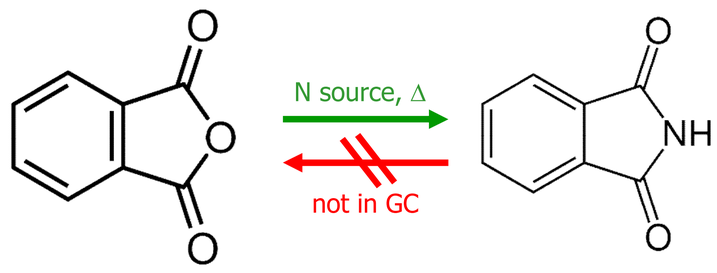
Phthalimide
A metabolite of Folpet or an artefact produced in the GC from other sources?

Hentschel, A., Löbner, J., Rothmeier, S., Kirchhoff, E.
Institut Kirchhoff Berlin GmbH, Oudenarder Str. 16, 13347 Berlin/D

Introduction - A new problem with an old pesticide

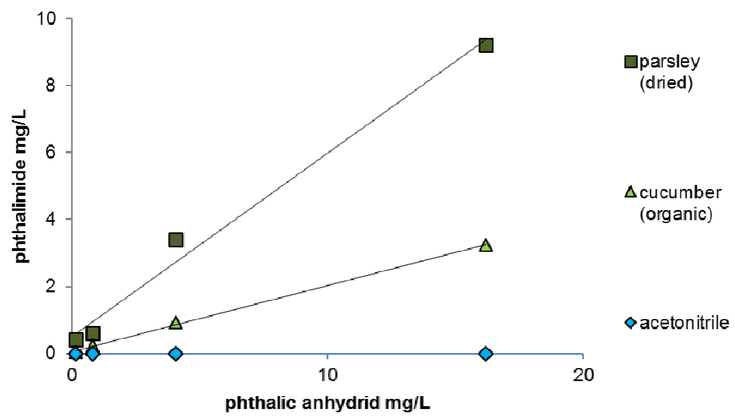
Since 2016, German laboratories detected a rising number of commodities which exceeded the maximum residue level of Folpet. This happened simultaneously with the new residue definition of Folpet, which entered into force as from August 26th 2016. Since this date, in addition to the parent compound also Folpet's metabolite phthalimide has to be considered for all commodities.

Creation of phthalimide in the GC injector



Phthalic anhydride **Phthalimide**

0.16, 0.8, 4 and 16 mg/L phthalic anhydride were spiked to acetonitrile and QuEChERS extracts of cucumber and dried parsley, respectively.



In subsequent GC experiments with QuEChERS extracts it could be shown that in the hot GC injector formation of phthalimide is possible when the contaminant phthalic anhydride and an additional source of nitrogen is present. The detected amount of phthalimide correlates with spiked concentration of phthalic anhydride. The "recovery" of this reaction depends on matrix (e.g. parsley > cucumber). In pure acetonitrile no phthalimide formation is observed.

Sources for phthalic anhydride

Phthalic anhydride and the corresponding phthalic acid are both widely used chemicals. They are needed for the production of alkyd resins and plasticisers. They are found in paints, PVC or paper coatings and used for printing newspapers. Residues of phthalic anhydride and phthalic acid are ubiquitous chemicals.

Influence of clean-up steps of QuEChERS

Inter-laboratory method study

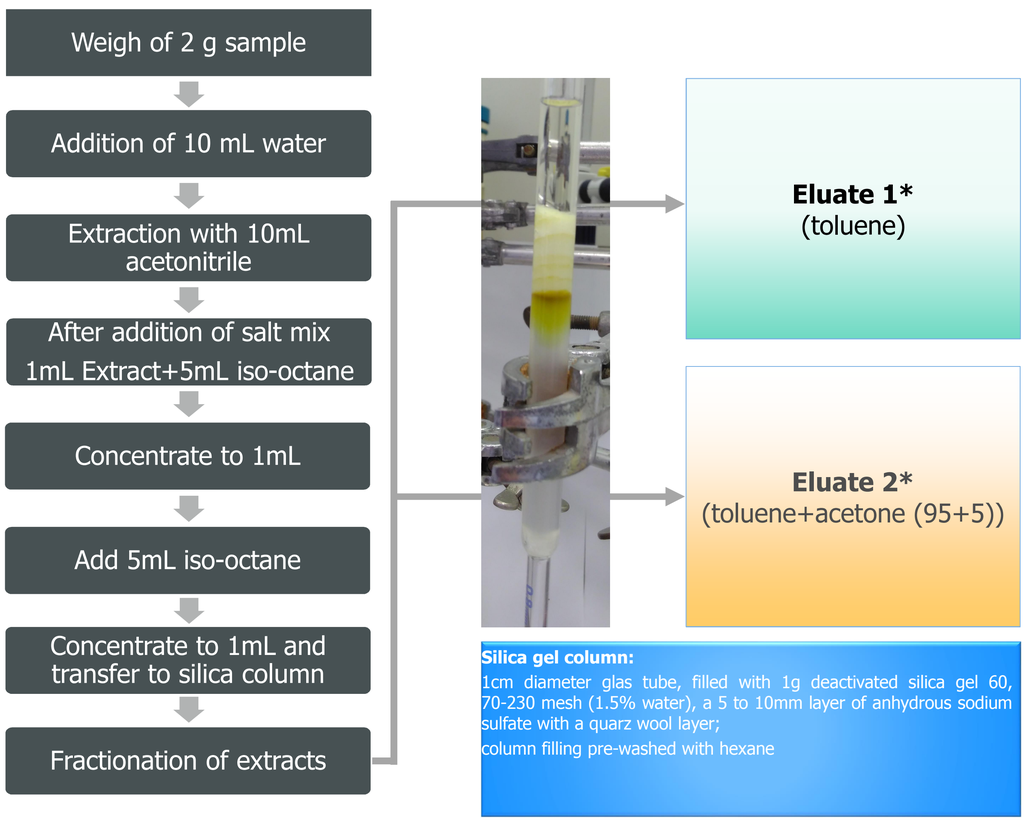
- Dried parsley with incurred residues (of ?) was used as sample material
- A subsample of this parsley (2nd sample) was spiked with phthalic anhydride before extraction
- The processing was mainly based on QuEChERS method with different use of buffers/clean-up steps
- Organized by WG Pesticides of the German Food Chemical Society in Sep./Oct.
- 21 labs participated (Germany, Austria)

Results of this study, when QuEChERS was used

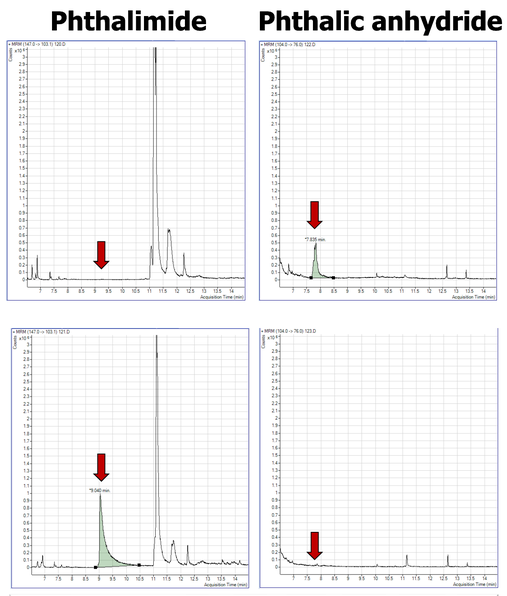
LabCode	unspiked [mg/kg]	spiked [mg/kg]	Ratio sp./unsp.	significant difference	method	extracting agent	clean up
7	0.000	0.000		*w/o evaluation*	QuEChERS	Acetonitrile	Citrate / PSA / GCB
14	0.000	0.000		*w/o evaluation*	QuEChERS	Acetonitrile	Citrate / PSA
20	0.000	0.000		*w/o evaluation*	QuEChERS	Acetonitrile	-NONE-
1	0.019	0.017	89%	no	QuEChERS	Acetonitrile	Citrate / PSA / GCB
1	0.014	0.014	107%	no	QuEChERS	Acetonitrile	Citrate / PSA / GCB
2	0.013	0.061	469%	YES	QuEChERS	Acetonitrile	PSA / GCB
3	0.016	0.054	338%	YES	QuEChERS	Acetonitrile	Citrate / PSA / GCB
5	0.026	0.138	531%	YES	QuEChERS	Acetonitrile	Citrate / PSA
6	0.105	0.066	63%	no	QuEChERS	Acetonitrile	Citrate / PSA / GCB
8	0.042	0.085	202%	YES	QuEChERS	Acetonitrile	Citrate / PSA
9	0.039	0.042	108%	no	QuEChERS	Acetonitrile	Citrate / PSA / GCB
9	0.069	0.100	145%	YES	QuEChERS	Acetonitrile	Citrate
9	0.069	0.112	162%	YES	QuEChERS	Acetonitrile	Citrate
10	0.027	0.104	385%	YES	QuEChERS	Acetonitrile	-NONE-
11	0,032	0.026	81%	no	QuEChERS	Acetonitrile	Citrate / PSA
12	0.027	0.179	663%	YES	QuEChERS	Acetonitrile	Citrate / PSA / GCB
15	0.100	0.183	183%	YES	QuEChERS	Acetonitrile	Citrate / PSA / GCB
15	0.113	0.415	367%	YES	QuEChERS	Acetonitrile	Citrate / PSA / GCB
16	0.021	0.041	195%	YES	QuEChERS	Acetonitrile	Citrate / PSA / GCB
17	0.003	0.015	500%	YES	QuEChERS	Acetonitrile	Citrate / PSA
17	0.003	0.024	800%	YES	QuEChERS	Acetonitrile	Citrate / PSA
17	0.003	0.024	800%	YES	QuEChERS	Acetonitrile	Citrate / PSA
18	0.003	0.007	206%	YES	QuEChERS	Acetonitrile	Citrate / PSA / GCB
21	0.008	0.044	550%	YES	QuEChERS	Acetonitrile	-NONE-

- 24 results were obtained using the QuEChERS method with GC-MS/MS detection
- No phthalimide was detected by 3 laboratories (neither in the incurred nor in the spiked sample)
- Fortification of phthalic anhydride significantly increased the observed levels of phthalimide in 16 out of remaining 21 experiments (76%).
- The amount of additional phthalimide found in spiked samples differed between labs
- Citrate buffering has no effect on observed levels
- PSA-cleanup may slightly reduce the observed phthalimide levels
- Purification with GCB has no effect on observed levels

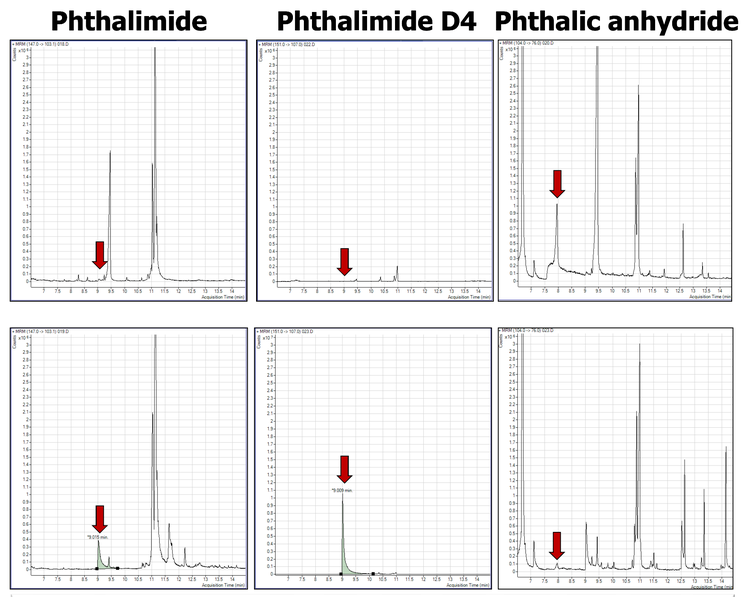
Special clean-up [1] to seperate phthalic anhydride from phthalimide



Silica gel column:
1cm diameter glas tube, filled with 1g deactivated silica gel 60, 70-230 mesh (1.5% water), a 5 to 10mm layer of anhydrous sodium sulfate with a quarz wool layer;
column filling pre-washed with hexane



1st experiment:
1mL of a mixture of phthalic anhydride (1mg/L) and phthalimide (1mg/L) in iso-octane was transferred to the silica column. Then the column was eluted with toluene (eluate 1) and after that eluted with a mixture of toluene/acetone (95+5).
⇒ Phthalic anhydride eluted in eluate 1, and phthalimide eluted in eluate 2



2nd experiment:
2g of dried parsley (same sample used in the above mentioned inter-laboratory study) was spiked with 100µL phthalic anhydride (1mg/L) and 100µL of the ILIS phthalimide-D4 (10mg/L). Further work-up as described on the left. Then the column was eluted with toluene (eluate 1) and after that eluted with a mixture of toluene/acetone (95+5).
⇒ Spiked Phthalic anhydride eluted in eluate 1, and spiked ILIS phthalimide-D4 eluted only in eluate 2; a small amount of phthalimide was detected, but this was confirmed by (APCI)LC-MS/MS, the sample was not free from phthalimide

*Eluate 1 corresponds to the Fraction 2 and Eluate 2 corresponds to the fraction 3 of ASU L 00.00-34:2010 module C

Conclusion

- As regulated, detection of phthalimide without Folpet may occur and has to be expected
- Problem: phthalimide can be formed during routine QuEChERS in GC-Liner from ubiquitous phthalic anhydride and other possible precursor
- Any QuEChERS clean-up does not offer a solution
- to avoid phthalimide occurrence during analyses additional clean-up is crucial

literature

[1] Combination of QuEChERS extraction and mini silica gel column chromatography for GC-µECD determination of organochlorine and pyrethroid matrices – Heike Pawelzick, Thomas Anspach - eurofins Dr. Specht Laboratorien GmbH Hamburg (D); Poster presented on EPRW 2014

www.institut-kirchhoff.de