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# Development of Green Onion and Cabbage Certified Reference Materials for Quantification of Organophosphorus and Pyrethroid Pesticides

Takamitsu Otake,*[,†] Takashi Yarita,[†] Yoshie Aoyagi,[†] Youko Kuroda,[†] Masahiko Numata,[†] Hitoshi Iwata,[‡] Kazushi Mizukoshi,[‡] Munetomo Nakamura,[‡] Masatoshi Watai,[‡] Hitoshi Mitsuda,[§] Takashi Fujikawa,[§] and Hidekazu Ota[§]

[†]National Metrology Institute of Japan (NMIJ), National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1, Umezono, Tsukuba, Ibaraki 305-8563, Japan

[‡]Japan Food Research Laboratories, 6-11-10, Nagayama, Tama, Tokyo 206-0025, Japan

[§]The General Environmental Technos (KANSO TECHNOS), 3-1-1, Higashikuraji, Katano, Osaka 576-0061, Japan

Supporting Information

**ABSTRACT:** Green onion and cabbage certified reference materials for the analysis of pesticide residues were issued by the National Metrology Institute of Japan, part of the National Institute of Advanced Industrial Science and Technology. Green onion and cabbage samples were grown so as to contain several kinds of organophosphorus and pyrethroid pesticides, and those were collected from a field in the Kochi Prefecture in Japan. The certification was carried out by using multiple analytical methods to ensure the reliability of analytical results; the values of target pesticides (diazinon, fenitrothion, cypermethrin, etofenprox, and permethrin for green onion and chlorpyrifos, fenitrothion, and permethrin for cabbage) were obtained by isotope dilution mass spectrometry. Certified values of target pesticides were 0.96−13.9 and 2.41−6.9 mg/kg for green onion and cabbage, respectively. These are the first green onion and cabbage powder certified reference materials in which organophosphorus and pyrethroid pesticides are determined.

**KEYWORDS:** Quality assurance/quality control, certified reference material, certification, pesticide, green onion, cabbage

## INTRODUCTION

In Japan, a Positive List System for Agricultural Chemical Residues in Foods (PL) was introduced in May, 2006, to prohibit the distribution of foods that contain agricultural chemicals, viz. pesticides, feed additives, and veterinary drugs, above a certain level,[1] and then, maximum residue limits (MRLs) were stipulated. Pesticides are extensively used to protect foods against pests and diseases.[2] However, if pesticides remain in foods more than MRLs, they may be ingested by humans through the food and may cause some adverse effects.[3−5] Thus, it is important to analyze and monitor the pesticides in foods to investigate the relationship between exposure and health risks. For accurate assessment of exposure level and health risks, accurate analytical results are needed.

The analysis of pesticides in food includes complex pretreatments of samples as well as highly selective instrumental analyses; thus, a quality control is required. In ensuring reliability of the analytical results, the validation for method performance of pesticide analysis is essential as written in some reports and studies.[6−8] In validation of an analytical method, matrix certified reference materials (CRMs) are one of the key elements. Testing recovery by spiking surrogates to food sample is necessary and widely used for the evaluation of analytical method in a lot of testing laboratories; furthermore, complementary use of CRMs is also useful because the conditions of analytes in CRM are more similar to those in actual sample.

The National Metrology Institute of Japan (NMIJ) has developed a green onion (NMIJ CRM 7507-a) and a cabbage (7508-a) CRM for the validation of pesticide residue analysis, and the certifications of target pesticides in NMIJ CRM 7507-a and 7508-a are described in this paper. Certifications of NMIJ CRM 7507-a and 7508-a were carried out by isotope dilution mass spectrometry (IDMS), which has a potential as the primary method of measurement.[9−11] To ensure the reliability of certification, certified values were decided from the analytical results obtained by two independent procedures for each pesticide.

## EXPERIMENTAL SECTION

**Preparation of Candidate Reference Materials.** Green onion samples were grown so as to contain the pesticides. Five pesticides, that is, *O*,*O*-diethyl *O*-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate (diazinon), *O*,*O*-dimethyl-*O*-4-nitro-*m*-tolyl phosphorothioate (fenitrothion), (*RS*)-α-cyano-3-phenoxybenzyl (1*RS*,3*RS*)-(1*RS*,3*RS*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (cypermethrin), 2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether (etofenprox), and 3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*RS*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (permethrin) were selected as the candidates for certification since they are registered pesticides available for green onion and widely used in Japan. The pesticides were applied by sprayer to green onion samples twice at 14 and 7 days before harvest for diazinon, fenitrothion, and etofenprox and at 10 and 3 days before

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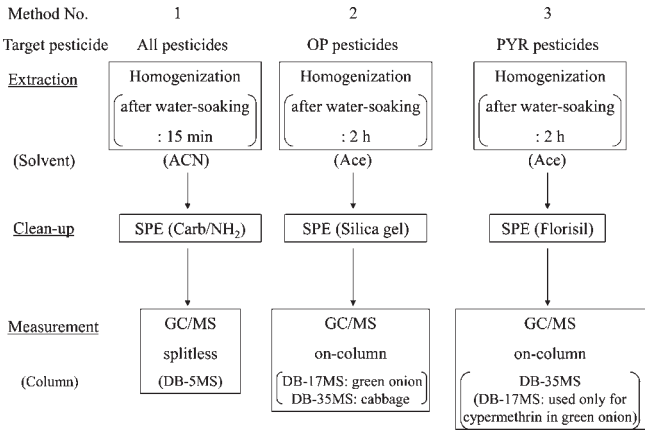


**Figure 1.** Analytical scheme for the certifications of pesticides in green onion (CRM 7507-a) and cabbage (7508-a). The matrix-matched calibration standards were used for quantification. The pesticides were quantified by IDMS. OP pesticides, organophosphorus pesticides; PYR pesticides, pyrethroid pesticides; ACN, acetonitrile; Ace, acetone; SPE, solid phase extraction; and Carb/$NH_2$, graphite carbon/aminopropylsilanized silica gel.

harvest for cypermethrin and permethrin. These green onions were collected from a field of Kochi Prefecture, Japan. The harvested green onions were freeze-pulverized, sieved, homogenized, and then placed into clean glass bottles. The samples were sterilized with $^{60}$Co $\gamma$ radiation (15 kGy) and stored at about −30 °C until analysis.

Cabbage samples were prepared as well as green onions, except for the applied pesticides. Four pesticides [*O*,*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate (chlorpyrifos), fenitrothion, etofenprox, and permethrin] were selected for cabbage, and these were applied by sprayer to samples twice at 10 and 3 days before harvest.

**Analytical Methods Used for Certification.** The analyses were carried out by NMIJ, and the scheme is shown in Figure 1. Acetonitrile, acetone, hexane, ethyl acetate, toluene, diethyl ether, anhydrous sodium sulfate (for pesticide residue and PCB analysis grade), and sodium chloride (reagent grade) were purchased from Kanto Chemical (Tokyo, Japan). Phosphate buffer solution (pH7.0) was prepared from dipotassium hydrogen phosphate (reagent grade; Kanto Chemical), potassium dihydrogen phosphate (reagent grade; Wako Pure Chemical, Osaka, Japan), and purified water (Milli-Q Integral 3; Millipore, Billerica, MA). Purified water (Millipore) was also used for the water-soaking process.

As NMIJ method 1, the analysis of all target pesticides was performed ($n$ = 5). This was based on an annex of the Director Notice about Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food (Syoku-An no. 0124001, 2005. Final amendments were made on 2006.[12]), and we partly modified it. The green onion or cabbage sample (0.4 g) was weighed in a glass vial (110 mL; Maruemu, Osaka, Japan), and the surrogate solution [gravimetrically prepared by dissolving in acetone from isotope-labeled pesticides: chlorpyrifos-$d_{10}$, diazinon-$d_{10}$, fenitrothion-$d_6$, etofenprox-$d_5$ (Hayashi Pure Chemical, Osaka, Japan), cypermethrin-$^{13}C_6$, *cis*-permethrin-$^{13}C_6$, and *trans*-permethrin-$^{13}C_6$ (Cambridge Isotope Laboratories, Andover, MA)] and purified water (10 mL) were added. After 15 min, this sample was homogenized [POLYTRON PT 1200 E (drive unit) with PT-DA 12/2EC-E157 (dispersing aggregates); KINEMATICA, Lucerne, Switzerland] for 2 min in acetonitrile (25 mL) and filtrated with a cellulose filter (diameter, 60 mm; no. 5A, Kiriyama glass, Tokyo, Japan). The residues on filter paper were re-extracted by homogenization (for 2 min) with acetonitrile (10 mL), and pesticide-containing filtrates were combined. This crude extract was shaken with sodium chloride (10 g) and 0.5 mol/L phosphate buffer solution (pH 7.0, 20 mL) in a separatory funnel for 10 min. After dehydration by an anhydrous sodium sulfate, the acetonitrile layer was concentrated and dried by a rotary evaporator and nitrogen gas stream, and then, 2.0 mL of toluene/acetonitrile (1:3, v/v) was added. This was cleaned up by a solid phase extraction (SPE) cartridge [graphite carbon/aminopropylsilanized silica gel layered cartridge (500 mg/500 mg); ENVI-Carb/LC-$NH_2$, Supelco, Division of Sigma-Aldrich, St. Louis, MO; conditioned with 10 mL of toluene/acetonitrile (1:3, v/v)]. Pesticides were eluted with toluene/acetonitrile (1:3, v/v; 20 mL) followed by concentration and drying processes using a rotary evaporator and nitrogen gas stream. Then, the syringe spike solution [gravimetrically prepared by dissolving in acetone from 2-chloro-2′, 6′-diethyl-*N*-(methoxymethyl) acetanilide (alachlor; GL Sciences, Tokyo, Japan); 0.5 mL] was added to this cleaned up extract. This sample was analyzed using a gas chromatograph with a mass spectrometer [GC/MS; an Agilent Technologies (Santa Clara, CA) 6890 GC equipped with a DB-5MS column (30 m × 0.25 mm i.d.; Agilent Technologies), and a 5973N MSD]. The GC/MS measurement was performed by using the splitless injection mode, and the injection volume was 1.0 $\mu$L. Helium was used as the carrier gas (1.0 mL/min), and the injector temperature was 220 °C. The GC oven was programmed to remain at 50 °C for the initial 1 min, ramped at 25 °C/min to 125 °C, then rose to 300 °C at a rate of 10 °C/min, and held for 6.5 min. Quantitative analysis was conducted by SIM mode, and the ions for quantification were as follows: chlorpyrifos, 314; chlorpyrifos-$d_{10}$, 324; diazinon, 304; diazinon-$d_{10}$, 314; fenitrothion, 277; fenitrothion-$d_6$, 283; cypermethrin, 181; cypermethrin-$^{13}C_6$, 187; etofenprox, 163; etofenprox-$d_5$, 168; permethrin, 183; permethrin-$^{13}C_6$, 189 (permethrin was quantified as the sum of *cis*- and *trans*-permethrin); and alachlor, 160. The pesticides were quantified by IDMS. The measurement was carried out twice per sample.

The NMIJ method 2 for the analysis of organophosphorus pesticides (chlorpyrifos, diazinon, and fenitrothion) was referred to a previous study,[13] and we partly modified it ($n$ = 5). The green onion or cabbage sample (0.4 g) was weighed in a glass vial (110 mL; Maruemu), and the surrogate solution (as with NMIJ method 1) and purified water (10 mL) were added. After 2 h, this sample was homogenized for 3 min in acetone (70 mL) and filtered with diatomaceous earth (reagent grade; Kanto Chemical). The residues on diatomaceous earth were re-extracted by homogenization (for 3 min) with acetone (50 mL), and pesticide-containing filtrates were combined. This crude extract was concentrated by a rotary evaporator and then was shaken with ethyl acetate/hexane (1:4, v/v; 100 mL) and saturated sodium chloride aqueous solution (100 mL) in a separatory funnel for 5 min. The upper [ethyl acetate/hexane (1:4, v/v)] layer was collected, and the lower (water) layer was re-extracted with 50 mL of ethyl acetate/hexane (1:4, v/v). This ethyl acetate/hexane (1:4, v/v) layer was combined and dried by a rotary evaporator after dehydration by an anhydrous sodium sulfate and filtration, and then, 5.0 mL of hexane/acetone (1:1, v/v) was added. This was cleaned up by using a SPE cartridge [silica gel cartridge (5 g); Bond Elut MEGA BE-SI, 5 GM, 20 ML, Varian, Palo Alto, CA; used by adding an anhydrous sodium sulfate (5 g) on this SPE cartridge; conditioned with 10 mL of hexane/acetone (1:1, v/v)]. Pesticides were eluted with hexane/acetone (1:1, v/v; 100 mL) followed by concentration and a drying processes using a rotary evaporator. Then, the syringe spike solution (as with NMIJ method 1) was added to this cleaned up extract. The pesticides were analyzed by GC/MS (Agilent Technologies 7890A GC/5975C MSD), and the columns used for the separation were DB-17MS and DB-35MS (30 m × 0.25 mm i.d.; Agilent Technologies) for green onion and cabbage, respectively. The GC/MS was performed by using on-column injection mode, and the injection volume was 0.5 $\mu$L. A deactivated fused silica capillary (length 1 m, 0.25 mm i.d.; Agilent Technologies) was placed as retention gap between the injector and the DB-17MS or 35MS column using a fused silica union (F.S. Union Universal, 2-way; Agilent Technologies) to eliminate peak broadening of chromatogram. The inlet temperature was set as oven track mode. The other conditions were the same with GC/MS for NMIJ method 1.

The NMIJ method 3 for the analysis of pyrethroid pesticides (cypermethrin, etofenprox, and permethrin) was also referred to a previous study,[13] and we partly modified it ($n$ = 5). The green onion or cabbage sample (0.4 g) was weighed in a glass vial (110 mL; Maruemu), and the surrogate solution (as with NMIJ method 1) and purified water (10 mL) were added. After 2 h, this sample was homogenized as well as NMIJ method 2. The crude extract was shaken with hexane (100 mL) and 10% sodium chloride aqueous solution (100 mL) in a separatory funnel for 5 min after concentration to about 30 mL by a rotary evaporator. The upper (hexane) layer was collected, and the lower (water) layer was re-extracted with 50 mL of hexane. This hexane layer was combined and dried by a rotary evaporator after dehydration by an anhydrous sodium sulfate and filtration, and then, 5.0 mL of hexane was added. The 2.0 mL taken from this extract was cleaned up by using a SPE cartridge [Florisil cartridge (5 g); Bond Elut MEGA BE-FL, 5GM, 20 ML, Varian; used by adding an anhydrous sodium sulfate (5 g) on this SPE cartridge; conditioned with 10 mL of hexane]. Pesticides were eluted with hexane (50 mL) and hexane/diethyl ether (3:1, v/v; 150 mL) followed by concentration and drying processes using a rotary evaporator. Then, the syringe spike solution (as with NMIJ method 1) was added to this cleaned up extract. The instrumental analysis was performed by GC/MS, and the conditions were the same with NMIJ method 2, except for GC column (as shown in Figure 1).

For all of the NMIJ methods, calibration solutions were prepared as follows: The pesticide solutions were gravimetrically prepared by dissolving in acetone from pesticide reagents chlorpyrifos, cypermethrin (for pesticide residue analysis grade; Wako Pure Chemical), diazinon, fenitrothion, etofenprox, *cis*-permethrin, and *trans*-permethrin (Traceable Reference Material grade; Wako Pure Chemical), and these were mixed with each other. This mixed pesticides solution was further mixed gravimetrically with surrogate and syringe spike solutions as prepared above. Moreover, by mixing with this final mixed solution and cleaned-up extracts of blank green onion or cabbage (confirmed to have no target pesticides detectable), the matrix-matched calibration solutions were prepared and used for quantification. These solutions were prepared to be almost the same as the final concentration of each pesticide in cleaned-up extract of the candidate CRM.

All of the NMIJ methods were used for certification after the validation by spiking pesticides. To validate the methods, target mixed pesticides solution was spiked to two types of blank green onions or cabbages with different production areas (confirmed to have no target pesticides detectable; purchased from a supermarket in Japan) to be almost the same concentration with candidate CRM. After 30 min,[8] target pesticides were analyzed by the NMIJ methods (Figure 1).

**Assay for Purity of Pesticide Reagents Used for Certification.** The purities of pesticide reagents were evaluated using a GC with a flame ionization detector (HP 6890 Series System, Agilent Technologies) and a high-performance liquid chromatograph with an ultraviolet detector (HPLC-UV; LC-20AB system, Shimadzu, Kyoto, Japan). The inorganic residue was determined with a Q 5000 IR thermogravimetric analyzer (TA Instruments, Tokyo, Japan) with platinum crucibles. The mass fraction of water was measured by a coulometric Karl Fisher titrator (MKC-510, Kyoto Electronics Manufacturing, Kyoto, Japan). The purities of the pesticide reagents were as follows: 99.48 ± 0.25% for chlorpyrifos, 99.74 ± 0.15% for diazinon, 99.53 ± 0.17% for fenitrothion, 96.02 ± 1.19% for cypermethrin, 99.57 ± 0.22% for etofenprox, 99.85 ± 0.12% for *cis*-permethrin, and 99.73 ± 0.15% for *trans*-permethrin (mean ± combined standard uncertainty).

**Stability Assessment.** Because the pesticides may be unstable, stability assessment for the target pesticides was performed on short (during transportation) and long-term (during storage). The short-term stability assessment was performed by using two delivery companies. The candidate CRMs were transported at three kinds of temperatures: ≤15 (normal temperature), ≤10, and ≤0 °C (≤10 and ≤0 °C were controlled by a refrigerator car). The concentrations of each pesticide were monitored on before and after transportation for two bottles at each temperature condition and evaluated the changes in the concentration. For a long-term stability assessment, the concentrations were monitored on a periodic basis for about 1 year before the certification by using the bottles stored about −30 °C in the dark. The analysis was performed as described in the Supporting Information.

**Table 1. Results of Spiking Test for Target Pesticides[a]**

| pesticides | method 1 | | method 2 or 3 | |
|---|---|---|---|---|
| green onion | sample A | sample B | sample A | sample B |
| diazinon | 99.9 ± 1.6 | 100.4 ± 0.5 | 98.8 ± 0.5 | 99.7 ± 3.6 |
| fenitrothion | 101.8 ± 1.9 | 101.5 ± 1.3 | 98.7 ± 1.0 | 99.0 ± 0.7 |
| cypermethrin | 99.3 ± 0.2 | 99.5 ± 1.4 | 98.2 ± 3.4 | 100.3 ± 1.9 |
| etofenprox | 100.0 ± 0.4 | 99.9 ± 0.4 | 98.8 ± 2.4 | 99.3 ± 0.5 |
| permethrin | 100.8 ± 0.6 | 100.1 ± 0.5 | 101.1 ± 1.2 | 100.5 ± 0.7 |
| cabbage | sample C | sample D | sample C | sample D |
| chlorpyrifos | 101.2 ± 0.4 | 100.6 ± 1.0 | 98.3 ± 0.4 | 98.7 ± 0.5 |
| fenitrothion | 103.6 ± 0.7 | 100.3 ± 1.2 | 100.9 ± 0.4 | 100.2 ± 1.3 |
| etofenprox | 100.6 ± 0.9 | 99.8 ± 0.4 | 98.7 ± 0.3 | 96.6 ± 4.9 |
| permethrin | 100.7 ± 1.0 | 100.4 ± 0.3 | 100.8 ± 2.3 | 99.9 ± 2.7 |

[a] The values represent the means ± standard deviations, and the unit of values is %. The method numbers correspond to Figure 1. Chlorpyrifos, diazinon, and fenitrothion were analyzed by methods 1 and 2. Cypermethrin, etofenprox, and permethrin were analyzed by methods 1 and 3. The two types of blank green onions (samples A and B) and cabbages (samples C and D) with different production areas were used. The results of etofenprox in cabbages are indicated as information because this pesticide was not certified as described in the text; $n$ = 3.

**Homogeneity Assessment.** The between-bottle homogeneity of the CRM was assessed by quantifying target pesticides in two subsamples taken from 10 bottles randomly selected from 200 bottles. Quantification of target pesticides was performed as described in the Supporting Information. Analysis of variance (ANOVA) was used for the analysis of differences of concentration between bottles.

## RESULTS AND DISCUSSION

**Analytical Methods Used for Certification.** Some analytical techniques were applied for certification (Figure 1) to avoid the bias associated with a certain analytical method. The results for validation of these methods by spiking pesticides are shown in Table 1, which are described as percentage by the quantification results of IDMS (a unit of mass) relative to the spiked amount of pesticides (a unit of mass). As a result, observed values by IDMS were nearly 100% as mean value for each pesticide, and the repeatability of the analysis, represented as standard deviations (SDs), was satisfactory. Thus, this result indicates that the NMIJ methods in Figure 1 were sufficiently optimized and can be applied for certification.

Since the degradation of thermolabile pesticides can be caused in the hot GC injection port, full attention should be paid for accurate analytical results. Therefore, an on-column injection technique,[14] which has been considered to be recommended one for thermolabile pesticides, was applied. Furthermore, in pesticide analysis, it is suggested that the occurrence of matrix effects has a major effect on the quantitative value. Matrix effects can cause enhancement or suppression in observed chromatographic response for pesticide residues in a matrix extract as compared with the same concentration in a matrix-free solution.[15] In fact, it was observed that there was a matrix effect for organophosphorus pesticides (both in green onion and cabbage) in our study. It is suggested that the use of

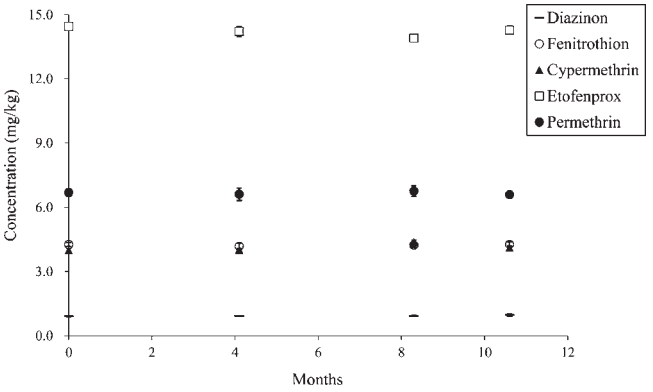


**Figure 2.** Trends of the observed pesticide concentrations during long-term stability assessment [green onion (CRM 7507-a)]. Plots and error bars represent the mean values and standard deviations, respectively; $n = 4$.

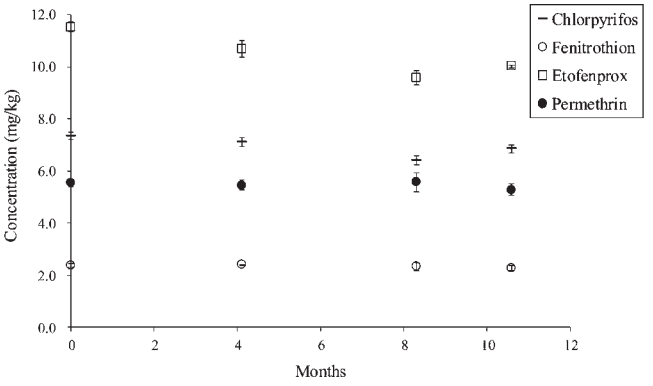


**Figure 3.** Trends of the observed pesticide concentrations during long-term stability assessment [cabbage (CRM 7508-a)]. Plots and error bars represent the mean values and standard deviations, respectively; $n = 4$.

matrix-matched standard for the calibration is effective for preventing matrix effects.[15] In addition, the use of internal standards, for example, isotope-labeled pesticides, can correct for matrix-induced enhancement.[15] Thus, these techniques were also applied.

**Stability Assessment and the Expiration Date of CRMs.** To our knowledge, no similar CRMs have been available at present. Thus, to determine if the target pesticides are stable or not, the concentrations had to be monitored for the short-term (during transportation) and long-term (during storage).

As the results of short-term stability assessment, no significant changes were detected for the concentration of target pesticides when the CRMs were transported at ≤0 °C. Also, the results for long-term stability assessment are shown in Figures 2 (for green onion) and 3 (for cabbage). The linear gradients $b_1$ in Figures 2 and 3 were calculated in accordance with the ISO guide 35[16] by using eq 1:

$$b_1 = \frac{\sum_{i=1}^{n}(X_i - \overline{X})(Y_i - \overline{Y})}{\sum_{i=1}^{n}(X_i - \overline{X})^2} \quad (1)$$

where $X_i$ and $Y_i$ represent the elapsed time (months) and the relative concentration at $i$ month to that at 0 month, respectively, and $\overline{X}$ and $\overline{Y}$ represent the average of $X_i$ and that of $Y_i$, respectively. The standard deviation of $b_1$ [$s(b_1)$] was calculated by using following eq 2:[16]

$$s(b_1) = \frac{s}{\sqrt{\sum_{i=1}^{n}(X_i - \overline{X})^2}} \quad (2)$$

The $s$ and $b_0$ were calculated by using eqs 3 and 4, respectively:[16]

$$s^2 = \frac{\sum_{i=1}^{n}(Y_i - b_0 - b_1X_i)^2}{n-2} \quad (3)$$

$$b_0 = \overline{Y} - b_1\overline{X} \quad (4)$$

The significance of the instability for target pesticides was tested by eq 5:[16]

$$|b_1| < t_{0.95,n-2} \times s(b_1) \quad (5)$$

where $t_{0.95,n-2}$ equals 4.30 (with 2 degrees of freedom at level of confidence $p = 0.95$), and the $|b_1|$ and $s(b_1)$ were calculated by using eqs 1 and 2, respectively. As a result, there were no significant decreases for the concentration of target pesticides both in green onion and cabbage because the requirement of eq 5 was satisfied. However, etofenprox in cabbage has been removed from certification because the rate of decrease in concentration was considerably larger than the others (about 17%). The reason for decrease in the concentration of etofenprox in cabbage (although not statistically significant as described above) might be due to a hydrolysis because there was a small difference for moisture content between green onion and cabbage CRM (green onion, about 8%;[17] cabbage, about 12%[18]).

The uncertainty due to long-term instability [$u$(lts)] have a high correlation with the expiry date of CRM, and that was calculated by eq 6:[16]

$$u(lts) = t \times s(b_1) \text{ (or } t \times |b_1| \text{ : the larger value between them were used)} \quad (6)$$

where $t$ represents the expiry date (months). From the results of calculation, if its storage period is shorter than 19 months (under the storage condition of about −30 °C in the dark), the $u$(lts) satisfied the requirement for the accuracy of the validation guideline for testing method of agricultural chemicals in food (in the range of 70−120%[8]). Thus, the $u$(lts) for 19 months of the expiry date was used as the relative uncertainty due to long-term instability. The calculated results for green onion (7507-a) were as follows: diazinon, 8.6% relative; fenitrothion, 2.7% relative; cypermethrin, 11.0% relative; etofenprox, 3.7% relative; and permethrin, 3.2% relative. Similarly, those for cabbage (7508-a) were as follows: chlorpyrifos, 17.0% relative; fenitrothion, 9.0% relative; and permethrin, 5.5% relative.

**Homogeneity Assessment.** The sample homogeneity was assessed for each pesticide as described above [$n$ = 20 (two subsamples taken from 10 bottles)]. No statistically significant differences for pesticide concentration values between bottles were observed in both green onion and cabbage. This result indicates that the materials are homogeneous enough for target pesticide analysis.

**Table 2. Analytical Results for Certified Pesticides in Green Onion (CRM 7507-a) and Cabbage (7508-a)[a]**

| pesticides | method 1 | method 2 or 3 |
|---|---|---|
| | green onion (CRM 7507-a) | |
| diazinon | 0.96 ± 0.02 | 0.96 ± 0.01 |
| fenitrothion | 4.38 ± 0.06 | 4.46 ± 0.12 |
| cypermethrin | 3.97 ± 0.03 | 4.00 ± 0.10 |
| etofenprox | 13.96 ± 0.16 | 13.89 ± 0.27 |
| permethrin | 7.15 ± 0.08 | 7.14 ± 0.11 |
| | cabbage (CRM 7508-a) | |
| chlorpyrifos | 6.95 ± 0.08 | 6.94 ± 0.07 |
| fenitrothion | 2.42 ± 0.03 | 2.41 ± 0.05 |
| permethrin | 5.74 ± 0.09 | 5.76 ± 0.04 |

[a] The values represent the mean concentrations ± standard deviations, and the unit of values is mg/kg. The method numbers correspond to Figure 1. Chlorpyrifos, diazinon, and fenitrothion were analyzed by methods 1 and 2. Cypermethrin, etofenprox, and permethrin were analyzed by methods 1 and 3; $n = 5$.

For the uncertainty due to inhomogeneity of the material, the $s_{bb}$ was calculated by using eq 7:[16]

$$s_{bb} = \sqrt{\frac{MS_{among} - MS_{within}}{n}} \quad (7)$$

where $MS_{within}$ and $MS_{among}$ represents the mean squares within a group and among groups, respectively. If the variability of measurement is not small enough to detect difference between bottles, the influence of analytical variation on the standard deviation between units ($u_{bb}$) was calculated and used as the estimate for the inhomogeneity instead of $s_{bb}$.[19] The $u_{bb}$ was calculated by using following eq 8:[19]

$$u_{bb} = \sqrt{\frac{MS_{within}}{n}} \sqrt[4]{\frac{2}{\nu_{MS_{within}}}} \quad (8)$$

where $\nu_{MS_{within}}$ represents the number of degrees of freedom of $MS_{within}$. The $s_{bb}$ and $u_{bb}$ were calculated by using eqs 7 and 8, respectively, and the results were used as the relative uncertainty due to inhomogeneity. The calculated results for green onion (7507-a) were as follows: diazinon, 1.0% relative; fenitrothion, 1.0% relative; cypermethrin, 1.7% relative; etofenprox, 0.5% relative; and permethrin, 1.3% relative. Those for cabbage (7508-a) were as follows: chlorpyrifos, 0.9% relative; fenitrothion, 1.7% relative; and permethrin, 1.1% relative.

**Analytical Results and Certified Values.** The concentrations of target pesticides in green onion and cabbage were calculated by the following eq 9.

$$C = F_{ext} \times \left(\frac{R_{sample}}{R_{cal}} - \frac{R_{blank}}{R_{cal}}\right) \times \frac{F_{cal} \times M_{cal} \times C_{cal} \times M_{spike(sample)}}{M_{sample} \times M_{spike(cal)}} \quad (9)$$

where $C$ is a concentration of analyte in the sample, $F_{ext}$ is a factor concerning extraction and cleanup step, $R_{sample}$ is a ratio of peak area of analyte/surrogate observed for the sample solution, $R_{blank}$ is a ratio of peak area of analyte/surrogate observed for the blank solution, $R_{cal}$ is a ratio of peak area of analyte/surrogate observed for the calibration solution, $F_{cal}$ is a correction factor of preparing calibration solution, $M_{cal}$ is a mass of the standard solution of analytes taken for preparation of the calibration solution, $C_{cal}$ is a concentration of analyte in the calibration solution, $M_{spike(sample)}$ is a mass of the surrogate solution added to the sample, $M_{sample}$ is a mass of the sample taken for analysis, and $M_{spike(cal)}$ is a mass of the surrogate solution taken for preparation of the calibration solution.

**Table 3. Certified Values and Expanded Uncertainties for Green Onion (CRM 7507-a) and Cabbage (7508-a)[a]**

| pesticides | certified value | expanded uncertainty |
|---|---|---|
| | green onion (CRM 7507-a) | |
| diazinon | 0.96 | 0.19 |
| fenitrothion | 4.41 | 0.29 |
| cypermethrin | 3.98 | 0.91 |
| etofenprox | 13.9 | 1.3 |
| permethrin | 7.14 | 0.59 |
| | cabbage (CRM 7508-a) | |
| chlorpyrifos | 6.9 | 2.4 |
| fenitrothion | 2.41 | 0.45 |
| permethrin | 5.75 | 0.68 |

[a] The unit of values is mg/kg. The expanded uncertainty was determined by using coverage factor ($k = 2$), corresponding to a 95% confidence interval.

The analytical results for the certification obtained by the respective methods (Figure 1) are summarized in Table 2, and the concentrations between method 1 and method 2 or 3 were in good agreement with each other.

The certified values are the weighted means of the analytical results obtained by the two methods for each pesticide, where $1/u_i$ ($u_i$: uncertainty of the result obtained by each method) was used as the weight, and they are shown in Table 3.

**Uncertainties of the Certified Values.** The uncertainties of the certified values were calculated from uncertainties due to respective factors according to the Guide to the Expression of Uncertainty in Measurement,[20] and they are shown in Table 3. The uncertainty budget is summarized in Table 4. The ISO guide 35[16] specifies that uncertainty of CRMs is estimated from standard uncertainty due to characterization, $u$(char); standard uncertainty due to long-term (during storage) instability, $u$(lts); short-term (during transportation) instability, $u$(sts); and inhomogeneity of the material, $u$(bb). The $u$(char) was estimated from $u(C_{ind})$, $u(C_{com})$, and $u(C_{bm})$. The $u(C_{ind})$ associated with each analytical method was obtained from the uncertainty of $R_{sample}$, $R_{blank}$, $R_{cal}$, $F_{ext}$, $M_{sample}$, and $M_{spike(sample)}$. The $u(C_{com})$ that is common to analytical methods was estimated from the uncertainty of $F_{cal}$, $M_{cal}$, $C_{cal}$, and $M_{spike(cal)}$ (The uncertainty of $C_{cal}$ was combined the uncertainty for purity of neat pesticides and for weighing). The uncertainty for between-method variance [$u(C_{bm})$] was calculated by performing an ANOVA on the result obtained from the analytical methods in Figure 1. The $u$(lts) was included for the uncertainties by using the result of long-term stability assessment as described above; however, we did not include the uncertainties for $u$(sts) in the uncertainties of certified values because no significant differences were detected for the concentration of target pesticides when the CRMs were transported at ≤0 °C as described above. The $u$(bb) derived from the inhomogeneity of the material was estimated in

**Table 4. Uncertainty Budget for the Certified Values of Green Onion (CRM 7507-a) and Cabbage (7508-a)**

| Uncertainty component | Values | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Green onion 7507-a | Green onion 7507-a | Green onion 7507-a | Green onion 7507-a | Green onion 7507-a | Cabbage 7508-a | Cabbage 7508-a | Cabbage 7508-a |
| | Diazinon | Fenitrothion | Cypermethrin | Etofenprox | Permethrin | Chlorpyrifos | Fenitrothion | Permethrin |
| Relative standard uncertainty (%) | | | | | | | | |
| $u$(char): combined $u(C_{ind})$, $u(C_{com})$, and $u(C_{bm})$ as calculated below | 4.72 | 1.56 | 2.64 | 2.45 | 2.37 | 2.82 | 1.84 | 1.93 |
| $u(C_{ind})$ | 0.77 | 0.93 | 0.78 | 0.91 | 0.59 | 0.50 | 0.69 | 0.45 |
| $u(C_{com})$ | 4.66 | 0.91 | 2.52 | 2.27 | 2.29 | 2.78 | 1.70 | 1.87 |
| $u(C_{bm})$ | 0 | 0.86 | 0 | 0 | 0 | 0 | 0 | 0.15 |
| $u$(lts) | 8.59 | 2.71 | 11.00 | 3.74 | 3.15 | 17.03 | 8.96 | 5.47 |
| $u$(sts) | | | | (not included) | | | | |
| $u$(bb) | 1.02 | 1.03 | 1.66 | 0.49 | 1.25 | 0.92 | 1.69 | 1.06 |
| Combined uncertainty, $u_c$ | | | | | | | | |
| Relative standard uncertainty (%) | 9.85 | 3.29 | 11.43 | 4.49 | 4.13 | 17.29 | 9.30 | 5.89 |
| × certified value (mg/kg) | 0.09 | 0.14 | 0.45 | 0.63 | 0.29 | 1.20 | 0.22 | 0.34 |
| Expanded uncertainty, $U$ ($k$=2) (mg/kg) | 0.19 | 0.29 | 0.91 | 1.3 | 0.59 | 2.4 | 0.45 | 0.68 |

homogeneity assessment as described above. The expanded uncertainty $U$ of the certified value is equal to $ku_c$, where $u_c$ is the combined standard uncertainty with coverage factor $k = 2$, corresponding to a 95% confidence interval. From the uncertainty budget (Table 4), the uncertainty of chlorpyrifos in cabbage (7508-a) was slightly larger than that of the other pesticides, which was mainly attributed to the $u$(lts).

The NMIJ CRM 7507-a (green onion; certified for five pesticides) and 7508-a (cabbage; certified for three pesticides) were issued by NMIJ. These are the first green onion and cabbage powder CRMs in which organophosphorus and pyrethroid pesticides are determined. These CRMs would be useful tools for the validation of the analytical methods and for quality assurance/quality control of organophosphorus and pyrethroid pesticides analyses in green onion and cabbage or similar sample matrices.

## ■ ASSOCIATED CONTENT

**Ⓢ Supporting Information.** Analytical method used for stability and homogeneity assessment. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Corresponding Author

*Tel: +81-29-861-4271. Fax: +81-29-861-6866. E-mail: t-ootake@aist.go.jp.

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