*Desalination and Water Treatment*
www.deswater.com

doi: 10.5004/dwt.2018.22001

117 (2018) 32–41
June

# Behavior of imidacloprid contamination in fruiting vegetables and their impact to human health

Patrycja Mojsak, Izabela Hrynko, Ewa Rutkowska, Julia Szabuńko, Bożena Łozowicka, Piotr Kaczyński*

*Plant Protection Institute, National Research Institute, Chelmonskiego 22, 15-195 Białystok, Poland, emails: p.kaczynski@iorpib.poznan.pl (P. Kaczyński), p.mojsak@iorpib.poznan.pl (P. Mojsak), i.hrynko@iorpib.poznan.pl (I. Hrynko), e.rutkowska@iorpib.poznan.pl (E. Rutkowska), j.szabuńko@iorpib.poznan.pl (J. Szabuńko), b.lozowicka@iorpib.poznan.pl (B. Łozowicka)*

Received 4 January 2018; Accepted 4 February 2018

**ABSTRACT**

The aim of this study was to investigate the degradation behavior and dietary intake risk for imidacloprid and its metabolites (imidacloprid-guanidine, imidacloprid-olefin and imidacloprid-urea) in fruiting vegetables (tomatoes and cucumbers) growing in greenhouse conditions. A simple, rapid analytical method for the quantification of these insecticide residues was developed using liquid chromatography coupled with tandem mass spectrometry. The dissipation of tested compounds was described according to a first-order kinetic equation with $R^2$ between 0.7130 and 0.9861. The results showed that the time after which 50% of the substance degraded was within the range 1.7–33.0 d. Residues of imidacloprid and its metabolites in tomato and cucumber samples varied from 0.001 to 0.521 mg/kg, respectively. Theoretical maximum residue contribution for imidacloprid was calculated and found to be well below maximum permissible intake (0.001 mg/kg) on tested fruiting vegetables on day 0 (1 h after spraying) for a single dose. No significant differences were found between the hazard quotient (below 3% of the acceptable daily intake after 9 d) values calculated for the residue of imidacloprid and for the sum of imidacloprid and its metabolites. The final residues of imidacloprid were much lower than the maximum residue limits. Our results indicate that harvested fruiting vegetable samples are safe for human consumption at the recommended dose (0.75 L/ha).

*Keywords:* Dissipation; Imidacloprid; Tomatoes; Cucumbers; Risk assessment

## 1. Introduction

Fruiting vegetables encompass a broad variety of plants, including *Solanacea* (peppers, tomatoes), cucurbits with edible peel (cucumbers, courgettes) and cucurbits with inedible peel (melons, pumpkins) [1]. In Poland, cultivation of tomatoes (*Lycopersicon esculentum* Mill.) and cucumbers (*Cucumis sativus*) is very common. These vegetables are an important component of the human diet and they are mostly consumed raw by children and adults [2]. They are rich sources of minerals, vitamins (A, E), essential amino acids, sugars and dietary fibers. According to WHO/FAO report recommends a minimum of 400 g of fruit and vegetables per day (excluding potatoes and other starchy tubers) for the prevention of chronic diseases such as heart disease, cancer, diabetes and obesity [3].

Despite many benefits of eating vegetables, their cultivation is susceptible to insect and disease attacks. Additionally, pests have a significant influence on the quality and quantity of their production. Therefore, a large number of insecticides are applied to control them in vegetables [4]. Imidacloprid (1-(6-chloro-3-pyridylmethyl)-*N*-nitr oimidazolidin-2-ylideneamine) is a systemic, chloronicotinyl insecticide with excellent systemic properties [5], used to

* Corresponding author.

*Presented at the 13th Conference on Microcontaminants in Human Environment, 4–6 December 2017, Czestochowa, Poland.*

control a broad range of sucking, soil and chewing insects that attack vegetable crops [6]. This compound acts on the central nervous system, interfering with synaptic transmission of nerve impulses in the central nervous system of insect pests such as aphids, white flies, thrips, scales, plant bugs, flies, etc. [7].

When applied to crops, systemic pesticides like imidacloprid reach the inner parts of the plant and undergo conversion into a variety of metabolites, many of which are toxic and show insecticidal activity. In the case of imidacloprid, degradation produces several kinds of metabolites, and some of these biologically active metabolites are retained to a considerable extent by plants and exhibit high toxicity (Fig. 1). Imidacloprid converts into imidacloprid-guanidine, imidacloprid-olefin and imidacloprid-urea. According to Codex regulations, the residue definition for risk assessment was proposed as the "sum of imidacloprid, imidacloprid-olefin, imidacloprid-guanidine and imidacloprid-urea, expressed as imidacloprid" [8]. The maximum residue limits (MRLs) of total imidacloprid in an agricultural commodity are regulated by the EU (0.05–5.0 mg/kg) [9].

Besides their positive effects on vegetable cultivation, chloronicotinyl compounds also pose various health risks to the consumer. Residue of such pesticides and their metabolites in raw foods could affect ultimate consumers, especially when freshly consumed [10]. To prevent the presence of pesticide residues above the MRL, the time between pesticide application and harvest must be determined, since the pesticide decay time depends on crop type, the pesticide applied and environmental conditions. So it is important to study the degradation behavior and dietary intake risk of imidacloprid, including its metabolites, in cucumber and tomato vegetables grown in greenhouse conditions.

Several studies have been reported for the residues and dissipation kinetic behavior of imidacloprid in different crops, including: lettuce [5], tea [11], white psyllium [12], zucchini [6], green beans and chili peppers [13], cotton [14], rice [15], cucumber [4,16,17] and tomato [18, 19], as well as soil [20].

There are few publications describing dissipation of this chloronicotinyl insecticide together with its metabolites. The dissipation of imidacloprid and its metabolites has been studied in cardamom [21], sugarcane leaves [22] and soil [23]. However, to our best knowledge, there is no published data reporting dissipation of this insecticide and its metabolites in fruiting vegetables from greenhouse cultivation.

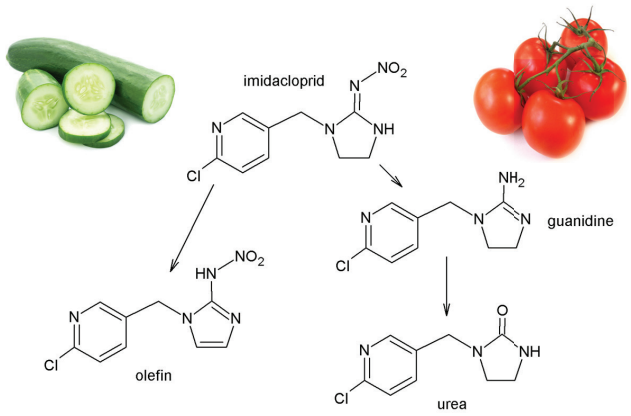


Fig. 1. Metabolic pathway of imidacloprid.

In this study, a rapid, robust and sensitive QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method coupled with LC-MS/MS was developed for simultaneous analysis of imidacloprid and its metabolites in fruiting vegetables. Moreover, an experimental trial was conducted under greenhouse conditions to evaluate the dissipation of imidacloprid and its metabolites in tomato and cucumber samples for the first time. Finally, the dietary intake risk was evaluated through dietary exposure assessment based on residue, food consumption and toxicology data.

## 2. Experimental

### 2.1. Materials and reagents

The pesticide standards were purchased from Dr. Ehrenstorfer Laboratory (Augsburg, Germany). The purities of all standard pesticides (imidacloprid, imidacloprid-guanidine, imidacloprid-olefin and imidacloprid-urea) were >99.0% (Fig. 1). Acetonitrile was obtained from J.T. Baker (Deventer, The Netherlands), methanol for LC-MS was purchased from POCH (Gliwice, Poland). LC-MS grade formic acid (98% purity) and ammonium formate (>99%) were obtained from Fluka (Seelze-Hannover, Germany). LC-grade water (18 MΩ cm) was obtained from a MilliQ water purification system (Millipore Ltd., Bedford, MA, USA). QuEChERS Extract Pouches containing 4 g magnesium sulfate, 1 g sodium chloride and sodium citrate dihydrate, and 0.5 g sodium hydrogen citrate sesquihydrate were purchased from Agilent Technologies (Santa Clara, USA).

### 2.2. Preparation of pesticide standard solutions

Stock standard solutions of pesticides (around 1,000 mg/mL) were prepared separately by dissolving an accurately weighed amount of each reference standard in acetone for imidacloprid, imidacloprid-olefin, imidacloprid-urea and in methanol for imidacloprid-guanidine. The combined working standard solutions were generated by serial dilution of the stock solutions with methanol. The working standard solutions were used for the preparation of matrix-matched standards within the concentration range of 0.001–0.50 mg/mL and for spiking of samples in validation studies. All stock and working standard solutions were stored in a freezer at about –20°C until analysis.

### 2.3. Sample preparation

Ten grams of homogenized cucumber or tomato samples were transferred into a 50 mL centrifuge tube. The sample was extracted with 10 mL of acetonitrile, shaken vigorously for 1 min and frozen for 15 min. A mixture of salts: 4 g magnesium sulfate, 1 g sodium chloride, 1 g sodium citrate dihydrate, 0.5 g sodium hydrogen citrate sesquihydrate was added into centrifuge tubes. The tubes were immediately shaken for 1 min and then centrifuged for 5 min at 4,500 rpm. The extract (1 mL) was filtered through a 0.2 μm hydrophilic PTFE filter, then transferred into the autosampler vial and analyzed via LC-MS/MS (Fig. 2).

### 2.4. Chromatographic LC-MS/MS conditions

An Eksigent Ultra LC-100 (Eksigent Technologies, Dublin, CA, USA) liquid chromatography system was operated at a flow rate of 0.5 mL/min without split using a KINETEX XB-C18 2.6 µm, 2.1 × 50 mm (Phenomenex, Torrance, USA) analytical column, maintained at 40°C during the experiments. The volume injected into the LC-MS/MS system was 10 µL. The binary mobile phase consisted of water with 0.5% formic acid and 2 mM ammonium formate (phase A) and methanol with 0.5% formic acid and 2 mM ammonium formate (phase B). The gradient elution started at 99% A and 1% B, was held for 1.0 min, rose linearly to 10% A and 90% B over 5 min, and was held for 5 min after ramping. Then, the mobile phase composition was returned to the initial condition over 2 min, and this was held for 2 min for re-equilibration.

An MS/MS 6500 QTRAP (AB Sciex Instruments, Foster City, CA) system equipped with an electrospray ionization source was used for mass spectrometric analysis. The capillary voltage was maintained at 5,000 V for positive ion mode, and the temperature of the turbo heaters was set to 400°C. Nitrogen was used as the nebulizer gas (GS1), auxiliary gas (GS2) and curtain gas (CUR) at pressures of 60, 50 and 30 psi, respectively. Nitrogen was used as the collision gas. Optimization of the compounds was performed by injecting individual standard solutions directly into the source (flow injection analysis methods). All pesticides were detected in multiple reaction monitoring (MRM) mode. The precursor ion and two products, one for quantification and one for qualification, were determined for each pesticide (Table 1).

### 2.5. Experimental field trials

The field experiment was conducted from July to September 2017 in a greenhouse located in the Podlasie region of Poland (53.139'N, 23.159'E). The field was divided into 10 $m^2$ sized blocks, which were separated by a 1-m wide buffer zone between plots to minimize possible cross-contamination between treatments. The tomato and cucumber samples were grown with a plant spacing of 0.5 m × 0.5 m. The greenhouse plants were cultivated under controlled conditions with a drip irrigation system.

These fruiting vegetables were sprayed with a dose 0.75 L/h of the plant protection product according to Directive WE 1107/2009 [24], when the plants were at fruiting stage (BBCH code: 50–80, ripening of fruit and seed). One plot of each cultivated plant was not treated with pesticides to serve as an untreated control. The temperature in the greenhouse ranged from 15°C to 25°C, and humidity ranged from 80% to 100% from the day of spraying until harvest.

To investigate dissipation and the terminal residue of imidacloprid, whole tomato and cucumber vegetables (about 1 kg) were collected randomly from the control and treated plots of each treatment at 0 (1 h), 1, 2, 3, 5, 7, 8, 9, 11, 13, 15, 21 and 28 d after application of the imidacloprid. All collected samples were stored in a freezer at –20°C until analysis.

### 2.6. Dissipation kinetics

The dissipation kinetics of imidacloprid under greenhouse conditions were determined using the first-order kinetics equation $C_t = C_0 e^{-kt}$, where $C_t$ (mg/kg) is the concentration of pesticide at time $t$, $C_0$ (mg/kg) is the initial concentration and


Fig. 2. QuEChERS preparation procedure for LC-MS/MS analysis of fruiting vegetables.

Table 1
Monitoring ions and reference collision voltage

| Active substance | Quantification | | | Confirmation | | | DP (V) | EP (V) |
|---|---|---|---|---|---|---|---|---|
| | MRM transition *m/z* | CE (V) | CXP (V) | MRM transition *m/z* | CE (V) | CXP (V) | | |
| Imidacloprid | 256 > 209.1 | 21 | 12 | 256 > 175.1 | 27 | 10 | 80 | 10 |
| Imidacloprid-guanidine | 212 > 127 | 31 | 10 | 212 > 177 | 23 | 10 | 30 | 10 |
| Imidacloprid-olefin | 254 > 236 | 13 | 10 | 254 > 171 | 27 | 10 | 36 | 10 |
| Imidacloprid-urea | 212 > 128 | 27 | 10 | 212 > 99 | 25 | 10 | 30 | 10 |

CE, collision energy; CXP, cell exit potential; DP, declustering potential; EP, entrance potential.

$k$ is the rate constant in $d^{-1}$. The persistence of a pesticide in an environmental compartment can be characterized by the pesticide half-life ($t_{1/2}$). The half-life is the time required for a concentration of pesticide to be reduced to one half and was calculated using equation $t_{1/2} = \ln(2)/k$.

### 2.7. Dietary risk assessment

The chronic/long-term consumer health risk (hazard quotient, HQ) was calculated based on the estimated daily intake (EDI) and the acceptable daily intake (ADI). According to Directive 08/116 [25], the ADI value for imidacloprid has been observed to be 0.06 mg/kg body weight per day. The EDI of each pesticide residue was calculated by multiplying the mean concentration of pesticide residue (mg/kg), the food consumption rate (kg/d) and based on the average body weight of adults (A), infants (I) and toddlers (T). Meanwhile, the EDIs of pesticide residues were calculated as follows: EDI = ΣRL × F/BW × 100%, where RL is the residue level of the vegetable; F is the food consumption data (tomatoes – A: 0.283 kg/d, I: 0.060 kg/d and T: 0.0905 kg/d; cucumbers – A: 0.1077 kg/d, I: 0.013 kg/d and T: 0.0856 kg/d ); BW is the body weight (A = 70 kg, I = 5 kg and T = 12 kg).

The dietary risk was expressed as HQs calculated as follows: HQ = EDI/ADI, where the food involved should be considered as a risk to consumers if the HQ is >100; meanwhile, if the index is % <100, this would indicate that the food involved is considered acceptable [26,27].

## 3. Results and discussion

### 3.1. Method validation

Sample preparation was validated using untreated cucumber and tomato samples with respect to the following parameters: linearity, limit of detection (LOD), limit of quantification (LOQ), recovery and relative standard deviation.

Five-point calibration curves (0.001, 0.01, 0.05, 0.10 and 0.50 mg/kg) were constructed for quantitative analysis of imidacloprid and its metabolites (imidacloprid-guanidine, imidacloprid-olefin and imidacloprid-urea). The calibration curves showed good linearity and strong correlation between concentration and peak area within the studied range ($r^2 \geq 0.9995$). The LOD and LOQ were measured as the analyte concentration at signal to noise ratio of 3:1 and 10:1, respectively. The LOD was estimated at 0.0003 mg/kg and the LOQ at 0.001 mg/kg (Fig. 3).

Recovery experiments were conducted by fortification of blank cucumber and tomato samples at three different concentrations (0.001, 0.01 and 0.50 mg/kg) with three replicates per level. The recoveries ranged from 71% to 111% with RSDs below 11%.

All results were considered satisfactory as they readily met the acceptable criteria in the European SANTE guideline [28].

The linearity, LOD, LOQ, recovery and relative standard deviation values obtained from the validation study are shown in Table 2.

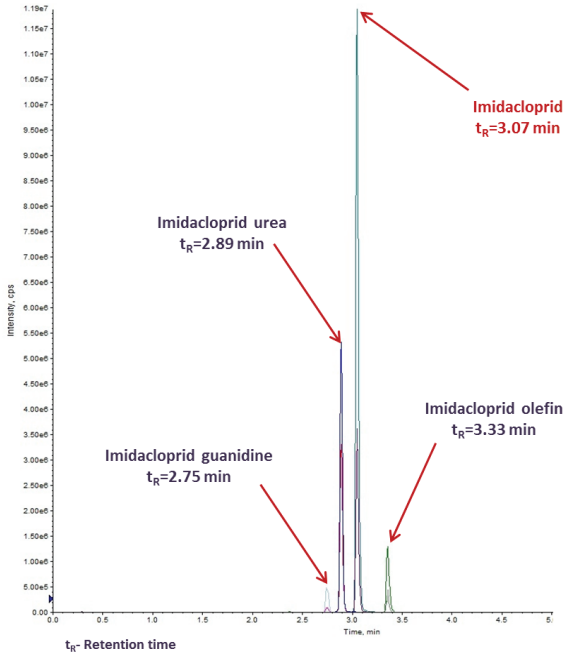


Fig. 3. Chromatogram of imidacloprid and its metabolites (imidacloprid guanidine, imidacloprid urea and imidacloprid olefin) at 0.05 mg/mL in tomato matrix.

Table 2
Validation parameters: linearity, recovery and relative standard deviation of imidacloprid and metabolites for cucumber and tomato samples

| Commodity | Analyte | Linear equation | $R^2$ | Recoveries % (RSD%) | | |
|---|---|---|---|---|---|---|
| | | | | 0.001 | 0.05 | 0.50 |
| | | | | mg/kg | | |
| Cucumber | Imidacloprid | $y = 8.6003e^8 + 5.6847e^5$ | 0.99962 | 108 (4) | 78 (6) | 81 (4) |
| | Imidacloprid-guanidine | $y = 2.2283e^7 + 20575.7$ | 0.99970 | 109 (8) | 80 (7) | 76 (6) |
| | Imidacloprid-olefin | $y = 7.3270e^7 + 5.2433e^4$ | 0.99978 | 102 (6) | 86 (9) | 71 (8) |
| | Imidacloprid-urea | $y = 7.7027e^8 + 8.8106e^5$ | 0.99966 | 105 (7) | 79 (10) | 74 (5) |
| Tomato | Imidacloprid | $y = 8.2412e^8 + 4.3986e^5$ | 0.99976 | 108 (6) | 82 (5) | 84 (8) |
| | Imidacloprid-guanidine | $y = 7.9333e^7 + 1803.0$ | 0.99959 | 104 (8) | 87 (8) | 89 (10) |
| | Imidacloprid-olefin | $y = 2.1922e^7 + 3.3464e^4$ | 0.99954 | 110 (5) | 86 (4) | 96 (7) |
| | Imidacloprid-urea | $y = 2.1458e^8 + 2.3539e^5$ | 0.99951 | 111 (11) | 96 (10) | 74 (8) |

### 3.2. Dissipation kinetics of imidacloprid and its metabolites in tomato and cucumber

The study of dissipation is an important part of full evaluation and is helpful for the proper and safe use of a pesticide. To better understand the possible hazardous impacts of pesticide residues, dissipation studies are necessary to examine the appropriateness of pesticide application strategies [29]. Changes of imidacloprid concentration in tomato and cucumber samples were studied and are presented in Fig. 4. Residues of imidacloprid and its metabolites were detected according to the aforementioned modified QuEChERS method. Imidacloprid concentration was determined in samples of tomato and cucumber that were collected at 0 d (1 h after spraying) as well as 1, 2, 3, 5, 7, 8, 9, 11, 13, 15, 21 and 28 d.

The initial concentration of imidacloprid (1 d) was 0.356 mg/kg in tomato, but was relatively high in cucumber and equal to 0.521 mg/kg, reaching its maximum concentration on the 1st day. The initial deposit of imidacloprid was higher in cucumber, which might be due to the different planting densities. Between 1 and 28 d after application, the imidacloprid residue decreased to the minimum values of 0.039 mg/kg for tomato and 0.001 mg/kg for cucumber, respectively.

The initial concentrations of imidacloprid olefin and imidacloprid guanidine were similar in tomato and cucumber (Fig. 3). Moreover, the residue concentrations of imidacloprid olefin after 2 d were much higher in cucumber than determined in tomato. The initial concentration of imidacloprid olefin in cucumber was 0.16 mg/kg, reaching a maximum of 0.70 mg/kg after 3 d. Moreover, a decrease of this metabolite was observed after the concentration reached the highest point, which could probably be due to the plant's growth [15]. As presented in Fig. 4, a similar trend was not observed for imidacloprid guanidine. In the case of tomato, 23 d after application, the terminal residue concentration was equal to the LOD (0.001 mg/kg), whereas in the case of cucumber, the terminal residue concentration was reached 28 d after treatment.

The final residues of imidacloprid metabolites in tomato samples were all greater than imidacloprid after 19 d, which may be caused by the degradation of imidacloprid to imidacloprid guanidine and olefin. The guanidine and olefin metabolites were detected on the 1st day and persisted up to the 21st day (Table 3). The imidacloprid urea metabolite was not detectable in the tomato and cucumber samples during the period of study. This might indicate that degradation of imidacloprid to imidacloprid urea is longer [23]. According to the metabolic pathway presented in Fig. 1, imidacloprid-guanidine degrades to imidacloprid urea, which was detected in samples even after 21 d.

Imidacloprid demonstrated a varied persistence in tomato and cucumber (Table 4). On the 2nd day after application of the insecticide, the percentage of imidacloprid degradation in tomato was 20%, and 51% in cucumber. In the case of tomato, the biggest increase of the concentration value was observed between the 2nd and 5th day, where the percentage of imidacloprid degradation differed by 35%. Results indicated that imidacloprid residues in cucumber decreased the fastest. On the 5th day, the percentage of degradation was equal to 89% and 56% for cucumber and tomato, respectively.

Fig. 5 shows the kinetic curves of first-order reaction for imidacloprid and its metabolites in tomato and cucumber samples. The kinetic curves in tomato were described by the following equations: $y = 0.03478e^{-0.13x}$ (imidacloprid), $y = 0.00113e^{-0.097x}$ (imidacloprid-guanidine) and $y = 0.00214e^{-0.021x}$ (imidacloprid-olefin). In turn, the kinetic curves in cucumber samples were described by the following equations: $y = 0.04134e^{-0.405x}$ (imidacloprid), $y = 0.0196e^{-0.05x}$ (imidacloprid-guanidine) and $y = 0.00592e^{-0.187x}$ (imidacloprid-olefin).

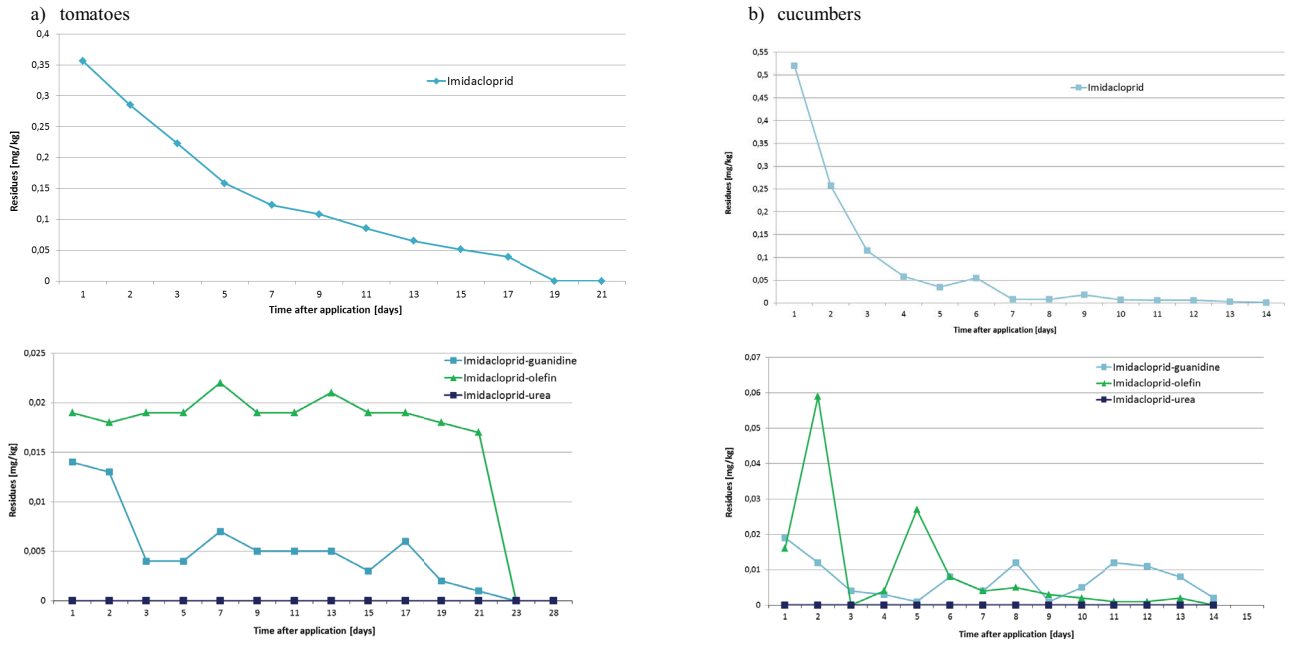


Fig. 4. Concentration changes of imidacloprid and its metabolites in tomato and cucumber samples.

Table 3
Final residues of imidacloprid and metabolites (mg/kg) determined in tomato and cucumber samples

| Time (d) | Imidacloprid | Imidacloprid-guanidine | Imidacloprid-olefin | Imidacloprid-urea | Total |
|---|---|---|---|---|---|
| Tomato | | | | | |
| 1 | 0.356 | 0.014 | 0.019 | ND | 0.389 |
| 2 | 0.285 | 0.013 | 0.018 | ND | 0.316 |
| 3 | 0.223 | 0.004 | 0.019 | ND | 0.246 |
| 5 | 0.158 | 0.004 | 0.019 | ND | 0.181 |
| 7 | 0.123 | 0.007 | 0.022 | ND | 0.151 |
| 9 | 0.108 | 0.005 | 0.019 | ND | 0.132 |
| 11 | 0.085 | 0.005 | 0.019 | ND | 0.109 |
| 13 | 0.065 | 0.005 | 0.021 | ND | 0.091 |
| 15 | 0.051 | 0.003 | 0.019 | ND | 0.073 |
| 17 | 0.039 | 0.006 | 0.019 | ND | 0.064 |
| 19 | ND | 0.002 | 0.018 | ND | 0.020 |
| 21 | ND | 0.001 | 0.017 | ND | 0.018 |
| 23 | ND | ND | ND | ND | ND |
| 28 | ND | ND | ND | ND | ND |
| Cucumber | | | | | |
| 1 | 0.521 | 0.019 | 0.016 | ND | 0.556 |
| 2 | 0.257 | 0.012 | 0.059 | ND | 0.328 |
| 3 | 0.115 | 0.004 | 0.070 | ND | 0.189 |
| 5 | 0.058 | 0.013 | 0.044 | ND | 0.115 |
| 7 | 0.035 | 0.011 | 0.027 | ND | 0.073 |
| 9 | 0.055 | 0.018 | 0.008 | ND | 0.081 |
| 11 | 0.008 | 0.014 | 0.004 | ND | 0.026 |
| 13 | 0.008 | 0.012 | 0.005 | ND | 0.025 |
| 15 | 0.018 | 0.011 | 0.003 | ND | 0.032 |
| 17 | 0.007 | 0.015 | 0.002 | ND | 0.024 |
| 19 | 0.006 | 0.012 | 0.001 | ND | 0.019 |
| 21 | 0.006 | 0.011 | 0.001 | ND | 0.018 |
| 23 | 0.003 | 0.008 | 0.002 | ND | 0.013 |
| 28 | 0.001 | 0.002 | ND | ND | 0.003 |

ND, not detected.

Dissipation is a complicated process influenced by different physicochemical and biological transformations, as a result of which the content of the active substance decreases over time [30,31]. The half-life times of imidacloprid, imidacloprid-guanidine and imidacloprid-olefin were calculated (Table 5) to be 5.3, 7.2 and 33.0 in tomato, and 1.7, 13.9 and 3.7 in cucumber, respectively. These field half-lives of imidacloprid are greater than those reported in other studies (which range from 2.7 to 3.4 d, e.g., Hassanzadeh et al. [16] under controlled conditions). According to Nasr et al. [4], the calculated half-life value of imidacloprid in cucumber was 2.2 d, which was similar to our results.

According to available data, the dissipation behavior of imidacloprid residues was also studied in other matrices. For example, dissipation in okra has been studied by Karthik et al. [32] and Sahoo et al. [33]. The half-life values obtained by the former were 1.04 and 1.13 d at 24.5 and 49 g active ingredient/ha (a.i./ha) [31], respectively. Meanwhile, Sahoo et al. [33] observed a half-life period of 0.85 and 0.96 d at the rate of 60 and 120 g a.i./ha, respectively. The half-life periods for imidacloprid in brinjal were found to be 2.31 and 2.18 d at single and double the application rate, respectively, and residues took 10 d for both the dosages [34]. $t_{1/2}$ of this active substance in tea amounted to 1.20–1.39 d at recommended and double the recommended doses [11], 1.03–1.23 d [35] and 0.96 to 1.16 d when applied at 30 and 60 g a.i./ha, respectively [36].

The dissipation kinetics of imidacloprid has been studied in a few vegetable matrices and soil but only a few publications concerning dissipations of this active substance and its metabolites are available. Therefore, to our knowledge, this is the first study to investigate the dissipation of imidacloprid and its metabolites in fruiting vegetables.

In addition, we can find few studies about monitoring and optimization methods for determination of imidacloprid and its metabolites in different matrices such as cucumber and soil [17], lettuce [37], tomato [19] and greenhouse air [38].

Table 4
Degradation (in %) of imidacloprid in tomato and cucumber cultivated under greenhouse conditions

| Time (d) | Tomato | | Cucumber | |
|---|---|---|---|---|
| | mg /kg | Loss % | mg /kg | Loss % |
| 1 | 0.356 | – | 0.521 | – |
| 2 | 0.285 | 19.94 | 0.257 | 50.70 |
| 3 | 0.223 | 37.4 | 0.115 | 77.93 |
| 5 | 0.158 | 55.62 | 0.058 | 88.87 |
| 7 | 0.123 | 65.45 | 0.055 | 89.44 |
| 9 | 0.108 | 69.67 | 0.055 | 89.44 |
| 11 | 0.085 | 76.12 | 0.008 | 98.46 |
| 13 | 0.065 | 81.74 | 0.008 | 98.46 |
| 15 | 0.051 | 85.65 | 0.008 | 98.46 |
| 17 | 0.039 | 89.04 | 0.007 | 98.66 |
| 19 | ND | 100 | 0.006 | 98.85 |
| 21 | ND | 100 | 0.006 | 98.85 |
| 23 | ND | ND | 0.003 | 99.42 |
| 28 | ND | ND | 0.001 | 99.81 |

Oliva et al. [6] studied imidacloprid residues in zucchini, and the level 3 d after spraying was 0.015 mg/kg when applied at the recommended dose (650 L/ha). Hanafi et al. [13] observed that the residues in green beans increased after pestigation to reach 0.148 mg/kg on the 15th day and then declined to 0.102 mg/kg 21 d after pestigation. Meanwhile,

Table 5
Regression equation, correlation coefficients and half-life time of imidacloprid and metabolites in tomato and cucumber plants

| Compound | Regression equation | $R^2$ | $t_{1/2}$ |
|---|---|---|---|
| Tomato | | | |
| Imidacloprid | $0.03478e^{-0.13x}$ | 0.9861 | 5.3 |
| Imidacloprid-guanidine | $0.00113e^{-0.097x}$ | 0.7539 | 7.2 |
| Imidacloprid-olefin | $0.00214e^{-0.021x}$ | 0.7130 | 33.0 |
| Cucumber | | | |
| Imidacloprid | $0.04134e^{-0.405x}$ | 0.9101 | 1.7 |
| Imidacloprid-guanidine | $0.01960e^{-0.05x}$ | 0.6004 | 13.9 |
| Imidacloprid-olefin | $0.00592e^{-0.187x}$ | 0.8431 | 3.7 |

$R^2$, correlation coefficients; $t_{1/2}$, half-life time.

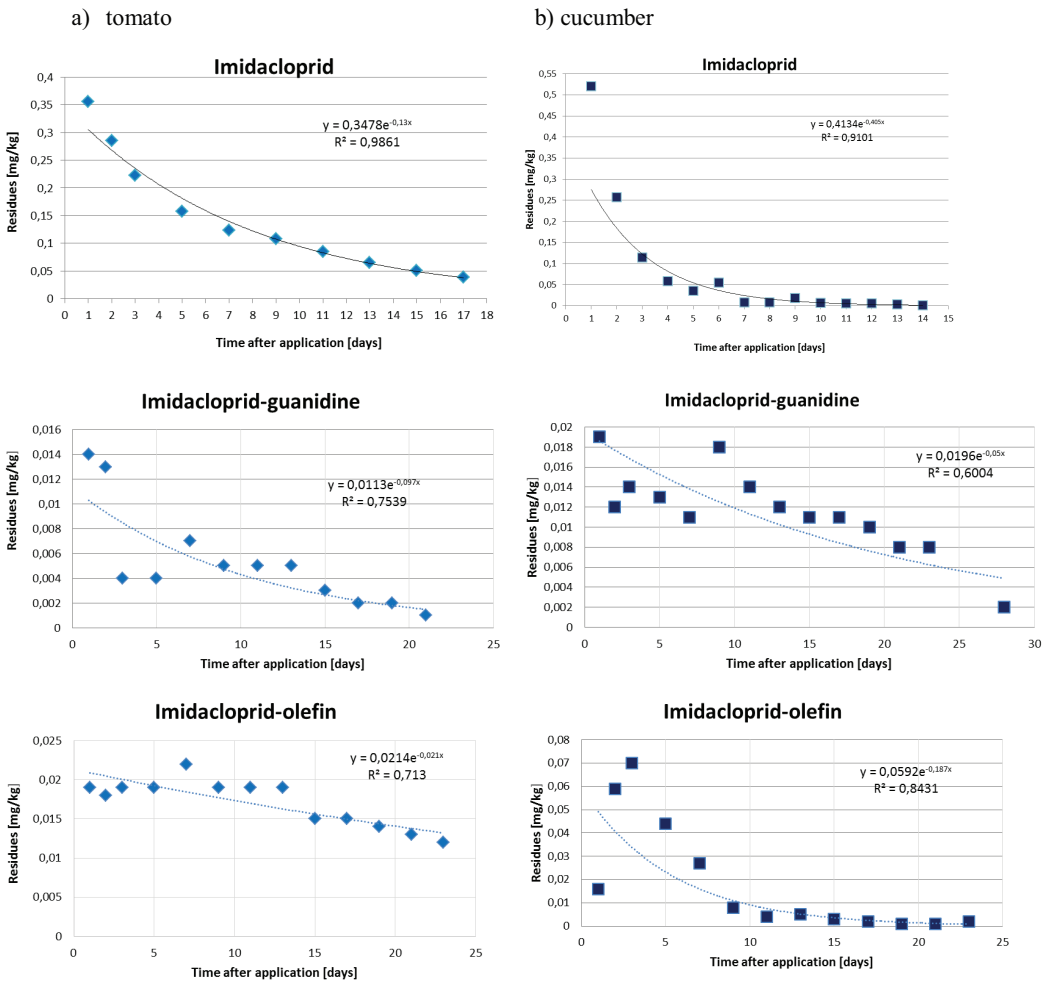


Fig. 5. Dissipation kinetics designated for imidacloprid and its metabolites in tomato and cucumber samples.

in the case of chili pepper, residues were at trace level (<0.001 mg/kg) until 15 d after treatment and then reached 0.010 mg/kg at 21 d.

Dissipation of imidacloprid and its metabolites was studied only in cardamom [21], sugarcane leaves [22] and soil [23]. Pratheeshkumar et al. [21] reported that the residues in cardamom dissipated below the quantification level of 0.01 mg/kg after 28 d, and the half-lives were 4.02 and 3.63 d in fresh and cured cardamom, respectively, at lower dose, and 3.61 d for both at higher dose. Among the metabolites of imidacloprid, urea had maximum residues in fresh and cured cardamom, followed by 5-hydroxy and guanidine. Other metabolites, such as 6-chloronicotinic acid, olefin and nitrosimine were not detected in either fresh or cured cardamom. Also, Sharma and Singh [22] studied the persistence of imidacloprid and its metabolites in sugarcane leaves and found that residues were mostly constituted by the parent compound and persisted up to the 7th day after application.

### 3.3. Risk assessment of imidacloprid and its metabolites

The use of pesticides on food crops leads to unwanted residues, which may constitute barriers to exporters and domestic producers when they exceed MRLs. European MRLs for imidacloprid are 0.5 mg/kg for tomato and 1.0 mg/kg for cucumber [9]. Imidacloprid residues in tested fruiting vegetables at the recommended dosage dissipated to below their respective MRLs. Hassanzadeh et al. [16] reported that residues of this active substance dissipated below the MRL of 1 mg/kg in 3 d, and $t_{1/2}$ in cucumber was observed to be 3.40 and 2.70 d at the single and double dosages, respectively. The usage of pesticides may raise serious health concerns when fresh tomatoes are consumed, especially for children, as their central nervous system is not fully developed and particularly susceptible to the hazards caused by pesticide residues [25].

As was presented in Table 6, the HQ% was within the range of 1.34%–7.78% of the ADI on the 1st day after application for all tested subpopulations in both cultivations. On the 9th day after application, the HQ% was below 3%. No significant differences were found between the HQ values calculated for the residue of imidacloprid and for the sum of imidacloprid and its metabolites. The risk to consumers posed by the use of this insecticide was assessed by comparing dietary exposure with maximum permissible intake (MPI). The ADI values of imidacloprid are 0.06 mg/kg body weight. The MPI was calculated by multiplying the ADI by the body weight of an average adult, infant and toddler (70, 5 and 12 kg). The calculated MPI values of imidacloprid were 4,200 mg/adult per d, 300 mg/infant per d and 720 mg/toddler per d. The values of dietary exposure in terms of Theoretical Maximum Residues Contribution (TMRC) were calculated by considering the observed maximum residue levels (for cucumbers 0.621 mg/kg, for tomatoes 0.596 mg/kg at 0-d after application) and average per capita daily consumption 283, 60 and 90.5 g of tomatoes and 107.7, 13 and 85.6 g of cucumbers for adults, infants and toddlers, respectively. TMRC

Table 6
Assessment of chronic dietary exposure (HQ%) to imidacloprid residues and to the sum of imidacloprid and its metabolites in analyzed samples of fruiting vegetables

| Time after application (d) | Concentration of imidacloprid (mg/kg) | HQ% | | | Total concentration of imidacloprid and its metabolites (mg/kg) | HQ% | | |
|---|---|---|---|---|---|---|---|---|
| | | Adults | Infants | Toddlers | | Adults | Infants | Toddlers |
| Tomatoes | | | | | | | | |
| 1 | 0.356 | 2.40 | 7.12 | 4.47 | 0.389 | 2.62 | 7.78 | 4.89 |
| 2 | 0.285 | 1.92 | 5.70 | 3.58 | 0.316 | 2.13 | 6.32 | 3.97 |
| 3 | 0.223 | 1.50 | 4.46 | 2.80 | 0.246 | 1.66 | 4.92 | 3.09 |
| 5 | 0.158 | 1.06 | 3.16 | 1.99 | 0.181 | 1.22 | 3.62 | 2.28 |
| 7 | 0.123 | 0.83 | 2.46 | 1.55 | 0.151 | 1.02 | 3.02 | 1.90 |
| 9 | 0.108 | 0.73 | 2.16 | 1.36 | 0.132 | 0.89 | 2.64 | 1.66 |
| 11 | 0.085 | 0.57 | 1.70 | 1.07 | 0.109 | 0.73 | 2.18 | 1.37 |
| 13 | 0.065 | 0.44 | 1.30 | 0.82 | 0.091 | 0.61 | 1.82 | 1.14 |
| 15 | 0.051 | 0.34 | 1.02 | 0.64 | 0.073 | 0.49 | 1.46 | 0.92 |
| Cucumbers | | | | | | | | |
| 1 | 0.521 | 1.34 | 2.26 | 6.19 | 0.556 | 1.43 | 2.41 | 6.61 |
| 2 | 0.257 | 0.66 | 1.11 | 3.06 | 0.328 | 0.84 | 1.42 | 3.90 |
| 3 | 0.115 | 0.29 | 0.50 | 1.37 | 0.189 | 0.48 | 0.82 | 2.25 |
| 5 | 0.058 | 0.15 | 0.25 | 0.69 | 0.115 | 0.29 | 0.50 | 1.37 |
| 7 | 0.055 | 0.14 | 0.24 | 0.65 | 0.093 | 0.24 | 0.40 | 1.11 |
| 9 | 0.055 | 0.14 | 0.24 | 0.65 | 0.081 | 0.21 | 0.35 | 0.96 |
| 11 | 0.008 | 0.02 | 0.03 | 0.10 | 0.026 | 0.07 | 0.11 | 0.31 |
| 13 | 0.008 | 0.02 | 0.03 | 0.10 | 0.025 | 0.06 | 0.11 | 0.30 |
| 15 | 0.007 | 0.02 | 0.03 | 0.08 | 0.021 | 0.05 | 0.09 | 0.25 |

values of imidacloprid in tomatoes at 0-d were found to be 16.9 mg/adult/d, 3.60 mg/infant/d and 5.40 mg/toddler/d, which were also below MPI. Whereas TMRC values of imidacloprid in cucumbers at 0-d were found to be 6.7 μg/adult/d, 0.80 μg/infant per d and 5.30 μg/toddler/d. The present results showed that the TMRC values were below MPI values for both cultivations when a single dose of insecticide was applied, and the consumer health risks are minimal at the recommended dose of imidacloprid on tomato and cucumber cultivations. According to Mukherjee and Gopal [7], the TMRC value calculated from residue of imidacloprid in eggplant, cabbage and mustard are also lower than MPI. Therefore, the application of imidacloprid could be considered safe from the perspective of crop protection and environmental contamination [39].

Furthermore, the long-term exposure of consumers to pesticide residues through the consumption of raw vegetables is not associated with health risk. Moreover, the estimated risk assessment via long-term exposure is based on toxicological evaluation of individual compounds and not based on an evaluation of cumulative exposure to multiple pesticide residues in crops.

## 4. Conclusion

This study was designed to investigate dissipation of imidacloprid and its metabolites in fruiting vegetables grown under greenhouse conditions. The results showed that imidacloprid half-lives in tomato and cucumber where approximately 5.3 and 1.7 d, respectively. The terminal residues of imidacloprid (expressed as the sum of imidacloprid, imidacloprid-guanidine, imidacloprid-olefin and imidacloprid-urea) were much lower than the MRLs. Therefore, a single dosage of 0.75 L/ha is recommended. The HQs of imidacloprid for both cultivations were lower than 1% of ADI, and the TMRC value was significantly lower than the MPI for three subpopulations and both cultivations. Imidacloprid can be considered safe for application to vegetables belonging to *Solanacea* and cucurbits with edible peel at the recommended dosage, for the purpose of controlling a broad spectrum of insects.

## References

[1] PPDB Pesticide Properties DataBase, The FOOTPRINT Pesticide Properties Database, University of Hertfordshire, 2017. Available at: http://sitem.herts.ac.uk/aeru/footprint/es/index2.htm (Accessed 23 October 2017).
[2] J.L. Slavin, B. Lloyd, Health benefits of fruits and vegetables, Adv. Nutr., 3 (2012) 506–516.
[3] FAO/WHO, Human Vitamin and Mineral Requirements, Report of a Joint FAO/WHO Expert Consultation Bangkok, Thailand, Food and Nutrition Division FAO Rome, 2001. Available at: https://searchworks.stanford.edu/view/7696463 (Accessed 23 October 2017).
[4] H.M. Nasr, M.A. Abbassy, M.A. Marzouk, A.S. Mansy, Determination of imidacloprid and tetraconazol residues in cucumber fruits, J. Pollut. Eff. Control, 2 (2014) 1.
[5] E. Sevigné Itoiz, P. Fantke, R. Juraske, A. Kounina, A.A. Vallejo, Deposition and residues of azoxystrobin and imidacloprid on greenhouse lettuce with implications for human consumption, Chemosphere, 89 (2012) 1034–1041.
[6] J. Oliva, S. Cermeño, M.A. Cámara, G. Martínez, A. Barba, Disappearance of six pesticides in fresh and processed zucchini, bioavailability and health risk assessment, Food Chem., 229 (2017) 172–177.
[7] I. Mukherjee, M. Gopal, Environmental behaviour and translocation of imidacloprid in eggplant, cabbage and mustard, Pest Manage. Sci., 56 (2000) 932–936.
[8] Codex Alimentarius (FAO/WHO), Codex International Food Standard. Available at: http://www.fao.org/fao-who-codexalimentarius/codex-home/en/ (Accessed 24 October 2017).
[9] EC 2005, Commission Regulation No 396/2005 of the European Parliament and of the Council of 23 February 2005 on Maximum Residue Levels of Pesticides in or on Food and Feed of Plant and Animal Origin and Amending Council Directive 91/414/EEC, Official Journal of the European Union, Vol. 70, p. 1.
[10] F. Ding, W. Peng, Biological assessment of neonicotinoids imidacloprid and its major metabolites for potentially human health using globular proteins as a model, J. Photochem. Photobiol., B, 147 (2015) 24–36.
[11] M. Paramasivam, M. Deepa, C. Selvi, S. Chandrasekaran, Dissipation kinetics of beta-cyfluthrin and imidacloprid in tea and their transfer from processed tea to infusion, Ecotoxicol. Environ. Saf., 144 (2017) 531–536.
[12] T.S. Varghese, V. Tripathy, J. Kumar, Dissipation of imidacloprid on white psyllium (*Plantago ovata*) under semiarid conditions, Proc. Natl. Acad. Sci., India, (2016) 1–5. doi: 10.1007/s40011-016-0786-z
[13] A. Hanafi, M. Dasenaki, A. Bletsou, N.S. Thomaidis, Dissipation rate study and pre-harvest intervals calculation of imidacloprid and oxamyl in exported Egyptian green beans and chili peppers after pestigation treatment, Food Chem., 240 (2018) 1047–1054.
[14] E.S. El-Hamady, R. Kubiak, A.S. Derbalah, Fate of imidacloprid in soil and plant after application to cotton seeds, Chemosphere, 71 (2008) 2173–2179.
[15] J. Ge, K. Cui, H. Yan, Y. Li, Y. Chai, X. Liu, J. Cheng, X. Yu, Uptake and translocation of imidacloprid, thiamethoxam and difenoconazole in rice plants, Environ. Pollut., 226 (2017) 479–485.
[16] N. Hassanzadeh, S.A. Esmaili, N. Bahramifar, Dissipation of imidacloprid in greenhouse cucumbers at single and double dosages spraying, J. Agric. Sci. Technol., 14 (2012) 557–564.
[17] M.F. Abdel-Ghany, L.A. Hussein, N.F. El Azab, A.H. El-Khatib, M.W. Linscheid, Simultaneous determination of eight neonicotinoid insecticide residues and two primary metabolites in cucumbers and soil by liquid chromatography–tandem mass spectrometry coupled with QuEChERS, J. Chromatogr. B, 1031 (2016) 15–28.
[18] R. Juraske, F. Castells, A. Vijay, P. Munoz, A. Antón, Uptake and persistence of pesticides in plants: measurements and model estimates for imidacloprid after foliar and soil application, J. Hazard. Mater., 165 (2009) 683–689.
[19] C.R. Bojacá, L.A. Arias, D.A. Ahumada, H.A. Casilimas, E. Schrevens, Evaluation of pesticide residues in open field and greenhouse tomatoes from Colombia, Food Control, 30 (2013) 400–403.
[20] B. Mahapatra, T. Adak, N.K.B. Patil, G.G.P. Pandi, G.B. Gowda, N.N. Jambhulkar, M.K. Yadav, P. Panneerselvam, U. Kumar, S. Munda, M. Jena, Imidacloprid application changes microbial dynamics and enzymes in rice soil, Ecotoxicol. Environ. Saf., 144 (2017) 123–130.
[21] N. Pratheeshkumar, M. Chandran, S. Naseema Beevi, T. Biju Mathew, T. George, A. Paul, G. Xavier, K.P. Ravi, S.V. Kumar, R. Rajith, Dissipation kinetics and effect of processing on imidacloprid and its metabolites in cardamom (*Elettaria cardamomum* Maton), Environ. Monit. Assess., 188 (2016) 53.
[22] S. Sharma, B. Singh, Persistence of imidacloprid and its major metabolites in sugarcane leaves and juice following its soil application, Int. J. Environ. Anal. Chem., 94 (2014) 319–331.
[23] M.A. Sarkar, S. Roy, R.K. Kole, A. Chowdhury, Persistence and metabolism of imidacloprid in different soils of West Bengal, Pest Manage. Sci., 57 (2001) 598–602.
[24] Regulation (EC) No 1107/2009 REGULATION (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 Concerning the Placing of Plant Protection Products on the Market and Repealing Council Directives 79/117/EEC and

91/414/EEC. Available at: http://eur-lex.europa.eu/legalcontent/EN/TXT/PDF/?uri=CELEX:32009 R1107&from=EN (Accessed 1 July 2017).
[25] EC 2008, Commission Regulation No 149/2008 of 29 January 2008 amending Regulation (EC) No 396/2005 of the European Parliament and of the Council by Establishing Annexes II, III and IV Setting Maximum Residue Levels for Products Covered by Annex i thereto.
[26] B. Łozowicka, E. Rutkowska, M. Jankowska, I. Hrynko, P. Kaczyński, Toxicological evaluation of multi-class pesticide residues in vegetables and associated human health risk study for adults and children, Hum. Ecol. Risk Assess., 22 (2016) 1480–1505.
[27] B. Łozowicka, P. Mojsak, M. Jankowska, P. Kaczyński, I. Hrynko, E. Rutkowska, J. Szabunko, A. Borusiewicz, Toxicological studies for adults and children of insecticide residues with common mode of action (MoA) in pome, stone, berries and other small fruit, Sci. Total Environ., 566–567 (2016) 144–156.
[28] SANTE 11945/2015, Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed.
[29] M.X. Lu, W.J. Wayne, W. Jia-Lei, J. Qiu, S. Yan, L. Xian-Jin, Y. Xiang-Yang, Persistence and dissipation of chlorpyrifos in *Brassica chinensis*, lettuce, celery, asparagus lettuce, eggplant, and pepper in a greenhouse, PLoS One, 9 (2014) 100556.
[30] P. Kaczynski, B. Lozowicka, I. Hrynko, E. Wolejko, Behaviour of mesotrione in maize and soil system and its influence on soil dehydrogenase activity, Sci. Total Environ., 571 (2016) 1079–1088.
[31] B. Łozowicka, P. Mojsak, P. Kaczyński, A. Borusiewicz, The fate of spirotetramat and dissipation metabolites in *Apiaceae* and *Brassicaceae* leaf-root and soil system under greenhouse conditions estimated by modified QuEChERS/LC-MS/MS, Sci. Total Environ., 603–604 (2017) 178–184.
[32] P. Karthik, S. Venugopal, K.D. Murthy, S. Lokesh, G. Karthik, U. Sharmila, M. Paramasivam, K. Senguttuvan, K. Gunasekaran, S. Kuttalam, Bioefficacy, phytotoxicity, safety to natural enemies and residue dynamics of imidacloprid 70 WG in okra (*Abelmoschus esculenta* (L) Moench) under open field conditions, Crop Prot., 71 (2015) 88–94.
[33] S.K. Sahoo, G.S. Chahil, K. Mandal, R.S. Battu, B. Singh, Estimation of β-cyfluthrin and imidacloprid in okra fruits and soil by chromatography techniques, J. Environ. Sci. Health, Part B, 47 (2012) 42–50.
[34] K. Mandal, G.S. Chahil, S.K. Sahoo, R.S. Battu, B. Singh, Dissipation kinetics of beta-cyfluthrin and imidacloprid in brinjal and soil under subtropical conditions of Punjab, India, Bull. Environ. Contam. Toxicol., 84 (2010) 225–229.
[35] M. Gupta, A. Sharma, A. Shanker, Dissipation of imidacloprid in Orthodox tea and its transfer from made tea to infusionm, Food Chem., 106 (2008) 158–164.
[36] N. Sanyal, D. Hazra, R. Pal, A.K. Somchaudhury, A. Chowdhury, Imidacloprid in processed tea and tea liquor, J. Zhejiang Univ. Sci. B, 7 (2006) 619–622.
[37] A.-Y. Ko, Md. M. Rahman, A.M. Abd El-Aty, J. Jang, J.-H. Park, S.-K. Cho, J.-H. Shim, Development of a simple extraction and oxidation procedure for the residue analysis of imidacloprid and its metabolites in lettuce using gas chromatography, Food Chem., 148 (2014) 402–409.
[38] A. Segura Carretero, C. Cruces-Blanco, S. Pérez Durán, A.F. Gutiérrez, Determination of imidacloprid and its metabolite 6-chloronicotinic acid in greenhouse air by application of micellar electrokinetic capillary chromatography with solid-phase extraction, J. Chromatogr. B, 1003 (2003) 189–195.
[39] S. Dharumarajan, A.K. Dikshit, Maximum residue limit and risk assessment of beta-cyfluthrin and imidacloprid in combination formulation on Tomato (*Lycopersicon esculentum*), J. Indian Chem. Soc., 90 (2013) 1189–1194.