Asia Pacific Metrology Program (APMP) -
Asia Pacific Laboratory Accreditation Cooperation (APLAC)
Joint Proficiency Testing Programme
<APLAC PT T094>

Pesticide Residues in Cabbage

Jointly coordinated by:
Korea Research Institute of Standards and Science (KRISS)

&

Korea Laboratory Accreditation Scheme (KOLAS)

July 2014
1. Introduction

Kimchi cabbage, a type of cabbage and a main ingredient of kimchi, a fermented Asian dish, is widely cultivated and the most popular Brassica vegetable in the Asia-Pacific region. It is a good source of nutrients but it is prone to be contaminated with pesticides. Many countries set food safety regulations limiting the amount of pesticide residues in agricultural products. With increasing international trade of food and agricultural products, traceable measurements of pesticide residues in agricultural products have become one of the essential requirements for ensuring food safety.

With the aim of enhancing the quality and traceability of measurements in various economies of the Asia-Pacific region through a better regional scientific infrastructure, the Asia-Pacific Metrology Programme (APMP) and the Asia Pacific Laboratory Accreditation Cooperation (APLAC) agreed to strengthen bilateral cooperation and established the APMP-APLAC Joint Proficiency Testing Working Group (PTWG) in November 2013. As an initiative of the joint proficiency testing (PT) programme Korea Research Institute of Standards and Sciences (KRISS), a member of APMP, proposed a PT scheme jointly with Korea Laboratory Accreditation Scheme (KOLAS), a member of APLAC, on the determination of pesticide residues ($p,p'$-DDE and $\alpha$-endosulfan) in freeze-dried kimchi cabbage. The purpose of this study is to demonstrate the capability of participating laboratories in measuring pesticide residues in a test sample of dried kimchi cabbage. A PT round for National Agricultural Products Quality Management Service (NAQS) in Korea will be conducted in parallel with the APMP-APLAC joint PT.

Reference values provided by KRISS for $p,p'$-DDE and $\alpha$-endosulfan in the test sample will be used as the assigned values for evaluating measurement results of participants. The relevant Calibration and Measurement Capabilities (CMCs) of KRISS are registered in the Key Comparison Data Base (KCDB) of the Comité International des Poids et Mesures (CIPM, International Committee for Weights and Measures) Mutual Recognition Arrangement (MRA). The use of reference values traceable to the International System of Units (SI, Système international d’unités) provided by NMIs with appropriate CMCs as PT reference values at this and forthcoming APMP-APLAC Joint PTs will allow the rigorous evaluation of the accuracy of participants’ results. It will enhance the quality of the PT programme and also help build the measurement capabilities of the participants through a better regional linkage between the NMIs/DIs and the analytical laboratories in the Asia-Pacific region.
2. Objectives

The aim of this study is to demonstrate the capability of participating laboratories in measuring the amount of \( p,p'-\text{DDE} \) and \( \alpha\text{-endosulfan} \) at mg/kg levels in the test sample of kimchi cabbage by various analytical techniques.

3. Organisers of the joint PT Programme

KRISS(Address: 267Gajeong-Ro, Yuseong-Gu, Daejeon 305-340, Republic of Korea) is the National Metrology Institute(NMI) of Korea. KRISS takes responsibility for all tasks in the development and operation of the proficiency testing programme, including preparation and distribution of proficiency test samples, data analysis and evaluation of results, preparation of interim and final reports, and communications with participants.

KOLAS(Address: 93, Isu-ro, Maengdong-myeon, Eumseong-gun, Chungcheongbuk-do, 369-811, Republic of Korea) is the governmental accreditation body of Korea. KOLAS is responsible for proposing the proficiency testing programme, inviting participants, circulating the interim report, the draft final report and the final report to participants and acting as a contact point between participating accreditation bodies/participants and KRISS.

4. Fee for participation

Free of charge.

5. Call for participation

APLAC members as well as non-APLAC members will be invited to participate in the programme. Invitations will be sent to all APLAC members and other accreditation bodies. Participating accreditation bodies will be asked to nominate laboratories to participate and indicate the accreditation status of the nominated laboratories for the test. The number of laboratories shall be limited to 100 due to the limitation of the test sample prepared in KRISS(participants for the parallel NAQS PT round will not be included in this number). APLAC members are invited to nominate up to a maximum of 3 laboratories and non-APLAC members are invited to nominate up to a maximum of 2 laboratories. When enrolment exceeds the limit, the number of participating laboratories from each accreditation body will be limited upon discussion with APLAC.

6. Test sample
Approximately 240kg of kimchi cabbage cultivated in greenhouses was purchased from the local farm in Korea. The outer leaves of cabbage were peeled off and the cabbage top was removed, and then it was sliced. Sliced cabbage was rinsed 4 times with tap water to remove dirt and foreign particles. Thoroughly washed cabbages were soaked in distilled water and sieved to remove excess water. The washed cabbages were frozen for 2 hours and then dried for 90 hours using a freeze dryer (freeze drying was carried out by Dongrim food, Ltd.). The weight loss due to freeze-drying was about 95%. Freeze-dried kimchi cabbages were ground using a laboratory mill (Pulvurisette 14; Fritsch, Idar-Oberstein, Germany) with a 0.5 mm sieve ring. The speed of rotor was 12000rpm. Dried cabbage powder was sieved using a vibrating sifter (V/Sifter-141, Daega, Korea) to collect powder with limited range of particle size, 50μm~250μm. An aqueous solution containing several pesticides as well as \( p,p' \)-DDE and \( \alpha \)-endosulfan was prepared. An appropriate amount of the aqueous solution was added into the prepared kimchi cabbage powder to make it into a paste form. This kimchi cabbage paste was move to mixing bowl and mixing for about 4 hours. After mixing, this paste was re-freeze-dried in freeze-dryer (PVRFD 100R, Ilshin Lab, Korea) and re-sieved. It was further homogenized with V-blender (Daega Powder, Korea) for over 10 hours and then bottled into pre-cleaned 60mL amber bottles in 15 g per unit. The sample bottles were purged with argon gas and sealed. Sample bottles were stored at -70 ℃ prior to distribution or use.

The homogeneity study of the proficiency test sample was carried out using Isotope dilution Gas Chromatography Mass Spectrometry (ID-GC/MS) analysis after sample preparation. At least ten samples will be taken with even interval following the bottling order and one subsample from each bottle will be analysed with minimum sampling size of 1.0g. Homogeneity test for \( p,p' \)-DDE and \( \alpha \)-endosulfan was already finished. The relative standard deviations of \( p,p' \)-DDE and \( \alpha \)-endosulfan among bottles were 0.5 % and 0.4 %, respectively, which are satisfactory for the present proficiency testing.

Based on stability tests of several batches of Chinese cabbage CRMs and PT materials produced by KRISS, \( p,p' \)-DDE and \( \alpha \)-endosulfan were stable for more than three years at − 70 ℃ and more than 6 months at − 20 ℃ (storage condition recommended in the instruction for participants). The stability of \( p,p' \)-DDE and \( \alpha \)-endosulfan in the PT material will be tested after the due date of reporting results for participants.

### 7. Reporting and submission of results

Participants should complete the Result Report (Annex C). The guideline of reporting results is as followings:

- For each analyte, the mean value of at least three independent measurements and its associated expanded uncertainty with 95% level of confidence should be reported on
a dry mass basis;

- Report the mass fractions of analytes in mg/kg for p,p'-DDE and α-endosulfan; and
- Participants should provide information about the methods of analysis.

Participants should be aware that any submitted results are considered final and accordingly such results and units should be thoroughly checked before submission. Participants should submit the Result Report electronically to the coordinator of the proficiency testing programme (E-mail: aplacpt.org@kriss.re.kr) before the deadline. Results submitted after the deadline will not be accepted. Participants are reminded that the ability to report results in the specified unit and within the given time scale are part of the proficiency test. Under no circumstances, correction or adjustment of analytical data will be accepted after the issue of the interim report.

8. Measurement uncertainty

Measurement uncertainty is best estimated within the individual laboratory environment. An estimate of measurement uncertainty is normally based on the combination of a number of influencing parameters (components of uncertainty). As stipulated in ISO Guide to the Expression of Uncertainty in Measurement [1], the uncertainty of each individual parameter should be quantified and expressed numerically as a standard uncertainty. These values are then combined according to the rules of the propagation of uncertainty and the combined standard uncertainty is multiplied by a coverage factor to produce an expanded uncertainty at the 95% level of confidence.

9. Evaluation of performance of participants

Performance of the participating laboratories will be assessed using z-score, which is calculated as follows:

\[ Z = \frac{x_i - x}{\sigma} \]

where
- \( x_i \) = the reported result of individual participant
- \( x \) = the assigned value*
- \( \sigma \) = the standard deviation for proficiency assessment estimated from the Horwitz Equation

*Note: The certified reference values determined by KRISS will be used as the assigned values. This is in accordance with the ISO/IEC 17043 recommendations on the determination of assigned values for proficiency testing schemes.
z-Score is commonly interpreted as:

(i) \(|z| \leq 2\) Satisfactory
(ii) \(2 < |z| < 3\) Questionable
(iii) \(|z| \geq 3\) Unsatisfactory

Laboratories having a \(|z|\) score equal to or larger than 3 shall thoroughly investigate their results for the discrepancy and those having a \(z\)-score in the range \(2 < |z| < 3\) are also encouraged to review their results.

10. Issue of reports

An interim report will be issued to participants and their respective accreditation bodies for checking the correctness of results submitted. The draft final report will be then prepared and submitted to APMP-APLAC PTWG for comments and approval. Upon approval, an electronic copy of the final report will be distributed to the participants and their respective accreditation bodies.

11. Proposed program schedule

The proposed time schedule for the various phases of the proficiency testing programme is as follows:

<table>
<thead>
<tr>
<th>Time schedule</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 14, 2014</td>
<td>Call for participation</td>
</tr>
<tr>
<td>July 24, 2014</td>
<td>Deadline for registration</td>
</tr>
<tr>
<td>July 31, 2014</td>
<td>Distribution of samples</td>
</tr>
<tr>
<td>September 5, 2014</td>
<td>Deadline for submission of results</td>
</tr>
<tr>
<td>November 14, 2014</td>
<td>Issue of the interim report</td>
</tr>
<tr>
<td>February 28, 2015</td>
<td>Issue of the final report</td>
</tr>
</tbody>
</table>
12. Confidentiality

The concerned parties (APMP, APLAC, KOLAS and KRISS) strive to maintain strict confidentiality of the characteristic properties of the proficiency test sample distributed and the performance of all participating laboratories. To preserve the confidentiality, participants receive reports giving all results for assessment but without identifying individual laboratories. The code number assigned to a participant in the proficiency testing programme will be provided only to the contact person/authorized person of the participating laboratory and/or the respective accreditation body.

13. Contact

For more information on the proficiency testing programme, please contact the coordinator by e-mail (E-mail: aplacpt.org@kriss.re.kr).

If you have any query or comment, please send it to the following contact points:
   Dr. Byungjoo Kim or Dr. Seonghee Ahn, aplacpt.org@kriss.re.kr
   Mr. Kyunghee Lee, kolas1@korea.kr

14. References

Instructions for Participants

1. Analysis of the proficiency test sample

(1) Sample information

• Participating laboratories will be provided with ONE bottle containing about 15g of dried Kimchi cabbage powder. The date of dispatching of sample will be informed to participants. Participants are required to confirm the receipt of the samples (including the serial number of the sample, date of receipt, any damages found in the sample package) by e-mail (aplacpt.org@kriss.re.kr) using the sample receipt form attached in annex B. If any damage on the sample is observed on arrival, please contact us immediately.

• Analytes and their approximate mass fractions are given as follows:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mass fraction (expected range of values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p,p'-DDE</td>
<td>0.5 –10mg/kg</td>
</tr>
<tr>
<td>α-endosulfan</td>
<td>0.5 –10mg/kg</td>
</tr>
</tbody>
</table>

• Homogeneity: ID-GC/MS analysis of one subsample taken from each of 10 bottles was carried out. The relative standard deviations of p,p'-DDE and α-endosulfan among bottles were 0.5 % and 0.4 %, respectively, which are satisfactory for the present proficiency testing.

• Several other pesticides can be detected in the test sample, but please report only the mass fractions of the two target measureands (p,p'-DDE and α-endosulfan).

(2) Sample storage

• The proficiency test sample should be kept sealed in its original bottle and stored at -20 °C or lower temperature.

• According to our stability test, storing the sample at room temperature for a week did not change the level of the target analytes. However, storing at room temperature for a long term period should be avoided.

• Opening of the sample bottle should be carefully planned to avoid contamination and deterioration of the sample.
For safety considerations, the proficiency test sample should be handled with care to prevent inhaling the sample powder and getting into eyes. In the case of accidental exposure to sample, wash the exposed areas with plenty of water and consult physicians when necessary.

For this proficiency testing programme, it is not required to return remaining sample.

(3) Guidelines for sample preparation and dry-mass correction

Sample preparation:
- Sample handling in a laboratory with well-controlled humidity and temperature is recommended and any kinds of contamination should be avoided.
- Sample bottle should be equilibrated to ambient temperature prior to opening the cap (by placing the sample bottle to the weighing room at least overnight before sampling).
- Sample in the bottle should be mixed thoroughly before taking a subsample from a bottle.
- It is recommended that the minimum subsampling size for a single analysis is 1.0g (Homogeneity test was carried out with 1.0g of sampling).
- Sample taken for analysis should be reconstituted with water before further sample preparation (equivalent to 10mL of water for 1g of dried kimchi cabbage powder).
- Participants are recommended to perform at least triplicate measurements and report the mean and associated measurement uncertainty. A single result with its associated measurement uncertainty for each analyte shall be reported as specified in the Result Report. Participants should estimate the measurement uncertainty using their own practice.

Dry-mass correction:
- Participants should also carry out the dry mass correction.
- Sampling for dry mass correction should be carried out at the same time with sampling for analysis.
- At least three separate portions(with a recommended sample size of about 0.5g for each portion) of the sample should be taken and placed over P₂O₅ in a desiccator at room temperature for 7 days (168 hours).
- Calculate the moisture content from the mass change observed in the three aliquots and use it for dry-mass correction.
(4) Measurement method

- Participants are expected to use the test method of their choice, which should be consistent with their routine procedures. If the laboratory is accredited, it is recommended to use the method listed in the scope of its accreditation.

2. Reporting and submission of results

Participants should complete the Result Report (Annex C). The manners of reporting test results are as followings:

- For each analyte, the mean value of at least three independent measurements and its associated expanded uncertainty with 95% level of confidence should be reported on a dry mass basis;
- Report the mass fractions of analytes in mg/kg for \( p,p' \)-DDE and \( \alpha \)-endosulfan; and
- Participants should provide information about the methods of analysis.

Participants should be aware that any submitted results are considered final and accordingly such results and units should be thoroughly checked before submission. Participants should submit the Result Report electronically to the coordinator of the proficiency testing programme (E-mail: aplacpt.org@kriss.re.kr) before the deadline. Results submitted after the deadline will not be accepted. Participants are reminded that the ability to report results in the specified unit and within the given time scale are part of the proficiency test.

The proficiency testing programme is conducted in the belief that participants will perform the analysis and report results with scientific rigour. Collusion and falsification of results are clearly against the spirit of the proficiency testing programme.

3. Contact

Participants may wish to contact the coordinator of the proficiency testing programme for any enquires, (E-mail: aplacpt.org@kriss.re.kr).

Dr. Seonghee Ahn
Principle Research Scientist
Center for Organic Analysis
Division of Metrology for Quality of Life
Korea Research Institute of Standards and Science (KRISS)
267 Gajeong-Ro, Yuseong-Gu, Daejeon 305-340, Republic of Korea
Tel: +82-42-868-5652
Fax: +82-42-868-5800
Institute/Laboratory: _____________________________________________________________________
Postal address: _______________________________________________________________________
Contact person: _______________________________________________________________________  
Title   Given name   Surname
E-mail: _______________________________________________________________________________
Print name / Signature: ___________________________________________________________________
Date of receipt: _________________________________________________________________________

Confirmation of Package Content

Please choose the state of the sample:  □ intact  □ broken  □ others: _______________________

Please write the serial numbers of the sample bottle: _________________________

Please complete this form and return it to Dr. Seonghee Ahn, KRISS(E-mail: aplacpt.org@kriss.re.kr) by email after receipt of the sample.
Annex C

Result Report

Institute/Laboratory: ____________________________________________________________

Postal address: ______________________________________________________________

Contact person: ______________________________________________________________

Title | Given name | Surname
---- | ---------- | -----

E-mail: _________________________________________________________________

Laboratory accreditation: YES (based on ISO 17025/ ISO 9000 series/ GLP/ under the law of your economy(please specify))/NO

If yes, please specify the name of accreditation body. ________________________

1. Analytical results

1) Mass fractions of \( p,p' \)-DDE and \( \alpha \)-endosulfan in Kimchi cabbage

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Analytical result (mg/kg)</th>
<th>Combined standard uncertainty (mg/kg)</th>
<th>Coverage factor ( k ) (95% level of confidence)</th>
<th>Expanded uncertainty (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p,p' )-DDE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \alpha )-endosulfan</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Notes: (i) Report the analytical results and associated uncertainties in the unit mg/kg; (ii) Report the analytical results on a dry mass basis.
2) Results from individual aliquots (dry-mass corrected)

<table>
<thead>
<tr>
<th>Aliquot number</th>
<th>$p,p'$-DDE (mg/kg)</th>
<th>α-endosulfan (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation of the mean</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please insert more lines for more determinations.

3) Dry Mass Correction

Moisture contents (weight %): ________________________________
Correction factor: ________________________________

2. Methods of analysis

1) Sample size used for analysis ________ g

2) Extraction method (e.g., Liquid/Liquid extraction, Soxhlet, etc.)
(Please briefly describe the extraction procedures)

3) Solvent used for extraction ________________________________

4) Duration of extraction ________________________________

5) Post extraction clean-up method and the transformation procedure, if any.
6) Analytical instrument(s) used
   ________________________________
   (Specify the model)

7) Chromatographic column used
   ________________________________
   (Specify the dimensions)

8) The chromatographic conditions
   (Please describe GC oven temperature programme, LC Mobile phase gradient, etc)

9) Methods of quantification(s)
   (e.g., single-point, bracketing, three-point calibration curves, etc.)

10) Internal standard (if used)
    (Specify the compounds)
11) Sources of calibration standards (with the purity/concentration and associated uncertainties)

12. Other information, observation or evidences, if any, please describe.

Print name/ Signature of responsible person:

Date(dd/mm/yyyy): / /