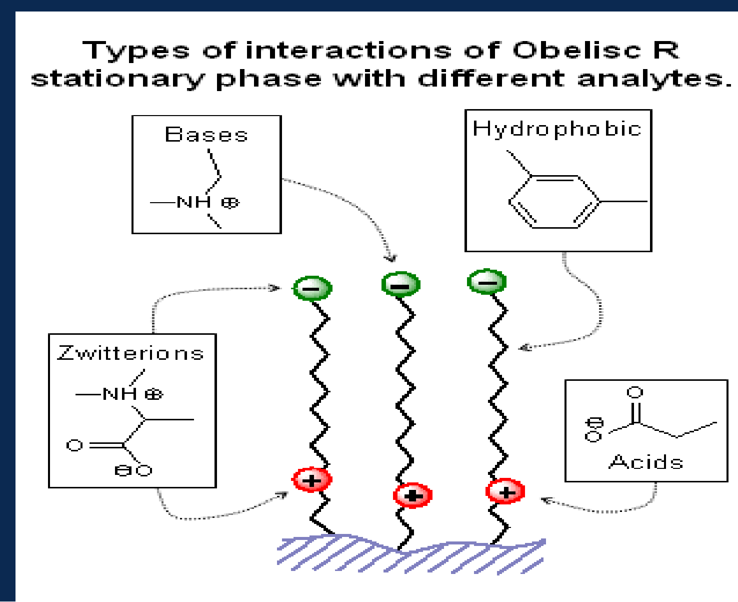
Column used for „Fosetyl andMH“: **ObeliscR** by Sielc

R stands for RP-Charakter, but we have **used it in NP-mode!**

LiSC (Liquid Separation Cell)

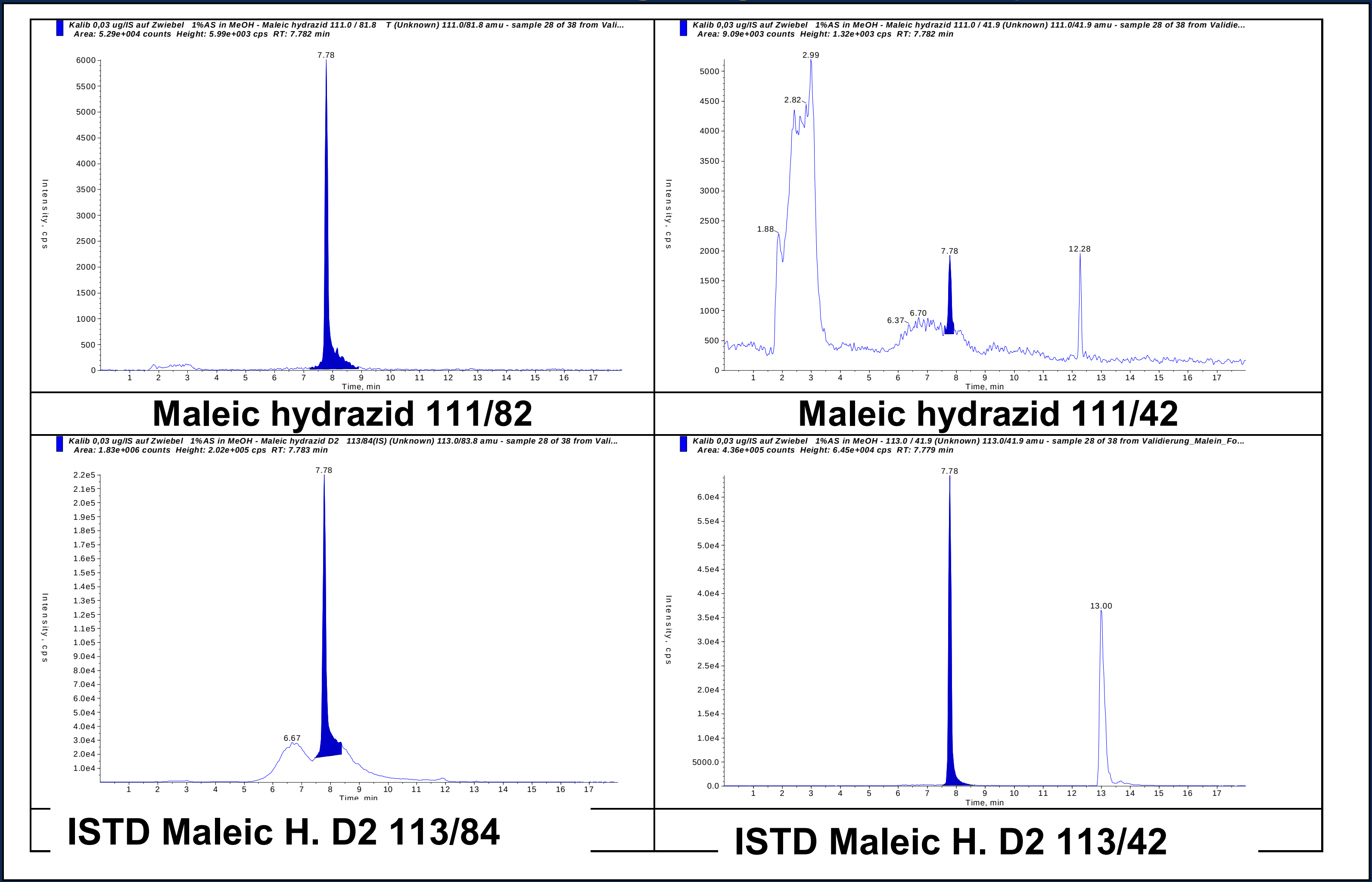
- Zwitterionic Ligands
- „Small pores act like living cells„
- „Pore-openings act as a membrane“
- „Different environment inside and outside the cells“





LC-MS/MS Maleic Hydrazide

Onion with 0.06 mg/kg maleic hydrazide

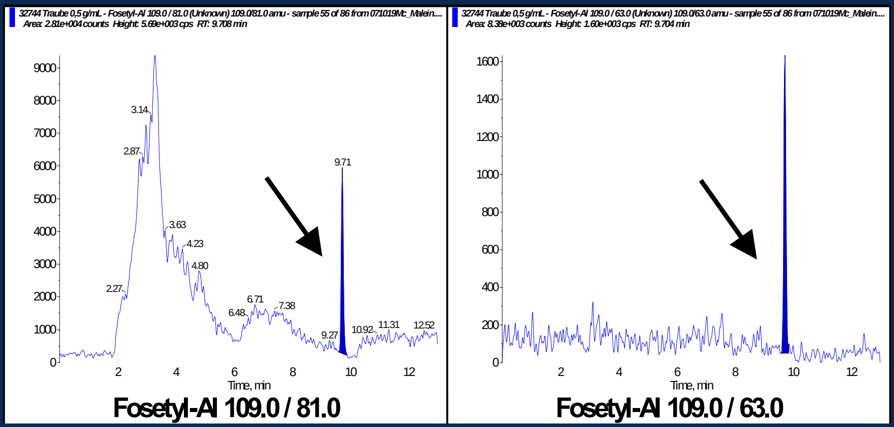


LC-MS/MS of Fosetyl

Longer RT, less suppression with neutral extracts

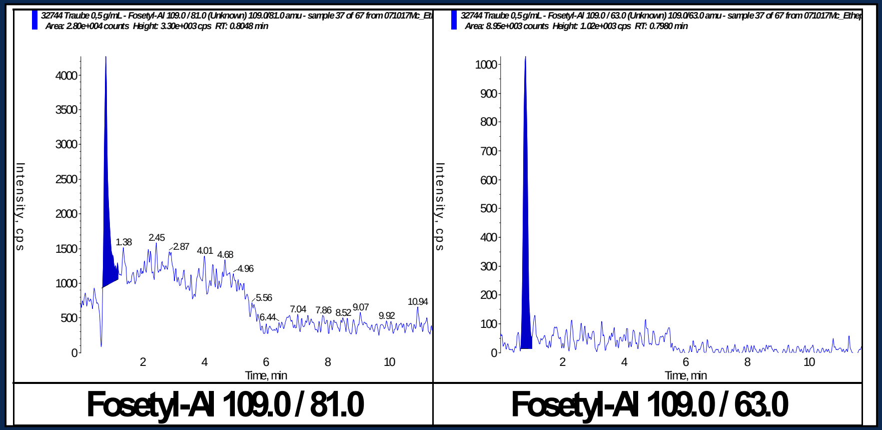
Grapes 0,5 g/mL with 0,03 mg/kg

- Atlantis HILIC - late RT



Moderate matrix effects
(20% suppression)

- Dionex-AS11 - early RT

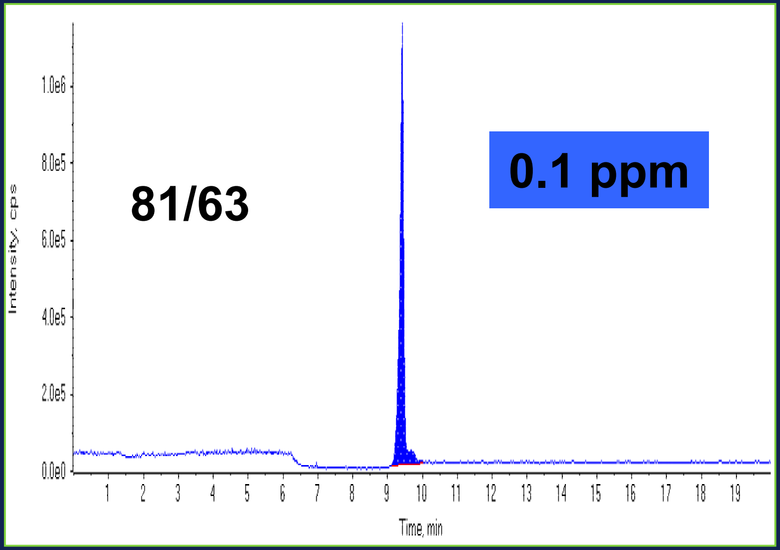


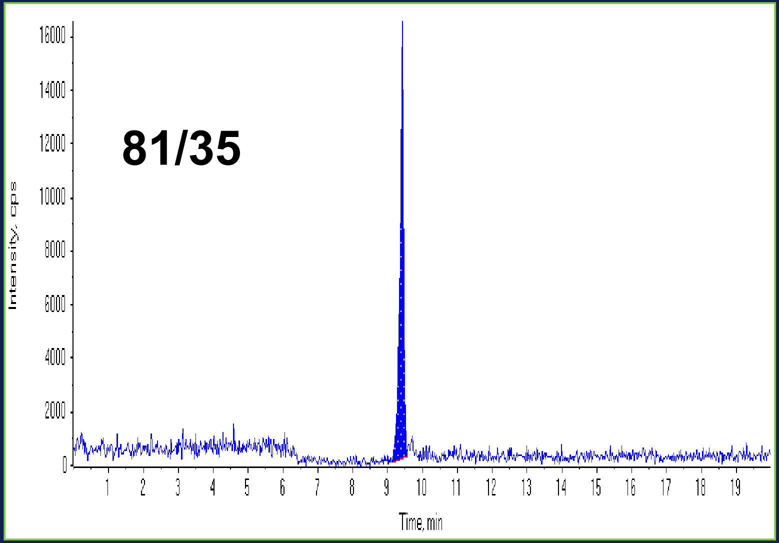
Strong matrix effects
(80% suppression!)

- HILIC also works well for Fosetyl

- „Glyphosate & Co.-Method„ not optimal but suitable for Fosetyl SCREENING

LC-MS/MS of Phosphorous acid

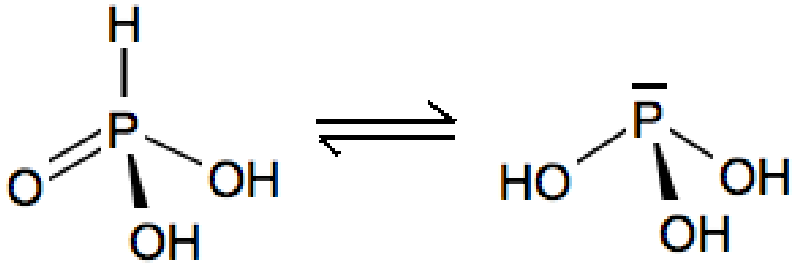




w. "Glyphosate+co" method

Problem: no isotopically labelled ISTD

Phoshorous acid (Phosphonic acid)



Shifts of H between O and P
Exchange of OH in aqueous media

Recoveries - „Fosetyl & MH“- method

Fosetyl: Strawberry 0.1 mg/kg

Maleic Hydrazide: Onion 0.1 mg/kg

Compound	Matrix mached cal. (with ISTD)		Matrix mached cal. (No ISTD)		Matrix Effects cal. in solv.= 100%	
	Rec.	RSD	Rec.	RSD	Compound	ISTD
Fosetyl-Al	98	4.6	96	4.3	54	44
Maleic Hydrazide	105	3.0	112	1.9	62	72

LODs

Limits of detection in fruits, vegetables and cereals

Method	Pesticide	Cucumber	Orange	Barley
M1	**Amitrol**	0.01	0.01	0.02
M 1	**ETU**	0.01	0.02	0.02
M1	**PTU**	0.01	0.02	0.02
M1/M2	Chlormequat	0.005/0.005	0.005/0.005	0.01/0.01
M1/M2	**Mepiquat**	0.005/0.01	0.005/0.01	0.001/0.02
M1/M2	**Cyromazine**	0.01/0.01	0.01/0.01	0.02/0.02
M1/M2	**Daminozide**	0.01/0.02	0.01/0.02	0.02/0.04
M1/M2	**Trimethylsulfonium-Cation**	0.01/0.005	0.01/0.005	0.02/0.01
M1/M2	**Morpholine**	0.01	0.01	n.a.
M2	**N,N-Dimethylhydrazine**	0.005	0.005	0.01
M2	**Diquat**	0.005	0.005	0.005
M2	Paraquat	0.005	0.005	0.005
M3	**Ethephon**	0.01	0.01	0.02
M3	**HEPA**	0.01	0.01	0.02
M3	**Glyphosate**	0.01	0.02	0.02
M3	**AMPA**	0.01	0.02	0.02
M3	**Glufosinate**	0.01	0.02	0.02
M3	**MPPA**	0.01	0.02	0.02
M3	**N-AG**	0.02	0.02	0.02
M3/M4	**Fosetyl**	0.005/?	0.005/?	0.005/?
M4	**Maleic Hydrazide**	0.01	0.01	0.02
M5	**Streptomycin**	0.01	n.a.	n.a.
M5	**Kasugamycin**	0.01	n.a.	n.a.

Use of ISTDs

Approx. cost of ISTDs

Name of ISTD	€-cent per portion (exemplary)	
	If added to sample (2µg)	If added to 1 mL sample extract aliquot (0.1µg)
Chlormequat D4	6 c	0.3 c
Mepiquat D3	**136 c**	**7 c**
Diquat D4	8 c	2 c
Paraquat D6	**146 c**	**7 c**
Glyphosate 1,2-$^{13}C2$ ^{15}N	**660 c**	**33 c**
Ethephon D4	13 c	0.6 c
Fosetyl-Al D15	8 c	0.4 c
Maleic hydrazide D2	12 c	0.6 c

Notes:

If detections are rather seldom ISTD can be added in a 2nd analysis in case of positives

If RTs are shifting, adding ISTD is highly recommended, at least to the 1 mL aliquot (vial)
- RT of ISTD gives additional certainty to identification

Residue findings of polar pesticides in real samples

Chlormequat and Mepiquat - Residues

Data from CVUA Stuttgart 2005-2008

Commodities	Chlormequat	Mepiquat
Mushrooms* (champignons, oyster)	+++ (also in organic*)	+++ (also in organic*)
Pears	++ (decreasing)	++ (decreasing)
Cereals	+++ (also in organic**)	+++ (also in organic**)
Fruiting vegetables (tomato, sweet pepper)	+	+

* From the cereals used as a substrate for fungi growth

** In organic often due to cross-contamination in mills

Glyphosate / Glufosinate - Residues

- Glyphosate:

Analyzed: **261 samples** (28 different commodities) from 16 countries

Positive: 5 samples (asparagus, shallots, oats, bran) from 3 countries

MRL-violations: none

	Samples analyzed	with residues	Min (mg/kg)	Max (mg/kg)
Asparagus	37	2	0,01	0,04
Onions, shallots	4	1	0,02	
Cereals	24	2	0,52	0,61

- Glufosinate: 1x in potatoes
- AMPA, 3-MPPA: No detections

Ethephon - Residues

Analyzed: 1162 samples (79 different commodities) from 45 countries

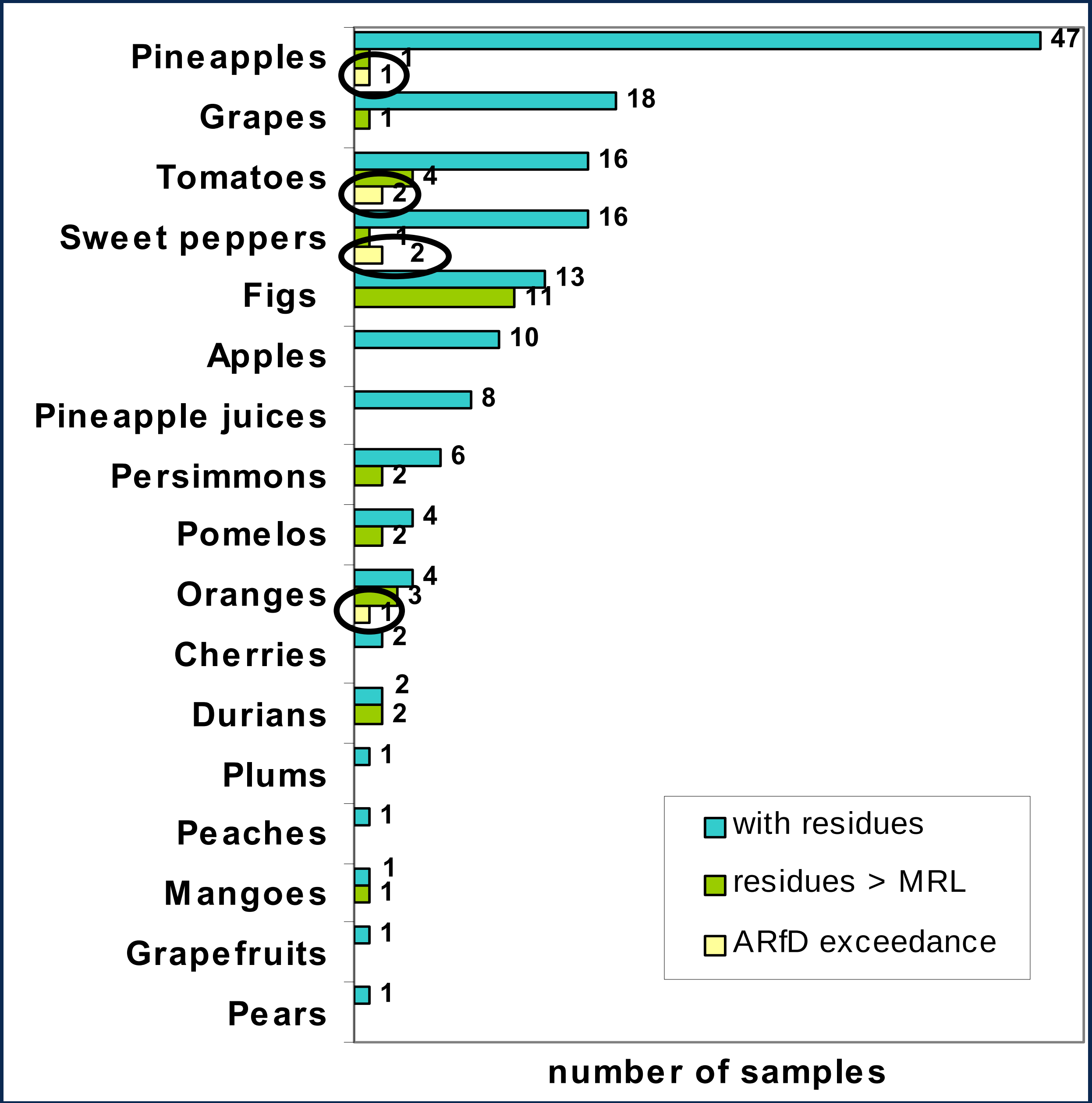
Positive: 74 samples (12 commodities) from 18 countries, **MRL-violations:** 11

Matrix	Country of origin	No. of sam-ples	No. of findings	Ethephon mg/kg	Organic growth	>MRL
Pineapple	Africa	1	1	0.54		
	Brazil	1	1	0.03		
	Costa Rica	16	10	0.04 to 1.0		
	Ghana	5	1	0.29		
	Honduras	1	1	0.30		
	Kamerun	4	3	0.031 to 1.25	2 x yes	
	Unknown	1	1	0.17		
	Panama	2	2	0.016 to 0.4		
Pineapple Juice	Unknown	10	7	0.005 to 0.22	4 x yes	
Apple	Germany	75	6	0.005 to 0.1		
Pear	Italy	11	1	0.01		
Durian	Thailand	2	2	0.31 to 10.1		2
Figgs	Brazil	3	3	0.22 to 0.55		3

Ethephon – Residues cont.

Matrix	Country of origin	No. of sam-ples	No. of findings	Ethephon mg/kg	Organic growth	>MRL
Sweet Pepper	Spain	28	4	0.049 to 1.2		
Kaki	Italy	3	1	0.12		1
	Spain	27	4	0.024 to 0.61		1
Pomelo	China	5	3	0.032 to 0.24		2
Cherry	Germany	12	1	0.022		
Grapes	Argentina	5	1	0.063		
	Italy	48	7	0.28 to 0.78		
	Namibia	2	1	1.43		1
	Spain	2	1	0.016		
	South Africa	25	6	0.004 to 0.4	1 x yes	
Tomato	Belgium	7	2	0.12 and 1.3		1
	The Netherlands	11	4	0.18 to 0.68		
Total (positiv matrices)		307	74		7	11

Ethephon Residues – newer data (1890 samples)



6 ARfD exceedances !!

Ethephon and HEPA in real samples

Sample	Ethephon mg/kg	HEPA mg/kg
Pomelo	0.24	0.032
Figs	0.22	0.071
Grapes	1.43	0.220
Pomelo	0.23	0.063
Figs	0.55	0.120
Figs	0.52	0.096
Figs	0.81	0.122
Figs	0.13	0.038
Figs	0.42	0.093
Orange	0.55	0.040
Pineapple	1.2	0.0092
Pineapple	0.65	0.024
Oat	-	0.034
Champignons	-	0.17
Champignons	-	0.23
Champignons	-	0.33

From straw used to grow on mushrooms

Maleic Hydrazide - Residues

Analyzed: **177 samples** (15 commodities) from 13 countries

Positive: 15 samples (3 commodities garlic, onion and shallots) from 4 countries.

MRL-violations: 1 MRL-violation in shallots from France with 17.2 mg/kg.

1 organic onion sample from Italy with 1.7 mg/kg.

Commodity	Country of Origin	No. of findings	Minimum Value (mg/kg)	Maximum Value (mg/kg)	Remarks
Garlic	France	1	14,3		
Onions	France	4	0.27	15	
	Germany	1	1.4		Illegal use
	Italy	1	1.7		1 organic
	The Netherlands	2	4.1	8.4	
	Unknown	1	0,2		
Shallots	France	5	6.6	17.2	1 > MRL

Fosetyl Aluminium – Residue Findings

Analyzed: **1241 samples** (85 commodities) from 44 countries
Positive: 37 samples (in 9 dif. commodities) from 9 countries
MRL-violations: None

Matrix	Country of origin	No. of samples	No. of fin-dings	Fosetyl mg/kg	Organic growth
Lettuce, ice	Spain	4	3	0.017 to 0.085	
Lettuce, cabbage	Germany	20	1	0.009	
Lettuce, lollo	France	2	1	0.008	
Strawberry	Germany	29	13	0.019 to 0.067	
	Greece	2	1	0.047	
	Spain	59	2	0.82 to 1.1	
Cucumber	Germany	12	2	0.68 to 2.6	
	Italy	4	3	0.008 to 0.3	3 x yes
	Maroc	2	1	0.045	1 x yes
	Spain	3	2	0.032 and 0.43	2 x yes
Litchi	Thailand	1	1	0.027	
Maracuja	Columbia	7	1	0.006	
Melon	Italy	2	1	0.024	
Rucola	Germany	6	1	0.01	
	Italy	2	1	0.055	
Tomato	Italy	9	1	0.020	
	Spain	33	1	0.014	
Grape	The Nether-lands	1	1	0.040	
Total		198	37		6

Intermediate Conclusion…


Ethephon
Fosetyl-Al
Chlormequat
Glyphosate
Maleic hydrazide


Do SRM!
Do SRM!
Do SRM!
Do SRM!
Don't do SRM
Don't do SRM
Don't do SRM
Don't do SRM
GASPIRTZ

III.
Partitioning Losses
(due to unfavorable pH)

Nicotine in Mushrooms

Nicotine in Mushrooms - Background Information:

- In 2008 high levels of Nicotine detected in mushrooms at CVUA Sigmaringen
- CVUA Stuttgart consulted for confirmation (LC-TOF, -MS/MS ✓ GC-MS ✓)
- Since then many findings by various labs in mushrooms mainly from China
- Most affected dried Porcini (Boletus edulis) but also Truffles and Chanterelles
- Porcini are reported to be not cultivable
- China (Yunnan Region) largest producer (80% of EU-imports from CN)
- Chinese authorities say : tobacco is also widely cultivated in Yunnan region
- Nicotine is a naturally occurring alkaloid in tobacco (*Nicotiana tabacum*) where it occurs at concentrations ranging from 2% to 8%

- Cross-contamination in drying/packing sites may be an issue
- Intentional use of nicotine as pesticides is also speculated

Nicotine in Mushrooms –
Risk assessment/management

EFSA

ARfD : 0.0008 mg/kg body weight;
ADI: 0.0008 mg/kg bw per day

99% of dried mushroom samples contained Nicotine (conc. often above 1 ppm)

Provisional MRL-Proposal:

Dried ceps with a nicotine > 2,3 mg/kg should be withdrawn from the market and safely disposed of.

- **M**onitoring program for European wild mushrooms initiated

NICOTINE PROPERTIES:

(K. Chamberlain et al., Pestic. Sci., 47, 265 (1996)

- **Basic:**

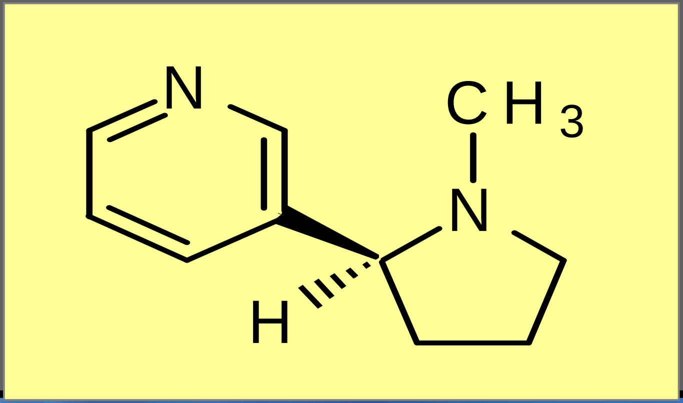
$pKa_1 = 3.1$; $pKa_2 = 8.2$
(i.e. predominantly protonated at pH<8.2 and double protonated at pH<3.1)

- **Polar:**

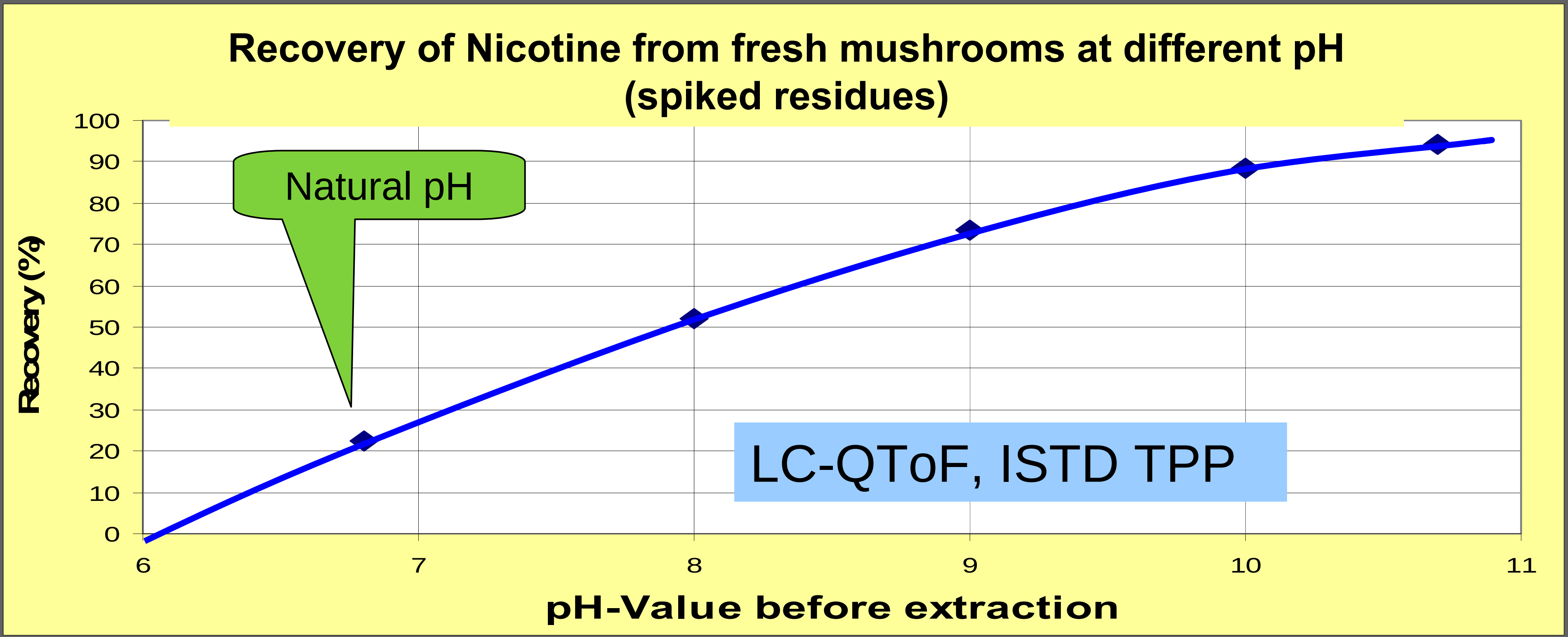
logP = 0.93 (25 °C/unionised) , the lower the pH the lower the logP

- **Volatile:**

Pvap = 5.6 Pa (25 °C). Evaporation losses reduced at low pH (ionized)



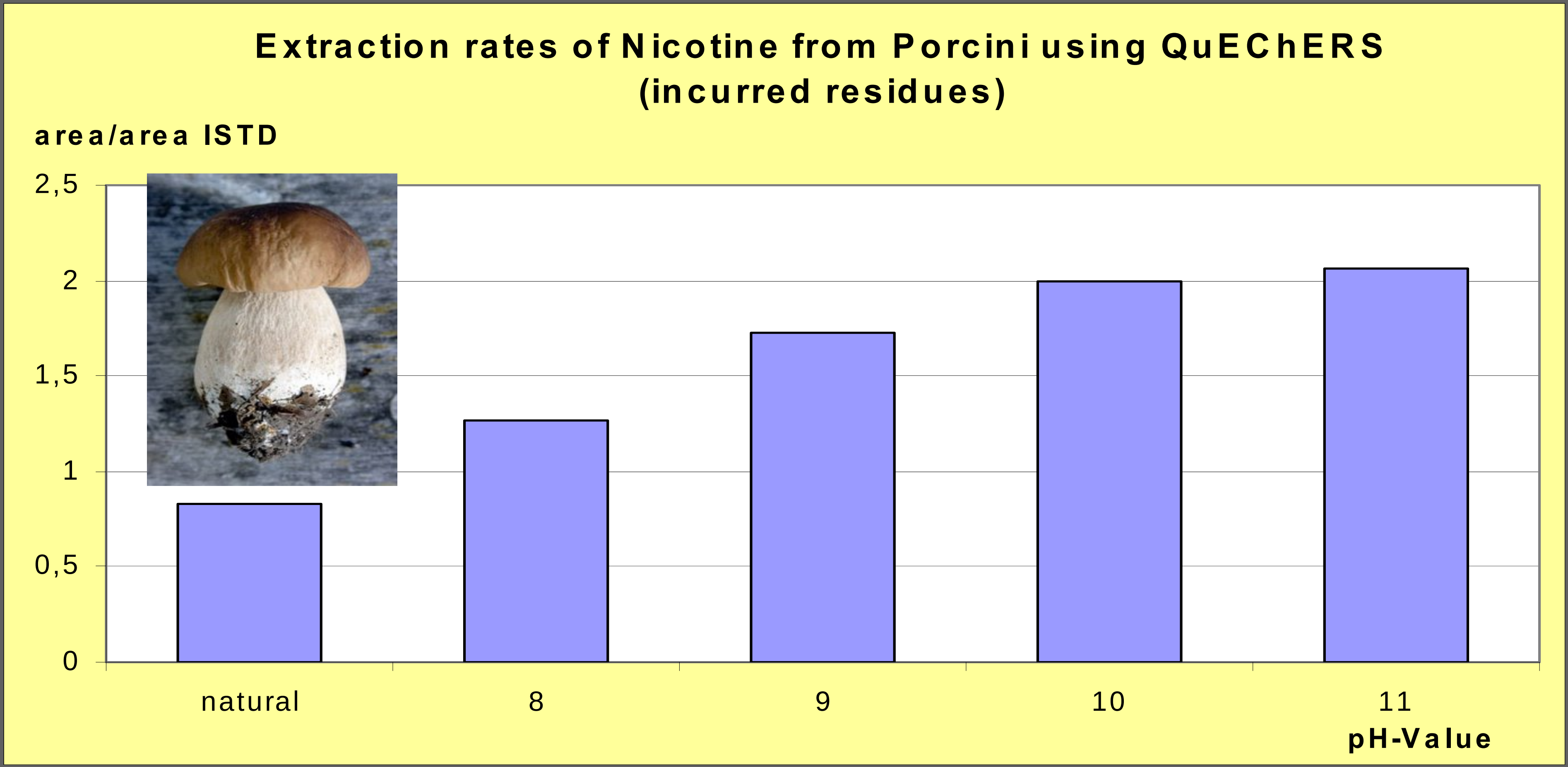
Nicotine by QuEChERS –
Optimisation of pH at extraction/partitioning-step



Using Nicotine D3 as ISTD losses were compensated irrespective of pH (Rec. 93 - 120 %)

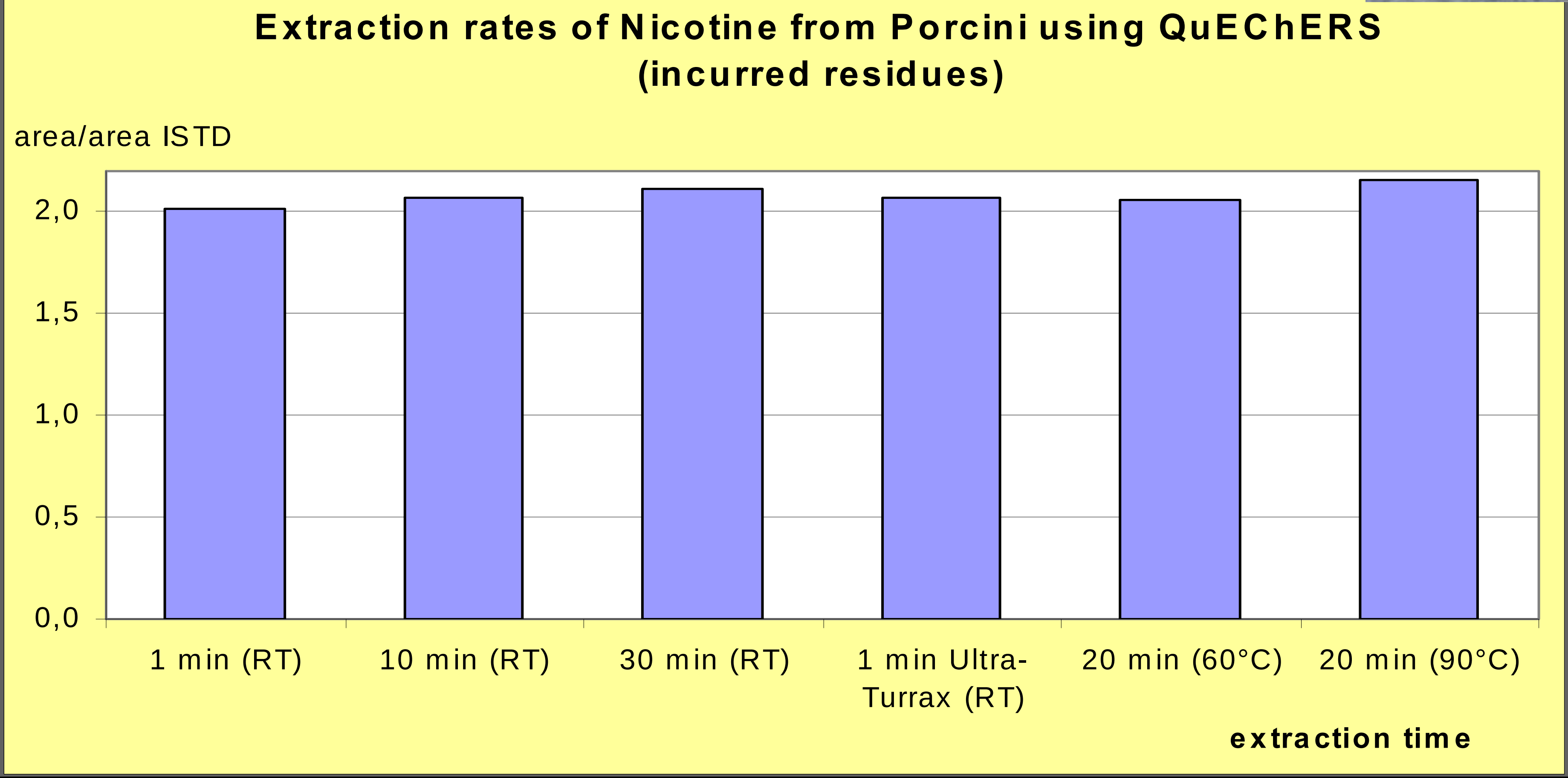
Extraction of incurred residues from Dried Porcini

QuEChERS - impact of pH



Extraction of incurred residues from Dried Porcini

QuEChERS pH 10, Variation of Temperature and Time



QuEChERS modification for Nicotine

10 g Fresh mushrooms or
2 g dried sample + 10 mL water

Bring pH to 10-11
by addition of NaOH 5N

Add 4 g $MgSO_4$ + 1g NaCl

Shake 1 min by hand

Centrifugue 5 min.
at 3500 r.p.m.

Take alicuot

Add 150 mg $MgSO_4$ anh.
+ 50 mg PSA per mL extract

Shake for 30s

Centrifugue 5 min.
at 3500 r.p.m.

Take aliquot and acidify
w. 5% Formic Acid to pH~5

Analysis via GC or LC

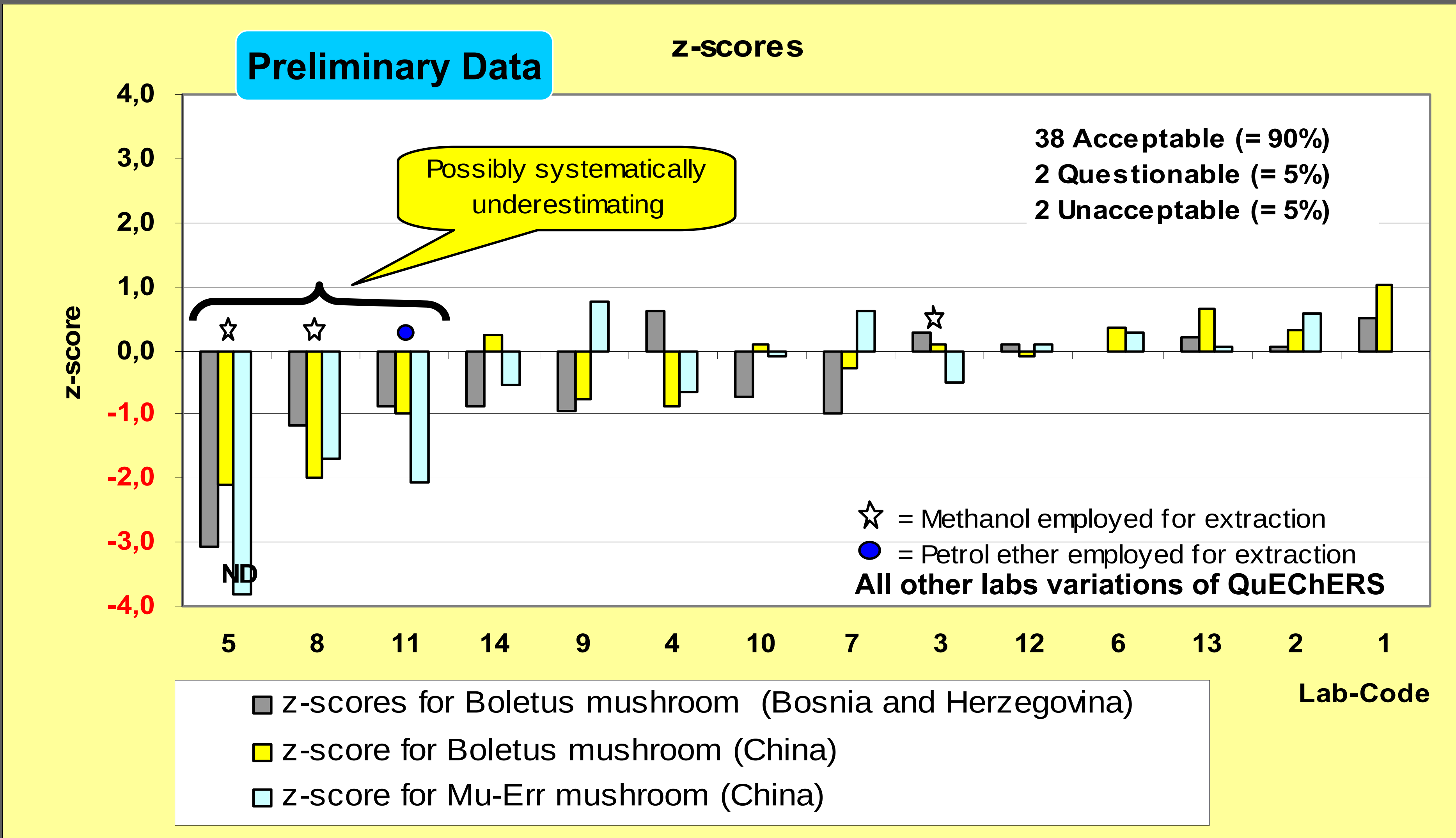
Validation (n=5)
using Champignon 10 g

Mean Recovery: 106%

RSD: 4,1%

Ad-hoc PT on Nicotine in dried mushrooms

- in collaboration with COOP-Switzerland
- 2 Boletus (CN, BiH) + 1 Mu-Err (CN) w. incurred Nicotine (~1.0/0.7/2.5 mg/kg)



V. Pesticides requiring inclusion of metabolites or reaction products

Acidic Pesticides

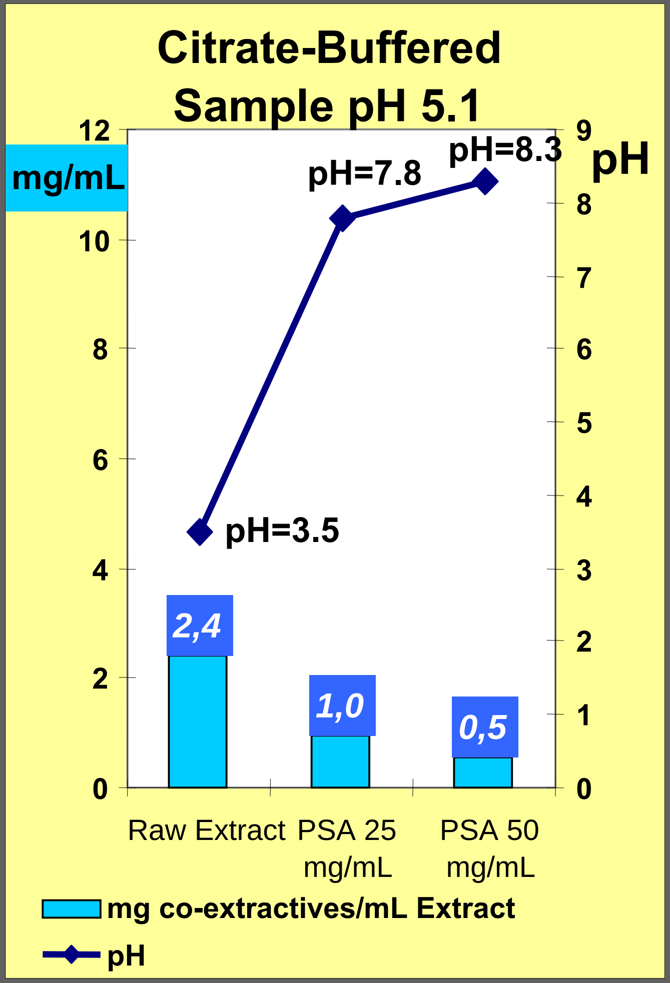
-

Bound Residues

Acidic pesticides – cleanup issue

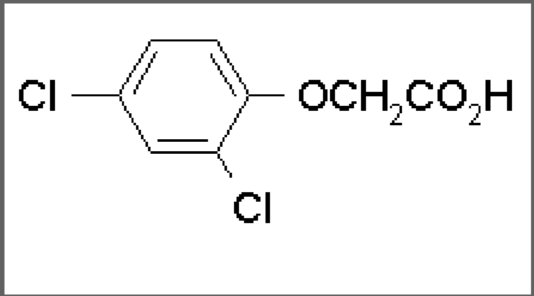
Example: Extract of red currant

- Losses of acidic components after cleanup
- Acidic compounds interact with PSA. Thus skip PSA cleanup



QuEChERS – alkaline hydrolysis

Example 2,4-D



- Selective systemic herbicide
- Control of broad leaved weed
- Plant growth regulator used to prevent premature fruit drop
- Formulations include free acid, salts, esters
- May form conjugates
- Method designed to convert all possible residues to free acid

- Acids are often covalently bound to matrix components and thus their concentration underestimated!

Acidic pesticides

pH-issue: Ionization of pesticides at low or high pH-values

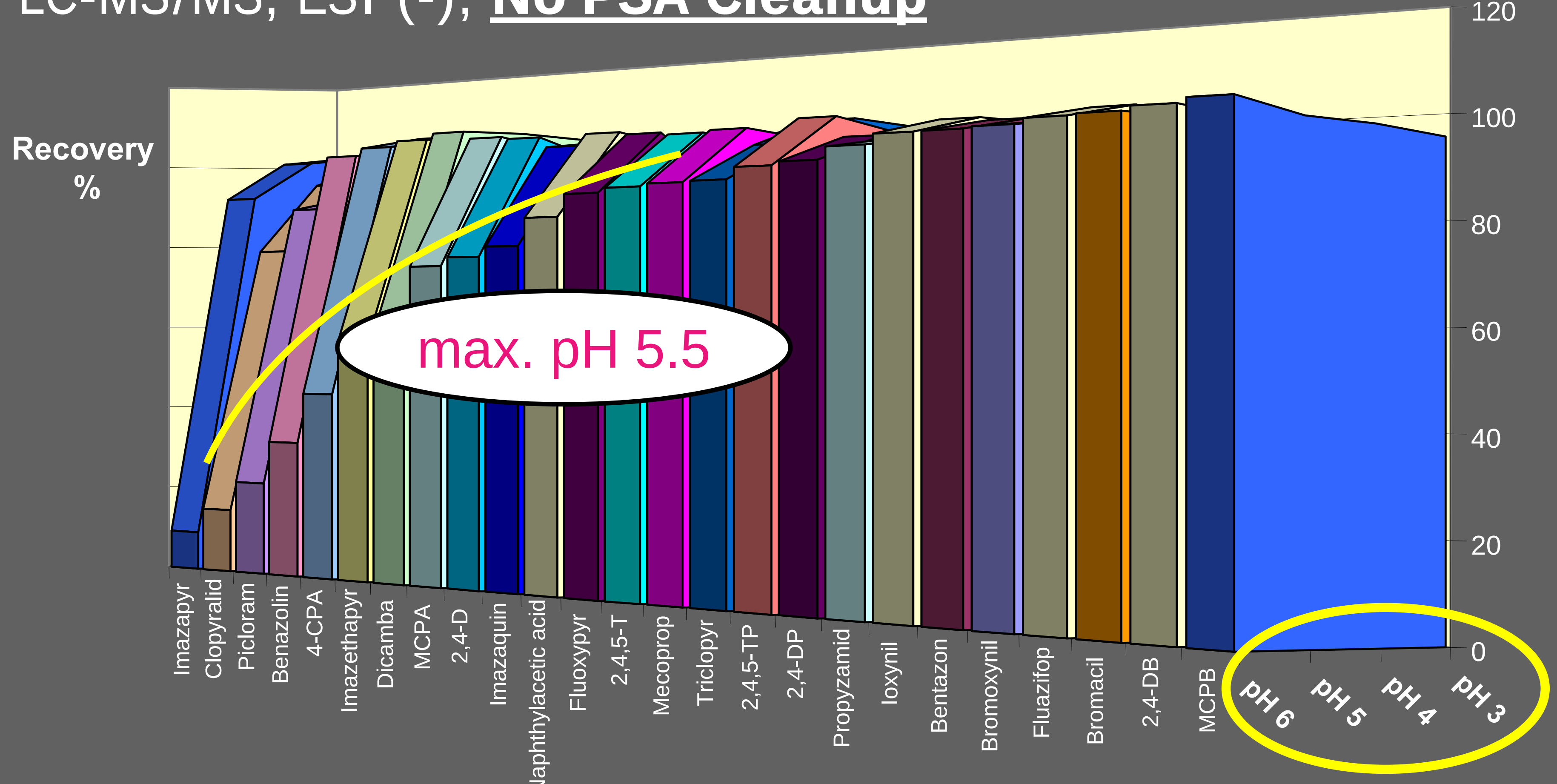
- **Acids:** $HX \leftrightarrows H^+ + X^-$
- **Bases:** $B + H^+ \leftrightarrows BH^+$

⇨ **Ionic form prefers to stay in the water phase**

pH-range of agricultural samples: ~2.5 – 7

Acidic pesticides – influence of pH on recovery

LC-MS/MS, ESI (-), **No PSA Cleanup**



lower pKa ← general trend → higher pKa

QuEChERS – schematic description with AH

Citrate-Buffered QuEChERS

Weigh 10 g of Frozen Sample

Shake

> **alkaline hydrolysis:**
> **Add NaOH and store for 30 min at RT, then neutralize w. H_2SO_4**

Add 10 mL Acetonitrile

Add ISTD-Solution

Shake

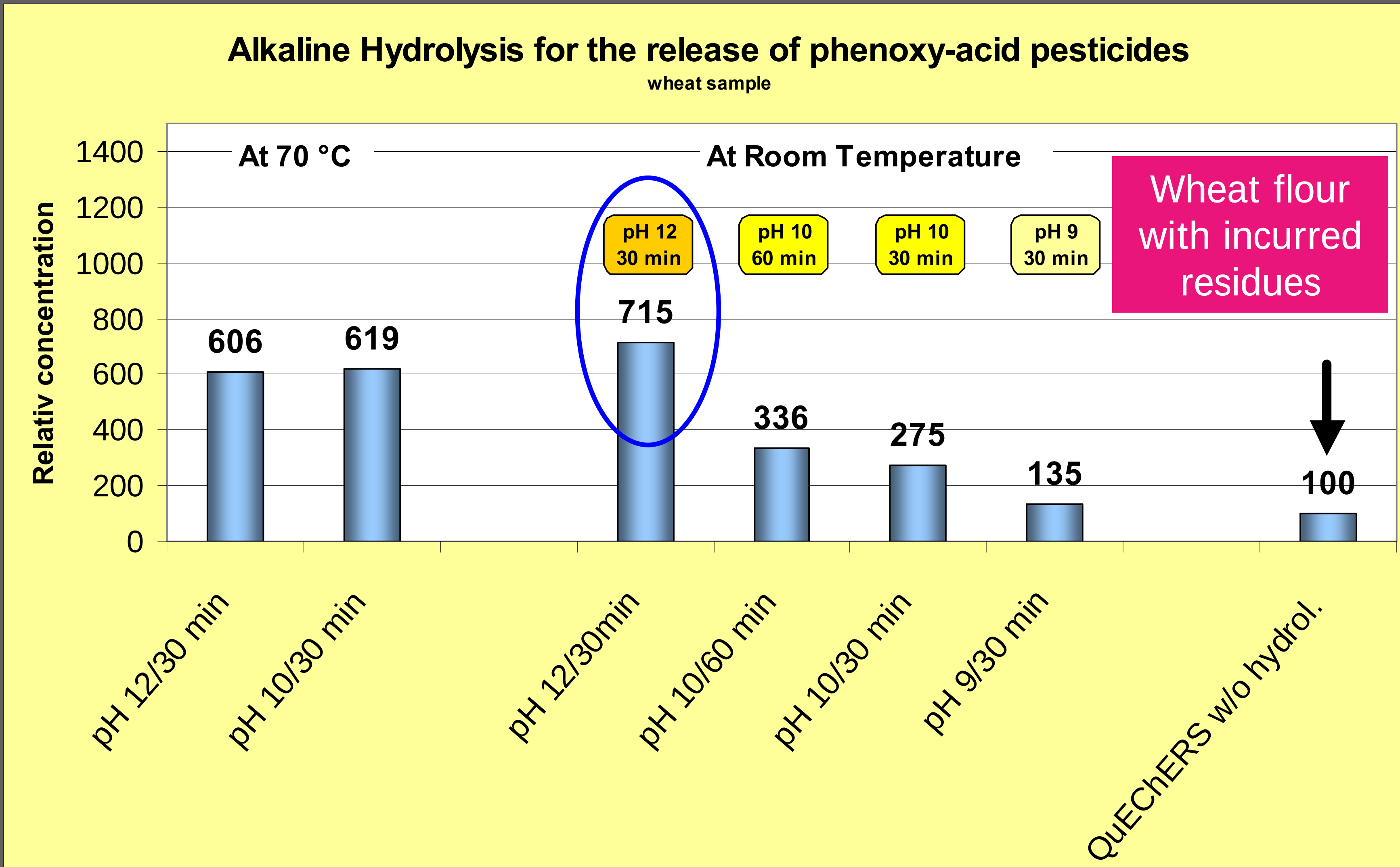
Add 4 g $MgSO_4$ / 1 g NaCl / Citrate Buffer (pH 5-5.5)

Shake and Centrifuge

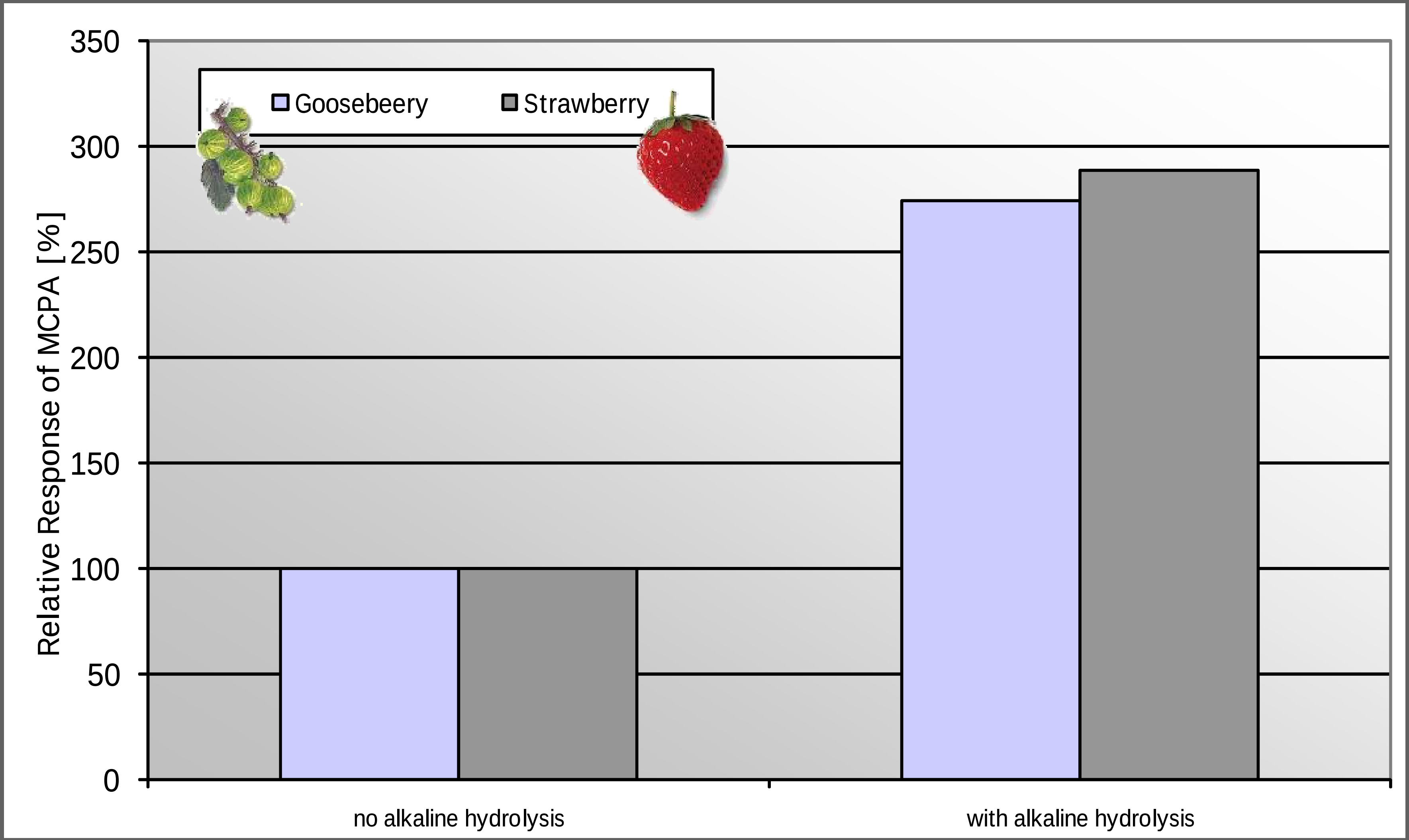
> **optionally:**
> **Freeze-out of extracted fat over night**

Analysis of acidic pesticides by LC-MS/MS

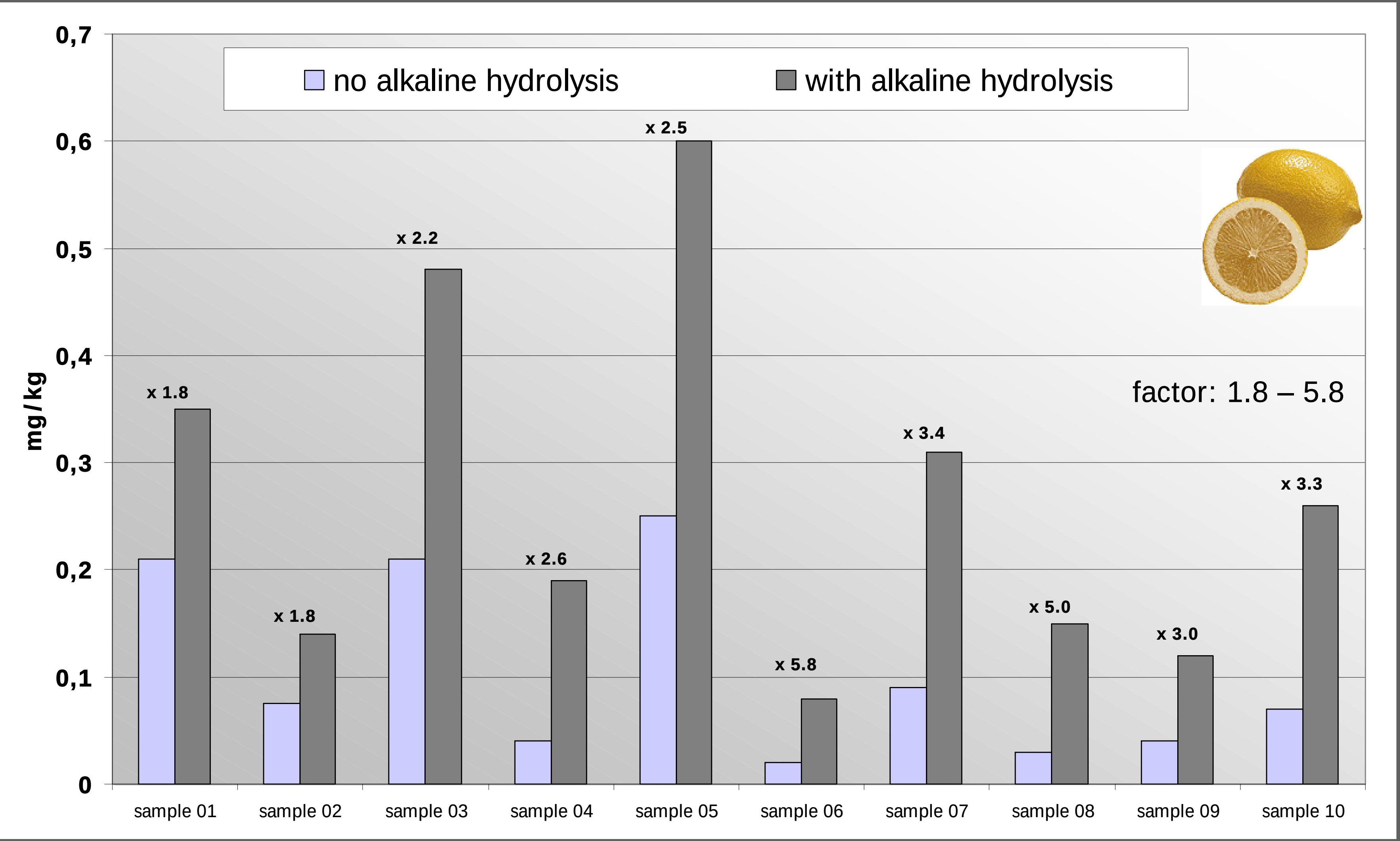
Alkaline cleavage or the release of phenoxy-acids



Alkaline Hydrolysis – Level of MCPA in berries



Alkaline Hydrolysis – Level of 2,4-D in different citrus samples



Matrix:	Cucumber	Redidue Definition				Experiments performed in advance to QuEChERS							
Method:	QuEChERS					I	II	III	IV	V	VI	VII	VIII
Acid	Ester	Acid	Esters	Conjug.	Residue Definition	AH RT 30 min	AH 80°C 30 min	AH RT 16 h	EH (1 mg) RT 3 h	EH (2 mg) RT 3 h	EH (5 mg) RT 3 h	EH (2 mg) RT 16 h	VII + II
2,4,5-T	2-butyl	x			2,4,5-T (F)	63	33	19	0	0	0	0	0
	butoxyethyl					7	0	0	0	0	0	0	0
	ethylhexyl					103	47	26	7	4	2	0	0
	isooctyl					104	48	26	8	5	3	0	0
	isopropyl					15	0	0	0	0	0	0	0
	methyl					1	0	1	2	2	2	1	0
	octyl					38	10	3	3	2	1	1	0
2,4-D	methyl	x	x		2,4-D (sum of 2,4-D and its esters exp. as 2,4-D)	0	0	0	0	0	0	0	0
	butyl						0			0	0	0	
	ethyl						0			0	0	0	
	isobutyl						0			0	0	0	
	isooctyl						9			2	3	0	
	isopropyl						0			0	0	0	
2,4-DP	methyl	x			Dichlorprop, incl. Dichlorprop-p	0	0	0	0	0	0	0	0
Carfentrazone	ethyl	x	x		Carfentrazone-ethyl (determined as carfentrazone and expr. as carfentrazone-ethyl)	0	0	0	0	0	0	0	0
Chlorthal	dimethyl		x		Chlorthal-dimethyl	100	61	78	93	89	78	57	24
Cinidon	ethyl		x		Cinidon-ethyl (sum of cinidon ethyl and its E-isomer)	10	0	0	2	1	0	0	0
Clodinafop	propargyl	x			Clodinafop and its S-isomers, expr. as clodinafop (F)	2	0	0	0	0	0	0	0
Cyhalofop	butyl	x	x		Cyhalofop-butyl (sum of cyhalofop butyl and its free acids)	79	2	0	0	0	0	0	0
Dicamba	methyl	x			Dicamba		32			5	1	0	
Dichlorprop	2-ethylhexyl	x			Dichlorprop, incl. Dichlorprop-p	70	58	41	8	3	1	1	0
Diclofop	methyl	x	x		Diclofop (sum diclofop-methyl and diclofop acid expr. as diclofop-methyl)	60	1	0	1	1	1	0	0
Diethatyl	ethyl					8	0	0	12	2	0	0	0
Dinoseb	acetate	x			Dinoseb	8	0	0	0	0	0	0	0
Fenoprop	isooctyl						64			11	11	0	
	methyl						0			0	0	0	
Fenoxaprop	P-ethyl					37	0	0	1	0	0	0	0
Flamprop	isopropyl					103	33	20	97	92	76	53	15
Fluazifop	butyl					77	3	1	1	0	0	0	0
	methyl					1	0	0	0	0	0	0	0
Flumiclorac	pentyl					13	0	0	0	0	0	0	0

remaining esters 5%
remaining esters 5-20%
remaining esters > 20%

AH = alkaline hydrolysis, pH 12, re-neutralized with H_2SO_4

EH = incubation of buffered samples with esterase from porcine liver (buffering at pH 6-9 with phosphate buffer

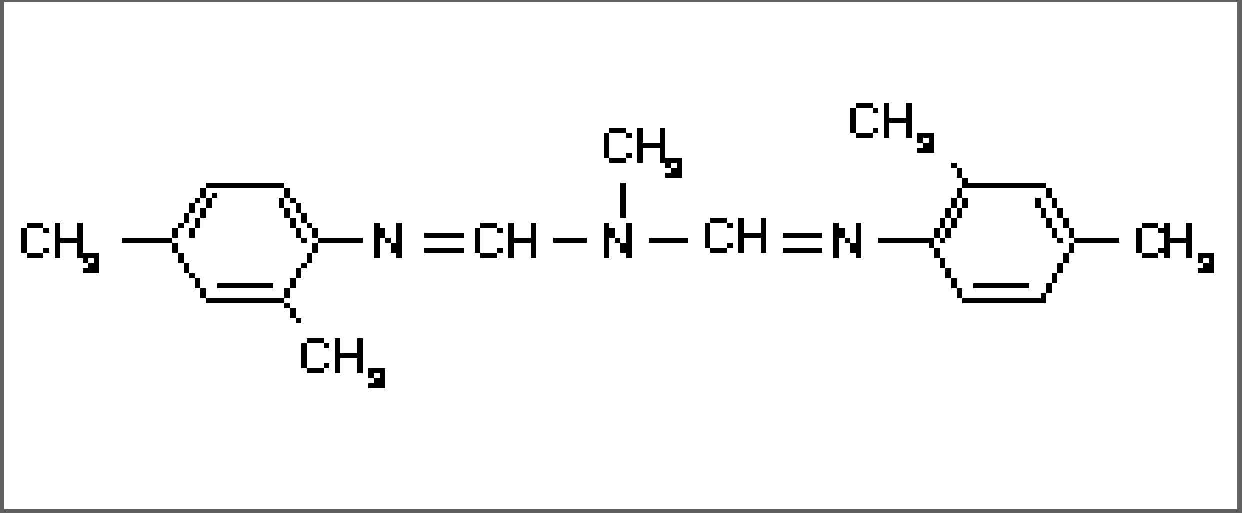
Matrix:	Cucumber	Rediduce Definition				Experiments performed in advance to QuEChERS							
Method:	QuEChERS					I	II	III	IV	V	VI	VII	VIII
Acid	Ester	Acid	Esters	Conjug.	Residue Definition	AH RT 30 min	AH 80°C 30 min	AH RT 16 h	EH (1 mg) RT 3 h	EH (2 mg) RT 3 h	EH (5 mg) RT 3 h	EH (2 mg) RT 16 h	VII + II
Haloxyfop	ethoxyethyl	x	x	x	Haloxyfop incl. haloxyfop-R (Haloxyfop-R methyl ester, haloxyfop-R and conjugates of haloxyfop-R expr. as haloxyfop-R) (F) (R)	9	0	0	0	0	0	0	0
	methyl					6	0	0	1	1	1	0	0
Ioxynil	octanoat	(x)	x		Ioxynil, incl. its esters expr. as ioxynil (F)	70	16	0	3	2	1	0	0
MCPA	1-butyl	x	x	x	MCPA and MCPB (MCPA, MCPB incl. their salts, esters and conjugates expr. as MCPA) (F) (R)		1			0	0	0	
	butoxyethyl						0			0	0	0	
	ethyl						0			0	0	0	
	ethylhexyl						21			3	3	0	
	thioethyl					3	0	0	0	0	0	0	0
MCPB	ethyl	x	x	x	MCPA and MCPB (MCPA, MCPB incl. their salts, esters and conjugates expr. as MCPA) (F) (R)		3			1	1	1	
Mecoprop	methyl	x			Mecoprop (sum of mecoprop-p and mecoprop expressed as mecoprop)	0	0	0	0	0	0	0	0
	1-octylester					103	85	54	8	5	3	2	1
	2,4,4-trimethylpentyl					104	83	44	7	4	3	0	0
Mefenpyr	diethyl					2	0	0	0	0	0	0	0
Nitrothal	di-isopropyl					19	0	0	2	1	0	0	0
Picloram	isooctyl	x			Picloram		14			1	1	0	
Triclopyr	2-butoxyethyl	x			Triclopyr		0			0	0	0	
Trinexapac	ethyl	x			Trinexapac	8	0	0	82	64	29	10	0

- remaining esters 5%
- remaining esters 5-20%
- remaining esters > 20%

AH = alkaline hydrolysis, pH 12, re-neutralized with H_2SO_4

EH = incubation of buffered samples with esterase from porcine liver (buffering at pH 6-9 with phosphate buffer

Amitraz



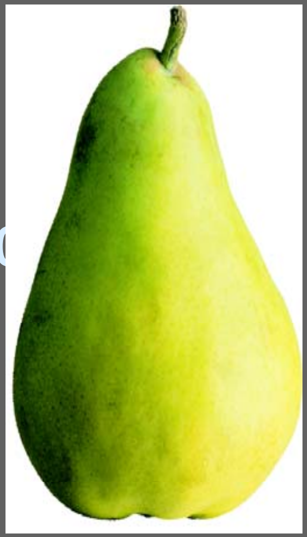
Amitraz

❖ **Use:**

- Acaricide and Insecticide
  - ➢ mite control in many different crops
    pome fruit, citrus, stone fruit, strawberries, cucurbits, solanaceous etc.
    To control varroa mite in Honey-production

❖ **Mode of Action:**

- Non-systemic
- Interacts with the nervous system of mites and

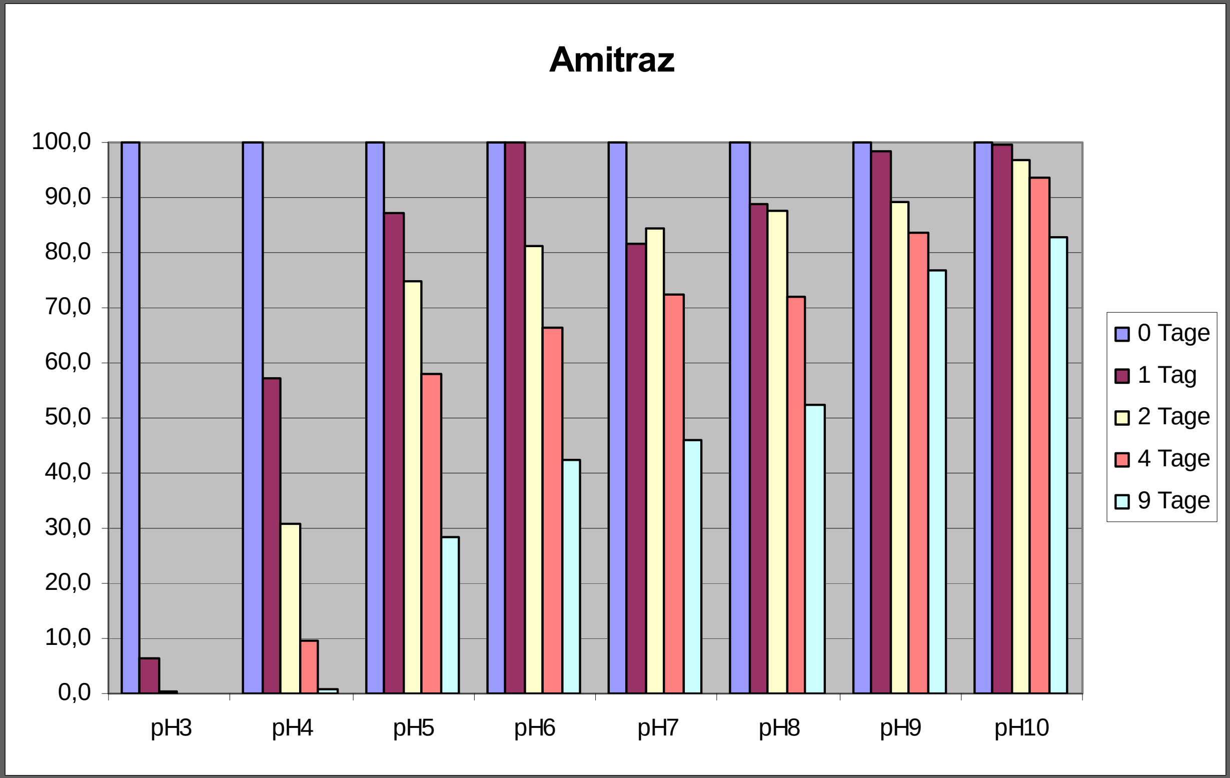


❖ **Toxicity:**

Relatively high toxicity to mammals

Amitraz

- Recoveries with QuEChERS quite good
- Better stability of Amitraz in extracts at high pH
  - PSA cleanup (w/o acidification) increases pH and thus stability

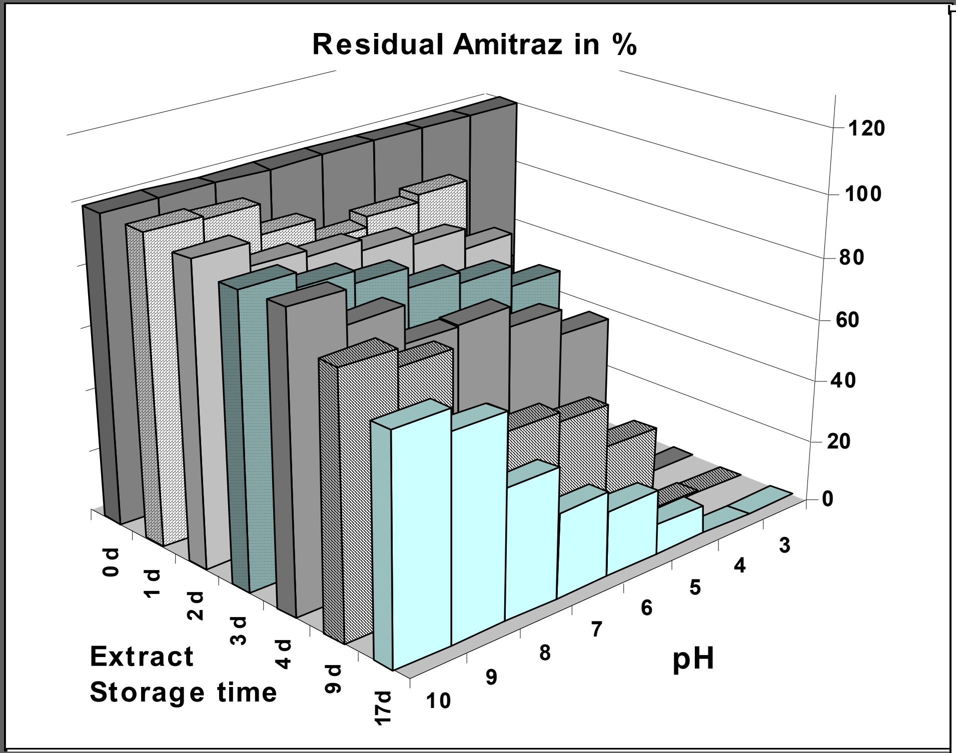


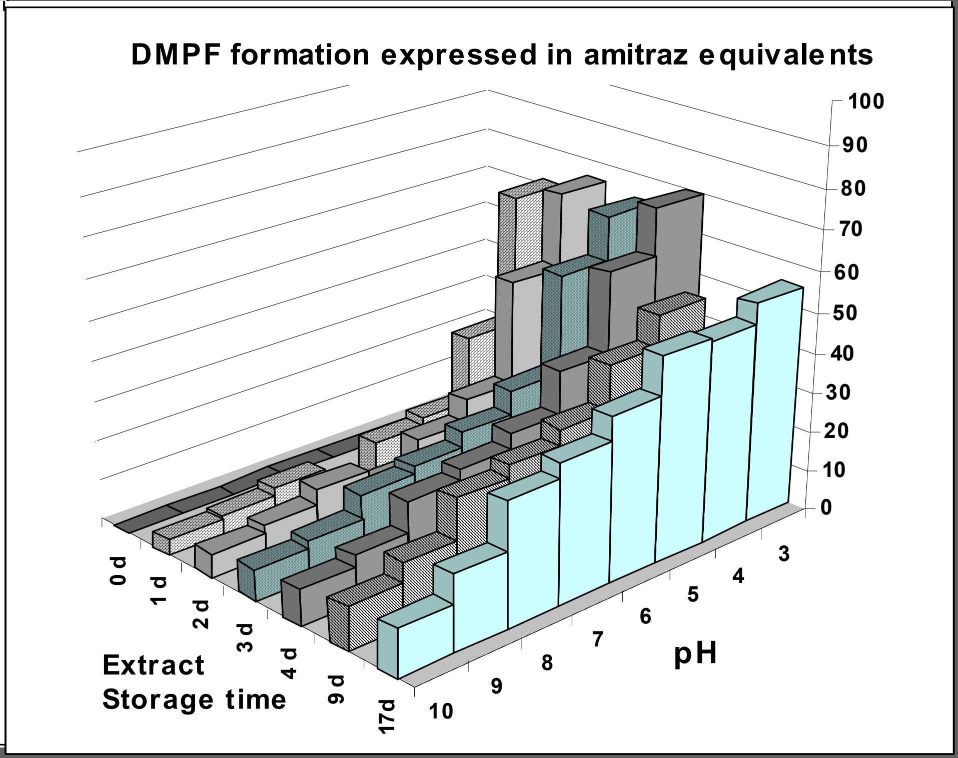
Measurement of acidified extract should be performed within one day.

Better don't acidify QuEChERS extract !

Amitraz

Degradation of amitraz and formation of DMPF (amidine) during storage of QuEChERS extracts at diff. pH





Amitraz

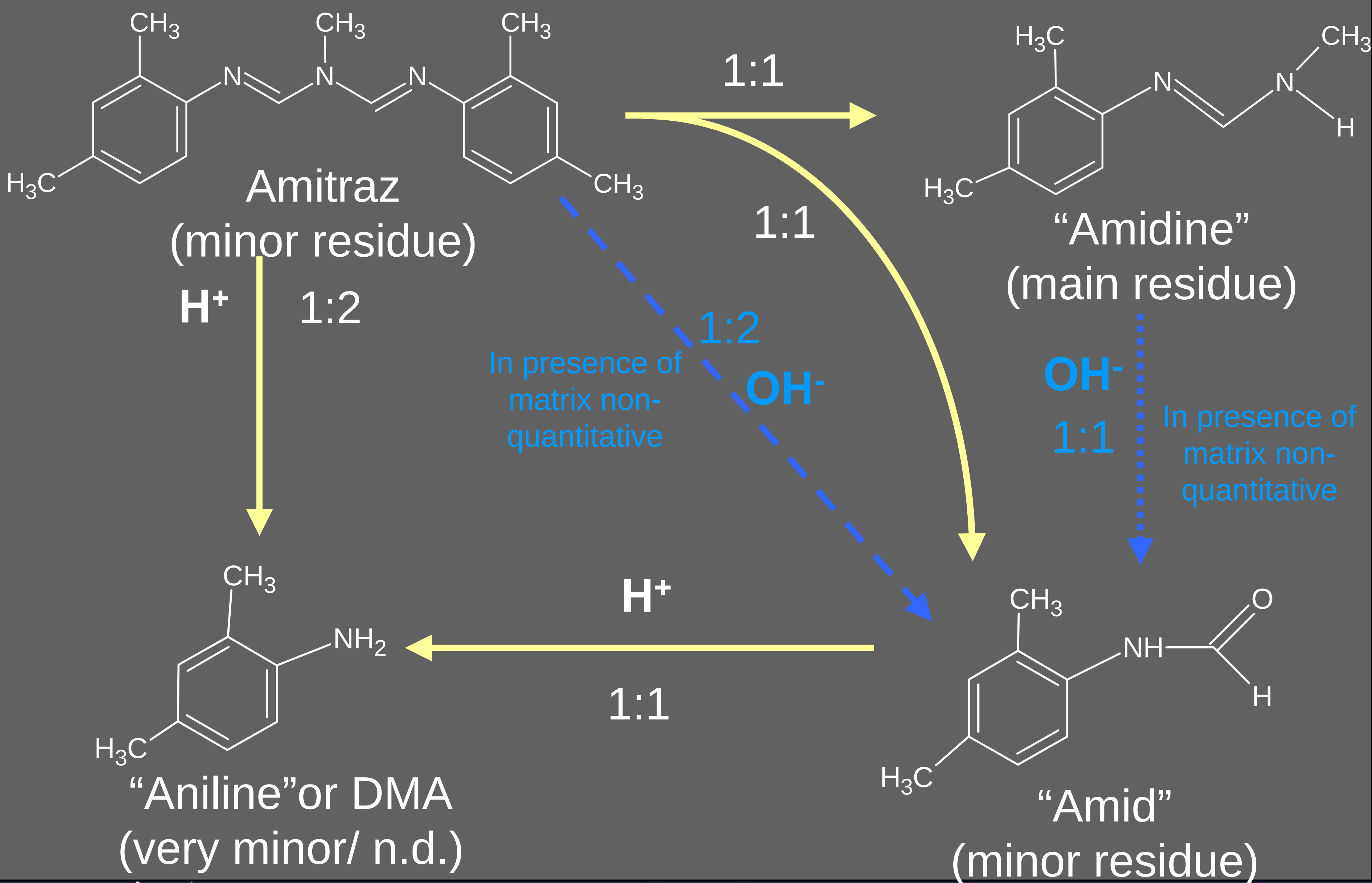
- Residue Definition:
  "Amitraz including all metabolites containing 2,4-dimethylaniline expressed as Amitraz"

- Traditional methods involve:
  Conversion of Amitraz and degrad. products to 2,4-dimethylanilin

  Different approaches:

  - Alkaline hydrolysis,
  - Acidic hydrolysis,
  - Combination of both

Degradation paths of Amitraz



Amitraz

- Acidic hydrolyses were quantitative:
  - Amitraz ⇨ "Aniline"
  - "Amid" ⇨"Aniline"
  - "Amidin" remains stable
  - "Aniline" remains stable
- Alkaline hydrolyses were not quantitative (especially in presence of matrix)
  - "Amitraz" ⇨"Amid"
  - "Amid" ⇨"Aniline"
  - "Amidin" ⇨"Aniline"
  - "Aniline" remains stable (closed vessel to avoid losses!)

Amitraz

Amitraz and its 3 main metabolites well detectable by LC-MS/MS

So, “total DMA” can be calculated (virtual hydrolysis)

Virtual vs. chemical Hydrolysis:

Virtual hydr.: only covers the detected compounds.

Chemical hydr.: covers also other DMA-containing metab./conj.

Question: *Are there any significant levels of other metabolites/conjugates releasing DMA upon hydrolysis?*

Observation: Alkaline hydrolysis did not increase determined residue level in real samples

In any case:

- Acidic hydrolysis does not transform amidine to aniline
- Risk of losses alkaline hydrolysis is very high.

Amitraz - Calculating the Total Residue

Important: Mind stechiometries of degradation pathways

- Amidin ⇨ Amitraz 1:1
- Amid ⇨ Amitraz 2:1 or 1:1 (depending of pathway)
- DMA ⇨ Amitraz 2:1

CASE 1: Amidin is the only metabolite detected (as in pears)

☞ 1:1 stechiometry (conversion factor based on MW-difference)

CASE 2: Amidin + at least one of other metabolites present

1) Expression of all metabolites as DMA (1:1 stechiometry), and calculation of Amitraz from DMA (2:1 stechiometry)
2) Expression of Amidin as Amitraz (1:1), Amid + DMA are ignored as they can be formed as by-products during the degradation of Amitraz to Amidin.

Take the highest of both

Amitraz-Findings in pears (2008): CVUA Stuttgart*

Origin	Amitraz (parent)	Metabolite: N-2,4-Dimethylphenyl-N-methylformamidine	Amitraz (sum)	MRL-Exceedance	ARfD-Exceedance [%]
Turkey	ND	1,6	2,9	Yes	2647
Turkey	ND	1,31	2,4	Yes	2191
Turkey	0,001	1,2	2,2	Yes	2008
Turkey	<0,001	1,21	2,2	Yes	2008
Turkey	0,003	0,96	1,7	Yes	1552
Turkey	ND	0,84	1,52	Yes	1387
Turkey	ND	0,81	1,5	Yes	1369
Turkey	ND	0,79	1,4	Yes	1278
Turkey	ND	0,69	1,2	Yes	1095
Turkey	ND	0,59	1,1	Yes	1004
Turkey	ND	0,59	1,1	Yes	1004
Turkey	ND	0,49	0,89	Yes	812
Turkey	ND	0,49	0,89	Yes	812
Turkey	ND	0,48	0,87	Yes	794
Turkey	ND	0,47	0,85	Yes	776
Turkey	ND	0,42	0,76	Yes	694
Turkey	ND	0,28	0,51	Yes	465
Turkey	ND	0,27	0,49	Yes	447
Spain	ND	0,013	0,024	No	22
Italy	ND	0,32	0,58	Yes	529
Unknown	ND	0,9	1,6	Yes	1460

Amitraz parent not detected or at very low levels in 2008
→
Residue situation is clearly underestimated if only parent is targeted

⇒ Risk Assessment: German Child 2-5 y. old, 97.5 percentile (232 g/portion); Variability Factor: 7 (JMPR model); ARfD 0.01 (EFSA)

* www.cvuas.de; Amitraz-article: http://www.cvuas.de/pub/beitrag.asp?subid=1&Thema_ID=5&ID=881&Pdf=No

Amitraz-Findings in Pears (2008): CVUA Stuttgart

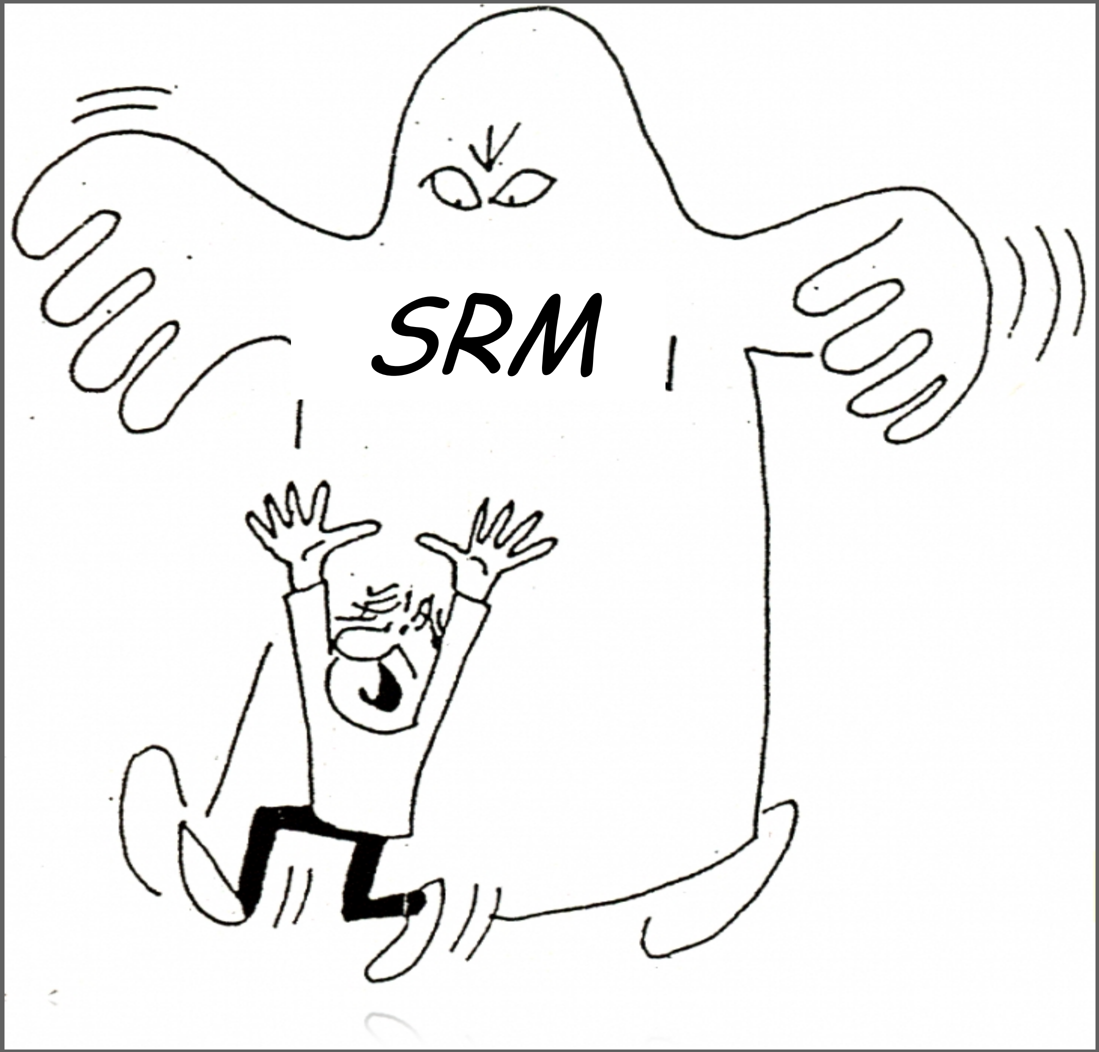
- Turkish Pear-samples: 18 analyzed; 18 >ARfD
- Other countries:
  - Italy (11/1 >ARfD),
  - Argentina (10 samples/0 findings),
  - Germany (7/0),
  - Spain (6/1 <ARfD),
  - South Africa (5/0),
  - China (4/0)
  - Chile (1/0),
  - France (1/0),

Amitraz-Findings in Pears (2009): CVUA Stuttgart

8 Turkish Pear-samples analyzed, 8 >ARfD

Total Amitraz	Amitraz parent	% ARfD
15.7	0.35	14327 (~140 x)
10.4	0.04	9491 (~100 x)
9.8	**1.3**	8943
6.0	0.02	5475
5.9	0.06	5384
4.3	0.007	3924
1.8	nd	1643
0.2	nd	183

Turkey has withdrawn amitraz registration.
Farmers have 2-year period of grace to get rid of stock.
This had obviously a negative effect in terms of agricultural practice



Thank you very much for your attention!