



TESTQUAL

PROFICIENCY TESTING SCHEMES

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(Proficiency Testing Schemes)

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PROTOCOL

TestQual 204

Multi-analysis in Avocado:

- ***Pesticides multiresidues***
- ***Fosetyl, Phosphonic acid***
- ***Glyphosate***
- ***AMPA***

INDEX

0. GLOSARY AND ABBREVIATIONS.....	3
1. INTRODUCTION	3
2. OBJECTIVE	3
3. CALENDAR	3
4. REGISTER AND PARTICIPATION REQUEST (APPLICATION FORM).....	4
5. TEST MATERIAL. CONTROL AND DISTRIBUTION.....	5
6. CONCENTRATION RANGES, SIGMA OBJECTIVE AND ANALYTES.....	7
7. RESULTS EXPRESSION.....	9
8. STATISTICAL EVALUATION	9
9. EVALUATION REPORT/S	13
10. CONTACT	13
11. BIBLIOGRAPHY AND REFERENCES OF INTEREST	14

Summary of changes:

Changes from previous revision are marked in *blue* or **highlighted blue**.

Rev00 a Rev01: updated period to appeal evaluation (30 days). Removed reserve deadline.

Rev01 a Rev02: technical department changes.

Rev02 a Rev03: Updated exercise deadlines.

Rev03 a Rev04: Changes in fiscal year dates.

Rev04 a Rev05: Updated exercise deadlines and correction of analytes.

Rev05 a Rev06: Changes in final date to submit AMPA results.

0. GLOSARY AND ABBREVIATIONS

Text	Abbreviation
TestQual	TQ
Proficiency test	PT / P.T.
Limit Of Quantification	LOQ
Not Analysed	NA
Aminomethylphosphonic acid	AMPA
Fosetyl-Al (Sum of fosetyl and phosphonic acid and its salts expressed as fosetyl)	Fosetyl-Al (sum)

1. INTRODUCTION

This document describes the **protocol** of the **TestQual 204** Proficiency Test (P.T.) in **Avocado**, for the analysis of the **participant's choice** from the following group of **analytes**:

Analyte-group code	Grouped analytes
A	Pesticides multiresidues
B	Fosetyl, Phosphonic acid & Fosetyl-Al (sum)
C	Glyphosate
D	AMPA

In the present document is detailed how to start working with TestQual, send your application to participate, the statistic that will be applied and information about the evaluation report.

TestQual, S.L. is committed to maintaining confidentiality with the information of each laboratory from the beginning of the proficiency test.

2. OBJECTIVE

The objective of the **TestQual 204** Proficiency Test is to evaluate the quality and accuracy of the results sent by the participating laboratories. Because of this, proficiency testing is an essential element of laboratory quality assurance. It will help to control and detect errors in their results or methods of analysis.

3. CALENDAR

The following table shows the program for this proficiency test:

Date (YYYY/mm/dd)	Activity	Carried out by
2024/11/18	Deadline to request participation*	Participants
2024/12/02-04	Sample delivery	TestQual
2024/12/20	Final date to submit AMPA results	Participants
2024/12/23	Final date to submit results	Participants
2025/01/20	Evaluation/global report issued	TestQual

*Acceptance of the participation between the deadline to reserve the PT sample and the deadline to request the participation will depend on availability of the proficiency test.

Participants are requested in the application to submit their LOQ/LOQs. For PTs with multiple groups of analytes, for each group that the participant has submitted LOQ/LOQs, if a certain percentage (described in our internal procedure) of the present/planned analytes is analysed, then the participation is accepted, and a laboratory code is generated and sent by email to confirm the participation acceptance to the user of the TestQual's account.

The dates of this calendar and the definitive shipping distribution might be changed depending on the development of the proficiency test. The definitive distribution date is confirmed when the sample instructions are sent, which, besides the instructions for the sample, contains the deadline to submit the results (which might update and override the here displayed deadline).

The sample instructions are exclusively sent to inscribed participants. These possible changes would be notified to all participants through our website www.TestQual.com and/or by email.

In the technical department of this proficiency test will be María Dolores García Valiente. Any question regarding the development of the proficiency test, the application status or any other query can be consulted by email to testqual@testqual.com.

4. REGISTER AND PARTICIPATION REQUEST (APPLICATION FORM)

NEW CLIENT

If your laboratory has not participated before in one of our proficiency tests you will have to register using the REGISTER form.

Once you have completed and sent the form you will have to wait until the activation of the account from the website administrator. If some more information is needed someone from our team will get in contact with you. You can find our contact data at the end of this protocol.

For those laboratories that require more than one contact per account or are in a situation not contemplated in this protocol will have to contact the organizer using the Contact tab to be instructed how to proceed.

APPLICATION FOR THE PROFICIENCY TEST

To participate in this proficiency test or to be updated via email of any changes regarding this this, it is required to have a laboratory code. To get your laboratory code is needed to apply through the website or have it arranged by the coordinator at the latest, on the deadline to request the participation.

In the “Proficiency Tests” tab on our website, you will have to select the proficiency test you want to participate, by clicking its name or the image you will enter the proficiency test page, which contains general information and a summary of that proficiency test, there you can find the present document (the protocol) and the button to start the application.

You will be required to log in to start the application and then the website will require you to submit your Limit Of Quantification (LOQ) for the parameters you will study. **The analytes left as NA (NOT ANALYSED) will NOT appear in the Results form** and therefore will not be able to send results for those parameters through the website.

Once the participation has been requested, as soon as possible, it will be checked by the website administrator, and you will be sent an email with the participation code. This code will be just known only by the organizer and the participant and will be kept confidential **at all times**, even after the proficiency test finishes.

You can check on the dashboard of your client area if an application you sent has been accepted or is still pending.

Just one application per exercise can be sent by each laboratory site, not being allowed for a laboratory site to participate with two different codes.

The applications of the laboratories will be studied and accepted in base of the quantification limits of the analytes of the proficiency test and if the logistics allow the sample shipping without risk of deterioration.

According to the experience, TestQual can anticipate that the number of participants of this proficiency test will be around **15-20**, being 8 the minimum participants for the proficiency test to take place.

5. TEST MATERIAL. CONTROL AND DISTRIBUTION.

TestQual **204** scheme is a proficiency test based in the analysis of Pesticides multiresidues, Fosetyl, Phosphonic acid, Glyphosate, AMPA in Avocado that has been spiked or were present in the matrix.

The material will be bought in a specialized shop in Spain and verified as adequate to prepare the samples by a subcontracted laboratory that holds the standard UNE-EN ISO/IEC 17025 into force.

Blank items will be reserved to be acquired along the proficiency test, the blank item can be shipped along the PT sample, saving the shipping costs. The blank items will be available at a reduced cost for the participants of this PT. Blank samples are guaranteed to have a concentration <FP/FN (concentration limit to determine False Negatives and/or False positives) for the analytes of your interest (according to the LOQ you submitted and intended analytes to be analysed).

BLANK ITEMS, if available (contact us to check), WILL BELONG TO A DIFFERENT LOT THAN THE USED TO PREPARE THE PROFICIENCY SAMPLES.

The subcontracted laboratory will analyse randomly selected sample to ensure the homogeneity and during and after the proficiency test more samples to check the stability of the parameters through the duration of the round.

Once the matrix is deemed adequate the material is cut in very small pieces or triturated, which might be assisted by dropping the matrix into liquid N₂, once it is fully frozen, it is triturated. The final texture will either be a fine powder (sieved to have below 6mm diameter), as puree or juice, always completely made of the matrix. This will be spiked with a solution with the analytes of the proficiency test and poured into a homogenizer with controlled temperature to ensure complete homogeneity.

Once the lot of samples is ready they will be stored in a temperature-controlled freezer below -20°C until the dispatch of the samples.

As previously mentioned, before the samples are distributed, the homogeneity of the lot will be assessed analysing in repeatability conditions randomly selected samples in duplicate. If the mean concentration obtained from this study is not within the planned range, the participants will be informed, and a new distribution day might be scheduled if another spiking is deemed as necessary.

For stability assessment purpose, a total of three samples will be analysed, in duplicate, before, during and at the end (once all laboratories have sent the results) of the proficiency test.

The quality controls subcontracted by TestQual, including verification of adequacy of the matrix, homogeneity/stability quality controls and any other analytical study required by TestQual will be subcontracted to an accredited laboratory in ISO/IEC 17025 into force.

In the evaluation report will be included the conclusions and if applies, any comment regarding homogeneity &/or stability. Additionally, the results of these tests and the spiking evaluation is available to all participants of this proficiency test upon request.

The shipment of the test materials will take place on the date shown in the calendar*, to the address provided by each laboratory in the application. The participant is responsible to communicating TestQual if the address is changed or update the delivery address in their client area at least 2 working days before the shipping.

*Specific delivery dates can be changed from the scheduled dates of the calendar, but all changes will be announced either in the website or by email to the registered laboratories. **Before shipping the samples TestQual will email the registered laboratories the sample instructions and will confirm the definitive distribution date**, deadline to submit results and date of the report, if necessary, the dates shown in the sample instructions override the calendar here shown and the new dates will be updated in the PT page.

This PT will consist in a single round in which will be sent a sample of approximately **150 g** of test material. The samples will be sent by courier service (MRW, FedEx, DHL or TNT, depending on the destination). The material will be sent with materials and package that ensures the samples arrives

correctly. The transit will be 1, 2 or 3 days to the destination country, depending on the location of the receiving laboratory.

The shipping conditions for this PT are:

FROZEN, isothermal boxes will be used and filled with either dry ice or cold packs (-21°C) to keep the temperature.

The shipping costs are not included in the price displayed on the website, which can only be seen if you are registered and logged in. To get an approximation of the shipping costs you can get your quotation by using the contact data at the end of this protocol.

The distribution of samples will be subcontracted to a courier previously homologated by TestQual. The main criteria being the courier's delivery time to ensure the receipt of the sample is correct in the participant's facilities.

In addition to this, TestQual established other characteristics important for a courier like shipping management (tracking, notifications, exceptions), and ensuring the delivery conditions are proper (low breakage/lost ratio, keeping of cold chain, required documentation), always checking and evaluating they are complying with TestQual's requirements.

A second test material can be requested if necessary. If the package and/or the sample arrived damaged, defective, or not valid the participating laboratory will have to notify of this to the coordinator before two working days to get another sample.

Before the shipment, TestQual will send the instructions for storage and analysis via email and confirm the distribution date. You can request a paper copy to be attached to the package and/or TestQual might decide to include it in addition to have it sent by email.

From TestQual we encourage our participants to read the samples instructions carefully and follow them, as it can help to correctly conserve the sample and increase the reproducibility of the analysis. You can request a digital copy of this document (sample instructions) by letting us know through any communication channel.

6. CONCENTRATION RANGES, SIGMA OBJECTIVE AND ANALYTES

For this proficiency tests the range of concentration for the target analytes might be between **10** and **400 µg/Kg approximately**. This range is not absolute and depending on the various factors some or no analyte might be within this range. This range is given to provide participants a sense of the concentration ranges we usually work and expect in these kind of proficiency tests (combination of analyte/matrix).

The maximum concentration can vary depending on the MRL of the analytes in the matrix.

The **sigma objective ($\hat{\sigma}$)** which works in this scheme can be checked on section 9 of this protocol.

In this proficiency test, for each group of analytes, a percentage of analytes will be present (being at least >1% (the % is determined internally in our procedure) to possibly all of the analytes of the

list). Therefore, this PT should be useful to check each of the groups, possibly in different degrees, depending on the number of selected/present analytes and those that your laboratory analyze.. In the following table you can check the groups of analytes for this proficiency test, below you can find the lists of possible positives and how the website and the results are expected to be sent:

Analyte-group code	Grouped analytes
A	Pesticides multiresidues
B	Fosetyl, Phosphonic acid & Fosetyl-Al (sum)
C	Glyphosate
D	AMPA

The list of **possible multiresidues pesticides** in the matrix are from the list below:

Abamectin	Acetamiprid	Acrinathrin	Aldrin
Atrazine	Azinphos-ethyl	Azoxystrobin	Bifenthrin
Boscalide	Bromopropylate	Carbendazin	Cymoxanil
Cyproconazole	Cyprodinil	Chlorfenvinphos	Chlorpyrifos
Chlorpyrifos-methyl	Chlorpropham	Deltamethrin	Desmethrin
Diazinon	Dieldrin	Dimetomorph	EMA B1a (Avermectin B1a)
EMA B1b (Avermectin B1b)	Endosulfan-alpha	Endosulfan-beta	Endosulfan-sulphate
Endrin	Epoxiconazole	Famoxadone	Phenazaquin
Fenbuconazole	Fenpropimorph	Fention	Fenvalerate
Fluquinconazole	Flusilazole	HCH-Beta	HCH-Delta
HCH-Gamma (Lindane)	Hexachlorobenzene	Hexythiazox	Imazalil
Imidacloprid	Indoxacarb	Linuron	Metazachlor
Methidathion	Myclobutanil	Nitrofen	Oxydemeton-methyl
Oxifluorfen	Parathion-ethyl	Parathion-methyl	Pendimethalin
Pyrazophos	Pirimiphos-methyl	Piriproxyfen	Profenofos
Propargite	Quintozene	Tebuconazole	Tebufenocide
Tecnacene	Terbutilacina	Terbutryn	Thiacloprid
Triazophos			

In addition to the previous, the following analytes will also be available to be quantified and evaluated:

Each participant shall report his results as the European legislation 2021/1807 (...) amending Annex III from No 396/2005 (...) levels for fosetyl (...):

- “Fosetyl_AI (Sum)” will be evaluated as “Fosetyl-Al (sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl)”.
- “Phosphonic Acid” as “Phosphonic acid and their salts”.
- “Fosetyl” as only “Fosetyl” ($C_2H_6O_3P^+$ MW= 109.04)

The results for Glyphosate will have to be reported as EU applicable legislation No 293/2013 of 20 March 2013 amending Annexes II and III to Regulation (EC) No 396/2005 (..), Glyphosate (..)

- “Glyphosate” as “Glyphosate”

The list of possible glyphosate’s metabolites and related compounds in the matrix will be:

Aminomethylphosphonic acid (AMPA)

7. RESULTS EXPRESSION

Each participant laboratory must analyse the sample received according to their routine procedure and fill up the RESULTS form of its client are of the website www.TestQual.com with just one value per analyte/parameter.

The results should be expressed in $\mu g/Kg$. The number of significant figures and the units are to be chosen by laboratories and will be displayed in the report as received by the participant.

The method used for the analysis of each compound informed should be sent when filling up the results form.

The organizer should get the results before the fixed data of the scheme.

If you have any problem logging in to your client area or submitting your results you can contact the coordinator of the PT for guidance or help.

Once the results are sent you can check if they were correctly recorded by accessing the detailed information of this proficiency test, which can be accessed in your client area. You are allowed to modify results within the stipulated period range, until the deadline to submit results, included. Currently our website, once the results are sent, cannot be changed, therefore, for any modification you will be required to open an issue in your client area for this PT and send the required modifications through that communication channel.

8. STATISTICAL EVALUATION

TestQual will develop the following statistical evaluation:

TestQual considers as an **extreme outlier** any data which differs more than **50 %** of the average of all results reported by the laboratories, according to the Harmonize Protocol of the IUPAC. These extreme values will not be included in the calculation of the assigned value.

Once received all the results, TestQual evaluates the unimodality of all the valid values with a test using the kernel’s density representation, being explained in the final report which is the followed procedure in case there is more than one distribution.

If enough valid results (determined in our internal procedure) are provided and the uncertainty is negligible the **assigned value (X)** will be determined using the robust average of the results considered valid for statistical computing (after eliminating the extreme outliers), according to the standard ISO 13528 into force.

In case of having a lower number of results than expected, the participants would be evaluated against an independent value from their own results, since the lower the number of results is used to obtain the assigned value, the higher is the chance of not identify necessary action signals in case the participants have/had an unknown bias. For this reason, both the assigned value obtained from robust statistic and the characterization value are compared between them, this way we check that there are not sustainable differences and depending on the number of results available and the uncertainty of the assigned value, TestQual will chose according to our internal procedure how the assigned value will be determined and evaluate participants, accordingly, showing it in the report.

If the consensus value is not used, then the characterization of the spiking and the uncertainty will be calculated based in a suitable model and according to ISO 13528 into force.

The evaluation against the characterization is not accredited, analytes evaluated with this assigned value will not be accredited.

Likewise, and as mentioned, the characterization value will be compared to the participants' consensus and only used if there are not sustainable differences between assigned values. If the assigned values are too different, no evaluation or informative evaluation would be issued, explaining the reason in the report.

The **standard uncertainty (u_x)** when the assigned value is obtained from the consensus it is calculated using robust statistics from the following formula:

The **standard uncertainty (u_x)** is calculated using robust statistics from the following formula:

$$u_x = s^*/\sqrt{p}$$

Being s^* the robust standard deviation of the data and p the number of results considered.

When the assigned value is obtained from the spiking done, the uncertainty is calculated accounting all sources of uncertainty according to our internal procedure and according to ISO 13528 into force.

The following condition must be fulfilled in order to discard the contribution of the uncertainty:

$$u_x \leq 0,3 \hat{\sigma}$$

The **standard deviation for proficiency assessment**, also named **target standard deviation, ($\hat{\sigma}$)**, comes from this formula:

$$\hat{\sigma} = b_i \cdot X$$

Being $b_i = \%_{trSD} / 100$, and $\%_{trSD}$ is the target relative standard deviation.

In this case, the assigned relative standard deviation is **25 %**.

This value is fixed previously by the organizer based in the experience of TestQual.

Proficiency assessment (z-score): This parameter shows the competence and accuracy of the laboratory. It is calculated using the following formula:

$$z = (x_i - X) / \hat{\sigma}$$

Where x_i is the value reported by the laboratories, X is the assigned value, and $\hat{\sigma}$ is the target standard deviation for each analyte.

The criteria for defining the z-score values are:

	$ z $	≤ 2	Satisfactory
$2 <$	$ z $	≤ 3	Questionable
	$ z $	> 3	Unsatisfactory

In case the inequation $u_x \leq 0,3 \hat{\sigma}$ is not fulfilled, the participants of the scheme will be informed in the report that the uncertainty is not negligible. For the parameters/analytes in which this situation occurs, the following calculation will be made:

$$z'\text{-score} = (x_i - X) / \sqrt{\hat{\sigma}^2 + U_x^2}$$

Where x_i is the value reported by the laboratories, X is the assigned value, $\hat{\sigma}$ is the target standard deviation for each analyte and U_x is the uncertainty of the assigned value.

The criteria for defining the z'-score values are:

	$ z' $	≤ 2	Satisfactory
$2 <$	$ z' $	≤ 3	Questionable
	$ z' $	> 3	Unsatisfactory

The z'-score is a underestimation of the z-score, for this reason, for those analytes in which the uncertainty of the assigned value cannot be neglected and a z'-score is issued, it will be accompanied by the percentual difference against z-score, this way participants should be able to complete evaluate their performance.

The evaluation could be informative if the difference between scores surpasses the limit contemplated in our procedure. If any analyte or evaluation is informative it will be indicated in the report through marking and a legend.

Both z-score and z'-score will be issued accredited if the criteria of our technical annex are met.

False negatives: Any analyte not reported in the results that is in the sample above the limit of quantification previously established for this proficiency test by the organization and above the LOQ of the participant laboratory (**10 µg/Kg**). TestQual assigns to all false negatives a result equal to half the laboratory limit of quantitation (LOQ/2). If no LOQ is available, the result evaluated will be 0. If <LOQ was reported, and the assigned value is above the LOQ a false negative will be evaluated.

False positives: Those analytes reported in the results, which is not present in the test material, and is reported by the participant at concentrations higher than the limit of quantification of the P.T. (**10 µg/Kg**).

Testing for sufficient homogeneity:

Ten samples will be chosen at random and sent to be analysed by TestQual's subcontracted laboratory in duplicate in repeatability conditions.

Once received the results, a statistical evaluation will be performed, with the homogeneity evaluation of the Harmonic Protocol published by IUPAC and based in ISO 13528 into force.

The acceptance criterion to ensure that the randomly chosen samples are homogeneous is that the square of the estimated sampling standard deviation is below the critical value for accepting proper homogeneity:

$$S_{sam}^2 < c$$

Being S_{sam}^2 the estimated sampling standard deviation, obtained from the variance of the results sums and the experimental estimate of analytical standard deviation (S_{an}), which in turn is obtained from the differences between replicates of the same sample. Lastly, c is the limit value, which is obtained as ISO 13528 states, it takes into account two constants, obtained from two significance groups, which are multiplied with a term related to the target standard deviation and the S_{an} .

If $S_{sam}^2 < c$ is true, then the lot prepared will be considered sufficiently homogeneous and only then it would be distributed. With this test what we achieve is to check if the intra-sample deviation is lower than the inter-sample deviation.

If the results from the homogeneity test does not meet the criteria TestQual would communicate any change required in the proficiency test (new lot will be prepared, new calendar, etc.).

Testing for sufficient stability:

Three samples will be analysed, in duplicate, before, during and at the end (once all laboratories have sent the results) of the proficiency test.

The acceptance criteria to ensure the samples have been stable during the whole proficiency test are the following:

$$\begin{aligned} |(X_{t1} - X_{t2}) / X_{t1}| \cdot 100 &\leq 10\% \\ |(X_{t1} - X_{t3}) / X_{t1}| \cdot 100 &\leq 10\% \end{aligned}$$

Being $|(X_{t1} - X_{tn}) / X_{t1}|$ the difference between the average of the samples analysed before, during and at the end of the proficiency test.

If this criterion is not met, TestQual will review if the instability can be neglected using statistical, data driven and/or technical approaches.

9. EVALUATION REPORT/S

Upon completion of the analysis period TestQual will conduct a comprehensive statistical evaluation of all participant laboratories' results. Subsequently, TestQual will either issue an individual report, summarizing each participant's performance, or a global report **according to the published calendar**. Regardless of the type of report, the reports will be sent via email in PDF format or made available for download from the client area on www.TestQual.com.

Either if a global report is issued or an individual report is issued, participants are given a 30-day period to review and, if necessary, lodge an appeal against the evaluation of the proficiency program or report any identified mistakes **from TestQual side**. Following this appeal window, TestQual will proceed to release the final global report within the **upcoming 30** days, with the complete performance of all laboratories **and appellations accepted (detailed in report if accepted)**.

This allows participants to communicate concerns or disputes related to results, evaluation received, or any mistakes identified **from TestQual side**. Reports can be appealed for mistakes found even outside the formal appellation period, ensuring a mechanism for ongoing quality assurance.

Participants have the option of requesting analyte-group reports, specific to the groups of analytes given in the proficiency test. It's important to note that these individual reports are not accredited and serve as a summary, cross-referencing the global report to ensure comprehensive information is given. All participants that send results will receive at least the global report.

This structured process ensures fast reporting, transparency, accountability, and the provision of comprehensive reports tailored to the specific needs and interests of each participant in TestQual's proficiency testing program.

10. CONTACT

TestQual puts at your disposal any of the following means to contact our team:

Website:	<u>Contact</u>
Email:	<u>testqual@testqual.com</u>
Office phone:	+34 868 94 94 86
Mobile phone:	+34 676 367 555

11. BIBLIOGRAPHY AND REFERENCES OF INTEREST

TestQual Proficiency Testing Schemes are based on the following standards:

UNE-EN ISO/IEC 17043, into force. Conformity assessment- General requirements for proficiency testing.

ISO13528 into force. Statistical methods for use in proficiency testing by interlaboratory comparison.

THE INTERNATIONAL HARMONIZED PROTOCOL FOR THE PROFICIENCY TESTING OF ANALYTICAL CHEMISTRY LABORATORIES, into force.

EU Pesticides database (v.2.2) Search Pesticides residues

SANTE/11312/2021 v2 (Implemented by 01/01/2024) Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed.

Commission Regulation (EU) 2021/1807 of 13 October 2021 amending Annexes II, III and IV to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for acibenzolar-S-methyl, aqueous extract from the germinated seeds of sweet Lupinus albus, azoxystrobin, clopyralid, cyflufenamid, fludioxonil, fluopyram, **FOSETYL**, metazachlor, oxathiapiprolin, tebufenozide and thiabendazole in or on certain products

OJ L 16, 23.1.2016, p. 8–20: ES // EN

Commission Regulation (EU) No 293/2013 of 20 March 2013 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for emamectin benzoate, etofenprox, etoxazole, flutriafol, **GLYPHOSATE**, phosmet, pyraclostrobin, spinosad and spirotetramat in or on certain products.

DO L 96 de 5.4.2013, p. 1/30: ES // EN



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