Influence of various parts of sweet corn ears on pesticide residue levels

Tomonari Yajima,* Masahiro Fujita, Kazuaki Iijima, Kiyoshi Sato and Yasuhiro Kato

The Institute of Environmental Toxicology (IET), 4321 Uchimoriya-machi, Joso-shi, Ibaraki 303–0043, Japan

(Received January 15, 2017; Accepted March 21, 2017)

Pesticide residue levels in various parts of sweet corn ears were analyzed. For this purpose, five pesticides were sprayed on corn in two different fields, and the harvested samples were separated into four portions, namely kernels, cobs, silks, and husks. Each of these portions was then separately analyzed. Pesticide residues were predominantly distributed in the silk and husk portions, which constituted ≥91% of the whole crop, whereas relatively minimal residues remained in the kernel and cob portions. Further, residue distributions in the silks and husks were found to differ between the two fields. The calculated residue levels in kernels with the cob and silk were obviously higher than the residue levels in the kernel alone (max. >62 times different). This result suggests that the silk portion could greatly affect pesticide residue levels in the edible portion of corn.

Keywords: analytical portion, sweet corn, kernel, cob, silk, husk.

Electronic supplementary materials: The online version of this article contains supplementary material (Supplemental Tables S1, S2, S3 and S4), which is available at http://www.jstage.jst.go.jp/browse/jpestics/.

Introduction

Standardizing the analytical portions of raw agricultural commodities that should be analyzed for pesticide residues (henceforth referred to as the analytical portion) is one of the most important tasks for facilitating international trade and effectively utilizing pesticide residue data. Japan has been one of the largest corn importing countries in the world in recent years. Kernels of sweet corn are edible and are sold in retail markets as various commodities such as fresh ears of corn with the husk, corn on or off the cob without the husk, and frozen or canned corn. Therefore, laboratory samples of sweet corn would be different depending on the analytical objectives.

Further, definitions of the analytical portion for sweet corn are different in different test guidelines and are sometimes unclear, especially regarding whether to include the silk or cob. According to the enforcement of Japanese regulations for maximum residue limits (MRLs), the analytical portion of corn (maize, including popcorn and sweet corn) is defined as "kernels without husk, silk, or cob." On the other hand, according to the FAO manual, the analytical portion of fresh corn and sweet corn is defined as "kernels plus cob without husk." There are also no clear guidelines as to whether silk and cob are included or not in the analytical portion under international standards.

Many papers estimate the levels of pesticide residues in sweet corn. However, the analytical portions in those investigations included any one part of an ear of corn. Not much data is available on evaluating pesticide residue levels in different analytical portions—such as kernels, cob, silk, and husk—of one ear of sweet corn. Particularly, we were not able to find pesticide residue data for the silk portions of sweet corn. Because silks are such a small part of the weight of the whole commodity, they might not be recognized as an important analytical portion of sweet corn. Therefore, we could not estimate the influence of different parts of sweet corn ears on pesticide residue levels.

In this context, the present study was undertaken to understand the influence of different analytical portions on determining pesticide residues in sweet corn ears. Test samples were obtained from two test fields in Japan that had been sprayed with five types of pesticide formulations registered in Japan. The pesticides were selected to cover a wide range of physicochemical properties. For example, acephate, an organophosphate insecticide, has a relatively low log $P_{OW}$ of −0.89 and a relatively high water solubility of 790 g/L. In contrast, etofenprox, a synthetic pyrethroid insecticide, has a relatively high log $P_{OW}$ of 6.9 and relatively low water solubility of 22.5 µg/L. Three other pesticides, acetamiprid, chromafenozide, and tolclofos-methyl, are insecticides or fungicides with intermediate log $P_{OW}$ and water solubility properties. Harvested sweet corn ears were separated into kernel, cob, silk, and husk portions, and the samples were separately analyzed. This investigation provides valuable information on the influence of different analytical portions on pesticide residue levels in sweet corn ears.

* To whom correspondence should be addressed.
E-mail: yajima@iet.or.jp
Published online #M# #D#, 2017
© Pesticide Science Society of Japan
Materials and Methods

1. Field experiments

Field experiments were conducted at Ibaraki and Chiba fields of the Japan Plant Protection Association in accordance with the Japanese Guidelines for Crop Field trials. Five pesticides were applied in the cornfields at the maximum label rates, maximum number of applications, and minimum preharvest intervals (PHIs). After dilution with water in ratios ranging from 1:2000 to 1:1000, the pesticides were sprayed two to four times, with approximately 7-day intervals between applications. Pesticides were sprayed with a tank-mix combination of Ortran® wettable powder composed of acephate, 50.0% a.i. (Arysta LifeScience Corporation, Tokyo, Japan); Mospilan® emulsifiable concentrate (80%), water soluble powder of acetamiprid, 20.0% a.i. (Nippon Soda Co., Ltd., Tokyo, Japan); Matric® flowable of chromafenozide, 5.0% a.i. (Nippon Kayaku Co., Ltd., Tokyo, Japan); Trebon® emulsifiable concentrate of etofenprox, 20.0% a.i. (Mitsui Chemicals Agro, Inc., Tokyo, Japan); and Rizolex® wettable powder of tolclofos-methyl, 50.0% a.i. (Sumitomo Chemical Co., Ltd., Tokyo, Japan); using backpack sprayers with corn nozzles. The application rates ranged from 200 to 286 L/ha.

The detailed pesticide application schedule is summarized in Table 1. Pesticide applications started 28 days before harvest, which is the tassel stage of corn growth. On the other hand, the silking stage of corn growth is approximately 21 days before harvest. The final applications of Ortran® were kept frozen at −20°C until analysis. The frozen samples were then homogenized using a Blixer-5Plus (Robot Coupe, Vincennes Cedex, France) or GM-200 (Verder Scientific Co., Ltd., Tokyo, Japan) before analysis.

2. Sample preparation

After measuring the sample weight, the sweet corn ears were separated into kernels, cobs, silks, and husks. These samples were kept frozen at −20°C until analysis. The frozen samples were then homogenized using a Blixer-5Plus (Robot Coupe, Vincennes Cedex, France) or GM-200 (Verder Scientific Co., Ltd., Tokyo, Japan) before analysis.

Table 1. Information on pesticide application

| Pesticides       | log \( P_{ow} \) | Dilution factors | Application \( a \) |
|------------------|------------------|------------------|---------------------|
|                  |                  |                  | 1-d                 |
| Acetate          | −0.89            | 1000 times       | ○                   |
| Acetamiprid      | 0.80             | 2000 times       | ○                   |
| Chromafenozide   | 2.7              | 2000 times       | ○                   |
| Etofenprox       | 6.9              | 2000 times       | ○                   |
| Toclofos-methyl  | 4.56             | 1500 times       | ○                   |
|                  |                  |                  | 7-d                 |
| Acetate          | −0.89            | 1000 times       | ○                   |
| Acetamiprid      | 0.80             | 2000 times       | ○                   |
| Chromafenozide   | 2.7              | 2000 times       | ○                   |
| Etofenprox       | 6.9              | 2000 times       | ○                   |
| Toclofos-methyl  | 4.56             | 1500 times       | ○                   |
|                  |                  |                  | 14-d                |
| Acetate          | −0.89            | 1000 times       | ○                   |
| Acetamiprid      | 0.80             | 2000 times       | ○                   |
| Chromafenozide   | 2.7              | 2000 times       | ○                   |
| Etofenprox       | 6.9              | 2000 times       | ○                   |
| Toclofos-methyl  | 4.56             | 1500 times       | ○                   |
|                  |                  |                  | 21-d                |
| Acetate          | −0.89            | 1000 times       | ○                   |
| Acetamiprid      | 0.80             | 2000 times       | ○                   |
| Chromafenozide   | 2.7              | 2000 times       | ○                   |
| Etofenprox       | 6.9              | 2000 times       | ○                   |
| Toclofos-methyl  | 4.56             | 1500 times       | ○                   |
|                  |                  |                  | 28-d                |
| Acetate          | −0.89            | 1000 times       | ○                   |
| Acetamiprid      | 0.80             | 2000 times       | ○                   |
| Chromafenozide   | 2.7              | 2000 times       | ○                   |
| Etofenprox       | 6.9              | 2000 times       | ○                   |
| Toclofos-methyl  | 4.56             | 1500 times       | ○                   |

\( a \) The day refers to the time until harvest.

3. Residue analysis

The residue analysis methods were optimized to allow the rapid analysis of the five pesticides in each portion of the sweet corn ears, as described below.

3.1. Chemicals and reagents

Analytical standards (purity ≥99.1%) of acephate, acetamiprid, chromafenozide, etofenprox, and tolclofos-methyl were purchased from Kanto Chemical (Tokyo, Japan) and Wako Pure Chemical Industries (Osaka, Japan). Pesticide analysis-grade acetone, acetoniitrite, and toluene; LC-MS-grade acetonitrile and analytical-grade ammonium acetate were purchased from Wako Pure Chemical Industries. Water used for the experiments was purified with a Milli-Q system (Merck KGaA, Darmstadt, Germany).

Standard stock solutions (200 mg/L) of each pesticide were separately prepared with acetonitrile. The acetamiprid and chromafenozide stock solutions were diluted with acetonitrile/water (80:20, v/v) to obtain standard solutions with concentrations in a range of 0.05–2 µg/L. On the other hand, the etofenprox and tolclofos-methyl stock solutions were diluted with acetone to obtain standard solutions with concentrations in a range of 1–40 µg/L. The acetate stock solution was diluted with acetonitrile/water (80:20, v/v) to obtain standard solutions with concentrations in a range of 0.25–10 µg/L. These standard solutions were used to prepare LC-MS/MS or PTV-GC-MS calibration curves.

3.2. Extraction

For kernels, cobs, and husks, 20 g of the homogenized sample was weighed into an Erlenmeyer flask and extracted with 100 mL of acetone by shaking for 30 min using a reciprocal shaker. The mixture was filtered by vacuum suction, and the residual cake was washed with 50 mL of acetone and filtered again. The filtrates were combined with acetone to a volume of 200 mL.

For analyzing the corn silk, 10 g of the homogenized sample was weighed into an Erlenmeyer flask and extracted with 70 mL of acetone using a Polytron® disperser (PT3100, Kinematica AG, Luzern, Switzerland). The shaft of the disperser was washed with 30 mL of acetone, and the washing solution was combined with the dispersed sample. The mixture was shaken for 30 min using a reciprocal shaker. The extract was filtered, and the residual cake was washed with 50 mL of acetone, after which it was filtered again. The filtrates were combined and acetone added to a

---

**Table 1.** Information on pesticide application

| Pesticides       | log \( P_{ow} \) | Dilution factors | Application \( a \) |
|------------------|------------------|------------------|---------------------|
|                  |                  |                  | 1-d                 |
| Acetate          | −0.89            | 1000 times       | ○                   |
| Acetamiprid      | 0.80             | 2000 times       | ○                   |
| Chromafenozide   | 2.7              | 2000 times       | ○                   |
| Etofenprox       | 6.9              | 2000 times       | ○                   |
| Toclofos-methyl  | 4.56             | 1500 times       | ○                   |
|                  |                  |                  | 7-d                 |
| Acetate          | −0.89            | 1000 times       | ○                   |
| Acetamiprid      | 0.80             | 2000 times       | ○                   |
| Chromafenozide   | 2.7              | 2000 times       | ○                   |
| Etofenprox       | 6.9              | 2000 times       | ○                   |
| Toclofos-methyl  | 4.56             | 1500 times       | ○                   |
|                  |                  |                  | 14-d                |
| Acetate          | −0.89            | 1000 times       | ○                   |
| Acetamiprid      | 0.80             | 2000 times       | ○                   |
| Chromafenozide   | 2.7              | 2000 times       | ○                   |
| Etofenprox       | 6.9              | 2000 times       | ○                   |
| Toclofos-methyl  | 4.56             | 1500 times       | ○                   |
|                  |                  |                  | 21-d                |
| Acetate          | −0.89            | 1000 times       | ○                   |
| Acetamiprid      | 0.80             | 2000 times       | ○                   |
| Chromafenozide   | 2.7              | 2000 times       | ○                   |
| Etofenprox       | 6.9              | 2000 times       | ○                   |
| Toclofos-methyl  | 4.56             | 1500 times       | ○                   |
|                  |                  |                  | 28-d                |
| Acetate          | −0.89            | 1000 times       | ○                   |
| Acetamiprid      | 0.80             | 2000 times       | ○                   |
| Chromafenozide   | 2.7              | 2000 times       | ○                   |
| Etofenprox       | 6.9              | 2000 times       | ○                   |
| Toclofos-methyl  | 4.56             | 1500 times       | ○                   |

\( a \) The day refers to the time until harvest.
volume of 200 mL.

3.3. Cleanup

3.3.1. Acephate

A portion of the extract (10 mL as 1 g of the samples of kernel, cob, and husk portions, or 20 mL as 1 g of the sample of the corn silk portion) was cleaned using solid-phase extraction with a styrene-divinylbenzene copolymer (SDB) cartridge (1 g/6 mL, InertSep PLS-2; GL Science Inc., Tokyo, Japan, conditioned with acetonitrile and water) and a PSA cartridge (500 mg, InertSep Slim PSA; GL Science Inc., conditioned with acetonitrile and water). The extract was diluted with 5 mL of water and concentrated to a volume below 5 mL, using a rotary evaporator, by removing the organic solvent. The concentrated extract was loaded onto the SDB cartridge, following which the PSA cartridge was connected to the outlet of the SDB cartridge. Ten mL of acetonitrile/water (40:60, v/v) was passed through tandem SDB-PSA cartridges, and the eluate was collected in a measuring flask. The eluate was increased to 20 mL with water, and diluted with acetonitrile/water (20:80, v/v) as needed. An aliquot (5 µL) of the diluted test solution was injected into the LC–MS/MS system.

3.3.2. Acetamiprid and chromafenozide

A portion of the extract (1 mL as 0.1 g of the samples of kernel, cob and husk portions, or 2 mL as 0.1 g of the sample of the corn silk portion) was diluted with water (10 mL) and loaded onto a graphite carbon black cartridge (0.5 g/6 mL, InertSep GC; GL Science Inc., conditioned with acetonitrile and water) for solid-phase extraction. After washing the cartridge with 10 mL of acetonitrile/water (20:80, v/v), 10 mL of acetonitrile/water (80:20, v/v) was passed through the cartridge, and the eluate was collected in a measuring flask. The eluate was increased to 20 mL with acetonitrile/water (80:20, v/v) and further diluted with the mixture, as necessary. An aliquot (5 µL) of the test solution was injected into the LC–MS/MS system.

3.3.3. Etofenprox and tolclofos-methyl

A portion of extract (5 mL as 0.5 g of the samples of kernel, cob, and husk portions, or 10 mL as 0.5 g of the sample of the corn silk portion) was diluted with water (5 mL), and the mixture was loaded onto the graphite carbon black cartridge for solid-phase extraction. The cartridge was washed with 10 mL of acetonitrile/water (80:20, v/v). After suction of the cartridge for 1 min, 10 mL of acetonitrile/toluene (75:25, v/v) was passed through the cartridge, and the eluate was collected in a round-bottom flask. The eluate was evaporated to a dry state under a gentle stream of nitrogen, and the residue was dissolved in a suitable volume of acetone. An aliquot (5 µL) of the diluted test solution was then injected into the PTV-GC-MS system.

3.4. LC–MS/MS analysis

A liquid chromatography (LC, Model 1290 Infinity Pumping System; Agilent Technologies, Inc., CA, USA)-tandem mass spectrometer (MS/MS, Model 6460 Triple Quadrupole Tandem Mass Spectrometer; Agilent Technologies, Inc.) equipped with an electrospray interface (ESI) operating in the positive ion mode was used to determine the acephate, acetamiprid, and chromafenozide. The data were processed with Agilent MassHunter software.

The LC-MS/MS measurement conditions for acephate were as follows. LC separation was performed on an Atlantis dC18 (150 mm × 2.1 mm i.d., 3 µm; Waters Co., MA, USA) at 40 °C. Acetonitrile and a 5 mM ammonium acetate aqueous solution were used as the mobile phase at a flow rate of 0.3 mL/min. In the gradient elution analysis, the initial mobile phase consisted of 2% acetonitrile (held for 4 min), after which the amount of acetonitrile increased linearly to 50% for 4 min. The retention time was 5.5 min. The MS/MS parameters were as follows: capillary voltage, 2000 V; nebulizer gas, 45 psi; drying gas flow rate, 10 L/min (350°C); sheath gas flow rate, 121 L/min (400°C); and fragmentor voltage, 50 V. Nitrogen was used as the collision gas at 1 eV. The monitoring ions (precursor ion→product ion) in the multiple-reaction monitoring mode had an m/z of 184.0→143.1.

The LC-MS/MS conditions for acetamiprid and chromafenozide were as follows. LC separation was performed on a ZORBAX Eclipse Plus C18 (100 mm × 2.1 mm i.d., 1.8 µm; Agilent technologies, Inc.) at 40°C. Acetonitrile and a 5 mM ammonium acetate aqueous solution were used as the mobile phase at a flow rate of 0.3 mL/min. In the gradient elution analysis, the initial mobile phase consisted of 20% acetonitrile (held for 1 min), after which the amount of acetonitrile increased linearly to 90% for 4 min. Retention times for acetamiprid and chromafenozide were 3.2 min and 5.4 min, respectively. The MS/MS parameters were as follows: capillary voltage, 3500 V; nebulizer gas, 45 psi; drying gas flow rate, 5 L/min (300°C); sheath gas flow rate, 11 L/min (400°C); and fragmentor voltage, 100 V (for each compound). Nitrogen was used as the collision gas at 15 eV (for acetamiprid) and 10 eV (for chromafenozide). The monitoring ions (precursor ion→product ion) in the multiple-reaction monitoring mode had an m/z of 223.0→126.1 for acetamiprid, and an m/z of 395.2→175.1 for chromafenozide.

3.5. PTV-GC-MS analysis

A gas chromatography (GC, Model 6890N; Agilent Technologies, Inc.)-mass spectrometer (MS, Model 5973 inert; Agilent Technologies, Inc.) equipped with a programmed temperature vaporization inlet system (PTV system, Model Optic 3; GL Sciences B.V., Eindhoven, The Netherlands) and a multifunctional auto injector (Model Focus; GL Sciences B.V.) was used to determine etofenprox and tolclofos-methyl. The data were processed using Agilent ChemStation software.

A multifunctional auto injector was employed twice for sequential injection during one measured cycle. In the case of the injected sample test solution, 5 µL of the test solution was injected following 5 µL of acetone. In the case of the injected matrix-matched standard, 5 µL of the standard solution was injected following 5 µL of the blank sample solution. The GC conditions were as follows. GC separation was performed on an HP-5 ms (length 30 m, 0.25 mm i.d., film 0.25 µm; Agilent Technologies, Inc.). The helium carrier gas flow was constant at 1.0 mL/min, and the oven temperature program was 70°C–20°C/min–300°C (held for 3.5 min). The PTV system temperature program was
66°C (held for 1.5 min)–5°C/sec–250°C. The transfer line temperature was set to 300°C. Injection was performed using the at-column method. Retention times for etofenprox and tolclofos-methyl were 12.9 min and 8.7 min, respectively. The MS parameters were as follows: ion source, electron impact (EI); ionization energy, 70 eV; and ion source temperature, 250°C. The monitoring ions in the selected ion monitoring mode had an \( m/z \) of 163 or 376 for etofenprox and an \( m/z \) of 265 for tolclofos-methyl.

**Results and Discussion**

1. **Validity of the analytical method**

Results of the recovery and stability tests for the kernel, cob, silk, and husk portions of the sweet corn ears are summarized in Supplemental Tables S1, S2, S3, and S4, respectively. The accuracy and precision of the analytical methods were evaluated by recovery tests, which were conducted using samples at more than two spiked levels, including a level at a limit of quantification of 0.01 mg/kg and a level above the maximum residue level. The mean recoveries of the spiked samples measured in triplicate or sixfold ranged from 76% to 103%, and their relative standard deviations were \( \leq 7.4\%\). The specificity of the analytical method was evaluated by analyzing duplicate blank samples, which were obtained from both test fields. No interference peak was observed around the retention time of each pesticide on chromatograms from the blank samples.

Stability tests were conducted at a fortification level of 0.1 mg/kg, with the samples stored for 76 (Ibaraki) or 88 (Chiba) days at \(-20°C\). Recoveries from the stability samples \( (n=60)\) were proven to be within the acceptable range of 70–120%, except in the case of corn silk. The stability tests for the corn silk samples could not be performed due to running out of the blank sample. Furthermore, although we attempted to obtain reliable residue data for methamidophos, which is a major metabolite of acephate, we were unsuccessful because of this component's poor recoveries in stability tests (19–31%, \( n=12\)).

From the results presented in this section, the analytical methods used in this study were confirmed to generate adequate data to meet the current study objectives.

2. **Information on the sweet corn samples**

Honey-Bantam and Gold-Rush varieties of sweet corn plants were cultivated in the Ibaraki and Chiba fields, respectively. The mean weights of whole sweet corn ears were similar in the two fields, at 312 g (4.67 kg/15 ears) in the Ibaraki field and 313 g (9.38 kg/30 ears) in the Chiba field. Furthermore, the weight ratios of the various analytical portions of the sweet corn ears were also similar for the samples from the two fields (kernel/cob/silk/husk = 56:27:2:15 in the Ibaraki field samples, and 56:25:3:17 in the Chiba field samples).

The appearance of corn ears harvested from both test fields was similar to that commonly seen in the retail market. However, there was an apparent difference in the length of the corn silks (Fig. 1) between samples harvested from the two sites. The silks of the Ibaraki field samples were longer than those of the Chiba samples and protruded from the tops of the corn ears. On the other hand, the silks of the Chiba field samples almost wrapped around their husks and appeared slightly from the top of the corn ears. This difference may be due to weather conditions during the growing stage of the sweet corn plants in both fields. The corn silks emerge after the tassel stage (flowering of staminate flowers of corn) and participate in cross-pollination by wind. The corn silks are functional stigmas of the female flowers, whose bottoms are ovules, which develop into kernels on the cob. The silks elongate by as much as 3.8 cm per day during the first few days and continue to elongate until the pollen grains are captured. The length of the corn silk depends on the duration of the silking stage until pollination, which is influenced by field conditions such as wind intensity or wind direction. Therefore, the length of silks in the corn ear units varies widely. Except for the length of corn silks, there were no differences in form and information from the two test fields, including weather conditions.

3. **Measured residue data**

The residue data obtained from the kernel, cob, silk, and husk portions of the sweet corn ears are summarized in Table 2. Four of the five pesticides were not detected in the kernel portion, which is the edible portion of the sweet corn ears. Only the insecticide acephate was detected in the kernel portion. Acephate
is the most polar pesticide of those analyzed in this study. The residue level of acephate was 0.02 or 0.07 mg/kg in the kernel portion of the sweet corn samples, which is far below the Japanese MRL (for acephate; 0.5 mg/kg), as specified by the Japanese Food Sanitation Law.13)

Three of the five pesticides were not detected in the cob portions of the sweet corn ears. Acephate and acetamiprid, which are relatively popular among the pesticides analyzed in this study, were detected in the cob portions. The residue level of acephate was 0.03 or 0.08 mg/kg in the cob portion, which was similar to the levels in the kernel portions.

All pesticides examined were detected in the corn silk portions, except for tolclofos-methyl in the Chiba field samples. The residue levels of the pesticides in the corn silk samples ranged from <0.01 to 25.9 mg/kg. The residue levels in the corn silk portions from the Ibaraki field were obviously higher than those in the Chiba field samples.

All pesticides investigated were also detected in the husk portions, except for tolclofos-methyl in the Ibaraki field sample. The pesticide residue levels in the husk portions ranged from <0.01 to 5.20 mg/kg. The residue levels in the husk portions from the Chiba field were higher than those in the Ibaraki field sample.

From the results described in this section, the individual pre-harvested residue data sets obtained in this study were considered to adequately provide representative pesticide residue data for sweet corn ears, according to normal Japanese agricultural practices.

4. Overview of residue data

The total residue levels of the five pesticides in the whole corn ears are shown in Fig. 2. The majority of pesticide residues were detected in the silk and husk portions (≥91% of the whole crop), while relatively minimal fractions of the residues were detected in the kernel portions, which are the edible parts of sweet corn ears. There was no significant difference between samples from the Ibaraki and Chiba fields in terms of total residue levels for each pesticide in whole corn ears. This result indicates that the pesticides were applied to the corn plants in both test fields in a similar manner. On the other hand, there was a significant difference between the samples from the two fields in terms of the residue levels in the corn silk and husk portions. This result suggests that the relatively long silks of the Ibaraki samples effectively captured the sprayed pesticides as compared to the Chiba field samples.

Furthermore, the residue levels of tolclofos-methyl were lower than those of the other four pesticides in all analyzed parts of the corn ears because this pesticide had the longest interval between application and harvest. The long interval allowed pesticide degradation and/or dilution during the rapid growth of the corn ears after the silking stage.

The results in this section suggest that differences in the residue levels of the five pesticides in the whole corn ears from Ibaraki field (left bar for each pesticide) and Chiba field (right bar for each pesticide). The relative bar length for each analytical portion indicates the distribution of pesticide residues in the various portions.

Table 2. Pesticide residue levels in the kernel, cob, silk, and husk portions of the sweet corn samples

| Field location | Pesticide | Measured residue levels | Calculated residue levels$^a$ |
|---------------|-----------|-------------------------|-------------------------------|
|               | kernel (K) | cob (C) | silk (S) | husk | K+C | K+C+S |
| Ibaraki       | Acephate   | 0.07   | 0.08   | 14.2 | 2.24 | 0.07 | 0.41 |
|               | Acetamiprid| <0.01  | 0.03   | <0.01| 25.9 | 2.80 | 0.01 | 0.62 |
|               | Chromafenozide| <0.01  | <0.01  | 8.96 | 1.08 | <0.01| 0.22 |
|               | Etofenprox | <0.01  | <0.01  | 12.0 | 0.79 | <0.01| 0.29 |
|               | Tolclofos-methyl| <0.01  | <0.01  | 0.05 | <0.01| <0.01|<0.01|
| Chiba         | Acephate   | 0.02   | 0.03   | 1.14 | 4.56 | 0.02 | 0.06 |
|               | Acetamiprid| <0.01  | 0.02   | 0.56 | 5.20 | 0.01 | 0.03 |
|               | Chromafenozide| <0.01  | <0.01  | 0.15 | 1.76 | <0.01| 0.01 |
|               | Etofenprox | <0.01  | <0.01  | 0.20 | 1.34 | <0.01| 0.01 |
|               | Tolclofos-methyl| <0.01  | <0.01  | <0.01| <0.01|<0.01|<0.01|

Mean of residue levels for measurements in duplicate (mg/kg). $^a$Calculated residue levels in the kernel with cob (K+C) and kernel with cob and silk (K+C+S). A half-value of the limit of quantification (0.005 mg/kg) was used for the calculations, where the residue level is <0.01 mg/kg.

Fig. 2. Total residue levels of the five pesticides in the whole corn ears from Ibaraki field (left bar for each pesticide) and Chiba field (right bar for each pesticide). The relative bar length for each analytical portion indicates the distribution of pesticide residues in the various portions.
due distribution pattern between samples from the two fields might reflect the influence of complex factors such as the manner of plant growth as well as the application schedule of pesticides.

5. Handling effect of the analytical portion in sweet corn
To evaluate the pesticide residue levels in various parts of the sweet corn samples, the residue levels in the kernels with cob and kernels with cob and silk were calculated separately, as shown in Table 2. In the calculations, a half value for the limit of quantification (0.005 mg/kg) was used, where the residue level was <0.01 mg/kg. The calculated residue levels for pesticides in the kernels with the cob portions were similar to the measured residue levels in the kernel portions alone.

Although the degree was different, the handling of the silks affected the calculated residue levels in kernels with both cob and silk. Six of the eight undetected measured values in the kernel portions were calculated as detected values. In sweet corn samples from the Chiba field, the effect of adding silk to the samples was lower. Calculated pesticide residue levels in kernels with cob and silk were similar to the measured residue levels in kernel portions of the Chiba field samples. In contrast, calculated pesticide residue levels in kernels with cob and silk were obviously higher than the measured residue levels in kernels of the Ibaraki field samples. The maximum ratio of the calculated residue level in kernels with cob and silk to the residue level in kernels was 62 times higher than for acetamiprid in the Ibaraki field sample. Results from the two fields suggest that the presence of silk in the sweet corn samples affects pesticide residue levels in the edible portion of the samples.

Conclusions
Pesticide residues were predominantly distributed in the silk and husk portions, while relatively minimal amounts remained in the kernel, which is the edible part of sweet corn ears. There is little difference between the residue data in the kernel and that in the kernel with cob of the sweet corn samples. However, the residue levels in the kernel with cob and relatively long silk were estimated to be higher than the residue levels in the kernel portions. This result suggests that the silk could sometimes greatly affect pesticide residue levels in the edible portion of corn. Therefore, in the analytical reports, it is necessary to clearly state whether the analytical portion of the sweet corn sample did or did not include silk.

Acknowledgements
We thank the staff at the Japan Plant Protection Association for their cooperation in the field experiments. This research was performed as part of the "Shokuhin-no-Anshin-Anzenkaku-Suishin-Jigyou," sponsored by the Japanese Ministry of Health, Labour, and Welfare (MHLW). The opinions expressed in this paper are solely those of the authors and do not necessarily reflect the views of MHLW.

References
1) Grain: World Markets and Trade; Foreign Agricultural Service, United States Department of Agriculture, August 2016; p 22, https://apps.fas.usda.gov/psdonline/circulars/grain.pdf (Accessed 15 Jan., 2017).
2) Notification of Ministry of Health, Labour and Welfare of Japan, No. 499, Nov. 29 (2005).
3) Food and Agriculture Organization of the United Nations: "FAO Plant Production and Protection Paper 197, Submission and Evaluation of Pesticide Residues Data for the Estimation of Maximum Residue Levels in Food and Feed," Rome, Italy, 2009.
4) R. Frank, H. E. Braun and B. D. Ripley: J. AOAC Int. 70, 1081–1086 (1987).
5) M. Zhong, T. Wang and J. Hu: Environ. Monit. Assess. 187, 390 (2015).
6) P. Wang, M. Rashid, J. Liu, M. Hu and G. Zhong: Food Chem. 212, 420–426 (2016).
7) C. D. S. Tomlin (eds.): "The e-Pesticide Manual version 5.0.1," British Crop Protection Council, Hampshire, U.K., 2010.
8) Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries of Japan. The guidelines related to the study reports for the registration application of pesticide. Appendix to Director General Notification No. 12-Nousan-8147. Nov. 24, 2000 (Final amendments were made on April 1, 2011).
9) S. de Koning, M. Kurano, H.-G. Janssen and U. A. Th Brinkman: J. Chromatogr. A 1023, 165–174 (2004).
10) T. Yajima, K. Iijima, M. Saka, Y. Odanaka, K. Sato and Y. Kato: Synopsis of 31st. Special Committee Meeting on Pesticide Residue Analysis of Pesticide Science Society of Japan, 188–194 (2008) (in Japanese).
11) O. Tanaka: "Shokubutu-wa-Sugoi, Nana-Fushigi-hen," Chuko shinsho 2328, Chuokoron-Shinsha, Tokyo, pp. 151–154, 2015 (in Japanese).
12) R. L. Nielsen: "Silk Development and Emergence in Corn", Corny News Network, July 2016, Purdue University Department of Agronomy, https://www.agry.purdue.edu/ext/corn/news/timeless/Silks.html (Accessed 15 Jan., 2017).
13) The Japan Food Chemical Research Foundation: "Maximum Residue Limits (MRLs) List of Agricultural Chemicals in Foods", http://www.m5.ws001.squarestart.ne.jp/foundation/search.html (Accessed 15 Jan., 2017).