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Pesticide residues in leafy vegetables and human health risk assessment in North Central agricultural areas of Chile

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Abstract

To investigate pesticide residue concentrations and potential human health risk, a study was conducted in 118 leafy vegetable samples collected in 2014-2015 from the North Central agricultural areas of Chile. The pesticides residues were determined using the multiresidue QuEChERS method using gas chromatography as well as high-performance liquid chromatography. The results indicated that 27% of the total samples contained pesticide residues above the maximum residue limits of each active ingredient. The maximum estimated daily intake obtained for carbon disulphide (CS_2), methamidophos, azoxystrobin and cypermethrin were 0.57, 0.07, 0.06, and 0.05 mg kg^{-1}, which was higher than their acceptable daily intake. It is concluded that inhabitants of the North Central agricultural area of Chile are not exposed to health risks through the consumption of leafy vegetables with the exception of methamidophos. Nevertheless, the high levels of methamidophos detected in leafy vegetables could be considered a potential chronic health risk.

Keywords: Pesticide residues, Maximum residue limits (MRLs), Acceptable daily intake (ADI), Hazard quotient (HQ).

Introduction

The demand for food is increasing worldwide because of the high global population and improvement of living standards. The use of pesticides in modern agriculture is necessary for most crops to guarantee the food supply. However, improper and excessive use of pesticides in horticultural processes has resulted in environmental pollution and increasing human health risk (Oerke & Dehne 2004; Hernández et al. 2013; Köck-Schulmeyer et al. 2013). The World Health Organization (WHO) promotes the consumption of vegetables and fruit as an important source of essential vitamins, minerals and antioxidants. This food group has active compounds that can reduce the effect of cardiovascular diseases (Ivey et al. 2015). However, this food group can be a source of contamination, because the ingestion of pesticide residues is a potential source of diseases and negative effects (Parrón et al. 2014; Lemos et al. 2016).

It is reasonable to assume that vegetables and fruit may contain pesticide residues in harmless concentrations (Chen et al. 2012; Li et al. 2015). The maximum residue limits (MRLs), expressed as the highest concentration of pesticide residues in vegetables and fruits (mg kg^{-1}) are used to determine the maximum intake legally permitted by regulations introduced by different Countries and internationally by the Codex Alimentarius Commission (Darko & Akoto 2008; Codex Alimentarius Commission 2011; Gad Alla et al. 2015). The human intake of pesticide residues in vegetables can be much higher than the intake related to water consumption and air inhalation. It is very important to monitor such chemicals in vegetables and to assess their potential risks to human health (Szpyrka et al. 2015). Some indicators can estimate the amount of chemicals in food, which is represented as the acceptable daily intake (ADI) and expressed as mg kg^{-1} body weight (bw) day $^{-1}$. The ADI can be used to predict the daily intake during a lifetime that would prevent health risks to the consumer (FAO/WHO, 1997; Akoto et al., 2013) and is used to determine the estimated daily intakes (EDIs).

The aim of obtaining data on pesticide residue in food is to improve pest management programs (Bhanti & Taneja 2005). Most organophosphate pesticides such as methamidophos, parathion-methyl, parathion, monocrotophos and phosphamidon are toxic. Therefore, numerous types have been banned since 2007 for agricultural use in China (Chen et al. 2011; Yu et al. 2016). In Brazil, the registration of methamidophos was recently cancelled and the phase-out period ended in July 2012 (Jardim et al. 2014). In some countries including Guatemala, Spain, Jamaica, and China the use of methamidophos is still allowed but adverse effects have been detected (Galt 2010). In Chile there are no international scientific articles reporting the monitoring of pesticide residue in food and their health risk assessment. The lack of information on the health risk associated with the exposure of the population to pesticides through consumption of vegetables is of important concern. Since 2009, Chile has been part of the Organization for Economic Co-Operation and Development (OECD) that promotes policies to improve the economic and social well-being of people. The statistical analysis by the OECD reports that pesticides are used at a total average of 0.21 kg ha^{-1}. Chile has one of the highest levels of pesticide use with a total of 10.7 kg ha^{-1} used in 2009. The high level of pesticide use can be explained by the severe phytosanitary food certification standards that international markets have imposed on Chile (Engler et al. 2012; Handschuch et al. 2013; Melo et al. 2014).

Vegetables are produced in all regions in Chile, with nearly 34,000 farms, 65% of which have less than 5 ha, indicating that this sector is composed mainly of small growers (INE, 2016). The National Institute of Statistics in Chile estimated that the national horticultural area consisted of almost 70,000 ha by 2014, 84% of which is between the regions of Coquimbo and Maule (30°00′S-72°30′W to 36°05′S-72°30′W). Leafy vegetables such as lettuce (*Lactuca sativa* L.), chard (*Beta vulgaris* subsp. *vulgaris*) and spinach (*Spinacia oleracea*) are among the main leafy vegetables produced in Chile. The production of lettuce in Chile has been estimated at over 75,000 tons and is concentrated (74%) mainly in the Valparaiso (33°2′S-71°40′W) to the Metropolitana (33°27′S-70°40′W) regions (Oficina de Estudios y Políticas Agrarias, ODEPA). The purposes of this study were 1) to quantify the concentration of pesticide residues in leafy vegetables in three regions of Chile and 2) to assess the health risks associated with the consumption of potentially contaminated vegetables. The relevance of this work is to enhance the understanding of the importance of pesticide residues in food and its effects on human health, which would improve the methods of pesticide use in integrated pest management programs in Chile.

Materials and methods

Study area

The Coquimbo, Valparaiso, and Metropolitana regions are located in North Central Chile, which is the most important growing area for leafy vegetables. The local climate is characterized by short and rainy winters and warm summers. The average annual temperature ranges from 12 to 14°C, whereas the average annual rainfall is from 78 mm in Coquimbo to 312 mm in the Metropolitan Region. More than 75% of the rainfall occurs in June, July, and August.

Leafy vegetable sampling

To conduct a representative study it was necessary to determine the appropriate sample size (n) based on the number of leafy vegetables (lettuce, chard, and spinach) growers in the area under study (Table 1), using the following formula (INE, 2002):

$$n = (N \times Z^2 ά \times p \times q)/(d^2 \times [N-1] + Z^2 ά \times p \times q)$$

where, N is the total number of growers, $Z^2ά$ is 1,962 (security 95%), p is the expected proportion (5% = 0.05), q = 1-p (1-0.05=0.95), and d is the precision (3%). A total of 118 samples of leafy vegetables consisting of lettuce (74), chard (26), and spinach (18) from all regions were taken and analysed during the 2014-2015 cultivation season. The sample size was 2 kg of leaves collected from the sites, wrapped in aluminium foil and transported to the laboratory. Samples were milled and homogenized, 20 gr were transferred into 100mL glass containers and stored at -20°C until the analysis.

Chemicals and reagents

Analytical grade pesticide standards (99% purity) were obtained from Chem Service (West Chester, PA, USA) and Pestanal (Sigma-Aldrich, Santiago, Chile). The standard working solutions were prepared by diluting the stock solutions with acetone/isooctane. All analytical reagents were purchased from Merck and J.T Baker (Santiago, Chile). All the solutions were prepared with ultra-high purity water (UHP) and filtered using a Milli-Q system from Millipore (IADET, Santiago, Chile).

Multiresidue method and instrumentation

The pesticides analysed in this study are the most frequently detected and used in pest management programs by growers in Chile. The MRLs in Chile were established by the RES-33/2010-02-16 and RES-76272011-10-02 based on the MRLs of Codex Alimentarius, the European Union (EU) and the USA. All pesticide residues present in the leafy vegetables were determined using gas chromatography-mass spectrometry (GC-MS). The standard method EN 15662 of the European Committee for Standardization for food material of plant origin, known as the quick, easy, cheap, effective, rugged and safe (QuEChERS) method was used in this study (CEN, 2008). This method was previously described by Anastassiades et al. (2003) and Payá et al. (2007). Two tubes in the QuEChERS system Waters (Milford, USA) were used for extraction and clean-up. The first tube contained 4 g magnesium sulphate ($MgSO_4$), 1 g sodium chloride (NaCl) and 1.5 g citrate. The second tube contained 900 mg $MgSO_4$, 150 mg primary secondary amine (PSA) and 150 mg C18. Briefly, 10 g of each homogenized leafy vegetable sample was weighed, placed into a 50-mL centrifuge tube and extracted with 10 mL acetonitrile with vigorous shaking for 1 min. After 3 min the mixture was centrifuged at 2500 rpm for 5 min and the total acetonitrile supernatant phase was transferred to a new 15-mL tube for the clean-up. The tube was shaken vigorously for 1 min and then centrifuged for 1 min at 2500 rpm. Then 6 mL of the supernatant was placed in a glass vial with ethyl acetate acidified with 100µL formic acid, evaporated to dryness, re-dissolved in 1 mL acetone/isooctane (1:9) and filtered through a 0.20-µm polyvinylidene fluoride (PVDF) syringe filter into an autosampler vial for the analysis.

Organophosphate residues were quantified using an Agilent 7890-Autosampler (Wilmington, USA) gas chromatography system with a nitrogen-phosphorus detector (NPD) detector. The GC system was equipped with a fused silica capillary column HP-5MS (30 m × 0.25 mm × 0.25-µm; Agilent, Santa Clara, USA). For the halogenated pesticides a GC-electron capture detector (ECD) and a Perkin Elmer auto-system XL (Waltham, MA, USA) were used. The GC-ECD system was equipped with a fused silica capillary column (DB-1 and DB-17, 30 m × 0.25 mm × 0.25 µm; Sigma-Aldrich (St. Louis, MO, USA). The methyl-carbamates were quantified using a high-performance liquid chromatography (HPLC) system with a Merck Hitachi LaChrom D-7000-autosampler (Dartford, United Kingdom) with ultraviolet (UV) and fluorescence (FL) detectors and a 655A-B reaction pump. All samples were filtered through a 0.22-µm PVDF syringe filter into an auto-sampler vial before analysis. The HPLC was equipped with a UV detector set to 270 nm and a column (RP-18 X-Terra, 3.9 × 150 mm; Waters, Milford, USA). The mobile phase was acetonitrile/water (30/70, % v/v). The samples were filtered through a 0.22-µm PVDF syringe filter into an auto-sampler vial before analysis. For imidacloprid and carbendazim, a HPLC system with a Merck Hitachi D-6000 (Burladingen, Germany) was used. The dithiocarbamate levels were determined using distillation and quantification with a Thermo-Genesys 10VIS detector (Madison, USA), using the method of Perz et al. (2000). The results were expressed as milligrams of carbon disulphide per kilogram (mg CS_2 kg^{-1}).

Method performance and quantification

The methods were validated according to the SANCO document 12571/2013 guidelines. The limit of quantification (LOQ), which was defined as the lowest concentration that could be quantified with an acceptable recovery (70-105%), started at 0.01 mg kg^{-1} and was further increased depending on the pesticide (Table 2). The precision, which was expressed as the relative standard deviation (RSD, %) was determined by analysing replicate samples at 3 levels (n = 5/level) and was set at <20%). The average recovery of the different pesticides varied between 70 and 105%. The limit of detection (LOD) was calculated according to the Environmental Protection Agency (EPA) recommendations. The quantification was performed using a mixture containing known amounts of the pesticides and the detector response for each compound was determined. The area of the corresponding peak in the sample was compared with that of the known standard (Bempah et al. 2012; Gad Alla et al. 2015).

Human health risk assessment

Pesticide residue concentrations on the leafy vegetables were determined as the arithmetic mean of all results obtained. The estimated daily intake (EDI) was calculated by multiplying the pesticide concentration of each pesticide (mg kg^{-1}) with the food consumption rate (kg day^{-1}) and dividing this by the bw (Gad Alla et al. 2015). Food consumption was based on data from the WHO/Global Environment Monitoring (GEMS)/Food Cluster Diet 2012, a part of the FOSCOLLAB, the WHO Global platform for food safety data and information. Cluster G05 for Chile reports a leafy vegetable consumption of 2.046 g day^{-1}. The average bw used for Chileans was defined as 72.3 kg (ENS Chile, 2009-2010). The EDI (mg/ kg bw/day) was calculated as follows: EDI = $\sum RL_i \times F_i$ / bw; where RL_i is the residue level of leafy vegetable i and F_i is the food consumption rate of food item i.

The hazard quotient (HQ) was considered as the potential risk of adverse health effects from a mixture of pesticides to indicate the long-term risk assessment and was calculated by dividing the EDI by the relevant acceptable daily intake. The ADI values are given by the European Commission and the European Food Safety Authority (EFSA). HQ was calculated using the formula HQ = EDI/ADI × 100, a percentage > 100 indicating that the exposed people were unlikely to experience obvious toxic effects. Furthermore, an index below 100 indicated the possibility that the exposure could induce toxic effects (Yu et al., 2016). HQ was calculated for the pesticides and leafy vegetables and the results were summed up to obtain a chronic hazard index (cHI) where, cHI = ∑HQ. cHI > 100 indicated that the leafy vegetables should be considered a risk to the consumers, whereas an index below 100 indicated that the consumption of the leafy vegetable was considered acceptable (Gad Alla et al. 2015).

Results and Discussion

Analytical results

The accuracy of the QuEChERS method was expressed as recovery (%) and RSD (%) at the levels 0.01, 0.1, and 0.5 mg kg^{-1} in leafy vegetables. The recovery values varied at 70-105%. These results are concordant with 70-120% as given in the EU guidance document on pesticides analysis (SANTE 2015). In addition, the RSD obtained was 0.5-13.4%, which is lower than the 15% required to validate extraction and clean-up (WHO/FAO 2013). The LOD and LOQ for the analytical method varied at 0.005-0.02 mg kg^{-1} for the leafy vegetables. Similar results were previously obtained by Srivastava et al. (2010) for 20 vegetables including leafy samples. They extracted their samples using the QuEChERS method, measured with a GC-ECD/NPD and obtained a recovery of 70-96% and RSD of 15% for the 35 pesticides analysed.

Table 3 gives an overview of the obtained occurrence data. Pesticide residues were found in 27, 9, and 5 samples of lettuce (36%), chard (35%), and spinach (72%), respectively. 47, 17, and 13 samples of lettuce (64%), chard (65%), and spinach (72%) showed a mixture of pesticide residues. Among the leafy vegetables, 27% of the total samples contained pesticide residues above the MRLs of each active ingredient, whereas 42, 28, and 22% of the pesticides detected were above the MRLs for chard, spinach, and lettuce, respectively. In brief, 2 or more pesticide residues were detected in 65% of the samples. These results can be are attributed to the high frequency of pesticide application on farms, the short time intervals allowed between the numerous applications (McEwen et al. 1980) and the pesticide retention capacity of the leafy vegetable surfaces (Li et al. 2014; Gad Alla et al. 2015; Yu et al. 2016).

These results are in accordance with those of Yu et al. (2016) who monitored organophosphorus pesticides (OPs) in leafy vegetables in Changun, China. In that study, only 7% of the total samples were free from pesticide residues, whereas 23 and 68% of the total samples contained OPs above and below the MRLs, respectively. In addition, Gonzales-Rodriguez et al. (2008) monitored pesticide residues in 75 leafy vegetable samples (chard, spinach and lettuce). They reported that pesticide residues were above the MRLs in 15 of 75 analysed samples. The highest concentrations of the insecticides procymidone and cypermethrin (12 and 6 mg kg^{-1}) were found in lettuce and chard, respectively. Esturk et al. (2011) detected a high occurrence of pesticide residues in 120 lettuce and spinach samples, most of which were not authorised for use in Turkey.

Occurrence and levels

Table 4 illustrates the occurrence and concentrations of pesticide residues in the analysed samples. Methamidophos, chlorpyrifos, boscalid and lambda-cyhalothrin were the most predominant compounds found in all the samples, followed by carbendazim and imidacloprid, which were found in lettuce and chard. Methamidophos showed the highest concentration (29.47 mg kg^{-1}), followed by chlorpyrifos (6.86 mg $kg^{-1)}$, which were detected in lettuce, chard and spinach. The levels of methamidophos found in this study could induce serious adverse effects on human health and the environment. Goh et al. (1990) provided the first evidence that people could be affected by consumption of leafy vegetables with high levels of methamidophos and profenofos, as they showed that blood cholinesterase levels of hospitalised patients were depressed by 26-81%.

The results showed that cypermethrin, cyfluthrin, difenoconazole, esfenvalerate, metalaxyl, permethrin and thiamethoxam were found in 1% of the samples at values below the MRLs. However, metalaxyl exceeded the MRL for chard (0.13 mg kg^{-1}). Moreover, boscalid, chlorothalonil, difenoconazole and metalaxyl found in the chard samples are not authorized for use in this vegetable. Their presence could be attributed to a wrong pesticide choice made by the farmers for pest control in chard or wrong advice from pesticide retailers (Wang et al. 2013). Furthermore, boscalid was also detected in spinach and lettuce, although it is an unauthorized active ingredient for these vegetables. Similar results have also been found in Thailand and China (Sapbamrer and Hongsibsong 2014; Yu et al. 2016).

The results also showed the presence of methamidophos residues in lettuce, chard and spinach with a frequency of 25% and a mean of 2.61 mg kg^{-1}, which exceeded the MRLs for leafy vegetables. Similarly chlorpyrifos, detected at a frequency of 14% and mean of 0.54 mg kg^{-1} and carbendazim, showing a frequency of 11% and a mean of 0.20 mg kg^{-1}, in both cases exceeding the MRLs. Mancozeb, difenoconazole, lambda-cyhalothrin and thiamethoxam exceeded the MRLs for lettuce, whereas only difenoconazole and metalaxyl exceeded the MRLs in chard. The sum of the mean pesticide residues was highest in lettuce, followed by chard and spinach.

Similar situations to those observed in our study have been described due to the use of unauthorised or a high amount of pesticides, leading to high residue levels. De Putter and Van Sauers-Muller (2008) presented several reports of values as high as 1800 times the MRLs for methamidophos in spinach over the period 2004-2005. In addition, Akoto et al. (2015) found that methamidophos exceeded the MRLs in other commodities.

Human risk assessment

As shown in Table 5, the maximum EDI was obtained for mancozeb, methamidophos, azoxystrobin and cypermethrin at 0.57, 0.07, 0.06 and 0.05 mg kg^{-1}, which is 11-, 73-, 30- and 0.3-fold, respectively, higher than the corresponding ADI values. Freeman et al. (2015) found methamidophos exceedances of the ADI at the 25th percentile in vegetables in Israel, demonstrating potential exposure of the population to pesticide residues. Park et al. (2016) revealed for leafy vegetables in South Korea EDI and ADI exceedances of the MRLs in a range of 0.003-30%. Similar results were obtained by Chen et al. (2011), who detected cypermethrin in 18.7% of the investigated vegetable samples and EDI values ranging from 0.1% of the ADI for cyfluthrin to 2.61% of the ADI for omethoate. Yu et al. (2016) found methamidophos and phorate to be key components contributing to the potential health risks of inhabitants of the agricultural areas in China.

HQs for methamidophos, cypermethrin, mancozeb, cyfluthrin and lambda-cyhalothrin were 73.9, 30.4, 11.5, 4.5, and 3.0, respectively. HQs decreased in the order: methamidophos > cypermethrin > cyfluthrin > chlorpyrifos > chlorothalonil > pyraclostrobin > esfenvalerate > methomyl > boscalid > carbendazim imidacloprid > propiconazole. The results show a potential health risk associated mainly with methamidophos and cypermethrin in leafy vegetables in Chile. The HQs were summed up and the cHI of all residues was 135%, which was above 100%, including methamidophos and therefore should be considered a risk for consumers. When methamidophos was excluded from the risk assessment cHI was 61%, which was below 100% and therefore not considered a health risk. Chen et al. (2011) found no health risk for consumers in fruit and vegetables, although the highest detected cypermethrin level (13.92 mg kg^{-1} in cabbage) resulted in an intake that was determined to be 400% of the ARfD value of 40 µg/kg bw /day when the WHO/GEMS high consumption diet was used. Lozowicka et al. (2015) found cHI values of 6.89, 12.57, 12.73% for organochlorines (endosulfan and dicofol), organophosphorus compounds (trizophos, chlorpyrifos and dimethoate) and fungicidal azoles (triadimefon, tebuconazole, triamenol, flusilazole and prochloraz), respectively in tomatoes and cucumbers from Kazakhstan.

Conclusions

This study showed the presence of pesticide residues alone or in combination in leafy vegetables in 3 important regions of Chile. Furthermore, 65% of the samples showed 2 or more active ingredients and 27% of them were above the MRLs. Fungicides and insecticides were the most common pesticide types detected. The results provide important information on the current contamination status of leafy vegetables in Chile. The results showed that the population was not exposed to health risks with the consumption of leafy vegetables, except for methamidophos, of which high levels could be considered a potential risk. Therefore it is advisable to modify pest control management to reduce use of methamidophos containing products because of their potential effect on human health.

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Table 1. Number of growers of leafy vegetables in North-Central areas of Chile (INE; 2002).

Regions	Leafy vegetables		
	Chard	Spinach	Lettuce
	Number of growers		
Coquimbo	46	7	263
Valparaiso	160	128	767
Metropolitana	210	60	483
Total	416	195	1513

Table 2. Validation parameters (n=5) for QuEChERS multiresidue method at 3 levels in leafy vegetables and MRLs.

Pesticide	Recovery %	RSD %	LOQ (mg kg^{-1})	LOD (mg kg^{-1})	MRL mg kg^{-1}	Instrument
Chlorothalonil	75.7	13.4	0.01	0.005	0.01	GC-ECD
Chlorpyrifos	92.5	1.1	0.01	0.005	0.05	
Difenoconazole	103.6	7.1	0.01	0.005	0.05	
Lambda-cyhalothrin	98.0	3.5	0.01	0.005	0.05	
Biphenthrin	92.8	3.2	0.01	0.005	0.05	
Boscalid	99.8	3.6	0.01	0.005	5	
Cyfluthrin	92.6	3.1	0.02	0.01	0.02	
Deltamethrin	95.0	7.8	0.01	0.005	0.02	
Cypermethrin	93.6	8.1	0.01	0.005	0.7	
Azoxystrobin	94.9	3.0	0.01	0.005	0.01	
Esfenvalerate	89.9	3.7	0.02	0.005	0.02	
Endosulfan	91.4	3.3	0.01	0.005	0.05	
Linuron	93.4	12.7	0.01	0.005	0.01	
Permethrin	91.4	0.8	0.01	0.005	2	
Propiconazole	103.4	4.6	0.01	0.005	0.05	
Azinphos-methyl	100.0	4.0	0.01	0.005	0.5	GC-NPD
Metalaxyl	96.1	2.2	0.01	0.005	0.05	
Methamidophos	89.3	4.4	0.01	0.005	0.01	
Diazinon	86.3	5.4	0.01	0.005	0.5	
Dimethoate	103.0	5.9	0.01	0.005	0.02	
Fludioxonil	100.7	4.5	0.01	0.005	0.05	
Phosmet	103.2	4.2	0.01	0.005	0.05	
Malathion	95.7	3.2	0.01	0.005	0.02	
Methidathion	93.9	2.9	0.01	0.005	0.02	
Penconazole	90.4	4.1	0.01	0.005	0.05	
Pyrimethanil	101.7	3.1	0.01	0.005	0.05	
Pyraclostrobin	87.6	7.1	0.01	0.005	0.5	
Thiabendazole	95.3	5.6	0.01	0.005	0.05	
Thiamethoxam	92.2	3.0	0.01	0.005	0.01	
Triadimefon	97.4	0.5	0.01	0.005	0.1	
Abamectin	104.3	5.8	0.002	0.001	0.01	HPLC- FL
Aldicarb	86.9	4.4	0.01	0.005	0.02	
Methomyl	94.4	5.1	0.01	0.005	0.01	
CS_2 (mancozeb)	91.8	5.3	0.01	0.005	0.05	Spectro-photometer
Carbendazim	70.0	1.57	0.02	0.01	0.05	HPLC-UV
Imidacloprid	95.3	1.3	0.02	0.01	0.05	

Table 3. Pesticide residues in leafy vegetables in North Central agricultural areas in Chile. All samples above LOD contained multiple residues.

Vegetable	n	n < LOD		n > LOD		n > MRL	
Lettuce	74	27	36%	47	64%	16	22%
Chard	26	9	35%	17	65%	11	42%
Spinach	18	5	28%	13	72%	5	28%
total	118	41	35%	77	65%	32	27%

Table 4. Pesticide residues in leafy vegetables in North Central agricultural areas in Chile.

Active substance	Category	N > LOD / frequency		Min-max (mg kg^{-1})	Mean (mg kg^{-1})	MRLs Chile (mg kg^{-1})	
Cypermethrin	I	1	1%	0-1.61	1.61	0.70	(L. C. S)
Azoxystrobin	F	3	3%	0.39-3.38	2.25	not available	not available
Boscalid	F	15	13%	0.01-3.06	0.52	30.00	(L. C. S)
Carbendazim	F	13	11%	0.01-1.38	0.20	0.10	(L. C. S)
Cyfluthrin	I	1	1%	0-0.48	0.48	3.00	(L. C. S)
Chlorothalonil	F	2	2%	0.11-1.42	0.77	0.01	(L. C. S)
Chlorpyrifos	I	16	14%	0.01-6.86	0.54	0.05	(L. C. S)
CS2 (Mancozeb)	F	5	4%	0.34-40	20.32	10.00	(L)
	F	4	3%	0.38-5.88	2.20	0.05	(S)
Difenoconazole	F	1	1%	0-0.08	0.08	0.05	(C)
	F	5	4%	0.32-3.14	0.95	2.00	(L)
Esfenvalerate	I	1	1%	0-0.3	0.30	0.02	(L. C. S)
Imidacloprid	I	6	5%	0.02-0.18	0.11	3.50	(L)
	I	4	3%	0.02-1.07	0.52	0.05	(C)
Lambda-cyhalothrin	I	14	12%	0.029-1	0.22	2.00	(L)
	I	5	4%	0.01-0.80	0.53	0.50	(C)
Metalaxyl	F	1	1%	0-0.13	0.13	0.05	(C)
	F	5	4%	0.02-1.76	0.50	2.00	(L)
Methamidophos	I	30	25%	0.01-29.47	2.61	0.01	(L. C. S)
Methomyl	I	3	3%	0.01-0.02	0.03	0.20	(L. C. S)
Permethrin	I	1	1%	0-1.45	1.45	20	(L. C. S)
Pyraclostrobin	F	2	2%	0.31-1.25	0.78	29.00	(L. C. S)
Propiconazole	F	2	2%	0.03-0.05	0.04	0.05	(L. C. S)
Thiamethoxam	I	1	1%	0-0.71	0.71	5	(L)

I- Insecticide, F- Fungicide, L=lettuce, C=chard, S=spinach

Table 5. Estimated daily intake (mg/kg bw/day) and potential health risk of pesticide residues in leafy vegetables.

Pesticide	ADI	EDI	HQ
Cypermethrin	0.002	0.05	30.4
Azoxystrobin	0.200	0.06	0.3
Boscalid	0.040	0.01	0.4
Carbendazim	0.080	0.01	0.1
Cyfluthrin	0.003	0.01	4.5
Chlorothalonil	0.015	0.02	1.4
Chlorpyrifos	0.010	0.02	1.5
CS2 (Mancozeb) L	0.050	0.57	11.5
CS2 (Mancozeb) S	0.050	0.06	1.2
Difenoconazole (C)	0.010	0.00	0.2
Difenoconazole (L)	0.010	0.03	2.7
Esfenvalerate	0.020	0.01	0.4
Imidacloprid (L)	0.060	0.00	0.0
Imidacloprid (C)	0.060	0.01	0.2
Lambda-cyhalothrin (L)	0.005	0.01	1.2
Lambda-cyhalothrin (C)	0.005	0.02	3.0
Metalaxyl (C)	0.080	0.00	0.0
Metalaxyl (L)	0.080	0.01	0.2
Methamidophos	0.001	0.07	73.9
Methomyl	0.003	0.00	0.4
Pyraclostrobin	0.030	0.02	0.7
Propiconazole	0.040	0.00	0.0
Thiamethoxam	0.026	0.02	0.8