Ensemble pour s'améliorer

TECHNICAL STUDY No. 9

Reliability evaluation of metabolites of chloroacetamides measurement in natural waters and in bottled waters

This document is issued for information and is based on results and observations from proficiency tests of A.G.L.A.E.

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ABSTRACT

In this technical note, we report the reliability evaluation of measurement of metabolites of chloroacetamides carried out in 2016. To achieve this, a proficiency test has been carried out by AGLAE upon request of the Heath Regional Agency of Aquitaine Limousin Poitou Charentes which was concerned by the question of sanitary control of water. This proficiency test focused on 6 metabolites of chloroacetamides in river water and Evian water with several concentration levels. We observed that values targeted by spiking were overall well recovered by participants. Reproducibility values were also consistent for the concentration level targeted. Performances of laboratories are overall satisfactory.

This proficiency test enabled us to prove that dosage of these 6 molecules didn't present more difficulties than pesticides dosage in general and that laboratories control the whole process (reliability of analyses, uncertainty estimation).

CONTENT

1. INTRODUCTION

As part of the health-Environment regional plan 2011-2014, the Health Regional Agency of Aquitaine Limousin Poitou Charentes ordered and partially financed a proficiency test carried out by AGLAE, aimed to evaluate the reliability of metabolites of chloroacetamides measurement within the framework of sanitary control of water. Indeed, during the first stage of "3RSDE" (national action of research and reduction of hazardous substance release in waters), positive results had been found for these molecules. The objective was to insure the reliability of measurement results for these molecules by excluding potential analytical problems.

2. PRESENTATION OF THE PROFICIENCY TEST

The proficiency test was held from February to March 2016 and gathered 22 laboratories.
This test focused on 6 metabolites of chloroacetamides:

- Metolachlor OA (metolachlor oxanilic acid - CAS 152019-73-3)
- Metolachlor ESA (metolachlor ethane sulfonic acid - CAS 171118-09-5)
- Alachlor OA (alachlor oxanilic acid - CAS 171262-17-2)
- Alachlor ESA (alachlor ethane sulfonic acid - CAS 140939-15-7)
- Metazachlor OA (metazachlor oxanilic acid - CAS 1231244-60-2)
- Metazachlor ESA (metazachlor ethane sulfonic acid - CAS 172960-62-2)

Five batches were prepared, involving different matrices and different concentration levesl:

	Batch 1 (Bottles A, B)	Batch 2 (Bottles C, D)	Batch 3 (Bottles E, F)	Batch 4 (Bottles G, H)	Batch 5 (Bottles I, J)
Matrix	River water sieved at 2mm		Evian water		
Modality of spiking of the 6 metabolites	Low level 0,1µg/L	High level 0,4µg/L	No spiking	Low level 0,1µg/L	High level 0,4µg/L

Laboratories had to carry out an analysis on each bottles in repeatability conditions.

For all the molecules and all the batches, we had between 90% and 100% of results returned. Results are mainly quantitative (up to 90%) for low level of spiking and exclusively quantitative for high level of spiking.
For batch 3 for which there was no spiking, all results are such as <LOQ.

The homogeneity of the batches is assessed using the study of the deviation between bottles as observed by the participants.
Concerning the risk of instability, laboratories were required to start their analyses as quickly as possible and in any case upon receipt of the test samples; this in order to limit the effect of potential instabilities.
Test materials were found to be homogeneous enough for usage in the proficiency testing scheme.
The value assigned to the test material (consensus) and standard deviation for proficiency assessment (standard deviation used for z-score calculation) were estimated from participants' results. These values were calculated with an improved version of algorithm A from ISO 13528 standard.

Abbreviations

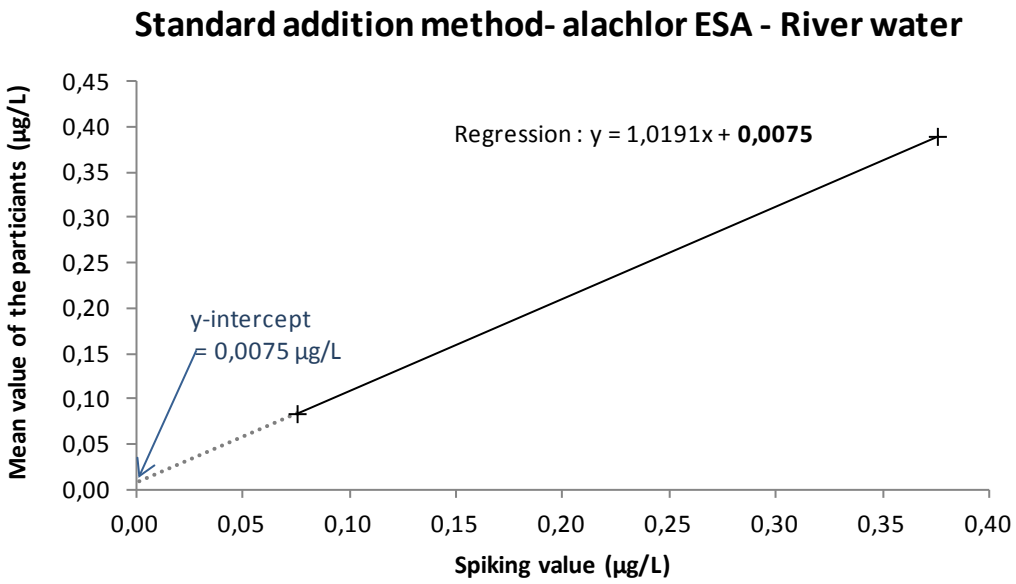
AGLAE : Association générale des laboratoires d'analyse et d'essai
S/L Extraction: Solid/Liquid extraction
LC/MS/MS : liquid phase chromatography coupled to tandem mass spectrometry

3. EVALUATION OF RESULTS' RELIABILITY

3.1 Determination of inital concentrations

The spiking of each matrix with two concentration levels has enabled us to check the initial concentration of the matrix by a standard addition method. To achieve this, a linear regression was made between the spiking values and the mean values of the participants. Y-intercept of this regression enabled us to calculate the parameter concentration before spiking.

Example of alachlor ESA in river water:



Thanks to uncertainties estimated on spiking values and means of participants, we have defined a confidence interval on y-intercepts which had enabled us to check if initial concentrations were significantly different from 0 or not.

Matrix	Parameter	Lower bond of confidence interval (k=2)	**Initial concentration recalcalulated by standard addition method (in µg/L)**	Upper bond of confidence interval (k=2)	**Initial concentration significantly different from 0 (at error risk of 5%) ?**
River water	Alachlor ESA	-0,012	**0,007**	0,026	**NO**
	Alachlor OA	-0,018	**0,010**	0,038	**NO**
	Metazachlor ESA	0,004	**0,019**	0,034	**YES**
	Metazachlor OA	-0,002	**0,015**	0,033	**NO**
	Metolachlor ESA	0,004	**0,022**	0,040	**YES**
	Metolachlor OA	-0,014	**0,005**	0,023	**NO**
Evian water	Alachlor ESA	-0,010	**0,005**	0,021	**NO**
	Alachlor OA	-0,018	**0,006**	0,030	**NO**
	Metazachlor ESA	-0,011	**0,003**	0,016	**NO**
	Metazachlor OA	-0,014	**0,002**	0,019	**NO**
	Metolachlor ESA	-0,008	**0,006**	0,019	**NO**
	Metolachlor OA	-0,012	**0,003**	0,018	**NO**

It appears that only the initial concentration of the metazachlor ESA and metolachlor ESA in river water are significantly different from 0 at error risk of 5%. Besides, initial concentrations calculated by standard addition method are higher in river water. However, they remain consistent with those measured by our sub-contractor laboratory (<0,05µg/L). In Evian water, these calculations enabled us to confirm the hypothesis of absence of these molecules in Evian water. So, we have considered that the initial concentrations in Evian water were equal to 0.

3.2 Comparison of values targeted by spiking and values of participants.

The table below compares the value targeted by spiking with the mean observed by participants, considering confidence interval of the two values (unit in µg/L)

	Parameter	Initial concentration in the matrix	Spiking		Participants' results		Deviation from the target in %	Value targeted by spiking recovered ?
			Value targeted by spiking[(1)]	Expanded uncertainty (k=2)	Value observed by the participants	Expanded uncertainty (k=2)		
River water	Alachlor OA Batch 1	<0,05	0,1004	0,0291	0,1065	0,0198	6,0%	YES
	Alachlor ESA Batch 1	<0,05	0,1003	0,0291	0,0842	0,0118	-16,0%	YES
	Metazachlor OA Batch 1	<0,05	0,1004	0,0289	0,0753	0,0129	-25,0%	YES
	Metazachlor ESA Batch 1	<0,05	0,1009	0,0291	0,0840	0,0102	-16,7%	YES
	Metolachlor OA Batch 1	<0,05	0,1001	0,0291	0,0762	0,0120	-23,9%	YES
	Metolachlor ESA Batch 1	<0,05	0,1008	0,0291	0,0966	0,0135	-4,2%	YES
	Alachlor OA Batch 2	<0,05	0,4000	0,0325	0,4901	0,0706	22,5%	YES
	Alachlor ESA Batch 2	<0,05	0,4004	0,0325	0,3901	0,0512	-2,6%	YES
	Metazachlor OA Batch 2	<0,05	0,4026	0,0331	0,3151	0,0398	-21,7%	NO
	Metazachlor ESA Batch 2	<0,05	0,4003	0,0325	0,3399	0,0332	-15,1%	YES
	Metolachlor OA Batch 2	<0,05	0,4003	0,0325	0,3618	0,0493	-9,6%	YES
	Metolachlor ESA Batch 2	<0,05	0,4000	0,0325	0,3904	0,0378	-2,4%	YES
Evian water	Alachlor OA Batch 4	0,000	0,0754	0,0070	0,0996	0,0161	32,1%	NO
	Alachlor ESA Batch 4	0,000	0,0753	0,0070	0,0799	0,0103	6,0%	YES
	Metazachlor OA Batch 4	0,000	0,0754	0,0061	0,0648	0,0105	-14,0%	YES
	Metazachlor ESA Batch 4	0,000	0,0759	0,0070	0,0724	0,0079	-4,6%	YES
	Metolachlor OA Batch 4	0,000	0,0751	0,0071	0,0756	0,0074	0,7%	YES
	Mtolachlor ESA Batch 4	0,000	0,0758	0,0070	0,0831	0,0075	9,5%	YES
	Alachlor OA Batch 5	0,000	0,3750	0,0160	0,4720	0,0627	25,9%	NO
	Alachlor ESA Batch 5	0,000	0,3754	0,0161	0,3771	0,0362	0,4%	YES
	Metazachlor OA Batch 5	0,000	0,3776	0,0173	0,3145	0,0485	-16,7%	YES
	Metazachlor ESA Batch 5	0,000	0,3753	0,0161	0,3467	0,0325	-7,6%	YES
	Metolachlor OA Batch 5	0,000	0,3753	0,0161	0,3658	0,0450	-2,5%	YES
	Metolachlor ESA Batch 5	0,000	0,3750	0,0161	0,3888	0,0298	3,7%	YES

(1) **Attention :** the values targeted by spiking are the values aimed for during material preparation; the concentration levels reached are likely close, however they must not be used as a reference.

Note: the uncertainty of the value targeted by spiking was calculated metrologically according to NF ISO/IEC Guide 98-3 (Guide to the expression of uncertainty in measurement).

For information, a deviation from the target was calculated for each participant.

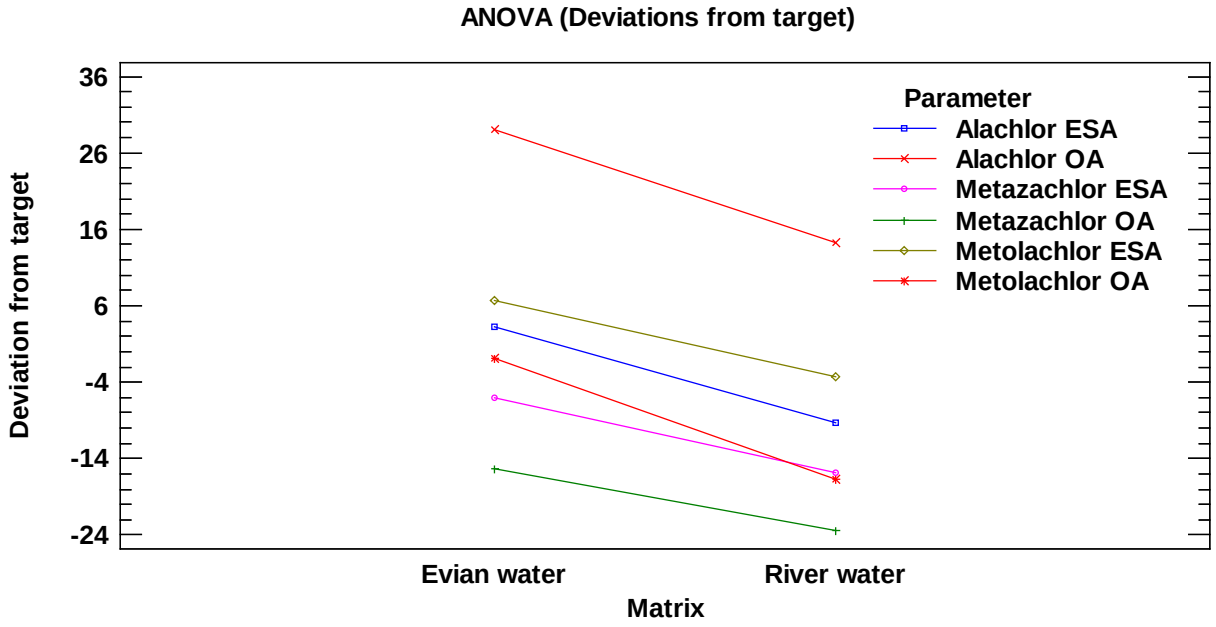
Overall, the values targeted by spiking are recovered by participants. Only 3 values targeted by spiking are not recovered considering the uncertainties: alachlor OA in Evian water (low and high levels) and metazachlor OA in river water high level.

After analysing deviations from the target as a function of the parameter, of the matrix and of the concentration level with an ANOVA (analysis of variance), we can see that they vary significantly as a function of the parameter and the matrix. The matrix effect is also variable depending on the parameter (interaction of factors matrix/parameter).

Analysis of variace for Deviation from the target

Source	*Sum of squares*	*Df*	*Mean square*	*F-Ratio*	*P-value*
MAIN EFFECTS					
A:Matrix	835,058	1	835,058	55,79	0,0007
B:Levels	24,5792	1	24,5792	1,64	0,2562
C:Parameter	3974,89	5	794,977	53,11	0,0002
INTERACTIONS					
AB	249,27	1	249,27	16,65	0,0095
AC	47,3538	5	9,47077	0,63	0,6861
BC	52,8008	5	10,5602	0,71	0,6444
RESIDUAL	74,8355	5	14,9671		
TOTAL (CORRECTED)	5258,78	23			

The graph below presents the deviation from target for each molecule in river water and Evian water.
We can note that for alachlor OA, participants tend to find values higher than the value targeted by spiking. The molecule the most recovered is metolachlor ESA. Values observed for metazachlor OA are lower than the value targeted by spiking.
The values recovered by participants are lower in river water than in Evian water. We can note that the deviation between river water and Evian water is comparable for metazachlor OA, metazachlor ESA, metolachlor ESA, alachlor ESA while it is wider for alachlor OA and metolachlor OA.



3.3 Reproducibility

The table below lists reproducibility values observed:

Parameter	m (in µg/L)	CV_R%	Parameter	m (in µg/L)	CV_R%
River water			Evian water		
Alachlor ESA batch 1	0,0842	24,0	Alachlor ESA batch 4	0,0799	22,5
Alachlor OA batch 4	0,1065	33,0	Alachlor OA batch 4	0,0996	28,5
Metazachlor ESA batch 1	0,0840	22,0	Metazachlor ESA batch 4	0,0724	20,0
Metazachlor OA batch 1	0,0753	31,0	Metazachlor OA batch 4	0,0648	29,5
Metolachlor ESA batch 1	0,0966	25,5	Metolachlor ESA batch 4	0,0831	16,5
Metolachlor OA batch 1	0,0762	28,5	Metolachlor OA batch 4	0,0756	18,5
Alachlor ESA batch 2	0,3901	22,5	Alachlor ESA batch 5	0,3771	16,5
Alachlor OA batch 2	0,4901	25,5	Alachlor OA batch 5	0,4720	23,5
Metazachlor ESA batch 2	0,3399	18,0	Metazachlor ESA batch 5	0,3467	17,5
Metazachlor OA batch 2	0,3151	23,0	Metazachlor OA batch 5	0,3145	28,0
Metolachlor ESA batch 2	0,3904	17,5	Metolachlor ESA batch 5	0,3888	14,0
Metolachlor OA batch 2	0,3618	24,5	Metolachlor OA batch 5	0,3658	22,5

For batch 3, laboratories returned too many results such as <LOQ to be able to calculate reproducibility values.

We can note that the CVR% are in reasonable orders of magnitude regarding concentration levels targeted.

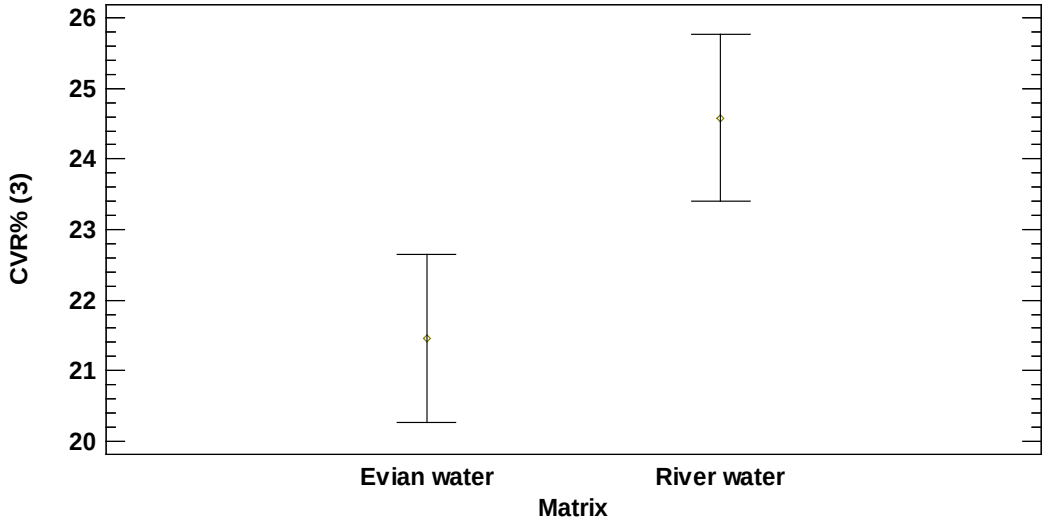
An ANOVA enabled us to show that results reproducibility varies depending on parameter, matrix and concentration level (see table below). However, interactions between these factors are not significant.

ANOVA for CVR%

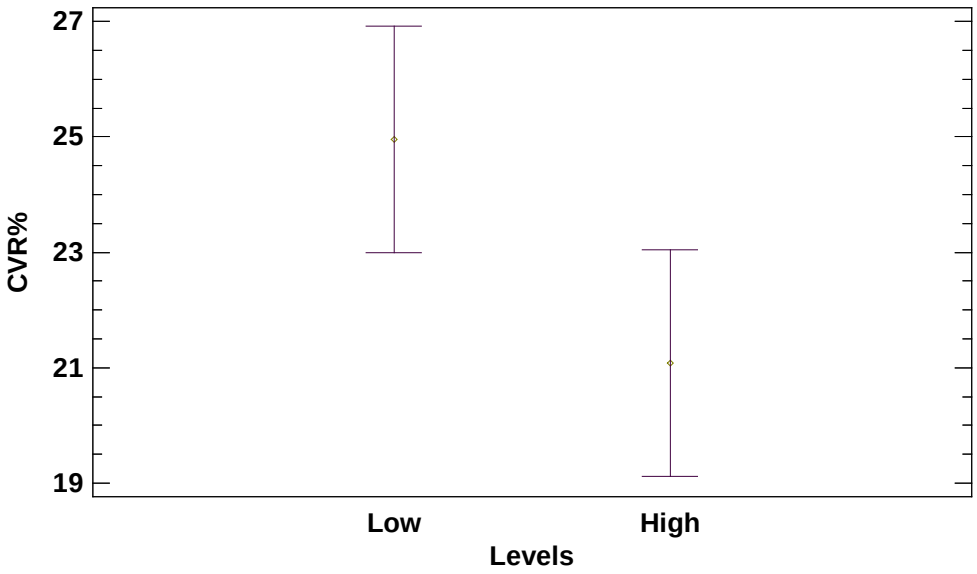
Source	*Sum of squares*	*Df*	*Mean square*	*F-ratio*	*P-value*
MAIN EFFECTS					
A:Matrix	58,5937	1	58,5937	11,50	0,0194
B:Levels	90,0938	1	90,0938	17,69	0,0084
C:Parameter	330,302	5	66,0604	12,97	0,0069
INTERACTIONS					
AB	15,8437	1	15,8437	3,11	0,1381
AC	45,7187	5	9,14375	1,80	0,2682
BC	23,7187	5	4,74375	0,93	0,5302
RESIDUAL	25,4687	5	5,09375		
TOTAL (CORRECTED)	589,74	23			

The graphs below present mean CVR and their confidence intervals at 95% as a function of studied factors.

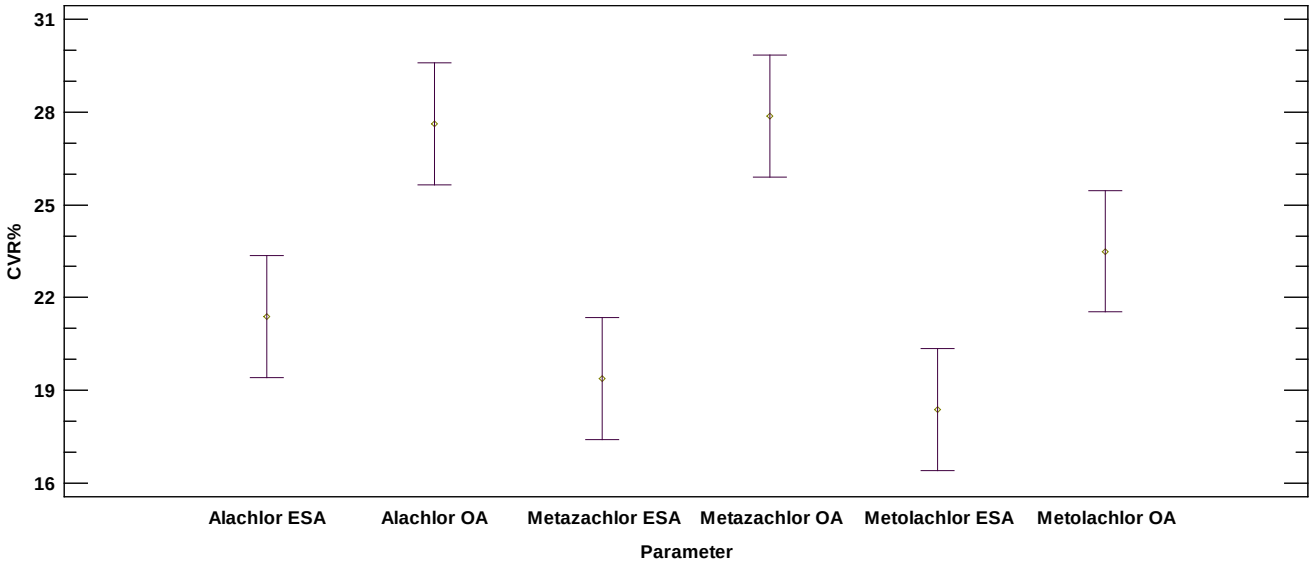
Results reproducibility is worst in river water than in Evian water.



Results' reproducibility is better when concentration level is higher.

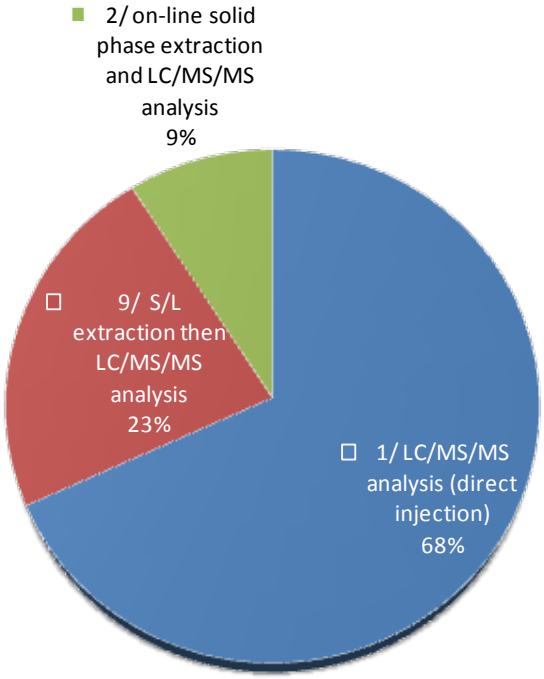


Results' reproducibility of oxalic acids (OA) is worst than sulfonic acids (ESA).



3.4 Analytical methods carried out

The data processing from the combined methods was not disrupted by potential deviations due to the analytical modalities. However, please note that we did not have sufficient data in order to carry out a specific analysis of the participants' results for each method.
For information, here is the distribution of analytical techniques used for this test.

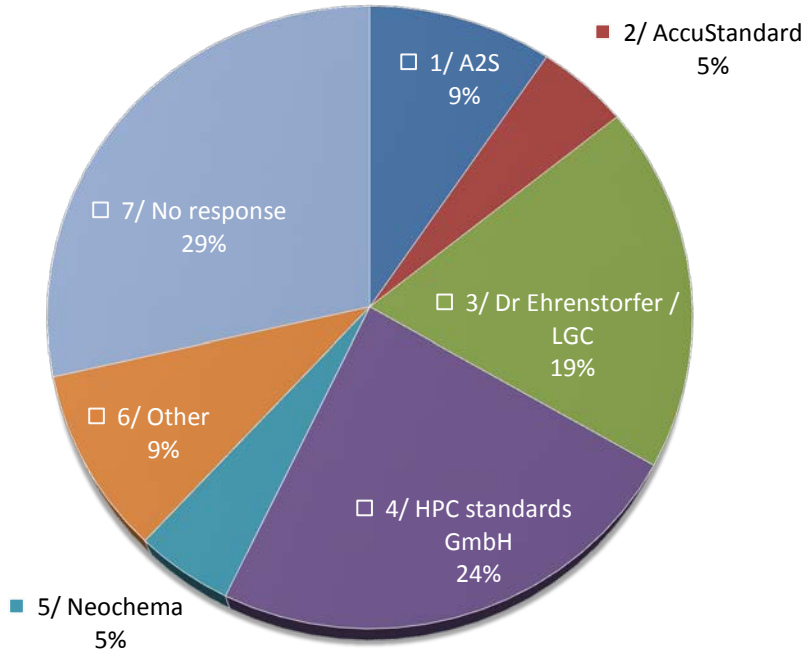


15M69.1 - Analytical line carried out

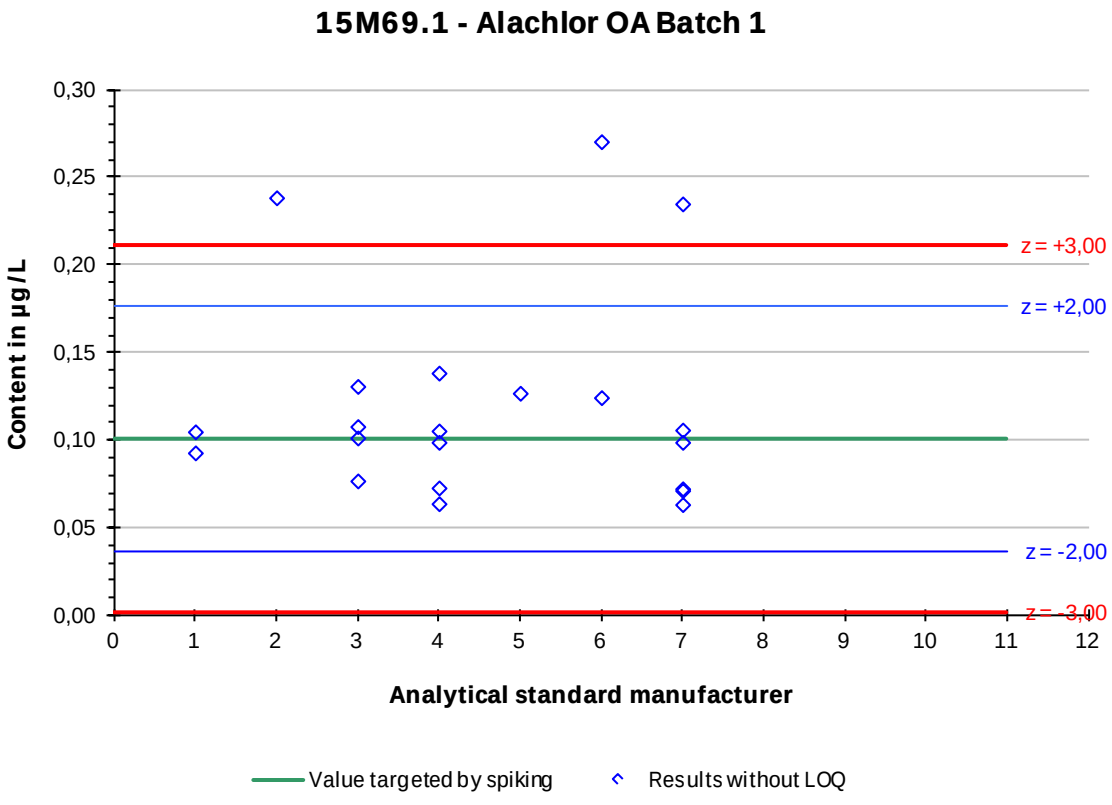
3.5 Standards used by the participants

The review of results' distribution as a function of standards used didn't highlight deviation due to standards (study carried out on batch 1). The number of results by manufacturer/product reference was very reduced.

Example for alachlor OA – batch 1:



15M69.1 - Alachlor OA Batch 1 - Standard manufacturer



Standard manufacturer	Number of results
1/ A2S	2
2/ AccuStandard	1
3/ Dr Ehrenstorfer / LGC	4
4/ HPC standards GmbH	5
5/ Neochema	1
6/ Other	2
7/ No response	6

Note: Standard references don't explain the results' distribution within each manufacturer.

4. LABORATORIES' PERFORMANCE

4.1 Z-score

Z-scores obtained for each molecule by the 22 participants are generally satisfactory :

Parameter	Satisfactory Z-score	Questionnable Z-score	Unsatisfactory Z-score
Alachlor OA Batch 1	18 (86%)	0 (0%)	3 (14%)
Alachlor ESA Batch 1	16 (80%)	2 (10%)	2 (10%)
Metazachlor OA Batch 1	20 (91%)	0 (0%)	2 (9%)
Metazachlor ESA Batch 1	21 (95%)	0 (0%)	1 (5%)
Metolachlor OA Batch 1	19 (86%)	0 (0%)	3 (14%)
Metolachlor ESA Batch 1	19 (86%)	0 (0%)	3 (14%)
Alachlor OA Batch 2	19 (90%)	0 (0%)	2 (10%)
Alachlor ESA Batch 2	19 (95%)	0 (0%)	1 (5%)
Metazachlor OA Batch 2	19 (86%)	1 (5%)	2 (9%)
Metazachlor ESA Batch 2	22 (100%)	0 (0%)	0 (0%)
Metolachlor OA Batch 2	20 (91%)	0 (0%)	2 (9%)
Metolachlor ESA Batch 2	18 (82%)	2 (9%)	2 (9%)
River water	**230 (89%)**	**5 (2%)**	**23 (9%)**
Alachlor OA Batch 4	18 (86%)	0 (0%)	3 (14%)
Alachlor ESA Batch 4	18 (90%)	0 (0%)	2 (10%)
Metazachlor OA Batch 4	19 (86%)	2 (9%)	1 (5%)
Metazachlor ESA Batch 4	21 (95%)	0 (0%)	1 (5%)
Metolachlor OA Batch 4	16 (73%)	3 (14%)	3 (14%)
Metolachlor ESA Batch 4	18 (82%)	1 (5%)	3 (14%)
Alachlor OA Batch 5	19 (90%)	0 (0%)	2 (10%)
Alachlor ESA Batch 5	18 (90%)	1 (5%)	1 (5%)
Metazachlor OA Batch 5	19 (86%)	1 (5%)	2 (9%)
Metazachlor ESA Batch 5	22 (100%)	0 (0%)	0 (0%)
Metolachlor OA Batch 5	20 (91%)	0 (0%)	2 (9%)
Metolachlor ESA Batch 5	19 (86%)	1 (5%)	2 (9%)
Evian water	**227 (88%)**	**9 (3%)**	**19 (7%)**
Total	**457 (89%)**	**14 (3%)**	**45 (9%)**

Only 3 laboratories have recurring anomalies.

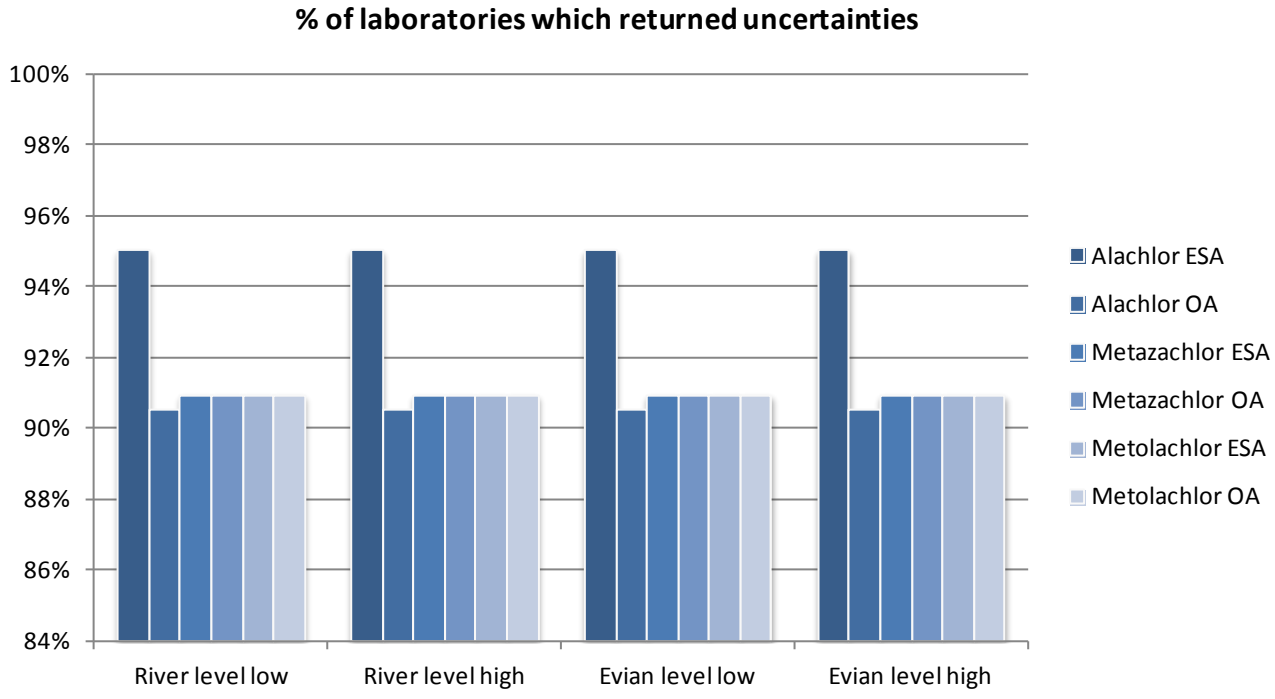
For batch 3 (Evian water not spiked), only a qualitative evaluation of results could be done because of the high number of results such as <LOQ returned by laboratories. The assigned value for this batch was estimated at < 0,020µg/L for all parameters. No laboratory had an unsatisfactory or questionable result (no false positives).

4.2 Zeta- score

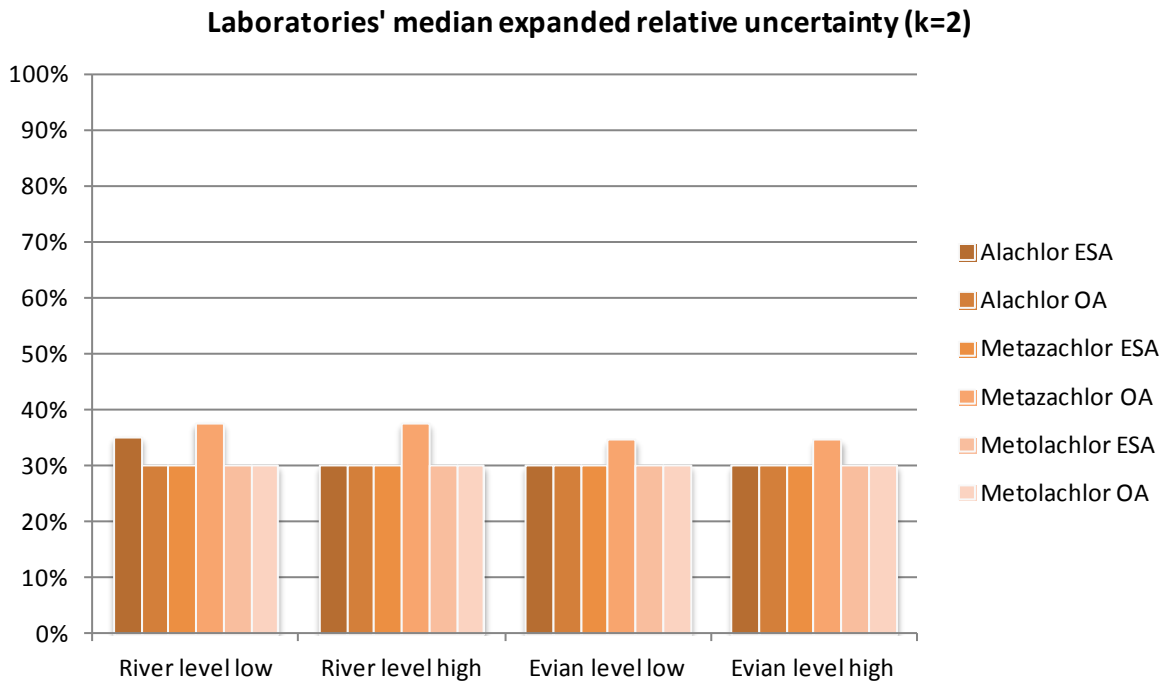
During this test, we suggested to laboratories which wished to indicate the uncertainty of their analysis. **The objective was to check the reliability of the estimations of measurement uncertainty.**

Having a satisfactory zeta-score with an unsatisfactory z-score, shows that you have managed to identify and integrate all the uncertainty sources of your analytical process within your calculations. However, should your analytical result vary too far from the assigned value (relative to the participants' results dispersion). Therefore, the identification of the error sources carried out for the calculation of the uncertainty of measurement should enable identification of the corrective actions to be implemented.
In summary, the zeta-score characterises the quality of the uncertainty assessment and its ability to recover the "true" value. The z-score characterizes the position of the laboratory in relation to the performance of other participants.

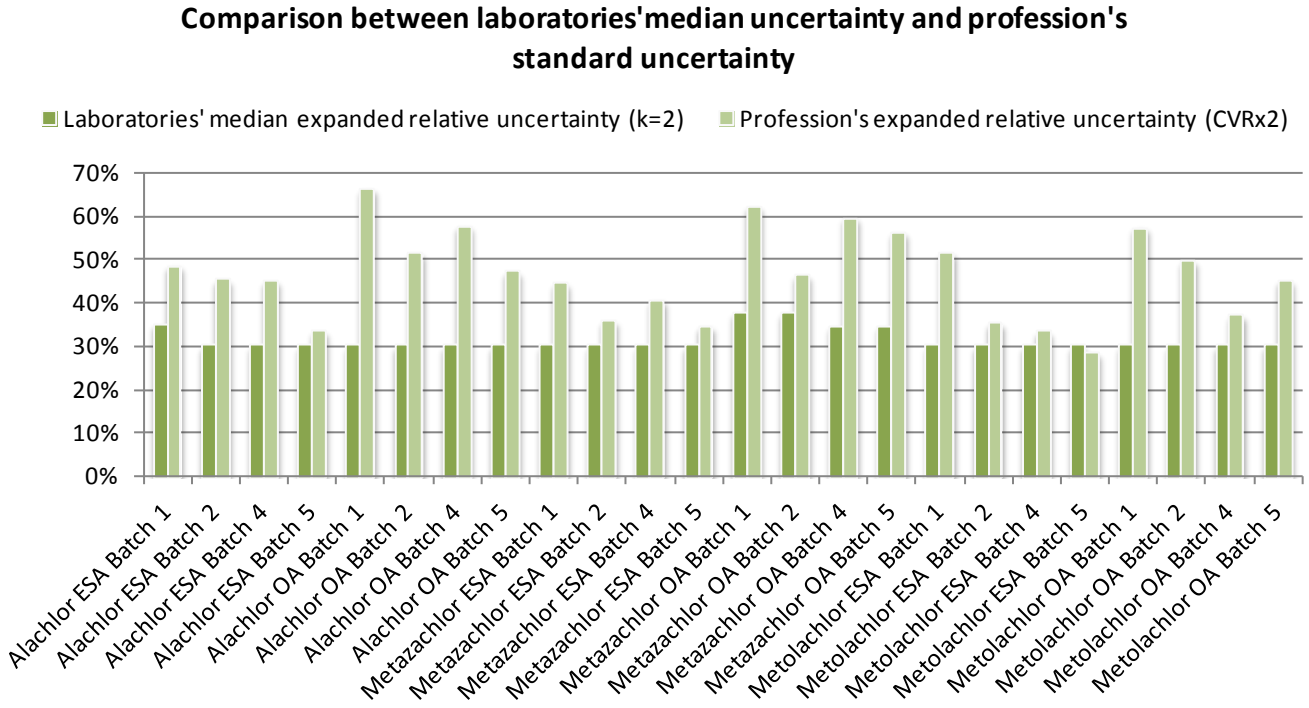
Almost 90% of participants returned results:



Uncertainty of laboratories is quite consistent and close to 15% (in terms of CV%, k=1):



Laboratories' median uncertainty is consistent with the profession's standard uncertainty.



5. CONCLUSION

As a conclusion, this proficiency test went well, with no false positive or false negative.

It has enabled us to demonstrate that:

- The measurement of the 6 molecules doesn't present more difficulties than pesticides dosage;
- Laboratories control the whole process (reliability of the analysis, uncertainty estimation).

Since this conclusive test, this test has been maintained in AGLAE's network. Two proficiency tests, one in 2016 (16M69.1) and one in 2017 (17M69.1) were carried out complemented with new molecules: Acetochlor ESA and OXA, dimethachlor OXA, dimethenamide ESA and OXA, flufenacet ESA and OXA.

For information, find below data from the 16M69.1 and 17M69.1 tests:

	Parameter	Assigned value	Value targeted by spiking	Value targeted by spiking recovered?	Reproducibility value (CVR %)	Repeatability value (CVr %)
16M69.1	Acetochlor ESA	0,2743	0,331	YES	36,5	3,0
	Acetochlor OXA	0,2529	0,2798	YES	21,5	2,5
	Alachlor ESA	0,1671	0,1808	YES	31,5	5,5
	Alachlor OXA	0,1608	0,2001	YES	29	3,0
	Dimethachlor OXA	0,1379	0,15	YES	37,5	6,5
	Dimethenamide ESA	0,2903	0,3199	YES	32,5	2,5
	Dimethenamide OXA	0,2067	0,2498	YES	57	2,0
	Flufenacet ESA	0,2591	0,3127	YES	38,5	6,0
	Flufenacet OXA	0,2643	0,3496	YES	53,5	5,0
	Metazachlor ESA	0,2171	0,2604	YES	28,5	5,5
	Metazachlor OXA	0,3172	0,3602	YES	22	2,5
	Metolachlor ESA	0,1592	0,2019	NO	29	5,5
	Metolachlor OXA	0,1225	0,1401	YES	26,5	4,5
17M69.1	Acetochlor ESA	0,3785	0,4	YES	14,5	4,0
	Acetochlor OXA	0,1965	0,1997	YES	22	3,0
	Alachlor ESA	0,2554	0,2504	YES	18	7,5
	Alachlor OXA	0,2753	0,2999	YES	14,5	4,0
	Dimethachlor OXA	0,346	0,4404	YES	38	2,0
	Dimethenamide ESA	0,2878	0,3198	YES	14	3,0
	Dimethenamide OXA	0,3216	0,3496	YES	22,5	3,5
	Flufenacet ESA	0,3776	0,3897	YES	18	2,0
	Flufenacet OXA	0,4633	0,4701	YES	24,5	4,5
	Metazachlor ESA	0,2618	0,2803	YES	24,5	4,0
	Metazachlor OXA	0,1843	0,2001	YES	25	6,0
	Metolachlor ESA	0,22	0,2301	YES	20,5	4,0
	Metolachlor OXA	0,1498	0,1796	YES	23,5	5,5

Unit in µg/L