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Effect of Sampling Size on the Determination of Accurate Pesticide Residue Levels in Japanese Agricultural Commodities

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Supporting Information

ABSTRACT: The uncertainty in pesticide residue levels (UPRL) associated with sampling size was estimated using individual acetamiprid and cypermethrin residue data from preharvested apple, broccoli, cabbage, grape, and sweet pepper samples. The relative standard deviation from the mean of each sampling size ($n = 2^x$, where $x = 1-6$) of randomly selected samples was defined as the UPRL for each sampling size. The estimated UPRLs, which were calculated on the basis of the regulatory sampling size recommended by the OECD Guidelines on Crop Field Trials (weights from 1 to 5 kg, and commodity unit numbers from 12 to 24), ranged from 2.1% for cypermethrin in sweet peppers to 14.6% for cypermethrin in cabbage samples. The percentages of commodity exceeding the maximum residue limits (MRLs) specified by the Japanese Food Sanitation Law may be predicted from the equation derived from this study, which was based on samples of various size ranges with mean residue levels below the MRL. The estimated UPRLs have confirmed that sufficient sampling weight and numbers are required for analysis and/or re-examination of subsamples to provide accurate values of pesticide residue levels for the enforcement of MRLs. The equation derived from the present study would aid the estimation of more accurate residue levels even from small sampling sizes.

KEYWORDS: *sampling size, pesticide residue, raw agricultural commodity*

INTRODUCTION

Since May 2006, a positive list system for pesticides in foods has been enforced in Japan.[1] Foods in which pesticide residues are found in excess of the Japanese maximum residue limits (MRLs, including the uniform limits of 0.01 mg/kg) shall not be produced, imported, processed, used, cooked, or stored for sale or sold in Japan. This strict enforcement with punishment for food safety has necessitated more accurate residue analysis for a wide range of pesticides and raw agricultural commodities (RAC). This analytical requirement for RAC has a great impact on Japan, which is one of the largest importing countries in the world trade of RAC and also influences other exporting countries. The sum of agricultural products imported into Japan from the United States (27%), the Association of South East Asian Nations (15%), the European Union (15%), China (11%), Australia (8%), Canada (6%), and other countries (18%) in 2010 was 4.8 trillion yen.[2]

In January 2008, at least 10 consumers in Japan became sick after ingesting frozen dumplings produced in China that were contaminated with an organophosphate insecticide, methamidophos.[3,4] In consideration of the increasing consumer concern for food safety, the Food Safety Commission of Japan (FSCJ) publicized an acute reference dose (ARfD) of 0.003 mg/kg body weight (bw) per day for methamidophos as official information on May 1, 2008.[5] Subsequently, the FSCJ publicized an ARfD of 0.1 mg/kg bw per day for acetamiprid on August 29, 2008.[6] This food poisoning incident with pesticide contamination has led to stricter pesticide residue analysis.

From 2007 to 2010, we conducted studies to estimate the acetamiprid and cypermethrin residue variations in apple, broccoli, cabbage, grape, and sweet pepper.[7,8] Acetamiprid ((*E*)-*N*-[(6-chloro-3-pyridyl)methyl]-*N*-cyano-*N*-methylethanimidamide) is a neonicotinoid insecticide used worldwide. Acetamiprid has a relatively low log P_{OW} of 0.80 and a relatively high water solubility of 4250 mg/L.[9] Cypermethrin (cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate) is a synthetic pyrethroid insecticide used worldwide. Cypermethrin has relatively high log P_{OW} of 6.6 and a relatively low water solubility of 0.004 mg/L.[9] The two pesticides differ significantly in their respective physicochemical properties to facilitate an accurate determination of the variations in individual pesticide residues. These investigations provided valuable information on the estimation of variations in the pesticide residue levels in RAC under normal Japanese agricultural practices.

Variation in pesticide residue levels is an important factor for the risk assessment of acute dietary exposure of consumers to pesticides applied to a given RAC. Since 1994, the ARfD has been progressively established for particular pesticides by the Joint FAO/WHO Meeting on Pesticide Residues to address potential exposure to residues in food at relatively higher doses for short-term periods, due to accidental or incidental events.[10,11] Many papers discuss the various levels of pesticide residues in RAC, with the aim of predicting the ARfD for human health.[12−16] These papers evaluate the individual pesticide residue variations for an index of the variability factor (VF), which is calculated as the 97.5th percentile of the residue population divided by the average residue levels in the lot. The distribution of the preharvested RAC is not uniform and is

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Table 1. Measured Individual Residue Levels of Pesticides, Sample Weights, and Calculated Variability Factors

crop; field in Japan (pesticide)	no. of applications × PHI (dilution factor; application vol)[a]	sample wt[b], g (min−max)	results of Shapiro−Wilks test	residue[b], mg/kg (min−max)	VF[c] (97.5th percentile)
apple-I; Iwate (cypermethrin)	3 × 14 (1000 times; 400 L/10 a)	425 ± 61.6 (280−644)	$W = 0.97374$ $p < 0.0126$	0.21 ± 0.078 (0.04−0.43)	1.76 (0.37)
apple-F; Fukushima (cypermethrin)	3 × 14 (1000 times; 500 L/10 a)	416 ± 71.2 (245−608)	$W = 0.98658$ $p < 0.2335$	0.24 ± 0.081 (0.04−0.46)	1.75 (0.42)
broccoli; Ibaraki (acetamiprid)	3 × 14 (2000 times; 250 L/10 a)	523 ± 93.3 (301−786)	$W = 0.98817$ $p < 0.3283$	0.039 ± 0.013 (0.007−0.071)	1.72 (0.067)
cabbage; Ibaraki (acetamiprid)	5 × 7 (1000 times; 213−292 L/10 a)	1330 ± 260 (841−2032)	$W = 0.97517$ $p < 0.0173$	0.129 ± 0.056 (0.044−0.288)	2.00 (0.258)
cabbage; Ibaraki (cypermethrin)	5 × 7 (1000 times; 213−292 L/10 a)	same as above[d]		0.066 ± 0.042 (<0.005−0.266)	2.39 (0.158)
grape; Yamanashi (acetamiprid)	3 × 14 (2000 times; 300 L/10 a)	155 ± 12.5 (129−180)	$W = 0.97264$ $p < 0.0099$	1.34 ± 0.578 (0.114−3.12)	1.82 (2.44)
grape; Yamanashi (cypermethrin)	5 × 7 (1000 times; 300 L/10 a)	same as above[d]		1.87 ± 0.578 (0.574−3.38)	1.63 (3.05)
sweet pepper; Kochi (cypermethrin)	2 × 1 (2000 times; 250 L/10 a)	31 ± 2.6 (27−39)	$W = 0.96626$ $p < 0.0015$	0.55 ± 0.135 (0.22−0.85)	1.48 (0.81)

[a]Number of applications × PHI: days after final application (dilution factors of the formulations; application volumes). [b]Data are presented as mean values (±standard deviations). Minimum−maximum values are indicated in parentheses. [c]Variability factor (coefficient of variation at the 97.5th percentile of the mean residue value). [d]The two pesticides were applied on cabbage and grape samples using a tank-mix application technique.

influenced by many factors, such as the physical and chemical properties of the pesticide, application directions, agricultural conditions, weather, sampling procedures, and growth rates.

The sampling size requirements for pesticide residue analysis depend on the study objectives and/or experimental conditions. The importance of sampling size (including both weight and number) has been widely recognized, and various parameters that require consideration during sampling have been described in several guidelines. The sampling size necessary in crop field trials to estimate pesticide residue levels for chronic dietary risk assessment and to derive the MRL is regulated in detail for each RAC.[17−20] On the other hand, the recommended sampling size may differ with the research objectives because field and/or market samples are often required to satisfy other needs, such as monitoring programs for MRL enforcement.[21−23] In some cases, the sampling sizes used in field trials or market surveys are not sufficient to accurately represent residual pesticides left in the RAC after conventional handling prior to consumption. However, limited information is available for the effects of sampling size on a combination of RAC. For example, VFs are not useful to estimate the uncertainty in pesticide residue level (UPRL) in relation to sampling size. As a result, it is difficult to estimate the accuracy of residual amounts obtained from inadequate sampling sizes.

Most recently, a mass spectrometric technique has been developed, and an ionization technique for direct analysis in real time (DART-MS) was introduced to allow direct examination of various types of RAC units in the open atmosphere and at ground potential.[24] This novel technique, which uses foam swabs to recover multiclass pesticides from the surfaces of RAC units, requires little or no sample treatment before analysis. Edison et al. analyzed postharvest RAC using both the standard QuEChERS extraction[25] and DART-MS, and the results from the two techniques were found to be comparable.[26] Unfortunately, the effect of the variability in pesticide residue levels on preharvest RAC units has not been clarified by comparative testing with postharvest samples.

It should be noted that the current major tolerances for the enforcement of pesticide residues in RAC are the MRLs based on the acceptable daily intake, which are globally established for all pesticides, including those with uniform limits. On the other hand, the ARfDs are reference values, which have not been established for all pesticides and are not common tolerances for the enforcement of pesticide residue risk in RAC. On the basis of the above-mentioned requirements, this study was undertaken to establish a prediction method for estimating the UPRL in RAC and determining residue variations in a wide range of sampling sizes using individual preharvest pesticide residue data collected from apple, broccoli, cabbage, grape, and sweet pepper samples. The purpose of this study was to provide statistical information on appropriate practical sampling sizes for residue analysis in individual unit samples and bulk samples. The equation derived from this study would be helpful to estimate more accurate residue levels even from small sampling sizes. The simple and easy understanding of the uncertainty in pesticide residue levels associated with sampling size for RACs is provided in this paper.

MATERIALS AND METHODS

Overview of Applied Individual Preharvested Residue Data Sets. To estimate the effects of sampling size on the determination of pesticide residue levels in preharvested RAC, investigation data set results from supervised field trials were applied to this study. These studies were supervised by our institute to estimate the unit-to-unit variability in cypermethrin and acetamiprid residue levels in five

species of preharvested Japanese RAC. The individual cypermethrin residue variations in sweet peppers and apples were studied in 2007 and 2008, respectively. The individual acetamiprid residue variation in broccoli was studied in 2009. The individual acetamiprid and cypermethrin residue variations in cabbage and grape samples were studied in 2010, and then the two pesticides were applied using a tank-mix application technique. An overview of the supervised field trials is presented below.

Field Experiments. Field experiments were carried out at five test sites across Japan, in accordance with Japan's good agricultural practices.[27] For each RAC, the pesticides of interest were applied at the maximum label rates, the maximum number of applications, and the minimum preharvest intervals (Table 1). Samples were randomly selected from five locations according to simple random design. Each sample was collected in a separate polyethylene bag to prevent cross-contamination and then packed in hard boxes. Broccoli and cabbage samples were brought to our institute on the sampling day. The other samples were shipped to our institute by a commercial shipping service and were maintained at a standard temperature of 3 °C. The weights of individual commodities were measured, and all samples were extracted on the day following arrival at our institute, without storage. Details of the test site conditions at the apple orchard, cabbage field, and grape vineyard have been described in our previous studies.[7,8]

Residue Analysis. Residue analysis was performed using three analytical methods: a single method for cypermethrin in apples and sweet peppers,[7] a single method for acetamiprid in broccoli (see also the Supporting Information), and a method for simultaneous analysis of acetamiprid and cypermethrin in cabbage and grape samples.[8] The residue analytical methods were optimized for rapid analysis of each analyte as described below.

Acetamiprid and cypermethrin were extracted from the samples with acetone. Extracts were cleaned up using solid phase extraction with octadecyl silica cartridges (Inert Sep C18-C, 1 g/6 mL; GL Science, Japan) or graphite carbon black cartridges (GL-Pak Carbograph, 500 mg/6 mL; GL Science) and analyzed by liquid chromatography (LC, model 1290 Infinity Pumping System; Agilent, USA)−tandem mass spectrometry (MS/MS, model 6460 triple-quadrupole tandem mass spectrometer; Agilent).

Calculation of Variability Factor. The VFs of residues in the RAC test samples were calculated with Microsoft Excel, using the ratio of the 97.5th percentile to the mean residue values as the reference procedure.[11] Hamilton et al. showed that a sample size of 119 or more RAC units is required for a 95% certainty that at least 1 unit exceeds the 97.5th percentile of the sample population.[14] On this basis, an adequate sampling size of 130 was analyzed for each RAC sample in this study.

Estimation Procedure To Determine the Effects of Sampling Size. A table of numbers (n) was automatically generated by randomly selecting integers between 1 and 130, inclusively, which were used to represent individual values of a residue and sample weight of the RAC. Factors of 2^x, where $x = 1-6$, were selected to calculate the mean value of residue and the weight of each sampling size of RAC sample. This process was repeated 130 times to represent the total numbers of the actual RAC samples. These simulations were repeated five times for each RAC data set. The relative standard deviation (RSD) from the mean was calculated for each RAC sample number/weight pair that was randomly selected for the chosen RAC sample. The resultant RSD represents the residue variation for each sample population (number and mean weight × n) and was defined as the UPRL for each sampling size. Tables of random numbers were automatically generated in each simulation, and all of the data were calculated using Microsoft Excel without a macro program.

RESULTS AND DISCUSSION

Variation of Residue Analysis. Results of the recovery test and quality control test of each RAC, which were used to confirm the variations of residue analytical methods applied in this study, are summarized in Table 2. The accuracy and precision of the analytical methods were confirmed by recovery tests on acetamiprid or cypermethrin at more than three dose levels from the limit of quantification (LOQ) to exceeding the highest residue levels. The mean recoveries of spiked samples in triplicate (total of 25 sets) ranged from 74 to 107%, and their RSDs were ≤5.3%. The specificity of the analytical method was confirmed by analyzing duplicate blank samples, which were obtained from each of the five field sites. No interference peak was observed around the retention time of acetamiprid and cypermethrin on chromatograms from the blank samples.

Table 2. Results of the Recovery and Quality Control Tests

crop (pesticide)	spike level, mg/kg	recovery (mean ± RSD[a]), % (n = 3)	recovery range of QC samples, %
apple-I	2	87 ± 1.8	78−87
(cypermethrin)	0.2[b]	87 ± 0.7	(n = 7)
	0.01[c]	86 ± 3.1	
apple-F	2	88 ± 2.4	70−82
(cypermethrin)	0.2[b]	81 ± 4.3	(n = 7)
	0.01[c]	101 ± 6.0	
broccoli	5	91 ± 1.7	85−88
(acetamiprid)	0.2[b]	87 ± 4.3	(n = 7)
	0.005[c]	86 ± 2.7	
cabbage	5	107 ± 1.4	99−104
(acetamiprid)	0.1[b]	107 ± 3.5	(n = 7)
	0.005[c]	103 ± 4.8	
cabbage	1	95 ± 3.8	70−82
(cypermethrin)	0.1[b]	79 ± 2.5	(n = 7)
	0.005[c]	80 ± 3.3	
grape	5	102 ± 3.0	91−103
(acetamiprid)	0.1[b]	102 ± 2.0	(n = 7)
	0.005[c]	102 ± 2.5	
grape	4	98 ± 2.1	72−76
(cypermethrin)	2	96 ± 2.2	(n = 7)
	0.1[b]	74 ± 2.1	
	0.005[c]	80 ± 5.1	
sweet pepper	2	101 ± 1.6	103−105
(cypermethrin)	0.5[b]	107 ± 2.2	(n = 5)
	0.01[c]	84 ± 5.3	

[a]RSD, relative standard deviation. [b]Spike levels same as the quality control (QC) samples. [c]Spike levels same as the limits of quantitation.

Accurate and consistent instrument performance was ensured using additional recovery samples (quality control samples spiked at 10 times the LOQ for acetamiprid or cypermethrin) and blank samples and by running a control after every 20 samples. All of the recoveries from a total of 56 additional recovery samples were within the acceptable range (70−120%). No interference peak was observed around the retention time of acetamiprid or cypermethrin on chromatograms from the 54 additional blank samples.

From the results described in this section, the residue analytical methods applied to this study were confirmed to provide adequate data sets for the evaluation of UPRL in relation to the RAC sampling size.

Distribution of Sample Weights and Pesticide Residues in Primary Data Sets. The field experimental data, which were used as primary data sets in this study, are summarized in Table 1. The mean values of individually

measured weights of 130 samples for each commodity ranged from 31 g for sweet pepper to 1330 g for cabbage. Representative frequency distributions of the sample weights of broccoli, grape, and sweet pepper are shown in Figure 1. Statistical results from

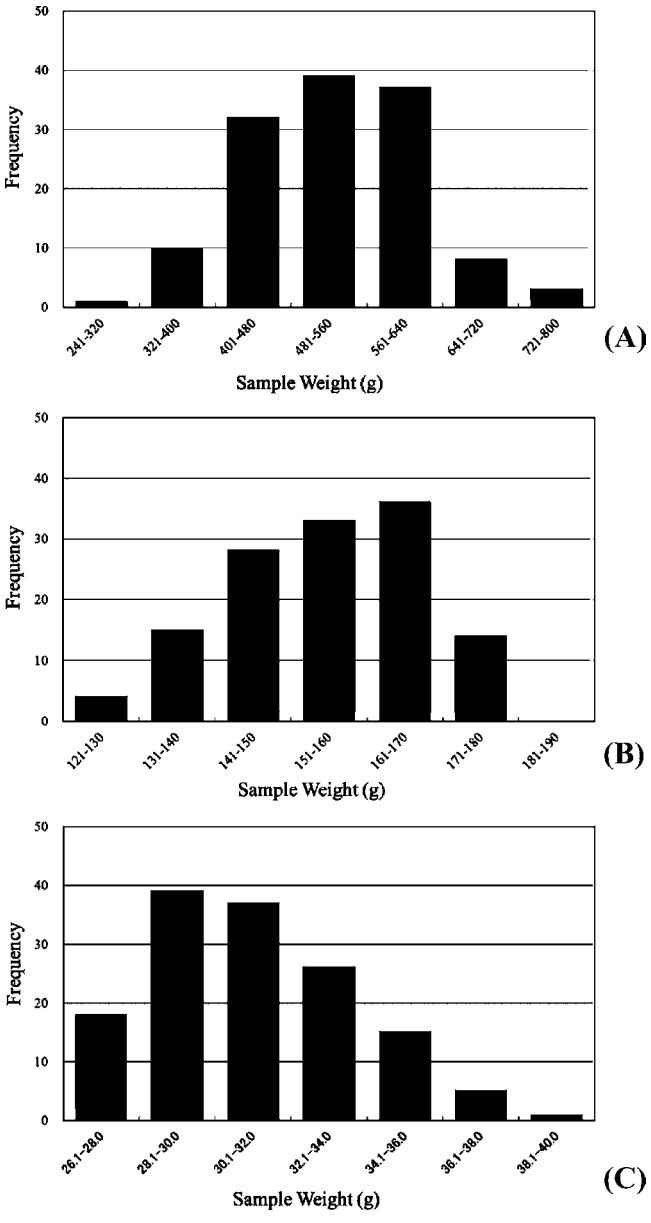

Figure 1. Representative frequency distributions of the sample weights of broccoli (A), grapes (B), and sweet peppers (C).

the Shapiro–Wilks test for the distribution of sample weights indicated that apple, broccoli, and cabbage samples exhibited a normal distribution. The distributions of the sample weights of grapes and sweet peppers were slightly skewed at the larger and smaller weights, respectively. The Shapiro–Wilks test revealed that the sample weights of grapes and sweet peppers were not normally distributed ($p < 0.01$), and the median weights of grapes and sweet peppers (155 and 30.8 g, respectively) were almost the same as their mean values (155 and 31.0 g, respectively).

The RSD of individually measured residue values in the primary data sets ranged from 24.7% for sweet pepper to 63.2% for cabbage (Table 3). Representative frequency distributions of acetamiprid residue levels in broccoli and cypermethrin residue levels in sweet pepper are shown in Figure 2. Residue levels were normally distributed in most primary data sets, except for cabbage.[8] The distributions of both acetamiprid and cypermethrin residues in cabbage were slightly skewed at the lower residue levels. The median residues of acetamiprid and cypermethrin in cabbage (0.116 and 0.056 mg/kg, respectively) were lower than their mean values (0.129 and 0.066 mg/kg, respectively).

The VFs of the residues in the tested RAC samples, the most conventional parameters for estimating individual pesticide residue variations for the risk assessment of dietary exposure like the ARfD, are shown in Table 1. The VFs ranged from 1.48 for cypermethrin in sweet peppers to 2.39 for cypermethrin in cabbage samples. The VFs in all tested commodities were clearly within the default VF value of 3, which was proposed by the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues.[19] The VFs are useful for the risk assessment of dietary exposure; however, they are not useful for estimating UPRL in relation to sampling size.

From the results described in this section, the individual pre-harvested residue data sets applied to this study were confirmed to adequately provide a representative frequency distribution of pesticide residue levels and sample weights for the evaluation of UPRLs in relation to the RAC sampling size.

Estimation of the UPRL in Relation to RAC Sampling Size. Representative scatter plots of the sample weights versus cypermethrin residues in grapes at various sampling sizes (individual values of the primary unit, $n = 2, 4, 8, 16, 32, 64$) are shown in Figure 3 (see also the Supporting Information). These scatter plots directly and visually express a simulation scenario corresponding to the UPRL in each sampling size of grape samples. The UPRL of acetamiprid and cypermethrin from five calculations versus the mean weights of each RAC sample are shown in Figure 4. The UPRLs were calculated from the mean number of randomly selected samples and related sample weights. The derived equations are shown in Table 3. The coefficients of correlation (γ^2) by log-normal power approximation were >0.8667 (cypermethrin residues in cabbage) for all of the tested commodities (Table 3). These results suggest that at least 8 RAC units would be necessary to obtain representative pesticide residue levels within 20% of the UPRL in RAC.

Codex MRLs for RAC take into account the maximum expected level to occur in a composite sample, which is derived from multiple units of the treated product and is intended to represent the average residue level.[28] The OECD Test Guidelines on Crop Field Trials specify that minimum field sampling sizes should be 12–24 units and should yield between 1 and 5 kg of sample.[20] The estimated UPRLs, which were calculated using the derived equations based on the regulatory sample weights, showed that the regulatory numbers ranged from at least 2.1% for cypermethrin in sweet pepper to 14.6% for cypermethrin in cabbage (Table 3).

In comparison with the supervised crop field trial described above, monitoring programs for market RAC samples are often applied to more convenient sampling sizes, because they have to analyze for multiclass pesticides in a wide range of agricultural commodities (including processed foods) and in huge numbers of samples. According to the Guides and Field Activities of the U.S. Food and Drug Administration, the sampling sizes of small and medium RACs are >1 kg (at least 10 units) and 2 kg for large commodities.[20] According to the Pesticide Data Program

Table 3. Estimated Residue Variations Based on the Sampling Size of Analyzed Samples

	numbers used for the calculation								UPRL at the regulatory sampling size[c]	
crop (pesticide)	primary[a]	$n = 2^b$	$n = 4^b$	$n = 8^b$	$n = 16^b$	$n = 32^b$	$n = 64^b$	derived eq		
apple-I	36.8%	25.7%	18.4%	12.1%	7.5%	4.5%	2.3%	$y = 25.166x^{-0.681}$	15.7%	**8.3**%
(cypermethrin)	0.425 kg	0.849 kg	1.70 kg	3.40 kg	6.79 kg	13.6 kg	27.2 kg	$\gamma^2 = 0.9721$	(2 kg)	(12 plants)
apple-F	34.1%	25.2%	18.2%	12.1%	7.6%	4.2%	2.3%	$y = 24.65x^{-0.704}$	15.1%	**7.9**%
(cypermethrin)	0.416 kg	0.832 kg	1.66 kg	3.33 kg	6.66 kg	13.3 kg	26.6 kg	$\gamma^2 = 0.9096$	(2 kg)	(12 plants)
broccoli	32.9%	22.7%	15.8%	10.8%	7.8%	5.5%	2.2%	$y = 25.113x^{-0.618}$	16.4%	**8.1**%
(acetamiprid)	0.52 kg	1.04 kg	2.09 kg	4.18 kg	8.36 kg	16.7 kg	33.4 kg	$\gamma^2 = 0.9424$	(2 kg)	(12 plants)
cabbage	43.4%	30.4%	20.9%	14.0%	9.7%	5.8%	2.0%	$y = 71.925x^{-0.741}$	21.8%	**9.2**%
(acetamiprid)	1.33 kg	2.66 kg	5.32 kg	10.6 kg	21.3 kg	42.6 kg	85.1 kg	$\gamma^2 = 0.895$	(5 kg)	(12 plants)
cabbage	63.2%	43.2%	31.0%	21.7%	15.3%	10.4%	3.4%	$y = 97.597x^{-0.687}$	32.3%	**14.6**%
(cypermethrin)				same as above				$\gamma^2 = 0.8667$	(5 kg)	(12 plants)
grape	43.3%	30.9%	22.0%	15.7%	11.0%	7.4%	2.5%	$y = 16.033x^{-0.678}$	16.0%	**10.5**%
(acetamiprid)	0.15 kg	0.31 kg	0.62 kg	1.24 kg	2.47 kg	4.95 kg	9.90 kg	$\gamma^2 = 0.8713$	(1 kg)	(12 bunches)
grape	32.6%	22.9%	16.0%	10.7%	7.3%	4.8%	1.7%	$y = 11.356x^{-0.696}$	11.4%	**7.4**%
(cypermethrin)				same as above				$\gamma^2 = 0.9116$	(1 kg)	(12 bunches)
sweet pepper	24.7%	17.5%	12.6%	8.9%	5.8%	3.4%	1.9%	$y = 3.2663x^{-0.635}$	**2.1**%	3.9%
(cypermethrin)	0.031 kg	0.062 kg	0.124 kg	0.248 kg	0.496 kg	0.993 kg	1.99 kg	$\gamma^2 = 0.9387$	(2 kg)	(24 plants)

[a]Relative standard deviations (RSDs) from 130 individual measurement values in primary units are given on the top row, and their corresponding mean sample weights are given on the bottom. [b]RSD values calculated from the mean number (n) of randomly selected samples are given on the top rwo, and their corresponding overall mean sample weights are given on the bottom. [c]The uncertainty in pesticide residue level (UPRL) at the regulatory sample weights and numbers are listed in the left column, and the lower values are expressed in boldface. The regulatory sampling size for each commodity according to the OECD Test Guideline No. 509[19] is expressed in parentheses.

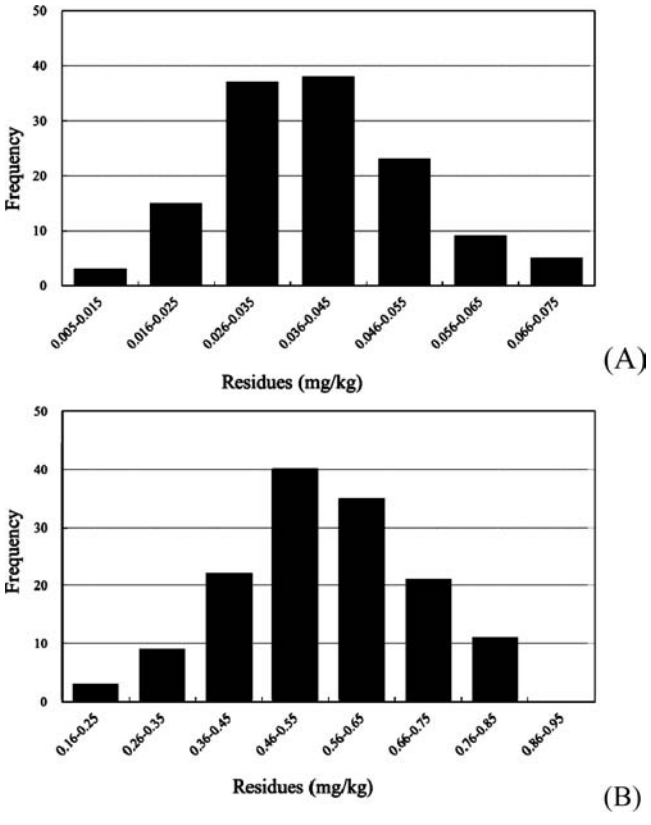

Figure 2. Representative frequency distributions of acetamiprid residues in broccoli (A) and cypermethrin residues in sweet peppers (B).

Annual Summary in 2009 reported by the U.S. Department of Agriculture, the sampling sizes of RAC are weighed at ca. 1.36 or 2.27 kg.[22] According to the annual monitoring plan of the Hyogo prefectural government in Japan, the sampling sizes of RAC are >1 kg with at least 5 units.[23] On the basis of the results from this study, the sampling sizes in the regulatory monitoring programs are almost suitable for enforced MRL.

Predicted Percentages of False Positives Based on Sampling Size. All of the mean residue values of acetamiprid and cypermethrin in the tested RAC samples were lower than the MRLs specified by the Japanese Food Sanitation Law. Individual residues were also lower than the MRLs, except for those of cypermethrin in grapes (Figure 3). The mean cypermethrin residue value in grape was 1.87 mg/kg, which was close to the MRL of 2 mg/kg. In grapes, 38% of individual cypermethrin residues exceeded the MRL. This higher residue level may be due to different growing conditions for the other RAC test samples compared to conventional orchards or fields without housing. In this study, only grapes were grown in greenhouses, thereby offering various degrees of protection against pesticide degradation and dilution from sunlight and/or rain.

The percentages of cypermethrin residues in grapes, which exceeded the MRL at various sampling sizes (individual values in the primary unit, $n = 2^x$, where $x = 1-6$) are shown as white bars in Figure 5. The predicted percentages of exceeded MRLs using the single-check procedure at the regulatory sampling size of 1 kg or 12 bunches are 31 and 24%, respectively. Samples that exceed the Japanese Food Sanitation Law MRLs are prohibited from market distribution. If a suspicious violation occurs during inspection surveys, a re-examination is conducted to confirm the

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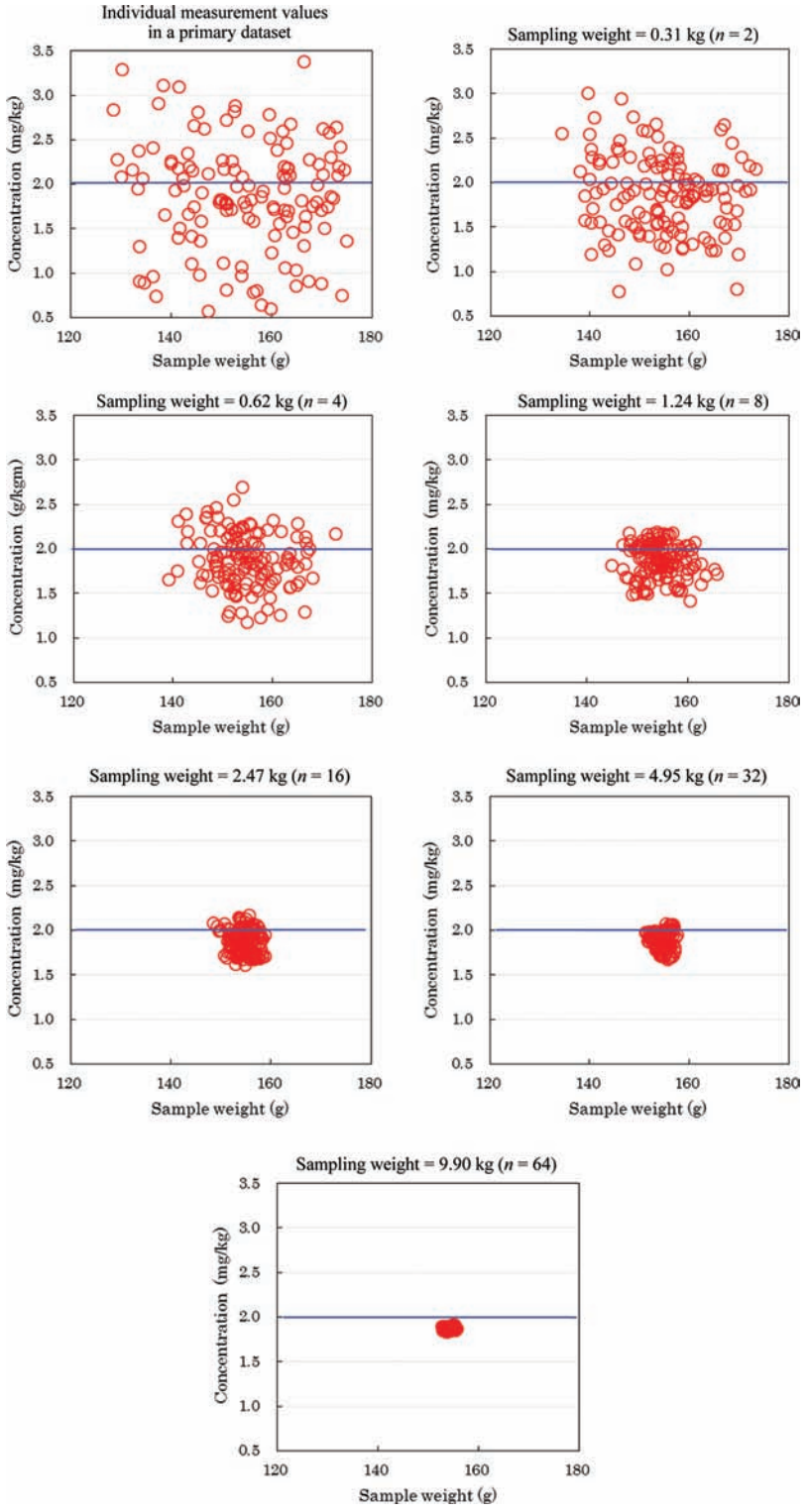

Figure 3. Scatter plots of sample weights versus cypermethrin residues in grapes. The blue line represents the MRL at 2 mg/kg in accordance with the Japanese Food Sanitation Law.

results.[23] The predicted percentages that exceeded the MRL for cypermethrin in grapes of various sampling sizes using the double-check procedure are also shown as black bars in Figure 5. Hence, use of the double-check procedure for a predicted percentage exceeding the MRL at the regulatory sampling size of 1 kg or 12 bunches dramatically reduced the false-positive risk by 10 or 6%, respectively.

It is important to note that the VFs of the data sets used in this study were calculated from supervised field data. The Scientific Panel on Plant Health of the European Food Safety Authority has compared field and market monitoring results and found that the estimated VFs for market monitoring tend to be higher and more variable than those of supervised field trials.[15] The predicted percentages of "false positives" for

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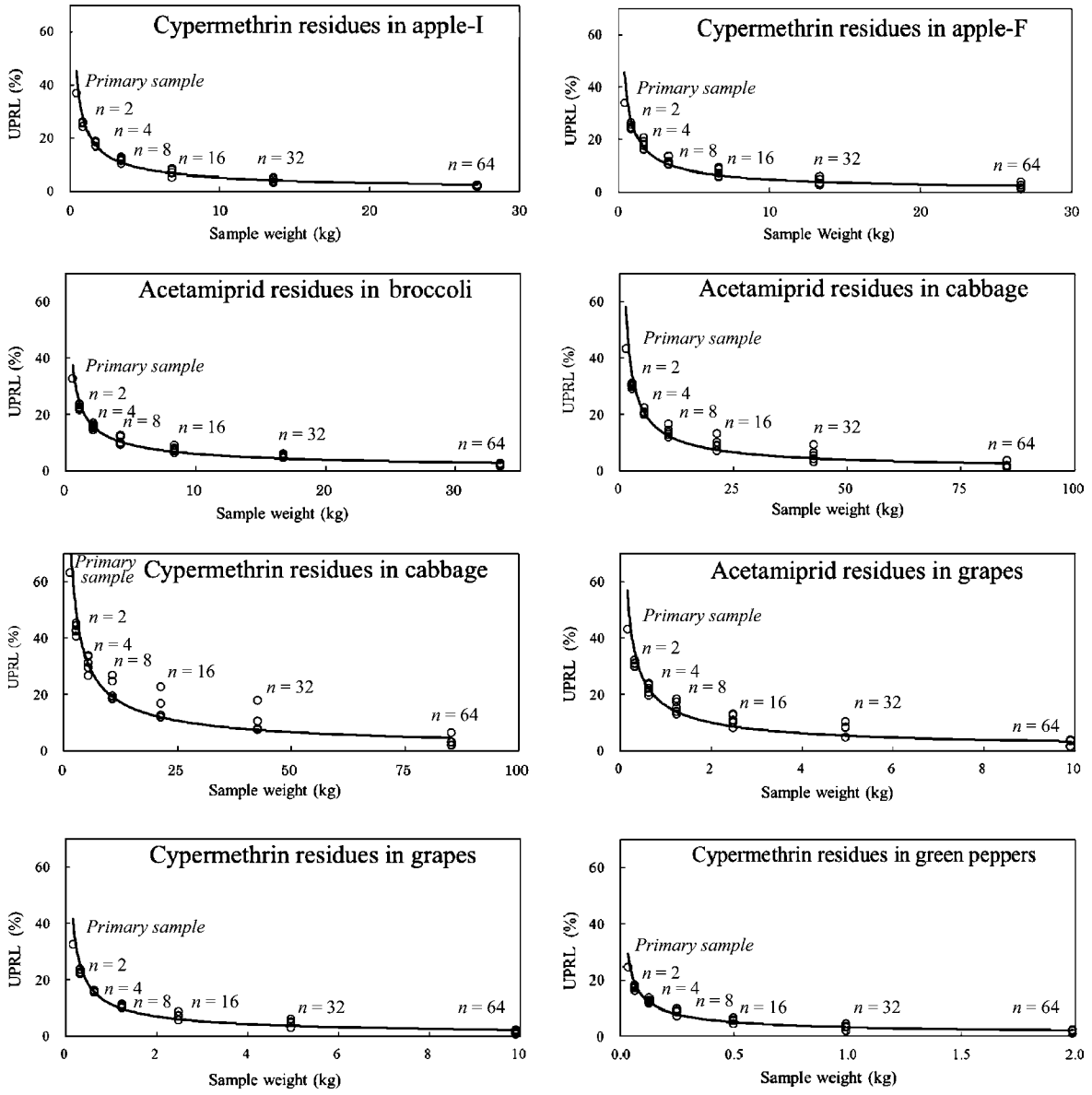

Figure 4. Plots of the uncertainty in pesticide residue levels (UPRL) of acetamiprid and cypermethrin from five calculations versus the corresponding weights of the preharvested apple, broccoli, cabbage, grape, and sweet pepper samples.

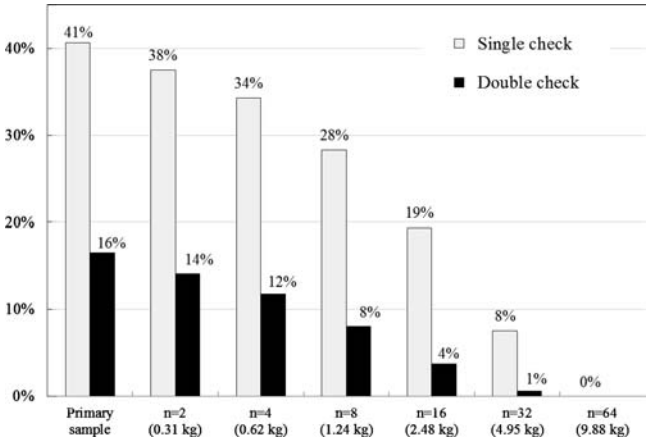

Figure 5. Predicted percentages exceeding the MRL for cypermethrin residues in grapes based on the sampling size.

residues compared to the MRLs are higher in the market monitoring surveys than in the field trials.

Conclusion. The results of this study indicate that a sufficient sampling size is required to obtain accurate analytical results for MRLs and to confirm that the residual pesticide levels in tested commodities are below the MRLs. The estimated results have confirmed that regulatory sampling sizes are suitable for residue analysis, providing accurate values of pesticide residue levels. The double-check procedure using subsamples improved the accuracy of pesticide residue analysis. In addition, the equation derived from the present study would be helpful for estimating residue levels with greater accuracy even from small sampling sizes, although further investigations should be performed.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional information regarding the scatter plots of acetamiprid and cypermethrin residues in the other commodities (S1) and a

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summary of the single analytical methods for acetamiprid in broccoli (S2; unpublished data); representative chromatograms of acetamiprid in broccoli and cypermethrin in sweet peppers (S3; unpublished data). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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