

Interesting observations made during the work of a private residue laboratory

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Stuttgart, 19 October 2023



- I. Procedure for the control of direct imports on behalf of the Bavarian state government
- II. Multiple Source Compound: Phthalimide
- III. Multiple Source Compound: Anthraquinone
- IV. Multiple Source Compound: 1,4-Dimethylnaphthalene
- V. Azadirachtin vs. Karanjin: ATTENTION – an example from everyday routine

Why we are part of this Joint Workshop?

The Bavarian State Office for Health and Food Safety (LGL) in Erlangen subcontracts the Friedle laboratory with investigations within the scope of the Regulation (EU) 2019/1793, i.e. “the control of direct imports”

Procedure

- Sampling at Munich Airport by the food monitoring staff of the responsible District Office (Erding)
- Sample transport: 120 km distance, by courier vehicle "Airportliner"
- Laboratory analysis for relevant pesticide residues
Turn Around Time: same day or next working day
- Evaluation and report
to LGL Erlangen and District Office Erding
final assessment (border cases) by LGL

Why we are part of this Joint Workshop?

Statistics

- Around 300 samples

78 % o.k.

5,5 % above mrl, within the expanded measurement uncertainty

16,5 % clearly above mrl

- 2023

17 samples, no complaints

mostly plant extracts (powder) and spices from India for ethylene oxide

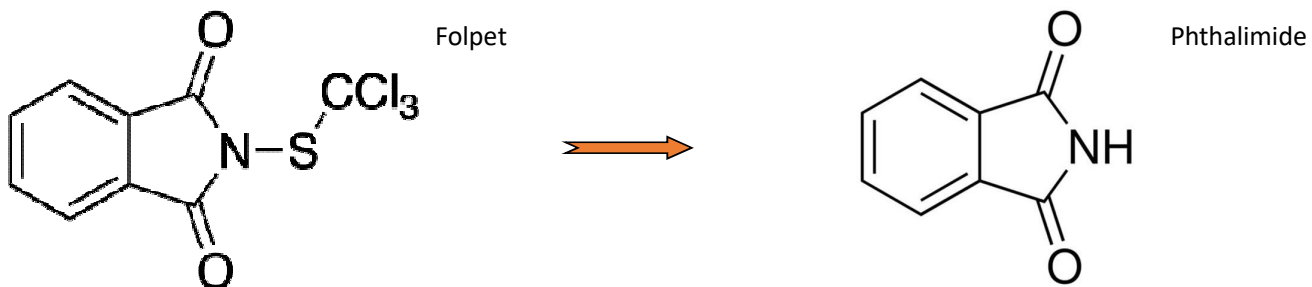


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Phthalimide

Regulation (EC) 2016/156 dated 18.01.16 newly regulates the residue definition for the fungicide *Folpet* from 26 August 2016 onward:

Folpet (sum of folpet and phthalimide, expressed as folpet)



➡ **Phthalimide is a metabolite of folpet and phosmet**



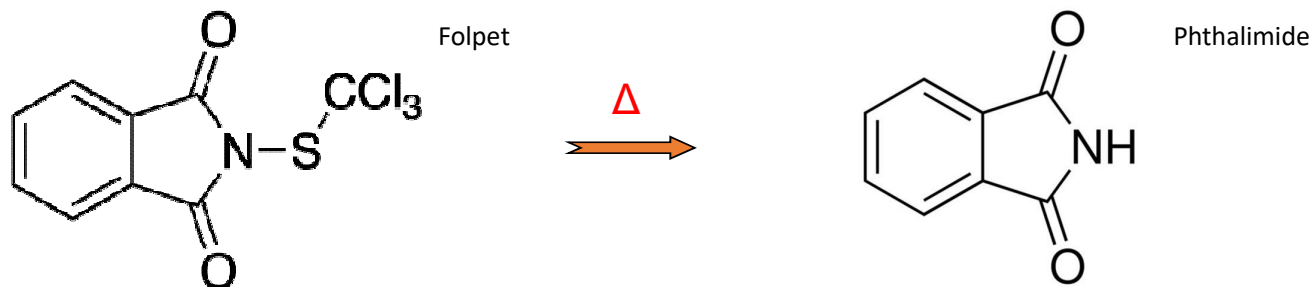
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Phthalimide

Determination of folpet by gas chromatography

Decomposition temperature is 177°C

(Perkow "Wirksubstanzen der Pflanzenschutz- und Schädlingsbekämpfungsmittel"
2. vollständig bearbeitete Auflage [Second completely revised edition] 1983/1992, Verlag Paul Parey)



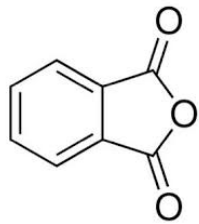
➡ **Phthalimide is a reaction product in the GC injector**



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Phthalimide

Phthalic anhydride



Industrial chemical and starting material for e.g.

- alkyd resins
- plasticisers
- dyes/colourants

Residues in:

- lacquers/paints
- paper coatings
- plastics (PVC)
- newspaper print

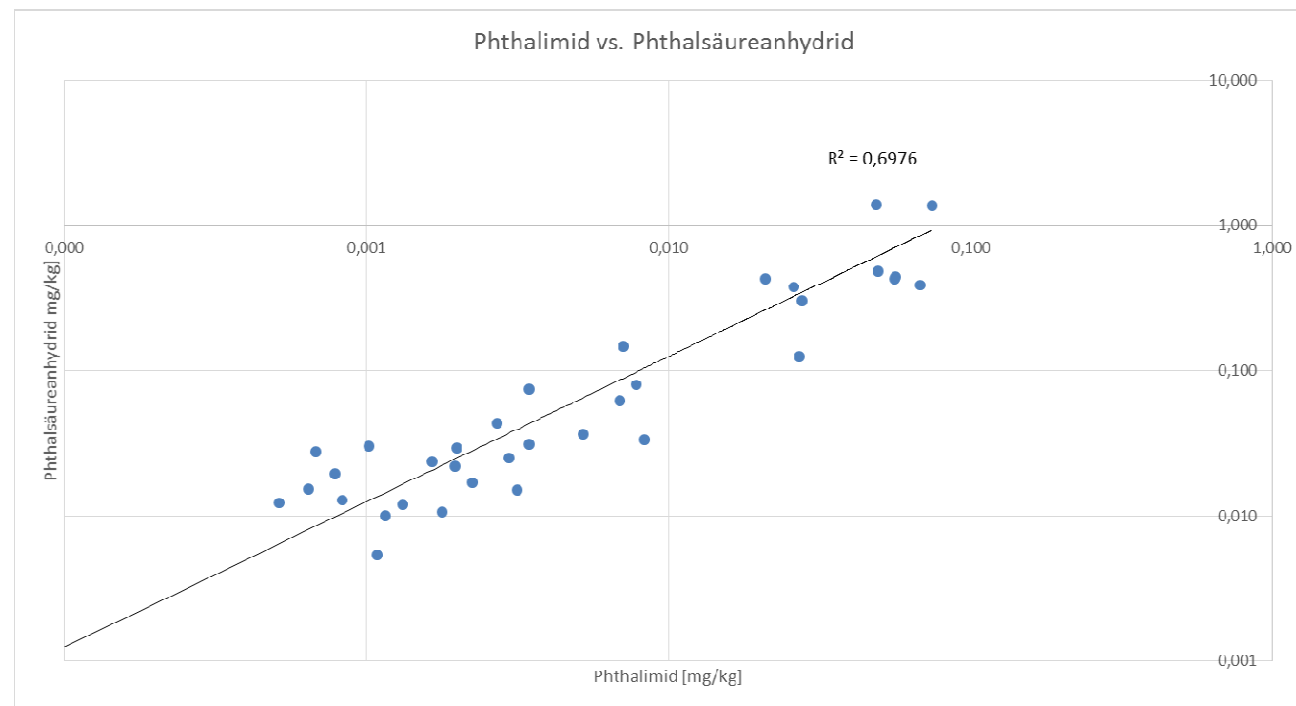
➡ **Ubiquitously found in house dust samples!**



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Phthalimide

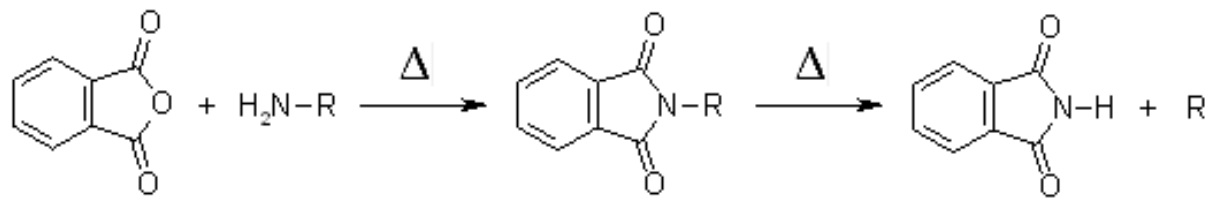
Correlation between phthalic anhydride and phthalimide





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Phthalimide



Phthalic anhydride

conjugate

phthalimide

- ✓ Phthalic anhydride is ubiquitous
- ✓ Food matrix provides primary amino group: amino acid, peptide, etc.

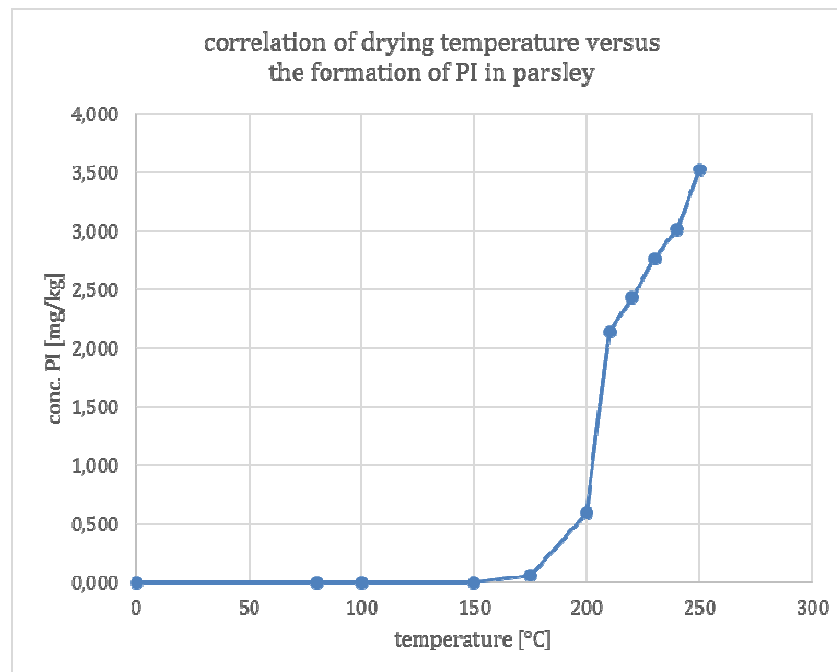
➡ **Phthalimide is an artefact in the GC injector**



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Phthalimide

Correlation between phthalimide and drying temperature



Matrix: Parsley

Relana POSITION PAPER No. 16 - 03

➡ **Phthalimide is a process contaminant**



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Phthalimide

Phthalimide is a ...

- ✓ **Metabolite** (folpet, phosmet)
- ✓ **Reaction product in the GC injector**
- ✓ **Artefact in the GC injector**
- ✓ **Process contaminant**

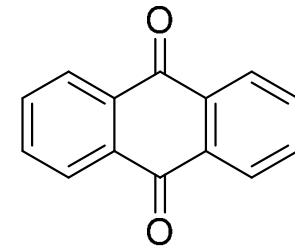
An evaluation according to the residue definition without simultaneous detection of folpet/phosmet is not possible!



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Anthraquinone

- Anthraquinone authorisation for plant protection products withdrawn by ,Commission Decision 2008/986/EC‘^[1]
- Residue concentrations exceeding the MRL are mostly found in teas^[2]
- Anthraquinone is a *multiple source compound*:
 - ✓ Paper pulp catalyst^[3]
 - ✓ Avian repellent^[4, 5]
 - ✓ Direct and indirect emission from incomplete pyrolysis and combustion of organic matter^[6, 7, 8]

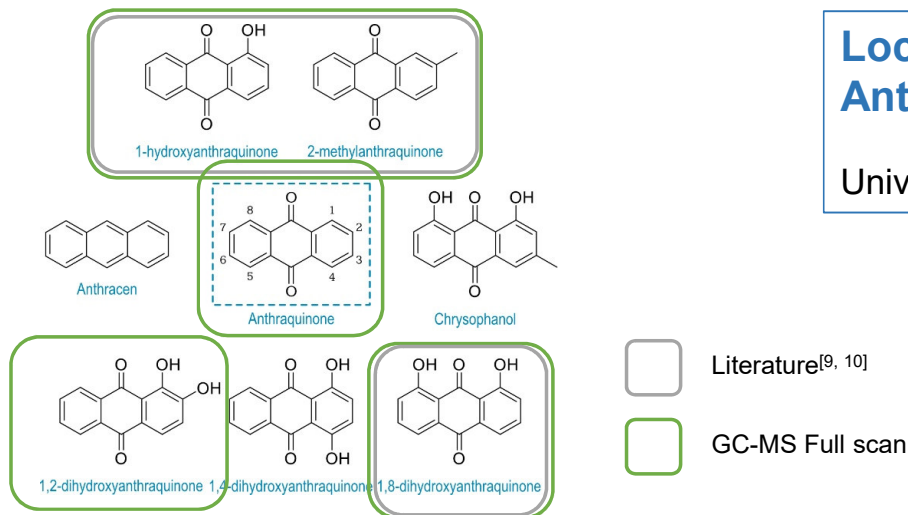




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Anthraquinone

- 2014 – 2022: 23 samples of oven dried leaves from Bosnian walnuts were analysed with QuEChERS
- All samples: Anthraquinone \geq mrl (0,02 mg/kg) in the range from 0,020 mg/kg to 0,084 mg/kg
- The literature indicates that walnuts show a plethora of anthraquinone derivatives in different parts of the plant, including the leaves of *Juglans regia*.^[9, 10]



Location and Quantification of Anthraquinone and Anthraquinone Derivatives in *Juglans regia*

University of Regensburg, Master Thesis, **Lucas Ferrando Plo**

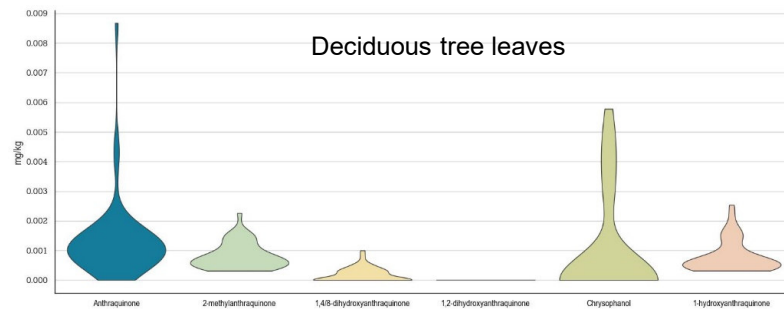
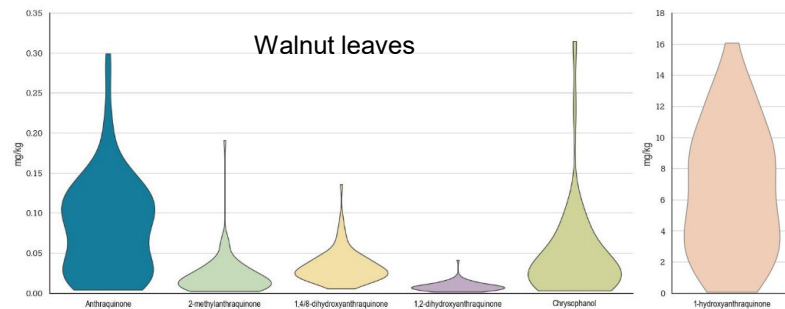


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Anthraquinone

Results:

- ✓ Oven drying of fresh leaves eliminates approx. 80% of anthraquinone content
- ✓ All deciduous tree leaves show levels approx. 50 times lower than *J. regia* leaves (factor 1.000 for 1-hydroxyanthraquinone)



➡ **An endogenous origin of anthraquinone in *Juglans regia* (walnut tree) is plausible!**

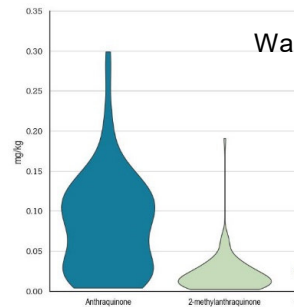


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Anthraquinone

Results:

- ✓ Oven drying of
- ✓ All deciduous t (factor 1.000 fc



Walnut leaves

Basenkräuter KRÄUTERTEEMISCHUNG

Zutaten: Brennnessel* (26 %), **Grünes Haferkraut***, **Walnussblatt***, Himbeerblatt*, Zitronengras*, Fenchel*, Schafgarbe*, Koriander*, Lindenblüte* (4 %), Löwenzahnkraut*, Salbei*, Brombeerblatt*, Ysop*, Ringelblume*

* AUS ÖKOLOGISCHEM LANDBAU

Zubereitung:

Einen Teebeutel pro Tasse mit **sprudelnd kochendem** Wasser übergießen und den Tee 8 Minuten ziehen lassen. So erhalten Sie ein sicheres Produkt.



200 ml
1 Beutel



100 °C



8 Min.

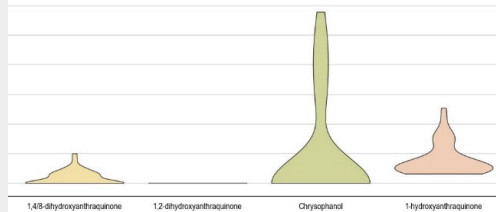


1 Liter
5 Beutel

quinone content

han *J. regia* leaves

Deciduous tree leaves



→ An endogen

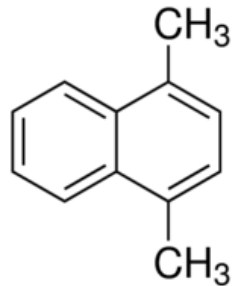
ia (walnut tree) is plausible!



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1,4-Dimethylnaphthalene

1,4-Dimethylnaphthalene (CAS 571-58-4)



- Plant growth regulator
- Natural content in potatoes (approx. 2 ppm), MRL = 15ppm
- Sprouting inhibitor (*1,4-Sight*[®], BASF)
- Replacement for *Chlorpropham*

Evidence: Parsley with 0.081mg/kg

mrl (2018): 0.01mg/kg (today: 0.05mg/kg) → **not marketable**

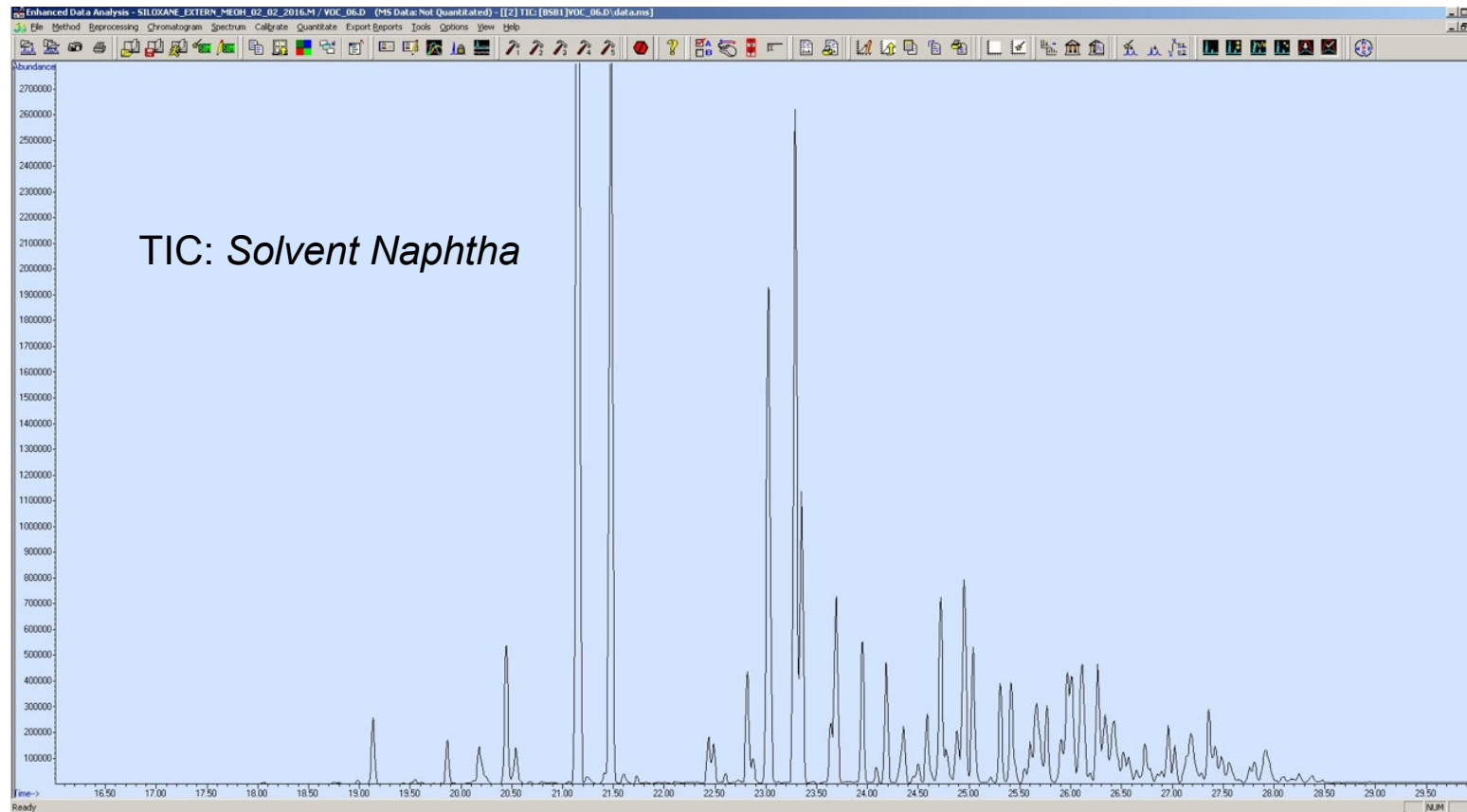
Research: Parsley had been treated with *Score*!

Formulation: *Difenoconazole* in "solvent naphtha (petroleum), highly aromatic"



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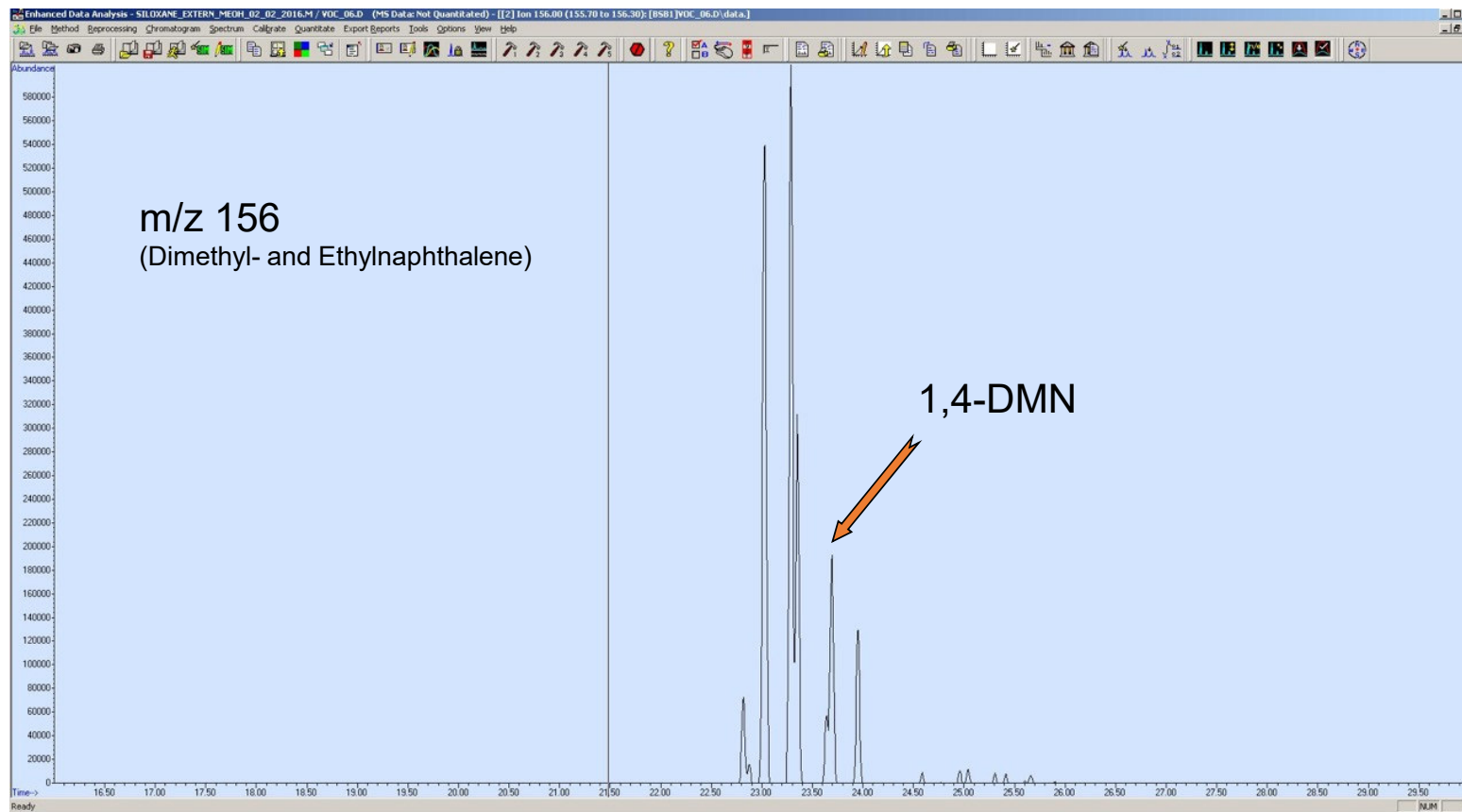
1,4-Dimethylnaphthalene





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1,4-Dimethylnaphthalene



The presence of isomers is an indication for the use of the petroleum fraction *Naphtha Solvent* as a formulation aid!

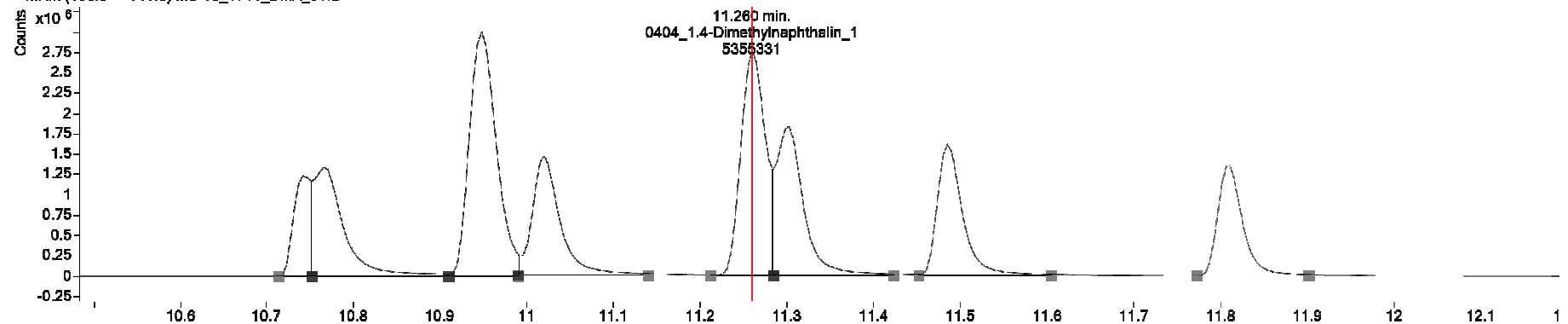


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1,4-Dimethylnaphthalene

Name	RT [min] (column: HP5-MS-UI 30m x 0,25mm ID x 0,25µm film)
2,6-Dimethylnaphthalin	10,746
2,7-Dimethylnaphthalin	10,775
1,7-Dimethylnaphthalin	10,943
1,3-Dimethylnaphthalin	10,957
1,6-Dimethylnaphthalin	11,020
1,4-Dimethylnaphthalin	11,260
2,3-Dimethylnaphthalin	11,274
1,5-Dimethylnaphthalin	11,303
1,2-Dimethylnaphthalin	11,490
1,8-Dimethylnaphthalin	11,811

+ MRM (156.0 -> 141.0) MS-10_1711_DMA_01.D

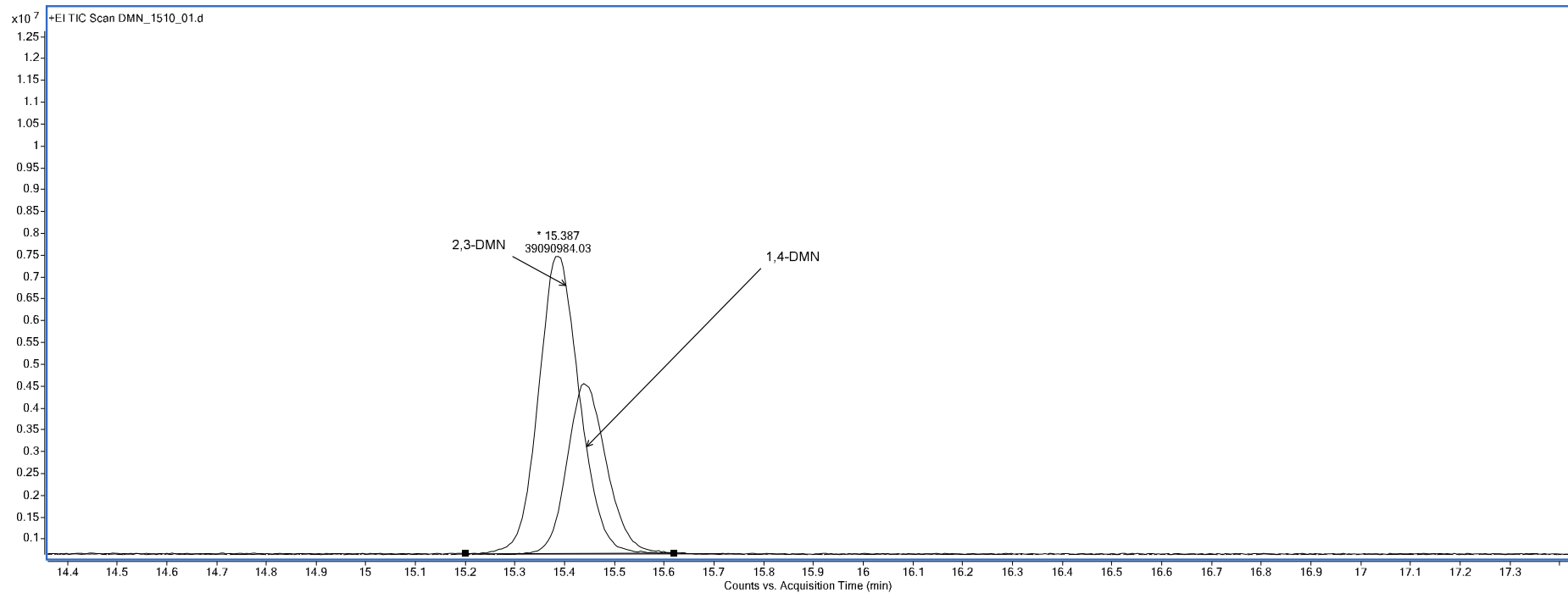


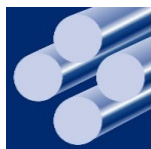


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1,4-Dimethylnaphthalene

chromatographic separation on a *Wax column* (ZB-WAX 30m x 0.25mm ID x 0.5µm film)





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Azadirachtin vs. Karanjin

Project: residue analysis

sample no.:	FL23-020653-0001
sample name:	██████████ Frozen Raspberry
description of sample:	raspberries defrosted, approx. 460 g in a sample bag
date of receipt:	Feb 9, 2023 period of analysis Feb 9, 2023 to Feb 13, 2023
sampler:	client

Scope of testing:	Plant protection products acc. current scope			
Method:	§64 LFGB, L00.00-115 (QuEChERS), GC-MS/MS + LC-MS/MS, 2018-10			
Substance	Unit	Result	MRL	RL
1,4-Dimethylnaphthalin	mg/kg	0.16 ←	¹	0.01
Azadirachtin (Neem)	mg/kg	0.045	1.00	0.01
Biphenyl	mg/kg	0.017 ←	0.01	0.01
Karanjin	mg/kg	0.65 ²	0.01	0.01

Scope of testing:	Fosetyl + phosphonic acid			
Method:	modif. QuPPE, (-ESI)LC-MS/MS / PA-ML-L-40, 2021-09			
Substance	Unit	Result	MRL	RL
Fosetyl-Al (sum of fosetyl and phosphonic acid, expressed as fosetyl)	mg/kg	-	300.00	0.01
Fosetyl	mg/kg	-		0.01
Phosphonic acid	mg/kg	-		0.01

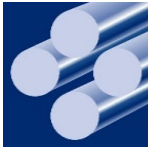
Scope: Feb 18, 2021

¹ see comment at evaluation

² validated via second determination and standard addition

RL = reporting limit MRL = maximum residue level according to Regulation (EC) No 396/2005

- = not detected (below reporting limit)





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Azadirachtin vs. Karanjin

Comment:

1,4-Dimethylnaphthalene (1,4-DMN) is a multiple source substance. It is found, for example, as a component of the petroleum fraction naphtha (crude petrol), which is used to formulate agricultural inputs. In contrast to the chemical synthetic germination inhibitor 1,4Sight, the chromatographic detection of naphtha differs in the presence of further isomers of 1,4-DMN. In the present case, further isomers of 1,4-DMN are detectable. This is a clear indication of a naphtha-containing agent. The limit value of Reg. (EC) no. 396/2005 is therefore not applicable.

Substance	Unit	Result	MRL	RL
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Fosetyl	mg/kg	-		0.01
Phosphonic acid	mg/kg	-		0.01

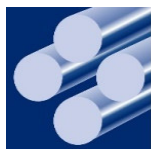
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Biphenyl	mg/kg	0.017	0.01	0.01
Karanjin	mg/kg	0.65  ²	0.01	0.01

Scope of testing:	Fosetyl + phosphonic acid			
Method:	modif. QuPPE, (-ESI)LC-MS/MS / PA-ML-L-40, 2021-09			
Substance	Unit	Result	MRL	RL
Fosetyl-Al (sum of fosetyl and phosphonic acid, expressed as fosetyl)	mg/kg	-	300.00	0.01
Fosetyl	mg/kg	-		0.01
Phosphonic acid	mg/kg	-		0.01

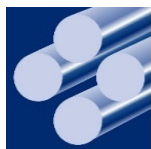
Scope: Feb 18, 2021

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Azadirachtin vs. Karanjin

Project: residue analysis

sample no.:	FL23-020653-0001
sample name:	██████████ Frozen Raspberry
description of sample:	raspberries defrosted, approx. 460 g in a sample bag

Evaluation:

The detected value of Karanjin is even in consideration of the expanded measurement uncertainty of 50% (Document No. SANTE/11312/2021) above the MRL. Due to this result the examined sample does not observe the requirements of the Reg. (EC) no. 396/2005 in its latest issue and is therefore not marketable.

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Azadirachtin (Neem)	mg/kg	0.045 ←	1.00	0.01
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Fosetyl-Al (sum of fosetyl and phosphonic acid, expressed as fosetyl)	mg/kg	-	300.00	0.01
Fosetyl	mg/kg	-		0.01
Phosphonic acid	mg/kg	-		0.01

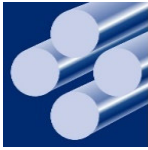
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Azadirachtin vs. Karanjin

Project: residue analysis

Sample No.:	L23-030968-01		
Your sample:	[REDACTED] 0.03% EC		
description of sample:	liquid, 200 ml, in an originally sealed container		
date of receipt:	23.03.2023 period of analysis: 23.03.2023 to 31.03.2023		
sampling:	client		
Scope of testing:	Plant protection products acc. current scope		
Method:	QuEChERS, GC-MS/MS, PA-ML-B-11, 2023-03		
Parameter	Unit	Result	RL
Active material (GC-MS/MS)			
Biphenyl	g/kg	0,29	0,01
1,4-Dimethylnaphthalene *	g/kg	3,0	0,01
Scope of testing:	Plant protection products acc. current scope		
Method:	QuEChERS, LC-MS/MS, PA-ML-B-11, 2023-03		
Parameter	Unit	Result	RL
Active material (LC-MS/MS)			
Azadirachtin (Neem)	g/kg	0,079	0,01
Karanjin	g/kg	3,3	0,01

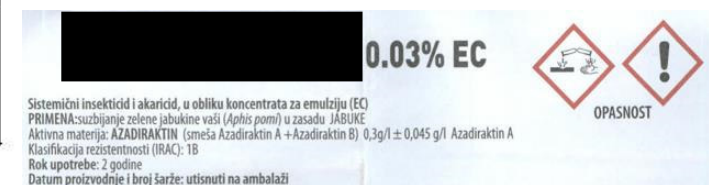
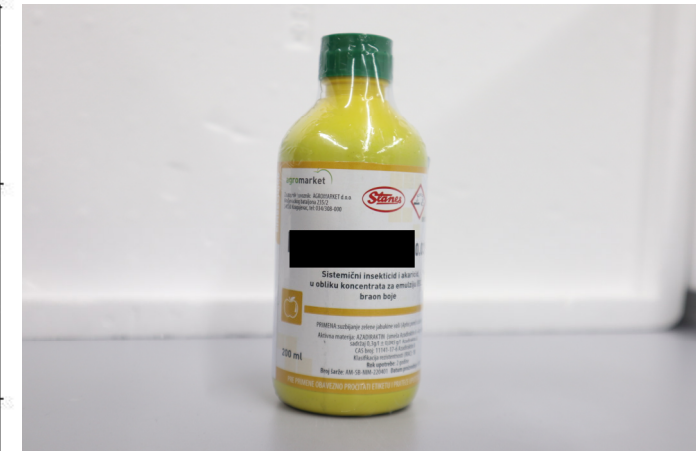
* further isomers present

Scope 2023/03

RL = reporting limit



Misdeclaration!



Thank you