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Original Research

Determining Pesticide Residues in Honey and their Potential Risk to Consumers

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Abstract

Forty-six organochlorine, organophosphorous, pyrethroid, and organonitrogen pesticides were analyzed in honey samples collected from 18 apiaries located in 9 centers in Kafr El-Sheikh governorate, Egypt, during 2011 by the QuEChERS method followed by gas chromatography. The recovery results ranged from 84.20 to 120.30%. The method provided limits of detection (LOD) in the range of 0.001-0.168 $mg \cdot kg^{-1}$. The results indicated that residues of the tested pesticides were detected in 55.6% of the collected samples and most of the detected pesticides belonged to the organochlorine and organophosphorous groups. Concerning the most detected pesticide residues, dicofol was found in 38.9% of the samples analyzed owing to its applications to control *Varroa destructor*. Other acaricides used by beekeepers against *Varroa destructor* were also detected (i.e., bromopropylate, tetradifon, malathion), indicating that the chemicals used by apiculturists inside the hives in order to control disease are the main pollutants of the produced honey. 81.8% of the detected pesticides exceeded the European Union maximum residue levels (EU MRLs). Data obtained were then used for estimating the potential health risks associated with exposure to these pesticides. Estimated daily intake (EDI) of the detected pesticides were much lower than acceptable daily intakes (ADIs), which show that honey consumption has a minimal contribution to toxicological risk. Our study suggests the need for regularly monitoring programs for pesticide residues in honey at the national level to protect consumer health.

Keywords: monitoring, pesticide residues, honey, QuEChERS, estimated daily intake

Introduction

Honey and bee products have the image of being natural, healthy, and clean [1]. It is often consumed by children, the eldery and ill people, particularly in developing countries. Therefore, honey must be free of any chemical contamination and safe for human consumption. However, the over-reliance on pesticides has caused several environmental problems, including pesticide residues in food, which constitutes a potential risk for human health. Cotton plants are very popular among the beekeepers due to their unique nectar secretion during the summer, a period with no other blooming of beekeeping importance. The main pitfall of cotton plants is the great number of different pesticide applications, resulting in bee population decline and contamination of honey [2]. Nowadays, bee products are produced in an environment contaminated by various pollutants. Pesticide application in crops can contaminate soil,

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air, water, and the flowers from which bees collect nectar for honey production, which may cause the introduction of those toxic chemicals into the food chain, affecting human health [3, 4].

In other words, hives could be contaminated by direct or indirect exposure. In the first case, the pesticide residues may originate from the treatment of bee hives with acaricides in the control of *Varroa destructor*. In the second case, the bees can get in touch with those pesticides during the foraging activities in an average radius of 3-6 km around the hive [5]. The honey benefits can be suppressed by pesticides introduced to honey during its processing and arising from both agricultural and beekeeping practices [6].

Therefore, the determination of contaminants and residues in honey and other bee products has become a growing concern in recent years, especially as these compounds may diminish the beneficial properties of honey and, if present in significant amounts, may pose a serious threat to human health. Monitoring pesticide residues in honey helps to assess the potential risk of this product to consumer health and gives information on the pesticide treatments that have been used in field crops surrounding the hives [7]. Several authors have indicated that bees and their products may be used as biological indicators of the environmental pollution present in the area where they fly [8-10].

There are three main purposes for monitoring bee products: consumer health protection, international commercial competition, and better product quality [11]. However, little has been done yet to monitor pesticide residues in honey in Egypt. In addition, attention must be given to evaluate the potential health risks associated with exposure to such residues in honey.

The determination of pesticide residues in honey at trace levels is a challenging task owing to the complex matrix of honey and its high sugar content [12]. The green approach to analytical chemistry, as well as environmental and economic concerns, have persuaded analysts to use smaller samples and reduced solvent volumes in analytical procedures [13]. Nowadays, the most universal extraction method to analyze a wide range of pesticides is the "QuEChERS method," which stands for quick, easy, cheap, efficient, rugged, and safe. Essentially, it is based on the extraction of the analytes from the sample matrix with an organic solvent (commonly acetonitrile) followed by the removal of interference using a clean-up sorbent; then, the purified extracts are finally analyzed by the appropriate analytical technique [14]. The QuEChERS method reduces the number of steps in the analytical procedure, thereby minimizing potential sources of error [15].

In light of these concerns, the aim of this study was to monitor pesticide residues in cotton honey samples collected from various apiaries located in 9 centers in Kafr El-Sheikh governorate, Egypt, during 2011's year by QuEChERS method. Risk assessment was also performed by calculating the estimated daily intake (EDI) and compared to the acceptable daily intake (ADI) for all the detected pesticides.

Materials and Methods

Chemicals

Pesticides standards were either purchased from Dr. Ehrenstorfer Gmbh. (Augsburg, Germany) or provided by the Food and Agriculture Organization of the United Nations (Rome, Italy), and most of them were of >99% certified purity. Individual stock standard solutions of pesticides were prepared by dissolving each compound in ace-

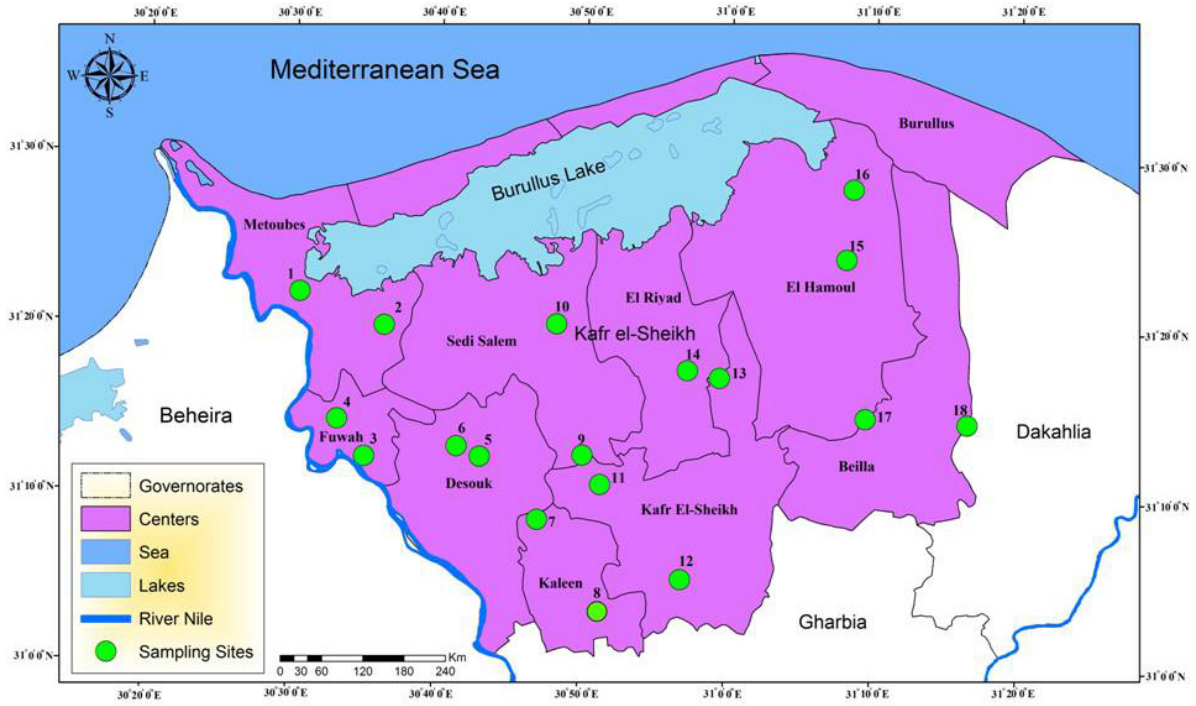

Fig. 1. Map of honey sampling sites in Kafr El-Sheikh governorate, Egypt.

tone and storing in glass flasks at -20ºC. Mixed compound calibration solutions, in acetone, were prepared from the stock solutions and used as spiking solutions as well. Matrix-matched standards were prepared in the same concentration as that of calibration solutions, by adding appropriate amounts of standards to the control matrix. All other organic solvents and reagents were of analytical grade and purchased from standard commercial suppliers.

Sample Collection

Honey samples represented the locally produced honey in 18 apiaries located in 9 centers in Kafr El-Sheikh governorate, Egypt, were directly collected just after harvesting of 2011 cotton honey (Fig. 1). All honey samples (weighing 500 g for each sample) were stored at -20ºC until extraction and analysis.

Sample Preparation

The sample preparation procedure usually involves homogenization, extraction, pre-concentration (when needed), cleanup, and final concentration prior to instrumental analysis. The procedure of Lehotay et al. [16] was used for extraction and purification of pesticide residues from honey samples as described below. Each honey sample (5 g) was weighed into a 50 ml PTFE tube and dissolved in 10 ml deionized water by shaking for one minute. Acetonitrile acidified with acetic acid (10 ml), 1.0 g sodium acetate, and 4.0 g anhydrous magnesium sulphate were added and shaken vigorously for one minute. The samples were centrifuged at 4,000 rpm for 2 min. Six ml of the upper clear solution (extracts) was transferred into 15 ml polyethylene tube containing 0.4 g primary secondary amine (PSA) sorbent and 0.6 g anhydrous magnesium sulphate. The tubes were capped, then the extract with the sorbent/dessicant mixed vigorously for one minute and centrifuged at 4,000 rpm for 2 min. Four ml of the clear solution was transferred into a 15 ml glass tube, and 50 µl tetradecan was added as a keeper and evaporated in a turbovab at 40ºC to dryness. The residues were dissolved in 2 ml of injection standard and 1 µl of the sample was injected into a gas chromatography-nitrogen phosphorous detector (GC-NPD) and gas chromatography-electron capture detector (GC-ECD).

Apparatus

Gas chromatographs: (1) Hewlett-Packard Model 5890 equipped with a double electron capture detector with 2 capillary columns, an injector at 225ºC, and a detector at 300ºC. Operating conditions: nitrogen carrier gas, 1.3 $ml \cdot min^{-1}$; carrier and makeup gas, 75-90 ml/min; and column head pressure, 82 kPa. (2) A Hewlett-Packard Model 5890 equipped with a double nitrogen-phosphorus detector, an injector at 225ºC, and a detector at 280ºC. Operating conditions: hydrogen, 3.5±0.1 $ml \cdot min^{-1}$; air, 100-200 $ml \cdot min^{-1}$; and nitrogen carrier gas, 25 $ml \cdot min^{-1}$. The information on chromatography columns was as follows:

(1) PAS-5 tested Ultra 2 Silicon, 25 m length × 0.32 mm id, and 0.52 mm film thickness.
(2) PAS-1701 tested 1701 Silicon, 25 m length × 0.32 mm id × 0.25 mm film thickness.

Temperature programs for both GC instruments were as follows: initial oven temperature, 90ºC hold for 2 min then a 20ºC min^{-1} ramp to 150ºC, followed by a 6ºC min^{-1} ramp to 270ºC hold for 15 min. For both GCs, the splitless injection mode was used with injection volume 1 µl.

Detection and confirmation of the presence of pesticide residues in honey samples depends on the use of chromatography columns of different polarities. An internal standard technique was used for quantitation. Aldrin for organochlorine and pyrethroids compounds with electron capture detection (ECD), and ditalimfos for organophosphorus and nitrogen-containing compounds with nitrogen-phosphorus detection (NPD) were used as internal standards.

Validation Studies

The analytical method and instruments were fully validated as part of a laboratory quality assurance system and were audited and accredited by the Centre for Metrology and Accreditation, Finnish Accreditation Service (FINAS), Helsinki, Finland. This quality system is referred to as SFS-EN ISO/IEC 17025:2005.

The method was validated in terms of recovery and limits of detection. A recovery study was performed in triplicate by adding known quantities of pesticide standard solutions to aliquots of 5 g of homogenized honey. The samples were then analyzed according to the proposed method in order to calculate extraction efficiency. The concentrations and recoveries were calculated from the matrix – matched calibration curves. Blank analyses were performed to determine possible interference from the sample.

Limit of detection (LOD) was determined considering it as 3 times the baseline noise, in a time close to the retention time of each analyte [17, 18]. LOD values were estimated by analyzing blank samples fortified with standards at levels producing signals at signal-to-noise ratios of 3. All the analyses were performed in triplicate. The average recoveries of the test compounds ranged from 84.20 to 120.30% at the spiking levels shown in Table 1. The limit of detection in honey samples ranged between 0.001 and 0.168 mg/kg.

Results and Discussion

This study was conducted to investigate the presence of pesticide residues (originating either from applications on agricultural crops or beehive treatments) in honey produced in the various parts of Kafr El Sheikh governorate, Egypt. Honey samples were analyzed for 46 pesticides, which included organophosphorus, organonitrogen, organochlorine, and certain pyrethroids compounds. As can be observed in Table 2, of the 90 analyzed samples (18 apiary; five samples from each apiary), pesticide residue monitoring showed that 44.4% of the samples contained no

Table 1. Mean recovery (%) and limits of detection (LOD) of the tested pesticides.

No.	Pesticide	Chemical family	Spiking level (µg/g)	Mean recovery (%)±SD (%)	Limit of detection (LOD)
1	α-HCH	Organochlorine	0.02	90.80±1.00	0.001
2	β-HCH	Organochlorine	0.03	103.0±10.1	0.017
3	γ-HCH	Organochlorine	0.02	86.70±5.40	0.003
4	δ-HCH	Organochlorine	0.05	101.3± 6.00	0.009
5	α-Endosulfan	Organochlorine	0.02	108.3±6.90	0.009
6	β-Endosulfan	Organochlorine	0.02	110.7±7.40	0.018
7	p,p`-DDD	Organochlorine	0.02	108.5±6.10	0.004
8	p,p`-DDE	Organochlorine	0.02	96.70±6.80	0.004
9	Dieldrin	Organochlorine	0.02	114.5±2.90	0.002
10	Endrin	Organochlorine	0.05	110.2±4.30	0.006
11	Heptachlor	Organochlorine	0.02	91.40±12.9	0.014
12	Heptachlorepoxid	Organochlorine	0.02	110.5±5.70	0.003
13	Chlorpyrifos-ethyl	Organophosphorus	0.05	105.3±6.70	0.010
14	Chlorpyrifos-methyl	Organophosphorus	0.05	96.70±1.60	0.002
15	Diazinon	Organophosphorus	0.05	97.20±1.70	0.003
16	Fenitrothion	Organophosphorus	0.05	113.2±6.50	0.010
17	Fenthion	Organophosphorus	0.05	97.00±6.20	0.009
18	Malathion	Organophosphorus	0.05	93.30±1.90	0.003
19	Parathion-ethyl	Organophosphorus	0.05	120.3±8.10	0.013
20	Parathion-methyl	Organophosphorus	0.05	91.80±4.00	0.006
21	Phosalone	Organophosphorus	0.05	91.80±13.4	0.020
22	Pirimiphos-ethyl	Organophosphorus	0.05	120.2±8.00	0.012
23	Pirimiphos-methyl	Organophosphorus	0.05	95.00±11.1	0.017
24	Profenofos	Organophosphorus	0.05	84.20±14.6	0.022
25	Prothiofos	Organophosphorus	0.05	103.4±5.10	0.060
26	Triazophos	Organophosphorus	0.05	89.30±3.80	0.006
27	Bendiocarb	Carbamate	0.10	101.8±7.50	0.011
28	Carbosulfan	Carbamate	0.05	107.3±5.60	0.008
29	Pirimicarb	Carbamate	0.05	108.5±5.10	0.008
30	α-Cypermethrin	Pyrethroids	0.20	103.7±5.20	0.168
31	Cyfluthrin	Pyrethroids	0.05	101.7±10.3	0.015
32	Fenvalerate	Pyrethroids	0.05	103.0±4.41	0.007
33	λ-Cyhalothrin	Pyrethroids	1.00	109.8±2.30	0.068
34	Permethrin	Pyrethroids	0.30	106.5±4.50	0.087
35	Dicofol	Organochlorine	0.07	106.0±5.80	0.025
36	Tetradifon	Organochlorine	0.05	109.8±9.60	0.014
37	Bromopropylate	Benzilate	0.05	97.70±7.50	0.011
38	Bupirimate	pyrimidinol	0.05	105.3±3.40	0.005
39	Chlorothalonil	Chloronitrile	0.02	109.7±8.60	0.012

Table 1. Continued.

No.	Pesticide	Chemical family	Spiking level (μg/g)	Mean recovery (%)±SD (%)	Limit of detection (LOD)
40	Pyrazophos	Phosphorothiolate	0.05	96.80±7.00	0.010
41	Tolclofos-methyl	Phosphorothioate	0.05	104.3±9.80	0.015
42	Iprodione	Dicarboximide	0.20	104.8±12.1	0.148
43	Procymidone	Dicarboximide	0.05	106.8±6.00	0.009
44	Vinclozolin	Dicarboximide	0.02	114.5±4.20	0.003
45	Atrazine	Triazine	0.05	86.70±7.50	0.011
46	Trifluralin	Dinitroaniline	0.02	105.8±7.10	0.004

Purpose of use (1-34) insecticides, (35-37) acaricides, (38-44) fungicides, (45-46) herbicides.

detectable residues of the target pesticides, and most of the pesticides found belonged to the organochlorine and organophosphorous groups. Residues of organophosphates viz. diazinon, chlorpyrifos, fenitrothion, and profenofos were detected. Acaricides used by beekeepers to combat varroatosis (i.e., dicofol, tetradifon, bromopropylate and malathion) also were detected, indicating that the chemicals used by apiculturists inside the hives in order to control disease are the main pollutants of the produced honey. Concerning the most frequently detected pesticide residues, dicofol was found in 38.9% of the samples analyzed owing to its applications to control *Varroa destructor*, a parasitic mite that affects honeybee colonies in the area studied. The use of synthetic pesticides for crop protection is the easiest and most effective way for beekeepers to control mites. A major problem could be the use of unauthorized products in order to control Varroatosis. Other pesticides such as β-HCH, γ-HCH, and carbamate pesticide pirimicarb were also found. The contamination of the area surrounding bee colonies markedly influences the type and concentration of contaminants found in the honey samples. The European maximum residue limits (MRLs) were followed due to lack of Codex MRLs of target pesticides on honey. The European regulation 396/2005 EC set the limit at 10 μg kg-1 for substances for which no MRL had been established. Since 1 September 2008 the European Commission has set new MRLs, which mostly are between 10 and 50 $ng \cdot g^{-1}$ in honey [19]. 81.8% of the detected pesticides exceeded the European Union maximum residue levels (EU MRLs).

Results revealed that organophosphorus were the most frequently detected pesticides in honey, followed by organochlorines. Although organochlorine pesticide usage has been completely prohibited by law since 1986 in Egypt [20] the results obtained could be expected, because those pesticides and their metabolites have been extensively used and are still present in the environment, owing to their high persistence. Organochlorine pesticides are lipophilic substances and consequently are soluble and stable in beeswax. Therefore, an amount of these substances gradually migrates from wax into the stored honey [21].

HCHs are mixtures of different isomers. Commercial HCH products mainly include technical HCHs and lindane. Technical HCHs primarily consist of α-HCH (55-80%, w/w), β-HCH (5-14%), γ-HCH (8-15%), and δ-HCH (2-16%), while lindane mainly contains γ-HCH (>98%). In addition, among the HCH isomers, β-HCH is the most persistent, is less volatile, and tends to be more bioaccumulative than the other HCH isomers [22]. Many studies have shown that residues of organochlorine pesticides (OCPs) bio-accumulate in plants from polluted soil from historical agricultural applications [23, 24]. Bio-accumulation levels in plant tissues can reach 10 to 1,000 times greater than those in ambient environmental media such as air and water [21]. OCPs can enter the food chain via not only fatty products [25], but also non-fatty products such as honey [26, 27].

Compounds detected and the range of concentrations is comparable with other studies. Antonescu and Mateescu [28] analyzed OCPs in 265 honey samples collected in Romania and found that 50% and 25% were positive for HCHs and dichlorodiphenyltrichloroethanes (DDTs), respectively. Blasco et al. [26] reported residues of hexachlorobenzene (HCB) and HCHs in 14, honey samples from Valencia, Spain. Wang et al. [22] found that honey samples from developing countries generally contained higher concentrations of HCHs, ΣDDTs, Σchlordanes, and HCB than those from developed countries. Malathion residues were detected in all the samples of locally produced honey, in Bauru (State of Sao Paulo, Brazil) during 2003-04, in a high concentration, owing to its applications to control dengue mosquitoes in the area studied [29]. Chlorpyrifos and λ-cyhalothrin residues were found in two of 11 honey samples from Brazil at concentrations below maximum residue limit (MRL $<1 \mu g \cdot g^{-1}$) established for food products [13]. A multi residue analysis was developed to quantify 80 environmental contaminants, pesticides and veterinary drugs belonging to different chemical classes, in honeys, honeybees, and pollens from France. In total, 36 compounds were detected but only 10 compounds were detected in all the matrices that can be used by beekeepers to combat varroa [30]. Concentration levels of 30 pesticide residues were measured in honey samples collected from apiaries in northern Poland (Pomerania) using a method based on QuEChERS extraction followed by liquid chromatography-tandem mass spectrometry with electron spray ionization (LC-ESI-MS/MS). 29% of the samples were found positive for at least some of the target compounds,

Table 2. Mean levels (mg/kg), and concentration ranges of pesticide residues detected in cotton honey samples collected from 18 apiaries in 9 centers in the Kafr El-Sheikh Governorate, Egypt.

Sampling sites*	Pesticides found	Range ($mg \cdot kg^{-1}$)	Mean ($mg \cdot kg^{-1}$)	EU MRLs ($mg \cdot kg^{-1}$)	No. of violated samples
Prempal[1)]	BDL	-	-	-	-
Elfath[1)]	BDL	-	-	-	-
Elkoum[2)]	BDL	-	-	-	-
Ourpan[2)]	BDL	-	-	-	-
Seifr Elbalad[3)]	diazinon	0.05-0.09	0.073	0.010	5
	tetradifon	0.09-0.13	0.106	0.050	5
Ezbet Elfar[3)]	BDL	-	-	-	-
Hesat Elghounami[4)]	dicofol	0.25-0.50	0.366	0.010	5
Ezbet Elarab[4)]	BDL	-	-	-	-
Elwarak[5)]	dicofol	1.30-2.79	1.988	0.010	5
Damrou[5)]	pirimicarb	0.08-0.2	0.133	0.050	5
	dicofol	0.49-0.72	0.583	0.010	5
El Hamra[6)]	diazinon	0.07-0.12	0.096	0.010	5
	γ-HCH	0.002-0.039	0.023	0.010	3
	dicofol	0.223-0.55	0.389	0.010	5
Fac. Agric.[6)]	pirimicarb	0.021-0.053	0.033	0.050	1
	chlorpyrifos	0.009-0.011	0.010	0.010	1
	dicofol	0.326-0.606	0.478	0.010	5
El Abbasia[7)]	BDL	-	-	-	
El Hasafa[7)]	BDL	-	-	-	
Abo Sekeen[8)]	fenitrothion	0.016-0.021	0.018	0.010	5
El Helmia[8)]	malathion	0.009-0.02	0.014	0.020	-
	bromopropylate	0.028-0.13	0.087	0.010	5
Abo Badawy[9)]	dicofol	0.406-1.87	0.999	0.010	5
El Garaida[9)]	profenofos	0.12-0.23	0.166	0.050	5
	diazinon	0.02-0.06	0.033	0.010	5
	β-HCH	0.01-0.021	0.014	0.005	5
	dicofol	0.78-2.748	1.581	0.010	5

*Kafr El-Sheikh Governorate Centers: [1)] Metoubes, [2)] Fuwah, [3)] Desouk, [4)] Kaleen, [5)] Sedi Salem, [6)] Kafr El-Sheikh, [7)] El Riyad, [8)] El Hamoul, [9)] Beilla.
Each value is the mean of five-sample analyses. BDL – below detection limit.

and profenofos was the most abundant pesticide [31].

This study indicates that in agricultural areas with developed apiculture, useful information about the occurrence and distribution of pesticide residues due to crop protection treatments can be derived from the analysis of randomly collected honey samples used as bioindicators. Because it is necessary to provide safe food to the consumers, it is essential that adequate monitoring should be in place to eliminate the possibility of the presence of the residues in food commodities in excess of the prescribed levels.

Dietary Intake Assessment and Risk Characterization

To evaluate the toxicological significance of human exposure to the pesticide residues found in honey, it is important to compare estimated daily intake (EDI) with the acceptable daily intakes (ADI) established by the FAO/WHO organization.The EDI was compared with the acceptable daily intake (ADI), meaning the daily dosage of a chemical which, during the entire lifetime, appears to be without appreciable risk on the basis of all the facts known

Table 3. Estimated daily intakes (EDIs) and ADIs of pesticide residues found in honey.

Pesticide	ADI* (μg/kg body weight/day)	EDI (μg/kg body weight/day)	Hazard index (EDI/ADI, %)
β-HCH	0.8	0.0013	0.1598
γ-HCH	5	0.0021	0.0420
Dicofol	2	0.0833	4.1643
Tetradifon	15	0.0097	0.0645
Bromopropylate	30	0.0079	0.0265
Chlorpyrifos	10	0.0009	0.0091
Diazinon	2	0.0061	0.3059
Fenitrothion	5	0.0016	0.0329
Malathion	30	0.0013	0.0040
Pirimicarb	35	0.0076	0.0217
Profenofos	30	0.0152	0.0505

*Established by Codex Alimentarius Commission on Pesticide Residues, JMPR (Joint FAO/WHO Meeting on Pesticide Residues), EPA (Environmental Protection Agency) and EFSA (European Food Safety Authority).

at the time [32]. Health risk estimations were done based on an integration of pesticide residue analysis data and food consumption assumptions, which aim to represent the actual residue levels in food consumed by the local population, with a bodyweight of 60 kg. Food consumption data was derived from the WHO/Global Environment Monitoring System-Food Contamination Monitoring and Assessment Program average consumption cluster B diets [33]. Results obtained were used to calculate EDI expressed as microgram pesticides per kilogram body weight per day (μg/kg b.w/day). The EDI is a realistic estimate of pesticide exposure that was calculated for each pesticide on honey in agreement with the international guidelines [34, 35], using the following equation:

$$EDI = \Sigma C \times F/D \times W$$

...where C is the mean of pesticide residues concentration in honey ($\mu g \cdot kg^{-1}$), F is mean annual intake of honey per person (2 kg per person approximately), D is number of days in a year (365), and W is mean body weight (60 kg).

As can be seen from Table 3, the estimated daily intakes of detected pesticides were much lower than ADIs, which show that honey consumption has a minimal contribution to toxicological risk. These findings are in coincidence with those obtained by Blasco et al. [21]. The consumer is considered to be adequately protected if the hazard index of a pesticide residue does not exceed unity. The hazard index values show that all the intakes of pesticide residues remains clearly below the safe limit. It should be emphasized that dietary pesticide intakes estimated in this study considered only exposures from honey and did not include other food products such as grains, vegetables, fruits, dairy, fish, and meats. As such, estimates are not considered as total dietary exposure to the pesticides, nor do we consider drinking water, residential, or occupational exposures.

Conclusions

The data obtained from this study pertaining to the detection of pesticide residues in honey samples are probably an indication of the widespread use of pesticides in the area of study. Since honeybees travel long distances and come close to many plants, honey may be an easily accessible environmental pollution indicator. In addition, this study revealed for the first time that the bees and/or hives in the study areas are exposed to chemical contaminants, which represents a risk to bees. Although the results show a negligible risk associated with exposure via honey consumption, a special precaution should be taken with the possible total exposure to these chemicals from various foods in the future. Tighter regulation in the production of pesticides, their sale, and application are needed as well as implementation of integrated pest management methods. Additionally, further monitoring studies must be performed to improve food safety and protect consumers' health.

References

1. BOGDANOV S. Contaminants of bee products. Apidologie, **37**, 1, **2006**.
2. KARAZAFEIRIS E., MENKISSOGLU-SPIROUDI I.U., THRASYVOULOU A. Assessment of Pesticide Residues in Cotton Honey from Greece. 4th MGPR International Symposium of Pesticides in Food and the Environment in Mediterranean Countries, Sept. 21-24, Kusadasi, Turkey, pp. 6, **2005**.
3. PORRINI C., SABATINI A., GIROTTI S., GHINI S., MEDRZYCKI P., GRILLENZONI F., BORTOLOTTI L., GATTAVECCHIA E., CELLI, G. Honey bees and bee products as monitors of the environmental contamination. Apiacta, **38**, 63, **2003**.
4. COLIN M.E., BONMATIN M., MOINEAU I., GAIMON C., BRUN S., VERMANDERE, J.P. A method to quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides. Arch. Environ. Con. Tox., **47**, (3), 387, **2004**.
5. BEEKMAN M., RATNIEKS F.L.W. Long range foraging in the honeybee Apis mellifera. Funct. Ecol., **14**, (4), 490, **2000**.
6. ALIFERIS K.A., TARANTILIS P.A., HARIZANIS P.C., ALISSANDRAKIS E. Botanical discrimination and classification of honey samples applying gas chromatography/mass spectrometry fingerprinting of headspace volatile compounds. Food Chem., **121**, (3), 856, **2010**.
7. FERNÁNDEZ M., PICÓ Y., MAÑES J. Analytical methods for pesticide residue determination in bee products. J. Food Protect., **65**, 1502, **2002**.
8. TADDIA M., MUSIANI A., SCHIAVI S. Determination of heavy metals in honey by Zeeman electrothermal atomic absorption spectrometry. Ann Chim., **94**, (1-2), 107, **2004**.

9. HERRERA A., PEREZ-ARQUILLUE C., CONCHELLO P., BAYARRI S., LAZARO R., YAGUE C., ARINO A. Determination of pesticides and PCBs in honey by solid-phase extraction cleanup followed by gas chromatography with electron-capture and nitrogen-phosphorus detection. Anal. Bioanal. Chem., **381**, (3), 695, **2005**.
10. JIN Z., LIN Z., CHEN M., MA Y., TAN J., FAN Y., WENG J., CHEN Z., TU F. Determination of multiple pesticide residues in honey using gas chromatography-mass spectrometry. Se Pu. **24**, (5), 440, **2006**.
11. YAKOBSON B.A. The monitoring of possible biological and chemical contaminants in bee products. In A. Mizrahi and Y. Lensky [Eds.], Plenum, New York, **1996**.
12. KUJAWSKI M.W., NAMIESNIK J. Levels of 13 multi-class pesticide residues in Polish honeys determined by LC-ESI-MS/MS. Food Control, **22**, 914, **2011**.
13. DE PINHO G.P., NEVES A.A., DE QUEIROZ M., SILVÉRIO F.O. Optimization of the liquid-liquid extraction method and low temperature purification (LLE-LTP) for pesticide residue analysis in honey samples by gas chromatography. Food Control, **21**, 1307, **2010**.
14. ANASTASSIADES M., LEHOTAY S.J., ŠTAJNBAHER D., SCHENCK F.J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solidphase extraction" for the determination of pesticide residues in produce. J. AOAC Int., **86**, 412, **2003**.
15. WILKOWSKA A. BIZIUK M. Determination of pesticide residues in food matrices using the QuEChERS methodology. Food Chem., **125**, (3), 803, **2011**.
16. LEHOTAY S.J., KASERINA M., LIGHTFIELD A.R. Use of buffering and means to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables. J. AOAC Int., **88**, (2), 615, **2005**.
17. RIBANI M., BOTTOLI C.B.G., COLLINS C.H., JARDIM I.C.S.F., MELO L.F.C. Validação em métodos cromatográficos e eletroforéticos (Validation for chromatographic and electrophoretic methods). Química Nova, **27**, (5), 771, **2004**.
18. KURZ M.H.S., GONÇALVES F.F., MARTEL S., ADAIME M.B., ZANELLA R., MACHADO S.L.O., PRIMEL E.G. Rapid and accurate hplc-dad method for the determination of the herbicide bispyribac-sodium in surface water, and its validation. Quim. Nova, **32**, (6), 1457, **2009**.
19. BLASCO C., VAZQUEZ-ROIG P., ONGHENA M., MASIA A., PICÓ Y. Analysis of insecticides in honey by liquid chromatography-ion trap-mass spectrometry: Comparison of different extraction procedures. J. Chromatogr. A, **1218**, 4892, **2011**.
20. DOGHEIM S.M., GAD ALLA S.A., EL-MARSAFY A.M. Monitoring pesticide residues in Egyptian fruits and vegetables in 1995. J. AOAC Int., **82**, (4), 948, **1999**.
21. BLASCO C., FERNANDEZ M., PENA A., LINO C., SILVEIRA M.I., FONT G. Assessment of pesticide residues in honey samples from Portugal and Spain. J. Agric. Food Chem., **51**, 8132, **2003**.
22. WANG J., KLIKS M.M., JUN S., LI Q.X. Residues of organochlorine pesticides in honeys from different geographic regions. Food Res. Int., **43**, 2329, **2010**.
23. GONZALEZ M., MIGLIORANZA K.S., AIZPUN DE MORENO J.E., MORENO V.J. Organochlorine pesticide residues in leek (*Allium porrum*) crops grown on untreated soils from an agricultural environment. J. Agric. Food Chem., **51**, 5024, **2003**.
24. GUZZELLA L., ROSCIOLI C., VIGANO L., SAHA M., SARKAR S.K., BHATTACHARYA A. Evaluation of the concentration of HCH, DDT, HCB, PCB and PAH in the sediments along the lower stretch of Hugli estuary, West Bengal, northeast India. Environ. Int., **31**, 523, **2005**.
25. QU W.Y., SURI R.P.S., BI X.H., SHENG G.Y., FU J.M. Exposure of young mothers and newborns to organochlorine pesticides (OCPs) in Guangzhou, China. Sci. Total Environ., **16**, 3133, **2010**.
26. BLASCO C., LINO C., PICÓ Y., PENA A., FONT G., SILVEIRA M.I. Determination of organochlorine pesticide residues in honey from the central zone of Portugal and the Valencian community of Spain. J Chromatogr. A., **1049**, 155, **2004**.
27. ERDOĞRUL Ö. Levels of selected pesticides in honey samples from Kahramanmaras, Turkey. Food Control, **18**, 866, **2007**.
28. ANTONESCU C., MATEESCU C. Environmental pollution and its effects on honey quality. Rom. Biotech. Lett., **6**, 371, **2001**.
29. RISSATO S.R., GALHIANE M.S., DE ALMEIDA M.V., GERENUTTI M., APON B.M. Multiresidue determination of pesticides in honey samples by gas chromatography-mass spectrometry and application in environmental contamination. Food Chem., **101**, 1719, **2007**.
30. WIEST L., BULETÉ A., GIROUD B., FRATTA C., AMIC S., LAMBERT O., POULIQUEN H., ARNAUDGUILHEM C. Multi-residue analysis of 80 environmental contaminants in honeys, honeybees and pollens by one extraction procedure followed by liquid and gas chromatography coupled with mass spectrometric detection. J. Chromatogr. A, **1218**, 5743, **2011**.
31. BARGANSKA Z., SLEBIODA M., NAMIESNIK J. Pesticide residues levels in honey from apiaries located of Northern Poland. Food Control, **31**, 196, **2013**.
32. FAO/WHO, Evaluation of the toxicity of pesticide residues in food. FAO Meeting Report No. PL/1965/10/1. WHO/Food Add./27.65. <http://www.inchem.org/documents/jmpr/jmpmono/v065pr01.htm>, **1965**.
33. WHO/GEMS/FOODS, (WHO/Global Environment Monitoring System-Food Contamination Monitoring and Assessment Program). Consumption cluster diets. http://www.who.int/foodsafety/chem/gems/en/, **2006**.
34. WHO, Guidelines for predicting dietary intake of pesticide residues (revised). Global Environment monitoring System-Food Contamination and Assessment Programme (GEMS/Food) in collaboration with Codex Committee on Pesticide Residues, **1997**.
35. FAO, Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed (1st ed.). Rome: Food and Agriculture Organization. http://www.fao.org/ ag/AGP/AGPP/Pesticid/p.htm, **2002**.