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# Effect of household and industrial processing on the levels of pesticide residues and degradation products in melons

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Two varieties of melons (*Cucumis melo*) were treated with two fungicides (carbendazim and maneb) and four insecticides (acetamiprid, cyromazin, imazalil and thiamethoxam) to quantify the effect of household processing on the pesticide residues. To ensure sufficiently high levels of residues in flesh and peel, the most concentrated formulations were applied observing good agricultural practice. The peeling step decreased the concentration of pesticide residues for maneb, imazalil and acetamiprid by more than 90%. Cyromazin, carbendazim and thiamethoxam were reduced by approximately 50%. The reduction of the pesticides could not be fully explained by the systemic character of the pesticides. However, the agricultural practices (time of application), solubility and mode of action (systemic versus contact pesticide) of the pesticides could be used to explain the difference in processing factors for the studied pesticides. Degradation products (melamine and ethylenethiourea) were also investigated in this study, but were not detected.

**Keywords:** LC/MS; GC; pesticide residues; fruit; vegetables

## Introduction

Melon (*Cucumis melo*) belongs to the same family as the cucumber, squash, pumpkin and gourd and it grows on the ground on a trailing vine. It is a sweet, juicy and nutritious fruit. The major producing countries are China (which produces more than 50% of the world's production), Turkey, Iran, Spain, the United States, Romania, Egypt and India (Food and Agriculture Organization of the United Nations (FAO) 2011). This crop is susceptible to *Fusarium* wilt disease (destructive vascular disease), downy mildew (killing plants prematurely and reducing the sugar content of melons) and gummy stem blight (lesions on stems and leaves). To protect plants particularly during autumn growing seasons and to avoid these types of diseases, fungicide applications are necessary (Keinath et al. 2007; Zhao et al. 2011). Furthermore, insecticides on melons are necessary to suppress high whitefly populations and aphids (Tong-Xian 2004).

Pesticides have been linked to a wide spectrum of human health hazards, ranging from short-term impacts such as headaches and nausea to chronic impacts such as cancer, reproductive harm and endocrine disruption. Chronic health effects may occur years after even minimal exposure to them in the environment, or result from their residues being ingested through food and water (Berrada et al. 2010; Keikotlhaile et al. 2010; Claeys et al. 2011). Research focuses on the proper use of pesticides in terms of authorisation and registration and on compliance with maximum residue limits (MRLs). Given the potential risk of pesticides for public health, the use of pesticides in agriculture is subjected to constant monitoring (Chen et al. 2011; Claeys et al. 2011). It is important for consumers to know their intake of pesticide residues. The processing factors (PF: the ratio between residues' concentration in the processed commodity and the same in the raw commodity) are useful to estimate the level of pesticide exposure at the point of consumption after processing (Ling et al. 2011). Until now, no processing factors have been available for melons. However, melon consumption is increasing in the European Union (AND International 2010). Indeed, among the EU-27, the volumes of import of melons increased twofold between 1999 and 2008.

It is well known that peeling plays a role in the reduction of residues (Holland et al. 1994; Timme and Walz-Tylla 2004; Kaushik et al. 2009). Several studies of peeling have been done on potatoes to measure some pesticides and a minimum 90% reduction of

pesticide residues was observed (Lewis et al. 1996; Schattenberg et al. 1996; Lentza-Rizos and Balokas 2001; Randhawa et al. 2007; Kaushik et al. 2009). The peeling of apples, tomatoes, peppers, carrots, bananas, oranges and lemons was also investigated in several studies (Burchat et al. 1998; Rasmusssen et al. 2003; Timme and Walz-Tylla 2004; Chavarri et al. 2005; Balinova et al. 2006). In these experiments, peeling decreased residues (70–100%). However, some pesticides were not influenced by this process because of their systemic activity (Krol et al. 2000).

Melons, frequently consumed in Belgium, were chosen in cooperation with the Belgian Federal Agency for Safety of the Food Chain (FASFC). Pesticides were chosen according to their frequency of MRL exceedance, frequency of detection and toxicity (FASFC 2007, 2008). Selected pesticides included acetamiprid, carbendazim, cyromazin, imazalil, maneb and thiamethoxam.

To establish of processing factors for these pesticide residues, melons were grown, sprayed, harvested, peeled, homogenised and analysed. The selected pesticides and the degradation products (ETU from maneb and melamine from cyromazin) were analysed before and after peeling with several analytical methods (GC-MS/MS, GC-ECD, UHPLC-MS/MS).

## Materials and methods

### Materials

#### Pesticides

Deuterated ethylenethiourea ($d_4$-ETU), ethylenethiourea, carbendazim and acetamiprid were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) with a certified purity of 99.0%. Thiram, cyromazin, melamine, imazalil and thiamethoxam were purchased from Sigma-Aldrich (Seelze, Germany) with certified purities of 99.0%, 99.9%, 99.0%, 99.8% and 99.7%, respectively. Carbon disulfide (99.9%) was obtained from Merck (Darmstadt, Germany).

#### Chemicals for analysis

Ultrapure water (<8 M cm resistance) was supplied by a Millipore purification system (Millipore Milli-Q Water System, Bedford, MA, USA). Methanol, dichloromethane and acetonitrile were of HPLC grade from Biosolve (Valkenswaard, the Netherlands). Iso-octane, sodium hydroxide and ammoniac (25%) came from Merck. Trifluoroacetic acid (TFA) and acid acetic (with certified purity of at least 99% for both) as well as ammonium acetate were purchased from Aldrich (Steinheim, Germany). Hydrochloric acid (37%) and tin(II)-chloride were obtained from VWR (Fontenay sous Bois, France). Ethanol (96%), ammonium chloride (99.5%),

Celite® 545, sodium sulphate (99%) and sodium chloride (99%) were purchased from VWR (Leuven, Belgium).

The stock solution of carbon disulfide ($CS_2$), for the analysis of maneb, was prepared at $4 \, \text{mg ml}^{-1}$ in iso-octane. Dilute standards at $40 \, \mu\text{g ml}^{-1}$ were prepared by dilution of the stock solution with iso-octane. A solution of thiram was prepared with ethanol to obtain an equivalent concentration in $CS_2$ of $63.23 \, \text{mg l}^{-1}$.

The stock solutions of acetamiprid, carbendazim, cyromazin, imazalil and thiamethoxam for LC-MS/MS were prepared at $1 \, \text{mg ml}^{-1}$ in acetonitrile containing 0.1% of acetic acid. Dilute standards at $10 \, \mu\text{g ml}^{-1}$, $1 \, \mu\text{g ml}^{-1}$ and $100 \, \text{ng ml}^{-1}$ were prepared by dilution of the stock solution with methanol.

The stock solution of internal standard, $d_4$-ETU was prepared at $100 \, \mu\text{g ml}^{-1}$ in methanol. The stock solution of ETU was prepared at $1 \, \text{mg ml}^{-1}$ in methanol. Dilute standards were prepared by dilution of the stock solution with water.

The stock solution of melamine was prepared at $1 \, \text{mg ml}^{-1}$ in NaOH 0.1%. Dilute standards at 10 and $1 \, \mu\text{g ml}^{-1}$ were prepared by dilution of the stock solution with water.

Solutions for calibration were prepared with spiking of diluted standard solution on blank extract of melon. The blank extract came from melons previously tested in which no pesticides were detected above the limit of detection. All solutions were stored at $-18°C$.

### Produce

#### Cultivation

The purpose of the field experiment was to produce in parallel two different melons varieties, Mohican and Pancha, exposed to six selected pesticides.

Field melon was grown at Villers-Perwin, Belgium, by Redebel SA under greenhouse conditions. Four applications of pesticides were performed on different crop growth stages, as shown in Table 1. The concentrations of the six active substances and the number of applications were as high as possible, but following good agricultural practices (pre-harvest time, time between two applications, etc.), to ensure sufficiently high pesticide levels for the effects of processing practices to be studied. It should be noted that some pesticides are not approved for melon crops in Belgium, but are in other countries. The formulations were applied in two passings using vertical spraying boom equipment at the proposed normal settings and timing.

#### Harvest

Fruit specimens were taken manually. Sterile disposable (nitrile) gloves were worn for the sampling.

Table 1. Pesticides with their product name, rate of application and the crop stage at the moment of application.

| Pesticides | Product name | Active ingredient ($g\,ha^{-1}$) | Application date | Crop stage (BBCH[a]) | Number of days before harvest | MRL ($mg\,kg^{-1}$) |
|---|---|---|---|---|---|---|
| Acetamiprid | Gazelle SP | 105 | 9 August 2010 | BBCH 83 = 30% of fruits show typical fully ripe colour (FTRC) | 10 | *0.01** |
| | | 98 | 16 August 2010 | BBCH 87 = 70% FTRC | 3 | |
| Carbendazim | Punch SE | 319 | 5 July 2010 | BBCH 65 = fifth flower open on main stem | 45 | *0.1** |
| Cyromazine | Trigard 100 SL | 58 | 2 August 2010 | BBCH 81 = 10% FTRC | 17 | *0.3* |
| | | 63 | 9 August 2010 | BBCH 83 = 30% FTRC | 10 | |
| | | 59 | 16 August 2010 | BBCH 87 = 70% FTRC | 3 | |
| Imazalil | Fungaflor EC | 97 | 2 August 2010 | BBCH 81 = 10% FTRC | 17 | 2 |
| | | 105 | 9 August 2010 | BBCH 83 = 30% FTRC | 10 | |
| | | 98 | 16 August 2010 | BBCH 87 = 70% FTRC | 3 | |
| Maneb | Trimangol 75 WG | 1950 | 2 August 2010 | BBCH 81 = 10% FTRC | 17 | *1* |
| | | 2108 | 9 August 2010 | BBCH 83 = 30% FTRC | 10 | |
| | | 1971 | 16 August 2010 | BBCH 87 = 70% FTRC | 3 | |
| Thiamethoxam | Actara 25 WG | 5 | 2 August 2010 | BBCH 81 = 10% FTRC | 17 | *0.2* |
| | | 5 | 9 August 2010 | BBCH 83 = 30% FTRC | 10 | |
| | | 5 | 16 August 2010 | BBCH 87 = 70% FTRC | 3 | |
| | | | 19 August 2010 | BBCH 89 = fully ripe: fruit have typical fully ripe colour | Harvest | |

Notes: [a]The BBCH scale is a system for a uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species.
*Lower limit of analytical determination (Reg. (EU) No 600/2010).
Values shown in italics are those substances not authorised on melon in Belgium.
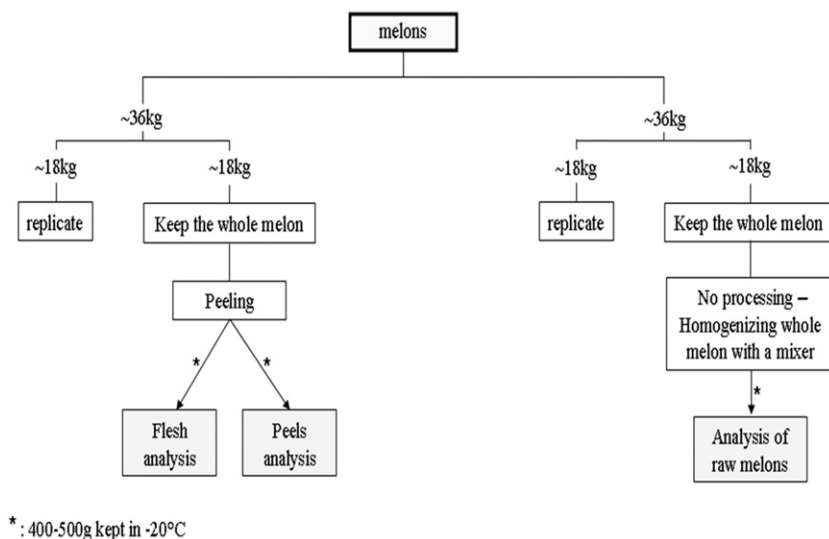


Figure 1. Flow diagram of the processing steps and sampling for one variety of melon.

The first and the last plants of plot, diseased and undersized fruits were excluded from the sampling. To obtain more than 70 kg of melons, 67 melons Pancha and 103 melons Mohican were randomly collected from the whole plot. A number identified each variety. Melons were stored in a refrigerator room at 4°C directly after the sampling. They were transported in a refrigerated condition to the laboratory on the day of harvest.

### Sample preparation and processing

#### Overview of processing and sampling steps

Figure 1 shows the sampling flow diagram. To minimise the factor of variability, the two melon varieties were each divided into two batches of 18 kg of raw melons, giving four batches in total (Figure 1). As shown in Figure 1, a part of the melons did not undergo any processing and was used as a 'positive

control sample'. The peeling step for all batches was executed on the same day.

At each step, melons were homogenised and 500 g were collected and stored at $-20°C$ until pesticide residue analysis. Frozen samples were analysed within 1 month.

### Unprocessed melon

These melons, which did not undergo any processing, were the 'positive control sample'. It is the raw melon used to calculate the PFs. The harvested melons (18 kg of melons taken randomly) were cut into pieces and comminuted with the Robot Coupe® R23 (Mont-Saint-Genevieve, Belgium). A sample was taken after a rough cut (approximately 1 cm) and frozen for the analysis of maneb. The rest was comminuted for a longer time and frozen until analysis.

### Peeling

Peeling was realised with a knife with a 10 cm blade and corresponded to the peeling done by consumers at home. The green part on the peels ($<0.5$ cm) was left. Melons Mohican were smaller than melons Pancha, but both varieties were highly variegated.

After removal of the peels, flesh (about 10 kg) and peels (about 7 kg) were comminuted separately with the Robot Coupe® R23 and frozen until analysis.

### Extraction and analysis of pesticides

#### Analysis of maneb by GC-ECD

The analysis of maneb as $CS_2$ (a compound obtained when dithiocarbamates are heated in the presence of acid and tin chloride) was based on methods normalised by the European Committee for Standardization (1998) and modified by de Kok (2001) to replace the existing headspace chromatography method by a liquid injection in GC-ECD.

The extraction of 25 g of matrix was performed in the presence of hydrochloric acid, iso-octane and tin chloride. A closed vessel was stirred for 2 h in a water bath at $80°C$. After cooling, 1 µl of the supernatant containing 0.28 g matrix ml$^{-1}$ was injected on a Varian 3400 gas chromatographic system coupled with a $^{63}$Ni electron capture detector and a Varian 8200 C autosampler. The GC separation of $CS_2$ was achieved on a DB-5 column (60 m, 0.53 mm and 1.5 µm) from Grace (Deerfield, IL, USA). The helium gas flow was set at 2.6 ml min$^{-1}$ at 5 psi. The injector and detector temperatures were 250 and $300°C$, respectively. The column temperature was $50°C$ for 5 min; after that it increased by $50°C$ min$^{-1}$ until $250°C$. This temperature was maintained for 5 min to obtain a total run time of 19 min. The retention time of $CS_2$ was 3.3 min.

#### Analysis of acetamiprid, carbendazim, cyromazin, imazalil and thiamethoxam by LC-MS/MS

This method followed the Granby principle (Granby et al. 2004) with some adaptations. A 10 g sample of melons was extracted with a mixture of an extraction solvent (5 mM ammonium acetate, 90% MeOH–10% water), blended for 1 min with an Ultra-turrax homogeniser (Ultra-turrax IKA, Boutersem, Belgium) and filtrated on Büchner. Oxfendazole, the internal standard, was added to the filtrate. The mixture of the extraction solvent was used to give an extract volume of 60 ml. The raw extract was diluted with mobile phase A (10% MeOH, 1% ammonium acetate 0.5 M and 89% water) to a final concentration equivalent to 0.1 g matrix ml$^{-1}$ and injected after filtration on 0.2 µm. LC separation was achieved on an Acquity UPLC BEH C$_{18}$ column (1.7 µm, $2.1 \times 100$ mm) from Waters (Ireland). A gradient of mobile phase A and mobile phase B (containing 10% water, 1% ammonium acetate 0.5 M and 89% of methanol) supplied at 0.45 ml min$^{-1}$ was applied. Starting from 99.9% of mobile phase A, the mobile phase was linearly increased to 99.9% of mobile phase B over 10 min and maintained for 2 min. The gradient was then returned to the initial conditions in 0.1 min and held there for 2.9 min for stabilisation. The mass spectrometer operated in a positive electrospray ionisation (ESI) mode and acquired two transitions for each pesticide. Typical optimised ESI voltage settings are presented in Table 2. Argon was used as the collision gas at a setting of 0.35 ml min$^{-1}$. The dwell time was 10 ms per channel for quantifier ions and 5 ms for second transition.

#### Analysis of ethylenethiourea by LC-MS/MS

The method of analysis is described by Bonnechère et al. (2011). Briefly, ETU was extracted from the homogenised melon (10 g), after thawing of the sample, by a 3:1 mixture of methanol–water. After 0.2 µm filtration, the extract containing 0.333 g matrix ml$^{-1}$ was injected in LC-MS/MS (the conditions are given in Table 2) on a Uptisphere 5MM1 mixed-mode chromatographic column ($100 \times 2.1$ mm, 5 µm) with two different silanes (C8/SCX) bonded on silica (Interchim, Montluçon, France), using an isocratic mobile phase of 0.1% trifluoroacetic acid/methanol (95/5). The injection volume was 5 µl in full loop; the run time was 2 min.

#### Analysis of melamine by LC-MS/MS

The method of analysis is described by Goscinny et al. (2011). The extraction of melamine was carried out using acetonitrile–water–dichloromethane in an acidic environment. After SPE and 0.2 µm filtration, the extract was injected in LC-MS/MS (the conditions are

Table 2. MS detection and selected ion for multi-residues analysis by LC-MS/MS.

| Selected ion | Parents (m/z) | Daughter (m/z) | Capillary (kV) | Source temperature (°C) | Desolvation temperature (°C) | Cone (V) | Collision (V) | Retention time (min) |
|---|---|---|---|---|---|---|---|---|
| Cyromazin | 167.01 | **84.9** | 0.6 | 130 | 400 | 28 | 16 | 1.53 |
| | | 60.3 | | | | | 18 | |
| Thiamethoxam | 292.3 | **211.0** | 0.6 | 130 | 400 | 19 | 12 | 2.49 |
| | 294.3 | 180.9 | | | | 19 | 24 | |
| | | 211.0 | | | | | 12 | |
| Acetamiprid | 223.0 | **125.8** | 0.6 | 130 | 400 | 26 | 21 | 3.65 |
| | | 89.9 | | | | | 35 | |
| Carbendazim | 192.1 | **160.1** | 0.6 | 130 | 400 | 25 | 18 | 3.92 |
| | | 132.1 | | | | | 30 | |
| Oxfendazole | 316.0 | **159.0** | 0.6 | 130 | 400 | 34 | 28 | 5.35 |
| Imazalil | 297.1 | **159.0** | 0.6 | 130 | 400 | 30 | 20 | 8.67 |
| | | 69.1 | | | | | 20 | |
| ETU | 102.9 | **44.3** | 0.3 | 130 | 450 | 31 | 15 | 0.91 |
| | | 85.9 | | | | | 15 | |
| d$_4$-ETU | 106.9 | 45.1 | 0.3 | 130 | 450 | 31 | 15 | 0.89 |
| | | **48.2** | | | | | 15 | |
| Melamine | 126.9 | **84.8** | 0.8 | 150 | 400 | 42 | 22 | 0.91 |
| | | 67.8 | | | | | 17 | |

Notes: Quantifier daughter ions are shown in bold.
*MS detection* Extractor (V): 4, RF lens (V): 0.2, Cone gas flow (l h$^{-1}$): 50, Desolvation gas flow (l h$^{-1}$): 800.

given in Table 2) on a Acquity HILIC column (100 × 2.1 mm, 1.7 μm) (Waters), using an isocratic mobile phase containing 2% ammonium acetate 0.5 M, 8% water and 90% of acetonitrile. The injection volume was 1 μl; the run time was 2 min.

### Quantification and quality control

Limits of quantification (LOQ) were estimated from the lowest calibration level. Based on a signal-to-noise ratio greater than or equal to 6, LOQs were at 40 μg kg$^{-1}$ for maneb, 2 μg kg$^{-1}$ for imazalil, 5 μg kg$^{-1}$ for cyromazin, 2 μg kg$^{-1}$ for thiamethoxam, and 1 μg kg$^{-1}$ for acetamiprid and carbendazim, 50 μg kg$^{-1}$ for ETU and 25 μg kg$^{-1}$ for melamine. The linearity of the instrument was evaluated with five or six points in the range of 50−1000 μg kg$^{-1}$ (wet weight basis) for ETU, 25–600 μg kg$^{-1}$ for melamine, 50–5000 μg kg$^{-1}$ for maneb, and 10–1000 μg kg$^{-1}$ for the pesticide residues analysed by LC-MS/MS. In all cases, good linearity was achieved with correlation coefficients >0.995.

For each batch of analysis, a control (a blank of melon) and a sample were spiked prior to extraction and run. In the control, no pesticide must be found with a concentration greater than the LOQ. If it is not the case, that means a contamination and the analysis of the samples must be redone. The spiked sample must have a recovery of extraction between 70% and 120% (European Commission 2009, SANCO/10684/ 2009) and the concentrations found in this sample were

reported on the control chart, which allows the trend to be followed. Recoveries during the analysis of melon were 95.7% for ETU, 73.2% for maneb, 83.4% for melamine, 87.3% for cyromazin, 87.3% for acetamiprid, 84.0% for carbendazim, and 83.4% for imazalil. The calibration was checked using a standard injected at the end of the sequence and it could not deviate by more than 20% from the injection of the same standard at the beginning of the sequence.

### Calculation of processing factors

Processing factors (PFs) were calculated for all transformation steps by a ratio between the pesticide residue concentration (mg kg$^{-1}$) in the processed commodity and the pesticide residue concentration (mg kg$^{-1}$) in the raw commodity.

### Results and discussion

Sprayed pesticides were detected at various concentrations. In spite of the high concentration of active substances applied on the raw melon, the concentrations for thiamethoxam, carbendazim and imazalil were rather low. The lowest concentrations in the raw product were observed for thiamethoxam and carbendazim (Table 3). Thiamethoxam was applied at the lowest concentration in this study and carbendazim is the only one which was applied once, as early as the opening of the main stem (Table 1).

Table 3. Concentrations (mg kg$^{-1}$) of pesticides and degradation products for the two melon varieties after the peeling step.

| Pesticides | LOQ (mg kg$^{-1}$) | Raw | Flesh Mean ($\pm$SD) | Peels |
|---|---|---|---|---|
| *Variety 1: Mohican* | | | | |
| Acetamiprid | 0.001 | 0.042 ($\pm$0.00057) | 0.0039 ($\pm$0.00007) | 0.081 ($\pm$0.0087) |
| Carbendazim | 0.001 | 0.0014 ($\pm$0.00049) | <LOQ | 0.0042 ($\pm$0.0018) |
| Cyromazin | 0.005 | 0.078 ($\pm$0.0022) | 0.029 ($\pm$0.0031) | 0.110 ($\pm$0.0024) |
| Imazalil | 0.002 | 0.0092 ($\pm$0.00085) | <LOQ | 0.017 ($\pm$0.0039) |
| Maneb | 0.040 | 0.480 ($\pm$0.075) | 0.043 ($\pm$0.032) | 0.820 ($\pm$0.086) |
| Thiamethoxam | 0.002 | 0.0045 ($\pm$0.0000) | <LOQ | 0.0074 ($\pm$0.0003) |
| ETU | 0.050 | <LOQ | <LOQ | <LOQ |
| Melamine | 0.025 | <LOQ | <LOQ | <LOQ |
| *Variety 2: Pancha* | | | | |
| Acetamiprid | 0.001 | 0.046 ($\pm$0.0029) | 0.0051 ($\pm$0.0018) | 0.083 ($\pm$0.00092) |
| Carbendazim | 0.001 | 0.0012 ($\pm$0.00021) | <LOQ | 0.0034 ($\pm$0.00014) |
| Cyromazin | 0.005 | 0.081 ($\pm$0.013) | 0.039 ($\pm$0.0026) | 0.084 ($\pm$0.00064) |
| Imazalil | 0.002 | 0.011 ($\pm$0.00014) | <LOQ | 0.017 ($\pm$0.0032) |
| Maneb | 0.040 | 0.55 ($\pm$0.050) | <LOQ | 0.91 ($\pm$0.062) |
| Thiamethoxam | 0.002 | 0.0036 ($\pm$0.0003) | <LOQ | 0.0074 ($\pm$0.0001) |
| ETU | 0.050 | <LOQ | <LOQ | <LOQ |
| Melamine | 0.025 | <LOQ | <LOQ | <LOQ |

Note: Means ($\pm$SD, $n=2$) of two melon batches are depicted.

Table 4. Means ($\pm$SD, $n=2$) of processing factors (PF) for the peeling step for six pesticides in two varieties of melons.

| Pesticides | PF peeling ($\pm$SD) | | Action mode | Log octanol–water partitioning coefficients | Water solubility at 20°C (mg l$^{-1}$) |
|---|---|---|---|---|---|
| | Mohican | Pancha | | | |
| Acetamiprid | 0.091 ($\pm$0.002) | 0.110 ($\pm$0.040) | Systemic | 0.8 | 2950 |
| Carbendazim | 0.37 ($\pm$0.14) | 0.43 ($\pm$0.08) | Systemic | 1.48 | 8 |
| Cyromazin | 0.38 ($\pm$0.04) | 0.48 ($\pm$0.08) | Non-systemic | 0.069 | 13,000 |
| Imazalil | 0.110 ($\pm$0.010) | 0.093 ($\pm$0.001) | Systemic | 3.82 | 22.4 |
| Maneb | 0.088 ($\pm$0.068) | 0.073 ($\pm$0.007) | Non-systemic | −0.45 | 178 |
| Thiamethoxam | 0.33 ($\pm$0.16) | 0.42 ($\pm$0.20) | Systemic | −0.13 | 4100 |

In this study, peeling was performed with a knife. Table 4 represents all processing factors for the peeling step for the six detected pesticides for both varieties. The peeling removed an important part of the pesticide residues: between 62% and 95% for melon Mohican and between 52% and 95% for melon Pancha.

Maneb and cyromazin are contact pesticides and form a deposit on the surfaces of the leaves and fruits (Table 4). After the peeling step, in spite of an important reduction for maneb (91% for Mohican and 93% for Pancha), the decrease of cyromazin did not exceed 62% and 52% for Mohican and Pancha, respectively. However, this smaller reduction could be explained by the higher solubility of cyromazin compared with the other pesticides (Table 4). Compounds applied to the plants (leaves) do not have to cross the symplast to arrive in the xylem, contrary to compounds passing by roots. On the other hand, they have to cross the more or less waxy cuticle according to the type of plant and its age. With age, fractures in the waxy coat form hydrophilic pores allowing the passage of the most hydrophilic molecules as cyromazin (Al-Sayeda 2007). With this particularity, cyromazin could enter the fruit by the flow of xylem and be found in the flesh. As Krol et al. (2000) have also confirmed, it is possible that pesticide residues are incorporated into plant tissue in proportion to the time they remain on biologically active crops in the field. This may even be true of pesticides that are not specifically labelled as systemic (Krol et al. 2000). The other four pesticides (acetamiprid, carbendazim, imazalil and thiamethoxam) are systemic. Nevertheless, acetamiprid and imazalil were strongly reduced by 91% for Mohican, 89% for Pancha and 91% for Mohican and Pancha, respectively. The reduction was somewhat less important for the thiamethoxam (67% and 58%) and carbendazim (63% and 47%) (Table 4). The difference of behaviour could be explained by the time of application of these four active substances (Table 1). Indeed, carbendazim was the first pesticide applied

when the melon plant had its fifth flower open on main stem. With this early application, carbendazim could enter the plant and was present in it before the apparition of fruit. The others were applied in the latest stage of development when 10% of fruits showed typical fully ripe colour (Table 1). Otherwise, thiamethoxam has a good solubility and an octanol–water partition coefficient which allows it at the same time to circulate in the phloem and in the xylem and consequently to be present in the fruit (Table 4). On one hand, more than 90% of acetamiprid, imazalil and maneb, contact as well systemic pesticides, was removed with this process. On the other hand, approximately 50% of carbendazim and thiamethoxam (systemic pesticide) and cyromazin (contact pesticide) were eliminated. These two types of reduction could not be explained by the systemic character of the pesticides. However, the agricultural practices (time of application) and the water solubility could explain statistically these differences between the pesticides. The octanol–water partition coefficient is also necessary to explain the behaviour of cyromazin. It is a particular case, not shown in the statistical test, which is a global approach. Indeed a systemic pesticide applied late on the plants is found with a low concentration in the flesh. Until now, several studies have explained the processing factor with the physicochemical properties, but they did not reflect the times of application on the harvest (Watts et al. 1974; Burchat et al. 1998; Rasmusssen et al. 2003; Lee and Jung 2009). These results show that it really important to take into account the time of application of active substances for a risk assessment. Indeed as Krol et al. (2000) stated, a systemic pesticide applied late could not act as a systemic substance. For risk assessment, it is also necessary to be aware that the levels of residues in melons depend on the time of application quite as the statistical test reflects it.

Reductions of 90% are consistent with previous studies on the peeling of various fruit and vegetables (Schattenberg et al. 1996; Lentza-Rizos and Balokas 2001; Rasmusssen et al. 2003; Timme and Walz-Tylla 2004; Boulaid et al. 2005; Chavarri et al. 2005; Balinova et al. 2006; Fernandez-Cruz et al. 2006; Kaushik et al. 2009). It was indeed demonstrated that a majority of the insecticides or fungicides applied directly to crops undergo very limited movement or penetration of the cuticle. However, residues of these materials are confined to the outer surfaces where they are amenable to removal by peeling (Holland et al. 1994; Burchat et al. 1998; Timme and Walz-Tylla 2004).

This study also investigated the degradation products of cyromazin and maneb, being melamine and ethylenethiourea (ETU), respectively. In the unprocessed melon, these two by-products were not detected (Table 3). In general, ETU is formed in an acidic, heated environment and it is not the case with peeling (Dubey et al. 1997; Kontou et al. 2001; Hwang et al. 2003; Sottani et al. 2003).

If we compare the concentrations applied on the plants and the concentrations found in melon, there is high variability between the active substances under investigation (Table 1). For example, the concentration

Table 5. Active substances (mg) accounting for the mass balance of pesticides and degradation products for the two melon varieties after the peeling step.

| Pesticides | Mean values (±SD) | | | |
|---|---|---|---|---|
| | Raw (measured) | Flesh | Peels | Raw (calculated) |
| *Variety 1: Mohican* | | | | |
| Acetamiprid | 0.75  (±0.058) | 0.37  (±0.0001) | 0.57  (±0.084) | 0.60  (±0.084) |
| Carbendazim | 0.024 (±0.0073) | 0.048 (±0.0001) | 0.029 (±0.014) | 0.034 (±0.014) |
| Cyromazine | 1.380 (±0.049) | 0.281 (±0.026) | 0.770 (±0.014) | 1.050 (±0.04) |
| Imazalil | 0.160 (±0.026) | 0.010 (±0.0001) | 0.120 (±0.032) | 0.130 (±0.032) |
| Maneb | 8.600 (±1.88) | 0.410 (±0.31) | 5.780 (±0.84) | 6.190 (±1.15) |
| Thiamethoxam | 0.080 (±0.0051) | 0.010 (±0.013) | 0.052 (±0.0001) | 0.061 (±0.013) |
| ETU | <LOQ | <LOQ | <LOQ | <LOQ |
| Melamine | <LOQ | <LOQ | <LOQ | <LOQ |
| *Variety 2: Pancha* | | | | |
| Acetamiprid | 0.860 (±0.0016) | 0.065  (±0.025) | 0.590 (±0.077) | 0.653 (±0.10) |
| Carbendazim | 0.022 (±0.0052) | 0.0063 (±0.0002) | 0.024 (±0.0019) | 0.030 (±0.0021) |
| Cyromazine | 1.500 (±0.15) | 0.490  (±0.050) | 0.590 (±0.067) | 1.086 (±0.12) |
| Imazalil | 0.200 (±0.015) | 0.013  (±0.0004) | 0.120 (±0.037) | 0.130 (±0.037) |
| Maneb | 10.17  (±0.31) | 0.50  (±0.017) | 6.46  (±1.21) | 6.97  (±1.23) |
| Thiamethoxam | 0.067 (±0.0012) | 0.019  (±0.0095) | 0.051 (±0.0066) | 0.070 (±0.016) |
| ETU | <LOQ | <LOQ | <LOQ | <LOQ |
| Melamine | <LOQ | <LOQ | <LOQ | <LOQ |

Note: Means (±SD, $n=2$) of two melon batches are depicted.

Table 6. Means (±SD, $n = 2$) with a mass balance of processing factors (PF) for the peeling step for six pesticides in two varieties of melons.

| Pesticides | PF peeling (±SD) | |
|---|---|---|
| | Mohican | Pancha |
| Acetamiprid | 0.049 (±0.0038) | 0.075 (±0.030) |
| Carbendazim | 0.20 (±0.062) | 0.29 (±0.072) |
| Cyromazine | 0.20 (±0.020) | 0.33 (±0.046) |
| Imazalil | 0.058 (±0.0091) | 0.063 (±0.0051) |
| Maneb | 0.048 (±0.038) | 0.050 (±0.0023) |
| Thiamethoxam | 0.12 (±0.16) | 0.29 (±0.14) |

in maneb applied on plants was more than 50 times higher than the concentration in cyromazin. The concentration retrieved in the melons (Table 3) showed that the concentration in maneb is 1.5 times lower than for cyromazin. However, the correlation of the results of both varieties was good.

Mass balance recoveries were calculated to compare the total mg of each pesticide in the whole commodity with the residue in the flesh and peels after peeling. Measured concentrations shown in Table 4 were corrected with the mass of the whole melons, the flesh and peels comminuted to obtain reconstituted samples. Taking into account the measured uncertainties, the measured concentration and calculated concentration were close (Table 5), Table 6 shows all corrected PFs. These PFs show some difference between varieties. An explanation could be the difference in the size of both varieties. The proportion of peels-to-flesh is more important and engenders variations between the PFs of both varieties for certain pesticides.

## Conclusion

During this study, processing factors for peeling of melons were determined for acetamiprid, carbendazim, cyromazin, imazalil, maneb and thiamethoxam. Mechanical peeling, a typical household process, will remove 52–91% of the pesticides on the melon. The reduction of the pesticides could not be fully explained by the systemic character of the pesticides. However, the agricultural practices (time of application), solubility and mode of action (systemic versus contact pesticide) of the pesticides allowed assumptions to be made to explain the difference in processing factors for the studied pesticides.

Two degradation products (melamine and ETU) with a higher toxicity than the parent compounds were also studied and were not detected before and after peeling.

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