

Glyphosate residues in pre-harvest glyphosate treated cereal grains

Workshop on pesticide fate in soil and water in the northern zone

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Picking of headlines

- **Glyphosate Herbicide Found in 14 Popular Beer Brands from Germany**
 - The Munich Environmental Institute found glyphosate readings between 0.46 and 29.74 micrograms per liter in 14 different popular beers.
 - It is nearly 300 times more than the allowable limit of 0.1 micrograms in water.
 - Posted on February 26, 2016 by Christina Sarich

<http://naturalsociety.com/>

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- Renewed call for bakers to end use of wheat sprayed with glyphosate
 - By Vince Bamford + Vince Bamford, 19th of July 2016
 - Campaigners are urging major UK bread manufacturers and supermarkets not to use flour from wheat sprayed with glyphosate immediately before harvest.

<http://www.bakeryandsnacks.com/Ingredients/Glyphosate-Bread-firms-urged-not-to-use-flour-from-treated-wheat>

Clarification of pre-harvest uses of glyphosate

In several north western European countries glyphosate can be applied before crop harvest for weed control, to enhance ripening on non-determinate crops to reduce crop losses, and to help manage determinate crops in wet seasons...

...the bulk grain sample must have dried to a maximum of 30% moisture content. At this point it is physiologically mature and the grain is filled, so glyphosate will not be translocated into the grain from the plant.

http://www.glyphosate.eu/system/files/sidebar-files/clarification_of_pre-harvest_uses_of_glyphosate_en_0.pdf

Facts about pre-harvest treatment with glyphosate in Finland

- Spraying must be done at least ten days before harvesting
- Moisture of grains must be below 30 %
- For destroying weeds, mainly couch grass
- Not allowed for grains used as seed or food
- Allowed for rape and turnip rape
- Allowed for oats and barley as animal feed
- Feed industry of Finland does not accept grains, which have been treated with glyphosate
- Treated grains may only be used as cattle feed in own farm or directly sold to other farms

<https://kasvinsuojeluaineet.tukes.fi/>

Glyphosate and AMPA residues in grains

1. Experiment: October 2015
2. Preliminary analysis: January 2016
- poster presentation in EPRW 2016: June 2016
3. Matrix match calibration for barley: April 2016
4. Residue analysis in barley and oats: June 2016

Experiment

- We studied if pre-harvest treatment will lead to any glyphosate and AMPA residues in the grain yield of barley and oats.
- An experimental plot growing oats and a plot growing barley were sprayed with Roundup Bio at the label dose of 3.0 l/ha (glyphosate 360 g/l).
- The moisture of oat and barley grains was 26.5% and 38.7%, respectively, on the spraying day.
- Grains from both untreated and treated plots were harvested for glyphosate and AMPA analysis ten days after spraying.
- The harvest moisture of oats was 18% and barley 21%.

Preliminary analysis

- Sample matrix: barley, oats and their sorting waste
- Grains were extracted with water by blender
- Crude extract was partitioned with methylene chloride.
- Calibration standards were diluted with harvesting waste extract for residues in field water
- Calibrants and the aliquots of the water phase of extracts were derivatized with 9-fluorenylmethylchloroformate (FMOC-Cl)
- FMOC-derivatives of analytes were identified and quantified with MRM technique by Waters Acquity UPLC Xevo TQ MS – instrument
- LOQ: 0.5 mg/kg

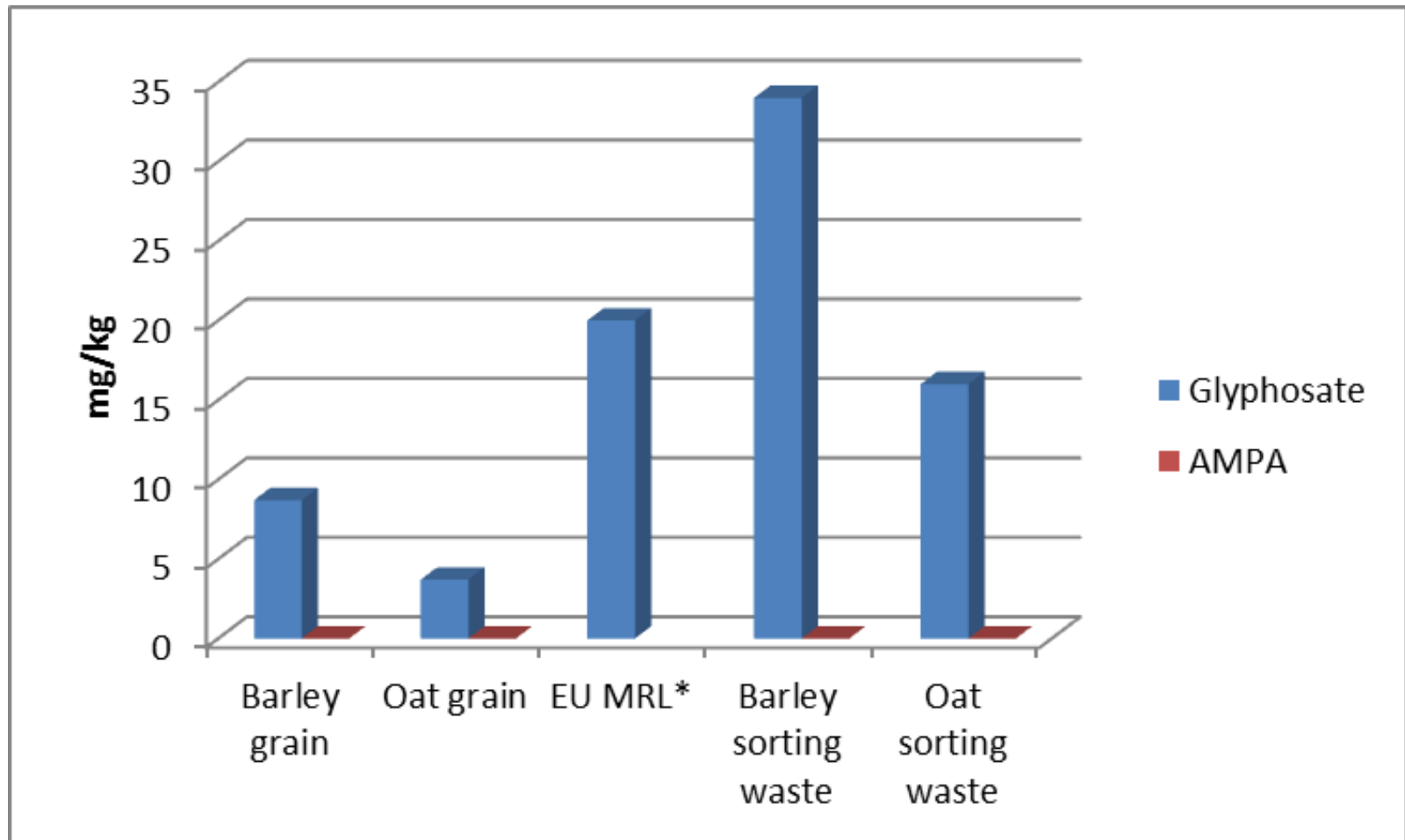
MRM reactions of ES+ ionisation:

IS = Internal standard, * = recovery standard of method, Q= Quantitative ion

Compound	Mother (m/z)	Daughter (m/z)	Dwell (s)	Cone (V)	Coll (V)
Glyphosate-FMOC	392.1	87.93 (Q)	0,019	20	16
	392.1	214	0,019	20	9
(IS) 13C2,15N-Glyphosate-FMOC	395.1	90.99 (Q)	0,019	20	16
	395.1	217	0,019	20	12
AMPA-FMOC	334.1	112	0,019	20	13
	334.1	156 (Q)	0,019	20	8
(IS)13C,15N-AMPA-FMOC	336.1	114	0,019	20	12
	336.1	158 (Q)	0,019	20	8
Glufosinate-FMOC*	404.1	136 (Q)	0,019	20	20
	404.1	182	0,019	20	16

The concentrations of glyphosate and AMPA in barley and oat grains along with their sorting wastes.

* The MRL value for glyphosate in barley and oat grains for human consumption is 20 mg/kg.

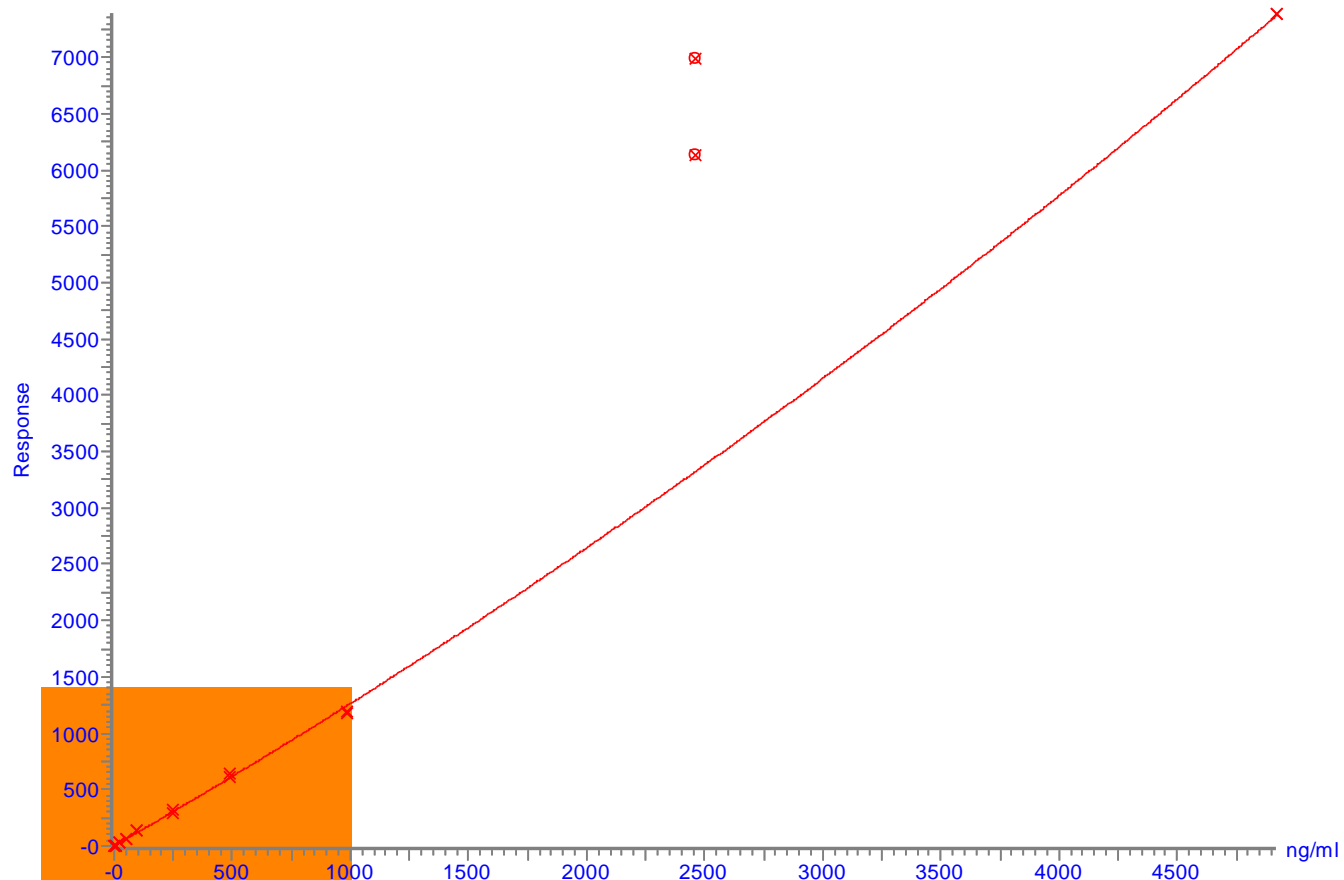


Estimation of reliability of the results

- No recovery tests were done in January
- => Matrix matched calibration : April 2016
- Grains were stored 3 months in room temperature before preliminary analysis
 - Were original concentrations even higher?
- => Subsamples were stored in freezer
- 3 months in room temperature : - 20 °C January 2016
 - 6 months in room temperature : - 20 °C April 2016
 - Residue analysis in barley and oats: June 2016

Glyphosate calibration curve for field water

Compound name: Glyphosate-FMOC
Coefficient of Determination: $R^2 = 0.999385$
Calibration curve: $6.00604e-005 * x^2 + 1.20239 * x + 0.201669$
Response type: Internal Std (Ref 4), Area * (IS Conc. / IS Area)
Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x, Axis trans: None

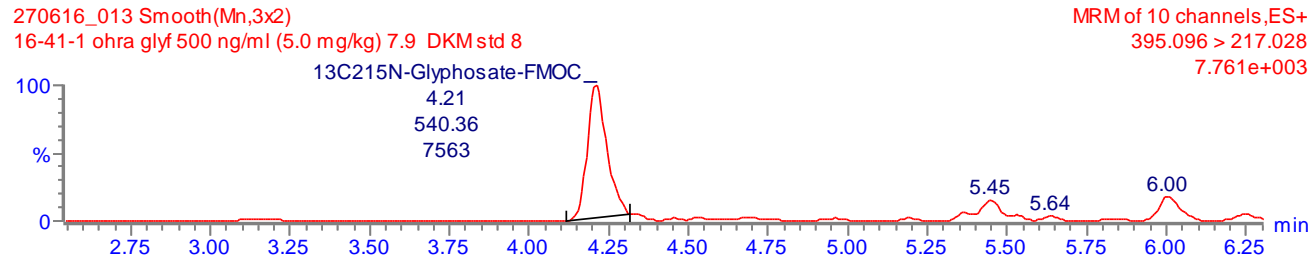
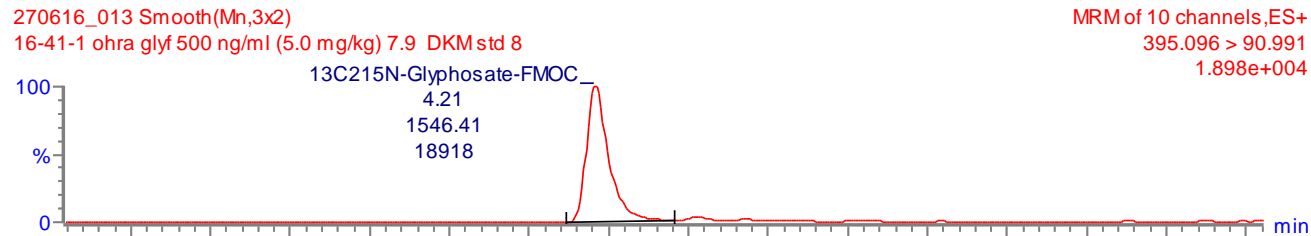
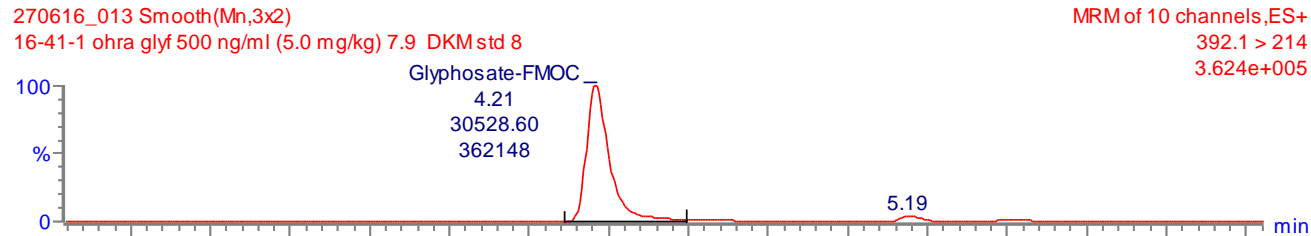
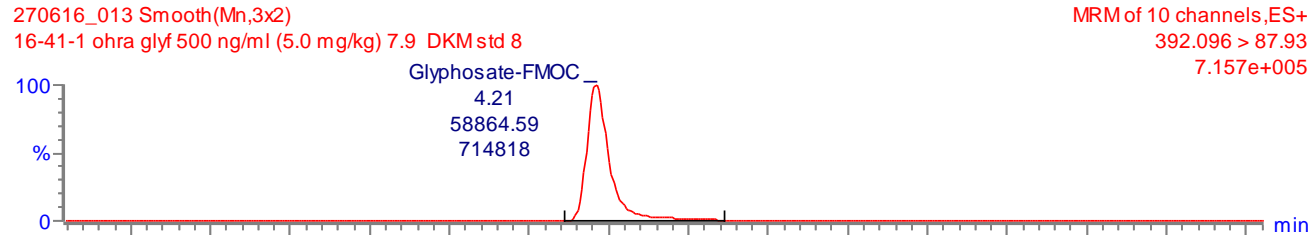


Uppsala, 7-8 September 2016

Matrix match calibration for barley

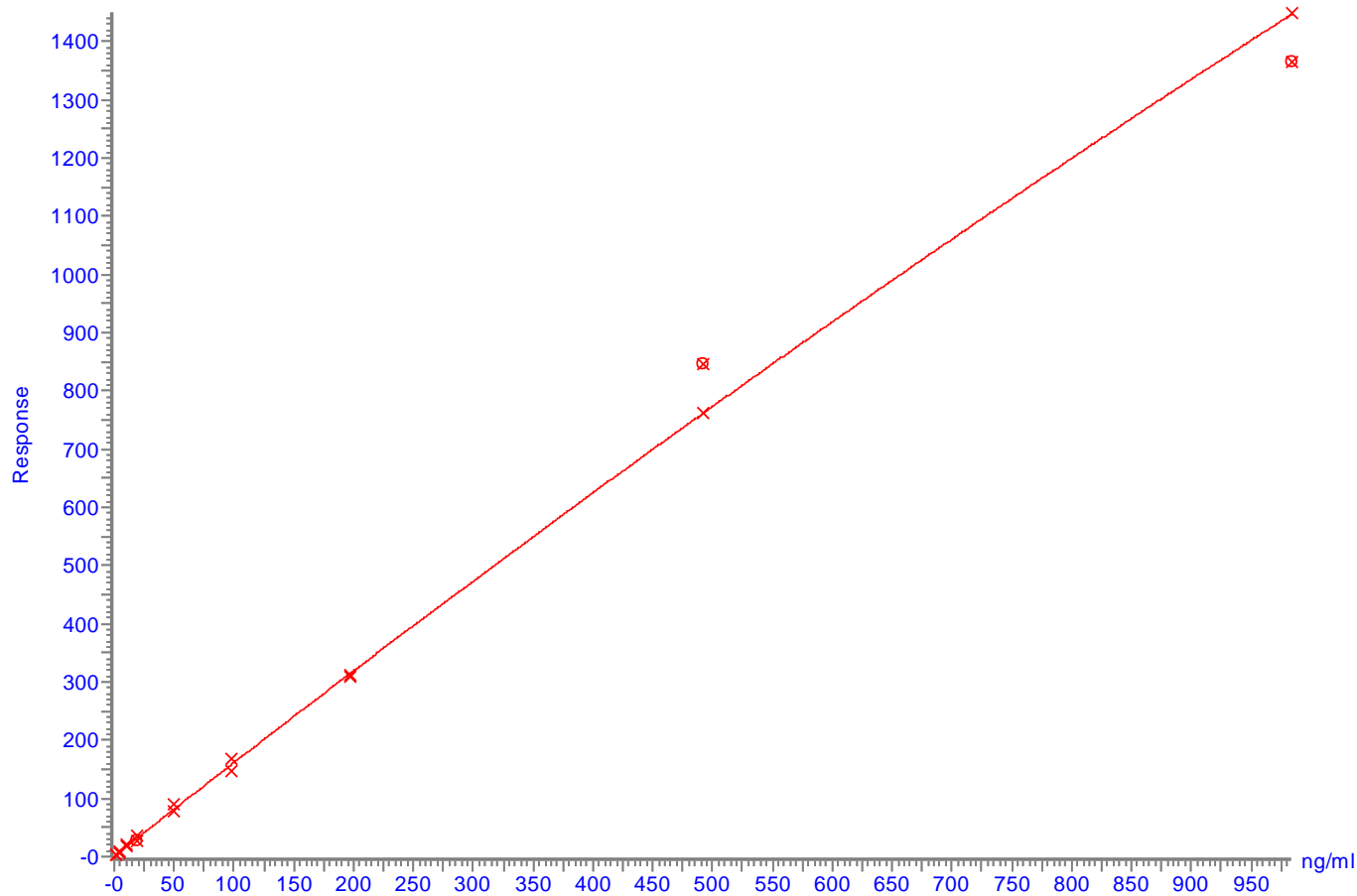
- Untreated barley grain were spiked with glyphosate and AMPA
 - 0.01 – 10 mg/kg
 - 0 mg/kg spiked with water
- Grains were extracted with water by blender
- Extract was used as itself or partitioned with DCM
 - Use DCM, if extract should storage for further use
- The aliquots of crude extract or the water phase of extract were derivatized as in preliminary analysis
- FMOC-derivatives were analyzed as in preliminary analysis
- LOQ 0.1 mg/kg (LOD 0.01 mg/kg)

Glyphosate-standard in barley 5.0 mg/kg



Glyphosate calibration curve for barley

Compound name: Glyphosate-FMOC
Coefficient of Determination: $R^2 = 0.998551$
Calibration curve: $-0.000155447 * x^2 + 1.62217 * x + 0.87404$
Response type: Internal Std (Ref 4), Area * (IS Conc. / IS Area)
Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x, Axis trans: None



Glyphosate in treated barley grain: 8.6 mg/kg

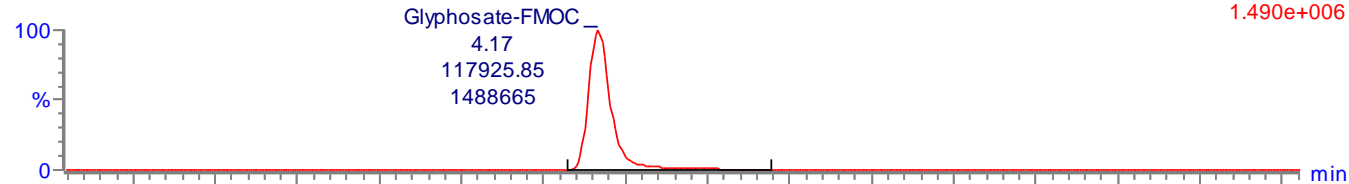
270616_023 Smooth(Mn,3x2)

16-41-2 ohra gly kasittely, 6 kk 25 C=> /pakastinkaappi 10.4DKM

MRM of 10 channels,ES+

392.096 > 87.93

1.490e+006



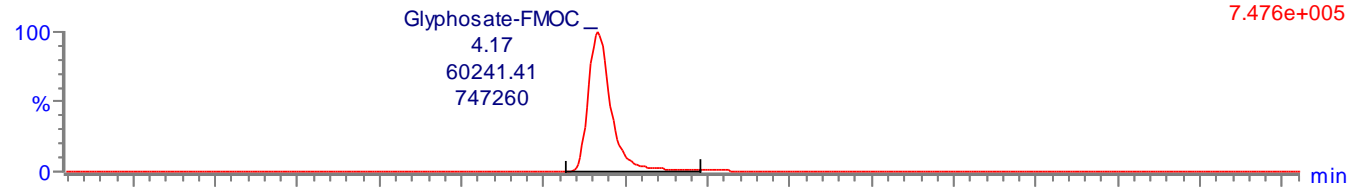
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MRM of 10 channels,ES+

392.1 > 214

7.476e+005



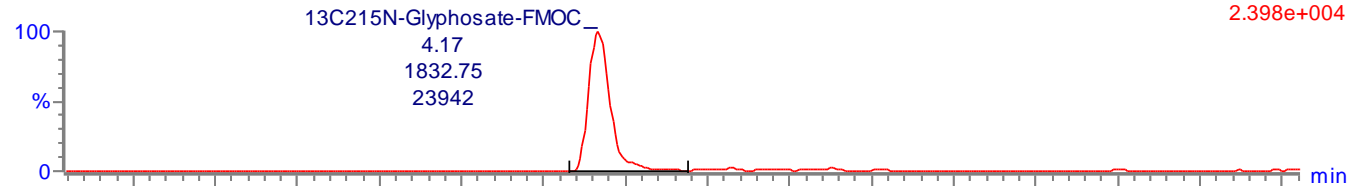
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MRM of 10 channels,ES+

395.096 > 90.991

2.398e+004



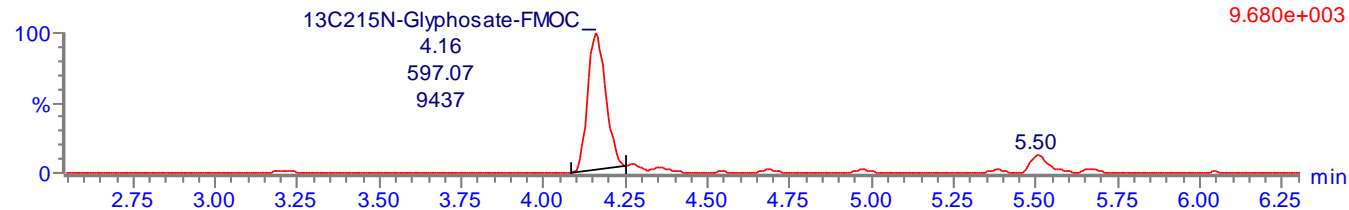
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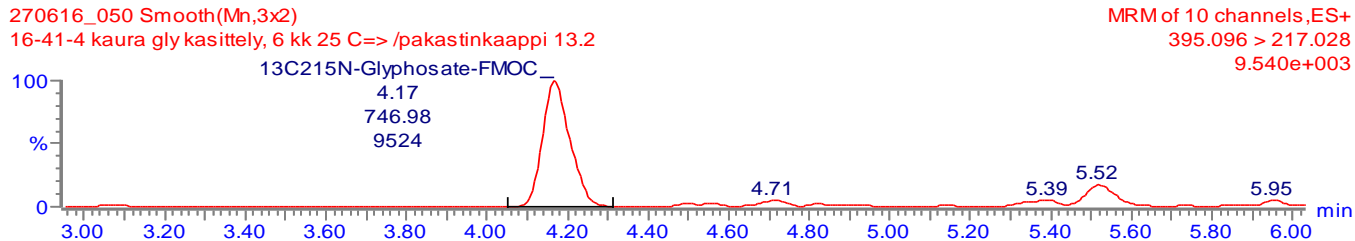
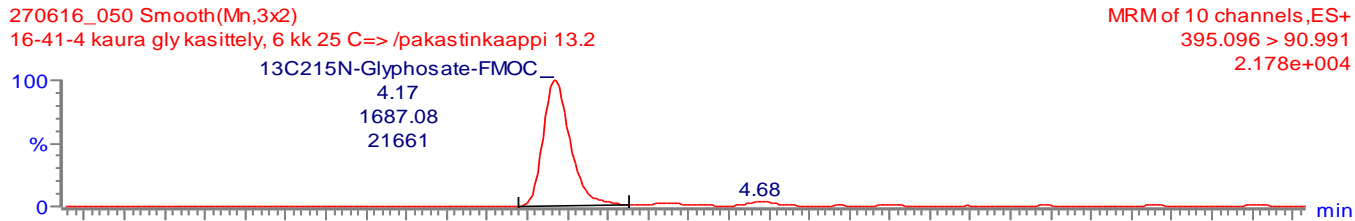
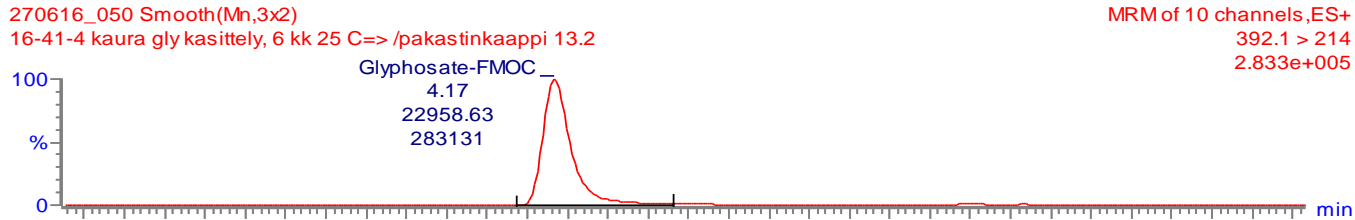
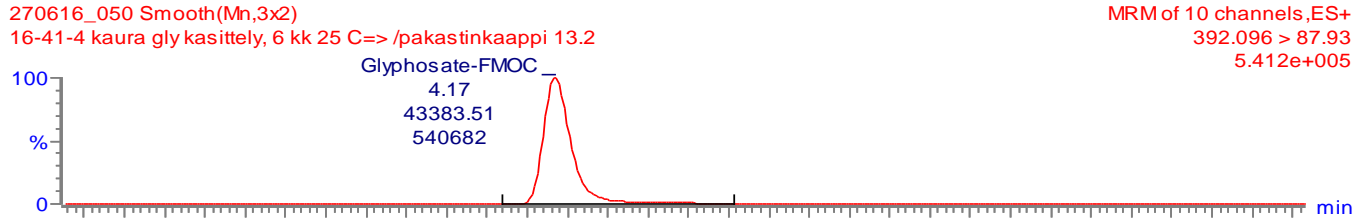
MRM of 10 channels,ES+

395.096 > 217.028

9.680e+003

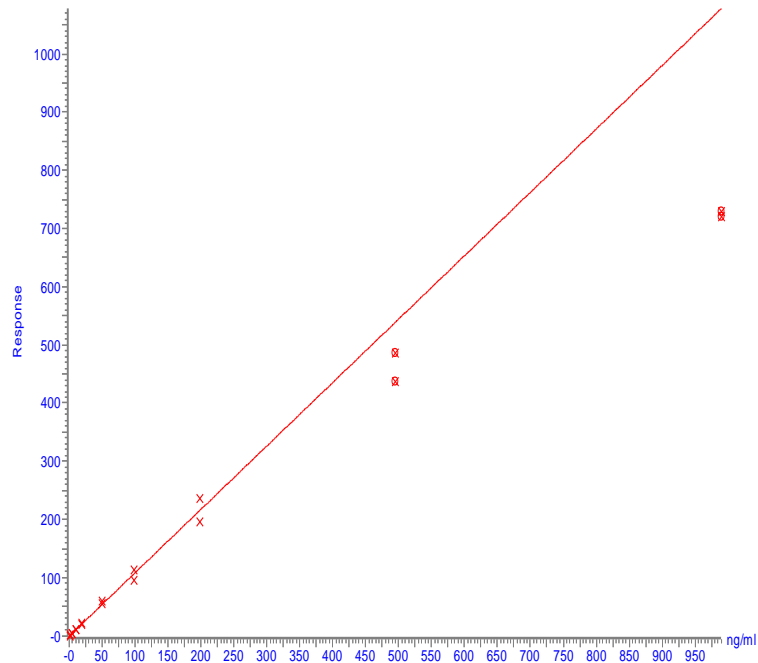


Glyphosate in treated oat grain: 3.1 mg/kg

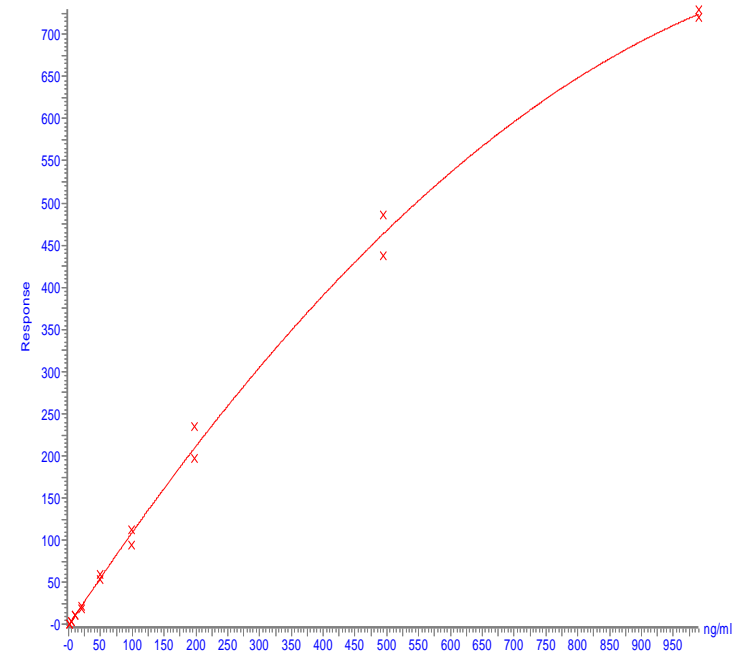


AMPA calibration curves for barley grain

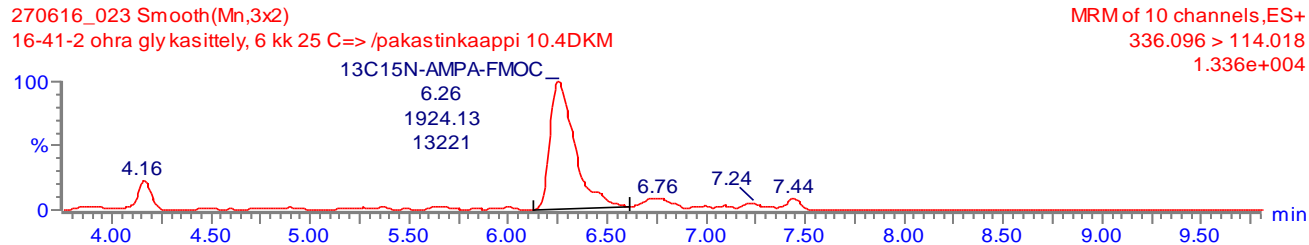
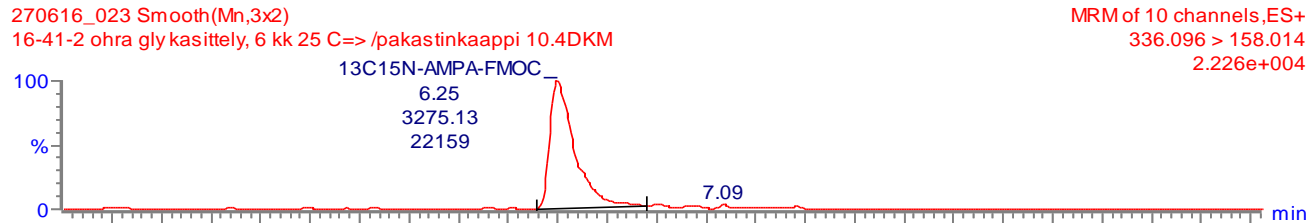
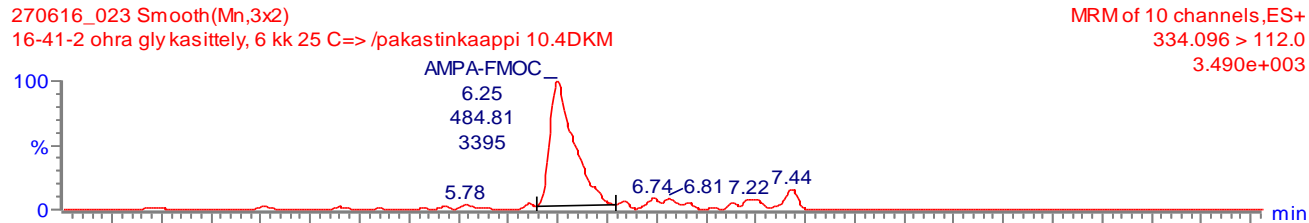
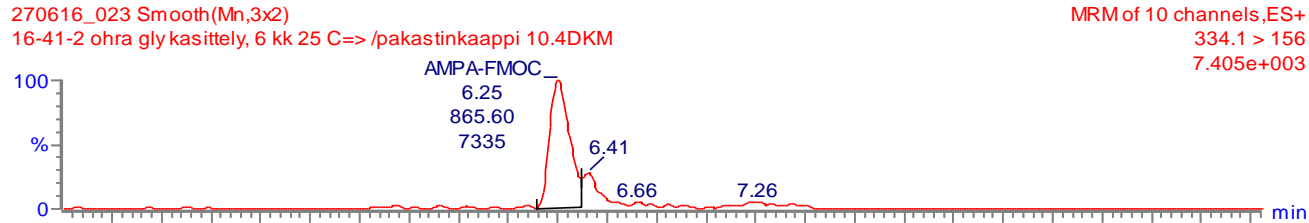
Compound name: AMPA-FMOC
Correlation coefficient: $r = 0.995835$, $r^2 = 0.991688$
Calibration curve: $1.08939 \cdot x + -0.562297$
Response type: Internal Std (Ref 3), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Compound name: AMPA-FMOC
Coefficient of Determination: $R^2 = 0.996122$
Calibration curve: $-0.00041739 \cdot x^2 + 1.14517 \cdot x + -0.823429$
Response type: Internal Std (Ref 3), Area * (IS Conc. / IS Area)
Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x, Axis trans: None

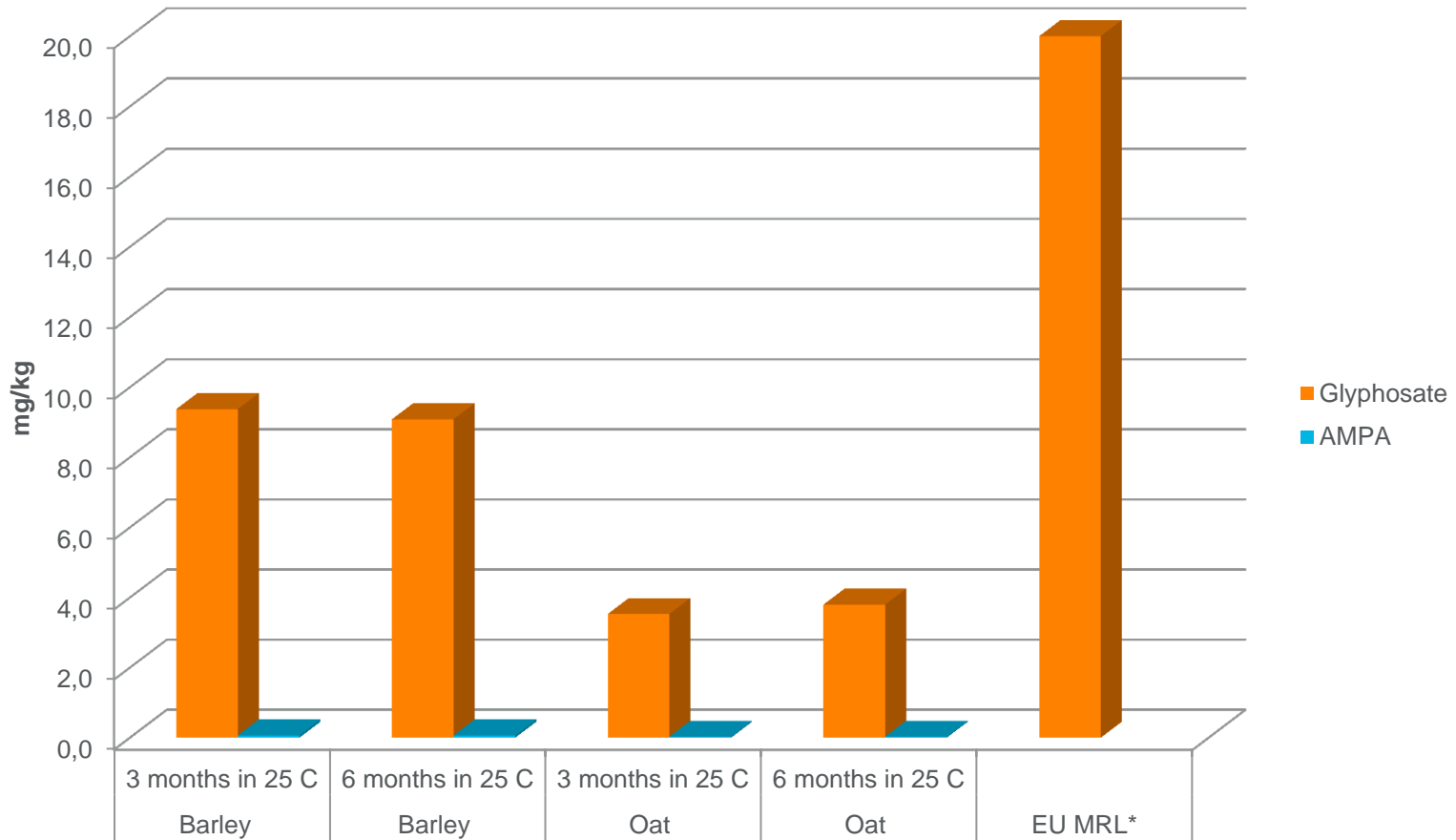


AMPA in treated barley grain: < 0.1 mg/kg



Residues with matrix matched calibration for barley

* The MRL value for glyphosate in barley and oat grains for human consumption is 20 mg/kg.



Conclusions

- Calibrations for both field water and barley grain worked well
- Residues were not detected in untreated grains
- Residues were quite stable:
 - no breakdown in room temperature
- AMPA residues were $<$ LOQ both in barley and oat grains
- Glyphosate residues were higher in barley than in oat grains
 - Higher moisture will explain it
 - $<$ 30% moisture will not prevent residues
- Is ten days period between spraying and harvesting too short?
- Was the spraying time so unusual (October)?

Method references

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Acknowledgements

- Pentti Ruuttunen about planning of the experiment
- Pentti Ruuttunen and Leena Ruokonen about spraying the fields
- Niko Jalava and Tuula Viljanen about harvesting grains
- Sari Rämö about residue analysis
- Jaana Uusi-Kämppe, the project leader of GLYFOS II

Thank you!



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