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An intercomparison study of the determination of glyphosate, chlormequat and mepiquat residues in wheat

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Abstract

An intercomparison study of the determinations of glyphosate, chlormequat and mepiquat residues in cereals was performed. Four samples comprising one blank, two incurred and one spiked sample were sent to six participating laboratories. For glyphosate, two laboratories reported considerably lower results than the other four. One of the two laboratories with low results also reported low recoveries. The results of a sample spiked with 0.80 mg/kg glyphosate and an incurred sample, ranged from 0.23-0.87 mg/kg and 0.11-0.25 mg/kg respectively. The strong correlation between the two samples ($r^2=0.95$) indicates a systematic between-laboratory variation. Several different principles were used for the analysis of glyphosate using different clean-up techniques and GC/MS, HPLC-fluorescence or LC/MS for detection. The results of the chlormequat residues showed more consistency. All but one laboratory obtained comparable results. However the correlation between the results for the sample spiked with 0.38 mg/kg (range: 0.26-0.65 mg/kg) and the incurred samples (range: 0.19-0.45 and 0.15-0.23 mg/kg, respectively) again showed a strong correlation ($r^2 = 0.99$ and 0.88) indicating a systematic component. For mepiquat, results above the limit of quantification were only reported for the spiked sample. The results ranged from 0.29-0.92 mg/kg (spiked concentration = 0.38 mg/kg). Three laboratories had results that deviated less than 25% from the fortified concentration. Two laboratories reported results 38% and 141% above the fortified concentration, respectively.

Keywords: Glyphosate, chlormequat, mepiquat, cereals, intercomparison, LC/MS, GC/MS

Introduction

Glyphosate is a widely used herbicide that is employed in weed control after sowing and before the harvest of different kinds of crops like cereals, peas and beans. Glyphosate is a systemic herbicide that is absorbed through the leaves of the weed and distributed in the plant. Glyphosate is the most frequently used pesticide in Denmark (Danish EPA 2004). Chlormequat and mepiquat are both plant growth regulators. Chlormequat is by far the most widely used plant growth regulator in Denmark (Danish EPA 2004) accounting for 85% of the total use of plant growth regulators in 2004. Both chlormequat and mepiquat are used on cereals, reducing the growth of the lowest part of the straw and thereby making it stronger. All three pesticides are sold and used as a salt of the compounds. Glyphosate is mostly sold as an ammonium salt and chlormequat and mepiquat as chloride salts.

Chlormequat is the most frequently found pesticide in the Danish monitoring of pesticide residues in cereals. In 1997, 83% of 46 cereal samples contained chlormequat (mean of all samples: 0.32 mg/kg) and in 1999 87% of 52 samples contained chlormequat (mean of all samples: 0.23 mg/kg) (Granby and Vahl 2001). In 2003, (Danish VFA 2004) and 2004 (DFVF 2006) the frequency decreased to 25% (of 124 samples) and 20% (of 161 samples), respectively. The frequency of glyphosate residues in cereals had decreased through the period 1998-2001 from >50% in 1998-1999 to <20% in 2000-2001. No residues of glyphosate were found in the 124 and 161 cereal samples analysed for glyphosate in the Danish pesticide monitoring program in 2003 and 2004, respectively (Danish VFA 2004, DFVF 2006). The decrease in glyphosate findings is at least partly due to statements from the Danish trade organizations for agriculture and bakeries, that they would produce bread without glyphosate for the consumers. The frequent use of glyphosate and chlormequat and the residues found in cereal samples makes it even more important to have reliable analytical results, and hence increase the need for intercomparison studies.

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Different methods are used to analyse the residues of glyphosate, chlormequat and mepiquat in food. HPLC with different detectors e.g. conductivity detector (Fegert et al. 1991), fluorescence detector (Hogendoorn et al. 1999) and MS/MS detection (Juhler and Vahl 1999, Granby et al. 2003, Riediker et al. 2002) are most common, but also other methods like GC with pulsed flame photometric detection (Tseng et al. 2004) and nitrogen-phosphorous detection (Roy and Konar 1990) as well as electrophoresis with ultra violet detection (Galceran et al. 1997) have been used to determine these residues.

Glyphosate, chlormequat and mepiquat have been part of pesticide monitoring programmes in some European countries for several years (European Commission 2004, European Commission 2003, European Commission 2002). To maintain accreditation for the analytical methods, laboratories have to undertake in-house validation of analytical methods. One part of this is participation in intercomparison studies (ISO/IEC 17025 2000). Very few intercomparison studies on glyphosate, chlormequat and mepiquat in cereals have been published, and organizations which offer proficiency testing e.g. FAPAS® have not been offering these compounds in their programme. CEN organised an intercomparison study in 2001 on the determination of chlormequat and mepiquat in different non-fatty matrices including wheat flour, using LC/MS/MS. The outcome of the intercomparisons was a standard which is still under development (prEN 15054/15055). Because of the few intercomparisons offered an intercalibration of glyphosate, chlormequat and mepiquat in wheat was organised by The Danish Institute for Food and Veterinary Research.

Organization of the ring test

Preparation of the materials

Three samples and a blind sample were prepared from wheat. From each sample, thirty-five sub-samples with 50-55 g were prepared in polypropylene bags, marked and stored at -18°C until dispatch on 18th of January 2005.

Blind sample. Kernels of organically grown wheat, bought from a domestic retailer, were used as blind sample material. The material was milled in a centrifugal mill (Retch Ultra ZM1000) with a 0.5 mm sieve to produce the final blind sample (sample C).

Samples. A solution with the concentration of 1.000 mg/kg, 0.777 mg/kg and 0.767 mg/kg of chlormequat, mepiquat and glyphosate, respectively, was prepared in water. 3.60 ml of the solution were added dropwise to 250 g of kernels from the blind sample material while slowly agitating the sample material in a polyethylene container. After mixing, the fortified portion was kept overnight in a dessicator to remove excess of water. The portion was mixed with an additional 1750 g of kernels from the blind sample material by thoroughly agitating the sample in a polyethylene bag, and the mixture was milled in the centrifugal mill with a 0.5 mm sieve. This sample was labelled sample A when sent to the participants.

Sub-samples of domestic and foreign samples of wheat kernels from a residue-monitoring project were selected, based on measurements of their content of chlormequat. The residue-monitoring project was undertaken by a contract laboratory. Neither glyphosate nor mepiquat had been detected in the sub-samples (quantification limits for the survey: glyphosate: 0.1 mg/kg; mepiquat: 0.01 mg/kg). Kernels from seven samples with measured contents from 0.5 to 1.4 mg/kg (approx. 1500 g) were mixed with 500 g of kernels from the blind sample material and milled in the centrifugal mill with a 0.5 mm sieve. When sent to the participants, this sample was labelled sample B.

Sub-samples of domestic and foreign wheat kernel samples from a residue-monitoring project were selected, based on measurements of their content of glyphosate. Some of the sub-samples also contained chlormequat. Mepiquat had not been detected in the sub-samples. Kernels from nine samples (with 0.08 – 0.9 mg/kg glyphosate) were combined (approx. 2000 g). Chlormequat had been found in three of the samples (0.16 – 0.6 mg/kg). The sub-samples were mixed and milled in the centrifugal mill with a 0.5 mm sieve and sent to the participants as sample D.

Homogeneity and stability

Homogeneity and stability tests were done on all samples except for the blind sample. The analytical methods used (Granby et al. 2003, Juhler and Vahl 1999) have been developed and validated at the Danish Institute for Food and Veterinary Research.

Method of analysis for glyphosate.

The homogenised sample (3 g) was extracted twice with 25 ml MilliQ water by ultrasonication. After centrifugation and filtration, glyphosate was cleaned up online on a polystyrene based reverse

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phase column and separated by ion chromatography-HPLC. Sodium ions were removed from the eluent by automatic ion suppression before quantification by MS/MS (negative electrospray). 2-¹³C¹⁵N-glyphosate was used as internal standard. Fragmentation for glyphosate: m/z 168→150. For the internal standard 2-¹³C¹⁵N-glyphosate: m/z 170→152.

Method of analysis for chlormequat and mepiquat.

The homogenised sample (10 g) was extracted with methanol/water/acetic acid by Ultra-Turrax. The centrifuged extracts were cleaned on a SPE-C₁₈ column with methanol/water/ ammonium acetate buffer. Chlormequat and mepiquat were quantified by LC-MS/MS using a reversed phase HPLC column and positive electrospray. ¹³C labelled chlormequat (MW=125) was used as internal standard. Fragmentation for chlormequat:

m/z 122→58, for ¹³C -chlormequat: m/z 125→61. Fragmentation for mepiquat: m/z 114→98.

Homogeneity test. The samples A, B and D were tested for homogeneity before dispatch to the participants. From each sample, six subsamples were randomly chosen: Two subsamples from the first ten subsamples, two from the middle fifteen and two from the last ten subsamples. Each of these subsamples were analysed in duplicate before being dispatched to the participants. No differences between sub-samples were found, when testing the single measurements by one-way Analysis of Variance (ANOVA) (Miller and Miller 1993) at 5% significance level, see table I.

Insert Table I

Stability test. After the date that was set as the last reporting date, three sub-samples from each sample were randomly chosen: One sub-sample from the first ten sub-samples, one from the middle fifteen and one from the last ten sub-samples. Each sub-sample was analysed in duplicate.

Insert Table II

As seen in table II, no differences between sub-samples were found when testing the single measurements by ANOVA at 5% significance level, confirming the homogeneity of the sub-samples. No differences between the average from the homogeneity test and the average from the

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4 stability test were found when testing the average from each sub-sample by ANOVA at 5%
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6 significance level, see table III.
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10 Insert table III
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14 Note that the sub-samples for the homogeneity test and the stability test are not the same. The
15 relative differences therefore include variation between sub-samples.
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18 19 *Sample dispatch and data return*

20 Six European laboratories participated in the test. The laboratories were allowed to use their own
21 method for the analysis. The participants were asked to analyse each sample in duplicate and report
22 the results on the spreadsheet dispatched with the samples. They were also asked to send
23 information about quantification limits and recovery check. Analysis and reporting of the blank
24 sample were optional. Finally information on the analytical principle applied was also requested.
25 The test materials were frozen and sent by mail to the laboratories on 18th of January 2005. Results
26 were reported to the organiser no later than 18th of February 2005.
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36 37 *Statistical treatment*

38 To preserve anonymity each laboratory was assigned a code which was known only to the
39 laboratory and the organizer. The mean and standard deviation were calculated for each data set.
40 All the results were tested using Grubb's test to see if any outliers or stragglers could be found.
41 When using Grubb's test the within-laboratory variation is assumed to be equal. To test this,
42 Cochran's test (ISO/EIC 5725-2 1995) was used. No outliers or stragglers were found. This could be
43 due to the very small data set.
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50 51 *Correlation plots*

52 *Correlation plot.* To check if the methods of analysis used in the different laboratories performed
53 equally, correlations plot for each compound was done for the results from each laboratory. A
54 strong correlation between the pair of results from each laboratory indicates a systematic
55 component (Youden 1967).
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Results

All results from the participating laboratories were received before the deadline (18th of February 2005). Five of the participants analysed the blind sample (C). No residues were reported.

Analytical methods employed

Several different method principles were used for the analysis of glyphosate: GC/MS, HPLC-fluorescence and LC/MS/MS. Five of the laboratories used LC/MS/MS methods for chlormequat and mepiquat analysis. One laboratory used LC-MS

Received results and correlation plot

Glyphosate. Results for glyphosate are listed in table IV.

Insert table IV

For sample A, two laboratories (2 and 4) had results that seem somewhat lower than the fortified level. Of these two, laboratory 4 reported a low recovery for their control sample (34%). The remaining four laboratories deviated less than 25% from the fortified level. Recoveries for all laboratories can be seen in table IV.

For sample B, glyphosate were detected by three laboratories (1, 3 and 6) at levels that were generally lower than the limit of quantification (LOQ) for the remaining laboratories. Thus the absence of detection by these laboratories could be in agreement with the low concentrations found by the other laboratories. Table VIII shows the LOQ's for all participating laboratories.

For sample D, five laboratories detected glyphosate. Three laboratories (1, 3 and 6) reported levels at 0.20-0.25 mg/kg while laboratory 2 and 4 reported levels at 0.07-0.09 mg/kg. The within-laboratory variation calculates to 14%, 5% and 12% for sample A, B and D, respectively. The between-laboratory precision (ISO/EIC 43 1997) is calculated to 39% and 48% for sample A and D, which is high compared to the Horwitz precision (Horwitz 1982) (17% at 0.8 mg/kg, 20% at 0.2 mg/kg).

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8 The strong correlation (figure 1) between results from sample A and D ($r^2=0.95$) indicates that the
9 between-laboratory variation has a highly systematic component. It seems like the participating
10 laboratories are divided in two groups: one with laboratories 1, 3 and 6 having high results and one
11 with laboratories 2 and 4 having low results. Laboratory 5 falls in the group with high results for
12 sample A, but did not detect any glyphosate in sample B and D. This could be due to fact that
13 laboratory 5 has the highest LOQ for glyphosate of all the participating laboratories.
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20 For glyphosate, there is a very high variation between the results obtained by the different
21 laboratories, indicating a need for improvement of the quality assurance and/or optimisation of the
22 method of analysis used for glyphosate.
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27 *Insert table V*
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31 When considering the systematic differences in relation to the different analytical techniques used
32 (see table V) by the participating laboratories there is no clear trend. All laboratories used aqueous
33 solutions for extraction, five of them used pure water and one laboratory used a borate buffer at pH
34 9. Laboratory 5 and 6 extracted the cereals twice with water while for the other laboratories the
35 extraction procedure is not mentioned. Validation of the extraction efficiency of the method used by
36 laboratory 5 and 6 on spiked samples had shown (Granby et al. 2003) that only 50-80% of the
37 glyphosate was extracted during the first extraction, perhaps due to some kind of sorption of the
38 glyphosate in a sort of equilibrium to the cereal flour. Hence insufficient extraction of glyphosate
39 from the cereal flour may be a possible explanation for systematic low glyphosate results. The
40 extraction study also showed that the extraction efficiency was not influenced by pH, e.g. extraction
41 with an aqueous solution at pH 9 or at pH 2 did not differ from extraction with pure water. The
42 clean-up techniques used by the participating laboratories were different e.g. ion-exchange
43 (laboratory 1 and 2), reverse phase (laboratories 3, 5 and 6) and liquid-liquid partitioning
44 (laboratory 4). Laboratory 1 used GC/MS detection after derivatisation, laboratory 2 used HPLC
45 fluorescence detection, while laboratory 3-6 all used LC/MS/MS.
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Chlormequat. Results for chlormequat are listed in table VI.

Insert table VI

For sample A, four laboratories reported results that deviated less than 25% from the fortification level. All laboratories reported results between 0.20 and 0.45 mg/kg for sample B. Laboratory 5 reported the highest level of chlormequat in all three samples, significantly higher than the remaining laboratories. Laboratory 5 was asked if this could be due to reporting of the results as chlormequat chloride instead of the chlormequat cation, as this would move their results to the same range as the remaining laboratories. This was not the case and it has not been possible to find the reason for the higher results.

For sample D, all laboratories reported results between 0.15 and 0.30 mg/kg. The within-laboratory variation calculates to 5-6% for all the samples. The between-laboratory precision is 28-31% for all the samples, which is somewhat higher than the Horwitz precision (18-20% at 0.4-0.2 mg/kg). Again the strong correlation (figure 2) between results from sample A and B ($r^2=0.99$) and sample A and D ($r^2=0.88$) indicates that the between-laboratory variation has a systematic component.

Insert figure 2

Concerning the results for chlormequat, there is more consistency in the results from the different laboratories. All but one laboratory obtained comparable results. However the one laboratory found systematically higher results than the others. It has not been possible to attribute differences in the results between the participating laboratories to the use of different methods or analytical principles.

Mepiquat. Results for mepiquat are listed in table 6. Laboratory 4 did not report results for mepiquat. For sample A, three laboratories had results that deviated less than 25% from the fortification level. One laboratory reported results 38% above the fortification level, one laboratory 141% above. For sample B and D, no laboratory reported results above their LOQ. The average within-laboratory precision for sample A is 14%. The between-laboratory precision is 50%, which is high compared to the Horwitz precision (18% at 0.4 mg/kg). As mepiquat was found in only one sample only, it has not been possible to test for systematic errors.

For the chlormequat and mepiquat analysis all the laboratories used LC/MS/MS, except laboratory 1 which used LC/MS (see table VII). The analytical procedures did not differ much. The extraction solvents used were methanol-water with or without addition of acetic acid. Laboratory 1-4 did not use any clean up while laboratory 5 and 6 used reverse phase clean up. The laboratory having somewhat higher results for chlormequat and mepiquat, compared to the other laboratories, was laboratory 5 and 2, respectively.

Insert table VII

Limit of quantification

The reported LOQ from the laboratories are given in table VIII.

Insert table VIII

Most calculated recoveries were in agreement with the recoveries reported by the participants in tables 4 and 7; in particular, the low reported recovery on glyphosate by laboratory 4 (34%) is in good agreement with the calculated recovery (29%). For laboratory 2, the calculated recovery for glyphosate (49%) was lower than the reported recovery (92%) and for mepiquat the calculated recovery (241%) were higher than the reported (100%). Also the calculated recoveries from laboratory 5 for chlormequat (173%) and mepiquat (138%) are somewhat higher than the reported recoveries (109% and 93%, respectively).

Conclusion

In general, good within-laboratory precision was seen for all compounds tested. However, the between-laboratory precision was higher than expected by the Horwitz estimation (Horwitz 1982). This could be due to the small data set, resulting in outliers not being pointed out or it could be caused by systematic differences between results from the different laboratories. The combination of a high total variation and relatively few participants makes a calculation of Z-scores meaningless. For glyphosate and chlormequat, the strong correlation of results from different samples indicates that the between-laboratory variation has a systematic component. It was not possible to correlate differences in the results between the laboratories to the use of different methods of analysis.

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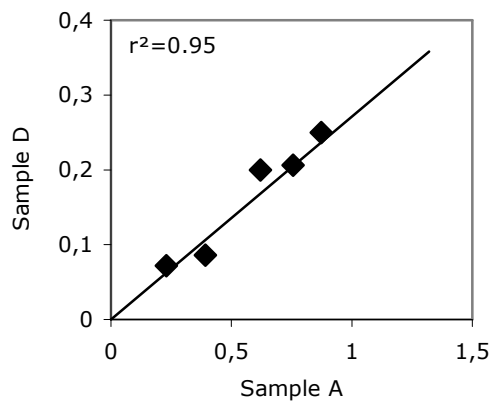


Fig. 1 Correlation between glyphosate results for sample A and D. Each point represents the pair of results from one laboratory. (Laboratory 5 has been excluded as the result for sample D was below LOQ). A line has been drawn through origin and the center of gravity for the population (the mean for each sample).

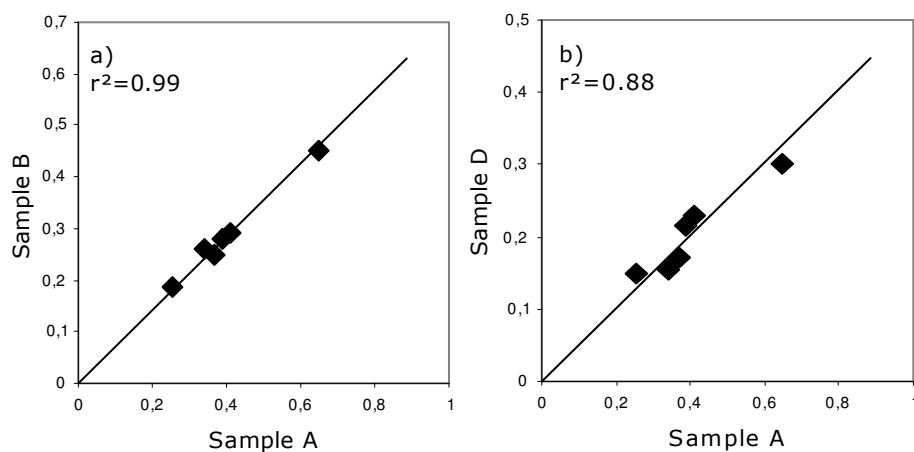


Fig. 2 a) Correlation between chlormequat results for sample A and B. b) Correlation between chlormequat results for sample A and D. Each point represents the pair of results from one laboratory. A line has been drawn through origin and the center of gravity for the population (the mean for each sample).

Table I Homogeneity test. Differences between the means of the subsamples were tested by one-way ANOVA. Each of the six subsamples were analysed in duplicate.

	Added (mg/kg)	Average (mg/kg, n=6)	Recovery (%)	SD * (mg/kg)	CV † (%)	P ‡ (%)
<i>Sample A</i>						
Glyphosate	0.800	0.715	89	0.057	8	68
Chlormequat	0.377	0.356	94	0.016	5	64
Mepiquat	0.384	0.380	99	0.027	7	8
<i>Sample B</i>						
Glyphosate		0.063		0.078	12	39
Chlormequat		0.254		0.021	8	41
Mepiquat		0.001 ¶		0.002	-	-
<i>Sample D</i>						
Glyphosate		0.218		0.008	4	79
Chlormequat		0.170		0.011	7	52
Mepiquat		0.001 ¶		0.001	-	-

* Standard deviation
† Coefficient of variation
‡ No significant difference between subsamples where P>5%
¶ Below quantification limit (0.01 mg/kg)

Table II Homogeneity in the stability test. Differences between the mean of the subsamples tested by one-way ANOVA. Each of the three subsamples were analysed in duplicate.

	Added (mg/kg)	Average (mg/kg, n=3)	Recovery (%)	SD * (mg/kg)	CV † (%)	P ‡ (%)
<i>Sample A</i>						
Glyphosate	0.800	0.769	96	0.034	4	74
Chlormequat	0.377	0.349	93	0.024	7	10
Mepiquat	0.384	0.346	90	0.019	5	9
<i>Sample B</i>						
Glyphosate		0.060		0.008	13	54
Chlormequat		0.248		0.007	3	21
Mepiquat		0.001 ¶		0.002	-	-
<i>Sample D</i>						
Glyphosate		0.225		0.011	5	58
Chlormequat		0.184		0.012	7	11
Mepiquat		0.001 ¶		0.001	-	-

* Standard deviation
† Coefficient of variation
‡ No significant difference between subsamples where P>5%
¶ Below quantification limit (0.01 mg/kg)

Table III Stability test. Difference between the measured contents before and after the test period tested by one-way ANOVA. See Table 1 and Table 2 for details.

	Relative difference * (%)	P † (%)
<i>Sample A</i>		
Glyphosate	7.6	18
Chlormequat	-1.9	63
Mepiquat	-9.0	9
<i>Sample B</i>		
Glyphosate	-5.1	57
Chlormequat	-2.2	66
Mepiquat	- ‡	-
<i>Sample D</i>		
Glyphosate	3.2	32
Chlormequat	8.2	13
Mepiquat	- ‡	-

* (Average_{Stability test} - Average_{Homogeneity test})/Average_{Homogeneity test}
† No significant difference between sample means before and after test period where P>5%:
‡ Below quantification limit (0.01 mg/kg)

Table IV Reported results for glyphosate. Sample A was fortified with 0.800 mg/kg.

Laboratory no.	<u>X1 (mg/kg)</u>	<u>X2 (mg/kg)</u>	<u>Average (mg/kg)</u>	<u>Reported recovery (%)</u>
<i>Sample A</i>				
1	0.81	0.702	0.756	77
2	0.382	0.402	0.392	92
3	0.574	0.661	0.62	96
4	0.21	0.25	0.23	34
5	0.69	0.77	0.73	113
6	0.995	0.75	0.873	112
<i>Sample B</i>				
1	0.022	0.018	0.02	77
2	<0.1	<0.1	<0.1	-
3	0.024	0.025	0.024	103
4	<0.05	<0.05	<0.05	34
5	<0.15	<0.15	<0.05	113
6	0.07	0.071	0.071	112
<i>Sample D</i>				
1	0.226	0.186	0.206	77
2	0.066	0.106	0.086	92
3	0.189	0.202	0.20	96
4	0.076	0.068	0.072	34
5	<0.15	<0.15	<0.15	113
6	0.246	0.254	0.25	112

Table V Method information for analysis of glyphosate

Laboratory no.	Sample amount (g)	Extraction Solvent	No. of extraction	Clean up	Detection principles	Internal standard
1	4	Water	-*	Dichloromethane, Chelex 100 and AG1X8 anion exchange	GC-MS after derivatization with TFAA and HFB	AEPA
2	30	Water	-*	Ion-exchange	HPLC-Fluorescence	-*
3	5	Water	-*	Envi-carb	LC-MS/MS	Glyphosate C13/N15
4	5	Water+borate buffer (pH 9)	-*	Liquid-liquid partition	LC-MS/MS ESI	None
5	3	Water	2	Polystyrene-based RP-column (online)	LC-MS/MS ESI negative mode	¹³ C ¹⁵ N-glyphosate
6	3	Water	2	Polystyrene-based RP-column (online)	LC-MS/MS ESI negative mode	¹³ C ¹⁵ N-glyphosate

*No information given

Table VI Reported results for chlormequat and mepiquat. Sample A was fortified with 0.3765 mg/kg and 0.3835 mg/kg of chlormequat and mepiquat, respectively.

Laboratory no.	Chlormequat				Mepiquat			
	X1 (mg/kg)	X2 (mg/kg)	Average (mg/kg)	Reported recovery (%)	X1 (mg/kg)	X2 (mg/kg)	Average (mg/kg)	Reported recovery (%)
<i>Sample A</i>								
1	0.236	0.274	0.255	103	0.342	0.317	0.33	106
2	0.406	0.372	0.389	106	0.962	0.887	0.924	100
3	0.378	0.364	0.37	83	0.26	0.322	0.29	82
4	0.41	0.41	0.41	96	n.a. [*]	n.a.	-	-
5	0.67	0.63	0.65	109	0.45	0.61	0.53	99
6	0.401	0.398	0.34	109	0.382	0.489	0.436	93
<i>Sample B</i>								
1	0.18	0.196	0.188	103	-	-	<0.05	106
2	0.277	0.284	0.28	106	0.0044 [†]	0.0046 [†]	0.0045 [†]	100
3	0.248	0.254	0.25	83	<0.01	<0.01	<0.01	82
4	0.29	0.28	0.29	96	n.a.	n.a.	-	-
5	0.44	0.47	0.45	109	<0.01	<0.01	<0.01	99
6	0.232	0.285	0.259	109	<0.01	<0.01	<0.01	93
<i>Sample D</i>								
1	0.14	0.159	0.15	103	-	-	<0.05	106
2	0.214	0.218	0.216	106	<0.005	<0.005	<0.005	-
3	0.177	0.158	0.17	83	<0.01	<0.01	<0.01	82
4	0.23	0.23	0.23	96	n.a.	n.a.	-	-
5	0.30	0.29	0.30	109	<0.01	<0.01	<0.01	99
6	0.142	0.166	0.154	109	<0.01	<0.01	<0.01	93

*Not analysed

[†]The laboratory reported this result as below Limit of Quantification (0.005 mg/kg)

Table VII Method information for analysis of chlormequat and mepiquat

Laboratory no.	Sample amount (g)	Extraction Solvent	No. of extraction	Clean up	Detection principles	Internal standard(s)
1	20	Water/methanol	- *	None	LC-MS	- *
2	10	Water/methanol	- *	None	LC-MS/MS	- *
3	5	Water/methanol (75/25)	- *	None	LC-MS/MS	Chlormequat-D4, chlormequat-D3
4	10	Water/methanol (20:40)	- *	None	LC-MS/MS ESI	None
5	10	Water/methanol/acetic acid (75:24:1)	1	Bond Elut 500 mg SPE C18	LC-MS/MS ESI positive mode	¹³ C-Chlormequat
6	10	Water/methanol/acetic acid (75:24:1)	1	Bond Elut 500 mg SPE C18	LC-MS/MS ESI positive mode	¹³ C-Chlormequat
*No information given						

Table VIII Reported Limit of Quantification (LOQ) and calculated recovery based on the results from spiked sample A.

Laboratory no.	Glyphosate		Chlormequat		Mepiquat	
	LOQ (mg/kg)	Recovery (%)	LOQ (mg/kg)	Recovery (%)	LOQ (mg/kg)	Recovery (%)
1	0.01	95	0.02	68	0.05	86
2	0.1	49	0.005	103	0.005	241
3	0.01	77	0.01	99	0.01	76
4	0.05	29	0.01	109	n.a. *	-
5	0.15	91	0.01	173	0.01	138
6	0.03	109	0.01	106	0.01	114
*Not analysed						