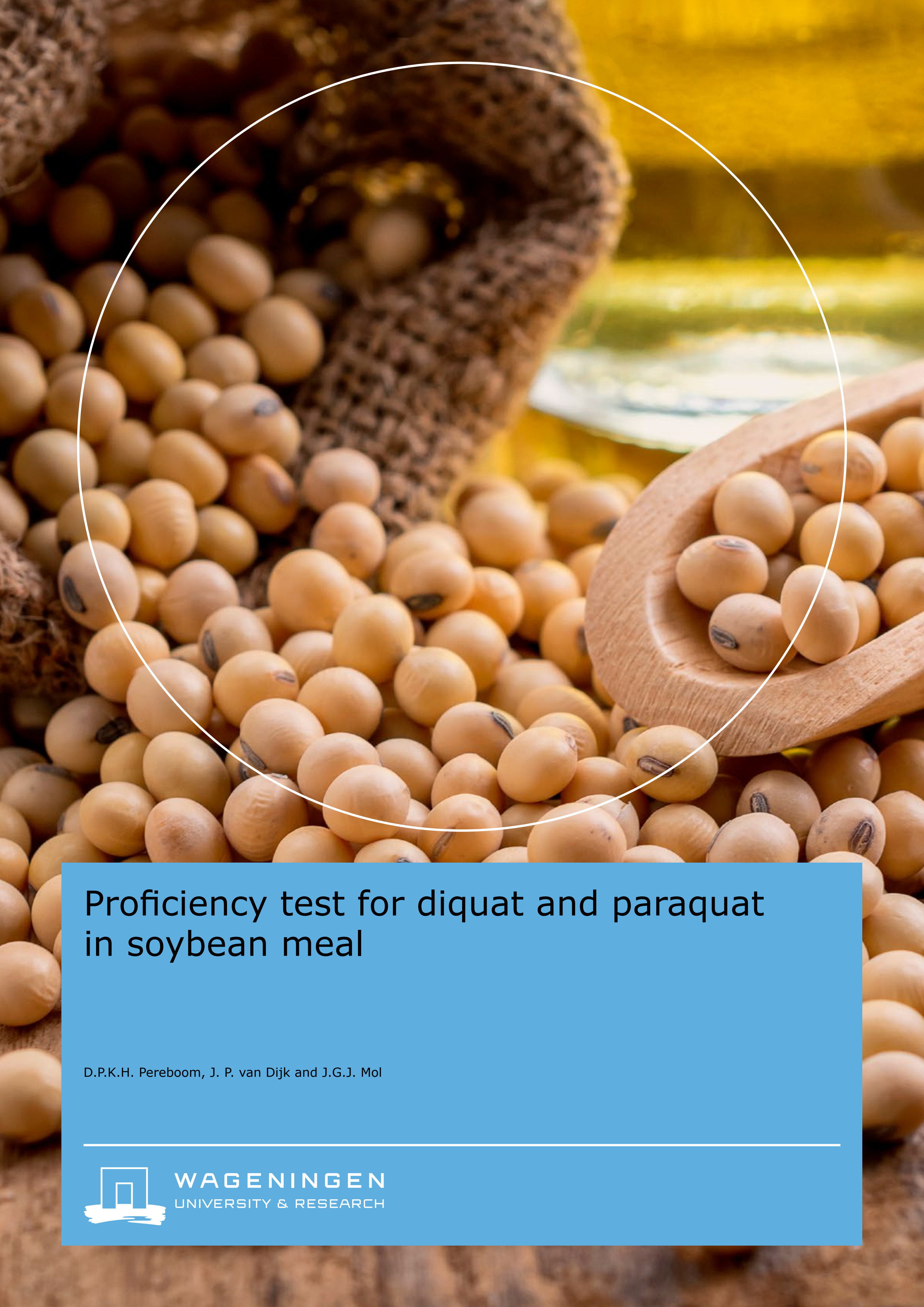

Proficiency test for diquat and paraquat in soybean meal

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WAGENINGEN UNIVERSITY & RESEARCH

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Distribution list:
- Participating laboratories

Contents

Summary

A proficiency test (PT) for diquat and paraquat (bipyridylium herbicides/desiccants) in soybean meal was organised by Wageningen Food Safety Research (WFSR), part of Wageningen University & Research between January and March 2024. This PT was performed by Wageningen Food Safety Research (WFSR) under accreditation (R013, Dutch Accreditation Council RvA, ISO/IEC 17043:2023).

For this proficiency test, two test materials were prepared:
- Soybean meal A containing diquat aimed at 400 µg/kg and paraquat at 40 µg/kg.
- Soybean meal B containing diquat aimed at 30 µg/kg and paraquat at 100 µg/kg.

Material A and B were prepared by spiking a solution of diquat and paraquat to soybean meal to the required target concentrations. The materials were mixed with water followed by extensive mixing, freeze-drying and milling of the samples. Both materials were sufficiently homogeneous and stable during the PT. Each participant received one test sample of 50 gram of each material. The participants were requested to report their results within 7 weeks after the dispatch of the samples.

Seventeen laboratories, of which 16 from EU Member States and one of the EFTA MS Norway participated in this PT. The participants were asked to quantify the pesticides diquat and paraquat in soybean meal and to report for each material two results. The participants' performance was assessed as z-score in both materials for the individual pesticides (maximum score 4 out of 4), calculated using the consensus values as the robust mean and a relative target standard deviation of 25%.

From the provided information on the identification and quantification of the pesticides all participants used LC-MS/MS and the reported Limit of Quantification (LOQ) values ranged from 4.77 to 20 µg/kg.

Characteristics of the PT materials and the outcome of this PT are summarised in Table 1a and 1b. For material A, the consensus values of diquat and paraquat in material A were, respectively 308 and 39.3 µg/kg and in material B, respectively 28.9 and 72.0 µg/kg. For material A and B, none of the RSD_R of the reported results were below the relative target standard deviation (25%) except for diquat in material B (20%).

For both materials (A and B) combined, 74% of the results for the pesticides were rated with satisfactory z-scores ($|z| \leq 2$), 8% of the results fell into the questionable range with $2<|z|<3$ and 14% of the results fell into the unsatisfactory range with $|z| \geq 3$. Four and a half percent were qualitative results of which 1.5% percent was a false negative result. Eleven participants showed a satisfactory performance for both materials by detecting diquat and paraquat with correct quantification, the absence of false negative results and reporting within the deadline. In this PT, one false negative result was reported for paraquat in material B.

From the results obtained in this PT on the pesticides diquat and paraquat it can be concluded that most participants have an analytical method available with sufficiently low LOQs for compliance testing of diquat and paraquat in soybeans. The results also reveal that for both materials relatively high robust RSD_R values were obtained, indicating at a relatively high variability of results amongst the laboratories. In this respect continued efforts need to be made by the participants to improve the robustness of the implemented methods, in order to produce reliable and comparable data.

Table 1a *Summary of proficiency materials parameters and participants' performance – number of laboratories reporting quantitative values, <LOQ and false negative (FN).*

		Consensus value	**Uncertainty**	**Robust RSDR1)**	**No of labs out of 17 reporting**		
pesticides	**Matrix**	**(µg/kg)**	**(µg/kg)**	**(%)**	**Quant. value**	**<LOQ**	**FN**
diquat	A	308	33.0	34	16		
	B	28.9	1.86	20	15	1	
paraquat	A	39.3	5.86	48	16	1	
	B	72.0	11.8	53	16		1

[1)] robust relative standard deviation (interlaboratory RSD based on participants' results).

Table 1b *Summary of proficiency materials parameters and participants' performance – evaluation of results, satisfactory, questionable and unsatisfactory z and z'-scores.*

		Consensus value	**z-scores[1)]**				
			Satisfact.	**Quest.**	**Unsatisf.**	**Qualitative results**	
pesticides	**Matrix**	**(ug/kg)**	**(%)**	**(%)**	**(%)**	**<LOQ %**	**FN %**
diquat	A	308	69	13	19		
	B	28.9	81	0	13	6	
paraquat	A	39.3	71	12	12	6	
	B	72.0	77	6	12		6

[1)] calculated using a fit-for-purpose target RSD for proficiency of 25%. False negatives were counted as unsatisfactory z-score.

1 Introduction

Diquat and paraquat are bipyridylium compounds that are used as herbicide or desiccant. Paraquat has been banned for use in the EU since 2007. Diquat was no longer approved in the EU from 2019 (max period of grace: 4 February 2020). Both compounds are however still used elsewhere. In the EU, the maximum residue level (MRL) for diquat in soybeans is 0.3 mg/kg and for paraquat in soybeans is 0.02 mg/kg and has been set in (Regulation (EC) No 396/2005 [3] and amendments thereof. For the matrix soybean meal as such, no specific MRLs have been set and MRLs derived from soybeans after taking processing factors into account apply.

Proficiency testing is conducted to provide participants with a powerful tool to evaluate and demonstrate the reliability of the data that are produced by the laboratory. Next to validation and accreditation, proficiency testing is an important requirement and demanded by ISO/IEC 17025:2017 [1].

The aim of this proficiency test was to give participants the opportunity to evaluate or demonstrate their competence for the analysis of diquat and paraquat in soybean meal. The preparation of the materials, including the homogeneity and stability testing of the materials, and the evaluation of the quantitative results were carried out under accreditation according to ISO/IEC 17043:2023 [2] accreditation by Wageningen Food Safety Research, part of Wageningen University & Research (R013).

2 PT material

2.1 Scope of the PT

This proficiency test (PT) focused on the pesticides diquat and paraquat in the feed matrix soybean meal. The target concentrations for the pesticides in this PT are presented in Table 2. The soybean meal is a by-product released during the extraction of oil from soybeans.

Table 2 *Target concentrations of polar pesticides in the PT materials.*

	Target concentrations (µg/kg)	
pesticides	**Material A**	**Material B**
diquat	400	30
paraquat	40	100

2.2 Material preparation

For preparation of the two PT materials A and B, soybean meal was used. Levels were artificially increased by spiking with diquat and paraquat. For each material, 2.5 kilograms were first fortified by adding a solution of a pesticide mix in water, aiming at the levels as presented in Table 2. The materials were mixed with approximately seven litres of water, homogenized using an industrial mixer (Topcraft, Belgium) according to an in-house standard operating procedure [4]. The fortified slurries were freeze-dried, homogenised in a Stephan cutter UM12, and stored in the freezer until use.

2.3 Sample identification

After homogenisation, materials A and B were divided into sub-portions of approximately 50 grams and stored in polypropylene, airtight closed containers in the freezer until use.

The samples for the participants were randomly selected and coded through the WFSR PT website application. The code used was “2024/pesticides/soybean/xxx”, in which the three-digit number of the code was automatically generated by the web application. One sample set was prepared for each participant. Each sample set consisted of one randomly selected sample of material A and one of material B. The codes of the samples for each sample set are shown in Annex 1. The samples for homogeneity and stability testing were also randomly selected out of the set of materials A and B.

2.4 Homogeneity study

To verify the homogeneity of the PT materials, 10 containers of each of the materials A and B were analysed in duplicate for diquat and paraquat.

The homogeneity of both materials was evaluated according to the International Harmonized Protocol for Proficiency Testing of Analytical Laboratories [5] and ISO 13528:2022 [6]. For homogeneity a target standard deviation for proficiency assessment (σ_P) of 25% was used as a fit-for-purpose standard deviation, in line with the target RSD used in proficiency tests on pesticides as organised by the pesticides EURLs [9]. With this procedure the between-sample standard deviation (s_s) and the within-sample standard deviation

(s_w) were compared with the standard deviation for proficiency assessment. The method applied for homogeneity testing is considered suitable if $s_w<0.5\times\sigma_P$ and a material is considered adequately homogeneous if $s_s<0.3\times\sigma_P$. Both materials proved to be sufficiently homogeneous for this PT.

The results of the homogeneity study (grand means with the corresponding RSD_r) are presented in Table 3. The statistical evaluation of materials A and B is presented in Annex 2.

Table 3 *Concentrations of pesticides in materials A and B obtained during homogeneity testing.*

Compound	Material A		Material B	
	Conc. (µg/kg)	RSD (%)	Conc. (µg/kg)	RSD (%)
diquat	416	3.98	34	7.60
paraquat	46.2	6.18	100	10.3

2.5 Stability of the materials

The stability of the pesticides in the materials was assessed according to [5, 6]. On January 22th, 2024, the day of distribution of the PT samples, 6 randomly selected containers of material A and B were stored in a freezer (-18 °C). Under these conditions it is assumed that diquat and paraquat are stable in the materials. In addition, 6 samples of each material were stored in a refrigerator.

On the 19th of April 2024, 88 days after distribution of the samples, 6 samples of materials A and B, stored in the freezer and refrigerator, were analysed in one batch. For each set of test samples, the average of the results and the standard deviation were calculated.

It was determined whether a consequential instability of the analytes had occurred [5,6] in the materials stored in the refrigerator. A consequential instability is observed when the average value of an analyte in the samples stored in the refrigerator is more than $0.3\sigma_P$ below the average value of the analyte in the samples stored in the freezer. If so, the instability has a significant influence on the calculated z-scores.

The results of the stability of materials A and B are presented in Annex 3. For the pesticides in both materials none of the tested storage conditions caused a consequential difference. Diquat and paraquat in the materials were, therefore, considered stable for the duration of the PT.

3 Organisational details

3.1 Participants

This PT focused on the determination of diquat and paraquat, using soybean meal as a feed matrix. Nineteen laboratories registered for the PT and 17 reported their results within the deadline. From two participants, no results were received, without an explanation. Each participant was free to use their method of choice reflecting their routine procedures. The participants were asked to report the results through the WFSR PT website application.

3.2 Material distribution and instructions

Each participant received a randomly assigned laboratory code, generated by the web application. The sample sets with the corresponding numbers, consisting of 2 coded samples (Annex 1) were sent to the participants on January 22th, 2024. The sample sets were packed in an insulating box containing dry ice and dispatched to the participants immediately by courier. The participants were asked to store the samples in the refrigerator and to analyse the samples according to their routine method. As reported by participants, all parcels were received in good order.

The samples were accompanied by a letter describing the requested analysis (Annex 5) and an acknowledgement of receipt form. In addition, each participant received instructions by e-mail on how to use the web application to report the results. Results should be reported as µg/kg product (no correction for moisture).

The deadline for submitting the quantitative results was March 11th, 2024, allowing the participants 7 weeks for analysis of the test samples. All results were submitted within the deadline.

4 Evaluation of results

The statistical evaluation was carried out according to the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories [5], elaborated by ISO, IUPAC and AOAC and ISO 13528:2015 [6] in combination with the insights published by the Analytical Methods Committee [7, 8] regarding robust statistics.

The evaluation of results was based on consensus values, its uncertainty, and the standard deviation for proficiency assessment (σ_P). If not negligible, the uncertainty of the assigned value and, if applicable, instability of analytes in the PT material, are taken into account in the determination of the z-scores.

4.1 Calculation of the consensus value (X)

The robust mean was used as consensus value in this PT [6, 7, 8]. The advantage of robust statistics is that all values are taken into account: outlying observations are retained, but given less weight. Furthermore, it is not expected to receive normally distributed data in a proficiency test. When using robust statistics, the data do not have to be normally distributed in contrast to conventional outlier elimination methods. The values and their uncertainties are summarised in Table 1 in the Summary section.

The robust mean of the reported results of all participants, calculated from an iterative process that starts at the median of the reported results using a cut-off value depending on the number of results, was used as the consensus value [6,7].

4.2 Calculation of the uncertainty of the consensus value (u)

The uncertainty of the consensus value is calculated to determine the influence of this uncertainty on the evaluation of the laboratories. A high uncertainty of the consensus value will lead to a high uncertainty of the calculated participants z-scores. If the uncertainty of the consensus value and thus the uncertainty of the z-score is high, the evaluation could indicate unsatisfactory method performance without any cause within the laboratory. In other words, illegitimate conclusions could be drawn regarding the performance of the participating laboratories from the calculated z-scores if the uncertainty of the consensus value is not taken into account.

The uncertainty of the consensus value (the robust mean) is calculated from the estimation of the standard deviation of the consensus value and the number of values used for the calculation of the consensus value [6,7]:

$$u = 1.25 * \frac{\hat{\sigma}}{\sqrt{n}}$$

where:
u = uncertainty of the consensus value;
n = number of values used to calculate the consensus value;
$\hat{\sigma}$ = estimate of the standard deviation of the consensus value resulting from robust statistics.

According to ISO 13528 [6] the uncertainty of the consensus value (u) is negligible and therefore does not have to be included in the statistical evaluation if:

$u \leq 0.3\sigma_P$

where:
u = the uncertainty of the consensus value;
σ_P = standard deviation for proficiency assessment (§4.3).

In case the uncertainty of the consensus value does not comply with this criterion, the uncertainty of the consensus value should be taken into account when evaluating the performance of the participants regarding the accuracy (§4.4). In case the uncertainty is > $0.7\sigma_P$ the calculated z-scores should not be used for evaluation of laboratories' performance and are presented for information only.

4.3 Calculation of the standard deviation for proficiency assessment (σ_P)

A target standard deviation for proficiency assessment (σ_P) of 25% was used as a fit-for-purpose standard deviation which is in line with the target RSD used in proficiency tests on mycotoxins as organised by the EURL.

$\sigma_P = 0.25c$

where:
σ_P = Expected standard deviation in proficiency tests for animal feed;
c = Concentration of the analyte (μg/kg).

4.4 Performance characteristics with regard to the accuracy

For illustrating the performance of the participating laboratories with regard to the accuracy a z-score is calculated. For the evaluation of the performance of the laboratories, ISO 13528 [6] is applied. According to these guidelines z-scores are classified as presented in Table 4.

Table 4 *Classification of z-scores.*

$\|z\| \leq 2$	Satisfactory
$2 < \|z\| < 3$	Questionable
$\|z\| \geq 3$	Unsatisfactory

If the calculated uncertainty of the consensus value complies with the criterion mentioned in §4.2, the uncertainty is negligible. In this case the accuracy z-score is calculated from:

$$z = \frac{x - X}{\sigma_p}$$ Equation I

where:
z = z-score;
x = the result reported by the laboratory;
X = consensus value;
σ_p = target standard deviation for proficiency testing.

However, if the uncertainty of the consensus value does not comply with the criterion mentioned in §4.2, it could influence the evaluation of the laboratories. Although, according to ISO 13528 in this case no z-scores can be calculated if a consensus value is used as the consensus value, we feel that evaluation of the participating laboratories is of main importance justifying the participating laboratories' effort. Therefore in this case, the uncertainty is taken into account by calculating the accuracy z-score [6]:

$$z' = \frac{x - X}{\sqrt{\sigma_p^2 + u^2}}$$ Equation II

where:
z' = z-score taking into account the uncertainty of the consensus value;
x = the result reported by the laboratory;
X = consensus value;
σ_p = target standard deviation for proficiency testing;
u = uncertainty of the consensus value.

If a consequential instability of the PT materials is observed, this can influence the evaluation of the laboratory performance. Therefore, in that case the consequential instability is taken into account when calculating z-scores. Because instability only regards one side of the confidence interval (a decrease of the concentration) this correction only applies to the lower 2s limit and results in an asymmetrical confidence interval.

In the case of a consequential instability the accuracy z-score for the laboratories that reported an amount below the consensus value is corrected for this instability by:

$$z_i = \frac{x - X}{\sqrt{\sigma_p^2 + \Delta^2}}$$ Equation III

where:
z_i = z-score taking into account the instability of the consensus value;
x = the result reported by the laboratory;
X = consensus value;
σ_p = target standard deviation for proficiency testing;
Δ = difference between average concentration of compound stored in the freezer and average concentration stored in refrigerator.

In some cases the uncertainty of the consensus value does not comply with the criterion in §4.2 and a consequential instability is observed. In this case the z' score for the laboratories that reported an amount below the consensus value is corrected for this instability by:

$$z'_i = \frac{x - X}{\sqrt{\sigma_p^2 + \Delta^2 + u^2}}$$ Equation IV

where:
z'_i = z-score taking into account the uncertainty and instability of the consensus value;
x = the result reported by the laboratory;
X = consensus value;
σ_p = target standard deviation for proficiency testing;
Δ = difference between average concentration of compound stored in the freezer and average concentration stored in refrigerator;
u = uncertainty of the consensus value.

In this PT, the uncertainty of the assigned value for diquat in material A and diquat and paraquat in material B were not negligible and taken into account in the assignment of the z-scores (z’). In the case of diquat in material B, the uncertainty of the assigned value was negligible. No instability of the analytes in the PT material was observed during the PT period.

4.5 Evaluation of non-quantified results

In cases where participant(s) reported ‘<[value] or ‘not detected’ (nd) (i.e. below their LOQ), ‘proxy-z-scores’ were calculated to assess possible false negatives.

A proxy-z-score was calculated by using the reported LOQ value as a result and indicated as a value between brackets. Proxy-z-score values [z<-2] were considered as false negatives.

Non-quantitative results, e.g. ‘detected’ or ‘not detected’, without specification of LOQ, were excluded from the evaluation. In these cases, the participant was considered to have no quantitative method available for the specific analyte or analyte group/matrix. Non-quantitative reported results for analytes or analyte groups are to be interpreted as unsatisfactory performance.

4.6 False positive and false negative results

A false positive is a quantitative result reported by the participant while the analyte is not detected in the PT material by the organiser, and/or not detected by most of the other participants. A threshold is then applied, above which results are considered false positives, indicated as FP. False positives are to be interpreted as unsatisfactory performance.

False negatives are indicated as ‘FN’. Also, when no LOQ values were reported and the compound was reported as ‘<[value]’ or (nd) a false negative result was assigned. False negatives are to be interpreted as unsatisfactory performance.

5 Performance assessment

5.1 Scope and LOQ

This PT was dedicated to the quantification of diquat and paraquat in soybean meal. Annex 6 summarises the quantitative scope of each participant, with an indication of the LOQ for each pesticide. Two participants provided no details on the LOQs of the method used.

Sixteen participants reported for both materials A and B a total of 2 results, comprising levels of diquat and paraquat, as was requested. One participant reported only paraquat for both materials. In material A, one participant reported for paraquat <LOQ and for material B, one participant reported for both pesticides <LOQ. For these results proxy z-scores were calculated.

The LOQs provided by the participants ranged from 4.77 to 20 µg/kg (Annex 6). Of these LOQs, one participant reported an LOQ of 0.01 for both pesticides and one participant reported an LOQ for diquat of 0.00477 µg/kg and for paraquat 0.00589 µg/kg which may have been unit errors.

5.2 Analytical methods

An overview of the information provided by the participants regarding the methods applied in this PT is presented in (Annex 7). Five participants provided no information about method details.

Most participants extracted diquat and paraquat under acidic conditions: Quick Polar Pesticides Method (QuPPe) (4) or similar (methanol in combination with hydrochloric acid (4); methanol in combination with hydrochloric acid and ethylene diamine tetra acetic acid (EDTA) (1); acetonitrile in combination with hydrochloric acid (1)). One participant used the QuEChERS method, one participant indicated liquid-liquid extraction (LLE).

For the identification and quantification of the pesticides all participants used LC-MS/MS.

Eleven participants used one or more isotopically labelled internal standards for diquat and paraquat quantification. Three participants provided only that the laboratory used deuterated standards. The internal standards used were: diquat-d4 dibromide, diquat-d8 dibromide, paraquat diodide D6 (dimethyl/dichloride) and paraquat-d8 dichloride.

5.3 Performance

The quantitative performance was assessed through z-scores. The individual z-scores obtained by each participant, including their graphical representation, for the pesticides in materials A and B are summarised in Annex 8. A summary of the performance of the participants in this PT is provided in Annex 9. The RSD_R values are included in Annex 8, in Tables 5 and in Table 1 (Summary section).

A summary of the statistical evaluation of the PT results is presented in Tables 5. These table include all relevant parameters: the consensus value (X), the uncertainty of the consensus value (u), the standard deviation for proficiency assessment (σ_p) and the robust (relative) standard deviation, based on participants' results. In case the uncertainty of the consensus value did not comply with the criterion $u \leq 0.3\sigma_p$, the uncertainty of the consensus value was taken into account in the evaluation of the z-scores (by calculating the z'-score). This was the case for diquat in material A and paraquat in both materials. For diquat in material B the uncertainty of the consensus value did comply with the criterion $u \leq 0.3\sigma_p$ and was therefore considered as negligible.

Table 5 *Summary of statistical evaluation of the PT results on diquat and paraquat in material A and B.*

	Material A		**Material B**	
	diquat	**paraquat**	**diquat**	**paraquat**
X (µg/kg)	308	39.3	28.9	72.0
lowest reported value (µg/kg)	3.11	1.004	0.678	1.191
highest reported value (µg/kg)	1200	136	62	290
u (µg/kg)	33.0	5.86	1.86	11.8
σ_p (µg/kg) (25%)	77.0	9.83	7.24	18.0
$u>0.3\sigma_p$	Yes	Yes	No	Yes
robust σ (µg/kg)	105	18.8	5.78	37.8
robust σ (%)	34.2	47.7	20.0	52.5
# reported	16	17	16	17
"<" (qualitative result)		1	1	1
# quantitative results	16	16	15	16
$\|z\| \leq 2$	11	12	13	13
$2<\|z\|<3$	2	2	0	1
$\|z\| \geq 3$	3	2	2	2
FN				1
S z-scores (%)	68.8	70.6	81.3	76.5

S z-scores = satisfactory z-scores.

FN = False negative result

The consensus values for diquat and paraquat in material A were respectively 308 and 39.3 µg/kg and in material B respectively 28.9 and 72.0 µg/kg.

The robust relative standard deviation (RSD_R) was calculated according to ISO13528:2022 [6] for informative purposes only. In this study it was used as a good estimation of the interlaboratory variability. The RSD_R values for each pesticide in both materials are shown in Annex 8 and in Table 5.
For material A, the robust standard deviations (RSD_R) of the reported results for diquat (34%) and for paraquat (48%) were above the target standard deviation (25%). For material B, the RSD_R of diquat (20%) was below the target standard deviation (25%) and for paraquat the RSD_R (53%) exceeded the target standard deviation.

For both pesticides in material A, 70% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 12% of the results fell into the questionable range with $2<|z|<3$ and 15% of the results fell into the unsatisfactory range with $|z| \geq 3$ (Table 6). Three percent of the reported results were qualitative results. For material B this was 79%, 3%, and 12%, respectively. Of the reported results 6% were qualitative results. Of these 6%, 3% were false negative results and interpreted as an unsatisfactory performance. Overall, 74% of the diquat and paraquat results obtained for both materials (A and B) were rated with satisfactory z-scores ($|z| \leq 2$), 8% of the results fell into the questionable range with $2<|z|<3$ and 14% of the results fell into the unsatisfactory range with $|z| \geq 3$. Five were qualitative results of which 2% was a false negative result.

In Annex 9 an overview of the overall performance for each participant in this PT is provided. For the 2 materials combined, a maximum of 4 satisfactory z-scores, could be obtained. Out of 17 participants, 10 participants achieved satisfactory performance for both materials by detecting diquat and paraquat with correct quantification, the absence of false negative results within their scope and reporting within the deadline. One participant achieved also satisfactory results for both materials but had only paraquat in their scope. For the other 6 participants, false negative (FN) results were reported or one or more non-satisfactory z-scores were obtained.

6 Conclusions

Seventeen laboratories participated in the PT on quantitative determination of diquat and paraquat in the feed matrix soybean meal.

Out of 17 participants, 10 participants achieved satisfactory performance for both materials by detecting both pesticides diquat and paraquat with correct quantification, the absence of false negative results within their scope and reporting within the deadline. One participant achieved also satisfactory results for both materials but had only paraquat in their scope. For the remaining 6 participants one or more non-satisfactory z-scores were obtained.

For the pesticides in material A, the percentage of satisfactory results for diquat was 69% and for paraquat 71%. The RSD_R of the reported results for diquat (34%) and paraquat (48%) were above the target standard deviation (25%). For material B, the satisfactory results were for diquat 81% and for paraquat 77%. The RSD_R was for diquat (20%) below the target standard deviation and for paraquat (53%) far above the target standard deviation.

High variation in the reported results of the pesticides for both materials resulted in relatively high robust RSD_R values (34 to 53%) except for diquat in material B (RSD_R 20%). No explanation can be given for the lower RSD_R of diquat in material B. The results imply that the methods used by some of the participants were not suitable enough for the determination of diquat and paraquat at the levels present in soybean meal and suggest that improved methods are required.

Overall, for diquat and paraquat in both materials combined (4 results), 74% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 8% of the results fell into the questionable range with $2<|z|<3$ and 14% of the results fell into the unsatisfactory range with $|z| \geq 3$. This included the results from participants only reporting quantitative results.

From the results obtained in this PT it can be concluded that most of the participants (65%) have an analytical method available for quantification of the pesticides diquat and paraquat with sufficiently low LOQs and satisfactory performance. Furthermore, the room for improvement remains, because the variation in the results is relatively high. For the pesticides in both materials, the interlaboratory reproducibility (RSD_R) was in most cases far above 30%.

References

[1] ISO/IEC 17025:2017(E). 2017. General Requirements for the Competence of Calibration and Testing testing Laboratories.

[2] ISO/IEC 17043:2023. Conformity assessment - General requirements for the competence of proficiency testing providers.

[3] Regulation (EC) No 396/2005 of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council 91/414/EEC.

[4] WFSR SOP-A-0989 – Preparation of PT materials and PT samples.

[5] Thompson M, Ellison SL, Wood R. 2006. The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories. Pure Applied Chemistry 78(1):145-196.

[6] ISO 13528:2022 (E). Statistical methods for use in proficiency testing by inter-laboratory comparison.

[7] Analytical Methods Committee. 1989. Robust statistics - How not to reject outliers Part 1. Basic concepts. Analyst 114:1693-1697.

[8] Analytical Methods Committee. 1989. Robust statistics - How not to reject outliers Part 2. Inter-laboratory trials. Analyst. 114:1699-1702.

[9] Target standard deviation for proficiency assessment (σ_P) of 25% used in proficiency tests on pesticides organised by the pesticides EURLs.
https://www.eurl-pesticides.eu/library/docs/allcrl/EUPT-General_Protocol_V9_2020.pdf

Annex 1 Codification of the samples

Participant's code	Material A*	Material B*
PT7861	953	534
PT7862	441	418
PT7863	401	619
PT7864	286	984
PT7865	877	198
PT7866	370	495
PT7867	156	925
PT7868	603	282
PT7869	656	600
PT7870	729	974
PT7871	402	248
PT7873	360	620
PT7874	771	669
PT7875	578	608
PT7876	298	549
PT7877	225	490
PT7878	905	307

* All sample codes start with 2024/pesticides/soybean/

Annex 2 Statistical evaluation of homogeneity data

Sample No.	diquat in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	424	417
Hom/B002	438	428
Hom/B003	393	422
Hom/B004	368	415
Hom/B005	408	411
Hom/B006	414	435
Hom/B007	419	410
Hom/B008	417	432
Hom/B009	389	424
Hom/B010	423	422
Grand mean	416	
Cochran's test		
C	0.426	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	104	
s_x	12.1	
s_w	16.0	
s_s	4.22	
Critical= 0.3 σ_P	31.2	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

sx = Standard deviation of the sample averages.
sw = Within-sample standard deviation.
ss = Between-sample standard deviation.

Sample No.	paraquat in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	48.9	45.7
Hom/B002	45.0	47.9
Hom/B003	44.0	42.7
Hom/B004	42.4	48.7
Hom/B005	46.4	45.9
Hom/B006	47.0	48.9
Hom/B007	52.1	44.2
Hom/B008	41.2	50.2
Hom/B009	46.9	48.4
Hom/B010	43.3	43.9
Grand mean	46.2	
Cochran's test		
C	0.393	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	11.5	
s_x	1.67	
s_w	3.23	
s_s	0.00	
Critical= 0.3 σ_P	3.46	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

sx = Standard deviation of the sample averages.
sw = Within-sample standard deviation.
ss = Between-sample standard deviation.

	diquat in B (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/B001	34.7	33.9
Hom/B002	31.8	33.6
Hom/B003	33.0	34.5
Hom/B004	33.3	33.0
Hom/B005	33.9	34.2
Hom/B006	34.9	28.1
Hom/B007	34.4	35.2
Hom/B008	35.5	38.1
Hom/B009	mistaken injection	mistaken injection
Hom/B010	34.9	41.2
Grand mean	34.3	
Cochran's test		
C	0.464	
Ccrit	0.638	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	8.59	
s_x	2.02	
s_w	2.37	
s_s	1.12	
Critical= 0.3 σ_P	2.58	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

	paraquat in B (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/B001	84.8	91.8
Hom/B002	85.7	97.1
Hom/B003	103	94.7
Hom/B004	91.7	98.5
Hom/B005	109	94.7
Hom/B006	115	86.6
Hom/B007	103	113
Hom/B008	106	110
Hom/B009	mistaken injection	mistaken injection
Hom/B010	104	118
Grand mean	100	
Cochran's test		
C	0.505	
Ccrit	0.638	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	25.1	
s_x	7.91	
s_w	9.51	
s_s	4.15	
Critical= 0.3 σ_P	7.53	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Annex 3 Statistical evaluation of stability data

Stability evaluation for diquat in material A.

Storage temperature	freezer	refrigerator
Time (days)	0	88
Calculated amounts (µg/kg)	419	395
	422	416
	416	410
	411	415
	405	398
	409	426
Average amount (µg/kg)	414	410
n	6	6
st. dev (µg/kg)	6.38	11.7
Difference		3.98
$0.3*\sigma_P$		31.0
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for paraquat in material A.

Storage temperature	freezer	refrigerator
Time (days)	0	88
Calculated amounts (µg/kg)	55.5	51.5
	56.7	57.6
	54.9	53.3
	53.5	56.6
	55.3	50.0
	54.2	54.4
Average amount (µg/kg)	55.0	53.9
n	6	6
st. dev (µg/kg)	1.09	2.90
Difference		1.14
$0.3*\sigma_P$		4.13
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for diquat in material B.

Storage temperature	freezer	refrigerator
Time (days)	0	88
Calculated amounts (µg/kg)	35.0	35.0
	36.6	35.6
	31.6	35.2
	32.1	34.2
	31.5	36.9
	36.0	33.5
Average amount (µg/kg)	33.8	35.1
n	6	6
st. dev (µg/kg)	2.33	1.18
Difference		-1.29
$0.3*\sigma_P$		2.53
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for paraquat in material B.

Storage temperature	freezer	refrigerator
Time (days)	0	88
Calculated amounts (µg/kg)	121	111
	114	121
	103	112
	105	116
	106	134
	118	104
Average amount (µg/kg)	111	116
n	6	6
st. dev (µg/kg)	7.62	10.6
Difference		-4.95
$0.3*\sigma_P$		8.35
Consequential difference? Diff < $0.3*\sigma_P$		No

Annex 4 Invitation letter

WAGENINGEN
UNIVERSITY & RESEARCH

P.O. Box 230 | 6700 AE Wageningen | The Netherlands

Wageningen Food Safety Research

DATE
December 13, 2023

SUBJECT
Invitation proficiency test polar pesticides paraquat and diquat in feed matrix soybean meal

Dear Madam/Sir,

Hereby, I would like to invite you to participate in the proficiency test (PT) for the polar pesticides diquat and paraquat in the feed matrix soybean meal. This PT is initiated by the Netherlands Ministry of Agriculture, Nature and Food Quality and will be organised by Wageningen Food Safety Research (WFSR), part of Wageningen University & Research.

Participation in relevant proficiency tests is one of the requirements for certification for both public and private laboratories. Opportunities to fulfil this requirement are not always frequently offered and the Netherlands Ministry of Agriculture, Nature and Food Quality has therefore taken the initiative to organise a relevant proficiency test and has the intention to continue this study on a regular base.

This PT will focus on the quantification of the pesticides diquat and paraquat in the feed matrix soybean meal, as included in Regulation (EC) No 396/2005 and will be organised under accreditation according to ISO 17043 (General requirements for proficiency testing - R013). The primary goal of this PT is to give laboratories the opportunity to evaluate and demonstrate their performance regarding the analysis of these compounds in feed. The maximum number of participants is 20. Laboratories located in the Netherlands and Europe are given priority and other laboratories can participate at a first come first serve basis.

The following matters are important for participation in this proficiency test:

1. Test materials
- Two soybean meal materials of approximately 50 g will be provided.
- The test materials may contain diquat and paraquat.

2. Shipment of the test materials
- Test materials will be sent in January/February 2024. The distribution of the test materials will be announced by e-mail.
- The participant should arrange the necessary import permits for the test materials (if necessary).
- The deadline for reporting will be at the latest six weeks after the shipment of the test materials.

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6700 AE Wageningen
The Netherlands

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INTERNET
www.wur.nl

COC NUMBER
09098104

HANDLED BY
Diana Pereboom

TELEPHONE
+31(0) 614 323 017

EMAIL
pt.wfsr@wur.nl

Wageningen Research Foundation/Wageningen Food Safety Research (WFSR) is part of Wageningen University & Research. WFSR carries out research and analysis contributing to the safety and reliability of food and feed.

3. Report
- A report of the proficiency test will be dispatched in September 2024.
- The results will be evaluated based on a consensus value according to ISO 13528, Algorithm A.
- Evaluation of the performance will be based on z-scores, false positive and false negative results.
- Results of the proficiency test will be presented anonymously.

4. Additional information
- WFSR is allowed to use the anonymous results of the proficiency test in presentations, seminars and publications.
- WFSR will never inform third parties (e.g. accreditation bodies) on specific laboratory results without informing the laboratory first.

5. Costs
- Participation is free of charge.
- If an extra batch of samples is needed after the first shipping, the courier costs will be charged.

If you would like to participate, please fill out the accompanying participation form (preferably digitally) and send it back **before January 15th 2024** to: pt.wfsr@wur.nl.

Looking forward to welcome you for this proficiency test,

Diana Pereboom
Proficiency tests

Wageningen Food Safety Research
The Netherlands

Annex 5 Instruction letter

WAGENINGEN
UNIVERSITY & RESEARCH

P.O. Box 230 | 6700 AE WAGENINGEN | The Netherlands

Dear Madam/Sir,

Thank you very much for your interest in the proficiency test for the analysis of the polar pesticides diquat and paraquat in the feed matrix soybean meal (PT 2023-17).

The parcel shipped to you should contain:

- Two randomly coded samples. Each sample unit contains approximately 50 grams of test material.

Instructions:

- After arrival the samples should be stored at +4 °C.
- Please fill in the accompanied 'acknowledgement of receipt form' and return it immediately upon receipt of the samples by e-mail (pt.wfsr@wur.nl).
- Before analysis, (re)homogenise the samples according to your laboratory's procedure.
- Treat the test material as a sample for routine analysis. Carry out a **single analysis** for each sample.
- Reporting:
  - Report all analytical results in **µg/kg for the material as received** (**do NOT report on 12% moisture basis**).
  - For pesticides included in your method but not found, so below your LOQ, report as '**<[value µg/kg]**', e.g. < 5 µg/kg. Do not use the option 'detected' from the web application.
  - In case a pesticide is not included in the scope of your method, so not measured, report as '**nt**' (not tested).
- Results reported in any other format (e.g. nd, detected, <LOQ, etc) will be regarded as not tested, 'nt'.

- Please use the web application for entering your results (https://crlwebshop.wur.nl/ordsp/f?p=307:LOGIN). Information about the use of this web application was sent to you earlier by e-mail.

100years 1918 — 2018

Wageningen Food Safety Research

DATE
January 22, 2024

SUBJECT
Instruction proficiency test polar pesticides diquat and paraquat in feed matrix soybean meal (PT2023-17).

OUR REFERENCE
WFSR/pesticides/20242716

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Wageningen Research Foundation/Wageningen Food Safety Research (WFSR) is part of Wageningen University & Research. WFSR carries out research and analysis contributing to the safety and reliability of food and feed.

DATE
January 22, 2024

OUR REFERENCE
WFSR/pesticides/2024

PAGE
2 of 2

- The deadline for submitting test-results for this test is **11th of March 2024.**

- Your username is:
- Your password is:
- Your lab code to enter this proficiency test is:

- Please inform us about your applied method and detection technique (via the web application).

Please contact me if you have any questions or need any assistance.

With kind regards,

Diana Pereboom
Proficiency tests

pt.wfsr@wur.nl

Annex 6 Scope and LOQ

Lab code	LOQ (µg/kg)	
	diquat	paraquat
PT7861	5	5
PT7862	10	10
PT7863		
PT7864	10	10
PT7865	20	20
PT7866		8
PT7867	10	10
PT7868	10	10
PT7869	0.00477 (probably mg/kg)	0.00589 (probably mg/kg)
PT7870	0.01 (probably mg/kg)	0.01 (probably mg/kg)
PT7871		
PT7873	20	20
PT7874	5	5
PT7875	20	20
PT7876	10	10
PT7877	10	10
PT7878	0.01 (probably mg/kg)	0.01 (probably mg/kg)

Annex 7 Overview of the applied methods

Lab code	Sample preparation	Internal standard	Detection technique
PT7861	QuPPe method	diquat-d4 dibromide; paraquat-d8 dichloride; aminomethyl phosphonic acid (AMPA) 13C; Glyphosate-13C; phosphonic acid-18O3	LC-MS/MS
PT7862			LC-MS/MS
PT7863			
PT7864	acidic methanol extraction; heat + cooldown extract: shaking + centrifugation	Deuterated standards	LC-MS/MS
PT7865			
PT7866	Extraction of 5 g sample with 25 mL MeOH/0,48 M HCl (60/40) for 15 min (shaking). No further clean-up. Only 1:2 dilution of an aliquot portion of supernatant.	paraquat diodide D6	LC-MS/MS
PT7867	H_2O+MeOH+HCl	Yes	LC-MS/MS
PT7868			
PT7869	2.5 g sample + (9 mL H_2O + 1mL EDTA) + 10mL (MeOH + HC1 0.1M (1:1)) 15 min. 80 °C	No	LC-MS/MS
PT7870			
PT7871	5g sample, extracted with a mixture of MeOH, HCl and H_2O through shaking and heating at 80 °C. After centrifugation an aliqout of the extract is brought to pH 3.2. After dilution the extract is filtered and injected	diquat-d4 dibromide; paraquat-d8 dichloride	LC-MS/MS
PT7873	Liquid-liquid extraction	Deuterated standards	LC-MS/MS
PT7874	HCl (40%, 0.5M) in ACN; Thermal cleanup	diquat-d4 dibromide; paraquat-d8 dichloride	LC-MS/MS
PT7875	QuPPe method	diquat-d4 dibromide; paraquat-d8 dichloride	LC-MS/MS
PT7876	QuEChERS method	Deuterated standards	LC-MS/MS
PT7877	QuPPe method	paraquat diodide D6	LC-MS/MS
PT7878	QuPPe method	Diquat-d8 dibromide and paraquat D6	LC-MS/MS

ACN = acetonitrile; MeOH = methanol; HCl = hydrochloric acid; EDTA = ethylenediaminetetra acetic acid.

Annex 8 Results material A and B (soybean meal)

	Material A				Material B			
	diquat X: 308 µg/kg u: 33.0 µg/kg σ_p: 77.0 µg/kg robust σ: 105 µg/kg (34.2%)		paraquat X: 39.3 µg/kg u: 5.86 µg/kg σ_p: 9.83 µg/kg robust σ: 18.8 µg/kg (47.7%)		diquat X: 28.9 µg/kg u: 1.86 µg/kg σ_p: 7.24 µg/kg robust σ: 5.78 µg/kg (20.0%)		paraquat X: 72.0 µg/kg u: 11.8 µg/kg σ_p: 18.0 µg/kg robust σ: 37.8 µg/kg (52.5%)	
Lab code	**Result (µg/kg)**	**z'-score**	**Result (µg/kg)**	**z'-score**	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z'-score**
PT7861	420	1.34	30	-0.82	40	1.53	50	-1.02
PT7862	352	0.52	44.9	0.49	32.8	0.53	95.6	1.10
PT7863	1200	**10.65**	71	**2.77**	62	**4.57**	290	**10.13**
PT7864	230	-0.93	28	-0.99	25	-0.55	53	-0.88
PT7865	410	1.22	34	-0.47	30	0.15	82	0.46
PT7866	nt		17.3	-1.92	nt		40.4	-1.47
PT7867	378	0.83	58	1.63	31	0.28	108	1.67
PT7868	350	0.50	27	-1.08	25	-0.55	58	-0.65
PT7869	3.11	**-3.64**	1.004	**-3.35**	0.678	**-3.91**	1.191	**-3.29**
PT7870	346.09	0.45	48.55	0.81	29.52	0.08	98.23	1.22
PT7871	34	**-3.27**	51	1.02	33	0.56	106	1.58
PT7873	71.4	**-2.83**	69.4	**2.63**	<20	[-1.24]	<20	**[-2.42]FN**
PT7874	342	0.40	32.6	-0.59	28.5	-0.06	68.1	-0.18
PT7875	311	0.03	<20	[-1.69]	32.3	0.46	22.8	**-2.29**
PT7876	319	0.13	33	-0.55	26	-0.41	68	-0.19
PT7877	138	**-2.03**	136	**8.44**	19	-1.37	109	1.72
PT7878	274	-0.41	27	-1.08	23	-0.82	49	-1.07

X = consensus value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency test.

robust σ = robust (relative) standard deviation based on participants' results.

nt = not tested.

FN = False negative result.

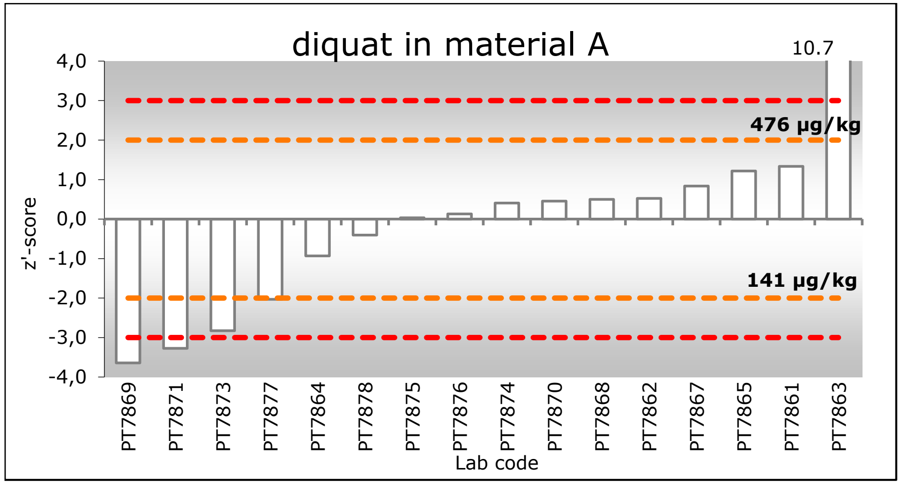

Figure 1 *Graphical representation of the z'-scores for diquat in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

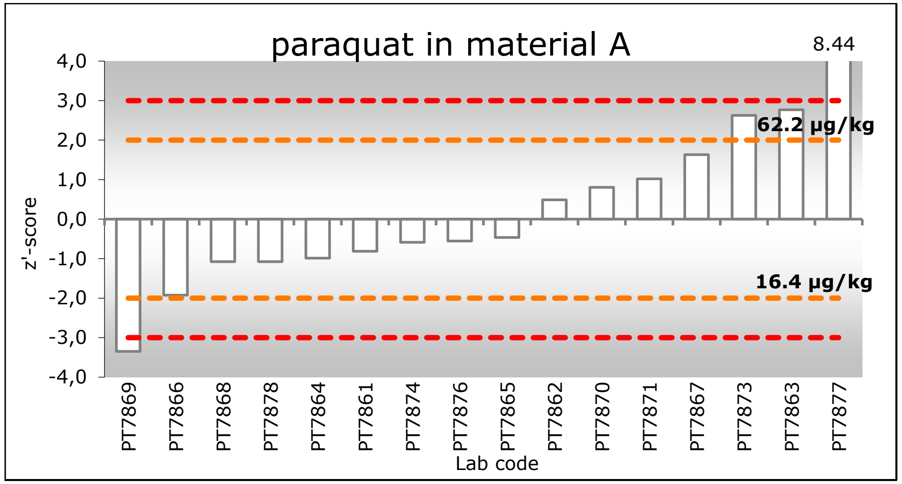

Figure 2 *Graphical representation of the z'-scores for paraquat in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

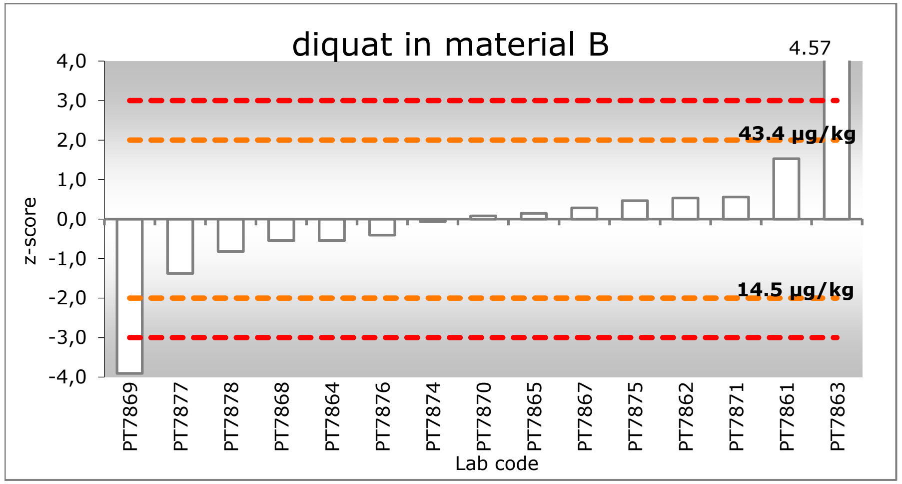

Figure 3 *Graphical representation of the z-scores for diquat in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

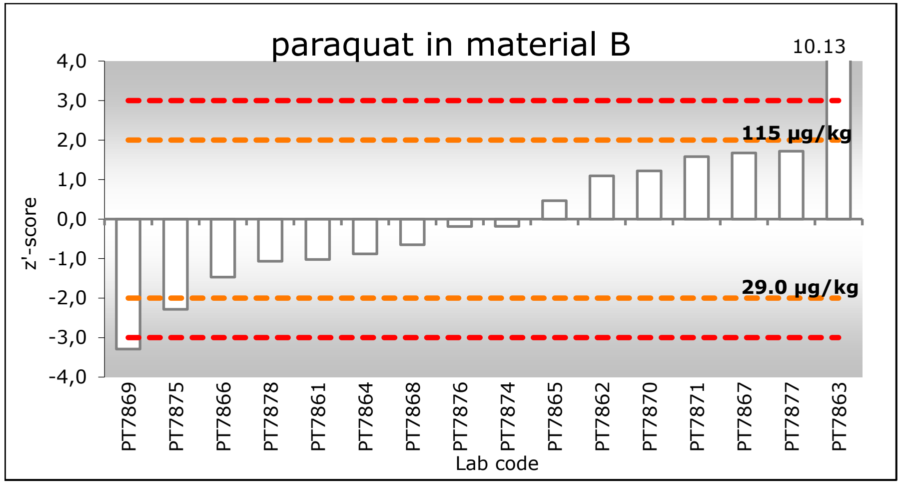

Figure 4 *Graphical representation of the z'-scores for paraquat in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

Annex 9 Overview performance per laboratory

Lab code	
PT7861	**4 s**
PT7862	**4 s**
PT7863	1 q, 3 u
PT7864	**4 s**
PT7865	**4 s**
PT7866	**2 s (diquat not in scope)**
PT7867	**4 s**
PT7868	**4 s**
PT7869	4 u
PT7870	**4 s**
PT7871	3 s, 1 u
PT7873	2 q, 1FN, 1 qualitative result
PT7874	**4 s**
PT7875	2 s, 1 q, 1 qualitative result
PT7876	**4 s**
PT7877	2 s, 1 q, 1 u
PT7878	**4 s**

s satisfactory z-score.

q questionable z-score.

u unsatisfactory z-score.

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WFSR Report 2024.011

The mission of Wageningen University & Research is “To explore the potential of nature to improve the quality of life”. Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to finding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 7,700 employees (7,000 fte), 2,500 PhD and EngD candidates, 13,100 students and over 150,000 participants to WUR’s Life Long Learning, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.

To explore the potential of nature to improve the quality of life

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WFSR report 2024.011

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