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Analytical Methods

Occurrence of fungicide and insecticide residues in trade samples of leafy vegetables

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Abstract

The aim of this work was to report on a total of 23 fungicides and insecticides residues in 75 green and leafy vegetables (Swiss chards, Spinaches and Lettuces) collected from Ourense (NW Spain) by Spring 2007. The pesticides in the study samples were extracted with acetonitrile; the extracts were then cleaned-up by solid-phase extraction and concentrated before determination by PTV (Programmable Temperature Vaporization Injector) – GC-ITMS. Use of analyte protectants mixtures provided the best results in terms of effective compensation for matrix-induced enhancement effect. Pesticide residues were determined above the maxima residue limits (MRL) in 15 of the 75 analyzed samples, with a total of 18 violations of the MRL (three of the samples did not fulfil with two different pesticide MRL). The highest concentrations of fungicides were found in lettuce (procymidone, 12 mg/kg) and the highest concentrations of insecticides were found in Swiss chard (cypermethrin, 6 mg/kg). More positives for fungicides were detected and at larger concentrations than insecticides, especially for lettuces. Accumulation of pesticides in lettuces is higher than for the other leafy vegetables. The findings of this study pointed to the following recommendation: the need for a monitoring program for residues of iprodione and procymidone, together with cypermethrin, in food crops at the national level.

Keywords: Fungicides; Insecticides leafy vegetables

1. Introduction

Pesticides are considered to be indispensable for the production of an adequate food supply for an increasing world population and for the control of insect-borne diseases. The use of pesticides is beneficial in decreasing crop loss both before and after harvest (Clarke, Levy, Spurgeon, & Calvert, 1997). Many pesticides are, however, toxic substances and persistent in character. Food is the main source of exposure of the general population to pesticides and accounts for more than 90% of total exposure (Mills, 1936). Pesticide residues in food and crops are a direct result of application of pesticides to crops growing in the field, and to a lesser extent from pesticide residues remaining in the soil (Businelli, Vischetti, & Coletti, 1992).

There is a growing social desire to reduce the use of pesticides in agriculture and horticulture (Beaumont, 1993; Freidberg, 2003; Pretty & Hine, 2005), which is supported at European Union by a Council Directive (Council Directive, 1991) concerning the placing of plant protection products on the market, which state that active substances cannot be used in plant protection products unless they are included in a positive EU list. Equally, supermarkets are under increasing pressure to adapt to a changing consumer preference that perceives pesticides as an undesirable component of food production. When seeking to rationalise pesticide use, both government (Council Directive, 1990) and supermarkets have tended to make

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the implicit assumption that any rationalisation is primarily an issue of decreasing the quantity of pesticides used, coupled with the banning of specific, highly toxic substances (Freidberg, 2003; Gallivan, Surgeoner, & Kovach, 2001; Levitan, 2000). Thus, pesticides with high toxicity to humans are targeted for rationalisation, irrespective of the quantity used. Such actions may not reflect the actual risk to humans or the full hazard profile of the pesticide. For these reasons, decisions as to which pesticides to target for reduction can be problematic unless they are made with full knowledge of their relative toxicological properties, environmental fate and mobility (Kovach, Petzoldt, Degnil, & Tette, 1992; Levitan, 2000). It is only in the presence of such information that more specific questions pertaining to the management of pesticide hazards can be answered.

The objective was to determine the current level of exposure of the local population of Ourense (NW Spain) to hazardous pesticides by the consumption of vegetables, taken into accounts that are often eaten raw. Fungicides and insecticides are the most widely used among the different classes of pesticides, and their levels were monitored in some leafy vegetables as they reach the consumer (whole product – range 1, and cut-and-washed product – range 4). These products were bought in big stores and also in small specialized shops for fruits and vegetables.

2. Materials and methods

2.1. Chemicals and materials

Pesticides tested were: acrinathrin, bifenthrin, carbofuran, cyfluthrin, cypermethrin, cyprodinil, chlorfenvinphos, deltamethrin, esfenvalerate, fenamiphos, fludioxonil, iprodione, λ-cyhalothrin, metalaxyl, methiocarb, penconazole, pyrimethanil, procymidone, tau-fluvalinate, tebuconazole, triadimefon, triadimenol and vinclozolin. Methiocarb standard, of certified purity of 99%, was obtained from Supelco (Bellefonte, pa, USA); all the rest were standards, of certified purity >98%, obtained from Riedel-de-Haën (Seelze, Germany). Fenpropathrin (Riedel-de-Haën) and hexachlorobenzene (Dr. Ehrenstorfer, Augsburg, Germany) were used as internal standards to correct for variability in gas chromatographic injection and mass spectrometric detection response. The 3-ethoxy-1,2-propanediol (98%), D-sorbitol (>99%) and L-gulonic acid γ-lactone of (>98%), used as analyte protectants, were obtained from Aldrich (Steinheim, Germany). Solvents (residue analysis grade) for dissolving and extracting were acetone, acetonitrile, toluene (Panreac, Barcelona, Spain) and methanol (Scharlau, Barcelona, Spain). Sodium chloride and anhydrous magnesium sulfate for residue analysis were purchased from Panreac. Solutions were prepared according to a previous work (González-Rodríguez, Rial-Otero, Cancho-Grande, & Simal-Gándara, submitted).

The sorbent material used for solid-phase extraction was Supelclean Envi-Carb II/PSA, 6 mL size (Supelco Corp., Bellefonte, PA, USA). For solid–liquid extraction, samples were placed in 125 mL glass containers. Organic extracts were placed in round-bottom flasks from Schott Duran (Germany) prior to evaporation on a Heidolph WB 2000 vacuum rotary evaporator (Germany). The final extracts were homogenized by vortex shaking on a Heidolph Reax Top apparatus (Germany) and placed via 350 μL glass inserts into 2 mL vials from Supelco (Bellefonte, PA, USA) prior to chromatographic analysis.

2.2. Chromatographic conditions

Gas chromatographic (GC) analyses were carried out on a Trace GC Thermo Finnigan gas chromatograph (Rodano, Italy) equipped with a PolarisQ ion trap mass spectrometric (ITMS) detection system, interfaced to a PC computer running the software XCalibur 1.4, from Thermo Electron Corporation (Italy). Chromatographic separations were done by using a SPB -5 fused-silica capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness) from Supelco. PTV was used for the 2 μL injection volume into a silcosteel liner (120 mm × 2 mm id). The temperature programming of the PTV was 85 °C for 0.3 min; 600 °C/min to 270 °C and hold for 2 min; 840 °C/min to 300 °C for 5 min. The GC was set to a constant head pressure of 100 kPa with He. The oven temperature was programmed as follows: 80 °C; 8 °C/min ramp to 200 °C; 1 °C/min ramp to 210 °C; and 1.2 °C/min ramp to 270 °C. The transfer line temperature was 270 °C, and the ion-trap manifold temperature was 250 °C. The ion energy for electron impact (EI) was always 70 eV. Mass detection was performed in the single ion monitoring (SIM) mode (with consideration of the relative intensities of selected ions). More details in a previous work (González-Rodríguez et al., submitted).

2.3. Samples

This study was conducted during the 3 months from March to May (47.2–88.2 mm rainfalls per month) - 2007. A total of 75 green and leafy vegetable samples (lettuce, Swiss chard and spinach) were collected from 6 major big food stores and 6 specialized individual vegetable and fruit sellers in Ourense (NW Spain). Ourense is the capital city of the province of Ourense with a population of approximately 0.4 million. It is located in Galicia at the NW of Spain. When possible, together with whole products – range 1, cut-and-washed products – range 4 – were also collected. A minimum of three samples were collected from the upper, middle, and lower shelves of each seller, put in sterile polythene bags, and transported to the laboratory where they were analyzed immediately or stored at 4 °C until analysis within 24 h.

2.4. Sample treatment

Use of an analyte protectants mixture provided the best results in terms of effective compensation for

matrix-induced enhancement effect (González-Rodríguez et al., submitted). To sum up the treatment followed, the chopped vegetable sample (10 g) was placed in a 125 mL glass container and extracted with 30 mL of acetonitrile. The glass container was vigorous homogenized in an ultrasounds bath for 10 min. Sodium chloride (3 g) and anhydrous magnesium sulphate (12 g) were added followed by vigorous shaking for 5 min. After phase partitioning for 15 min; an aliquot of 15 mL of the organic layer was transferred to a 100 mL round-bottomed flask and evaporated to 1–2 mL at 40 °C on the rotary evaporator (220 mbar).

For clean-up, a multi-layer Supelclean ENVI Carb-II/PSA SPE cartridge was conditioned with 5 ml of acetonitrile:toluene (3:1, v/v). Acetonitrile extract was loaded and the retained pesticides were eluted weakly, in a 50 mL round-bottomed flask, with a volume of 20 mL of acetonitrile:toluene (3:1, v/v). The eluate was evaporated down (40 °C, 75 mbar) and substituted the solvent with 0.5 mL of acetone containing hexachlorobenzene and fenpropathrin (at 500 μg/L; they were both used as internal standards) and containing the three analyte protectants (3-ethoxy-1,2-propanediol at 10 g/L; and D-sorbitol and L-gulonic acid γ-lactone at 10 g/L; respectively). Acetone extract was finally homogenized with vortex agitation.

2.5. Statistical analyses

Basic and descriptive statistics, and Pareto chart analyses were performed using SPSS v. 14.0 for Windows. The Pareto chart analysis is a simple but powerful way of identifying the causes of quality problems or loss. According to the so-called Pareto principle, the majority of the quality loss is caused by a small number of factors, and can easily be detected via the Pareto chart.

3. Results and discussion

3.1. Procedure performance

The performance of the SLE/SPE/GC-ITMS method was assessed by evaluating quality recovery and precision values for pesticides in different fresh vegetables, together with their limits of detection and quantitation (Table 1). Absolute recovery and precision (expressed as relative standard deviation) from fresh vegetables was measured by analysing three samples of each type of vegetable, fortified at a concentration of 0.050 mg/Kg for each pesticide. The recovery values ranged from 81 to 115%, and precision ranged from 2 to 12% for all fresh vegetables. Limits of detection (LOD) and quantitation (LOQ) were calculated from the signal-to-noise ratios obtained by analysing unspiked samples ($n = 10$); LOD and LOQ were taken to be the concentrations of pesticide resulting in a signal-to-noise ratio of 3 and 10, respectively. LODs ranged from 0.002 to 0.010 mg/Kg, whereas LOQs ranged from 0.006 to 0.020 mg/Kg for the tested pesticides.

Table 1
Performance of the proposed method for the determination of pesticides in leafy vegetables

Pesticide	Swiss chard				Spinach		Lettuce	
	Recov[a] (%)	RSD (%)	LOD[b] (mg/Kg)	LOQ[b] (mg/Kg)	Recov[a] (%)	RSD (%)	Recov[a] (%)	RSD (%)
Carbofuran	100	3	0.004	0.009	86	7	85	2
Methiocarb	98	4	0.002	0.004	85	5	81	5
Pyrimethanil	90	3	<0.001	0.001	86	4	84	4
Vinclozolin	95	5	0.001	0.002	91	2	107	6
Metalaxyl	93	3	0.001	0.001	87	5	82	3
Triadimefon	95	2	0.002	0.005	91	3	84	6
Cyprodinil	83	3	<0.001	0.001	80	10	79	9
Chlorfenvinphos	91	2	0.002	0.004	85	5	80	1
Penconazole	100	9	0.001	0.003	95	10	86	6
Triadimenol	85	3	0.005	0.010	83	4	83	8
Procymidone	98	4	0.003	0.007	88	8	87	3
Fenamiphos	96	4	<0.001	0.001	83	7	91	5
Fludioxonil	99	1	0.001	0.002	95	2	89	4
Tebuconazole	98	2	0.002	0.003	99	5	91	5
Iprodione	99	3	0.001	0.002	87	5	80	6
Bifenthrin	102	2	0.001	0.002	93	5	95	4
λcyhalothrin	104	5	0.002	0.006	100	4	96	8
Acrinathrin	103	10	0.003	0.007	100	8	106	15
Cyfluthrin	114	5	0.010	0.021	107	2	101	6
Cypermethrin	120	12	0.016	0.026	117	5	110	8
Esfenvalerate	107	5	0.003	0.007	111	2	95	12
t-fluvalinate	115	7	0.002	0.006	111	4	92	2
Deltamethrin	121	6	0.013	0.034	99	11	133	6

[a] Average absolute percent recovery ($n = 3$).
[b] Average LOD and LOQ ($n = 10$).

3.2. Occurrence of fungicide and insecticide residues in leafy vegetables

The degradation of the food quality by pesticides pollution is a cause of concern. As a result of the great sensibility of pest attack and the necessity to launch them quickly in the market, the fruits and vegetables need special attention regarding pesticide use. The occurrence and distribution of pesticides in the shopping areas of Ourense (NW Spain) was the main aim of this work. The reason for investigations in this area is the toxicity and persistence of pesticides. Therefore, controlling the pesticide levels seems to be a substantial contemporary public health problem to guarantee food quality and to evaluate alimentary risk.

Dicarboximide fungicides (procymidone and iprodione) followed by phenylamide and triazole fungicides (metalaxyl and tebuconazole, respectively) are the fungicides with more number of positives above the MRL in leafy vegetables (Table 2). Among the different insecticides in leafy vegetables, cypermethrin was the insecticide with the highest number of positives above the MRL in leafy vegetables. Cyfluthrin and methiocarb were also found above the

Table 2
Pesticide concentrations ranges found in samples, together with number of samples with levels above the maxima residue limits

Pesticide type	Chemical name	Concentration range[a]	Swiss chard + Spinach	Lettuce	MRL S.C.[b] + Spinach	MRL Lettuce	Samples above MRL	
							S.C. + S.[c]	Lettuce
Fungicide	Cyprodinil	0.01–*0.02*	1	1	0.02	2.0	0	0
		0.10–0.50		1				
	Fludioxonil	*0.05*–0.10		1	0.05	2.0	0	0
	Iprodione	0.01–*0.02*	1	1	0.02	10.0	*4*	0
		0.02–0.05	*1*					
		0.05–0.10	*1*					
		0.10–0.50		2				
		5.0–*10.0*	*2*					
	Metalaxyl	0.01–0.02	1	3	0.05	1.0	*2*	0
		0.02–*0.05*		2				
		0.05–0.10	*1*	3				
		0.10–0.50		3				
		1.0–2.0	*1*					
	Procymidone	0.01–*0.02*	4	4	0.02	5.0	*3*	*2*
		0.02–0.05	*1*	1				
		0.05–0.10		1				
		0.10–0.50		1				
		0.50–1.0	*1*					
		1.0–2.0	*1*	1				
		2.0–*5.0*		1				
		5.0–10.0		*1*				
		>10.0		*1*				
	Tebuconazole	0.01–0.02	1	1	0.05	5.0	*1*	0
		0.05–0.10		1				
		0.10–0.50	*1*					
	Vinclozolin	*0.05*–0.10		2	0.05	5.0	0	0
		0.20–0.50		1				
Insecticide	Acrinathrin	0.20–0.50		1	0.02	1.0	0	0
	Bifenthrin	0.02–*0.05*		1	0.05	2.0	0	0
	∑Cyfluthrin	*0.05*–0.10	*1*		0.02	0.50	*1*	0
	∑Cyhalothrin	0.01–0.02	2		0.50	1.0	0	0
		0.02–0.05		1				
		0.05–0.10	5					
		0.10–*0.50*	1					
	∑Cypermethrin	0.02–0.05	1	1	0.50	2.0	*4*	0
		0.10–*0.50*	4	1				
		0.50–1.0	*2*	1				
		2.0–5.0	*1*					
		5.0–10.0	*1*					
	∑Deltamethrin	0.02–0.05	1		0.50	0.50	0	0
		0.05–0.1		1				
		0.10–*0.50*	2					
	∑Methiocarb	*0.5*–1.0		*1*	0.05	0.05	0	*1*
Total >MRL							*15*	*3*

[a] Numbers in italics correspond to pesticide MRLs for either Swiss chards and Spinaches or lettuces.
[b] S.C. = Swiss chards.
[c] S. = Spinaches.

MRL in one sample. The largest number of violations has occurred in Swiss chard and spinaches (15/18) rather than in lettuces (3/18). Overall, a 20% of the samples analyzed did not comply with the regulations, but only a 9% of lettuces against a 29% of Swiss chards and spinaches. This is because MRLs for Swiss chards and spinaches can even be one hundred times lower than for lettuces; the reason is that lettuces are highly sensitive to pests and need for successive applications of pesticide treatments, leaving in consequence higher level of residues that are tolerated. Anyway, the pesticides concentrations found in the samples show the need for a monitoring program for the residues of the fungicides iprodione and procymidone, and the insecticide cypermethrin in food crops.

The degree of accumulated pesticide pollution is 17 times higher in the outer leaves of raw vegetables than in those cut-and-washed, and this degree of pollution is almost twice higher for small grocery shops than for big food stores (Fig. 1). This finding can be considered indicative of pesticide pollution being reduced by technological treatments of washing and by analytical control of residues. Commercial processing procedures, which are used in the case of qualified producers, led to large reductions in residue levels in the finished products. Packed and labelled vegetables are usually safer due to traceability controls. The extent to which pesticide residues are removed by commercial processing depends on a variety of factors, such as the chemical properties of the pesticide, the nature of the food commodity and the processing step, and the length of time the compound has been in contact with the food (Farris, Cabras, & Spanedda, 1992; Holland, Hamilton, Ohlin, & Skidmore, 1994). Higher pesticide concentrations in lettuces were found as compared to Swiss chards and spinaches, being fungicides (vs. insecticides) the main contributors to the total pesticide load, especially for lettuces (Fig. 2). It is therefore clear that patterns of fungicide and insecticide use are crop dependent: the predominant use of fungicides in lettuce is mainly to control *Botrytis*, especially in the case of a rainy season like Spring. The most reasonable explanation for the observed decreasing trend of pesticides for spinaches seems to be lower inputs.

Overall, fungicides and insecticides found in this study were similar to those found in other studies (Amoah, Drechsel, Abaidoo, & Ntow, 2006; Cabras & Conte,

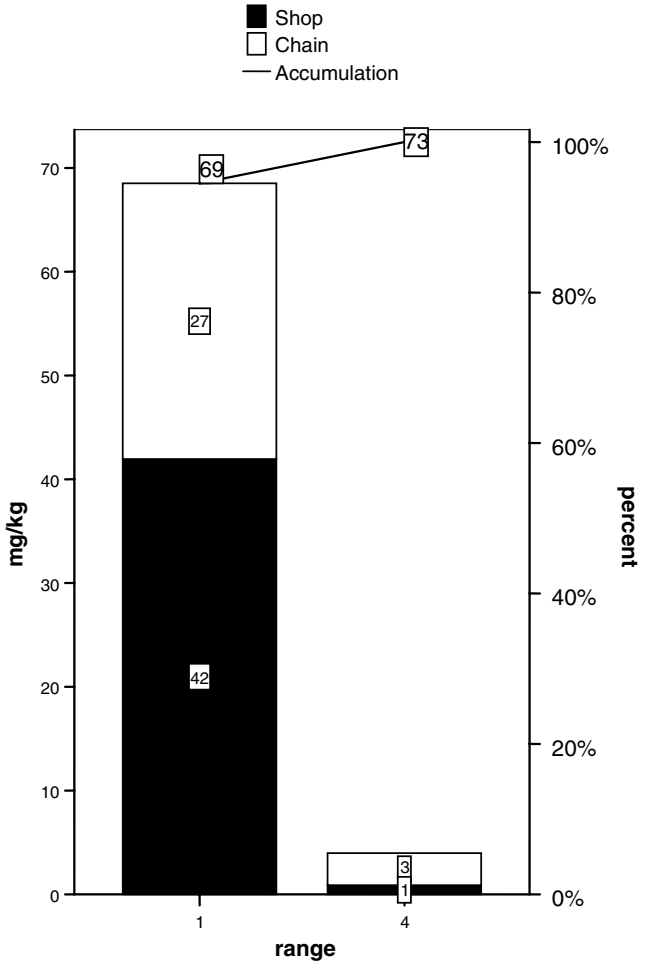

Fig. 1. Pareto chart of pesticide accumulated concentrations in vegetables, according to two factors: (1) type of sample (raw product-range 1-, and cut-and-washed product-range 4) plus (2) type of seller (small specialized shop, and big store or chain).

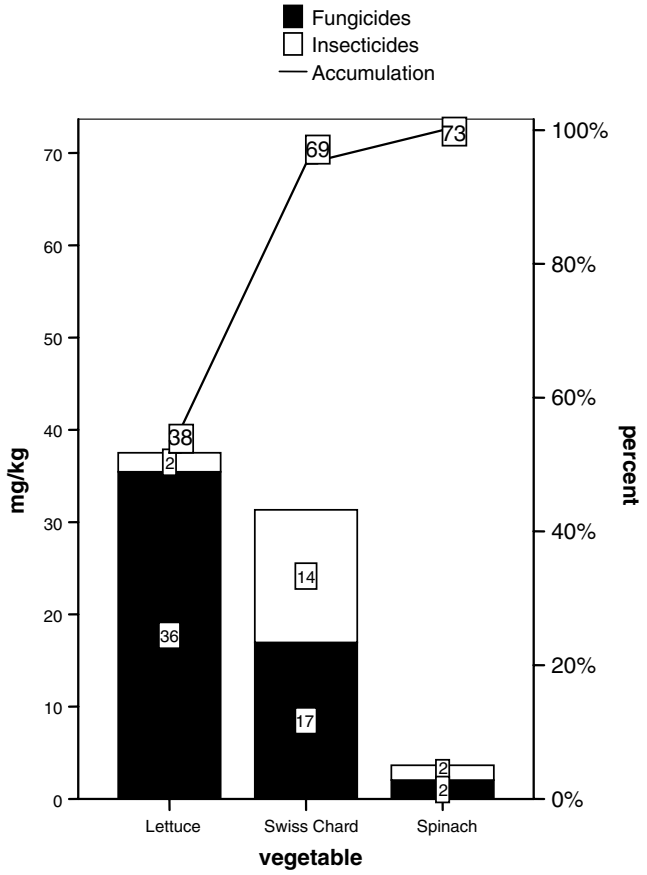

Fig. 2. Pareto chart of pesticide accumulated concentrations in vegetables, according to two factors: (1) type of leafy vegetable (lettuce, Swiss chard, and spinach) plus (2) type of pesticide (fungicides and insecticides).

2001; Dogheim, Ashraf, Alla, Khorshid, & Fahmy, 2004; Gebara, Ciscato, Ferreira, & Monteiro, 2005; Poulsen & Andersen, 2003), but 15 samples of 75 had residue levels >MRL (three of the samples did not fulfil with two different pesticide MRL). The selected plant foods will not give a chance for adverse biological effects to take place providing the residues of dicarboximide fungicides and pyrethroid insecticides are controlled to be kept to a minimum. Pesticide residue monitoring programs should then be implemented to assure the minimum allowable residue levels in plant foods, especially with regards to iprodione-procymidone and cypermethrin. The investigation on samples of a known history showed that most irregularities were due to a poor compliance of the pre-harvest interval, especially after repeated treatments with some active ingredients (Rial-Otero, Arias-Estévez, López-Periago, Cancho-Grande, & Simal-Gándara, 2005).

4. Conclusions

The proposed method helps to cover some of the most important research and development needs in this area of pesticides in crops to asses the state of food pollution. Pesticide pollution in crops was produced by their inputs. The results revealed that special attention should be paid to iprodione and procymidone, together with cypermethrin. Application, washing and analytical control appeared to be the most important factors conditioning their levels in leafy vegetables, indicating that the contribution of soil/water pesticides to crops (aerial part) is insignificant. Application is in the hands of the producer, but washing and analytical control is in the hands of the distributor.

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