Toxicology Letters 313 (2019) 91–100

Contents lists available at ScienceDirect

Toxicology Letters

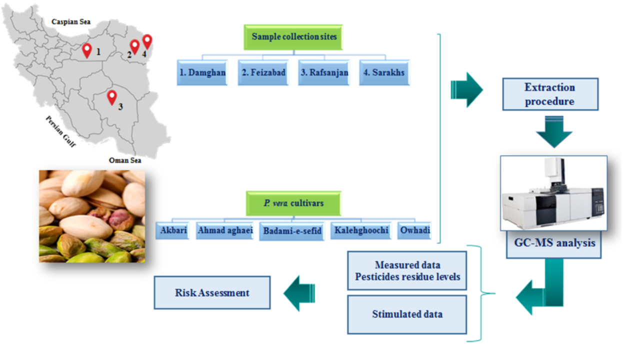
journal homepage: www.elsevier.com/locate/toxlet

Cumulative risk assessment of pesticide residues in different Iranian pistachio cultivars: Applying the source specific HQ_S and adversity specific HI_A approaches in Real Life Risk Simulations (RLRS)

Seyedeh Faezeh Taghizadeh[a,1], Marina Goumenou[b,1], Ramin Rezaee[c,d,e,f,1], Thanasis Alegakis[b], Venetia Kokaraki[b], Ourania Anesti[f], Dimosthenis A. Sarigiannis[e,f], Aristides Tsatsakis[b], Gholamreza Karimi[a,g,*]

[a] *Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran*
[b] *Center of Toxicology Science & Research, Medical School, University of Crete, Heraklion, Crete, Greece*
[c] *Clinical Research Unit, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
[d] *Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
[e] *Aristotle University of Thessaloniki, Department of Chemical Engineering, Environmental Engineering Laboratory, University Campus, Thessaloniki, 54124, Greece*
[f] *HERACLES Research Center on the Exposome and Health – Center for Interdisciplinary Research and Innovation, Aristotle University of Thessaloniki, Thermi, Greece*
[g] *Department of Pharmacodynamics and Toxicology School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Cumulative dietary exposure
Cumulative risk assessment
Mixtures
Pesticide residues
Pistacia vera L.

ABSTRACT

Iran is a major supplier of the world pistachio market. In this study, we collected five pistachio cultivars from four main pistachio-producing zones in August and September 2016, and determined the residues of 18 organophosphorus, carbamate, pyrethroid and nicotinoid pesticide in these samples using Gas Chromatography-Mass Spectrometry, as an efficient method for determination of pesticides' residues. Next, single-chemical and chronic cumulative risk assessment was done based on the new approaches of the food specific Hazard Quotient and adversity specific Hazard Index. Fifteen from eighteen food-specific Hazard Quotients were above 1 even in cases when the respective contamination was bellow MRLs. The adversity specific Hazard Indexes values were above 1 for five from six adversities indicating various risks in the resulted levels of pistachios' contamination from the

---

[*] Corresponding author at: Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, P. O. Box, 1365-91775. I.R., Iran.

E-mail address: KarimiG@mums.ac.ir (G. Karimi).

[1] The first three authors equally contributed to this work.

https://doi.org/10.1016/j.toxlet.2019.05.019
Received 9 February 2019; Received in revised form 20 May 2019; Accepted 21 May 2019
Available online 22 May 2019

pesticides' mixture. However, no risk for carcinogenicity was found. Our results indicate the necessity of taking appropriate measures to control/standardize pesticides practice in pistachio cultivation in Iran and the need to re-establish the MRLs based on cumulative exposure.

1. Introduction

Throughout life, people are exposed to numerous chemicals originating from different sources. Pesticides comprise a major group of these chemicals and are found more frequently in agricultural crops like fruits and vegetables rather than animal products and the environment. In recent years, food safety and risk assessment after simultaneous exposure to different chemicals have received considerable attention as in real life exposure, humans are not exposed to a single chemical but to mixtures of them (Akbari et al., 2012; Authority, 2008; Bopp et al., 2018; Pico et al., 2018; Tsatsakis et al., 2017). Recently, it was also reported that humans' exposure to combinations of chemicals and physical factors in real life, via consumption of food and water and through direct or indirect environmental sources, should be taken into account more seriously, especially when such exposures/data are evaluated for regulatory purposes (Tsatsakis et al., 2017). According to the 2016 European Union (EU) report on pesticide residues in food, multiple residues (more than one pesticide) were found in 27.3% of the samples analyzed with strawberries, peaches, apples and lettuce among the products with multiple residues. Even if these substances are found at very low concentrations, long-term exposure to combinations of pesticides may have the potential to induce adverse health effects (Kalliora et al., 2018; Tsatsakis et al., 2017). It was shown that exposure to pesticides is correlated with both acute and long-term effects (e.g. hepatotoxicity, nephrotoxicity, neurotoxicity, reproductive toxicity, cancers, behavioural changes, and immunological, cardiovascular and respiratory conditions as well as Alzheimer's and Parkinson's diseases) (Georgiadis et al., 2018; Guo et al., 2016; Hassani et al., 2015; Mrema et al., 2013). For this reason, frequent monitoring of pesticides residue levels in our environment, foods, etc. and performing risk assessment based on the obtained data, are of crucial importance (Moretto et al., 2015). In this regard, several organizations like the Joint FAO/WHO Expert Committee on Food Additives, the European Food Safety Authority (EFSA), the USA Environmental Protection Agency (USEPA) and the Australian Pesticides and Veterinary Medicines Authority have set safe exposure limits for different chemicals in foods and introduced maximum residue limits (MRLs) for the pesticides (López-Blanco et al., 2017; Zentai et al., 2016). In the EU, since the 1990s, all plant products are evaluated and authorized, and they undergo periodic post-authorization control through Directive Regulation (EC) No 1107/2009 (Regulation, 2009).

Among different classes of pesticides, organophosphorus (OP) insecticides which are effective broad-spectrum chemicals are of great concern as they may manifest serious adverse effects (Katsikantami et al., 2018; Vucinic et al., 2017). OP insecticides inhibit the enzyme acetylcholinesterase (AChE) in the central and peripheral nervous system (solely in humans), and may induce neurotoxicity, cardiotoxicity and tissue hypoxia (Abbasi Ghaeni et al., 2018; Georgiadis et al., 2018; Gupta, 2011; Hassani et al., 2015; Mohammadi et al., 2011). Carbamates (CBs) also inhibit AChE, and they are considered potential immunotoxic agents as they may affect several immune functions (Dhouib et al., 2016). To combat insects, pyrethroids (PYs), a class of synthetic insecticides, have been extensively used as a substitute for OP compounds (Li et al., 2016; Rezaee et al., 2013). PY residues are one of the most important pollutants of agricultural crops, and may potentially pose risk to public health (Saillenfait et al., 2015). Noteworthy, co-exposure to PY and other pesticides may cause serious toxicities (US EPA, 2011). Nicotinoid (NC) insecticides form a relatively new group with novel modes of action which may also induce serious risks for humans health and safety, act as agonists for nicotinic acetylcholine receptors in insects (Song et al., 2018).

The genus *Pistachio* which contains 11 species (e.g. *Pistacia vera* L., *P. terebinthus* L. *P. lentiscus*, etc.), belongs to Anacardiaceae family (Taghizadeh et al., 2018a, b; Taghizadeh et al., 2018c). *Pistacia vera* L. is an important horticultural product of Iran which is among the largest exporters of pistachio mainly to the European countries; therefore, regular monitoring and determination of chemical residues levels along with risk assessment for pistachio samples, are necessary (Taghizadeh et al., 2017b).

Though it is globally recognised that risk assessment after exposure to multiple chemical is of great importance, it should be noted that no universally applicable methodology could be found for such risk assessments. The International Programme on chemical safety of the World Health Organization (WHO IPCS) published guidelines with increased applicability (to a global level) but such initiatives are imperfect as they only deal with human health consequences. European Commission and its associated scientific authorities try to equally consider humans and environment health (Kortenkamp et al., 2009). A practical approach for policy/regulatory actions was suggested by Sarigiannis and Hansen (2012) for facilitating the investigation of the health effects of chemical mixtures present in the environment as well as consumer products. They proposed that when assessing the risk of exposure to xenobiotic mixtures, the following parameters should be taken into consideration: a) application of dose addition to calculate a hazard index which will address interactions among mixture constituents, and b) employment of the connectivity approach in data-rich cases to incorporate mechanistic data at different scales of biological system (Sarigiannis and Hansen, 2012). According to other researchers, to assess pesticides-associated risk, it is necessary to identify the substances with common mechanisms of toxicity (Gallagher et al., 2015). For example, some OP, CB and PY chemicals cause a common neurotoxic effect and have been identified by United States Environmental Protection Agency (USEPA) as members of the same cumulative group (USEPA, 2006; USEPA, 2007; USEPA, 2011). A similar grouping approach, applicable also to chemicals with dissimilar mode of action, is the Cumulative Assessment Groups (CAGs) developed by the European Food Safety Authority (EFSA). This approach assumes that all chemicals can produce joint cumulative toxicity if they manifest the same effects at higher levels of biological organization (Products and Residues, 2013).

In traditional risk assessment methodologies used for assessment of the risk posed by pesticide exposure, just a single chemical was considered. In cumulative risk assessment, methodologies utilize aggregate exposures, which refer to exposure to multiple compounds or mixtures causing similar toxicological effects, and use different risk characterization approaches (Ilyushina et al., 2019; Rotter et al., 2018; Tsakiris et al., 2015). Since there is no recent study on determination of commonly-used pesticide residues in Iranian pistachio orchard, the present study was conducted to 1) provide the levels of OP, CB, PY and NC pesticide residues, in whole samples, in each cultivar and each region of cultivation and 2) to perform risk assessment after dietary exposure to each single pesticide and their mixture. The risk characterization was done based on the new methodology of source-specific Hazard Quotients (HQ_S) and the adversity specific Hazard Index (HI_A) (Goumenou and Tsatsakis, 2019). A case study of this methodology was recently published (Renieri et al., 2019)

2. Materials and methods

2.1. Chemicals

Pesticide standards of 99% purity were procured from Sigma-Aldrich (Steinheim, Germany). For each pesticide, a stock standard solution (1000 mg/l) was prepared in methanol and all solutions were kept at 4 °C in the dark. Other chemicals and solvents used in this study were of analytical grade and were bought from Merck (Darmstadt, Germany) and Sigma (St. Louis, MO, USA).

2.2. Sample collection

The ripe fruits of five most important Iranian commercial *P. vera* cultivars namely, Akbari, Ahmad aghaei, Badami-e-sefid, Kalehghoochi and Owhadi, were collected from four major pistachio-producing sites namely, Damghan, Feizabad, Rafsanjan and Sarakhs, in Iran in August and September 2016. Samples were transported to the laboratory under cold conditions. Next, the kernels were air-dried, labelled and stored at − 20 °C until analyzed. Climatic and geographical features of the four cultivation sites are shown in Fig. 1.

2.3. Extraction procedure

After removal of the hulls and shells, kernels were collected and homogenized using a blender (Toos shekan Co., Iran) for 1.5 min. Then, 10 g of homogenized samples was placed in a 50-mL Falcon tube and 10 mL acetonitrile was added to the tubes; then, the mixture was vigorously shaken for 30 min by a mixer (Omni Mixer, USA). A mixture of 4 g $MgSO_4$, 1 g NaCl, 0.5 g $2Na_2C_6H_6O_7$ $1.5H_2O$ and 1 g $C_6H_9Na_3O_9$ was added to the tube and the tube was shaken for 3 min. The mixture was centrifuged at 3500 rpm for 3 min, and aliquots of the supernatant were transferred to a 2-mL dispersive solid-phase extraction (DSPE) tube containing 150 mg $MgSO_4$ and 50 mg primary-secondary amine (PSA) and 50 mg C_{18}. The DSPE tube was shaken for 30 s and then, centrifuged at 3500 rpm for 1 min (Bakırcı et al., 2014).

2.4. Apparatus

Gas chromatography-mass spectrometry (GC–MS) method was used in this study to determine the levels of pesticides residue. The GC–MS system was an Agilent 7890A Turbo MSD 5975C (Agilent, Santa Clara, USA) equipped with a PTV Inlet and 7683B auto injector (Agilent, Santa Clara, USA) used for measuring pesticide residues levels. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The system was equipped with a HP-5MS 30 m × 0.25 mm × 0.25 μm column (Agilent, Santa Clara, USA.).

2.5. Method validation

The extraction procedure and analytical methods used for determination of pesticide residues in pistachio samples, were validated in terms of linearity and limits of detection, respectively. The precision was expressed as percentage relative standard deviation (RSD%) by analysing three replicates of each sample. A linear calibration curve was plotted based on linear regression analysis. Recovery of pesticides was determined by spiking using standard pesticide solutions at four concentrations (50, 100, 150, and 200 μg/ml). Also, limit of detection (LOD) and limit of quantification (LOQ) were determined using the standard solutions calibration curves.

2.6. Cumulative risk assessment

2.6.1. Exposure assessment

Chronic exposure to the studied pesticides was calculated as the estimated daily intake (EDI) (mg / kg bw) of each residue found in pistcahio, corrected for the contribution of pistachios' consumption to the overall daily diet. For calculation of the EDI via consumption of pistachio, the following previously-reported equation was used (Lozowicka et al., 2014; Organization, 2009; Pico et al., 2018):

$$EDIi = \frac{F \times RLi}{mean\ body\ weight}$$

where RLi is the occurrence of each residue i (mg pesticide / kg pistachio) in percentile (median, 80th or 90th percentile) or the mean, and F is the daily pistachio consumption (kg). The value of consumption used in the current study is 0.3288 g/kg bw/day (97.5th of exposure distribution). This value was extracted by EFSA's Pesticide Residue Intake Model (Primo revision 3), and refers to the highest amount of edible raw commodity (EFSA, 2008). Previously used values such as 0.82 g/person/day for Iranian general population (Taghizadeh et al., 2017b) and 0.032 kg/person/day (FAO 2013 reports) were determined

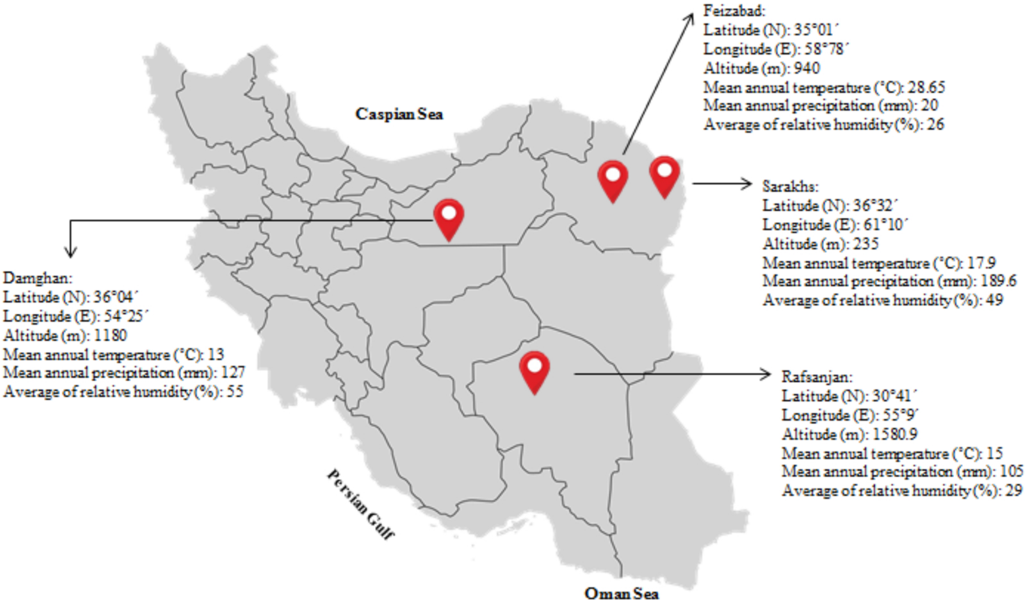


Fig. 1. Climatic and geographical characteristics of four main cultivation zones of pistachio in Iran.

by less accuarate methodologies than this reported by EFSA and they correspond to the average consumption based on tonnages of production and country population.

For the correction of the EDIs of the 18 pesticides via only pistachio consumption and the extrapolation of this exposure to the overall aggregated dietary exposure (from all possible dietary sources), we used a newly developed methodology (Goumenou and Tsatsakis, 2019). Based on this methodology, for each of the pesticides and based on the current EU MRLs (Table 1), we calculated the overall exposure from the consumption of the foods where the pesticide might be used and the exposure from pistachio only. The ratio of these two values was considered a surrogate of the contribution of pistachio to the overal intake of each pesticide and it was used as a correction factor of the calculated pistachio EDIs.

2.6.2. Risk characterisation

The risk characterisation was done by using the newly developed methodology of the Adversity Specific Hazard Index (HI_A) for cumulative risk assessment (Goumenou and Tsatsakis, 2019), based on which the classic hazard quotients (HQs) (Larsson et al., 2018; Lozowicka et al., 2014; Means, 1989; Pico et al., 2018; Taghizadeh et al., 2017a) of the pesticides in mixture are added after an adversity-based grouping.

$$cHI = \sum_{i=1}^{n} HQ_i$$

The hazard quotient (HQ) for each of the 18 pesticides was calculated by dividing the estimated daily intake (EDI) by the relevent acceptable daily intake (ADI; mg/kg bw):

$$HQ = \frac{EDI}{ADI}$$

The ADI values for the 18 pesticides (Table 1) were extracted from EU Pesticides Database (http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN accessed 19/1/2019) and the database "OpenFoodTox" of EFSA for chemical hazards data (https://www.efsa.europa.eu/en/data/chemical-hazards-data accessed 19/1/2019). For pesticides that were non-approved in the EU, additional sources of information were used (e.g. JECFA and EMEA). For the calculation of the HIs, the 18 pesticides were grouped in 6 groups (Table 1) depending on the type of toxicity they manifest. In order to accuratelly consider their main hazard, we considered the critical effect used for the derivation of the ADIs (i.e. the effect considered for the related NOAEL) (Goumenou and Tsatsakis, 2019). An HI ≤ 1 indicates no significant health risk, HI from 1.1 to 10 shows moderate risk, and HI $>$ 10 reflects high risk (Lemly, 1996).

2.7. Statistical analysis

IBM SPSS Statistics 24.0 and Excel 2010 for Windows spreadsheet were used for data analysis and graphical presentation of data. Residues levels, HQ_S and HI_A values were presented as means, median and 80th and 90th percentile. Comparisons of mean residue levels were made using Mann-Whitney or Kruskal-Wallis non-parametric tests. Due to the limited number of residue levels, a Monte Carlo simulation was performed for regenerating 10,000 samples for each pesticide using IBM SPSS Statistics simulation routines. A level of 0.05 was considered statistically significant.

Table 1

European Union values set as ADI and MRLs as well as critical effects and NOAEL reported for the identified pesticides.

Pesticides	Chemical Category	EU- ADI (mg/kg bw/ day)	EU- MRLs (mg/kg)	Critical Effect	NOAEL (mg/ kg bw/ day)
		Group A- Neurotoxicity			
Chlorpyrifos*	OP	0.001	0.01	Inhibition of ChE (RBC)	0.1
Deltamethrin*	PY	0.01	0.02	Neurotoxicity	1
Acetamiprid	NC	0.025	0.07	Neurotoxicity	2.5
Diazinon *	OP	0.000	0.02	Inhibition of ChE (RBC)	0.02
Fenthion *	OP	0.007	0.02	Inhibition of ChE (Plasma)	–
Phosalone *	OP	0.01	0.02	Inhibition of ChE (Brain)	0.9
Metasystox 1 *	OP	0.0003	0.01	Inhibition of ChE (Brain)	0.03
Aldicarb *	CB	0.003	0.05	Inhibition of ChE (RBC)	0.025
		Group B- Reproductive and Developmental Toxicity			
Glyphosate*	OP	0.5	0.1	Maternal toxicity (mortality), post- implantation losses	50
Cypermethrin*	PY	0.005	0.05	Systemic toxicity, changes in kidneys (weight) and testes (tubular atrophy and calcification)	0.5
Ethion *	OP	0.002	0.02	Embryotoxicity/ fetotoxicity	0.2
		Group C- Systemic Toxicity			
Cypermethrin*	PY	0.005	0.05	Systemic toxicity in rats included increased urea, changes in kidneys (weight) and testes (tubular atrophy and calcification)	0.5
Fenpyroximate	OP	0.01	0.01	Body weight changes	1
Fenvalerate *	PY	0.0125	0.05	Body weight gain of the F2b parents	1.25
Permethrin *	PY	0.01	0.05	Effects on liver weight	5
		Group D- Haematotoxicity			
Chloropropham*	CB	0.05	0.01	Haematological effects/ thyroid effects	5
		Group E- Thyroid Effects			
Chloropropham*	CB	0.05	0.01	Haematological effects/ thyroid effects	5
Thiophanate- methyl*	CB	0.08	0.2	Thyroid toxicity	2
Imidacloprid*	NC	0.06	0.05	Thyroid effects	5.7
		Group F- Carcinogenicity			
Fenoxycarb*	CB	0.053	0.05	Lungs and liver tumors	5.3

OP: organophosphorus, CB: carbamate, PY: pyrethroid and NC: nicotinoid.
ADI: Acceptable Daily Intake.
EU: European Union.
MRLs: maximum residue limits.
NOAEL: No- Observed- Adverse Effect Level.
* MRLs based on lower limit of analytical determination.

3. Results

3.1. Performance of the analytical method

Table 2 summerizes the recovery and RSD values confirming the appropriateness of this method for determination of pesticide residues in different pistachio cultivars. The LODs and LOQs, as well as the calibration curves (with correlation coefficients of 0.995-0.999%) are also presented in Table 2.

3.2. Pesticides residue levels in pistachio kernels

In Table 3, the pesticide levels measured in all cultivars collected from various locations and the simulated Monte Carlo results are presented. For most of the pesticides, the mean values of simulated data were the same as the measured data. Nevertheless, differences in measured and simulated data were observed, in 90th percentile, in many cases.

In Fig. 2, the pesticide residue levels in five pistachio cultivars collected from four sampling zones, are shown. Diagram is divivded

Table 2
Validation of GC–MS method used for determination of pesticide residues.

Pesticides	Correlation coefficient (r^2)	LOD range (mg/kg)	LOQ range (mg/kg)	Recovery (Mean ± RSD %)
OP Pesticides				
Chlorpyrifos	0.999	0.0004	0.0012	79.22 ± 3.86 - 89.22 ± 1.70
Diazinon	0.999	0.0009	0.0029	80.67 ± 2.20 - 93.77 ± 2.40
Ethion	0.998	0.0005	0.0015	82.71 ± 2.40 - 91.57 ± 2.20
Fenthion	0.999	0.0001	0.0003	74.03 ± 1.40 - 86.48 ± 1.40
Fenpyroximate	0.999	0.0003	0.0009	71.65 ± 1.90 - 90.44 ± 1.90
Phosalone	0.998	0.0001	0.0003	70.42 ± 3.40 - 82.12 ± 2.80
Glyphosate	0.998	0.0004	0.0015	80.88 ± 5.60 - 92.26 ± 3.60
Metasystox	0.999	0.0004	0.0020	72.12 ± 2.00 - 88.72 ± 2.30
CB Pesticides				
Aldicarb	0.997	0.0008	0.0027	90.13 ± 3.40 - 93.22 ± 3.80
Chlorpropham	0.995	0.0003	**0.0010**	72.45 ± 2.67 - 80.18 ± 2.30
Fenoxycarb	0.999	0.0003	0.0010	88.59 ± 3.66 - 90.37 ± 3.11
Thiophanate-methyl	0.999	0.003	0.009	74.58 ± 2.11 - 77.15 ± 2.98
PY Pesticides				
Cypermethrin	0.997	0.0006	**0.0010**	90.12 ± 2.88 - 91.65 ± 2.31
Deltamethrin	0.999	0.0010	**0.0030**	89.24 ± 2.13 - 90.83 ± 2.01
Fenvalerate	0.999	0.0003	**0.0010**	89.59 ± 2.47 - 90.91 ± 2.56
Permethrin	0.996	0.0007	**0.0025**	84.36 ± 2.01 - 86.41 ± 2.08
NC Pesticides				
Acetamiprid	0.999	0.007	0.049	81.32 ± 2.07 - 84.01 ± 2.06
Imidacloprid	0.997	0.013	0.040	80.26 ± 2.88 - 83.33 ± 2.38

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.

Table 3
Mean ± SD and quartiles of pesticide levels (mg/kg) of measured and simulated data.

	Simulated data*					Measured Data				
	Mean (mg/kg)	SD	Median	Percentiles		Mean (mg/kg)	SD	Median	Percentiles	
				75th	90th				75th	90th
OP Pesticides										
Chlorpyrifos	0.072	0.087	0.047	0.087	0.153	0.082	0.124	0.043	0.059	0.397
Diazinon	2.277	1.063	2.203	2.974	3.717	2.271	1.090	2.313	3.251	3.878
Ethion	1.809	0.733	1.677	2.179	2.771	1.812	0.709	1.684	2.436	2.845
Fenthion	0.490	0.334	0.420	0.675	0.958	0.490	0.337	0.465	0.698	1.136
Fenpyroximate	0.136	0.275	0.059	0.141	0.320	0.005	0.001	0.004	0.006	0.006
Phosalone	0.252	0.294	0.152	0.344	0.617	0.253	0.284	0.100	0.442	0.545
Glyphosate	1.440	1.045	1.172	1.803	2.681	1.449	0.963	1.180	2.251	3.104
Metasystox	0.172	0.109	0.153	0.249	0.336	0.164	0.119	0.163	0.230	0.315
CB Pesticides										
Aldicarb	0.004	0.001	0.004	0.004	0.005	0.004	0.001	0.004	0.004	0.005
Chlorpropham	0.005	0.001	0.005	0.006	0.007	0.006	0.001	0.005	0.006	0.008
Fenoxycarb	0.004	0.001	0.004	0.005	0.006	0.134	0.185	0.035	0.202	0.513
Thiophanate-methyl	0.012	0.002	0.012	0.014	0.015	0.012	0.002	0.012	0.014	0.015
PY Pesticides										
Cypermethrin	0.006	0.002	0.006	0.007	0.008	0.006	0.002	0.006	0.008	0.008
Deltamethrin	0.012	0.002	0.011	0.013	0.014	0.012	0.003	0.011	0.014	0.016
Fenvalerate	0.014	0.003	0.014	0.017	0.019	0.014	0.004	0.013	0.018	0.020
Permethrin	0.013	0.002	0.013	0.015	0.016	0.013	0.003	0.012	0.015	0.019
NC Pesticides										
Acetamiprid	0.131	0.032	0.127	0.149	0.173	0.131	0.034	0.123	0.142	0.196
Imidaclopride	0.120	0.015	0.117	0.130	0.142	0.119	0.017	0.115	0.129	0.151

OP: organophosphorus, CB: carbamate, PY: pyrethroid and NC: nicotinoid.
*As analyzed by monte carlo simulation.
SD: Standard Deviation.

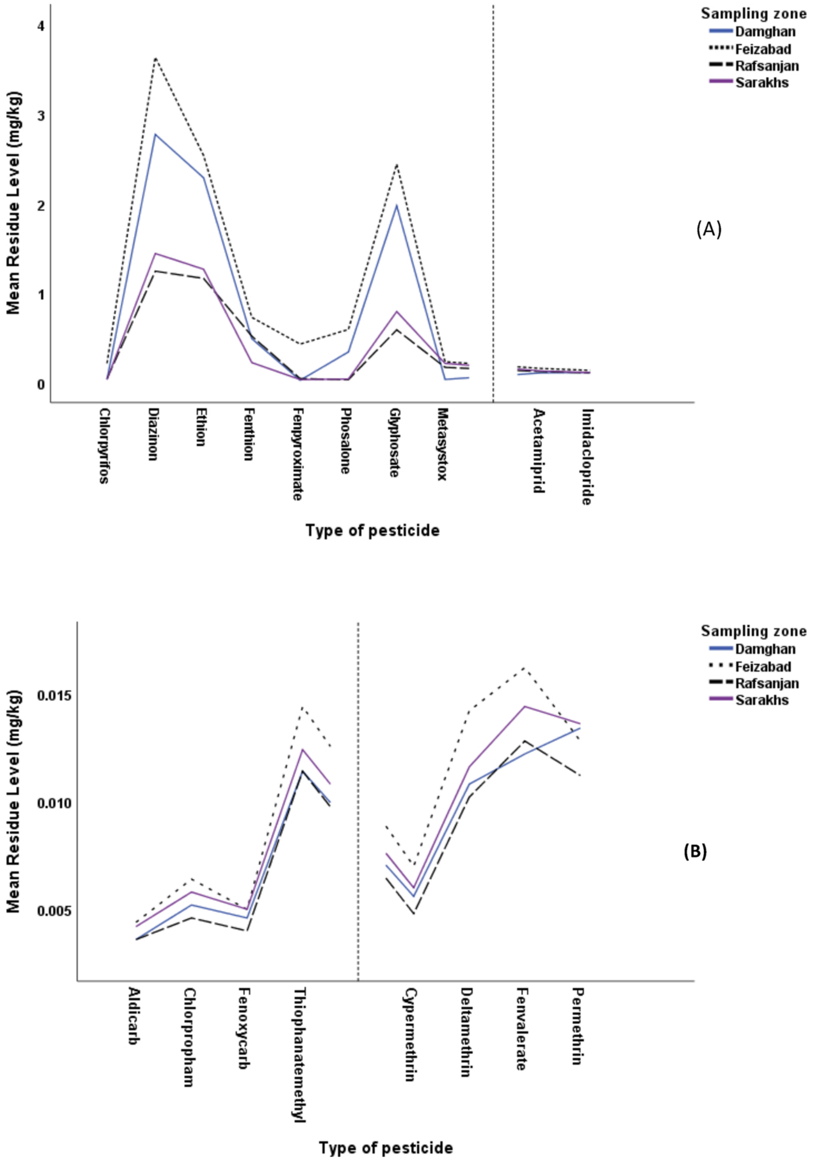


Fig. 2. Levels of OPs and NYs pesticides (A) and levels of CBs and PYs pesticides (B) in different sampling zones.

into four parts for OPs, CBs, PYs and NCs. All zones had similar levels of acetamiprid (p = 0.096) and imidaclopride (p = 0.105). Mean residue levels of PY were detectable in all samples but they were near LOD and LOQ values. The dispresion of data (standard deviation) was <0.01 mg/kg of pesticide residue. Sampling zones were not signficantly different with respect to cypermethrin (p = 0.205), deltamethrin (p = 0.085), fenvalerate (p = 0.424) and peremthrin (p = 0.513) levels.

A similar pattern was found in the pesticide residues in cultivars collected from different zones, for CBs levels. Levels of CBs were at least 3 to 10 times greater than the LOD values. None of the CBs showed statistically signficant differences among zones (aldicarb (p = 0.291), chlorpropharm (p = 0.102), fenoxycarb (p = 0.302) and thiophanate-methyl (p = 0.121)).

OPs comprised the only category of the pesticides that showed signficantly different levels among different zones, with the exception of chlorpyrifos (p = 0.075) and fenthion (p = 0.056). Feizabad had the highest residues levels for diazinon (3.63 ± 0.39 mg/kg, p = 0.002), ethion (2.53 ± 0.58 mg/kg, p = 0.002), fenpyroximate (0.43 ± 0.12 mg/kg, p = 0.009), phosalone (0.59 ± 0.30 mg/kg, p = 0.001), glyphosate (2.44 ± 0.97 mg/kg, p = 0.002), and metasystox (0.23 ± 0.18 mg/kg, p = 0.012).

Differences in pesticides residue levels among cultivars are shown in Fig. 3. None of the OPs showed statistically significant differences among five cultivars (p > 0.05) with the exception of fenvalerate (p = 0.006). All other pesticides in the category of OPs showed p values of 0.956 for chlorpyrifos, 0.652 for diazinon, 0.793 for ethion, 0.511 for fenthion, 0.598 for fenpyroximate, 0.667 for phosalone, 0.720 for glyphosate, and 0.473 for metasystox.

Within the category of CBs, the calculated p-values were 0.143 for aldicarb, 0.077 for chlorpropham, 0.059 for fenoxycarb, and 0.404 for thiophanate-methyl. PY levels also did not differ significantly (cypermethrin (p = 0.061), deltamethrin (p = 0.081) and permethrin (p = 0.061)). P-values for acetamiprid and imidaclopride were 0.598 and 0.158, respectively.

3.3. Risk assessment for oral exposure to pesticide residues via consumption of different pistachio cultivars

In this study, the potential risk of long-term health effects of consumption of Iranian pistachio was assessed based on the residue levels of 18 pesticides detected in our samples. In Table 4, the results of the

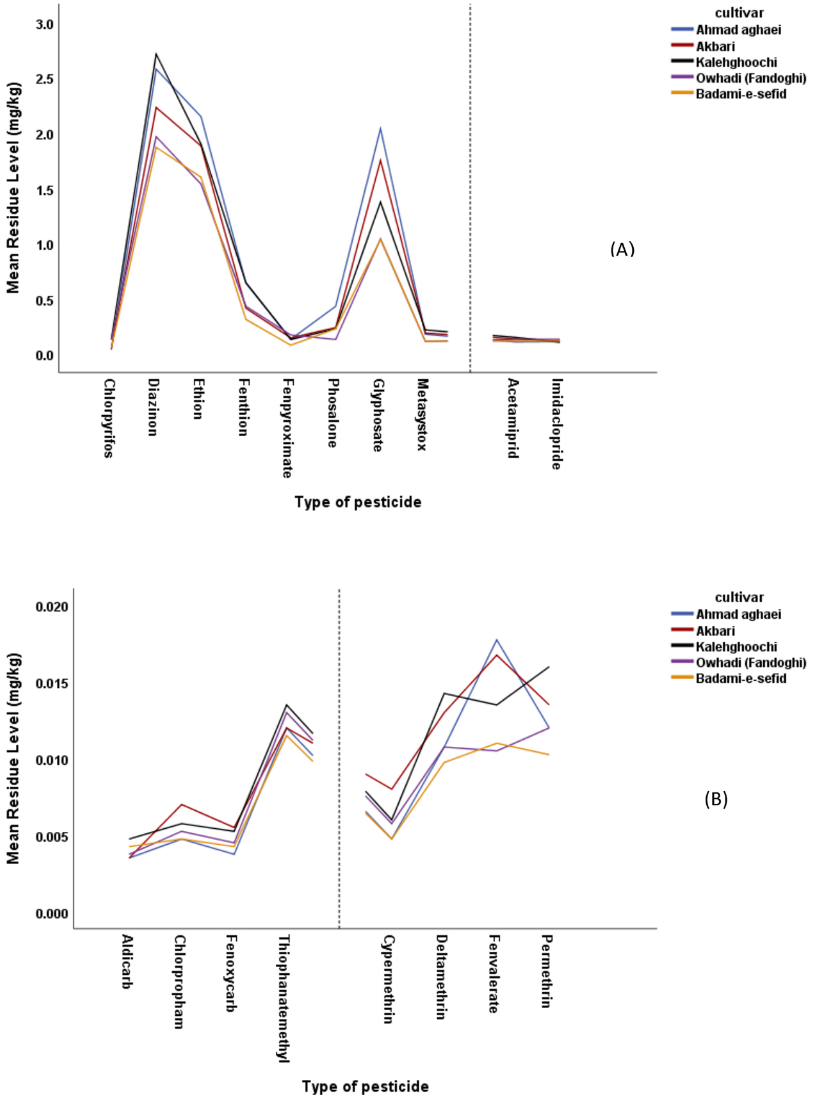


Fig. 3. Levels of OPs and NYs pesticides (A) and levels of CBs and PYs pesticides (B) in five pistachio cultivars.

HQs and HI_A based on pistachio consumption, are presented.

The contribution of pistachios' consumption to pesticides' exposure is generally low. Considering the 18 pesticides of the present study, the lowest contribution was for chlorpropham (0.0001%) while the highest was for thiophanate-methyl (0.164%). In relation to the dietary habits of various countries, in the Primo revision 3 tools, the highest contribution from pistachios consumption was observed for the children in Denmark followed by children and adults in France and the 11 countries participating in the Global Environment Monitoring System of WHO (including Iran). However, a higher consumption was reported for UK toddlers.

The exposure to 18 OP, CB, PY and NC pesticides via pistachio consumption was evaluated according to the measured pesticide levels and the simulated Monte Carlo procedures. The results showed the mean values of simulated data were the same as the measured data for both measured pesticide levels.

The HQ_S for the overall dietary intake were considerably higher than one for most of the pesticides, with the highest value for diazinon (4861 for median occurrence). Values below one were observed only for thiophanate-methyl, aldicarb and fenoxycarb (0.03, 0.6 and 0.8 for median occurrence, respectively).

The HI_A for the overall dietary intake was many times above one for five from six groups. The highest HI_A was observed for group A of neurotoxicity (5345 for median occurrence) followed by group B for reproductive and developmental toxicity (639 for median occurrence), group E for thyroid effects (134 for median occurrence), group C for systemic toxicity (113 for median occurrence), and group D for haematotoxicity (37 for median occurrence). Group F for carcinogenicity was the only group with HI_A below one for median occurrence (0.79), however approaching one (0.99) for occurrence at 90%.

4. Discussion

Pesticides are used for pre- and post-harvest protection of agricultural crops. Apparently, it is crucially important to employ fungicides, insecticides and pesticides either solely or in combination, for protection of nut trees from pests (Wang et al., 2017). These chemicals are also widely used in pistachio cultivation. Since most of the pesticides are hydrophobic chemicals, they may be markedly absorbed and accumulate in nuts with a lipidic nature (Cortés et al., 2008). Generally, humans are exposed to thousands of different chemicals which may exert additive or even synergistic effects (Tsatsakis et al., 2016). Beside their environmental effects, pesticide residues in crops might be transferred to humans via food consumption, creating global concern regarding their utilization (Quijano et al., 2016). Bioaccumulation of these chemicals may take place in many body organs and cause direct and indirect adverse effects on human health. Also, following their absorption from the water, soil, etc., by plants, pesticides can negatively

Table 4
Estimated HQs for single chemicals and cumulative HI_A for different classes of pesticides.

	Contribution %	Diet	HQ_S/HI_A			
			Mean	median	80%	90%
Group A - Neurotoxicity						
Chlorpyrifos	0.038%	FR adult	71	71	92	102
Deltamethrin	0.006%	DE child	7	7	8	9
Acetamiprid	0.034%	DE child	5	5	6	7
Diazinon	0.077%	DE child	4861	4862	5296	5526
Fenthion	0.082%	DE child	31	30	45	54
Phosalone	0.078%	DE child	14	13	22	27
Metasystox	0.057%	DE child	355	342	518	619
Aldicarb	0.068%	GEMS/Food G11	0.6	0.6	0.8	0.8
SUM			5345	5331	5988	6345
Group B – Reproductive and developmental toxicity						
Glyphosate	0.001%	GEMS/Food G11	204	199	298	349
Cypermethrin	0.001%	DK child	74	74	95	106
Ethion	0.083%	DE child	361	360	475	536
SUM			639	632	867	990
Group C – Systemic Toxicity						
Cypermethrin	0.001%	DK child	74	74	95	106
Fenpyroximate	0.023%	DE child	30	27	47	57
Fenvalerate	0.005%	DK child	7	7	9	10
Permethrin	0.027%	DE child	2	2	2	2
SUM			113	110	152	175
Group D- Haematotoxicity						
Chlorpropham	0.0001%	GEMS/Food G11	37	37	44	47
SUM			37	37	44	47
Group E – Thyroid effects						
Chlorpropham	0.0001%	GEMS/Food G11	37	37	44	47
Thiophanate-methyl	0.164%	FR adult	0.030	0.030	0.034	0.036
Imidaclopride	0.001%	NL child	97	97	108	114
SUM			134	134	152	162
Group F - Carcinogenicity						
Fenoxycarb	0.004%	DE child	0.79	0.79	0.92	0.99
SUM			0.79	0.79	0.92	0.99

HQs: Hazard Quotients.
HI: Hazard Index.

influence fruits and vegetables quality (Malarkodi et al., 2017). The levels of pesticide residues could be reduced by food processing methods such as storage, washing, drying, peeling, baking, canning and cooking, thermal processing, fermentation, infusion, juicing, malting, milling and parboiling (Kaushik et al., 2009). Most of the fruits and vegetables which are generally consumed raw or semi-processed, might also undergo the above-noted processing methods (Claeys et al., 2011), while nuts and especially pistachios are almost always consumed without any particular processing. Pistachio nuts are used in confectionary, and also in production of snack foods, ice-cream, bread, butter and sauces (Gentile et al., 2007); therefore, regular monitoring of the levels of chemical residues and/or contaminants is crucially important for improvement of food quality and safety.

Co-occurrence of pesticides in foods, particularly the neurotoxic ones, poses potential hazards to human health (García-Rodríguez et al., 2012). Combinations of pesticides of similar chemical structures do not necessarily exhibit additive effects; but, they may affect multiple organs and /or signalling pathways which consequently leads to different toxic effects (Hernández et al., 2013). In this study, we found various pesticides of different chemical categories in all analyzed samples and reported the 18 most-abundant pesticides which belong to four chemical classes.

Similar to our study, diazinon was the most frequently found pesticide in nuts analyzed in the study done by Cortés et al. (Cortés et al., 2008). In a previous study carried out in Rafsanjan, Iran, researchers reported that amitraz and parathion were applied more frequently for pistachio trees compared to other pesticides (Tavakolian Ferdosieyeh et al., 2012). Emami et al. also reported the pesticides residue levels in pistachios of different brands produced in Iran. In their report, the most abundant pesticides were fenitrothion (0.0486 mg/kg), carbaryl (0.0973 mg/kg) and diazinon (0.0432 mg/kg) (Emami et al., 2017). Similar findings were reported by Mousavi et al. in 2017 (Morteza et al., 2017).

European legislation (Regulation (EC) No 396/2005 and regulation (EC) No 1107/2009), permits food consumption when the residues of plant protection products are below the EC set MRLs (Larsson et al., 2018). The total levels of OP and NC pesticide residues found in our study, were above the MRLs in all samples, as in the study done by Bakırcı et al. in which pesticide residues in arugula, cucumber, lemon and grape, exceeded MRLs (Bakırcı et al., 2014). In a report from China, 26% of total food samples contained pesticide residues, and only in 3.62% of the samples, the residues' levels were above the MRLs (Li et al., 2016). Noteworthy, samples containing high levels of pesticide residues, may pose health hazards to consumers (Mahugija et al., 2017). Apparently, lack of proper training of agricultural workers, though being emphasized by the WHO, plays an important role in this issue (Salameh et al., 2004). Hence, to change the current situation, farmers have to be educated/ trained regarding safe use/practice of pesticides to employ these chemicals at appropriate doses. They also need to learn about the pre-harvest interval (i.e. the period/window between pesticides application and crop harvest) that will restrict/limit the use of pesticides before crop harvest, and may consequently reduce the level of pesticide residues.

For reason of comparison with other studies, where individual pesticides were assessed, and following the current regulatory practice of single-chemical risk assessment, we proceeded with characterization of the risk after exposure to single pesticides. In the present study and for reason of presentation, we preferred to apply the correction factor not in the HQ (for extrapolation from whole-diet to single food) but to the EDI (extrapolating from EDI for pistachios only to EDI from whole-diet) and we kept for the HQs the value of 1. The two approaches discussed in the original methodology are mathematically equivalent (Goumenou and Tsatsakis, 2019). Based on our results, the HQs were above 10 for eleven from the eighteen pesticides (diazinon > ethion > metasystox > glyphosate > imidochlopide > cypermethrin > chlorpyriphos > chlorpropham > fenthion > fenpyroximate > phosalone) indicating high risk and between 1 and 10 for four from the eighteen pesticides (deltamethrin > fenvalerate > acetamiprid > permethrin), indicating moderate risk. Only three pesticides had the respective HQs < 1 (fenoxycarb > aldicarb > thiophanate-methyl). Interestingly, though the level of fenoxycarb was higher than the respective MRL, it had HQs < 1, indicating no risk. On the contrary, chlorpropham and cypermethrin, both with HQs > 10, and deltamethrin, fenvalerate, and permethrin, with HQs between 1 and 10, were found in concentrations below the respective MRLs. However, these MRLs are set based on "lower limits of analytical determination" which are used for crops on which the pesticide has not been used or when its use had not left detectable residues and not based on risk assessment. The analytical limits of our method (both LOD and LOQ) were lower than the LODs used for setting these MRLs (see Table 2) and our study proves that, at least for certain pesticides, revision of the MRLs should be considered so as to ensure adequate protection of the consumers.

Grouping based on chemical stuctures is a well-known and globally used approach especialy in the cases of OPs and CBs, where neurotoxicity is considered the key adverse effect. However, this is not always the case as shown in Table 1. In this study, we followed the approach of the adversity specific HI_A, grouping the 18 pesticides according to their adversity and more precisely the adversity considered for the establishment of ADI (critical effect) and not the one related to their general mode of action. As a result, deltamethrin (PY) was grouped in group A of neurotoxicans, glyphosate and ethion (both OPs) were grouped in group B for reproductive and developmental toxicity and fenpyroximate (OP) in group C for systemic toxicity. Considering HI_A much higher than 10 in five from six advesrity groups, it is proved that the present level of contamination of pistachios is much higher than the acceptable level for this food commodity and extrapolated in the whole dieatry intake, it would lead to a considerable risk for neurotoxicty, reproductive and developmental toxicity, haematotoxicty, systemic toxicity and thyroid effects. Nevertheless, it should be highlighted that as our sampling was done during a limited period of time and samples were collected from limited cultivation zone, to have a clear picture of pesticides practice in pistachio cultivation in Iran, more frequent monitoring in higher numbers of cultivation zones should be done.

5. Conclusion

This paper reports the levels of eight OP, four CB, four PY and two NC pesticides in five pistachio cultivars collected from four different zones of Iran, as determined by GC—MS. Single-chemical and cumulative risk assessment were done to evaluate the effect of chronic consumption of these pistachios on consumers' health based on a newly proposed methodology for extrapolating from single food to exposure to whole diet and adversity groups based on critical effects. Our results showed that the HQs for single chemicals were above 1 for fifteen from the eighteen pesticides indicating the existence of health risk through chronic consumption. In a number of cases, the risk assessment results supported the existence of risk though the contamination levels were below the respective MRLs. This observation supports that the existing MRLs might need to be revised. Applying the HI_A approach for cumulative risk assessment, we concluded not only the existence of risk but also in which adversities this risk refers meaning neurotoxicity, reproductive and developmental toxicity, haematotoxicity, systemic toxicity and thyroid effects. However, no risk for carcinogenicity was indicated.

Our results ring the alarm bell that general awareness of Iranian farmers and pesticides-users need to be upgraded for a more cautious utilization of pesticides in agricultural practices. It is suggested to develop practicing biological pest control and organic agriculture, particularly in zones where pistachio-for-export is cultivated. Also, regulations should be updated, and pesticides usage must be limited as far as possible.

Conflict of interest

Authors declare that there is no conflict of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgment

The authors are thankful to Mashhad University of Medical Sciences, Iran.

References

Abbasi Ghaeni, F., Karimi, G., Mohsenzadeh, M.S., Nazarzadeh, M., Motamedshariaty, V.S., Mohajeri, S.A., 2018. Preparation of dual-template molecularly imprinted nanoparticles for organophosphate pesticides and their application as selective sorbents for water treatment. Sep. Sci. Technol. 1–10.

Akbari, B., Gharanfoli, F., Khayyat, M.H., Khashyarmanesh, Z., Rezaee, R., Karimi, G., 2012. Determination of heavy metals in different honey brands from Iranian markets. Food Addit. Contam. Part B 5, 105–111.

EFSA, 2008. Opinion of the Scientific Panel on Plant Protection products and their Residues to evaluate the suitability of existing methodologies and, if appropriate, the identification of new approaches to assess cumulative and synergistic risks from pesticides to human health with a view to set MRLs for those pesticides in the frame of Regulation (EC) 396/2005. EFSA J. 6, 705.

Bakırcı, G.T., Acay, D.B.Y., Bakırcı, F., Ötleş, S., 2014. Pesticide residues in fruits and vegetables from the Aegean region, Turkey. Food Chem. 160, 379–392.

Bopp, S.K., Barouki, R., Brack, W., Dalla Costa, S., Dorne, J.-L.C., Drakvik, P.E., Faust, M., Karjalainen, T.K., Kephalopoulos, S., van Klaveren, J., 2018. Current EU research activities on combined exposure to multiple chemicals. Environ. Int. 120, 544–562.

Claeys, W.L., Schmit, J.-F., Bragard, C., Maghuin-Rogister, G., Pussemier, L., Schiffers, B., 2011. Exposure of several Belgian consumer groups to pesticide residues through fresh fruit and vegetable consumption. Food Control 22, 508–516.

Cortés, J.M., Toledano, R.M., Villén, J.S., Vazquez, A., 2008. Analysis of pesticides in nuts by online reversed-phase liquid chromatography− gas chromatography using the through-oven transfer adsorption/desorption interface. J. Agric. Food Chem. 56, 5544–5549.

Dhouib, I., Jallouli, M., Annabi, A., Marzouki, S., Gharbi, N., Elfazaa, S., Lasram, M.M., 2016. From immunotoxicity to carcinogenicity: the effects of carbamate pesticides on the immune system. Environ. Sci. Pollut. Res. - Int. 23, 9448–9458.

Emami, A., Mousavi, Z., Ramezani, V., Shoeibi, S., Rastegar, H., Amirahmadi, M., Emami, I., 2017. Residue levels and risk assessment of pesticides in pistachio nuts in Iran. Iran. J. Toxicol. 11, 1–6.

Gallagher, S.S., Rice, G.E., Scarano, L.J., Teuschler, L.K., Bollweg, G., Martin, L., 2015. Cumulative risk assessment lessons learned: a review of case studies and issue papers. Chemosphere 120, 697–705.

García-Rodríguez, D., Cela-Torrijos, R., Lorenzo-Ferreira, R., Carro-Diaz, A., 2012. Analysis of pesticide residues in seaweeds using matrix solid-phase dispersion and gas chromatography–mass spectrometry detection. Food Chem. 135, 259–267.

Gentile, C., Tesoriere, L., Butera, D., Fazzari, M., Monastero, M., Allegra, M., Livrea, M.A., 2007. Antioxidant activity of Sicilian pistachio (Pistacia vera L. var. Bronte) nut extract and its bioactive components. J. Agric. Food Chem. 55, 643–648.

Georgiadis, N., Tsarouhas, K., Tsitsimpikou, C., Vardavas, A., Rezaee, R., Germanakis, I., Tsatsakis, A., Stagos, D., Kouretas, D., 2018. Pesticides and cardiotoxicity. Where do we stand? Toxicol. Appl. Pharmacol.

Guo, X., Mu, T., Xian, Y., Luo, D., Wang, C., 2016. Ultra-performance liquid chromatography tandem mass spectrometry for the rapid simultaneous analysis of nine organophosphate esters in milk powder. Food Chem. 196, 673–681.

Gupta, R.C., 2011. Toxicology of Organophosphate and Carbamate Compounds. Academic Press.

Hassani, S., Sepand, M., Jafari, A., Jaafari, J., Rezaee, R., Zeinali, M., Tavakoli, F., Razavi-Azarkhiavi, K., 2015. Protective effects of curcumin and vitamin E against chlorpyrifos-induced lung oxidative damage. Hum. Exp. Toxicol. 34, 668–676.

Goumenou, M., Tsatsakis, A., 2019. Proposing two new approaches for the risk characterisation of single chemicals and chemicals' mixtures: the Source Related Hazard Quotient (HQS) and the Adversity Specific Hazard Index (HIA). accepted for publication on. Toxicol. Lett.

Hernández, A.F., Parrón, T., Tsatsakis, A.M., Requena, M., Alarcón, R., López-Guarnido, O., 2013. Toxic effects of pesticide mixtures at a molecular level: their relevance to human health. Toxicology 307, 136–145.

Ilyushina, N., Goumenou, M., Stivaktakis, P.D., Vardavas, A.I., Masaltsev, G., Averianova, N., Dmitricheva, O., Revazova, Y., Tsatsakis, A.M., Rakitskii, V., 2019. Maximum tolerated doses and erythropoiesis effects in the mouse bone marrow by 79 pesticides' technical materials assessed with the micronucleus assay. Toxicol. Rep. 6, 105–110.

Kalliora, C., Mamoulakis, C., Vasilopoulos, E., Stamatiades, G.A., Kalafati, L., Barouni, R., Karakousi, T., Abdollahi, M., Tsatsakis, A., 2018. Association of pesticide exposure with human congenital abnormalities. Toxicol. Appl. Pharmacol. 346, 58–75.

Katsikantami, I., Colosio, C., Alegakis, A., Tzatzarakis, M.N., Vakonaki, E., Rizos, A.K., Sarigiannis, D.A., Tsatsakis, A.M., 2018. Estimation of daily intake and risk assessment of organophosphorus pesticides based on biomonitoring data–the internal exposure approach. Food Chem. Toxicol.

Kaushik, G., Satya, S., Naik, S., 2009. Food processing a tool to pesticide residue dissipation–a review. Food Res. Int. 42, 26–40.

Kortenkamp, A., Backhaus, T., Faust, M., 2009. State of the Art Review on Mixture Toxicity-final Report, Executive Summary. Report to the Commission of the European Union, Directorate General for the Environment.

Larsson, M.O., Nielsen, V.S., Bjerre, N., Laporte, F., Cedergreen, N., 2018. Refined assessment and perspectives on the cumulative risk resulting from the dietary exposure to pesticide residues in the Danish population. Food Chem. Toxicol. 111, 207–267.

Lemly, A.D., 1996. Evaluation of the hazard quotient method for risk assessment of selenium. Ecotoxicol. Environ. Saf. 35, 156–162.

Li, Z., Nie, J., Lu, Z., Xie, H., Kang, L., Chen, Q., Li, A., Zhao, X., Xu, G., Yan, Z., 2016. Cumulative risk assessment of the exposure to pyrethroids through fruits consumption in China–based on a 3-year investigation. Food Chem. Toxicol. 96, 234–243.

López-Blanco, R., Moreno-González, D., Nortes-Méndez, R., García-Reyes, J.F., Molina-Díaz, A., Gilbert-López, B., 2017. Experimental and theoretical determination of pesticide processing factors to model their behavior during virgin olive oil production. Food Chem.

Lozowicka, B., Kaczynski, P., Paritova, A., Kuzembekova, G., Abzhalieva, A., Sarsembayeva, N., Alihan, K., 2014. Pesticide residues in grain from Kazakhstan and potential health risks associated with exposure to detected pesticides. Food Chem. Toxicol. 64, 238–248.

Mahugija, J.A., Khamis, F.A., Lugwisha, E.H., 2017. Determination of levels of organochlorine, organophosphorus, and pyrethroid pesticide residues in vegetables from markets in Dar Es Salaam by GC-MS. Int. J. Anal. Chem. 2017.

Malarkodi, C., Rajeshkumar, S., Annadurai, G., 2017. Detection of environmentally hazardous pesticide in fruit and vegetable samples using gold nanoparticles. Food Control 80, 11–18.

Means, B., 1989. Risk-assessment guidance for superfund. Human Health Evaluation Manual. Part A. Interim Report (Final) Volume 1 Environmental Protection Agency, Washington, DC (USA). Office of Solid Waste ….

Mohammadi, H., Karimi, G., Mahdi Rezayat, S., Reza, A., Shafiee, H., Nikfar, S., Baeeri, M., Sabzevari, O., Abdollahi, M., 2011. Benefit of nanocarrier of magnetic magnesium in rat malathion-induced toxicity and cardiac failure using non-invasive monitoring of electrocardiogram and blood pressure. Toxicol. Ind. Health 27, 417–429.

Moretto, A., Di Renzo, F., Giavini, E., Metruccio, F., Menegola, E., 2015. The use of in vitro testing to refine cumulative assessment groups of pesticides: the example of teratogenic conazoles. Food Chem. Toxicol. 79, 65–69.

Morteza, Z., Mousavi, S.B., Baghestani, M.A., Aitio, A., 2017. An assessment of agricultural pesticide use in Iran, 2012-2014. J. Environ. Health Sci. Eng. 15, 10.

Mrema, E.J., Rubino, F.M., Brambilla, G., Moretto, A., Tsatsakis, A.M., Colosio, C., 2013. Persistent organochlorinated pesticides and mechanisms of their toxicity. Toxicology 307, 74–88.

Organization, W.H, 2009. Principles and Methods for the Risk Assessment of Chemicals in Food.

Pico, Y., El-Sheikh, M.A., Alfarhan, A.H., Barceló, D., 2018. Target vs non-target analysis to determine pesticide residues in fruits from Saudi Arabia and influence in potential risk associated with exposure. Food Chem. Toxicol. 111, 53–63.

Products, E.P.o.P.P., Residues, t, 2013. Scientific Opinion on the relevance of dissimilar mode of action and its appropriate application for cumulative risk assessment of pesticides residues in food. EFSA J. 11, 3472.

Quijano, L., Yusà, V., Font, G., Pardo, O., 2016. Chronic cumulative risk assessment of the exposure to organophosphorus, carbamate and pyrethroid and pyrethrin pesticides through fruit and vegetables consumption in the region of Valencia (Spain). Food Chem. Toxicol. 89, 39–46.

Regulation, E, 2009. 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. EU, Brussels.

Renieri, E.A., Goumenou, M., Kardonsky, D.A., Veselov, V.V., Alegakis, A.K., Buha, A., Tzatzarakis, M.N., Nosyrev, A.E., Rakitskii, V.N., Kentouri, M., 2019. Indicator PCBs in farmed and wild fish in Greece-Risk assessment for the Greek population. Food Chem. Toxicol. 127, 260–269. https://doi.org/10.1016/j.fct.2019.03.027.

Rezaee, R., Mahmoudi, M., Abnous, K., Rabe, S.Z.T., Tabasi, N., Hashemzaei, M., Karimi, G., 2013. Cytotoxic effects of crocin on MOLT-4 human leukemia cells. J. Complement. Integr. Med. 10, 105–112.

Rotter, S., Beronius, A., Boobis, A., Hanberg, A., van Klaveren, J., Luijten, M., Machera, K., Nikolopoulou, D., van der Voet, H., Zilliacus, J., 2018. Overview on legislation and scientific approaches for risk assessment of combined exposure to multiple chemicals: the potential EuroMix contribution. Crit. Rev. Toxicol. 1–19.

Saillenfait, A.-M., Ndiaye, D., Sabaté, J.-P., 2015. Pyrethroids: exposure and health effects–an update. Int. J. Hyg. Environ. Health 218, 281–292.

Salameh, P.R., Baldi, I., Brochard, P., Saleh, B.A., 2004. Pesticides in Lebanon: a knowledge, attitude, and practice study. Environ. Res. 94, 1–6.

Sarigiannis, D.A., Hansen, U., 2012. Considering the cumulative risk of mixtures of chemicals–a challenge for policy makers. Environ. Health 11, S18.

Song, S., Zhang, C., Chen, Z., He, F., Wei, J., Tan, H., Li, X., 2018. Simultaneous determination of neonicotinoid insecticides and insect growth regulators residues in honey using LC–MS/MS with anion exchanger-disposable pipette extraction. J. Chromatogr. A 1557, 51–61.

Taghizadeh, S.F., Davarynejad, G., Asili, J., Nemati, S.H., Rezaee, R., Goumenou, M., Tsatsakis, A.M., Karimi, G., 2017a. Health risk assessment of heavy metals via dietary intake of five pistachio (Pistacia vera L.) cultivars collected from different geographical sites of Iran. Food Chem. Toxicol. 107, 99–107.

Taghizadeh, S.F., Davarynejad, G., Asili, J., Riahi-Zanjani, B., Nemati, S.H., Karimi, G., 2018a. Chemical composition, antibacterial, antioxidant and cytotoxic evaluation of the essential oil from pistachio (Pistacia khinjuk) hull. Microb. Pathog. 124, 76–81.

Taghizadeh, S.F., Davarynejad, G., Nemati, S.H., Karimi, G., 2017b. Assessment of phenolic profile and antioxidant power of five pistachio (Pistacia vera) cultivars collected from four geographical regions of Iran. Avicenna J. Phytomed. 1–11.

Taghizadeh, S.F., Rezaee, R., Davarynejad, G., Asili, J., Nemati, S.H., Goumenou, M., Tsakiris, I., Tsatsakis, A.M., Shirani, K., Karimi, G., 2018b. Risk assessment of exposure to aflatoxin B1 and ochratoxin A through consumption of different Pistachio (Pistacia Vera L.) cultivars collected from four geographical regions of Iran. Environ. Toxicol. Pharmacol.

Taghizadeh, S.F., Rezaee, R., Davarynejad, G., Karimi, G., Nemati, S.H., Asili, J., 2018c. Phenolic profile and antioxidant activity of Pistacia vera var. Sarakhs hull and kernel extracts: the influence of different solvents. J. Food Meas. Charact. 1–7.

Tavakolian Ferdosieyeh, V., Karimifared, M., Esmaeili, A., Hassanshahi, G., Vazirinejad, R., 2012. Pesticide induced complications among pistachio farmers: in the rural area of Rafsanjan, Iran (2010). J. Occup. Health Epidemiol. 1, 111–117.

Tsakiris, I.N., Goumenou, M., Tzatzarakis, M.N., Alegakis, A.K., Tsitsimpikou, C., Ozcagli, E., Vynias, D., Tsatsakis, A.M., 2015. Risk assessment for children exposed to DDT residues in various milk types from the Greek market. Food Chem. Toxicol. 75, 156–165.

Tsatsakis, A., Kouretas, D., Tzatzarakis, M., Stivaktakis, P., Tsarouhas, K., Golokhvast, K., Rakitskii, V., Tutelyan, V., Hernandez, A., Rezaee, R., 2017. Simulating real-life exposures to uncover possible risks to human health: a proposed consensus for a novel methodological approach. Hum. Exp. Toxicol. 36, 554–564.

Tsatsakis, A.M., Docea, A.O., Tsitsimpikou, C., 2016. New challenges in risk assessment of chemicals when simulating real exposure scenarios; simultaneous multi-chemicals' low dose exposure. Food Chem. Toxicol. 96, 174–176.

Vucinic, S., Antonijevic, B., Tsatsakis, A.M., Vassilopoulou, L., Docea, A.O., Nosyrev, A.E., Izotov, B.N., Thiermann, H., Drakoulis, N., Brkic, D., 2017. Environmental exposure to organophosphorus nerve agents. Environ. Toxicol. Pharmacol. 56, 163–171.

Wang, J., Gong, Z., Zhang, T., Feng, S., Wang, J., Zhang, Y., 2017. Simultaneous determination of 106 pesticides in nuts by LC–MS/MS using freeze-out combined with dispersive solid-phase extraction purification. J. Sep. Sci. 40, 2398–2405.

Zentai, A., Szabó, I.J., Kerekes, K., Ambrus, Á., 2016. Risk assessment of the cumulative acute exposure of Hungarian population to organophosphorus pesticide residues with regard to consumers of plant based foods. Food Chem. Toxicol. 89, 67–72.