

Simple and Rapid Determination of Cypermethrin and Fenvalerate Residues in Kampo Products by Gas Chromatography-Mass Spectrometry with Negative Chemical Ionization

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In Japan, crude drugs that are used as ingredients of kampo formulae are mainly imported from China. In China, cypermethrin and fenvalerate, which are pyrethroid pesticides, are detected frequently in food exported to Japan. In fact, cypermethrin and fenvalerate have been detected in crude drugs distributed in Japan. There is concern that cypermethrin and fenvalerate will move from crude drugs to kampo products. Cypermethrin and fenvalerate in kampo products are controlled by a self-imposed residual pesticide limit set by the Japan Kampo Medicine Manufacturers Association. Several methods to analyze cypermethrin and fenvalerate in kampo products have been reported, but they are time-consuming, require expensive column clean-up, and use large amounts of organic solvents. In this study, a simple, rapid, and inexpensive sample preparation method was developed to determine cypermethrin and fenvalerate levels in kampo products by gas chromatography (GC)-mass spectrometry (MS) with negative chemical ionization (NCI). Twenty-two samples of kampo products were analyzed according to the proposed method. No samples were contaminated by cypermethrin and fenvalerate levels greater than the detection limit.

Key words — kampo product, pesticide residue, gas chromatography-mass spectrometry

INTRODUCTION

Kampo formulae are used for various illnesses, and these formulae should be confirmed to be safe because patients generally take kampo formulae directly over the long term. In recent years, pesticides have often been detected in imported food. In particular, cypermethrin and fenvalerate are detected most frequently in food exported to Japan through examination by a company in Tsingtao, China (First place: Cypermethrin, Second place: Fenvalerate).¹⁾ In Japan, crude drugs are mainly imported from China, and cypermethrin and fenvalerate have in fact been detected in crude drugs.^{2,3)} Crude drugs are the ingredients of kampo formulae, and there is concern that cypermethrin and fenvalerate will move from crude drugs to kampo products.

Cypermethrin and fenvalerate in kampo formulae are controlled by a self-imposed residual pesticide limit set by the Japan Kampo Medicine Manufacturers Association. Maximum residue levels were set for kampo formulae containing Polygala root, Perilla herb, Jujube, Citrus unshiu peel, and/or Loquat leaf (cypermethrin: 1.0 ppm, fenvalerate: 1.5 ppm). However, there have been few surveys of cypermethrin and fenvalerate in kampo products distributed in Japan and few analytical methods have been reported.^{4,5)} One method contains numerous time-consuming stages, including liquid-liquid partition 3 times, concentration under reduced pressure 3 times, expensive solid phase extraction using 20 g of Florisil, and elution with 300 ml of a mixture of hexane and acetone (9 : 1) at a rate of not more than 5 ml per min.⁴⁾ The other method is equally time-consuming, with 3 stages of concentration under reduced pressure, 2 stages of expensive solid phase extraction using macroporous diatomaceous earth column involving incubation for 30 min and elution with 200 ml of hexane and a Florisil mini-

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column comprising elution with 50 ml of a mixture of hexane and diethyl ether (17:3).⁵⁾ Both methods use gas chromatography (GC)-electron capture detection (ECD). It was reported previously that pyrethroid pesticides like cypermethrin and fenvalerate in crude drugs could be analyzed selectively by GC-mass spectrometry (MS) with negative chemical ionization (NCI).⁶⁾ In previous reported method, Florisil clean-up, liquid-liquid partition and dilution of working standard solution with blank sample was used.⁶⁾ In this study, a simple, rapid, and inexpensive sample preparation method to determine cypermethrin and fenvalerate in kampo products was investigated using GC-MS with NCI.

MATERIALS AND METHODS

Pesticide Standards — Pesticide standards were obtained from Dr. Ehrenstorfer G.m.b.H. (Augsburg, Germany). Each compound was dissolved in hexane to make a 0.5 mg/ml standard stock solution. Working standard solutions were diluted with hexane.

Reagents — Acetone, hexane, and anhydrous sodium sulfate of pesticide analysis grade were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Kampo Products — A self-imposed residual pesticide limit is set for 5 of the top 10 kampo formulae by volume of sales.⁷⁾ Five kampo products were obtained for each kampo formulae for which self-imposed residual pesticide limits are set. Saireito consisted of 2 samples because this kampo formula was distributed by only 2 companies. Finally, 22 kampo products of 5 kampo formulae were obtained (year: 2009).

Sample Preparation — Kampo products were powdered, 5 g was weighed out, and spiking solution (each pesticide: 5 µg) was added. After 60 min, 10 ml of acetone and 20 ml of hexane were added. The mixture was shaken for 30 min, centrifuged, and the supernatant was recovered. Water (20 ml) was added to the supernatant. The mixture was shaken for 5 min. The mixture was centrifuged if the mixture formed an emulsion (bakumondoto4). A portion (5 ml) of the upper layer was taken, transferred to a glass-stoppered test tube, and dried with 1 g of anhydrous sodium sulfate. A portion (2 ml) of the solution was taken, transferred to a glass-stoppered test tube, and 3 ml of hexane was added

and shaken. The solution was used as the sample solution.

NCI Mode GC-MS — A 5973MSD was connected to a GC6890 (Agilent, Wilmington, DE, U.S.A.). GC conditions: column, DB-1701 capillary column 30 m × 0.25 mm × 0.25 µm (J&W Scientific, Folsam, CA, U.S.A.); helium carrier gas flow, 1.7 ml/min; injection temperature, 200°C; interface temperature, 270°C; ion source temperature, 180°C; ion mode, NCI/selected ion monitoring (SIM) mode; reaction gas, methane; oven temperature program, 50°C for 1 min, 30°C/min to 100°C, and then 25°C/min to 270°C and held for 20 min; injection mode, splitless; injection volume, 2 µl.

GC-ECD — A Shimadzu GC2010 series was used. The GC conditions were same as that of NCI mode GC-MS.

RESULTS AND DISCUSSION

Selection of the Monitoring Ions for SIM Detection

The mass spectra of cypermethrin and fenvalerate in NCI mode are shown in Fig. 1. The selected monitoring ions of cypermethrin were 171(*m/z*) and 207(*m/z*). The selected monitoring ions of fenvalerate were 211(*m/z*) and 213(*m/z*). For quantitation, 207(*m/z*) and 211(*m/z*) were used, respectively.

Investigation of Extraction Method (Shaking and Homogenization)

The analytical method was investigated using hochuekkito, which is the biggest-selling kampo formula.⁷⁾ First, the extraction method was investigated. The extraction efficiency of shaking and homogenization were compared. Hochuekkito was powdered, 5 g was weighed out, and spiking solution (each pesticide: 5 µg) was added. A total of 10 ml of acetone and 20 ml of hexane were added. The mixture was shaken for 30 min or homogenized for 1 min, centrifuged, and the supernatant was recovered. The solution was analyzed. Shaking was slightly more efficient than homogenization (Table 1). Shaking is simple and can extract many samples simultaneously, so shaking was selected as the extraction method.

Investigation of Time Length of Extraction (Shaking)

Extraction volume with shaking for 5–40 min

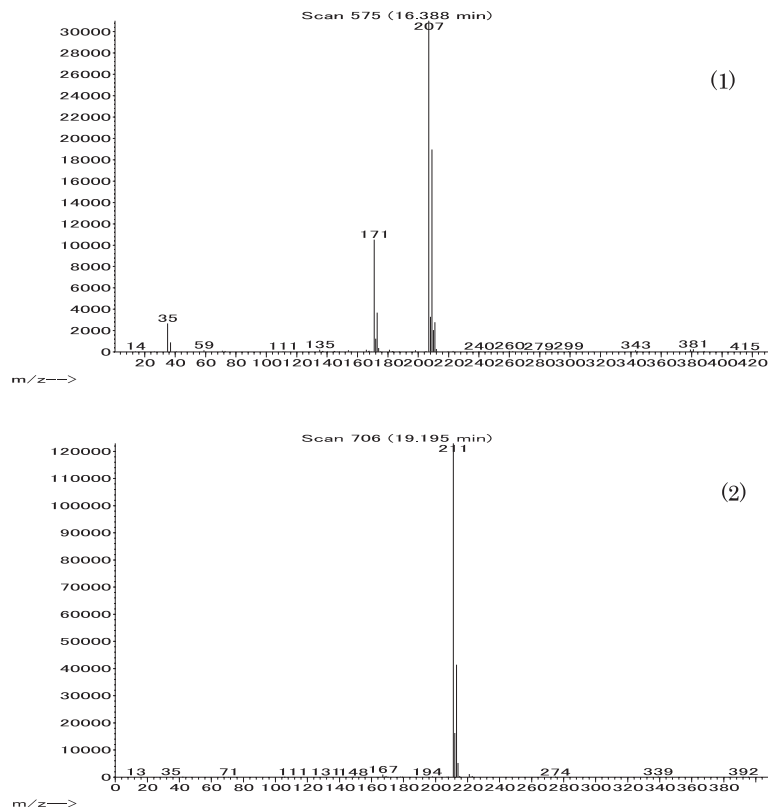


Fig. 1. Mass Spectrum of Pesticides in the NCI Mode. 1: Cypermethrin, 2: Fenvalerate

Table 1. Comparison of Extraction Efficiency between Homogenization and Shaking

Extraction method	Homogenization		Shaking	
	Extraction rates	RSD (%)	Extraction rates	RSD (%)
Cypermethrin	97.4	2.6	100.0	3.9
Fenvalerate	98.1	5.4	100.0	4.6

Extracted volumes for shaking were treated as 100.0% ($n = 3$).

was investigated. Hochuekkito was powdered, 5 g was weighed out, and spiking solution (each pesticide: 5 μ g) was added. A total of 10 ml of acetone and 20 ml of hexane were added. The mixture was shaken for 5–40 min, centrifuged, and the supernatant was recovered. The solution was analyzed. With a time length of shaking of 20 min or more, the extraction volume became constant (Table 2). It was considered that it is difficult to extract pesticides some kampo formulae, so a time length for shaking of 30 min was established.

Pesticides in the Residue of Extraction (Shaking)

Until now, all reported methods for analyzing cypermethrin and fenvalerate in kampo products have extracted pesticides by shaking three times with a mixture of acetone and water (5:2).^{4,5)}

Table 2. Comparison of Extraction Rates by Different Time of Shaking

Compound	Time length of shaking (min)				
	5	10	20	30	40
	Extraction rates (%)				
Cypermethrin	89	88	101	99	100
Fenvalerate	92	91	104	103	100

Extracted volumes when time length of shaking was 40 min were treated as 100% ($n = 1$).

Pesticides in the extraction residue were investigated. Hochuekkito was powdered, 5 g was weighed out, and spiking solution (each pesticide: 5 μ g) was added. A total of 10 ml of acetone and 20 ml of hexane were added. The mixture was shaken for 30 min, centrifuged, and the supernatant was recovered. A total of 5 ml of acetone and 10 ml

of hexane were added to the residue to wash out extraction liquid remaining in the residue. The mixture was mixed gently, centrifuged, and the supernatant was recovered. The supernatants were combined, transferred to a measuring flask, and hexane was added to make exactly 50 ml. This solution was used as the hochuekkito sample solution. A total of 10 ml of acetone and 20 ml of hexane were added to the centrifugation residue. Subsequent procedures were the same as for the hochuekkito sample solution. This solution was used as a residue of extraction solution. These solutions were analyzed. The peaks of cypermethrin and fenvalerate in the residue of extraction solution were less than the quantitation limit (peak height was less than 10 times the noise level of the major ions) and dramatically smaller than that of the hochuekkito sample solution. Thus, it was considered that a single extraction was sufficient to extract cypermethrin and fenvalerate.

Recovery Test for Hochuekkito (5 Products of 5 Companies)

Hochuekkito was powdered, 5 g was weighed out, and spiking solution (each pesticide: 5 µg) was added. A total of 10 ml of acetone and 20 ml of hexane were added. The mixture was shaken for 30 min, centrifuged, and the supernatant was recovered. Twenty milliliters of water was added to the supernatant, and the mixture was shaken for 5 min. A portion (5 ml) of the upper layer was taken, transferred to a glass-stoppered test tube, and dried with 1 g of anhydrous sodium sulfate. This solution was used as the sample solution. The recovery tests were conducted 3 times for 5 samples of hochuekkito that are distributed by 5 different companies. There were no interfering peaks. Furthermore, the recovery rates of 4 samples of hochuekkito were satisfactory, but the recovery rate of 1 sample (hochuekkito5) was unsatisfactory (Table 3). Kampo products are usually dry matter,

Table 3. Recovery Rates of Cypermethrin and Fenvalerate from Hochuekkito

Sample	Cypermethrin		Fenvalerate	
	Average (%)	RSD (%)	Average (%)	RSD (%)
Hochuekkito1	90.7	2.9	80.1	2.7
Hochuekkito2	100.3	1.8	92.7	3.3
Hochuekkito3	105.5	1.8	99.4	2.7
Hochuekkito4	102.8	5.1	92.4	6.1
Hochuekkito5	140.0	0.9	124.4	2.7

n = 3.

so it was considered that the extracts of kampo products are subject to a matrix enhancement effect in GC-MS analysis. There are some methods to prevent matrix enhancement effects,⁸⁾ including the dilution working standard solutions with sample solutions. When the standard solution was diluted with sample solution of hochuekkito5, the recovery rates were 86.5% (cypermethrin) and 84.6% (fenvalerate). Thus, it was considered that the high recovery rates of hochuekkito5 were obtained by a matrix enhancement effect. It was suggested that the differences in matrix enhancement effects between the 5 samples occurred because of differences in such as additives between companies.

Investigation to Prevent Matrix Enhancement Effect

When the matrix enhancement effect was overcome by the dilution working standard solution with sample solution, there was a problem. To dilute working standard solution with sample solution, exactly the same kampo product that contains no pesticides is needed. Because of the difference in such as additives between companies, it was difficult to obtain exactly the same kampo product. Another method to prevent matrix enhancement effects was required. The aim of this study is to develop a simple, rapid, and inexpensive sample preparation method. A simpler method to prevent matrix enhancement effects by diluting sample solution was investigated. The sample solution was diluted according to the proposed method with hexane, and recovery tests were conducted 3 times for 5 samples of hochuekkito1–5. The recovery rates and Relative Standard Deviation (RSD) were satisfactory (Table 4). Matrix enhancement was prevented by dilution of the sample solution with hexane. In this study, detection limits (DL) of cypermethrin and fenvalerate were 2.5 ppb (concentration in the

Table 4. Recovery Rates of Cypermethrin and Fenvalerate from Hochuekkito According to the Proposed Method

Sample	Cypermethrin		Fenvalerate	
	Average (%)	RSD (%)	Average (%)	RSD (%)
Hochuekkito1	85.4	4.9	82.7	5.7
Hochuekkito2	104.9	4.7	101.5	2.8
Hochuekkito3	99.3	3.3	95.3	2.9
Hochuekkito4	101.3	3.6	96.1	3.8
Hochuekkito5	100.9	8.6	93.5	6.0

n = 3.

injected solution; peak height was over 3 times the noise level of the major ions). In an analytical method using GC-ECD to analyze cypermethrin and fenvalerate in kampo products, DL (concentration in the injected solution; peak height was over 3 times the noise level) was reported to be 11 ppb for cypermethrin and 6 ppb for fenvalerate.⁴⁾ GC-MS with NCI is more sensitive at detecting cypermethrin and fenvalerate than GC-ECD. GC-MS with NCI had sufficient sensitivity to analyze cypermethrin and fenvalerate in the sample solutions.

Linearity

The correlation coefficients of linearity are shown in Table 5 and varied from 0.9978–0.9981. The standard solution showed good linearity.

Recovery Tests Using 4 Other Kampo Formulae

It was attempted to apply the proposed method to 4 other kampo formulae for which self-imposed residual pesticide limits are set. The recovery tests

were conducted 3 times for 17 kampo products (4 kampo formulae). The recovery rates of cypermethrin and fenvalerate were between 73.8% and 115.0%, and most RSD values were less than 10% (Table 6). The recovery rates and RSD were satisfactory. SIM chromatograms of Saireito (blank sample) and standard solution (0.1 ppm) are shown in Fig. 2. There were no interfering peaks near the pesticide peaks. GC-ECD chromatograms of Saireito (blank sample) and standard solution (0.1 ppm) are shown in Fig. 3. Many interfering peaks were detected by GC-ECD. Figure 2 and Fig. 3 show that GC-MS NCI can detect cypermethrin and fenvalerate more selectively than GC-ECD. This simple, rapid, and inexpensive sample preparation method was realized through the use of GC-MS with NCI. The proposed method was developed to analyze cypermethrin and fenvalerate in only kampo products. It is considered that further purification is needed to apply the proposed method to such as crude drugs.

Table 5. Linear Ranges and Correlation Coefficients of the Standard Solutions

Compound	Range (ppb)	Correlation Coefficient (γ)
Cypermethrin	10–1000	0.9981
Fenvalerate	10–1000	0.9978

Table 6. Recovery Rates of Cypermethrin and Fenvalerate from Kampo Products According to the Proposed Method

Sample	Cypermethrin		Fenvalerate	
	Average (%)	RSD (%)	Average (%)	RSD (%)
Saireito1	89.2	3.9	84.5	5.6
Saireito2	115.0	8.2	108.4	9.0
Shosaikoto1	104.0	2.2	95.5	1.7
Shosaikoto2	79.1	5.3	73.8	6.7
Shosaikoto3	97.1	2.9	93.9	3.6
Shosaikoto4	89.1	5.3	90.0	7.9
Shosaikoto5	95.1	7.6	87.0	6.8
Bakumondoto1	91.6	2.2	90.4	2.8
Bakumondoto2	87.6	8.2	84.3	10.1
Bakumondoto3	95.9	0.9	93.1	0.5
Bakumondoto4	95.3	1.7	91.8	1.6
Bakumondoto5	94.1	3.6	93.2	5.0
Rikkunshito1	101.8	2.5	99.8	1.6
Rikkunshito2	103.2	3.7	102.8	7.0
Rikkunshito3	95.3	3.9	91.7	4.2
Rikkunshito4	101.4	6.3	94.8	8.7
Rikkunshito5	112.8	3.8	101.1	5.3

$n = 3$.

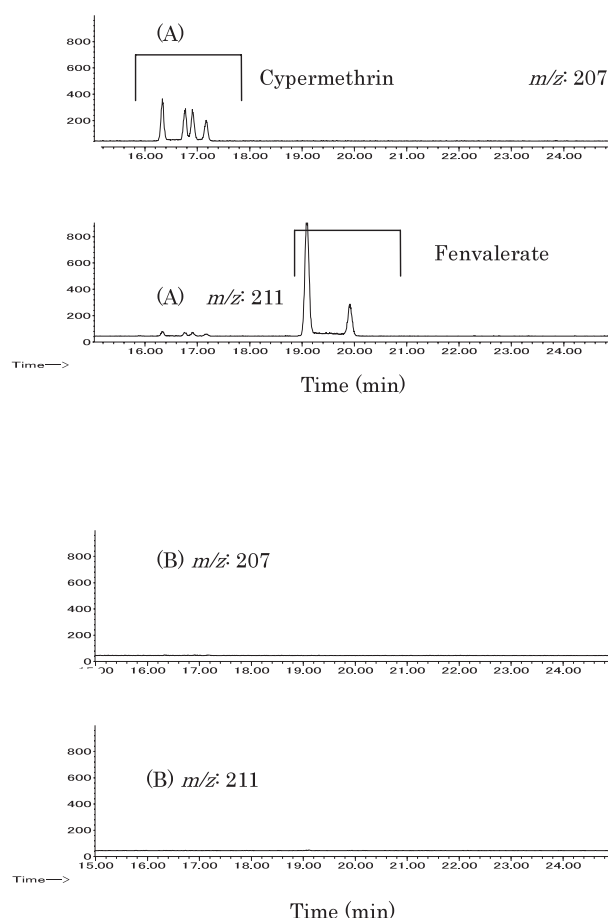


Fig. 2. SIM Chromatograms of (A) Standard Solution (Each Pesticide: 0.1 ppm), (B) Saireito (Blank Sample)

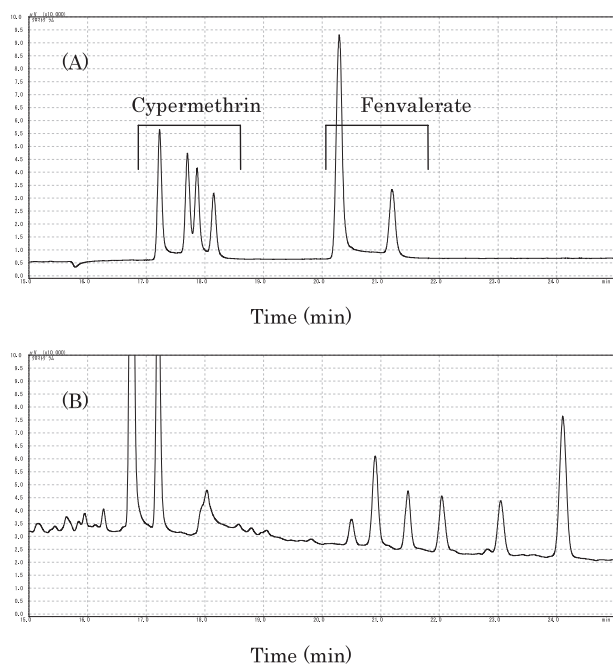


Fig. 3. GC-ECD Chromatograms of (A) Standard Solution (Each Pesticide: 0.1 ppm), (B) Saireito (Blank Sample)

Application of the Proposed Method for Determination of Cypermethrin and Fenvalerate in Kampo Products Distributed in Japan

Twenty-two samples of kampo products (5 kampo formulae), as shown in Table 4 and Table 6, were analyzed according to the proposed method. Kampo products were obtained in Japan (year: 2009). No samples were contaminated by cypermethrin or fenvalerate over the DL (2.5 ppb: concentration in the injected solution; peak height was over 3 times the noise level of the major ions). This result suggested that kampo products currently distributed in Japan were not contaminated by high levels of cypermethrin and fenvalerate.

In conclusion, a simple, reliable, rapid, and inexpensive sample preparation method was developed to determine cypermethrin and fenvalerate levels in kampo products. GC-MS with NCI could detect cypermethrin and fenvalerate more selectively than GC-ECD. This simple, rapid, and inexpensive sample preparation method was realized through the use of GC-MS with NCI. Twenty-two samples of kampo products were analyzed according to the pro-

posed method and no samples contained cypermethrin or fenvalerate levels over the DL. This result suggested that kampo products currently distributed in Japan were not contaminated by high levels of cypermethrin or fenvalerate.

REFERENCES

- 1) Sato, M. (2009) Food safety and inspection in China. *Journal of the Food Hygienic Society of Japan*, **50**, J-9–J-11.
- 2) Shioda, H., Hamano, T., Nakajima, J., Shimomura, J., Suetsugu, D. and Yasuda, I. (2004) Organophosphorous and pyrethroid pesticides in the crude drugs and the decoction. *Annual Report of Tokyo Metropolitan Institute of Public Health*, **55**, 43–47.
- 3) Nakajima, J., Hamano, T., Shioda, H., Yasuda, I., Kamakura, H. and Goda, Y. (2004) The factors of deviation in analyzing pyrethroid pesticides residues and their contents in crude drugs. *Annual Report of Tokyo Metropolitan Institute of Public Health*, **55**, 49–53.
- 4) Tamura, M., Endo, Y., Isozaki, T., Sugimoto, C., Maruta J., Ohashi, S., Yoshikawa, S., Okonogi, A., Shimizu, K. and Sasaki, H. (2008) Pesticide residues in kampo formulations, crude drug formulations and crude drugs (1). *Pharmaceutical Regulatory Science*, **39**, 63–75.
- 5) Shioda, H., Hamano, T., Nakajima, J. and Yasuda, I. (2007) Determination of organochlorine and pyrethroid pesticides in preparations of crude drug extract. *Annual Report of Tokyo Metropolitan Institute of Public Health*, **58**, 123–125.
- 6) Tagami, T., Kajimura, K., Satsuki, Y., Nakamura, A., Okihashi, M., Kitagawa, Y., Takatori, S. and Kitagawa, M. (2006) Simultaneous analysis of 10 pyrethroid pesticides in natural medicines by GC/MS with negative chemical ionization. *Yakugaku zasshi*, **126**, 991–995.
- 7) Japan Kampo Medicine Manufacturers Association (2004) *KANPOUSEIZAINADONOSEISANDOUTAI in Japanese*, http://www.nikkankyo.org/publication/movement/h16pdf/04_05.pdf
- 8) Pesticide Science Society of Japan (2005) *ZANRYUUNOUYAKUBUNSEKISITTEOKITAIMONDOUARE-KORE in (Japanese)*, 97–98.