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Residues levels of pesticides in walnuts of Iran and associated health risks

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ABSTRACT

The concentrations of 18 organophosphorus, carbamate, pyrethroid, and nicotinoid pesticides were measured, by use of gas chromatography coupled to mass spectrophotometry, in six cultivars of walnut from five geographical regions of Iran, including, Azarshahr, Damavand, Farouj, Shahmirzad, and Tuyserkan. Assessments of risks posed to humans were conducted by calculating the hazard indices (HIs), by use of the Monte Carlo Simulations. The 95th centile of HIs for humans based on exposure via ingestion of walnuts was estimated to be 1.68, which represented *di minimis* to moderate concern for human consumers. The most influential parameters, determined by sensitivity analysis conducted during the MCS, was concentration, which ranged from 0.71to 0.97. The results indicate that while the walnuts are, in general safe to eat, uses of organophosphorus, pesticides on walnut cultivation in Iran is not completely without risks so that guidelines should be established and a monitoring program should be established.

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contaminants; hazard index; Monte Carlo simulation; risk assessment; sensitivity analysis

Introduction

Despite application of modern protection techniques in agricultural practice, weeds, pathogens, and pests are still the most important problems. Due to the need for greater yields of crops and to preserve quality, pesticides, including insecticides, are used at various stages of plant growth (Liu et al. 2016). Pesticide residues in several fresh and processed products have posed risks to health of humans, such as increased risks of stillbirth and birth defects. To protect health of consumers from chronic risks of effects of pesticides extensively used in agriculture strict controls on when and how much can be applied to food items (Taghizadeh et al. 2019). Most countries have established maximum residue limits (MRLs) for pesticides, especially pesticides used in agriculture

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Table 1. European Union values set as ADI and MRLs as well as critical effects and NOAEL reported for observed pesticides.

Pesticides	EU ADI (mg/kg bm/ day)	MRLs (mg/kg)	JMPR ADI (mg/kg bm/ day)	ISIRI ADI (mg/kg bm/ day)	MRLs (mg/kg)	NOAEL (mg/ kg bm/ day)
OP pesticides						
Chlorpyrifos	0.001	0.05	0.01	0.01	0.2	0.1
Diazinon	0.0002	0.02	0.005	0.002	0.05	0.02
Ethion	0.002	0.02	0.002	–	–	0.2
Fenthion	0.007	0.02	–	–	–	–
Fenpyroximate	0.01	0.05	0.01	0.01	0.1	1
Phosalone	0.01	0.05	0.02	0.02	0.05	0.9
Glyphosate	0.5	0.1	–	0.3	0.2	50
Metasystox	0.0003	0.02	0.0003	0.0003	0.05	0.03
CB pesticides						
Aldicarb	0.003	0.05	–	–	–	0.025
Chlorpropham	0.05	0.01	–	–	–	5
Fenoxycarb	0.053	0.05	–	–	–	5.3
Thiophanate-methyl	0.08	0.2	0.08	–	–	2
PY pesticides						
Cypermethrin	0.05	0.05	0.04	–	–	0.5
Deltamethrin	0.01	0.02	0.05	–	–	1
Fenvalerate	0.0125	0.05	0.02	–	–	1.25
Permethrin	–	0.05	–	0.5	0.05	5
NC pesticides						
Acetamiprid	0.025	0.07	–	–	–	2.5
Imidacloprid	0.06	0.05	0.06	–	–	5.7

EU: European Union; JMPR: Joint FAO/WHO Meeting on Pesticide Residues; ISIRI: Institute of Standard and Industrial Research of Iran; ADI: acceptable daily intake; MRLs: maximum residue limits; NOAEL: no-observed-adverse effect level; OP: organophosphorus; CB: carbamate; PY: pyrethroid; and NC: nicotinoid.

(Codex Alimentarius Commission 2018, USEPA 2015). For instance, the European Food Safety Authority (EFSA) regulates maximum concentrations of pesticides permitted in foods, including walnut. The Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) follow the same general principles and methods for assessing risks of chemicals. The actual values are published in reports of both committees (WHO 2009). For food additives and for residues of pesticides in food, the health-based guidance value is termed the Acceptable Daily Intake (ADI) (Table 1) (Taghizadeh et al. 2019).

Organophosphorus pesticides (OPs) constitute a class of pesticides that act by inhibition of acetyl cholinesterase (AChE), which results in accumulation of the neurotransmitter acetylcholine, which can cause lethality by blocking transmission of impulses along nerves and in the brain. Therefore, quantifying OPs in foods and assessing risk is necessary (Songa and Okonkwo 2016). Carbamates (CBs), which are widely used in agricultural crops, also inhibit AChE. They are considered potential cytotoxic, genotoxic and immunotoxic agents that can affect several immune functions. Moreover, CBs have been associated with negative effects on cellular metabolic mechanisms, mitochondrial function, endocrine-disrupting activity, dementia, non-Hodgkin's lymphoma, and reproductive disorders (Della Pelle et al. 2018). Pyrethroids (PYs) are the most well-known and widely used representative pesticides. Due to their lipophilicity, PYs tend to accumulate into organisms and food items, and then separating PYs from matrices is difficult. Long-term exposure to pyrethroids, even at small doses, can cause chronic diseases including cardio-toxicity, immune-toxicity, and mutagenicity. Pyrethroids also have chronic effects on the male reproductive system, due to sperm aneuploidy, which is related to concentrations of metabolites of pyrethroids in urine. Another distinct mechanism of toxicity of PYs is allergenicity (Amjad et al. 2019). Nicotinoid (NC) pesticides with new modes of action and suitable selectivity are structurally distinct from the other classes of synthetic pesticides. They can cause serious effects on health and safety of consumers via developmental neurotoxicity (Sheets et al. 2016).

Usually in assessments of risk, single chemicals are considered, however EFSA has responsibility for considering cumulative risks, by use of aggregate estimates of exposures. This approach has been developed and codified into a mathematical model (Larsson et al. 2018). Modeling is a good alternative to environmental monitoring, which is often more costly and time-consuming. Due to inadequate frequency of sampling and spatial and temporal variability, monitoring alone can be unreliable. Monte Carlo Simulation (MCS) is a promising method that allows estimation of uncertainties associated with predicting risks to health. This method has been promoted by the United States Environmental Protection Agency (USEPA) and National Research Council (NRC) of the US National Academy of Sciences (NAS) (Ma et al. 2016; Razzaghi et al. 2018).

Objectives of this work were to: (1) determine concentrations of OP_S, CB_S, PY_S, and NC_S in various cultivars of walnut from various regions of Iran; (2) assess risks posed by these residue by use of MCS, which is proposed to be used for quantification of uncertainty and variability; and (3) use a sensitivity analysis to determine which input parameters most affected predicted risks to health of humans.

Materials and methods

Sample collection

Eastern black (*Juglans nigra* L.) and Persian walnuts (*Juglans regia* L.) were collected during September–October 2018, from five sites in Iran, including Azarshahr, Damavand, Farouj, Shahmirzad, and Tuyserkan (Figure 1). According to the United Nation Food and Agricultural Organization, Shahmirzad is home to the world's largest walnut orchard, with an area of 700 hectares. These are the most important cultivars of walnuts, followed by Chandler, Lara, Pedro and Vina, Jamal, and Damavand. Climate and topographic characteristics of the collection sites are shown (Figure 1).

Chemicals and reagents

Standards of pesticides of greater than 98% purity were purchased from Sigma-Aldrich (Steinheim, Germany). For each pesticide, a stock standard solution (1000 mg/l) was prepared in methanol and all solutions were kept in the dark at 4 °C. Other chemicals and solvents of analytical grade were supplied by Merck (Darmstadt, Germany) and Sigma (St. Louis, MO, USA).

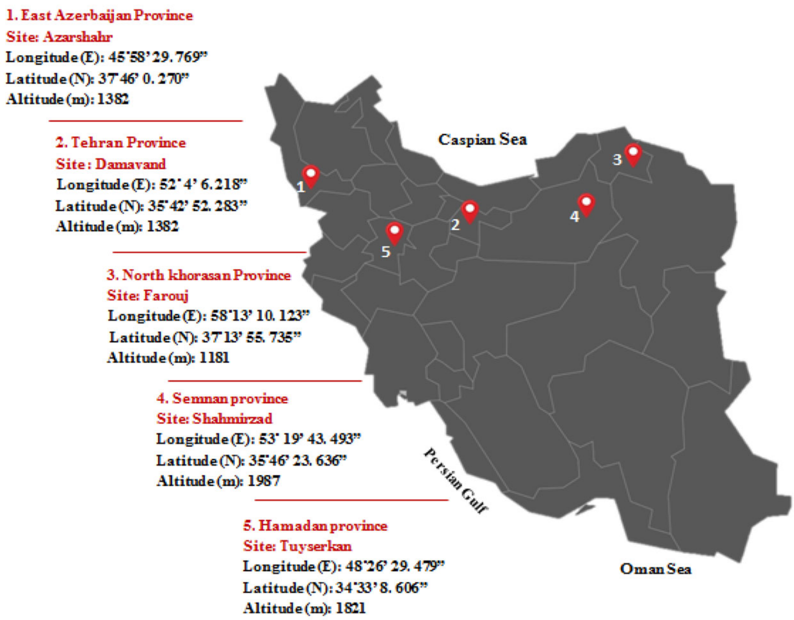

Figure 1. Climatic and geographical characteristics of four main cultivation site of pistachio in Iran.

Extraction procedure

Green husk and hard shells of walnuts were separated and then homogenized for 1.5 min, by use of a blender (Toos shekan Co., Iran). Then, 10 g of homogenized samples were put in a 50-mL falcon tube and 10 mL acetonitrile was added. The mixture was shaken well for 30 min by a mixer (Omni Mixer, USA). A mixture of 4 g $MgSO_4$, 1 g NaCl, 0.5 g $2Na_2C_6H_6O_7$ 1.5 H_2O and 1 g $C_6H_9Na_3O_9$ was added to the falcon tube and was shaken for 3 min. The mixture was centrifuged at 3500 rpm for 3 min. Aliquots of the supernatant were transferred to 2-mL dispersive solid-phase extraction (DSPE) tubes containing 150 mg $MgSO_4$ and 50 mg primary-secondary amine (PSA) and 50 mg C_{18}. DSPE tubes was shaken for 30 s and then, centrifuged at 3500 rpm for 1 min (Bakırcı et al. 2014).

Gas chromatography-mass spectrometry (GC-MS)

An Agilent 7890A Turbo MSD 5975C (Agilent, Santa Clara, USA), equipped with a PTV Inlet and 7683B auto injector (Agilent, Santa Clara, USA) and HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness) was used for identifying and quantifying pesticides. Helium, at a flow rate of 1.0 ml/min, was used as the carrier gas (Taghizadeh et al. 2018a, 2019). The quadrupole analyzer measured the abundance of ions of m/z from 50 to 490 and detector voltage was 1294 V. Electron ionization (70 eV) with selected ion monitoring mode was used, and the most abundant ion from the molecular ion cluster was measured for each analyzed compound (Szelewski 2005). Pesticides were identified based on comparisons of observed GC retention time with those of standard solutions of pesticides and use of characteristic ions.

Method validation

Accuracy and precision were assessed to assure the quality of the quantifications of pesticides. To validate analytical methods, limits of detection (LOD; 3 × background, signal to noise ratio) and limits of quantification (LOQ; 10 × background, signal to noise ratio) for pesticide residues were assessed. Quantification was done by use of an external, linear, standard calibration curve. The calibration curve was constructed before analysis of the samples, and linear regression equations used to quantify pesticides in walnut. Recoveries of pesticides were determined by spiking known amounts of pesticide standards four concentrations, 50, 100, 150, or 200 µg/ml into extracts. Precision was expressed as percentage relative standard deviation (RSD %) by analyzing three replicates of each sample (Taghizadeh et al. 2019).

Risk assessment

Health risks due to exposure to pesticides in food were estimated by comparing observed concentrations to the estimated daily intake (EDI) was determined (Eq. (1)) (Pico et al. 2018; Taghizadeh et al. 2018b).

$$EDI = \frac{IR \times C}{BW} \tag{1}$$

where IR is the rate at which walnuts were ingested (5.5 ± 1.5 g/person/day) (Preedy et al. 2011); C is the concentration of each pesticide in walnut (mg/kg), BM is the average body mass (75.61 ± 18.06 kg) (Portier et al. 2007).

The Target hazard quotient (THQ) for each of the 18 pesticides was calculated by dividing the estimated daily intake (EDI) by the relevent acceptable daily intake (ADI; mg/kg bm) (Eq. (2)) (Taghizadeh et al. 2017).

$$THQ = \frac{EDI}{ADI} \tag{2}$$

Protective residues, calculated as allowable daily intakes (ADIs), set by various jurisdictions and agencies for the 18 pesticides (Table 1) were extracted from the EU Pesticides Database (http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN, accessed January 19, 2019) and the database “OpenFoodTox” of EFSA for chemical hazards data (https://www.efsa.europa.eu/en/data/chemical-hazards-data, accessed January 19, 2019).

Cumulative hazard indices (HIs) were calculated as athe sum of THQ_n for classes of pesticides (Eq. (3)) (Taghizadeh et al. 2019).

$$\mathrm{HIs} = \sum_{i=1}^{n} \mathrm{THQn} \tag{3}$$

Uncertainty analysis for cancer risk

MCS (n = 10,000) was used to evaluate uncertainties and their effects on estimates of risk. This probabilistic model employed the entire range of input variables to develop a probability distribution of probability of exposure or risk rather than a single point

Table 2. Values and probability distributions of parameters in Monte Carlo Simulation.

Definition	Units	Distribution	Value	References
IR	g/day	LN	5.5 ± 1.5	(Preedy, et al. 2011)
BW	kg	LN	75.61 ± 18.06	(Portier et al. 2007)

IR: ingestion rate; BM: body mass; LN: log Normal.

estimate (Badibostan et al. 2019). The model input parameters applied in the simulation are shown (Table 2).

Sensitivity analyses

Sensitivity analyses were conducted to identify the most significant input data that affected the output values (Zhu et al. 2019). Input variables were concentration, ingestion rate, body weight, and acceptable daily intake.

Statistical analyses

Statistical analyses of data were carried out using IBM SPSS Statistics 24.0. Two-way ANOVA was employed to find the significant differences of concentrations of pesticides in samples of various cultivars of walnuts from various regions of Iran. Concentrations of pesticides are presented as means ± SD. Comparisons of mean concentrations were made using Mann-Whitney or Kruskal-Wallis, non-parametric tests. A level of 0.05 was considered statistically significant. The MCS and sensitivity analyses were conducted by use of Oracle Crystal Ball (version 11.1.4512.0). Values were extracted from Oracle Crystal Ball and then figures plotted by employing Excel (2010) software.

Results

Method validation

Correlation coefficients (r^2) up to 0.99, as well as recovery (mean ± RSD) confirmed appropriateness of this method for quantification of pesticides in various walnut cultivars. The LODs and LOQs of the proposed method for all pesticides were in ranges 0.0001–0.0130 and 0.0003–0.0490 mg/kg, respectively (Table 3).

Concentrations of pesticides in walnuts

Concentrations of pesticides in six cultivars of wallnuts collected from five regions of Iran (Figure 2). Mean concentrations of OPs were detactable in all samples, but were near LODs or LOQs in samples from Shahmirzad. All sampling sites except Shahmirzad contained similar concentrations of OPs, with no significant ($p = 0.096$) differnces among regions (Figure 2a). Concentrations of CBs were signficantly ($p<0.05$) different among regions. Concentrations of CBs were at least 3- to 10-fold greater than LODs, with the exception of Shahmirzad (Figure 2b). A similar pattern of differences ($p<0.05$)

Table 3. Validation concentrations of pesticides by use of GC-MS.

Pesticides	Correlation coefficient (r^2)	LOD range (mg/kg)	LOQ range (mg/kg)	Recovery (%)	RSD (%)
OP pesticides					
Chlorpyrifos	0.998	0.0004	0.0012	89–97	2.3
Diazinon	0.999	0.0009	0.0029	90–97	2.5
Ethion	0.999	0.0005	0.0015	89–93	1.9
Fenthion	0.999	0.0001	0.0003	89–94	2.3
Fenpyroximate	0.998	0.0003	0.0009	81–94	2.5
Phosalone	0.998	0.0001	0.0003	84–96	2.1
Glyphosate	0.999	0.0004	0.0015	88–95	2.4
Metasystox	0.998	0.0004	0.0020	84–96	2.4
CB pesticides					
Aldicarb	0.998	0.0008	0.0027	88–96	2.7
Chlorpropham	0.998	0.0003	0.0010	90–97	2.8
Fenoxycarb	0.998	0.0003	0.0010	89–94	2.3
Thiophanate-methyl	0.996	0.0030	0.0090	88–92	2.2
PY pesticides					
Cypermethrin	0.996	0.0006	0.0010	86–93	2.4
Deltamethrin	0.999	0.0010	0.0030	88–94	2.4
Fenvalerate	0.998	0.0003	0.0010	91–96	2.3
Permethrin	0.998	0.0007	0.0025	90–93	2.4
NC pesticides					
Acetamiprid	0.999	0.0070	0.0490	88–95	2.1
Imidacloprid	0.998	0.0130	0.0400	85–93	2.1

LOD: limit of detection (mg/kg); LOQ: limit of quantification (mg/kg); RSD: relative standard deviation; OP: organophosphorus; CB: carbamate; PY: pyrethroid and NC: nicotinoid.

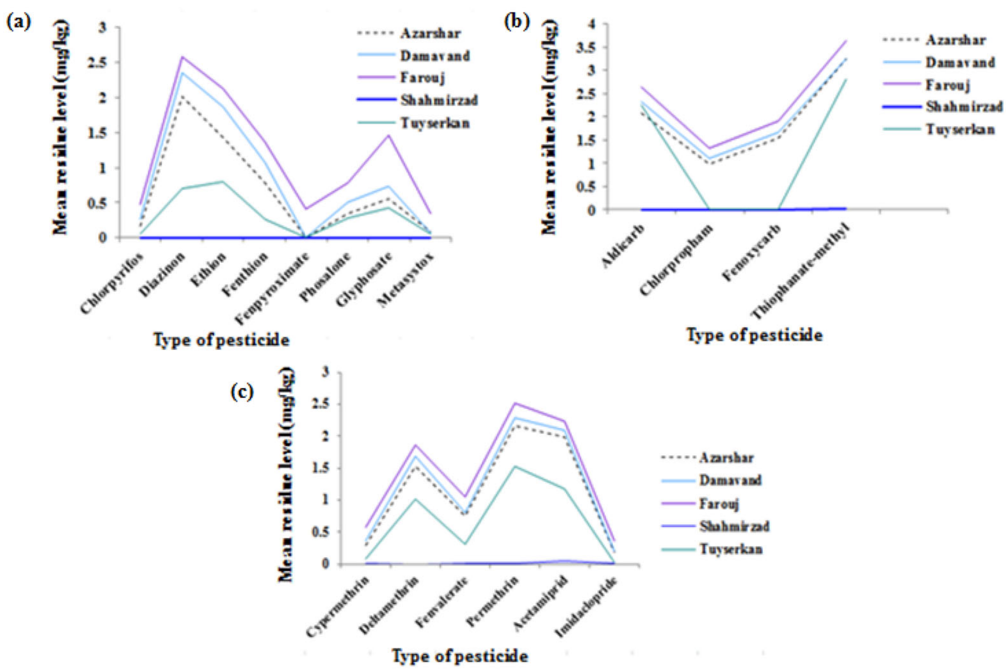

Figure 2. Concentrations of OPs (a), CBs (b), PYs and NYs (c) plotted for each region.

was observed for concentrations of PYs among regions. Concentrations of NCs were similar among regions except for Shahmirzad. There were no significant ($p = 0.123$) differences in concentrations of OP among regions (Figure 2c).

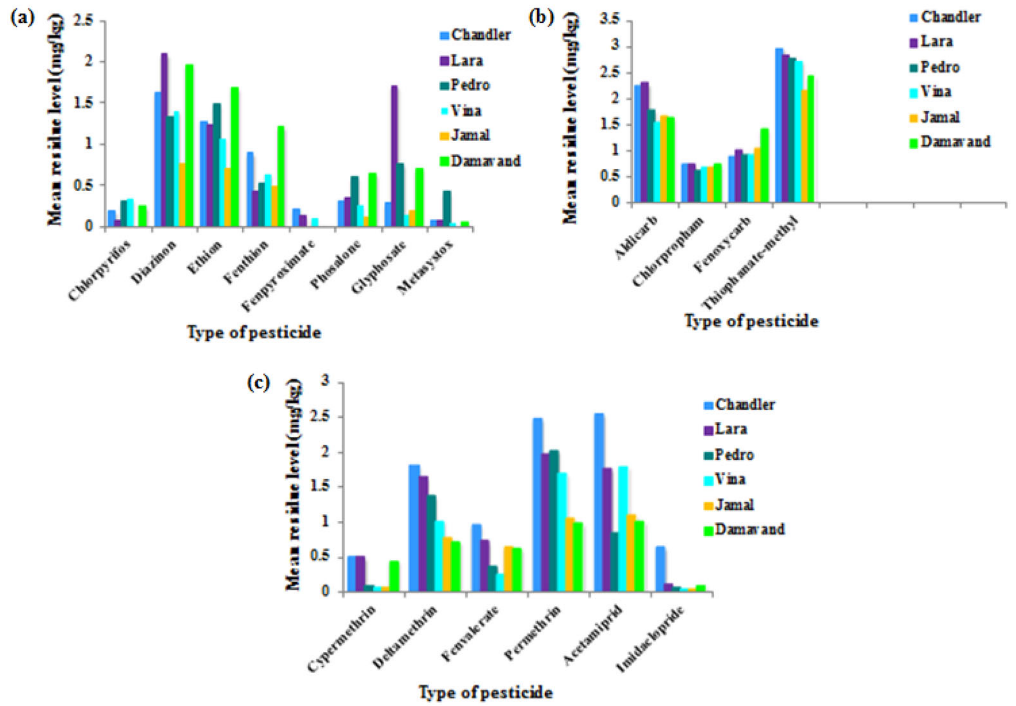

Figure 3. Concentrations of OPs (a), CBs (b), PYs and NYs (c) in various cultivars of walnuts.

Concentrations of pesticides varied among cultivars (Figure 3). Concentrations of all OPs varied significantly ($p<0.05$) among the six cultivars (Figure 3a). Within the category of CBs, the calculated p-values were 0.09, 0.130, 0.124, and 0.095 for aldicarb, chlorpropham, fenoxycarb, and thiophanate-methyl, respectively (Figure 3b). Concentrations of PY pesticides, cypermethrin ($p = 0.073$), deltamethrin ($p = 0.065$), and permethrin ($p = 0.065$)) did not differ one from the other. P-values for acetamiprid and imidaclopride were 0.095 and 0.172, respectively (Figure 3c).

Cumulative risk

THQs and HIs, based on consumption of walnuts are presented in Table 4. Employing MCS, 95th centiles for THQ based on consumption of walnut were estimated to range from 3.69×10^{-4} to 1.54 for OPs. For CBs, values ranged from 2.74×10^{-3} to 1.03×10^{-1}, while estimated THQs for PYs ranged from 9.63×10^{-3} to 8.54×10^{-2}. The 95th centiles of THQ for acetamiprid and imidaclopride were 1.16×10^{-2} and 1.08×10^{-3}, respectively (Table 4).

Cumulaive HIs (sums of His for all pesticides in a class, for collected walnut cultivars consumed by the population of Iran are given for the four classes of pesticides (Table 4). Values below one were observed for CBs, PYs and NCs (1.2×10^{-2}, 8.58×10^{-2} and 1.16×10^{-2} for 95th centiles occurrence, respectively). OP pesticides were the only class to have cumulative HI values exceeding 1.0 for 80th, 90th, and 95th centiles. This was due to the larger index for diazinon. Based on the cumulative risk

Table 4. Estimated THQs for single chemicals and HIs for the various classes of pesticides.

Pesticides	mean	median	50%	80%	90%	95%	Sensitivity analysis: Rank correlation		
							C	BW	IR
OP pesticides									
Chlorpyrifos	1.47×10^{-2}	7.96×10^{-3}	7.96×10^{-3}	2.68×10^{-2}	4.13×10^{-2}	5.58×10^{-2}	0.97	−0.10	0.09
Diazinon	5.84×10^{-1}	5.84×10^{-1}	5.84×10^{-1}	1.02	1.28	1.54	0.88	−0.25	0.28
Ethion	4.74×10^{-2}	4.68×10^{-2}	4.68×10^{-2}	7.74×10^{-2}	9.62×10^{-2}	1.14×10^{-1}	0.85	−0.28	0.32
Fenthion	7.81×10^{-3}	6.09×10^{-3}	6.09×10^{-3}	1.39×10^{-2}	1.85×10^{-2}	2.27×10^{-2}	0.94	−0.16	0.19
Fenpyroximate	6.43×10^{-4}	5.17×10^{-6}	5.17×10^{-6}	4.08×10^{-4}	1.57×10^{-3}	3.54×10^{-3}	0.91	−0.19	0.19
Phosalone	2.77×10^{-3}	2.43×10^{-3}	2.43×10^{-3}	4.96×10^{-3}	6.79×10^{-3}	8.50×10^{-3}	0.93	−0.20	0.23
Glyphosate	9.90×10^{-5}	5.31×10^{-5}	5.31×10^{-5}	1.85×10^{-4}	2.80×10^{-4}	3.69×10^{-4}	0.97	−0.08	0.10
Metasystox	3.02×10^{-2}	2.70×10^{-4}	2.70×10^{-4}	1.97×10^{-2}	7.90×10^{-2}	1.61×10^{-1}	0.92	−0.17	0.20
SUM (HI)	6.88×10^{-1}	6.48×10^{-1}	6.48×10^{-1}	1.16	1.52	1.58			
CB pesticides									
Aldicarb	4.83×10^{-2}	4.59×10^{-2}	4.59×10^{-2}	7.07×10^{-2}	8.73×10^{-2}	1.03×10^{-1}	0.77	−0.37	0.42
Chlorpropham	1.07×10^{-3}	1.07×10^{-3}	1.07×10^{-3}	1.82×10^{-3}	2.29×10^{-3}	2.74×10^{-3}	0.86	−0.27	0.29
Fenoxycarb	1.49×10^{-3}	1.48×10^{-3}	1.48×10^{-3}	2.66×10^{-3}	3.32×10^{-3}	3.97×10^{-3}	0.89	−0.24	0.28
Thiophanate-methyl	2.65×10^{-3}	2.48×10^{-3}	2.48×10^{-3}	3.75×10^{-3}	4.58×10^{-3}	5.33×10^{-3}	0.71	−0.43	0.48
SUM (HI)	5.35×10^{-2}	5.09×10^{-2}	5.09×10^{-2}	7.89×10^{-2}	9.75×10^{-2}	1.20×10^{-2}			
PY pesticides									
Cypermethrin	4.11×10^{-3}	4.29×10^{-5}	4.29×10^{-5}	3.07×10^{-3}	1.08×10^{-2}	2.28×10^{-2}	0.94	−0.14	0.16
Deltamethrin	9.34×10^{-3}	9.18×10^{-3}	9.18×10^{-3}	1.54×10^{-2}	1.93×10^{-2}	2.30×10^{-2}	0.85	−0.29	0.32
Fenvalerate	3.59×10^{-3}	3.61×10^{-3}	3.61×10^{-3}	6.44×10^{-3}	8.14×10^{-3}	9.63×10^{-3}	0.89	−0.25	0.27
Permethrin	1.33×10^{-2}	1.28×10^{-2}	1.28×10^{-2}	2.08×10^{-2}	2.57×10^{-2}	3.04×10^{-2}	0.82	−0.30	0.37
SUM (HI)	3.03×10^{-2}	2.56×10^{-2}	2.56×10^{-2}	4.57×10^{-2}	6.39×10^{-2}	8.58×10^{-2}			
NC pesticides									
Acetamiprid	4.54×10^{-3}	4.52×10^{-3}	4.52×10^{-3}	7.77×10^{-3}	9.74×10^{-3}	1.16×10^{-2}	0.86	−0.27	0.30
Imidacloprid	3.59×10^{-4}	4.34×10^{-5}	4.34×10^{-5}	1.89×10^{-4}	4.74×10^{-4}	1.08×10^{-3}	0.94	−0.19	0.23
SUM (HI)	4.90×10^{-3}	4.56×10^{-3}	4.56×10^{-3}	7.96×10^{-3}	1.02×10^{-2}	1.16×10^{-2}			

THQ: target hazard quotient; HI: hazard index; OP: organophosphorus; CB: carbamate; PY: pyrethroid and NC: nicotinoid; C: concentration; BW: body weight; IR: ingestion rate assessment, HIs were calculated for individual pesticides at the 95th centile was 1.68 (Table 4).

Sensitivity analysis

A quantitative sensitivity analysis was conducted to determine which input parameters had the greatest effect on the assessment of risks to health posed by consumption pesticides in walnuts. The most influential parameters affecting results of Monte Carlo simulations are shown (Table 4). Accordingly, concentration (0.71–0.97) was the parameter that had the greatest influence on exposure to pesticides in walnut, while body mass (BM) contributed in less (Table 4).

Discussion

In both more developed and developing countries, pesticides are used in almost all agricultural crops. Persistence and thus potential for exposure of humans of pesticides depends on their physiochemical characteristics as well as the food item and how it is prepared and consumed. Every pesticide used on fruits and vegetables needs some waiting period before harvesting, that depend on the type of pesticide, how it is applied and to what crop. Despite regulation of uses of pesticides, it is necessary to wait a particular period before harvesting. Alternatively, various processing methods can be applied to reduce concentrations of pesticides in crops or processed foods made from them. By means of physical and chemical treatments such as washing, drying, peeling, soaking in solutions of salt and acetic acid, the levels of pesticides can reduce in agriculture products. Thermal processing techniques including baking, steaming, cooking, canning, and pasteurization are effective at removing residues of pesticides from surfaces of crops before they are consumed as food by humans. However, magnitudes of reduction depend on the initial concentration at the time of harvest, crop, and type of pesticide. Because, some crops, including walnut are often consumed without the above-noted processing methods the potential for humans being exposed to residual concentrations of pesticides is greater (Bajwa and Sandhu 2014; Taghizadeh et al. 2019).

Exposure of humans, even to lesser doses of mixtures of pesticides can cause chronic adverse effects. Due to chemical properties and mechanisms of action of each pesticide, cumulative exposures to multiple pesticides or interactions can result in multiple responses (Hernández et al. 2013).

Deterministic methods provide single point estimates to evaluate hazard or risk, while probabilistic assessments can provide quantitative estimates of the probability of occurrence, either exposure or response or both (Calabrese 1996). Probabilistic models have usually been used to describe distributions as well as providing quantitative estimates of variability and uncertainties related to specific exposure concentrations. The MCS or bootstrapping technique is a statistical method that can generate hundreds of iterations with random sampling based on input parameters that describe distributions and generate estimates that can be used to describe a state space of possible outcomes (Yu et al. 2017).

In the present study, concentrations of 18 pesticides exceeded the MRLs set by EU (Reg. 396/2005). Although concentrations of all pesticides were greater than their respective MRLs, with the exception of diazinon, they had THQs < 1, at the 95th centile. Based on HIs the 95th centile (1.68), it can be concluded that concentrations of pesticides in walnut posed a *di minimis* to moderate risk. By definition, an HI ≤ 1 indicates that the threshold upon which it is calculated will not be achieved at a particular concentration in the diet or dose. When His are in the range of 1.1 to 10 shows moderate risk, and HI > 10 indicated greater risk (Lemly 1996). Similar to results of the study, results of which are presented here, results of a separate study (Zhan et al. 2015) suggested that application of PY pesticides to control spiders in walnuts resulted in greater risks to health of humans. In China, when concentrations of 29 pesticides, including OPs, PYs, OCs, and two fungicides were investigated in chestnut, walnut and pine nut, 20.5% of samples exceeded respective MRLs set by the EU. In that study, cumulative risks (HIs) for classes of pesticides were 8.43 for OPs, 0.42 for OCs, 12.82 for PYs, and 0.15 for fungicides. HIs was 21.82. There was no serious health risk for consumers via nuts consumption (Liu et al. 2016). Among the residue levels of pesticides which determined in nuts including pistachio, peanut, walnut, hazelnut, and sunflower seeds, diazinon is often applied more frequently on nuts than other pesticide (Cortés et al. 2008). Similar findings about nuts have been reported for Iran (Emami et al. 2017; Morteza et al. 2017).

In order to assess the greatest effect of input variables on the risk assessment the sensitivity analysis was performed during the Monte Carlo simulation (Yang et al. 2015). The results of sensitivity analysis showed that the most influential parameter was concentration of pesticide, of the total variance in the health risk assessment. Therefore uses of pesticides should be within standards set by international organizations. However, greater damages occur in post- harvest period which are caused by the pests that attack the stored crops, especially in the tropical sites. It should be mentioned that, extensive monitoring over a longer period of time gives us a more realistic picture of the status.

Conclusions

Results of this study showed that concentrations of four classes of pesticides in six cultivars of walnuts collected from five regions of Iran. Risk assessment was performed by Monte Carlo Simulation (MCS) method. The results of MCS showed that there is *di minimis* to moderate hazard and risk to humans who ingest walnuts from those regions. Furthermore, based on results of the sensitivity analysis concentrations of pesticides was the most influential factor in determining hazards and risks. Due to the presence of pesticides at concentrations greater than the MRLs in analyzed walnuts, particular attentions on uses of pesticides in this crop are required. Presence of pesticides in walnuts depends on pre and post- harvest conditions.

It is recommended that regulations should be updated and regular monitoring could be done in post- harvest. There is an urgent need to educate the farmers to employ these chemicals at appropriate manners. Because of small sample size in this study, further research on residues of pesticides in walnut is still required.

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Disclosure statement

Authors declare that there is no conflict of interest.

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