Medizinisches Labor Bremen

Gemeinschaftspraxis für
Laboratoriumsmedizin, Mikrobiologie
und Infektionsepidemiologie, Biochemie;
Umweltmedizin

Haferwende 12 • 28357 Bremen

Document Title	**Determination of Glyphosate residues in human urine samples from 18 European countries**
Test Compound	**Glyphosate and AMPA**
Study Initiation Date	**March 2013**
Study Completion Date	**June 6, 2013**
Test Facility	**Medical Laboratory Bremen, Haferwende 12, 28357 Bremen, Germany**
Sponsor	**BUND, FoE**
Date of the Document	**June 28, 2013**

STUDY LOCATION

The preparation of standards and analyses of samples were carried out at the Test Facility:

Medical Laboratory Bremen,
Haferwende 12,

28357 Bremen, Germany

Under the responsibility of the Study Director, Dr. Hans-Wolfgang Hoppe, according to the relevant Operating Procedures.

Contact Dr. Hoppe:

0049 (0) 421 2072 -251

Hans-Wolfgang.Hoppe@mlhb.de

The analytical phase of the study was started on March 28, 2013 and was completed June 6, 2013.

The hardcopy raw data will be scanned and stored as electronic media and kept at Bremen Lab for a period of at least 1 year. A copy of the electronic media and the original hardcopy raw data will be sent to sponsor for archival purposes on demand.

REPORT APPROVAL

TEST FACILITY

Medical Laboratory Bremen,
Haferwende 12,
28357 Bremen, Germany

	Signature	Date
Dr. rer. nat. Hans-Wolfgang Hoppe Study Director	H. W. Hoppe	6.6.2013

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1. OBJECTIVE

Determination of Glyphosate (2-[(phosphonomethyl)amino]acetic acid) and AMPA (Aminomethylphosphonic acid) residues in human urine samples. The goal of this study was to support the biomonitoring work of the BUND / FoE against the background of increasing Glyphosate use in some European countries.

2. Management of Biological Samples

We received shipments from 18 European countries during the period of March 22 (Belgium) to May 21 (Spain, Poland). Each shipment included 8-12 samples. The Spanish samples made a detour via Budapest. All urines were ice cold and in a good condition at receipt and were stored at –18°C until date of sample work-up. At reception, MLHB sent a confirmation of receipt to the Sponsor and has checked for any mismatch between the shipment and the list provided by the Sponsor. On completion of the assays, the samples were kept deep frozen. The study samples will be stored for a period of 1 year.

3. Analytical procedures

The human urine samples were analyzed by means of a validated GC-MSMS method. In addition we determined creatinine to correct for diurese, if needed. Brief descriptions and the relevant specifications are listed below.

Analytical Method for Glyphosate and AMPA

Sample preparation:

1) Evaporation and dissolution of the residue in methanol,
2) derivatization using trifluoroethanol and trifluoroactic acid anhydride

The sample preparation follows the method of Alferness and Iwata (J. Agric. Food Chem. 1994, 42, 2751-2759)

Internal Standards:
$^{13}C^{15}N$-Glyphosate; $^{13}C^{15}N$-AMPA

Measurement: GC-MSMS, NCI mode. Collision gas: Ar
Instrument: GC-System Agilent 7890A
Triple-Quad Agilent 7000
Ion transitions: Glyphosate 370 > 245 (Quantifier), 351 > 268 (Qualifier)
AMPA 351> 268 (Quantifier), 271>188 (Qualifier)

Capillary Column: HP-Innowax (30 x 0,25 x 0,25)

Figure 1 shows the Q1 mass spectra of derivatized Glyphosate and AMPA.

Figures 2 shows a typical GCMS chromatogram of a processed native urine sample (Belgium11). Figure 3 shows the chromatogram of the calibration standard spiked with 0,5 µg/L. The chromatogram of a reagent blank value is shown in Figure 4.

Method for Creatinine in urine: Jaffé-Reaction

Performance data

Precision: 8% each
Recovery: 95% each
Linearity: 0,2 - 20 µg/L each
Limit of Quantitation (LOQ): 0,15 µg/L each

Figure 5 shows the calibration curves for Glyphosate and AMPA in the relevant concentration range from 0.5 to 10 µg/L.

4. Reference Values

Table 1: Reference values

Parent compound	Biomarker	Reference Value	Literature
Glyphosate	Glyphosate	0,8 µg/L	Empirical value, MedLab 2012
Glyphosate	AMPA	0,5 µg/L	Empirical value, MedLab 2012
Creatinine	Creatinine	Range 0,3-3 g/L	

The reference values for Glyphosate and AMPA are only tentative. They were derived from an urban collective (n=90) and are defined as the 95. percentile of the measured values. They were established by *Medical Laboratory Bremen* in 2012 during the process of the method validation. Strictly speaking they are only valid to the region of Bremen.

5. Assay Results

A total of 182 humane urine samples were analyzed for residues of Glyphosate and the metabolite AMPA using a new GC-MSMS method. The results are presented in table 2. The samples to be analyzed were distributed across 6 runs. Besides the EU-Samples each run contained 2 QC-samples with known Glyphosate and AMPA concentrations. Creatinine concentrations were only used to check whether the spot urine samples are valid. All creatinine levels are inside the reference range (see table 2). The analytical results of Glyphosate and AMPA are not creatinine corrected. A mean blank value of 0.03 µg/L was subtracted from the measured concentrations of the urine samples and controls (Table 2 and 3).

Quality control samples showed good precision and accuracy throughout the measurement of the study samples as an indication of the good method performance (see table 3). A brief statistical evaluation including frequency of detection, mean and maximum values sorted by EU countries is given in table 4.

Table 2**:** Glyphosate and AMPA concentrations in human urine samples

Participant	Glyphosate	AMPA	Creatinine
	µg/L	µg/L	g/L
Belgium no 2	<0,15	0,195	0,43
Belgium no 3	<0,15	<0,15	0,9
Belgium no 4	0,256	0,197	2,27
Belgium no 5	<0,15	<0,15	0,81
Belgium no 6	0,17	1,256	1,84
Belgium no 7	0,18	0,684	1,64
Belgium no 8	0,190	<0,15	1,03
Belgium no 9	<0,15	<0,15	2,22
Belgium no 10	0,211	<0,15	1,28
Belgium no 11	0,575	0,373	2,7
Belgium no 12	<0,15	<0,15	0,98
Latvia No 1	<0,15	<0,15	0,79
Latvia No 2	<0,15	<0,15	0,96
Latvia No 3	0,896	0,391	1,9
Latvia No 4	0,208	0,150	2,33
Latvia No 5	<0,15	<0,15	1,95
Latvia No 6	1,821	0,15	2,75
Latvia No 7	0,636	0,706	2,01
Latvia No 8	0,203	0,220	2,28
Latvia No 9	0,339	<0,15	3,35
Latvia No 10	<0,15	<0,15	1,62
Latvia No 11	<0,15	<0,15	0,36
UK No 1	1,636	0,560	2,25
UK No 2	0,261	<0,15	0,7
UK No 3	0,205	<0,15	0,62
UK No 4	<0,15	<0,15	0,59
UK No 5	<0,15	<0,15	0,83
UK No 6	1,068	0,364	1,5
UK No 7	<0,15	<0,15	0,87

UK No 8	0,264	0,241	1,64
UK No 9	0,579	0,483	0,57
UK No 10	0,425	0,239	0,92
France No 1	<0,15	<0,15	1,65
France No 2	<0,15	<0,15	2,26
France No 3	<0,15	<0,15	0,96
France No 4	0,209	0,281	2,16
France No 5	0,200	0,408	2,4
France No 6	<0,15	0,209	1,19
France No 7	<0,15	<0,15	0,34
France No 8	<0,15	<0,15	1,33
France No 9	<0,15	<0,15	2,23
France No 10	0,232	<0,15	2,64
Czech Republic No 1	0,302	0,217	1,16
Czech Republic No 2	0,916	0,296	2,33
Czech Republic No 3	<0,15	<0,15	0,25
Czech Republic No 4	<0,15	<0,15	0,6
Czech Republic No 5	0,273	0,192	0,88
Czech Republic No 6	<0,15	<0,15	0,63
Czech Republic No 7	0,247	0,208	1,15
Czech Republic No 8	0,191	<0,15	1,07
Czech Republic No 9	0,159	0,212	0,52
Czech Republic No 10	<0,15	<0,15	0,34
Bulgaria No 1	<0,15	<0,15	0,96
Bulgaria No 2	<0,15	<0,15	0,96
Bulgaria No 3	<0,15	<0,15	0,75
Bulgaria No 4	<0,15	<0,15	1,04
Bulgaria No 5	0,176	<0,15	2,82
Bulgaria No 6	<0,15	<0,15	0,46
Bulgaria No 7	<0,15	0,201	1,53
Bulgaria No 8	<0,15	0,166	0,89
Bulgaria No 9	<0,15	<0,15	1,82
Bulgaria No 10	<0,15	<0,15	1,3
Malta No 1	0,363	0,180	2,62
Malta No 2	0,293	<0,15	1,08
Malta No 3	0,906	0,644	1,42
Malta No 4	1,555	0,886	2,17
Malta No 5	0,379	0,267	1,38
Malta No 6	1,242	0,387	1,19
Malta No 7	<0,15	<0,15	1,3
Malta No 8	0,992	0,397	2,6

Malta No 9	1,290	0,580	1,51
Malta No 10	1,127	0,552	1,52
Macedonia No 1	<0,15	<0,15	1,97
Macedonia No 2	<0,15	<0,15	1,63
Macedonia No 3	<0,15	<0,15	1,56
Macedonia No 4	0,239	<0,15	0,4
Macedonia No 5	<0,15	<0,15	0,96
Macedonia No 6	<0,15	<0,15	1,23
Macedonia No 7	<0,15	<0,15	1,72
Macedonia No 8	<0,15	<0,15	1,65
Macedonia No 9	<0,15	<0,15	3,15
Macedonia No 10	<0,15	<0,15	1,67
Austria No 1	0,198	<0,15	2,73
Austria No 2	<0,15	0,163	1,81
Austria No 3	<0,15	<0,15	2,97
Austria No 4	<0,15	<0,15	1,19
Austria No 5	<0,15	<0,15	0,33
Austria No 6	<0,15	<0,15	3,22
Austria No 7	<0,15	<0,15	1,15
Austria No 8	<0,15	<0,15	0,23
Austria No 9	<0,15	<0,15	0,28
Austria No 10	0,153	0,156	1,17
Croatia No 1	<0,15	<0,15	0,79
Croatia No 2	<0,15	<0,15	2,07
Croatia No 3	0,224	2,630	1,9
Croatia No 4	0,187	<0,15	1,57
Croatia No 5	<0,15	<0,15	1,65
Croatia No 6	0,158	<0,15	0,71
Croatia No 7	<0,15	<0,15	0,69
Croatia No 8	<0,15	<0,15	1,07
Croatia No 9	<0,15	<0,15	2,1
Croatia No 10	0,424	<0,15	1,02
Hungary No 1	<0,15	<0,15	0,9
Hungary No 2	<0,15	<0,15	1,13
Hungary No 3	<0,15	0,270	1,2
Hungary No 4	0,176	<0,15	1,21
Hungary No 5	0,171	0,153	0,49
Hungary No 6	0,171	<0,15	2,1
Hungary No 7	<0,15	<0,15	1,66
Hungary No 8	<0,15	<0,15	0,72
Hungary No 9	<0,15	<0,15	1,11

Hungary No 10	<0,15	<0,15	0,8
Switzerland 1	<0,15	<0,15	0,67
Switzerland 2	<0,15	<0,15	0,99
Switzerland 3	<0,15	<0,15	0,78
Switzerland 4	<0,15	<0,15	1,22
Switzerland 5	<0,15	<0,15	1,09
Switzerland 6	<0,15	<0,15	0,73
Switzerland 7	0,156	<0,15	1,9
Switzerland 8	<0,15	<0,15	1,57
Switzerland 9	0,159	<0,15	2,35
Switzerland 10	<0,15	<0,15	0,56
Switzerland 11	<0,15	<0,15	2,41
Switzerland 12	<0,15	<0,15	0,74
Netherlands 1	<0,15	<0,15	4,19
Netherlands 2	0,156	0,245	2,61
Netherlands 3	<0,15	<0,15	1,13
Netherlands 4	1,016	0,498	2,23
Netherlands 5	0,159	0,172	1,71
Netherlands 6	0,429	0,640	1,99
Netherlands 7	<0,15	<0,15	1,45
Netherlands 8	0,701	0,256	2,05
Germany 1	0,238	0,228	0,48
Germany 2	<0,15	<0,15	0,79
Germany 3	0,209	0,213	0,83
Germany 4	0,486	0,439	1,9
Germany 5	<0,15	<0,15	0,46
Germany 6	<0,15	0,202	1,7
Germany 7	0,460	<0,15	1,72
Germany 8	0,226	<0,15	1,38
Germany 9	0,445	0,700	1,64
Germany 10	0,204	0,205	2,6
Cyprus 1	0,199	<0,15	1,32
Cyprus 2	<0,15	<0,15	0,86
Cyprus 3	0,158	<0,15	2,33
Cyprus 4	<0,15	<0,15	0,65
Cyprus 5	0,180	0,674	0,99
Cyprus 6	0,223	0,228	2,01
Cyprus 7	<0,15	<0,15	0,85
Cyprus 8	<0,15	0,643	2,05
Cyprus 9	<0,15	<0,15	1,86
Cyprus 10	0,250	0,584	1,61

Georgia 1	<0,15	<0,15	1,91
Georgia 2	0,193	0,176	1,7
Georgia 3	0,353	0,178	1,61
Georgia 4	<0,15	<0,15	1,31
Georgia 5	<0,15	<0,15	0,81
Georgia 6	<0,15	<0,15	1,62
Georgia 7	<0,15	<0,15	1,5
Georgia 8	<0,15	0,185	1,6
Georgia 9	<0,15	<0,15	0,96
Georgia 10	<0,15	<0,15	0,62
Spain 1	<0,15	<0,15	1,25
Spain 2	<0,15	<0,15	0,74
Spain 3	0,160	<0,15	1,54
Spain 4	0,221	0,820	2,31
Spain 5	<0,15	<0,15	0,43
Spain 6	<0,15	<0,15	0,17
Spain 7	0,168	0,165	1,36
Spain 8	0,175	<0,15	0,68
Spain 9	<0,15	<0,15	0,76
Spain 10	<0,15	0,160	1,46
Poland 1	0,235	0,206	1,96
Poland 2	<0,15	<0,15	0,85
Poland 3	<0,15	<0,15	0,34
Poland 4	0,763	0,285	0,55
Poland 5	0,233	0,267	1,42
Poland 6	0,377	0,406	1,36
Poland 7	0,528	0,237	0,94
Poland 8	0,599	0,283	2,9
Poland 9	0,168	<0,15	0,91
Poland 10	<0,15	<0,15	0,92

Table 3: Results of quality controls

Run	Glyphosate	AMPA	Glyphosate	AMPA
Target (µg/L)	5	5	1	0,5
	Conc (µg/L)	Conc (µg/L)	Conc (µg/L)	Conc (µg/L)
Run 1	4,76	5,14	0,95	0,53
Run 2	5,10	5,48	1,18	0,50
Run 3	5,27	5,33	1,02	0,47
Run 4	5,35	5,29	1,02	0,47

Run 5	5,50	5,40	1,06	0,55
Run 6	5,30	5,10	1,04	0,53
Mean	5,21	5,29	1,05	0,51
CV (%)	4,52	2,55	7,34	6,76
Accuracy (%)	104,24	105,79	104,54	101,04

Table 4: Statistics
To calculate the mean values, results below the LOQ (0,15 µg/L) are replaced with ½ LOQ (0,075 µg/L).

			Glypho	Glypho	Glypho	Glypho	AMPA	AMPA	AMPA	AMPA
EU country		Subjects	Frequency of Detektion	Frequency of Detektion	Mean	Maximum Value	Frequency of Detektion	Frequency of Detektion	Mean	Maximum Value
		n	n	%	µg/L	µg/L	n	%	µg/L	µg/L
Belgium	B	11	6	54,55	0,18	0,57	5	45,45	0,29	1,26
Latvia	LV	11	6	54,55	0,41	1,82	5	45,45	0,19	0,71
Great Britain	GB	10	7	70,00	0,47	1,64	5	50,00	0,23	0,56
France	F	10	3	30,00	0,12	0,23	3	30,00	0,14	0,41
Czech Republic	CZ	10	6	60,00	0,24	0,92	5	50,00	0,15	0,30
Bulgaria	BG	10	1	10,00	0,09	0,18	2	20,00	0,10	0,20
Malta	M	10	9	90,00	0,82	1,56	8	80,00	0,40	0,89
Macedonia	MK	10	1	10,00	0,09	0,24	0	0,00	0,08	0,08
Austria	A	10	2	20,00	0,10	0,20	2	20,00	0,09	0,16
Croatia	HR	10	4	40,00	0,14	0,42	1	10,00	0,33	2,63
Hungary	H	10	3	30,00	0,10	0,18	2	20,00	0,10	0,27
Switzerland	CH	12	2	16,67	0,09	0,16	0	0,00	0,08	0,08
Netherlands	NL	8	5	62,5	0,34	1,02	5	62,50	0,25	0,64
Germany	D	10	7	70,00	0,25	0,49	6	60,00	0,23	0,70
Cyprus	CY	10	5	50,00	0,14	0,25	4	40,00	0,26	0,67
Georgia	GE	10	2	20,00	0,11	0,35	3	30,00	0,11	0,19
Spain	E	10	4	40,00	0,12	0,22	3	30,00	0,17	0,82
Poland	PL	10	7	70,00	0,31	0,76	6	60,00	0,20	0,41
Total		182	80	43,9	0,21	1,56	65	35,71	0,18	2,63

6. Conclusions

In this study, 182 urine samples received from 18 European countries were analyzed for Glyphosate and AMPA residues using a new GC-MSMS method (see table 2). With a LOQ of 0,15 µg/l, on average 44 % and 36 % of the urine samples analyzed were found to contain quantifiable levels of Glyphosate and AMPA, respectively. However the frequency of detection calculated for each individual EU-state ranged from 10% to 90% (see Table 4). The highest Glyphosate concentration was 1,8 µg/L (Latvia 6), the highest AMPA concentration was 2,6 µg/L (Croatia 3). All in all 12 (6,6%) participants of the study significantly exceeded the tentative reference value of 0,8 µg/L for Glyphosate (see section 4).

In general, Glyphosate and AMPA urinary level do not correlate very well. This is due to the finding that the ratio AMPA/Glyphosate (AGR) in human urine is very variable probably reflecting the variable AGRs in diet. A high AGR suggests an additional exposure against Aminopolyphosphonate based tensides like ATMT or EDTMP, which easily degrade to AMPA.

The results give a first idea to which extent adults in 18 European countries are exposed to Glyphosate. The regional and individual variations are large. Diet seems to be the main sources of exposure. However, more scientific work is needed to distinguish between different exposure situations.

7. Annex

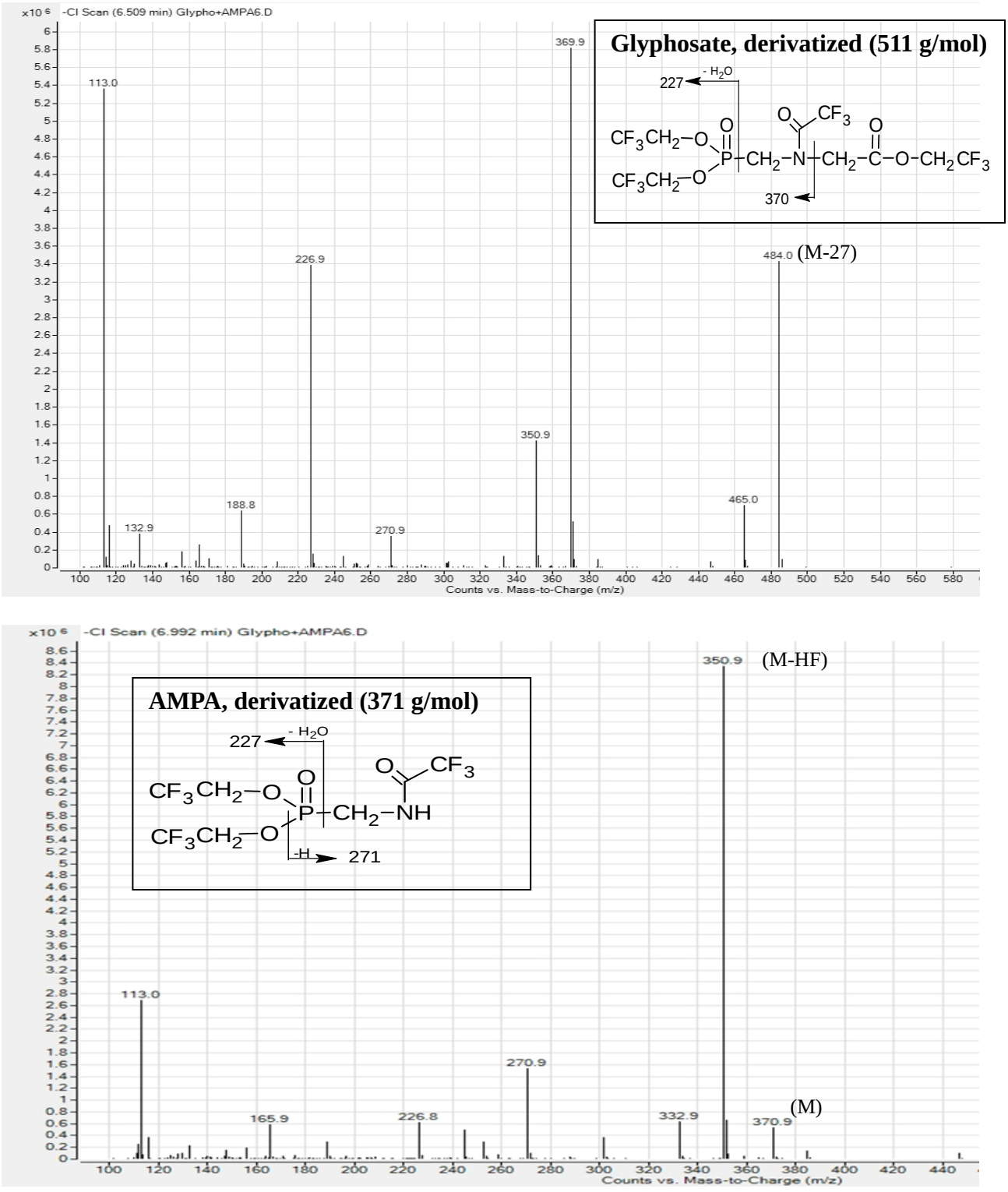

Fig 1 . Q1 mass spectra (NCI mode) and structures of derivatized Glyphosate and AMPA

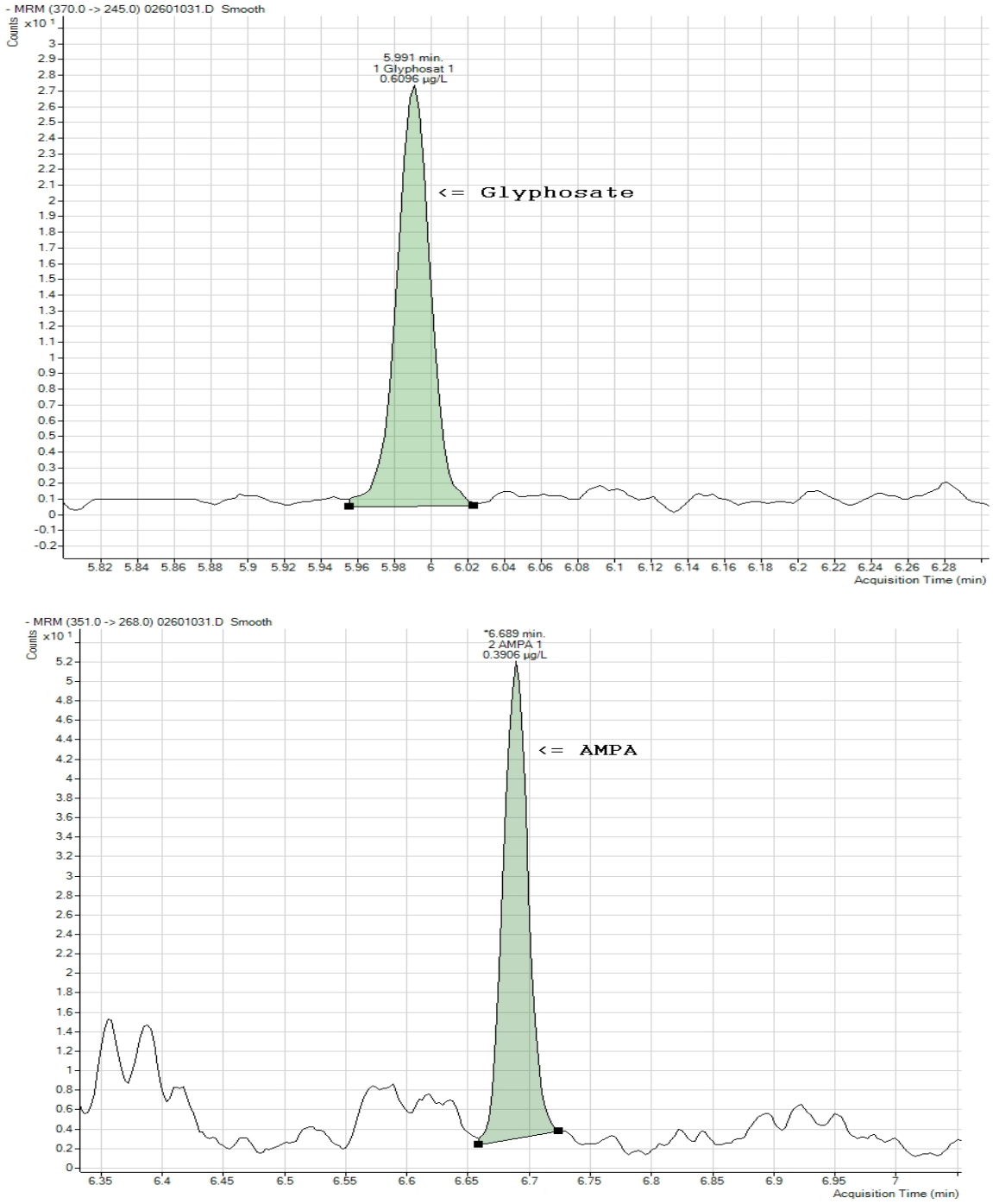

Fig 2. GC-MSMS chromatogram of a worked –up urine sample (Belgium 11; MRM mode). Only the quantifier traces of the daughter ions (Q3) of the analytes are shown. The concentrations were as follows: Glyphosate 0,6 µg/L; AMPA 0,4 µg/L.

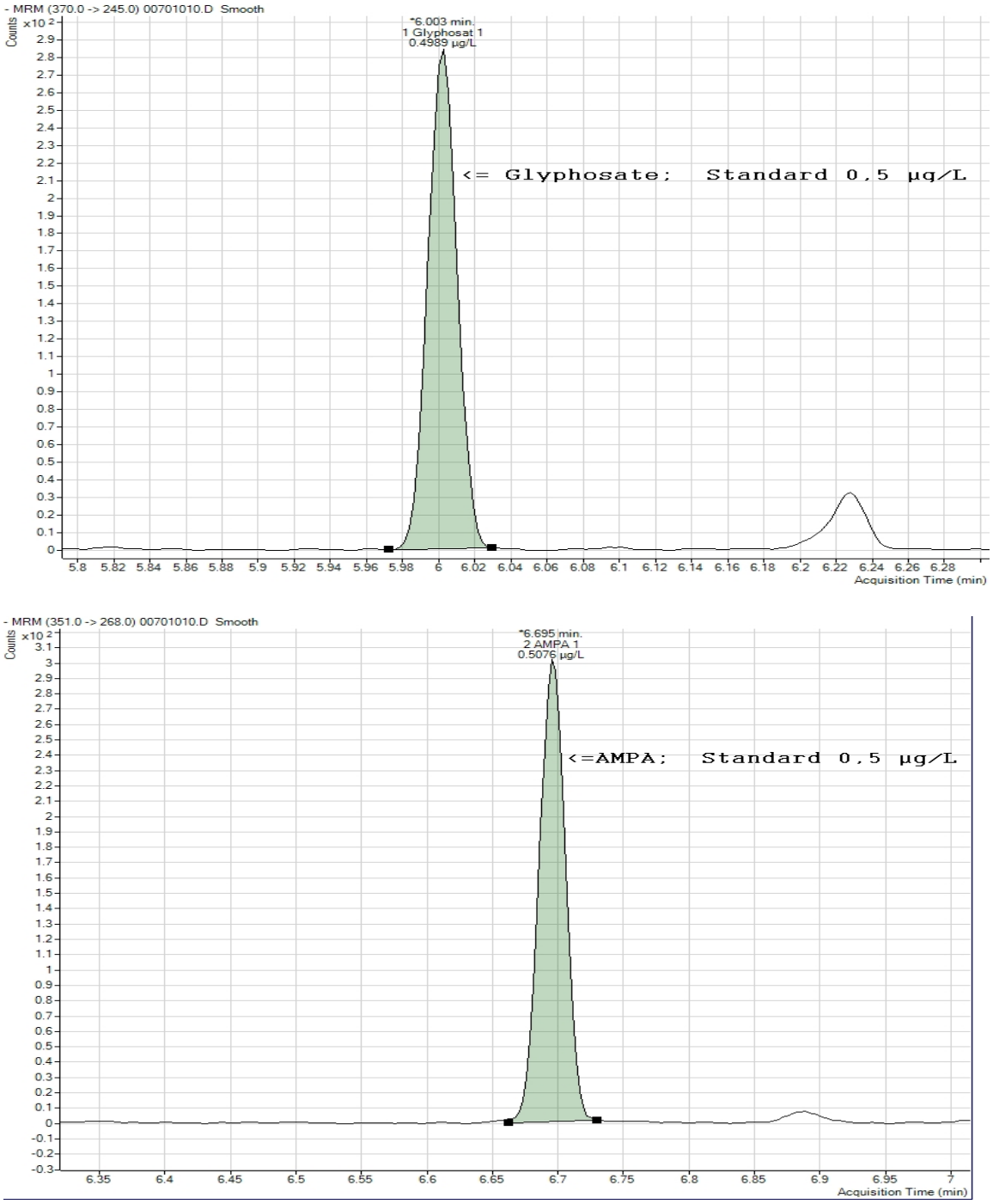

Fig 3. GC-MSMS chromatogram of a processed, aqueous calibration standard 0,5 µg/L. Only the quantifier traces of the daughter ions (Q3) of the analytes are shown.

Fig 4. Example of a chromatogram of a processed reagent blank (water). The concentrations of both Glyphosate and AMPA were below 0,01 µg/L.

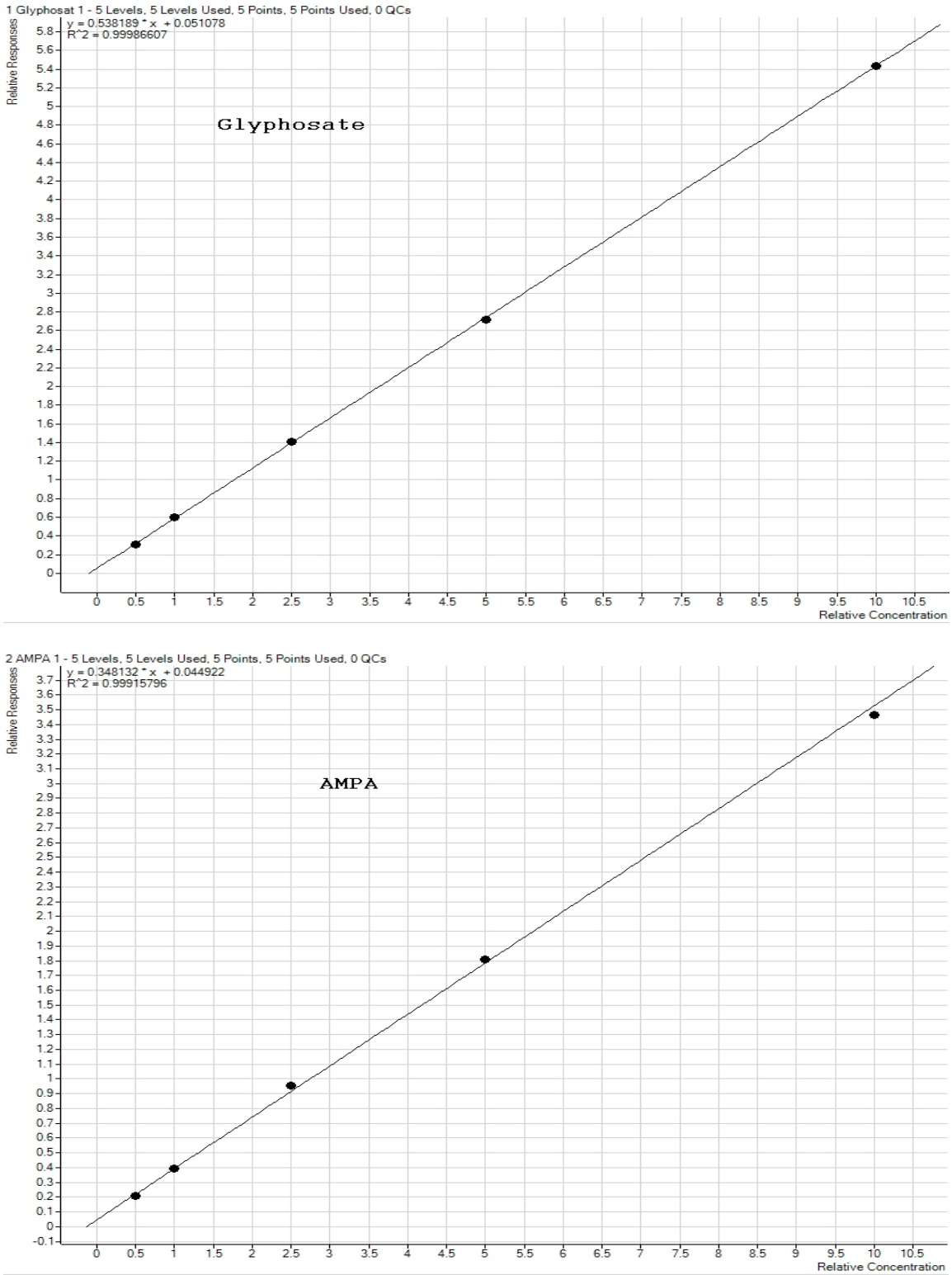

Fig 5. Calibration curves for Glyphosate and AMPA in the concentration range from 0,5 to 10 µg/L.