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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, Kamonrat Phopin, Somchai Boonpangrak, Virapong Prachayasittikul

Background The wide use of pesticides raises concerns on the health risks associated with pesticide exposure. For developing countries, pesticide residues in vegetables and fruits have not been totally monitored. 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 وعلم 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. It is unlikely that this will affect the health of tourists visiting Thailand because they do not consume these vegetables daily and in large amount. However, remarkably high incidence rates of detected pesticides give warning to the Thai authorities to implement proper regulations on pesticide monitoring programme. 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.

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33 ABSTRACT

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35 Background The wide use of pesticides raises concerns on the health risks associated with 36 pesticide exposure. For developing countries, pesticide residues in vegetables and fruits have not 37 been totally monitored. This study aims at providing comparison data on pesticide residues found in three commonly consumed vegetables (Chinese kale, pakchoi and morning glory) purchased 38 39 from some local markets and supermarkets in Thailand. Methods These vegetables were 40 randomly bought from local markets and supermarkets. Then they were analyzed for the content 41 of 28 pesticides by using GC-MS/MS. Results Types of pesticides detected in the samples either 42 from local markets or supermarkets were similar. The incidence of detected pesticides was 100% 43 (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 44 45 morning glory samples. The pesticides were detected exceeding their MRL at a rate of 48% 46 (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 47 48 morning glory. **Discussion** These rates are much higher than those seen in developed countries. 49 It should be noted that these findings were assessed on basis of using criteria (such as MRL) 50 obtained from developed countries. Our findings were also confined to these vegetables sold in a 51 few central provinces of Thailand and did not reflect for the whole country as sample sizes were 52 small. Risk assessment due to consuming these pesticide contaminated vegetables, still remains 53 to be evaluated. It is unlikely that this will affect the health of tourists visiting Thailand because 54 they do not consume these vegetables daily and in large amount. However, remarkably high incidence rates of detected pesticides give warning to the Thai authorities to implement proper 55 56 regulations on pesticide monitoring programme. Similar incidence of pesticide contamination 57 found in the vegetables bought from local markets and supermarkets raises question regarding 58 the quality of organic vegetables domestically sold in Thailand. This conclusion excludes Thai export quality vegetables and fruits routinely monitored for pesticide contamination before 59 60 exporting.

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

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INTRODUCTION

An enormous concern on toxic pesticides in foods has been raised because of its negative 66 health and environmental impacts. This is due to the widespread use of pesticides in agriculture. 67 In fact, the main exposure to pesticides for humans is via food, especially by vegetables and 68 69 fruits (Claeys et al., 2008; Drouillet-Pinard et al., 2011). Toxicity and human health risk 70 associated with pesticide contamination in foods has made it necessary to regulate pesticide 71 residues in our foods (Cervera et al., 2014). Detection and quantification of pesticide residues in 72 food samples are essential to verify whether these pesticides are within limits, so called 73 "maximum residue limits (MRL)". This regulation was established by the European Commission 74 and other regulatory authorities. Many developed countries have approved this regulation to 75 oversee and operate their food safety affair. Contrastingly, in developing countries such as Thailand, good agricultural practices (GAP) have not been fully implemented, nor has a pesticide 76 77 monitoring program been successfully implemented either. Pesticides have been greatly used in 78 agriculture in Thailand (Harnpicharnchai et al., 2013). The most popular classes of pesticides 79 imported into Thailand are herbicides, followed by insecticides and fungicides (Sapbamrer & 80 *Nata*, 2014). Among the insecticides, organophosphates and carbamates are very commonly used 81 for protecting crops from insects' invasion. The use of pesticides in agriculture has been linked 82 with occupational health of farmers, gardeners and consumers (Chan, 1990; Sapbamrer & Nata, 83 2014).

84 Chinese kale (Brassica oleracea) is also known as Chinese broccoli. Chinese kale is a leaf 85 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 86 87 kale is very like to that of broccoli, but somewhat bitterer. Chinese kale is used extensively in 88 Chinese cuisine, and especially in Cantonese cuisine. In Thailand, a number of admired Thai 89 dishes have Chinese kale as a principal ingredient. In some dishes, Chinese kale is consumed 90 fresh, without cooking. This possesses potential for toxicity if the vegetable is eaten freshly and 91 daily. Pakchoi [Brassica chinensis Jusl var parachinensis (Bailey) Tsen & Lee] is a species in 92 the Brassicaceae which is a popular vegetable consumed in Thailand, also in Southeast Asia and 93 southern China. Unlike napa cabbage (Brassica pekinensis), pakchoi does not form heads; 94 instead, they have smooth, dark green leaf blades forming a cluster reminiscent of mustard or

95 celery. Water morning glory (*Ipomoea aquatic* Forsk) is a semiaquatic, tropical plant grown as a 96 vegetable in East, South and Southeast Asia. It is also known as water spinach, water 97 convolvulus, or by the more ambiguous names Chinese spinach, Chinese convolvulus or swamp 98 cabbage (*Wikipedia, 2015*). It is known as pak bung in Thai, ong choy in Chinese and kangkong 99 in Tagalog. Water morning glory is one of the most popular vegetables constituted in Thai, 100 Burmese, Lao, Cambodian, Malay, Vietnamese, Filipino, and Chinese cuisines.

101 Pesticide residues have been found in many raw agricultural commodities such as vegetables 102 and fruits, and processed foods worldwide in the past decades (Chen et al., 2011; Chen et al., 103 2014; Huan et al., 2015; Li et al., 2014; Osei-Fosu et al., 2014; Sapbamrer & Hongsibsong, 104 2014; Wang et al., 2013; Wanwimolruk et al., 2015a; Wanwimolruk et al., 2015b). Presently, 105 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, 106 107 particularly in vegetables and fruits. Also, the current case of organic fruits and vegetables sold 108 in Thailand displays to consumers with no confidence in regard to quality whether the produce is 109 pesticide-free. Many supermarkets have placed labels on fruits and vegetables implying that they 110 are either organically grown or pesticide-free. Consequently, people are prepared to buy 111 vegetables and fruits from supermarkets at much higher price than those from local markets. This 112 is because they have a high expectation that supermarket produce is safe from pesticide 113 contamination. However, there is no scientific-based evidence to verify the supermarkets' claims 114 and people's beliefs. Therefore, the purpose of this study was to provide comparison data on 115 pesticide residues found in three commonly consumed vegetables (Chinese kale, pakchoi and 116 water morning glory) purchased from local markets and supermarkets. Also, we aimed to verify if these vegetables that are sold in supermarkets in Thailand are free of pesticides as they are 117 118 claimed to be.

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MATERIALS AND METHODS

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122 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

126 purchased from Merck (Darmstadt, Germany). Twenty eight pesticides and two metablolite 127 including aldrin, atrazine, captan, carbaryl, carbofuran (and its two metabolites standards 128 carbofuran-3-hydroxy and carbofuran-3-keto), carbosulfan, chlormefos, chlorpyrifos, chlorothalonil, λ -cyhalothrin, cypermethrin, deltamethrin, diazinon, dichlorvos, dicofol, 129 130 dimethoate, ethion, fenitrothion, fenvalerate, malathion, metalaxyl, methidathion, methomyl, paraoxon-methyl, phosalone, pirimicarb, pirimiphos-methyl and profenofos were purchased from 131 132 Dr. Ehrenstorfer (Augsburg, Germany). Purity of these pesticide standards was >98%. Individual stock of standard solutions (1000 mg/L) was prepared in acetonitrile. 133

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135 Vegetable samples

136 Three vegetables were selected for this study namely Chinese kale, pakchoi and water 137 morning glory. The selection was based on their high consumption in Thailand. These three 138 vegetables are widely consumed among Thai and Asian people. Chinese kale samples (n = 137) 139 were purchased randomly from local open-air markets (n = 69) and supermarkets (n = 68). For 140 pakchoi, a total of 125 samples were bought from local markets (n = 63) and supermarkets (n =141 62). Samples of water morning glory (n = 135) were purchased randomly from local markets (n 142 = 74) and supermarkets (n = 61). These markets were located in central provinces of Thailand 143 including Bangkok, Nakhon Pathom, Nonthaburi, Ayutthaya, Pathumthani, Samutsakorn and 144 Nakhon Ratchasima. These provinces are located surrounding Bangkok, Thailand, within a radial 145 distance of 260 km. The supermarkets which the vegetable samples were bought from were Big 146 C, Foodland, Jiffy Plus, Lemon Farm, Max Valu, Tesco Lotus, Tops and Villa Market. The study 147 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 148 149 farms and was not claimed to be 'organic produce'. Whereas the vegetable samples that were purchased from supermarkets mostly claimed to be 'organic produce' or "pesticide-free". 150 151 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 152 153 the vegetable sample was chopped into tiny pieces and homogenized using a food processor and 154 mixed carefully. The homogenized samples were then extracted and treated as described in following section. 155

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157 Sample preparation

158 The analysis of pesticide residues was performed using the pesticide multiresidue 159 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, 160 161 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 162 163 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), 164 7.5 mg graphite carbon black (GCB) and 150 mg magnesium sulphate. After shaking and 165 166 centrifugation, the extract supernatant was then transferred to an autosampler vial for direct injection into the Bruker GC/MS/MS system. 167

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169 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.

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177 Calibration and quantification

178 A working surrogate spiking standard solutions of pesticides were made by an appropriate

179 dilution of the stock solutions with acetonitrile. These standard solutions were guarded from light

180 and kept frozen at -20 °C until required. Calibration curves of each pesticide of interest were

181 conducted using an internal standard method according to the established procedure

182 (Koesukwiwat et al., 2011; Lehotay, 2007; Lehotay et al., 2010; Wanwimolruk et al., 2015b).

183 These were conducted using the same procedure each time when a new unknown sample set was

- 184 analyzed. Aldrin was used as an internal standard. The ratio of the peak area of the pesticide
- 185 standard to that of the internal standard was employed for quantification. Recovery studies for
- 186 method validation were conducted as previously described (Koesukwiwat et al., 2011;
- 187 Wanwimolruk et al., 2015b). The method validation in regard to reproducibility, calibration

188 linear range, limit of detection (LOD), limit of quantification (LOQ) was performed for each 189 vegetable matrix as expressed previously (Dong et al., 2012; Koesukwiwat et al., 2011). 190 Quantitation of pesticides in an unknown vegetable sample was carried out in duplicate unless 191 otherwise stated. MRL values for each pesticide in the vegetable of interest were quoted from 192 recommended MRL values established by Thailand Ministry of Agriculture and Cooperation 193 (2013), Codex Alimentarius Commission (2015), and European Commission (2015). 194 195 **Statistical analysis** 196 All results are presented as either mean \pm standard deviation (S.D.) or median. The 197 differences of parameter between two sample groups were assessed by either unpaired Student's 198 *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 199 200 software SPSS statistical package for Windows version 18.0 (SPSS Inc., Chicago, IL, USA). 201 202 **RESULTS** 203 204 The GC-MS/MS method was validated to determine efficiency and accuracy of the analytical 205 assay. Excellent linearity of calibration curves of each pesticide standards were attained as 206 illustrated by the coefficient of determination (r^2) values of >0.92. When the pesticides of interest 207 were assayed at 0.01 ppb the signal-to-noise ratio was well above 30 for all pesticides studied. 208 Therefore, detection limits were below 0.01 ppb using the sample preparation procedures 209 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 210 211 were in good precision. Their relative standard deviations (RSD) of repeatability were lower than 8% whereas the RSD of reproducibility were lower than 17%. The mean recoveries of all 212 213 pesticides studied from fortified samples in five replicated experiments were in the range of 75 -214 114%. These ranges of recovery fall within the typical acceptance criteria for quantitative 215 regulatory methods (Koesukwiwat et al., 2011). 216 Twenty eight pesticides studied were selected on the basis of their widespread use in agriculture in Thailand. The GC-MS/MS method employed in this study offered satisfactorily 217

separation with high sensitivity and selectivity for quantitation of all 28 pesticides of interest

219 (*Wanwimolruk et al., 2015b*). The absence of co-extracted interferences for all varieties of leaf

220 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

223 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.

226 Of 28 pesticides tested, 12 pesticides were detected in the Chinese kale samples purchased from supermarkets (Figure 1). These included carbaryl, carbofuran, chlorothalonil, chlorpyrifos, 227 228 λ -cyhalothrin, cypermethrin, deltamethrin, diazinon, dimethoate, malathion, metalaxyl and 229 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 230 231 samples bought from supermarkets. Most of Chinese kale samples (88% in local markets, 91% in 232 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 233 234 sources (Figure 1). The occurrence rate of metalaxyl in the local market samples was 91% 235 (63/69) and was 94% (64/68) for the supermarket samples. However, none of the Chinese kale 236 samples purchased from both local markets and supermarkets had metalaxyl that exceeded the 237 recommended MRL value (2000 ppb). Rates of occurrence for dimethoate in the Chinese kale 238 samples were 80% (55/69) and 88% (60/68) for the Chinese kale samples from local markets and 239 supermarkets, respectively. Of 69 samples from local markets, 23 of them had dimethoate 240 exceeding the MRL value (20 ppb). This corresponds to a rate of greater than dimethoate's MRL 241 of 33%. Eleven samples purchased from the supermarkets were found to contain dimethoate that 242 exceeded the MRL. These samples exceeded dimethoate's MRL by 16%. 243 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 244 supermarkets, respectively (Figure 1). None of both the local market and supermarket samples 245 246 had diazinon levels that exceeded the recommended MRL (50 ppb). Three other pesticides were 247 also detected in the Chinese kale samples which were profenofos, cypermethrin and carbaryl.

248 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

250 supermarket samples. Eleven of the samples purchased from the local markets had profenofos 251 levels exceeding the MRL (10 ppb), whereas twelve samples from the supermarkets contained 252 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 253 254 samples from both sources with a range from 0.1 - 2.095 ppb. Pyrethroid pesticide cypermethrin 255 was also detected in the Chinese kale samples at a relatively low rate of detection. Cypermethrin 256 was found in 16% (11/69) of the samples bought from the local market, similarly 15% (10/68) of 257 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 258 found to have cypermethrin that exceeded the MRL value (1000 ppb). Carbaryl was detected in 259 260 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 261 262 which two of the supermarket samples contained carbaryl exceeding its MRL value (50 ppb). 263 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 264 265 and malathion.

Figure 2 illustrates the incidence of pesticide detection in the Chinese kale samples 266 267 purchased from the local markets and supermarkets. The percentages of total pesticide detection 268 both in the two sources were extremely high, i.e., 100% and 99% for the samples bought from the local markets and the supermarkets, respectively. Of interest, the incidence of pesticides 269 270 detected exceeding the recommended MRL values was 48% in the Chinese kale samples 271 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 (Figure 2). 272 273 Very small samples were found to contain no pesticides; this represents a rate of free of pesticides of 1% in the supermarket samples (Figure 2). 274

Nine pesticides were detected in both the pakchoi samples purchased from the local markets and the supermarkets (Figure 3). These were carbaryl, carbofuran, chlorpyrifos, λ -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

281 multiple pesticide residues. Profiles of pesticide types detected in the pakchoi samples from both 282 sources were similar. Like the Chinese kale, occurrence of metalaxyl in pakchoi samples was 283 very high at 97% (61/63) for the samples purchased from the local markets, and 98% (61/62) for the samples from the supermarkets were found to have metalaxyl residues (Figure 3). Among 284 285 these local market samples, 13 samples (21%) had metalaxyl levels that exceeded the 286 recommended MRL (50 ppb). For the samples bought from the supermarkets, 11 samples (18%) 287 had metalaxyl that exceeded the MRL. Dimethoate was found in 94% (59/63) and 87% (54/62) 288 of the pakchoi samples from local markets and supermarkets, respectively (Figure 3). Thirty-four 289 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 290 291 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 3). 292 293 None of the pakchoi samples bought from both the local markets and the supermarkets had 294 diazinon levels above the MRL (50 ppb). Carbofuran, chlorpyrifos and cypermethrin were 295 detected in pakchoi samples from both the local market and supermarkets but with moderate 296 occurrence rates. Cypermethrin was found in 19% (12/63) of the pakchoi samples bought from 297 the local market samples, while 21% (13/62) of the supermarket samples contained cypermethrin 298 (Figure 3). Two of the pakchoi samples bought from the local markets were found to have 299 cypermethrin exceeding the recommended MRL (1000 ppb). Five of the pakchoi samples bought 300 from supermarkets had cypermethrin exceeding the MRL. Chlorpyrifos was detected in 11% 301 (7/63) of the pakchoi samples purchased from the local markets, whereas 16% (10/62) of the 302 supermarket samples were found to contain chlorpyrifos residues (Figure 3). Two of the pakchoi 303 samples purchased from the local markets and one supermarket sample had chlorpyrifos that 304 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 305 306 though other three pesticides including carbaryl, λ -cyhalothrin and profenofos were also detected 307 in the pakchoi samples but the occurrence rates were relatively low (Figure 3). 308 Figure 4 shows the overall incidence of pesticide detection in the pakchoi samples both from 309 the local markets and from supermarkets. The total incidence of pesticide detection in the

310 pakchoi samples was 98% and 100% for the samples bought from the local markets and from the

311 supermarkets, respectively. The incidence of pesticides detected exceeding the recommended

312 MRL values was 71% in the pakchoi samples purchased from the local markets (Figure 4).

- 313 While the incidence of MRL exceedance was 55% in the pakchoi samples bought from the
- 314 supermarkets. These left the proportions of pakchoi samples having pesticide residues of less
- than MRL and without pesticides to be approximately 30%.

316 Of 28 pesticides investigated, 12 different individual pesticides were detected in the water 317 morning glory samples purchased from both the local markets and the supermarkets (Figure 5). 318 Eight common pesticides detected in both the morning glory samples from the local markets and 319 the supermarkets were carbofuran, chlorpyrifos, λ -cyhalothrin, cypermethrin, diazinon, dimethoate, metalaxyl and profenofos. Few samples contained only one pesticide, but most of 320 them (90% in local markets, 89% in supermarket samples) had multiple pesticide residues. 321 322 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. 323 324 Occurrence rates of metalaxyl in pakchoi samples were 96% (71/74) and 93% (57/61) for the 325 local market and the supermarket samples, respectively. All of the morning glory samples tested 326 had metalaxyl levels below the recommended MRL (2000 ppb). Occurrence rates for dimethoate 327 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 328 329 dimethoate exceeding the MRL value (20 ppb). This represents a rate of greater than 330 dimethoate's MRL of 38%. Twenty-two samples purchased from supermarkets were found to 331 contain dimethoate that exceeded the MRL, denoting to a rate of greater than dimethoate's MRL 332 of 36%. For diazinon, the occurrence rates in the water morning glory samples were 53% (39/74) 333 and 79% (48/61) for the samples from local markets and supermarkets, respectively (Figure 5). Only one sample of the water morning glory purchased from the local markets had diazinon 334 335 exceeding the recommended MRL (10 ppb). None of the water morning glory samples from the 336 supermarkets had diazinon levels above the MRL value. Carbofuran and cypermethrin were 337 detected in the water morning glory samples from both the local markets and supermarkets with 338 moderate occurrence rates. Carbofuran was found in the morning glory samples with occurrence 339 rates of 32% (24/74) and 28% (17/61) for the local markets and supermarkets, respectively. All 340 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 341 342 local market samples, while 26% (16/61) of the supermarket samples contained cypermethrin

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343 (Figure 5). Five of the water morning glory samples (7%) from the local markets had 344 cypermethrin that exceeded its MRL (700 ppb). Out of the supermarket samples, four samples 345 (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 346 347 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 5). One sample 348 349 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 -350 cyhalothrin, malathion, methomyl and profenofos were detected in the water morning glory 351 samples, although their occurrence rates were very low (Figure 5). Of note, some of pesticides 352 353 mentioned were not detected in both the local market samples and the supermarket samples. For 354 example, carbaryl and chlorothalonil were detected only in the supermarket samples, but not 355 found in the water morning glory samples bought from the local markets.

Figure 6 compares the overall incidence of pesticide detection in the water morning glory 356 samples purchased from local markets and supermarkets. Small proportions of samples were 357 358 found to contain no pesticide residues; this represents a rate of free of pesticide-free residue of 359 1% and 3% in the local and supermarkets, respectively (Figure 6). Extremely high percentages of pesticide detection, i.e., 99% and 97% were observed in the morning glory samples bought from 360 361 the local markets and supermarkets, respectively. The incidence of pesticide residues detected 362 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 (Figure 6). 363 364 The profiles of pesticides detected in the three vegetables investigated are shown for comparison (Table 1). Of the 28 pesticides studied, there were 13 pesticides found in the fresh 365 366 samples of these three popularly consumed vegetables. Nine pesticides were found to be 367 common pesticides detected in all the three vegetables studied. These were carbaryl, carbofuran, 368 chlorpyrifos, λ -cyhalothrin, cypermethrin, diazinon, dimethoate, metalaxyl and profenofos. Methomyl was not detected in the Chinese kale and pakchoi samples. Chlorothalonil, 369 370 deltamethrin, metathion and methomyl were not found in the pakchoi samples, while 371 deltamethrin was also not detected in the morning glory samples. 372 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

374 data were evaluated and are presented in Table 2. All the pesticide concentrations detected in 375 these vegetables were found to be not normally distributed; therefore, the data was then 376 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 377 378 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 379 380 and the supermarkets. However, the median concentrations of diazinon and metalaxyl in the 381 Chinese kale samples purchased from the supermarkets were significantly greater than those 382 detected in the samples purchased from the local markets (P < 0.001, Table 2). With regard to results in pakchoi, the median concentrations of three pesticides, dimethoate, diazinon and 383 384 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 385 386 samples bought from the supermarkets were significantly higher than those observed in the local 387 markets (P < 0.001, Table 2). For the morning glory samples, there were no significant differences between the samples from the local markets and the supermarkets in median 388 389 concentrations of pesticides. The exception to this was for the median concentration of diazinon 390 in the supermarket samples was significantly higher than that seen in the local markets (P < 0.01, 391 Table 2).

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DISCUSSION

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395 The GC-MS/MS methods established in our laboratory (Wanwimolruk et al., 2015b) 396 involving QuEChERS sample preparation and GC-MS/MS analysis, were validated. The 397 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 398 399 results of assay validation which have illustrated good recovery, sensitivity, selectivity, linear 400 calibration curves, good reproducibility and accuracy. The utilizations of GC combined with 401 triple quadrupole MS technique not only aided the detection and quantitation of pesticides but it 402 also offered excellent sensitivity for pesticide detection. 403 The present study examined potential contamination of 28 pesticides in three leaf vegetables

404 (namely Chinese kale, pakchoi and morning glory sold in Thailand. Twelve pesticides were

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405 detected in fresh Chinese kale samples bought from the local markets and the supermarkets. 406 These included carbamates (carbaryl, carbofuran), organochlorines (chlorothalonil), 407 organophosphorus pesticides (chlorpyrifos, diazinon, dimethoate, malathion, profenofos), 408 pyrethroids (λ -cyhalothrin, cypermethrin, deltamethrin), and metalaxyl. Findings of so many 409 pesticides detected in this vegetable indicate that pesticides are widely and extensively used in 410 the agronomy of Chinese kale in Thailand. This observation is in agreement with our recent 411 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 412 contamination in vegetables in Thailand and other Asian countries (Chang et al., 2005: 413 414 Sapbamrer & Hongsibsong, 2014; Swarnam & Velmurugan, 2013). A study carried out in a northern part of Thailand (Sapbamrer & Hongsibsong, 2014) reported that vegetables bought 415 from markets contained organophosphorus pesticides greater than the recommended MRLs. 416 417 These vegetables included garlic, Chinese cabbage, spring onion, Vietnamese coriander and 418 Chinese kale. These findings with respect to Chinese kale agree with our observation in which 419 the levels of three organophosphorus pesticides (chlorpyrifos, dimethoate and profenofos) were 420 greater than their corresponding MRL values. In the present study, there were five pesticides, 421 namely carbofuran, chlorpyrifos, cypermethrin, dimethoate and profenofos, which were detected 422 in some samples at levels exceeded the MRLs (Figure 1). This implies that the Thai farmers used 423 these pesticides in excessive doses or did not follow the GAP in which an appropriate pre-424 harvest interval was not considered. Metalaxyl, dimethoate and diazinon appeared to be the most 425 often used pesticides in the agriculture of this vegetable. Although metalaxyl and diazinon were 426 among the most often detected in the Chinese kale samples, the pesticide residue concentrations found were not exceed their corresponding MRL values. In addition, it is of note that so many of 427 428 the Chinese kale samples (90%) tested contained multiple pesticide residues (Figure 1). This 429 clearly indicates that the Thai farmers are likely to use more than one pesticide during the cultivation of Chinese kale. 430

There were similarities in the profiles of pesticides found in the samples of the three commonly consumed vegetables studied, Chinese kale, pakchoi and morning glory, from both the local markets and the supermarkets (Table 1). Similar types of pesticides detected in these three individual vegetables indicates that Thai farmers cultivated these three commonly consumed vegetables in the same areas of their farm, as it is easy to water and protect these

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436 vegetables from pests by using the same mixture of pesticides. Among the pesticides used in cultivation of these three leafy vegetables, metalaxyl, dimethoate and diazinon were the most 437 438 often used pesticides. Moreover, the similarity in the profiles of pesticides detected in these three commonly consumed vegetables studied, i.e., Chinese kale, pakchoi and morning glory, suggests 439 440 that it is an advantage to reduce the cost for the pesticide monitoring by selecting to monitor the 441 pesticide residues in only one of these vegetables. The results from any of these three vegetables 442 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 443 444 sources the local markets and the supermarkets. For instance, most of the twelve pesticides found 445 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 446 447 samples from both sources, i.e., the local markets and the supermarkets. Chlorothalonil and 448 deltamethrin were not detected in the local market samples, while a few samples from the 449 supermarkets were contaminated with residues of these pesticides. Malathion was found in only 450 one Chinese kale sample from the local markets but not in the samples from the supermarkets. 451 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 452 453 supermarkets. These minor differences in the profiles of pesticides found in the three commonly 454 consumed vegetable samples from the local markets and the supermarkets may be related to the 455 sources (or farms) where the vegetables were cultivated, difference of usage of each type of 456 pesticides, and ignorance of GAP awareness. Traceability of the produces was hard to attain and 457 ultimately this was not the primary goal of the current study. The merchants were in fact asked where they bought the vegetables from and habitually many of them did not have an answer. For 458 459 those who provided an answer, it appeared that most of the vegetable samples tested were bought 460 from four different whole sale markets in Bangkok and Nakhon Pathom province situated near 461 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 462 463 pesticide detection and the MLR exceedance. Vitally, proper education such as GAP regarding 464 the appropriate use of pesticides must be provided to these farmers. The present study revealed overall incidence of pesticide detection in the three vegetables 465

466 studied was in a range from 97-100% (Figure 2). For the Chinese kale, this high incidence of

467 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 468 469 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 470 471 incidence of pesticide detection observed in the three commonly consumed vegetables are 472 noticeably higher than the tolerable detection rate in western or developed countries, such as 473 USA and European Commission (EC) countries like France, U.K., Norway and Germany. For 474 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 475 30% of the domestic vegetables and 21% of the imported vegetables. In Taiwan, between the 476 years 1997-2003, pesticide residues were detected in 14% of 9,955 vegetable samples tested 477 (Chang et al., 2005). A survey study conducted in India found residues of many 478 organophosphorus pesticides (e.g., chlorpyrifos, dimethoate, monocrotophos and profenofos) in 479 480 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 = 481 482 106) in various vegetables bought from the markets. This rate is nevertheless much lower than 483 the rates of pesticide detection in the Chinese kale, pakchoi and morning glory observed in this 484 study. The difference may be accounted for by differences in seasons of vegetable cultivation, 485 vegetable types, types of pesticides used and analytical methods employed.

486 Remarkably, the occurrence of pesticide detection exceeding the MRL in the three vegetables 487 studied ranged from 35 to 71% (Figure 2, Figure 4 and Figure 6). The incidence of pesticide 488 detection of exceeding the MRL in these three vegetables investigated was high in both samples 489 from the local markets and the supermarkets. These were noticeably high, as compared with the 490 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 491 492 vegetable samples (Granby et al., 2008). The European Union (EU) Monitoring Program for 493 pesticides declared that 5% of vegetable samples examined had the pesticide residue 494 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., 495 496 2005). Therefore, the incidence of pesticide detection of >MRL in our three vegetables, Chinese kale, pakchoi and morning glory samples at rates of 32 to 49% are unusually high when 497

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498 compared with acceptable rates reported in developed countries. Nevertheless, these incidence 499 rates are somehow similar to that found in Pakistan, an Asian country, in which 206 different 500 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 501 502 vegetable samples tested contained pesticide residues that exceeded the MLR values. In addition, 503 the incidence of pesticide detection of >MRL was stated to be 24% in several market vegetables 504 examined in northern districts of Thailand (Sapbamrer & Hongsibsong, 2014). This rate of 505 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 506 507 the pesticide monitoring program for vegetables and fruits in which more than 60,000 samples 508 were screened each year (Srithongkum, 2014). The report revealed that violations (with pesticide concentration >MRL) found in vegetables and fruits marketed in Thailand were in a range of 5% 509 510 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 511 (35-71%) found in this study. The conflicting findings are likely to be accounted for by the 512 513 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 514 of detecting two groups of pesticides, i.e., carbamates (carbofuran and methomyl) and 515 516 organophosphates (dicrotophos and EPN). Nevertheless, unlike our current GC-MS/MS method, 517 the GT-Test kit cannot offer a quantitative analysis like most analytical methods, such as UV 518 spectrophotometric assays, LC-MS/MS and GC-MS/MS. Because the kit assay is restricted in 519 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 520 521 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

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529 pakchoi. Using these low recommended MRLs yielded the remarkably high rate of MRL 530 exceedance observed in the present study. In addition, it should be noted that our findings were 531 limited to these three vegetables sold in a small number of central provinces of Thailand and did 532 not reflect the figure for the whole country. This is because the sample sizes were considerably 533 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 534 535 assessment due to consuming these pesticide contaminated vegetables, has not yet been 536 evaluated. A larger sample size would be necessary for that as well. Risk to health of tourists visiting Thailand is unlikely as they do not consume these vegetables daily nor in large amount. 537

538 There were substantial variations in the levels of pesticides found in the three vegetables 539 tested in this study. 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 540 541 carbaryl found in these samples ranged from 0.1 to 606 ppb. The large variation in the level of 542 pesticides detected in the vegetables may be due to many factors influencing the pesticide 543 residues that remained on the vegetables at the time of harvest. These factors include the dosage 544 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 545 546 crops is necessary. This education will assist to lessen the amount of pesticide residues 547 remaining in vegetables and fruits.

548 Critically, the remarkably high rate of exceedance of the MRL (ranged from 35 to 71%) 549 found in the three commonly consumed vegetables reported in the present study indicates that 550 these vegetables either purchased from both the local markets and the supermarkets are highly 551 contaminated with pesticide residues. Regarding Thai people's expectations of supermarket 552 produce, the findings in this study raises question to the quality of the vegetables marketed in 553 supermarkets in Thailand. Quality of vegetables sold in the supermarkets in Thailand is, in 554 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. 555 556 Most Thai consumers believe the labels placed on the produce sold in the supermarkets in which 557 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 558 559 contamination of vegetables sold in the local markets in Thailand is not guaranteed, as the

560 routine national monitoring programs of pesticide residues is not fully implemented 561 (Wanwimolruk et al., 2015b). The existing evidence points to considerable food safety problems, 562 since pesticide residues were noticeably detected in vegetables sampled from the local markets in Thailand (Sapbamrer & Hongsibsong, 2014; Wanwimolruk et al., 2015b). Findings derived from 563 the current study further document the evidence of significant pesticide residues found in the 564 565 three vegetables sold in Thailand. Such quality of these three commonly consumed vegetables 566 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 567 there was similarity in the profiles of pesticides detected in the three commonly consumed 568 569 vegetables from these two sources. By looking at the results of the current study, metalaxyl, 570 dimethoate and diazinon appear to be the most often detected pesticides in the three commonly 571 vegetables studied, bought both from local markets and supermarkets. The prices of vegetables 572 and fruits sold in supermarkets in Thailand are substantially higher (2-6 times) than the produce 573 sold in the local open-air markets. For example, the average price of Chinese kale from 574 supermarkets was 112 ± 44 Bahts/kg, (approximately US\$3.4/kg) which was more expensive 575 than those from local markets $(38 \pm 8 \text{ Bahts/kg}, \text{ approximately US}1.1/\text{kg})$. In spite of this, for some pesticides such as diazinon and metalaxyl, the levels of these pesticides in the Chinese kale 576 577 samples from the supermarkets were significantly higher than those seen in the samples from the 578 local markets (Table 1). A similar observation was also found in the other two vegetables 579 investigated, pakchoi and morning glory. This implies that the level of pesticide contamination 580 of these three commonly consumed vegetables cannot be warranted by the price of the produce. 581 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 582 583 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 584 585 on the produce labels. This problem is challenging the Thai government authorities such as Thai 586 FDA and the Department of Agriculture. The financial sponsor of this study, the Agricultural 587 Research Development Agency (Public Organization) of Thailand requested us, as researchers to 588 disseminate our findings through the Thai government authorities in order to facilitate the 589 implementation of regulations and laws on pesticide residues and food safety. The Thai 590 government authorities have been informed about the findings raised from this study. Further

591 actions have been planned: to rectify situation with the supermarket stakeholders, continue

592 pesticide monitoring programme, reinforce the laws, and properly instate the GAP system to the

593 farmers. These are very important not only to reduce the health risks of consumers associated

594 with pesticide residues in vegetables but also to protect consumers' rights. The consumers who

595 buy produce labeled organic pay more so should get a higher quality, pesticide free produce.

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CONCLUSIONS

600 There is considerable contamination of pesticides in these three commonly consumed 601 vegetables, 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 602 603 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 604 605 similar between the vegetables bought from local markets and supermarkets. These findings 606 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 607 608 conclusion excludes Thai export quality vegetables and fruits that are routinely monitored for 609 pesticide contamination before exporting. It is our recommendation for the Thai government 610 authorities to conduct a proper pesticide monitoring programme for these three commonly 611 consumed local vegetables to protect the health of domestic consumers. The findings arisen from 612 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 613 614 toward the safe and appropriate use of pesticides.

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630
631 Author Contributions
632 • Sompon Wanwimolruk conceived and designed the experiments, performed the experime
633 analyzed the data, contributed reagents/materials/analysis, prepared figures and/or tak
634 wrote the paper, reviewed drafts of the paper.
• Kamonrat Phopin collected the samples, prepared figures and tables, reviewed drafts of
636 paper.
637 • Somchai Boonpangrak collected the samples, prepared figures and tables, reviewed draft
638 the paper.
• Virapong Prachayasittikul reviewed drafts of the paper.
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757	Figure Legends
758	
759	Figure 1. Type of pesticides detected in the Chinese kale samples purchased from the local
760	markets $(n = 69)$ and the supermarkets $(n = 68)$. For each pesticide detected, the lower bars
761	are for samples from the local markets, and the upper bars are for samples from the
762	supermarkets.
763	
764	Figure 2. Overall incidence of detected pesticide residues in Chinese kale samples
765	purchased from the local markets and the supermarkets. This figure displays: samples with
766	no pesticide detected, total of samples with detected pesticide residues, samples with pesticide
767	residues detected at levels of $<$ MRL, and samples with pesticide residues detected at levels of $>$
768	MRL. Rate of detection for each pesticide in Chinese kale was expressed as percentage.
769	
770	Figure 3. Type of pesticides detected in the pakchoi samples bought from the local markets
771	(n = 63) and the supermarkets $(n = 62)$. For each pesticide detected, the lower bars are for
772	samples from the local markets, and the upper bars are for samples from the supermarkets.
773	
774	Figure 4. Overall incidence of detected pesticide residues in pakchoi samples purchased
775	from the local markets and the supermarkets. The figure displays: samples with no pesticide
776	detected, total of samples with detected pesticide residues, samples with pesticide residues
777	detected at levels of < MRL, and samples with pesticide residues detected at levels of > MRL.
778	Rate of detection for each pesticide in pakchoi was expressed as percentage.
779	
780	Figure 5. Type of pesticides detected in the morning glory samples bought from the local
781	markets $(n = 74)$ and the supermarkets $(n = 61)$. For each pesticide detected, the lower bars
782	are for samples from the local markets, and the upper bars are for samples from the
783	supermarkets.
784	
785	Figure 6. Overall incidence of detected pesticide residues in morning glory samples
786	purchased from the local markets and the supermarkets. The figure demonstrates: samples
787	with no pesticide detected, total of samples with detected pesticide residues, samples with

- 788 pesticide residues detected at levels of < MRL, and samples with pesticide residues detected at
- 789 levels of > MRL. Rate of detection for each pesticide in morning glory was expressed as
- 790 percentage.



1

Type of pesticides in 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.

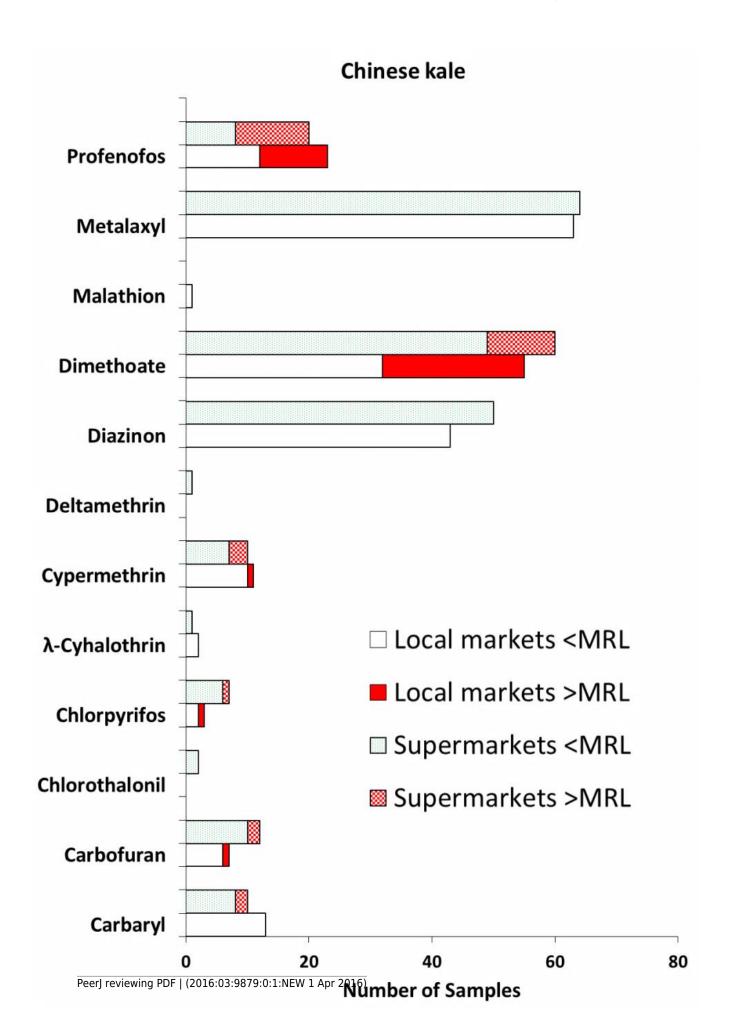


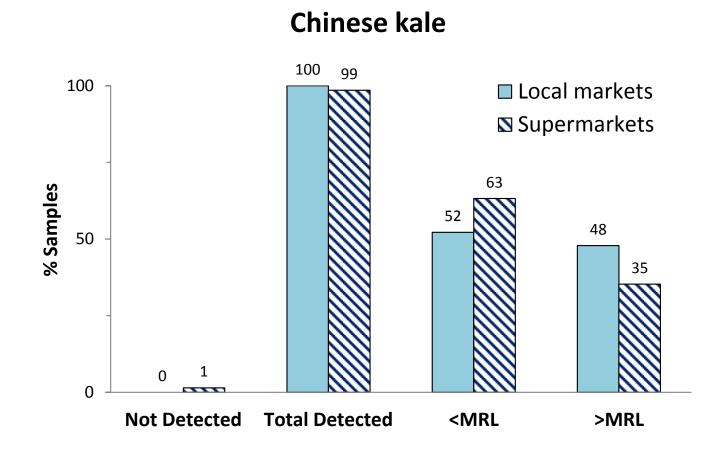


Figure 2(on next page)

Pesticide Incidence in Ch Kale

Figure 2. Overall incidence of detected pesticide residues in Chinese kale samples

purchased from the local markets and the supermarkets. This figure displays: samples with no pesticide detected, total of samples with detected pesticide residues, samples with pesticide residues detected at levels of < MRL, and samples with pesticide residues detected at levels of > MRL. Rate of detection for each pesticide in Chinese kale was expressed as percentage.





3

Type of pesticides detected in pakchoi

Figure 3. 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.

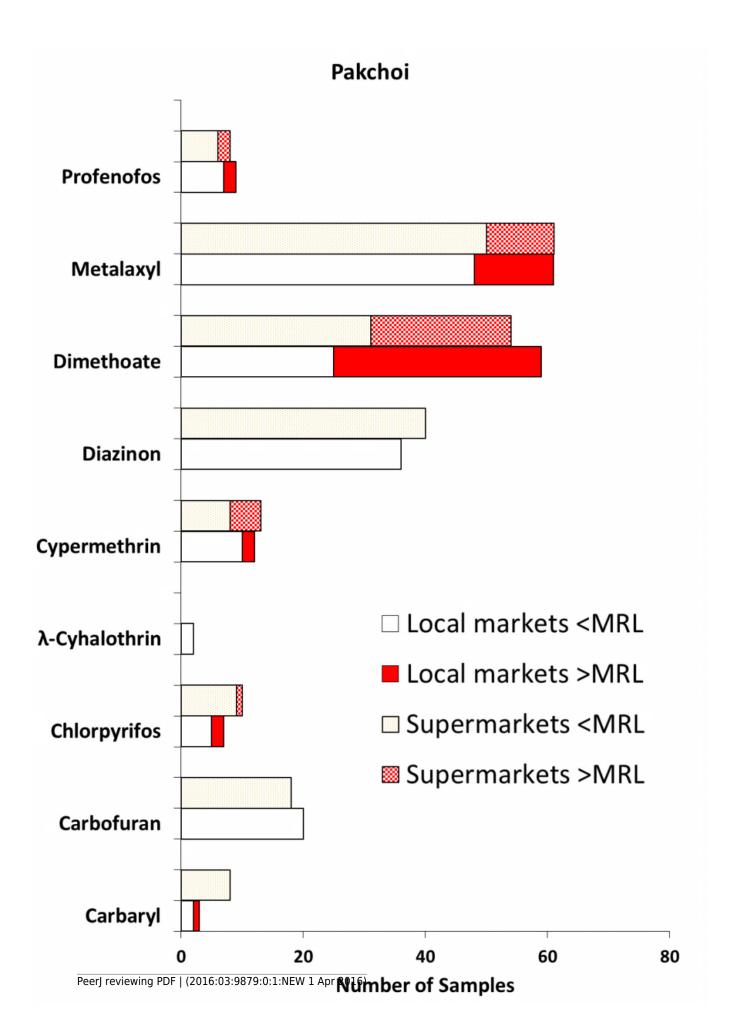


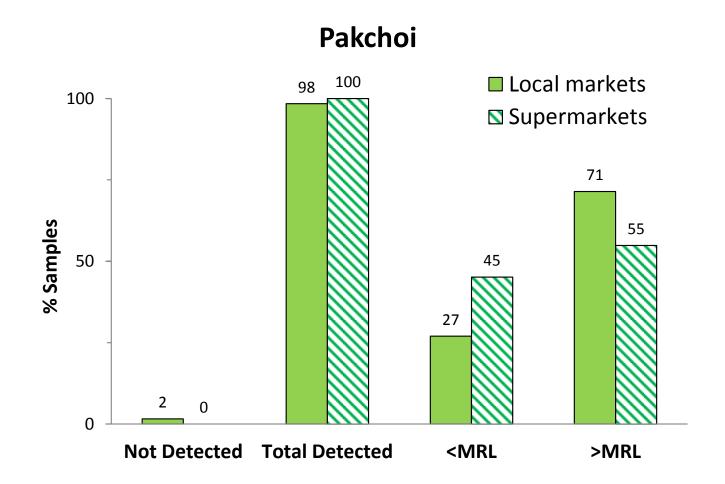


Figure 4(on next page)

Pesticide incidence in Pakchoi

Figure 4. Overall incidence of detected pesticide residues in pakchoi samples

purchased from the local markets and the supermarkets. The figure displays: samples with no pesticide detected, total of samples with detected pesticide residues, samples with pesticide residues detected at levels of < MRL, and samples with pesticide residues detected at levels of > MRL. Rate of detection for each pesticide in pakchoi was expressed as percentage.



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Type of pesticides in morning glory

Figure 5. 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 .

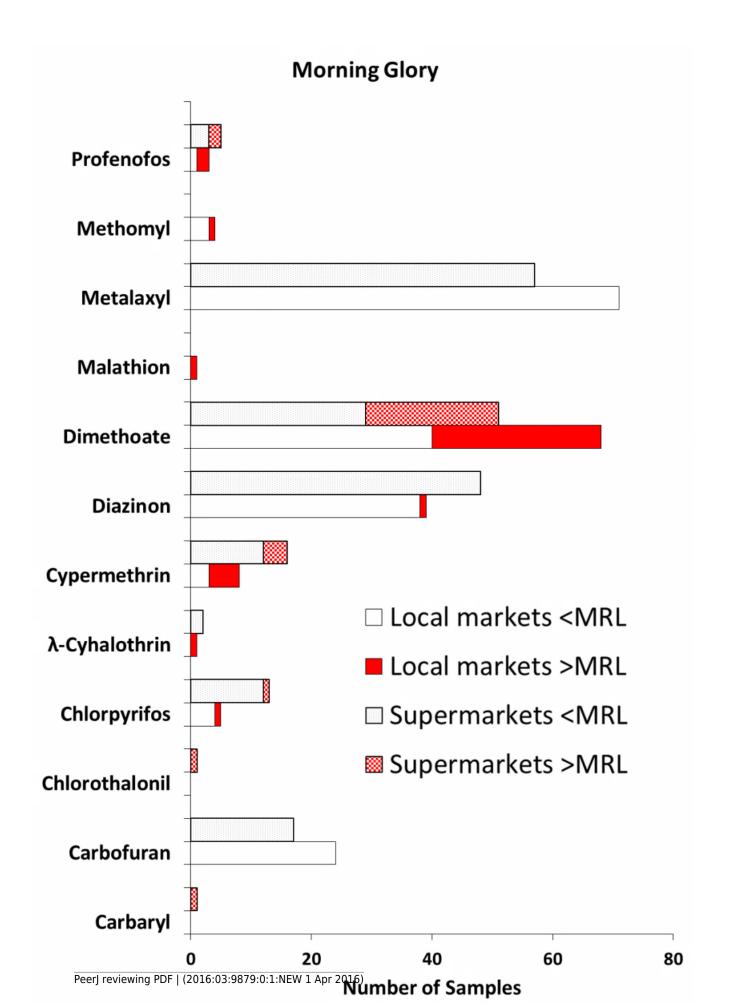




Figure 6(on next page)

Pesticide Incidence in Morning glory

Figure 6. Overall incidence of detected pesticide residues in morning glory

samples purchased from the local markets and the supermarkets. The figure demonstrates: samples with no pesticide detected, total of samples with detected pesticide residues, samples with pesticide residues detected at levels of < MRL, and samples with pesticide residues detected at levels of < MRL, and samples with morning glory was expressed as percentage.

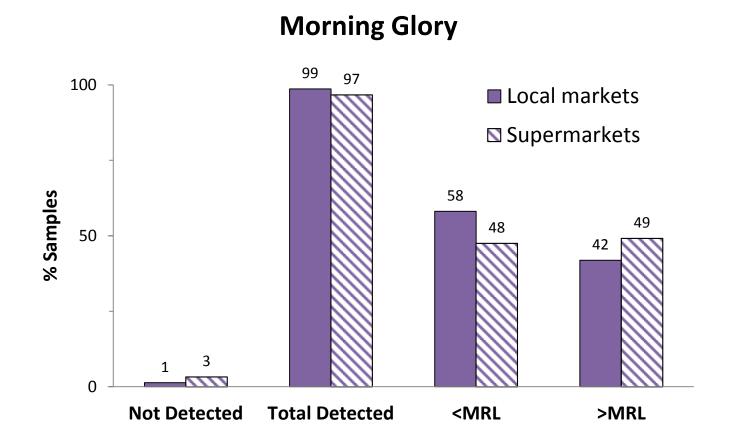




Table 1(on next page)

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

Table 1

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

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Vegetable	Carbaryl	Carbofuran	Chlorothalonil	Chlorpyrifos	λ-Cyhalothrin	Cypermethrin	Deltamethrin	Diazinon	Dimethoate	Malathion	Metalaxyl	Methomyl	Profenofos
Chinese kale	~	~	~	~	~	~	~	~	~	~	~	-	~
Pakchoi	~	~	-	~	√	~	-	~	~	-	~	-	\checkmark
Morning glory	~	~	~	~	~	~	-	~	~	~	~	~	~

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Table 2(on next page)

Mean and median concentrations of six commonly detected pesticides in three vegetables studied.

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Table 2

2 Mean and median concentrations of six commonly detected pesticides in three vegetables

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studied. n is number of samples in which the pesticide was detected.

	Vegetables									
Pesticide	Chin	ese kale	Pal	cchoi	Morning Glory					
	Local markets	Supermarkets	Local markets	Supermarkets	Local markets	Supermarkets				
Carbofuran	-	-	1.2 ± 2.2	2.6 ± 2.2	54.7 ± 80.1	25.6 ± 42.6				
			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)	<i>P</i> = 0.442				
				(n = 17)		(n = 57)				
Dimethoate	21.5 ± 35.1	11.4 ± 14.1	58.7 ± 88.3	28.9 ± 32.1	33.1 ± 52.4	19.4 ± 23.6				
	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)	<i>P</i> = 0.17	(n = 59)	<i>P</i> = 0.167	(n = 68)	<i>P</i> = 0.737				
		(n = 50)		(n = 55)		(n = 51)				
Diazinon	2.2 ± 4.3	54 ± 212	1.7 ± 1.3	2.6 ± 2.2	1.7 ± 1.9	2.2 ± 1.8				
	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)	<i>P</i> = 0.009				
		(n = 41)		(n = 40)		(n = 48)				
Metalaxyl	21.6 ± 72.9	23.2 ± 36.3	134 ± 791	44 ± 140	62.3 ± 118.1	44.1 ± 54.1				
	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)				

P = 0.562

(n = 27)

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PeerJ				Manuso	cript to b
	((1)	<i>P</i> = 0.0001	(n = 61)	P = 0.8	(n = 27)
	(n = 61)	(n = 52)		(n = 61)	
Profenofos	131 ± 393	192 ± 527	-	-	-
	9.3 (Med)	23.9 (Med)			
		(1			

9.3 (Med)	23.9 (Med)		
(0.26, 71.2)	(1.3, 36.3)		
(n = 28)	<i>P</i> = 0.39		
	(n = 18)		

^a Median value 4

5 $^{\rm b}$ 25 and 75 percentiles of the median value.

* Statistically significant differences in pesticide concentrations were observed between the local 6

market and the supermarket groups (P < 0.05). 7