A multinational joint project on the evaluation of residual pesticide analysis in tea in the Asia Pacific region

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ABSTRACT

This paper presents the results of a multinational joint proficiency testing (PT) programme on four pesticide residues (cypermethrin, α-endosulfan, β-endosulphan and bifenthrin) in two tea samples. The objectives were to evaluate the performance and to improve the testing capability of field laboratories in the Asia Pacific region for analysis of pesticide residues in tea. A total of 42 laboratories registered for the programme and 32 laboratories from 12 economies returned the results to the organizers. The assigned values of the pesticides were agreed to be the respective weighted mean determined by the three metrology institutes — the National Measurement Institute, Australia (NMIA), the National Institute of Metrology, China (NIM) and the Government Laboratory, Hong Kong (GLHK) in an Asia Pacific Metrology Programme (APMP) pilot study programme (APMP.QM-P15) using the same test materials. The performance of participating laboratories was expressed using the numerical indicators of z-scores and E_n-scores. Amongst the reported data, only 35.3% and 35.0% achieved satisfactory z-scores ($|z| \leq 2$) and E_n-scores ($|E_n| \leq 1$) respectively. Only two laboratories achieved satisfactory z-scores and E_n-scores for all the analytes. The results demonstrated that the performance of the majority of the laboratories participating in this study on the analysis of pesticide residues in tea was not satisfactory, and more capability building is required for further improvement.

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1. Introduction

Tea drinking has a long history and remains a popular social event in many countries around the globe. Tea contains antioxidant-rich natural substances such as catechins, proanthocyanidins and polyphenolic compounds (Luximon-Ramma et al., 2005). The common evergreen shrub, *Camellia sinensis* L. is the essential species used to produce black, green and Oolong tea. Production of these three main tea types is solely depending on fermentation treatment (Wan, Li, & Zhang, 2008). The majority of the tea producing countries are located in Asia where China, India, and Sri Lanka are amongst the key global stakeholders. The volumes of tea exported from Asia are projected to reach more than 800,000 tonnes in 2017 (Hicks, 2009), and the tea industry has made a significant contribution to the economy of some Southeast Asian countries like India, Indonesia and Sri Lanka. Similar to other modern cultivation processes, pesticides have been applied to tea plants for pest control before harvesting to increase production yield. As a result, various residual pesticides are often detected in raw tea and tea products (Chen, Cao, & Liu, 2011;

Huang et al., 2009). Analysis of the presence of residual pesticides in crops and foods is therefore vital for quality control, environmental monitoring, public health protection, legislative enforcement as well as building stronger brand images.

Accurate quantification of pesticide residues, however, is not always easy. There are a large number of possible pesticides and their concentrations in agricultural and food matrices are generally low (Jin, Xie, Guo, & Pang, 2012; Sinha, Rao, & Vasudev, 2012). To achieve a reliable measurement system, implementation of the use of sensitive and calibrated analytical instruments, efficient extraction procedures, well trained staff and other requirements as specified in the ISO/IEC 17025 is necessary. This is always a challenge not only to laboratories in the developing nations but also to well-equipped laboratories in the developed nations. Having observed the problems of pesticide analysis over the past few years in the region, the Developing Economies Committee (DEC) of APMP proposed a PT programme on pesticide residues in tea during a workshop held in Jakarta in 2008. The objectives of the programme were to set up a technical platform for a critical evaluation of the testing capability and, if possible with a view for further improvement, to identify the specific difficulties experienced by the participating field laboratories in the analysis under test. The programme was an international joint effort by

NMIA, NIM, GLHK, Industrial Technology Institute (ITI) of Sri Lanka and Physikalisch-Technische Bundesanstalt (PTB) of Germany. It was operated from 2009 to 2010 with a registration of 42 laboratories from 13 economies. Sample preparation, homogeneity and stability studies were performed by NIM and GLHK. Study protocol preparation, sample dispatch, data analysis and report writing were performed by ITI with the assistance from NMIA. A specific training session on the estimation of uncertainty in chemical measurement was also coordinated by NMIA and GLHK and supported financially by PTB prior to the commencement of the PT programme.

The consensus mean is commonly used as the assigned value by most PT providers due to simple and convenient manipulation. However, the major drawback of using consensus mean values is their possible bias from the 'true' values. Some recent PT programmes on residual pesticides in plant origin indicated that the overall performance of field laboratories was not satisfactory. For example, an interlaboratory comparison for diazinon and chlorpyrifos in Chinese cabbage (Dajes & Kim, 2009) under the framework of the Asia-Pacific Economic Cooperation (APEC) showed that the consensus mean values were significantly deviated by about 36% less than those of the IDMS derived reference values. Another PT example on the determination of organochlorine pesticides in ginseng root (Kong, Chan, Wong, Wong, & Sin, 2007) also showed a variation of about 10 to 54% between the consensus means and the assigned values. Having recognized that an inappropriate assigned value can undermine the validity of performance assessment, the organizers decided to use the assigned values that were derived from three experienced metrology institutes.

2. Materials and methods

2.1. Test materials

The test materials consisting of two tea types were separately prepared by NIM and GLHK in accordance with the ISO Guide 43 Part 1. Sample 1, a Chinese green tea, Mao Jian, contained trace level of incurred cypermethrin and Sample 2, an Oolong tea contained trace levels of incurred α-endosulphan, β-endosulphan and bifenthrin. The samples were purchased from the respective local tea markets and were ground, sieved (100 μm) and bottled prior to homogeneity studies. Participants were requested to report the dry weight mass fraction of the target analytes in ng/g for both samples. Instructions for assessing the moisture content and the subsequent dry mass correction were provided. For Sample 1, a minimum of three portions of tea powder with a recommended size of 1 g each was taken and placed over anhydrous calcium sulfate (e.g. DRIERITE®) in a dessicator at room temperature for a minimum of 10 days until a constant weight was reached. For Sample 2, a portion of minimum weight of 1 g of tea powder was dried in a ventilated oven at a temperature of 70 ± 2 °C for a minimum of 2 h. The sample was repeatedly weighed during drying until a constant weight was reached. The loss of mass corresponded to the moisture content of the samples and was applied in the dry mass calculation. Participants were advised to perform the above procedures at the same time as when the samples were weighed for analysis.

2.2. Homogeneity and stability tests

Homogeneity was assessed for each pesticide in the test materials provided for this PT programme scheme in accordance with the ISO13528:2005. For Sample 1, twelve randomly selected bottles were analyzed using an accredited GC–μECD method. Two portions of about 1 g were taken from each sample and Soxhlet extracted with 50 mL of ethyl acetate for 16 h. The extracts were dried with 5 g of anhydrous sodium sulfate and were centrifuged at 2000 r.p.m. for 10 min at 4 °C. The supernatant obtained was transferred to a 250 mL-flat bottom flask and concentrated to just dryness by a rotary evaporator. The residue was reconstituted with 10 mL of cyclohexane–dichloromethane (1:1) and the mixture was loaded onto a gel permeation chromatographic (GPC) column and eluted with a mixture (1:1) of cyclohexane:dichloromethane. The eluate was dried on a rotary evaporator and reconstituted with 1 mL of 15% petroleum ether prior to further cleanup with 25 mL of 15% petroleum ether using Florisil solid phase extraction (SPE). The eluent was pre-concentrated under nitrogen at room temperature and reconstituted with 1 mL of iso-octane prior to GC analysis. For Sample 2, homogeneity was assessed by duplicate analysis of fifteen random samples using a validated GC–MS method. Portions of 1 g of sub-sample were extracted twice with 20 mL of hexane and acetone (7:3) with shaking for 40 min. The extracts were centrifuged at 3000 r.p.m. for 10 min at 4 °C and the combined supernatants obtained were concentrated to about 2 mL. Cleanup was performed using two different SPE columns (ENVI-carb and alumina-N) that were connected in series. Analytes were eluted with 7 mL of hexane and acetone (9:1), and the eluate was dried under nitrogen and reconstituted with 0.5 mL of iso-octane for GC–MS analysis. The relative standard deviations (RSD) of the analysis were respectively 3.6% for cypermethrin in Sample 1 and 2.5% to 3.8% for α-endosulphan, β-endosulphan and bifenthrin in Sample 2. A one-way ANOVA for the duplicate sets of data showed insignificant within- and between-bottle variations. The results indicated that homogeneity status of the test materials was satisfactory and the samples were suitable to be used as test materials in PT.

The same GC–μECD and GC–MS methods were used in the stability test. Duplicate or triplicate analysis of random samples was performed at regular intervals over a 12-month period at room temperature (20 ± 5 °C) and 37 ± 1 °C for Sample 1; a 12-month and a 40-day period at 4 °C and 40 °C for Sample 2 respectively. The mean concentrations (X_{stab}) of triplicate analysis at different storage intervals were compared with the Horwitz standard deviation (σ_x) of mean values (X_{homo}) obtained from the homogeneity test. The absolute difference of X_{stab} and X_{homo} should be not more than 30% of σ_x in accordance with the ISO13528:2005. Results showed that the analytes in Samples 1 and 2 (Fig. 1) were stable over the study period.

2.3. Performance assessment

Participants were provided with two bottles of 20 g each of Sample 1 and Sample 2. They were requested to determine, at least in duplicate, the dry mass fraction of the target pesticides. Standard (u) and expanded (U) measurement uncertainties, standards used, details of analysis and approach to uncertainty calculations had to be reported. The performance of participants was evaluated using z-scores and E_n-scores according to the following equations:

$$z = (X_i - X_{av}) / \sigma$$

where X_i is the mean value from individual participant, X_{av} is the assigned value deriving from the weighted mean values determined by the three metrology institutes and σ is the target standard deviation,

$$E_n = (X_i - X_{av}) / \sqrt{Ui^2 + Uav^2}$$

where U_i and U_{av} are respectively the expanded uncertainty of individual participants and the assigned value.

2.4. Assigned values determination

The assigned values of cypermethrin, α-endosulphan and β-endosulphan were determined by gas chromatography isotope dilution mass spectrometry (GC–IDMS). When properly employed, IDMS is a highly accurate measurement technique (Vogl & Pritzkow,