Food safety in Thailand 4: Comparison of pesticide residues found in three commonly consumed vegetables purchased from local markets and supermarkets in Thailand

Sompon Wanwimolruk Corresp., 1, Kamonrat Phopin 1,2, Somchai Boonpangrak 1, Virapong Prachayasittikul 2

1 Center for Research and Innovation, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand
2 Department of Clinical Microbiology and Applied Technology, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand

Corresponding Author: Sompon Wanwimolruk
Email address: sompon-999@hotmail.com

Background The wide use of pesticides raises concerns on the health risks associated with pesticide exposure. For developing countries, like Thailand, pesticide monitoring program (in vegetables and fruits) and also the maximum residue limits (MRL) regulation have not been entirely implemented. The MRL is a product limit, not a safety limit. The MRL is the maximum concentration of a pesticide residue (expressed as mg/kg) recommended by the Codex Alimentarius Commission to be legally permitted in or on food commodities and animal feeds (Codex Alimentarius Commission, 2015; European Commission, 2008; European Commission, 2015). MRLs are based on supervised residue trial data where the pesticide has been applied in accordance with GAP (Good Agricultural Practice). This study aims at providing comparison data on pesticide residues found in three commonly consumed vegetables (Chinese kale, pakchoi and morning glory) purchased from some local markets and supermarkets in Thailand.

Methods These vegetables were randomly bought from local markets and supermarkets. Then they were analyzed for the content of 28 pesticides by using GC-MS/MS.

Results Types of pesticides detected in the samples either from local markets or supermarkets were similar. The incidence of detected pesticides was 100% (local markets) and 99% (supermarkets) for the Chinese kale; 98% (local markets) and 100% (supermarkets) for the pakchoi; and 99% (local markets) and 97% (supermarkets) for the morning glory samples. The pesticides were detected exceeding their MRL at a rate of 48% (local markets) and 35% (supermarkets) for the Chinese kale; 71% (local markets) and 55% (supermarkets) for the pakchoi, and 42% (local markets) and 49% (supermarkets) for the morning glory.

Discussion These rates are much higher than those seen in developed countries. It should be noted that these findings were assessed on basis of using criteria (such as MRL) obtained from developed countries. Our findings were also confined to these vegetables sold in a few central provinces of Thailand and did not reflect for the whole country as...
sample sizes were small. Risk assessment due to consuming these pesticide contaminated vegetables, still remains to be evaluated. However, remarkably high incidence rates of detected pesticides give warning to the Thai authorities to implement proper regulations on pesticide monitoring program. Similar incidence of pesticide contamination found in the vegetables bought from local markets and supermarkets raises question regarding the quality of organic vegetables domestically sold in Thailand. **This conclusion excludes Thai export quality vegetables and fruits routinely monitored for pesticide contamination before exporting.**
Original Article

Food safety in Thailand 4: Comparison of pesticide residues found in three commonly consumed vegetables purchased from local markets and supermarkets in Thailand

Sompon Wanwimolruk\textsuperscript{1*}, Kamonrat Phopin\textsuperscript{1,2}, Somchai Boonpangrak\textsuperscript{1}
and Virapong Prachayasittikul\textsuperscript{2}

\textsuperscript{1} Center for Research and Innovation, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand

\textsuperscript{2} Department of Clinical Microbiology and Applied Technology, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand

*Corresponding author: Sompon Wanwimolruk, Ph.D.
Professor, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand
E-mail: sompon.wan@mahidol.ac.th; sompon-999@hotmail.com, Phone +66 2 441 4370;
Fax: +66 2 441 4380

Running Title: Pesticide residues found in vegetables bought from local and supermarkets
Background The wide use of pesticides raises concerns on the health risks associated with pesticide exposure. For developing countries, like Thailand, pesticide monitoring program (in vegetables and fruits) and also the maximum residue limits (MRL) regulation have not been entirely implemented. The MRL is a product limit, not a safety limit. The MRL is the maximum concentration of a pesticide residue (expressed as mg/kg) recommended by the Codex Alimentarius Commission to be legally permitted in or on food commodities and animal feeds (Codex Alimentarius Commission, 2015; European Commission, 2008; European Commission, 2015). MRLs are based on supervised residue trial data where the pesticide has been applied in accordance with GAP (Good Agricultural Practice). This study aims at providing comparison data on pesticide residues found in three commonly consumed vegetables (Chinese kale, pakchoi and morning glory) purchased from some local markets and supermarkets in Thailand. Methods These vegetables were randomly bought from local markets and supermarkets. Then they were analyzed for the content of 28 pesticides by using GC-MS/MS. Results Types of pesticides detected in the samples either from local markets or supermarkets were similar. The incidence of detected pesticides was 100% (local markets) and 99% (supermarkets) for the Chinese kale; 98% (local markets) and 100% (supermarkets) for the pakchoi; and 99% (local markets) and 97% (supermarkets) for the morning glory samples. The pesticides were detected exceeding their MRL at a rate of 48% (local markets) and 35% (supermarkets) for the Chinese kale; 71% (local markets) and 55% (supermarkets) for the pakchoi, and 42% (local markets) and 49% (supermarkets) for the morning glory. Discussion These rates are much higher than those seen in developed countries. It should be noted that these findings were assessed on basis of using criteria (such as MRL) obtained from developed countries. Our findings were also confined to these vegetables sold in a few central provinces of Thailand and did not reflect for the whole country as sample sizes were small. Risk assessment due to consuming these pesticide contaminated vegetables, still remains to be evaluated. However, remarkably high incidence rates of detected pesticides give warning to the Thai authorities to implement proper regulations on pesticide monitoring program. Similar incidence of pesticide contamination found in the vegetables bought from local markets and supermarkets raises question regarding the quality of organic vegetables domestically sold in Thailand. This conclusion excludes Thai export
quality vegetables and fruits routinely monitored for pesticide contamination before exporting.

Keywords: Pesticide residues, Vegetables, Chinese kale, Pakchoi, Morning glory, Food safety

INTRODUCTION

An enormous concern on toxic pesticides in foods has been raised because of its negative health and environmental impacts. This is due to the widespread use of pesticides in agriculture. In fact, the main exposure to pesticides for humans is oral ingestion, especially by vegetables and fruits (Claeys et al., 2008; Drouillet-Pinard et al., 2011). Toxicity and human health risk associated with pesticide contamination in foods has made it necessary to regulate pesticide residues in our foods (Cervera et al., 2014). Detection and quantification of pesticide residues in food samples are essential to verify whether these pesticides are within limits, so called “maximum residue limits (MRL)”. This regulation was established by the European Commission and other regulatory authorities. Many developed countries have approved this regulation to oversee and operate their food safety affair. Contrastingly, in developing countries such as Thailand, good agricultural practices (GAP) have not fully been implemented, nor has a successful pesticide monitoring program. Exception is for those produce that will be exported. Pesticides have been greatly used in agriculture in Thailand (Harnpicharnchai et al., 2013). The most popular classes of pesticides imported into Thailand are herbicides, followed by insecticides and fungicides (Sapbamrer & Nata, 2014). Among the insecticides, organophosphates and carbamates are very commonly used for protecting crops from insects’ invasion. The use of pesticides in agriculture has been linked with occupational health of farmers, gardeners and consumers (Chan, 1990; Sapbamrer & Nata, 2014).

Chinese kale (Brassica oleracea) is also known as Chinese broccoli. Chinese kale is a leaf vegetable appearing thick and flat, with glossy blue-green leaves, thick stems and a small number of tiny, almost vestigial flower heads similar to those of broccoli. Flavour of Chinese kale is very like to that of broccoli, but somewhat bitterer. Chinese kale is used extensively in Chinese cuisine, and especially in Cantonese cuisine. In Thailand, a number of admired Thai dishes have Chinese kale as a principal ingredient. In some dishes, Chinese kale is consumed
fresh, without cooking. This possesses potential for toxicity if the vegetables are eaten freshly
every day without washing them properly. Pakchoi [Brassica chinensis Jusl var parachinensis
(Bailey) Tsen & Lee] is a species in the Brassicaceae which is a popular vegetable consumed in
Thailand, also in Southeast Asia and southern China. Unlike napa cabbage (Brassica pekinensis),
pakchoi does not form heads; instead, they have smooth, dark green leaf blades forming a cluster
reminiscent of mustard or celery. Water morning glory (Ipomoea aquatic Forsk) is a
semiaquatic, tropical plant grown as a vegetable in East, South and Southeast Asia. It is also
known as water spinach, water convolvulus, or by the more ambiguous names Chinese spinach,
Chinese convolvulus or swamp cabbage (Nagendra Prasad et al., 2008). It is known as pak bung
in Thai, ong choy in Chinese and kangkong in Tagalog. Water morning glory is one of the most
popular vegetables constituted in Thai, Burmese, Lao, Cambodian, Malay, Vietnamese, Filipino,
and Chinese cuisines.

Pesticide residues have been found in many raw agricultural commodities such as vegetables
and fruits, and processed foods worldwide in the past decades (Chen et al., 2011; Chen et al.,
2014; Huan et al., 2015; Li et al., 2014; Osei-Fosu et al., 2014; Sapbamrer & Hongsibsong,
2014; Wang et al., 2013; Wanwimolruk et al., 2015a; Wanwimolruk et al., 2015b). Presently,
information on pesticide contamination in vegetables in Thailand is limited and systemic
investigation is desired to verify the current status of pesticide contamination in foods,
particularly in vegetables and fruits. Also, the current case of organic fruits and vegetables sold
in Thailand displays to consumers with no confidence in regard to quality whether the produce is
pesticide-free. Many supermarkets have placed labels on fruits and vegetables implying that they
are either organically grown or pesticide-free. The supermarkets in Thailand sell not only organic
produce. The fresh vegetables sold in supermarkets in Thailand can be categorized into four
groups, i.e., conventional, organic, pesticide-free and safe vegetables. Regarding the latter
category, the Thai people have questioned if they are pesticide-free or organic vegetables.
Consequently, people are prepared to buy vegetables and fruits from supermarkets at much
higher price than those from local markets. This is because they have a high expectation that
supermarket produce is safe from pesticide contamination. However, there is no scientific-based
evidence to verify the supermarkets’ claims and people’s beliefs. Therefore, the purpose of this
study was to provide comparison data on pesticide residues found in three commonly consumed
vegetables (Chinese kale, pakchoi and water morning glory) purchased from local markets and
supermarkets. In addition, this study had no intention to compare the conventionally grown and organically grown vegetables. However, the present study raises questions about the quality of organically grown vegetables in Thailand.

MATERIALS AND METHODS

Chemicals and standards

Anhydrous magnesium sulphate, sodium chloride, primary and secondary amine (PSA, particle size 40 µm), graphite carbon black (GCB) and C18 sorbent (particle size 40 µm) were obtained from Supelco (Sigma-Aldrich Corp., St. Louis, USA). HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany). Twenty eight pesticides and two metabolite standards including aldrin, atrazine, captan, carbaryl, carbofuran (and its two metabolites carbofuran-3-hydroxy and carbofuran-3-keto), carbosulfan, chlormefos, chlorpyrifos, chlorothalonil, λ-cyhalothrin, cypermethrin, deltamethrin, diazinon, dichlorvos, dicofol, dimethoate, ethion, fenvalerate, malathion, metalaxyl, methidathion, methomyl, paraoxon-methyl, phosalone, pirimicarb, pirimiphos-methyl and profenofos were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Purity of these pesticide standards was >98%. Individual stock of standard solutions (1000 mg/L) was prepared in acetonitrile.

Vegetable samples

Three vegetables were selected for this study namely Chinese kale, pakchoi and water morning glory. The selection was based on their high consumption in Thailand. These three vegetables are widely consumed among Thai and Asian people. Chinese kale samples (n = 137) were purchased randomly from local open-air markets (n = 69) and supermarkets (n = 68). For pakchoi, a total of 125 samples were bought from local markets (n = 63) and supermarkets (n = 62). Samples of water morning glory (n = 135) were purchased randomly from local markets (n = 74) and supermarkets (n = 61). These markets were located in central provinces of Thailand including Bangkok, Nakhon Pathom, Nonthaburi, Ayutthaya, Pathumthani, Samutsakorn and Nakhon Ratchasima. These provinces are located surrounding Bangkok, Thailand, within a radial distance of 260 km. The supermarkets which the vegetable samples were bought from were Big C, Foodland, Jiffy Plus, Lemon Farm, Max Valu, Tesco Lotus, Tops and Villa Market. The study...
was carried out over a year period from November 2013 to December 2014. At the local markets at which vegetable samples were bought, the produce that was for sale came from conventional farms and was not claimed to be ‘organic produce’. The reason we purchased conventional from the local markets was because almost all produce sold in the local markets are known to be conventionally grown. There are only a few produce sold in the local markets that are labelled as organic, whereas the vegetable samples purchased from supermarkets were sorted into four groups, i.e., conventional, organic, pesticide-free and safe vegetables. Most of vegetable samples bought from the supermarkets were claimed to be organic and pesticide-free vegetables. Like for our previous study (Wanwimolruk et al., 2015b), it is very difficult to get the information on the suppliers as most of the workers in both supermarkets and local markets had no idea where the vegetables were bought from. Therefore, we could not obtain accurate information on suppliers. Approximately 500 g of vegetables were purchased and the samples were transported to the laboratory for analysis which was done within 24 hr. The representative portion (150-200 g) of the vegetable sample was chopped into tiny pieces and homogenized using a food processor and mixed carefully. The homogenized samples were then extracted and treated as described in following section.

Sample preparation

The analysis of pesticide residues was performed using the pesticide multiresidue QuEChERS (Quick Easy Cheap Effective Rugged and Safe) method as explained previously (Anastassiades et al., 2003; Lehotay, 2007; Lehotay et al., 2010; Paya et al., 2007). Briefly, extraction of pesticides was performed by extracting 15 g of homogenized vegetable with 15 ml acetonitrile saturated with 6 g of magnesium sulphate and 1.5 g of sodium chloride. This extraction process was pursued by a cleaning up procedure. This was achieved by transferring the supernatant (1 mL) into another tube comprising 50 mg of primary-secondary amine (PSA), 7.5 mg graphite carbon black (GCB) and 150 mg magnesium sulphate. After shaking and centrifugation, the extract supernatant was then transferred to an autosampler vial for direct injection into the Bruker GC/MS/MS system.

GC-MS/MS analysis
Detection of pesticides was accomplished by using a Bruker 456 gas chromatography (GC) coupled with Bruker Scion Triple Quadrupole mass spectrometer (GC-MS/MS). Details of GC-MS/MS conditions were referred to as in the previous reports (Duff and Voglino, 2012; Wanwimolruk et al., 2015b). Multiple reaction monitoring (MRM) acquisition method and two ion transition at the experimentally optimized collision energy (CE) were monitored for each pesticide analyte.

**Calibration and quantification**

A working surrogate spiking standard solutions of pesticides were made by an appropriate dilution of the stock solutions with acetonitrile. These standard solutions were guarded from light and kept frozen at -20 °C until required. Calibration curves of each pesticide of interest were conducted using an internal standard method according to the established procedure (Koesukwiwat et al., 2011; Lehotay, 2007; Lehotay et al., 2010; Wanwimolruk et al., 2015b). These were conducted using the same procedure each time when a new unknown sample set was analyzed. Aldrin was used as an internal standard. The ratio of the peak area of the pesticide standard to that of the internal standard was employed for quantification. Recovery studies for method validation were conducted as previously described (Koesukwiwat et al., 2011; Wanwimolruk et al., 2015b). The method validation in regard to reproducibility, calibration linear range, limit of detection (LOD), limit of quantification (LOQ) was performed for each vegetable matrix as expressed previously (Dong et al., 2012; Koesukwiwat et al., 2011).

Quantitation of pesticides in an unknown vegetable sample was carried out in duplicate unless otherwise stated. MRL values for each pesticide in the vegetable of interest were quoted from recommended MRL values established by Thailand Ministry of Agriculture and Cooperation (2013), Codex Alimentarius Commission (2015), and European Commission (2015). These three references were used because not all pesticides’ MRLs were listed in the individual reference.

**Data treatment**

Vegetable samples were grouped into two categories, according to the sources where the samples were purchased, i.e., local markets and supermarkets. Pesticide concentrations obtained from the GC-MS/MS analysis were treated separately for each vegetable studied. These data were further evaluated to determine % total detection of pesticide residues, % of samples which
pesticides were not detected, % of samples contained pesticide residues of < MRL, and % of samples contained pesticide residues of > MRL. For each vegetable of interest the number of samples (or frequency) containing individual pesticide was counted with aid of using Excel Microsoft program. Also the bar graphs were plotted (Excel Microsoft program) from these data to show frequency distribution or bar graphs illustrating types of pesticides in Chinese kale, pakchoi and morning glory, separately. Numbers of samples containing pesticide residues of > MRL were determined by using the MRL reference values for each pesticide and for particular commodity. These were also performed by using Excel Microsoft program.

**Statistical analysis**

All results are presented as either mean ± standard deviation (S.D.) or median. The differences of parameter between two sample groups were assessed by either unpaired Student’s *t*-test or the Mann-Whitney *U*-test, depending on their normality of distribution. The statistical significance level was customary to *P* < 0.05. All statistical analyses were assessed using the software SPSS statistical package for Windows version 18.0 (SPSS Inc., Chicago, IL, USA).

**RESULTS**

The GC-MS/MS method was validated to determine efficiency and accuracy of the analytical assay. Excellent linearity of calibration curves of each pesticide standards were attained as illustrated by the coefficient of determination (*r*²) values of >0.92. For instance, for Chinese kale, the linearity of calibration curves of all twelve pesticides detected (e.g., cypermethrin, deltamethrin, diazinon, dimethoate, metalaxyl, and profenofos) were excellent with *r*² > 0.93. When the pesticides of interest were assayed at 0.01 ppb the signal-to-noise ratio was well above 30 for all pesticides studied. Therefore, detection limits were below 0.01 ppb using the sample preparation procedures described previously. The precision of the method was verified by the reproducibility of the retention time and peak area. It was noticed that the retention time and peak area of all pesticides were in good precision. The relative standard deviations (RSD) of repeatability for cypermethrin and metalaxyl in Chinese kale samples were 5.2% and 4.6%, respectively. While the RSD of reproducibility for cypermethrin and metalaxyl in Chinese kale samples were 12.3% and 9.1%. Overall, their relative RSD of repeatability were lower than 8%
whereas the RSD of reproducibility were lower than 17%. In general, the mean recoveries of all pesticides studied from fortified samples in five replicated experiments were in the range of 75 - 114%. For example, the mean recovery of carbaryl and metalaxyl in Chinese kale were 102 ± 11% and 97 ± 7% at a concentration of 100 ppb. These ranges of recovery fall within the typical acceptance criteria for quantitative regulatory methods (Koesukwiwat et al., 2011). Similar observations of assay validations were found with respect to other two vegetables studied, i.e. pakchoi and morning glory.

Twentyeight pesticides studied were selected on the basis of their widespread use in agriculture in Thailand. Although the most popular classes of pesticides imported into Thailand are herbicides (Sapbamrer & Nata, 2014), only one herbicide namely atrazine was studied. This was simply due to lack of budget to purchase other pesticide standards. Glyphosate is very commonly used herbicide in Thailand but the analytical assay for glyphosate is rather expensive and not yet available in our laboratory. The GC-MS/MS method employed in this study offered satisfactorily separation with high sensitivity and selectivity for quantitation of all 28 pesticides of interest (Wanwimolruk et al., 2015b). The absence of co-extracted interferences for all varieties of leaf vegetables, Chinese kales, pakchoi and water morning glory, was demonstrated by blank extract analysis showing there was no interfering peak co-eluted with analytes of interest. Moreover, in all vegetable samples tested, there were no identifiable peaks detected with the same retention time as aldrin (retention time = 16.02 min) that was used as an internal standard in our GC-MS/MS assay. This supports the rationality of employing aldrin as the internal standard for the assays.

Of 28 pesticides tested, 12 pesticides were detected in the Chinese kale samples purchased from supermarkets (Figure 1). These included carbaryl, carbofuran, chlorothalonil, chlorpyrifos, \(\lambda\)-cyhalothrin, cypermethrin, deltamethrin, diazinon, dimethoate, malathion, metalaxyl and profenofos. Nevertheless, chlorothalonil and deltamethrin were not detected in those Chinese kale samples purchased from local markets (Figure 1), while malathion was not found in the samples bought from supermarkets. Most of Chinese kale samples (88% in local markets, 91% in supermarket samples) had multiple pesticide residues. Overall, metalaxyl, dimethoate and diazinon appeared to be the most often found pesticides in the Chinese kale samples from both sources (Figure 1). The occurrence rate of metalaxyl in the local market samples was 91% (63/69) and was 94% (64/68) for the supermarket samples. However, none of the Chinese kale...
samples purchased from both local markets and supermarkets had metalaxyl that exceeded the recommended MRL value (2000 ppb). Rates of occurrence for dimethoate in the Chinese kale samples were 80% (55/69) and 88% (60/68) for the Chinese kale samples from local markets and supermarkets, respectively. Of 69 samples from local markets, 23 of them had dimethoate exceeding the MRL value (20 ppb). This corresponds to a rate greater than dimethoate’s MRL of 33%. Eleven samples purchased from the supermarkets were found to contain dimethoate that exceeded the MRL. These samples exceeded dimethoate’s MRL by 16%.

Diazinon was other commonly pesticide detected in the Chinese kale samples studied. It was detected in 62% (43/69) and 74% (50/68) of the Chinese kale samples from local markets and supermarkets, respectively (Figure 1). None of both the local market and supermarket samples had diazinon levels that exceeded the recommended MRL (50 ppb). Three other pesticides were also detected in the Chinese kale samples which were profenofos, cypermethrin and carbaryl.

Profenofos was detected in the Chinese kale samples from both sources, with moderate occurrence rates of 33% (23/69) for the local market samples and 29% (20/68) for the supermarket samples. Eleven of the samples purchased from the local markets had profenofos levels exceeding the MRL (10 ppb), whereas twelve samples from the supermarkets contained profenofos at concentrations greater than the recommended MRL. Of note, profenofos concentrations detected in the Chinese kale samples was found to vary widely among the samples from both sources with a range from 0.1 – 2,095 ppb. Pyrethroid pesticide cypermethrin was also detected in the Chinese kale samples at a relatively low rate of detection. Cypermethrin was found in 16% (11/69) of the samples bought from the local market, similarly 15% (10/68) of the supermarket samples contained cypermethrin (Figure 1). One of the local market samples had cypermethrin that exceeded the MRL, while all of the supermarket samples (3 samples) were found to have cypermethrin that exceeded the MRL value (1000 ppb). Carbaryl was detected in 19% (13/69) and 15% (10/68) of the Chinese kale samples from local markets and supermarkets, respectively (Figure 1). Levels of carbaryl found in these samples ranged from 0.1 to 606 ppb, in which two of the supermarket samples contained carbaryl exceeding its MRL value (50 ppb).

The rest of pesticides found in the Chinese kale samples were detected with relative low rate of occurrence. These include carbofuran, chlorothalonil, chlorpyrifos, deltamethrin, λ-cyhalothrin and malathion.
The incidence of pesticide detection, i.e., the % of total pesticide detection in the Chinese kale samples from both sources were extremely high, that is 100% and 99% for the samples bought from the local markets and the supermarkets, respectively. Of interest, the incidence of pesticides detected exceeding the recommended MRL values was 48% in the Chinese kale samples purchased from the local markets. This was slightly higher than the incidence of pesticide detected exceeding the MRL of 35% observed in the samples from the supermarkets. Very small samples were found to contain no pesticides; this represents a rate of free of pesticides of 1% in the supermarket samples.

Nine pesticides were detected in both the pakchoi samples purchased from the local markets and the supermarkets (Figure 2). These were carbaryl, carbofuran, chlorpyrifos, \( \lambda \)-cyhalothrin, cypermethrin, diazinon, dimethoate, metalaxyl and profenofos. Similar to findings observed in the Chinese kale, three pesticides namely metalaxyl, dimethoate and diazinon, were the most often detected in the pakchoi samples collected from both sources. Few pakchoi samples had only one pesticide whereas others (92% in local markets, 97% in supermarket samples) had multiple pesticide residues. Profiles of pesticide types detected in the pakchoi samples from both sources were similar. Like the Chinese kale, occurrence of metalaxyl in pakchoi samples was very high at 97% (61/63) for the samples purchased from the local markets, and 98% (61/62) of the samples from the supermarkets were found to have metalaxyl residues (Figure 2). Among these local market samples, 13 samples (21%) had metalaxyl levels that exceeded the recommended MRL (50 ppb). For the samples bought from the supermarkets, 11 samples (18%) had metalaxyl that exceeded the MRL. Dimethoate was found in 94% (59/63) and 87% (54/62) of the pakchoi samples from local markets and supermarkets, respectively (Figure 2). Thirty-four samples from the local markets (54%) had dimethoate levels of greater than the recommended MRL (20 ppb), whereas 23 supermarket samples (37%) had dimethoate that exceeded its recommended MRL. Rates of occurrence for diazinon in the pakchoi samples were 57% (36/63) and 65% (40/62) for the samples from local markets and supermarkets, respectively (Figure 2). None of the pakchoi samples bought from both the local markets and the supermarkets had diazinon levels above the MRL (50 ppb). Carbofuran, chlorpyrifos and cypermethrin were detected in pakchoi samples from both the local market and supermarkets but with moderate occurrence rates. Cypermethrin was found in 19% (12/63) of the pakchoi samples bought from the local market samples, while 21% (13/62) of the supermarket samples contained cypermethrin
Two of the pakchoi samples bought from the local markets were found to have cypermethrin exceeding the recommended MRL (1000 ppb). Five of the pakchoi samples bought from supermarkets had cypermethrin exceeding the MRL. Chlorpyrifos was detected in 11% (7/63) of the pakchoi samples purchased from the local markets, whereas 16% (10/62) of the supermarket samples were found to contain chlorpyrifos residues (Figure 2). Two of the pakchoi samples purchased from the local markets and one supermarket sample had chlorpyrifos that exceeded the recommended MRL (1000 ppb). For carbofuran, the pesticide detection rate was 32% (20/63) in the local market samples, and 29% (18/62) in the supermarket samples. Even though other three pesticides including carbaryl, \( \lambda \)-cyhalothrin and profenofos were also detected in the pakchoi samples but the occurrence rates were relatively low (Figure 2).

The total incidence of pesticide detection in the pakchoi samples was 98% and 100% for the samples bought from the local markets and from the supermarkets, respectively. The incidence of pesticides detected exceeding the recommended MRL values was 71% in the pakchoi samples purchased from the local markets. While the incidence of MRL exceedance was 55% in the pakchoi samples bought from the supermarkets. These left the proportions of pakchoi samples having pesticide residues of less than MRL and without pesticides to be approximately 30%.

Of 28 pesticides investigated, 12 different individual pesticides were detected in the water morning glory samples purchased from both the local markets and the supermarkets (Figure 3). Eight common pesticides detected in both the morning glory samples from the local markets and the supermarkets were carbofuran, chlorpyrifos, \( \lambda \)-cyhalothrin, cypermethrin, diazinon, dimethoate, metalaxyl and profenofos. Few samples contained only one pesticide, but most of them (90% in local markets, 89% in supermarket samples) had multiple pesticide residues. Again, similar to Chinese kale and pakchoi, metalaxyl, dimethoate and diazinon appeared to be the most often found pesticides in the water morning glory samples from both sources.

Occurrence rates of metalaxyl in morning glory samples were 96% (71/74) and 93% (57/61) for the local market and the supermarket samples, respectively. All of the morning glory samples tested had metalaxyl levels below the recommended MRL (2000 ppb). Occurrence rates for dimethoate in the water morning glory samples were 92% (68/74) for the local market samples, and 84% (51/61) for the samples from supermarkets. Of 74 samples from local markets, 28 of them had dimethoate exceeding the MRL value (20 ppb). This represents a rate of greater than dimethoate’s MRL of 38%. Twenty-two samples purchased from supermarkets were found to
contain dimethoate that exceeded the MRL, denoting to a rate of greater than dimethoate’s MRL of 36%. For diazinon, the occurrence rates in the water morning glory samples were 53% (39/74) and 79% (48/61) for the samples from local markets and supermarkets, respectively (Figure 3). Only one sample of the water morning glory purchased from the local markets had diazinon exceeding the recommended MRL (10 ppb). None of the water morning glory samples from the supermarkets had diazinon levels above the MRL value. Carbofuran and cypermethrin were detected in the water morning glory samples from both the local markets and supermarkets with moderate occurrence rates. Carbofuran was found in the morning glory samples with occurrence rates of 32% (24/74) and 28% (17/61) for the local markets and supermarkets, respectively. All of the water morning glory samples tested had carbofuran levels below its recommended MRL. Cypermethrin was found in 11% (8/74) of the water morning glory samples bought from the local market samples, while 26% (16/61) of the supermarket samples contained cypermethrin (Figure 3). Five of the water morning glory samples (7%) from the local markets had cypermethrin that exceeded its MRL (700 ppb). Out of the supermarket samples, four samples (7%) had cypermethrin exceeding the recommended MRL. Chlorpyrifos was also detected in the water morning glory samples from the local markets and supermarkets with low rates of occurrence. It was found in 7% of the water morning glory samples bought from the local market samples, while 21% of the supermarket samples contained chlorpyrifos (Figure 3). One sample of the water morning glory from both the local markets and the supermarkets had chlorpyrifos that exceeded the MRL (50 ppb). Other six pesticides including carbaryl, chlorothalonil, λ-cyhalothrin, malathion, methomyl and profenofos were detected in the water morning glory samples, although their occurrence rates were very low (Figure 3). Of note, some of pesticides mentioned were not detected in both the local market samples and the supermarket samples. For example, carbaryl and chlorothalonil were detected only in the supermarket samples, but not found in the water morning glory samples bought from the local markets.

The overall incidence of pesticide detection in the water morning glory samples purchased from local markets and supermarkets. Small proportions of samples were found to contain no pesticide residues; this represents a rate of free of pesticide-free residue of 1% and 3% in the local and supermarkets, respectively. Extremely high percentages of pesticide detection, i.e., 99% and 97% were observed in the morning glory samples bought from the local markets and supermarkets, respectively. The incidence of pesticide residues detected exceeding the
recommended MRL values in the morning glory samples from local markets was 42% whereas the incidence rate of 49% was observed in the supermarket samples.

The profiles of pesticides detected in the three vegetables investigated are shown for comparison (Table 1). Details of the pesticides detected in the three studied vegetables and their concentrations can be accessed online from the Supplementary data. From 28 pesticides studied, 13 were found in the fresh samples of these three popularly consumed vegetables. Nine pesticides were found to be common pesticides detected in all the three vegetables studied. These were carbaryl, carbofuran, chlorpyrifos, \( \lambda \)-cyhalothrin, cypermethrin, diazinon, dimethoate, metalaxyl and profenofos. Methomyl was not detected in the Chinese kale and pakchoi samples. Chlorothalonil, deltamethrin, metathion and methomyl were not found in the pakchoi samples, while deltamethrin was also not detected in the morning glory samples.

Table 2 shows comparison of pesticide concentrations in the three vegetables studied found in the samples bought from the local markets and the supermarkets. Both mean as well as median data were evaluated and are presented in Table 2. All the pesticide concentrations detected in these vegetables were found to be not normally distributed; therefore, the data was then statistically evaluated by the non-parametric Mann-Whitney test. Subsequently, the median data was used to compare the differences in concentrations of pesticides between the two groups, the local market and the supermarket samples. For the Chinese kale, the median concentrations of dimethoate and profenofos were similar \( (P > 0.1) \) between the samples from the local markets and the supermarkets. However, the median concentrations of diazinon and metalaxyl in the Chinese kale samples purchased from the supermarkets were significantly greater than those detected in the samples purchased from the local markets \( (P < 0.001, \text{ Table 2}) \). With regard to results in pakchoi, the median concentrations of three pesticides, dimethoate, diazinon and metalaxyl in the local market samples were not significantly different from those found in the supermarket samples \( (P > 0.05) \). Though, the median concentrations of carbofuran in the pakchoi samples bought from the supermarkets were significantly higher than those observed in the local markets \( (P < 0.001, \text{ Table 2}) \). For the morning glory samples, there were no significant differences between the samples from the local markets and the supermarkets in median concentrations of pesticides. The exception to this was for the median concentration of diazinon in the supermarket samples was significantly higher than that seen in the local markets \( (P < 0.01, \text{ Table 2}) \).
DISCUSSION

The GC-MS/MS methods established in our laboratory (Wanwimolruk et al., 2015b) involving QuEChERS sample preparation and GC-MS/MS analysis, were validated. The methods were proven to be suitable and appropriate for determination of pesticide residues in the three leaf vegetables namely Chinese kale, pakchoi and morning glory. This was verified by results of assay validation which have illustrated good recovery, sensitivity, selectivity, linear calibration curves, good reproducibility and accuracy. The utilizations of GC combined with triple quadrupole MS technique not only aided the detection and quantitation of pesticides but it also offered excellent sensitivity for pesticide detection.

The present study examined potential contamination of 28 pesticides in three leaf vegetables namely Chinese kale, pakchoi and morning glory sold in Thailand. Twelve pesticides were detected in fresh Chinese kale samples bought from the local markets and the supermarkets. These included carbamates (carbaryl, carbofuran), organochlorines (chlorothalonil), organophosphorus pesticides (chlorpyrifos, diazinon, dimethoate, malathion, profenofos), pyrethroids (λ-cyhalothrin, cypermethrin, deltamethrin), and metalaxyl. Findings of so many pesticides detected in this vegetable indicate that pesticides are widely and extensively used in the agronomy of Chinese kale in Thailand. This observation is in agreement with our recent finding in which many pesticide residues were detected in Chinese kale sold in Thailand (Wanwimolruk et al., 2015b). Also, this is consistent with those previously observed pesticide contamination in vegetables in Thailand and other Asian countries (Chang et al., 2005; Saphamrer & Hongsibsong, 2014; Swarnam & Velmurugan, 2013). A study carried out in a northern part of Thailand (Saphamrer & Hongsibsong, 2014) reported that vegetables bought from markets contained organophosphorus pesticides greater than the recommended MRLs. These vegetables included garlic, Chinese cabbage, spring onion, Vietnamese coriander and Chinese kale. These findings with respect to Chinese kale agree with our observation in which the levels of three organophosphorus pesticides (chlorpyrifos, dimethoate and profenofos) were greater than their corresponding MRL values. In the present study, there were five pesticides, namely carbofuran, chlorpyrifos, cypermethrin, dimethoate and profenofos, which were detected in some samples at levels exceeded the MRLs (Figure 1). This implies that the Thai farmers used
these pesticides in excessive doses or did not follow the GAP in which an appropriate pre-
harvest interval, i.e., the time period between the last pesticide application and a safe harvest of
the treated crop, was not considered. Although metalaxyl and diazinon were among the most
often detected in the Chinese kale samples, the pesticide residue concentrations found were not
exceed their corresponding MRL values. Moreover, finding with large proportion of the Chinese
kale samples (90%) contained multiple pesticide residues (Figure 1), clearly indicating that the
Thai farmers are likely to use more than one pesticide during the cultivation of Chinese kale.

Observation on similar types of pesticides detected in these commonly three individual
vegetables (Table 1) indicates that Thai farmers cultivated these vegetables in the same areas of
their farm, as it is easy to water and protect these vegetables from pests by using the same
mixture of pesticides. Another reason could be that Thai farmers producing these vegetables are
neighbors and grouped their farms together. Among the pesticides used in cultivation of these
three leafy vegetables, metalaxyl, dimethoate and diazinon were the most often used pesticides.
Moreover, the similarity in the profiles of pesticides detected in these three commonly consumed
vegetables studied, suggests that it is an advantage to reduce the cost for the pesticide monitoring
by selecting to monitor the pesticide residues in only one of these vegetables. The results from
any of these three vegetables will be eventually applied to those of the other two counterparts.
Both extent and incidence of pesticide contamination observed in each vegetable were similar
between the samples from both sources the local markets and the supermarkets. For instance,
most of the twelve pesticides found in the Chinese kale were detected in both samples from the
local markets and the supermarkets. The exception was three pesticides detected in the Chinese
kale samples were not found in the samples from both sources, i.e., the local markets and the
supermarkets. Chlorothalonil and deltamethrin were not detected in the local market samples,
while a few samples from the supermarkets were contaminated with residues of these pesticides.
Malathion was found in only one Chinese kale sample from the local markets but not in the
samples from the supermarkets. Similar findings were seen in the other two vegetables (pakchoi
and morning glory) regarding minor differences in pesticides detected in the samples from the
local markets and the supermarkets. These minor differences in the profiles of pesticides found
in the three commonly consumed vegetable samples from the local markets and the supermarkets
may be related to the sources (or farms) where the vegetables were cultivated, difference of
usage of each type of pesticides, and ignorance of GAP awareness. Traceability of the produces
was hard to attain and ultimately this was not the primary goal of the current study. If available, the traceability would have been very useful for interpretation of the data obtained from this study. The merchants were in fact asked where they bought the vegetables from and habitually many of them did not have an answer. For those who provided an answer, it appeared that most of the vegetable samples tested were bought from four different whole sale markets in Bangkok and Nakhon Pathom province situated near Bangkok. Future studies are required to trace the farms where the vegetables are cultivated and to identify the factors or farmers’ behaviors that attribute to the differences in rates of the pesticide detection and the MLR exceedance. Vitally, proper education such as GAP regarding the appropriate use of pesticides must be provided to these farmers.

The present study revealed overall incidence of pesticide detection in the three vegetables studied was in a range from 97-100%. For the Chinese kale, this high incidence of pesticide detection is consistent with our previous study published recently (Wanwimolruk et al., 2015b). In that study, an incidence of pesticide detection of 85% was reported in the Chinese kale collected in Nakhon Pathom province of Thailand. Characteristics and sources of the samples were similar to those tested in the present study. It is obvious that these figures of the incidence of pesticide detection observed in the three commonly consumed vegetables are noticeably higher than the tolerable detection rate in western or developed countries, such as USA and European Commission (EC) countries like France, U.K., Norway and Germany. For example, the US FDA carried out a monitoring program of vegetables with thousands of domestic samples and imported samples (Granby et al., 2008). Pesticide residues were found in 30% of the domestic vegetables and 21% of the imported vegetables. In Taiwan, pesticide residues were detected in 14% of 9,955 vegetable samples tested (Chang et al., 2005). A survey study conducted in India found residues of many organophosphorus pesticides (e.g., chlorpyrifos, dimethoate, monocrotophos and profenofos) in 54% of the vegetable samples (Swarnam & Velmurugan, 2013). The latest study from Thailand (Sapbamrer & Hongsibsong, 2014) conveyed an overall pesticide detection rate of 25% (N = 106) in various vegetables bought from the markets. This rate is nevertheless much lower than the rates of pesticide detection in the Chinese kale, pakchoi and morning glory observed in this study. The difference may be accounted for by differences in seasons of vegetable cultivation, vegetable types, types of pesticides used and analytical methods employed.
Remarkably, the occurrence of pesticide detection exceeding the MRL in the three vegetables studied ranged from 35 to 71%; it was high in both samples from the local markets and the supermarkets. These were noticeably high, as compared with the incidence testified in developed countries. For instance, the US FDA declared that violations (with pesticide concentration >MRL) were found in 2% of the domestic and 7% of the imported vegetable samples (Granby et al., 2008). The European Union (EU) Monitoring Program for pesticides declared that 5% of vegetable samples examined had the pesticide residue concentrations that exceeded the MRL (Granby et al., 2008). In Asia, a study carried out in Taiwan reported that of 9,955 samples tested, 1.2% were violating the MRL (Chang et al., 2005). Therefore, the incidence of pesticide detection of >MRL in our three vegetables, at rates of 32 to 49% are unusually high when compared with acceptable rates reported in developed countries. Nevertheless, these incidence rates are somehow similar to that found in Pakistan, an Asian country, in which 206 different vegetables were analyzed for 24 pesticides, and 46% had levels greater than the MRL (Parveen et al., 2005). Also, a study from India (Swarnam & Velmurugan, 2013) reported that 15% of vegetable samples tested contained pesticide residues that exceeded the MRL values. In addition, the incidence of pesticide detection of >MRL was stated to be 24% in several market vegetables examined in northern districts of Thailand (Sapbamrer & Hongsibsong, 2014). This rate of pesticide detection is quite comparable to the rates reported in the present study. Recently, the Food and Drug Administration (FDA), Ministry of Public Health of Thailand issued a report on the pesticide monitoring program for vegetables and fruits in which more than 60,000 samples were screened each year (Srithongkum, 2014). The report revealed that violations found in vegetables and fruits marketed in Thailand were in a range of 5% in the year 2011 to 4% in the year 2013. These rates reported by the Thailand FDA were approximately 7-14 times lower than the incidence of pesticide detection exceeding the MRL (35-71%) found in this study. The conflicting findings are likely to be accounted for by the difference in methods utilized in the two survey studies. The survey by the FDA of Thailand was done by using a cholinesterase inhibition assay kit called GT-Test kit. This assay kit is competent of detecting two groups of pesticides, i.e., carbamates (carbofuran and methomyl) and organophosphates (dicrotophos and EPN). Nevertheless, unlike our current GC-MS/MS method, the GT-Test kit cannot offer a quantitative analysis like most analytical methods, such as UV spectrophotometric assays, LC-MS/MS and GC-MS/MS. Because the kit assay is restricted in detection to only four individual pesticides, it
has less sensitivity and does not provide a quantitative determination of pesticide concentration.

Thus, these restrictions of the kit assay can underestimate the incidence of MRL violations. Unusually high rate of exceedance of the MRL found in the three vegetables investigated may be due to the fact that we used the recommended MRLs adopted from those employed in developed countries, i.e. Codex Alimentarius Commission (2015), and European Commission (2015). Some of MRLs for pesticides used may be too low and made the incidence unnecessarily high. For examples, MRL values for carbofuran were 20 ppb (0.02 ppm) for Chinese kale and pakchoi; and 10 ppb (as a default value) for the morning glory. The MRLs for profenofos were 10 ppb (0.01 ppm) as a default value, for Chinese kale and morning glory; and 50 ppb for the pakchoi. Using these low recommended MRLs yielded the remarkably high rate of MRL exceedance observed in the present study. In addition, it should be noted that our findings were limited to these three vegetables sold in a small number of central provinces of Thailand and did not reflect the figure for the whole country. This is because the sample sizes were considerably rather small. Larger sample sizes collected from many provinces of different regions in Thailand would be required to verify the incidence of pesticide contamination. Importantly, health risk assessment due to consuming these pesticide contaminated vegetables, has not yet been evaluated. A larger sample size would be necessary for that as well.

There were substantial variations in the levels of pesticides found in the three vegetables studied. For instance, profenofos levels found in the Chinese kale samples varied widely among the samples from both sources ranging from 0.1 to 2,095 ppb; and levels of carbaryl found in these samples ranged from 0.1 to 606 ppb. In addition, the large S.D. values (relative to their corresponding means) were found for each pesticides detected in the Chinese kale and also in the other two vegetables. This reflects the huge variation in concentrations of each pesticide detected in the three commonly consumed vegetables. The large variation in the level of pesticides detected in the vegetables may be due to many factors influencing the pesticide residues that remained on the vegetables at the time of harvest. These factors include the dosage of pesticides applied, dosing frequency and the pre-harvest interval for crops (Banerjee et al., 2006; Zhang et al., 2012). Appropriate education on pesticide use and the pre-harvest interval for crops is necessary. This education will assist to lessen the amount of pesticide residues remaining in vegetables and fruits.
Critically, the remarkably high rate of exceedance of the MRL (ranged from 35 to 71%) found in the three commonly consumed vegetables reported in the present study indicates that these vegetables either purchased from both the local markets and the supermarkets are highly contaminated with pesticide residues. Regarding Thai people’s expectations of supermarket produce, the findings in this study raises question to the quality of the vegetables marketed in supermarkets in Thailand. Quality of vegetables sold in the supermarkets in Thailand is, in general, thought to be good with regard to levels of pesticide contamination. Thai people’s perception of supermarket vegetables and fruits is high with respect to quality and freshness. Most Thai consumers believe the labels placed on the produce sold in the supermarkets in which they are claimed to be pesticide-free or organic produce. However, these labels and claims are made without scientific evidence and testing to support them. The quality, in terms of pesticide contamination of vegetables sold in the local markets in Thailand is not guaranteed, as the routine national monitoring programs of pesticide residues is not fully implemented (Wanwimolruk et al., 2015b). The existing evidence points to considerable food safety problems, since pesticide residues were noticeably detected in vegetables sampled from the local markets in Thailand (Sapbamrer & Hongsibsong, 2014; Wanwimolruk et al., 2015b). Such quality of these three commonly consumed vegetables marketed in Thailand appears to be similar regardless where the vegetables are purchased from, i.e. from local open-air markets or supermarkets. The present study has also demonstrated that there was similarity in the profiles of pesticides detected in the three commonly consumed vegetables from these two sources. In addition, the current study did not aim to compare organically grown and conventional grown vegetables but rather to compare the quality of the three commonly consumed vegetables bought from local markets and from supermarkets, in term of pesticide contamination. As previously mentioned, the vegetables sold from supermarkets of Thailand were categorized into four groups, i.e., conventional, organic, pesticide-free and safe vegetables. However, most of vegetables sold in the supermarkets have claimed to be either organic and pesticide-free produce. Such statements issued by the supermarkets are not always reliable. Our study did not test all organically grown vegetables in Thailand, so the findings are limited to the three vegetables studied. Future studies are warranted to verify if the produce sold in the supermarkets claiming to be organic and pesticide-free are of better quality than the conventionally grown produce (in terms of pesticide contamination).
The prices of vegetables and fruits sold in supermarkets in Thailand are substantially higher (2-6 times) than the produce sold in the local open-air markets. For example, the average price of Chinese kale from supermarkets was $112 ± 44 Bahts/kg, (approximately US$3.4/kg) which was more expensive than those from local markets ($38 ± 8 Bahts/kg, US$1.1/kg). In spite of this, for some pesticides such as diazinon and metalaxyl, the levels of these pesticides in the Chinese kale samples from the supermarkets were significantly higher than those seen in the samples from the local markets (Table 1). A similar observation was also found in the other two vegetables investigated, pakchoi and morning glory. This implies that the level of pesticide contamination of these three commonly consumed vegetables cannot be warranted by the price of the produce. However, it may be correct that vegetables and fruits purchased from the supermarkets are fresher than those from local open-air markets.

Our findings also emphasize the fact that these three commonly consumed vegetables, namely Chinese kale, pakchoi and morning glory, sold in the supermarkets in Thailand are not pesticide-free or organically grown as the merchants stated on the produce labels. This problem is challenging the Thai government authorities such as Thai FDA and the Department of Agriculture. The financial sponsor of this study, the Agricultural Research Development Agency (Public Organization) of Thailand requested us, as researchers to disseminate our findings through the Thai government authorities in order to facilitate the implementation of regulations and laws on pesticide residues and food safety. The Thai government authorities have been informed about the findings raised from this study. Further action has been planned: to rectify situation with the supermarket stakeholders, continue pesticide monitoring program, reinforce the laws, and properly instate the GAP system to the farmers. These are very important not only to reduce the health risks of consumers associated with pesticide residues in vegetables but also to protect consumers’ rights. The consumers who buy produce labeled organic pay more so should get a higher quality, pesticide-free produce.

**CONCLUSIONS**

There is considerable contamination of pesticides in these three commonly consumed vegetables in Thailand, i.e., Chinese kale, pakchoi and morning glory. Nine to twelve pesticides were detected in these vegetables at detection rates of 97-100%. The rate of pesticide residues
exceeding the MRL in these vegetables studied were remarkably high as compared to those reported in developed countries. The incidence of pesticide contamination was found to be similar between the vegetables bought from local markets and supermarkets. These findings questioned the quality of vegetables claimed to be pesticide-free sold in the supermarkets and urged the attention of the Thai government authorities to solve this important problem. This conclusion excludes Thai export quality vegetables and fruits that are routinely monitored for pesticide contamination before exporting. It is our recommendation for the Thai government authorities to conduct a proper pesticide monitoring program for these three commonly consumed local vegetables to protect the health of domestic consumers. The findings arisen from this study would be also useful for the Thai government to ascertain the MRL of pesticides in these three commonly consumed vegetables, and to incorporate other pest management strategies toward the safe and appropriate use of pesticides.

ACKNOWLEDGEMENTS

We are thankful to Dr. Nipa Rojroongwasinkul, Institute of Nutrition, Mahidol University for her guidance in the statistical analysis, and Ms. Onnicha Kanchanamayoon for her help in GC-MS analysis.

REFERENCES

Anastassiades M, Lehotay SJ, Stajnbaher D, and Schenck FJ. 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. Journal of AOAC International 86:412-431.

Banerjee K, Upadhyay AK, Adsule PG, Patil SH, Oulkar DP, and Jadhav DR. 2006. Rate of degradation of lambda-cyhalothrin and methomyl in grapes (Vitis vinifera L.). Food Additives and Contaminants 23:994-999. DOI:10.1080/02652030600838613

Cervera MI, Portoles T, Lopez FJ, Beltran J, and Hernandez F. 2014. Screening and quantification of pesticide residues in fruits and vegetables making use of gas chromatography-quadrupole time-of-flight mass spectrometry with atmospheric pressure chemical ionization. Analytical and Bioanalytical Chemistry 406:6843-6855. DOI:10.1007/s00216-014-7853-1

Chan PK. 1990. 2-5. Practical Considerations for the Development and Risk Assessment of Pesticides. The Journal of Toxicological Sciences 15:176-182. DOI:10.2131/jts.15.SupplementIV_176
Chang J, Chen T, and Fang T. 2005. Pesticide residue monitoring in marketed fresh vegetables and fruits in Central Taiwan (1999-2004) and an introduction to the HACCP system. *Journal of Food and Drug Analysis* 13:368-376.

Chen C, Qian Y, Chen Q, Tao C, Li C, and Li Y. 2011. Evaluation of pesticide residues in fruits and vegetables from Xiamen, China. *Food Control* 22:1114-1120. DOI:http://dx.doi.org/10.1016/j.foodcont.2011.01.007

Chen MF, Chen JF, Syu JJ, Pei C, and Chien HP. 2014. Insecticide residues in head lettuce, cabbage, Chinese cabbage, and broccoli grown in fields. *Journal of Agricultural and Food Chemistry* 62:3644-3648. DOI:10.1021/jf405555w

Claeys WL, De Voghel S, Schmit JF, Vromman V, and Pussemier L. 2008. Exposure assessment of the Belgian population to pesticide residues through fruit and vegetable consumption. *Food Additives and Contaminants Part A, Chemistry, Analysis, Control, Exposure and Risk Assessment* 25:851-863. DOI:10.1080/02652030701854741

Codex Alimentarius Commission. 2015. Pesticide Residues in Food and Feed. Available at http://www.codex-alimentarius.net/pestres/data/pesticides/index.html (accessed 15 March 2015).

Dong F, Chen X, Liu X, Xu J, Li Y, Shan W, and Zheng Y. 2012. Simultaneous determination of five pyrazole fungicides in cereals, vegetables and fruits using liquid chromatography/tandem mass spectrometry. *Journal of Chromatography A* 1262:98-106. DOI:http://dx.doi.org/10.1016/j.chroma.2012.08.100

Drouillet-Pinard P, Boisset M, Periquet A, Lecerf JM, Casse F, Catteau M, and Barnat S. 2011. Realistic approach of pesticide residues and French consumer exposure within fruit and vegetable intake. *Journal of Environmental Science and Health Part B, Pesticides, Food Contaminants, and Agricultural Wastes* 46:84-91. DOI:10.1080/03601234.2011.534413

Duff M., Voglino M. 2012. Rapid and robust multi-residue pesticides analysis using the Bruker 320-MS GC triple quadrupole mass spectrometer. 2012, www.bruker.com/chemicalanalysis, (accessed 20 November 2014)

European Commission. 2015. Pesticide EU-MRLs. Available at http://ec.europa.eu/sanco_pesticides/pub-lic/?event=substance.selection (accessed 7 March 2015).

Granby K, Petersen A, Hermann S, and Poulsen M. 2008. Levels of pesticides in food and food safety aspects. In: Tadeo J, ed. *Analysis of Pesticides in Food and Environmental Samples*. Boca Raton, FL, USA: CRC Press, 287-318.

Harnpicharnchai K, Chaiear N, and Charerntanyarak L. 2013. Residues of organophosphate pesticides used in vegetable cultivation in ambient air, surface water and soil in Bueng Niam Subdistrict, Khon Kaen, Thailand. *The Southeast Asian Journal of Tropical Medicine and Public Health* 44:1088-1097.

Huan Z, Xu Z, Jiang W, Chen Z, and Luo J. 2015. Effect of Chinese traditional cooking on eight pesticides residue during cowpea processing. *Food Chemistry* 170:1118-122. DOI:10.1016/j.foodchem.2014.08.052

Koesukwiwat U, Lehotay SJ, and Leepipatpiboon N. 2011. Fast, low-pressure gas chromatography triple quadrupole tandem mass spectrometry for analysis of 150 pesticide residues in fruits and vegetables. *Journal of Chromatography A* 1218:7039-7050. DOI:10.1016/j.chroma.2011.07.094
Lehotay SJ. 2007. Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: collaborative study. *Journal of AOAC International* 90:485-520.

Lehotay SJ, Son KA, Kwon H, Koesukwiwat U, Fu W, Mastovska K, Hoh E, and Leepipatpiboon N. 2010. Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. *Journal of Chromatography A* 1217:2548-2560. DOI:10.1016/j.chroma.2010.01.044

Li W, Tai L, Liu J, Gai Z, and Ding G. 2014. Monitoring of pesticide residues levels in fresh vegetable form Hebei Province, North China. *Environmental Monitoring and Assessment* 186:6341-6349. DOI:10.1007/s10661-014-3858-7

Nagendra Prasad K, Shivamurthy GR, and Aradhya SM. 2008. *Ipomoea aquatica*, An underutilized green leafy vegetable: a review. *International Journal of Botany* 4: 123-129.

Osei-Fosu P, Donkor AK, Nyarko S, Nazzah NK, Asante IK, Kingsford-Adabo R, and Arkorful NA. 2014. Monitoring of pesticide residues of five notable vegetables at Agbogbloshie market in Accra, Ghana. *Environmental Monitoring and Assessment* 186:7157-7163. DOI:10.1007/s10661-014-3917-0

Parveen Z, Khuhiro MI, and Rafiq N. 2005. Monitoring of pesticide residues in vegetables (2000--2003) in Karachi, Pakistan. *Bulletin of Environmental Contamination and Toxicology* 74:170-176.

Paya P, Anastassiades M, Mack D, Sigalova I, Tasdelen B, Oliva J, and Barba A. 2007. Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection. *Analytical and Bioanalytical Chemistry* 389:1697-1714. DOI:10.1007/s00216-007-1610-7

Sapbamrer R, and Hongsibsong S. 2014. Organophosphorus pesticide residues in vegetables from farms, markets, and a supermarket around Kwan Phayao Lake of Northern Thailand. *Archives of Environmental Contamination and Toxicology* 67:60-67. DOI:10.1007/s00244-014-0014-x

Sapbamrer R, and Nata S. 2014. Health symptoms related to pesticide exposure and agricultural tasks among rice farmers from Northern Thailand. *Environmental Health and Preventive Medicine* 19:12-20. DOI:10.1007/s12199-013-0349-3

Srithongkum W. 2014. Situation of food safety on consumption of vegetables and fruits in Thailand. The 1st National Conference on Food and Nutrition for Health Fruits and Vegetables for Nutrition Security. Bangkok, Thailand.

Swarnam TP, and Velmurugan A. 2013. Pesticide residues in vegetable samples from the Andaman Islands, India. *Environmental Monitoring and Assessment* 185:6119-6127. DOI:10.1007/s10661-012-3012-3

Thailand Ministry of Agriculture and Cooperation. 2013. Pesticide residues: maximum residue limits. Thai Agricultural Commodity and Food Standards. TAS 9002-2013, ICS 67.040. Available at [http://www.acfs.go.th/standard/download/MAXIMUM_RESIDUE_LIMITS_new.pdf](http://www.acfs.go.th/standard/download/MAXIMUM_RESIDUE_LIMITS_new.pdf) (accessed 12 January 2015).

Wang S, Wang Z, Zhang Y, Wang J, and Guo R. 2013. Pesticide residues in market foods in Shaanxi Province of China in 2010. *Food Chemistry* 138:2016-2025. DOI:10.1016/j.foodchem.2012.11.116
Wanwimolruk S, Kanchanamayoon O, Boonpangrak S, and Prachayasittikul V. 2015a. Food safety in Thailand 1: it is safe to eat watermelon and durian in Thailand. *Environmental Health and Preventive Medicine* 20:204-215. DOI:10.1007/s12199-015-0452-8

Wanwimolruk S, Kanchanamayoon O, Phopin K, and Prachayasittikul V. 2015b. Food safety in Thailand 2: Pesticide residues found in Chinese kale (*Brassica oleracea*), a commonly consumed vegetable in Asian countries. *Science of the Total Environment* 447-455. DOI:10.1016/j.scitotenv.2015.04.114

Zhang F, Wang L, Zhou L, and Pan C. 2012. Dissipation and residues of monosultap in rice plant and environment. *Bulletin of Environmental Contamination and Toxicology* 88:362-367. DOI:10.1007/s00128-011-0437-2
Figure Legends

Figure 1. Type of pesticides detected in the Chinese kale samples purchased from the local markets (n = 69) and the supermarkets (n = 68). For each pesticide detected, the lower bars are for samples from the local markets, and the upper bars are for samples from the supermarkets.

Figure 2. Type of pesticides detected in the pakchoi samples bought from the local markets (n = 63) and the supermarkets (n = 62). For each pesticide detected, the lower bars are for samples from the local markets, and the upper bars are for samples from the supermarkets.

Figure 3. Type of pesticides detected in the morning glory samples bought from the local markets (n = 74) and the supermarkets (n = 61). For each pesticide detected, the lower bars are for samples from the local markets, and the upper bars are for samples from the supermarkets.
Figure 1

Chinese kale

**Figure 1. Type of pesticides detected in the Chinese kale samples purchased from the local markets (n = 69) and the supermarkets (n = 68).** For each pesticide detected, the lower bars are for samples from the local markets, and the upper bars are for samples from the supermarkets.
Figure 2

Pakchoi

Figure 2. Type of pesticides detected in the pakchoi samples bought from the local markets (n = 63) and the supermarkets (n = 62). For each pesticide detected, the lower bars are for samples from the local markets, and the upper bars are for samples from the supermarkets.
The bar chart shows the number of samples for different pesticides in Pakchoi:

- **Profenofos**: Few samples with no pesticide found (0% MRL), some samples exceeding MRL.
- **Metalaxyl**: A significant number of samples with MRL (40% MRL) and some exceeding MRL.
- **Dimethoate**: Most samples are below MRL (50% MRL), with a few exceeding MRL.
- **Diazinon**: A substantial number of samples below MRL (80% MRL), with some samples exceeding MRL.
- **Cypermethrin**: A small number of samples below MRL (10% MRL), with a few exceeding MRL.
- **λ-Cyhalothrin**: Only a few samples below MRL, no samples exceeding MRL.
- **Chlorpyrifos**: A small number of samples below MRL, no samples exceeding MRL.
- **Carbofuran**: All samples are below MRL.
- **Carbaryl**: All samples are below MRL.
Figure 3

Morning glory

Figure 3. Type of pesticides detected in the morning glory samples bought from the local markets (n = 74) and the supermarkets (n = 61). For each pesticide detected, the lower bars are for samples from the local markets, and the upper bars are for samples from the supermarkets.
Morning Glory

- Profenofos
- Methomyl
- Metalaxyl
- Malathion
- Dimethoate
- Diazinon
- Cypermethrin
- λ-Cyhalothrin
- Chlorpyrifos
- Chlorothalonil
- Carbofuran
- Carbaryl

Legend:
- Local markets <MRL
- Local markets >MRL
- Supermarkets <MRL
- Supermarkets >MRL

Number of Samples

0  20  40  60  80
Table 1 (on next page)

Table & MRL
Table 1
Profile showing types of pesticide residues found in three commonly consumed vegetables studied, Chinese kale, pakchoi and morning glory

| Vegetable       | Carbaryl | Carbofuran | Chlorothalonil | Chlorpyrifos | λ-Cyhalothrin | Cypermethrin | Deltamethrin | Diazinon | Dimethoate | Malathion | Metalaxyl | Methomyl | Profenofos |
|-----------------|----------|------------|----------------|--------------|----------------|--------------|--------------|-----------|------------|-----------|------------|-----------|-----------|
| Chinese kale    | ✓        | ✓          | ✓              | ✓            | ✓              | ✓            | ✓            | ✓         | ✓          | ✓         | ✓          | ✓         | ✓         |
| MRL* (ppb)      | 50       | 20         | 4000           | 50           | 1000           | 1000         | 500          | 50        | 20         | 3000      | 2000       | -         | 10        |
| Pakchoi         | ✓        | ✓          | -              | ✓            | ✓              | ✓            | -            | ✓         | ✓          | ✓         | ✓          | ✓         | ✓         |
| MRL* (ppb)      | 50       | 20         | -              | 1000         | 1000           | 1000         | -            | 50        | 20         | -         | 50         | -         | 50        |
| Morning glory   | ✓        | ✓          | ✓              | ✓            | ✓              | ✓            | -            | ✓         | ✓          | ✓         | ✓          | ✓         | ✓         |
| MRL* (ppb)      | 10       | 10         | 10             | 50           | 20             | 700          | -            | 10        | 20         | 20        | 2000       | 20        | 10        |

* MRL values for each pesticide in the vegetable studied were cited from recommended MRL values established by Thailand Ministry of Agriculture and Cooperation (2013), Codex Alimentarius Commission (2015), and European Commission (2015).
Table 2 (on next page)

Table Median & Statistical
Table 2
Mean and median concentrations of six commonly detected pesticides in three vegetables studied. n is number of samples in which the pesticide was detected.

| Pesticide | Chinese kale |  |  |  |  |
|-----------|--------------|-----------------|-----------------|-----------------|-----------------|
|           | Local markets | Supermarkets | Local markets | Supermarkets | Local markets | Supermarkets |
| Carbofuran | - | - | 0.28 (Med)\(^a\) | 1.6 (Med)* | 8.2 (Med) | 7.7 (Med) |
|           | | | (0.11, 0.96)\(^b\) | (1.1, 3.5) | (2.4, 83.2) | (3.2, 33.4) |
|           | | | (n = 21) | P = 0.0002 | (n = 71) | P = 0.442 |
| Dimethoate | 10.5 (Med) | 6.4 (Med) | 27.2 (Med) | 18.6 (Med) | 10.4 (Med) | 12.1 (Med) |
|           | (2.3, 30.1) | (1.9, 15.4) | (9.8, 46.2) | (4.7, 40.2) | (3.4, 34.6) | (4.0, 28.8) |
|           | (n = 57) | P = 0.17 | (n = 59) | P = 0.167 | (n = 68) | P = 0.737 |
|           | | | (n = 50) | | (n = 55) | |
| Diazinon | 0.28 (Med) | 2.0 (Med)* | 1.4 (Med) | 1.8 (Med) | 1.1 (Med) | 1.7 (Med)* |
|           | (0.13, 1.7) | (0.82, 4.2) | (0.67, 2.6) | (1.1, 3.6) | (0.62, 1.6) | (0.97, 2.7) |
|           | (n = 42) | P = 0.0004 | (n = 36) | P = 0.07 | (n = 39) | P = 0.009 |
|           | | | (n = 41) | | (n = 40) | |
| Metalaxyl | 0.93 (Med) | 10.8 (Med)* | 8.8 (Med) | 6.4 (Med) | 29.4 (Med) | 24.8 (Med) |
|           | (0.32, 3.5) | (2.1, 23.9) | (2.1, 36.1) | (2.5, 31.4) | (12.2, 71.3) | (5.8, 64.0) |
|           | (n = 61) | P = 0.0001 | (n = 61) | P = 0.8 | (n = 27) | P = 0.562 |
|           | | | (n = 52) | | (n = 61) | |
| Profenofos | 9.3 (Med) | 23.9 (Med) | - | - | - | - |
|           | (0.26, 71.2) | (1.3, 36.3) | | | | |
|                | \( P = 0.39 \) |
|----------------|---------------|
| (n = 28)       |               |
| (n = 18)       |               |

4  a Median value

5  b 25 and 75 percentiles of the median value.

6  * Statistically significant differences in pesticide concentrations were observed between the local market and the supermarket groups \((P < 0.05)\).