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Occurrence of pesticide residues in Italian honey from different areas in relation to its potential contamination sources

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ABSTRACT

Agricultural contamination with pesticides is a challenging problem that needs to be fully addressed. Bee products, such as honey, are widely consumed as food and their contamination may carry health hazards. In this study 28 pesticides selected as representative of different contamination sources were measured in 72 honey samples using methods based on SPE clean-up and GC–MS/MS detection. Particular emphasis was given to the pesticides utilised in intensive apple orchards in order to elucidate and relate the honey contamination and its potential sources. Residues of many pesticides were found in most of the samples investigated. The majority of honey samples (94%) contained at least one of the pesticides even if their concentrations were found to be lower than its MRL. DDT, DDD and DDE were the compounds isolated with higher frequency in honey samples produced in the industrialised area. Chlorpyrifos and quinoxyfen were the residues frequently detected in samples coming from the apple orchard area. No residues were isolated in honey coming from the mountain area dedicated to organic production. The results of this study show that pesticide contamination of honey is strictly related to the contamination source and could reflect the specific pollution of a given environment, confirming honey bee and beehive matrices as appropriate sentinels for monitoring contamination in the environment. This could represent an effective tool for beekeepers to select production areas especially for organic honey production.

1. Introduction

Honey is defined as a substance made of nectar and sweet deposits from plants, gathered, modified and stored in honeycombs by honey bees and it has always been considered a natural and healthy food, free of any trace of impurities (Atkins & Kellum, 1984; Garcia, Fernandez, & Melgar, 1995; Giorgi, Madeo, Baumgartner, & Lozzia, 2011; Wilczynska & Przybylowski, 2007). Pesticides, because of their extensive utilization and usual persistence in the environment, may be introduced to honey by bees during production (Debayle, Dessalces, & Grenier-Loustalot, 2008). The contamination of honey by pesticides may occur through direct contamination from beekeeping practices as well as indirect contamination from environmental sources (Anderson & Wojtas, 1986; Kujawski, Pinteaux, & Namieśnik, 2012).

Pesticide residues have been shown to cause mutations and cellular degradation. Besides being a risk to public health, the presence of pesticides in raw materials (nectar, pollen, plant exudates) or bee products (raw products, honey, royal jelly) decreases quality. Contaminants can reach the raw materials through air, water, plants and soil and then be transported into the beehive by the bees. The direct pollution of honey can originate from beekeeping practices using insecticides, fungicides, and acaricides and especially to control the bee disease Varroasis (Amendola, Pelosi, & Dommarco, 2010). In general, the threat of infestation by the parasitic mite *Varroa jacobsoni* Oud. forced beekeepers to treat their colonies with acaracides. These compounds are able to migrate from the wax comb into the stored honey and they can accumulate due to their fat-soluble and non-volatile characteristics (Panseri et al., 2008). The indirect contamination of honey from the environment is a result of pesticide utilization in agriculture or environmental contamination (Giorgi, Panseri, & Chiesa, 2012; Giorgi, Panseri,Mattara Andreis & Chiesa, 2013; Rissato, Galhiane, Knoll, & Apon, 2004).

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Among environmental contaminants, organochlorine pesticides (OCPs) represent the most important groups of dangerous organic contaminants and their occurrence has been widely documented in several studies. Blasco C. et al. described a method for determining nine organochlorine pesticide residues (hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), aldrin, p′,p′-DDE, p′,p′-DDD, o′,p′-DDT, and p′,p′-DDT) in forty-nine samples of honey collected in markets in Portugal and Spain during 2001 and 2002. The study stated that 14 Valencian samples contained residues of HCB or/and HCH isomers and in Portugal 23 samples were contaminated (Blasco, Fernandez, Pico, & Font, 2004; Blasco, Lino, et al., 2004; Blasco, Vazquez-Roig, Onghena, Masia, & Picó, 2011; Tsipi, Triantafyllou, & Hiskia, 1999). OCPs, effective against a variety of insects, were widely used worldwide in agriculture and animal production until restrictions were introduced by developed countries in the 1970s. These persistent organic pollutants have a highly stable, low volatile, lipophilic nature, and consequently exhibit considerable environmental persistence with a tendency to accumulate and bioaccumulate, leading to the contamination of foodstuffs, even those not directly treated. Residue concentrations have decreased in monitored foods since the ban of these chemicals in most countries, although trace levels are still detected in many foodstuffs. European regulations establishing pesticide residue levels in food have prompted EU members to monitor OCPs (Blasco, Fernandez, et al., 2004; Blasco, Font, & Pico, 2008; Blasco, Lino, et al., 2004; LeDoux, 2011; Regulation (EC) NO 396/2005). Insecticides used in an intensive agricultural production context like orchard areas in which a relatively high level of insecticides is generally applied may also contaminate honey. Few studies, however, have focused on pesticides used for crop protection introduced into hives by contaminated bees and wax. At present, fruit trees represent the second crop in terms of quantity of pesticides used *per* hectare (Tresnik & Parente, 2007: Pesticide Action Network (PAN) Europe May 2007). The amount and the number of pesticide applications during the growing season is related to the frequent presence of residues in apples, often exceeding the Maximum Residue Limits (MRLs) (Regulation (EC) NO 396/2005) with potentially adverse effects for human health. Organic production, despite having none of these drawbacks, currently represents a very small percentage of total apple production. Therefore, the use of pesticides in conventional production should be considered only when every other method of crop protection fails and particular attention must be paid in terms of selection of type of pesticide, time, dosage, and frequency of applications according to the Integrated Pest Management (IPM) principles (Tresnik & Parente, 2007). In North-east Italy, the mountainous Trento Province is considered one of the major apple growing areas of Europe with about 12,000 *ha* of commercial orchards distributed at between 150 and 950 m a.s.l (Marini, Quaranta, Fontana, Biesmeijer, & Bommarco, 2012). In this contest, an average of about 58 kg/ha/year of pesticides is used, compared to a national average of about 9 kg/ha for a total of over 2,150,000 kg of pesticides sold in Trentino in 2007 (Pesticide Action Network (PAN) Europe May 2007). On the basis of the above mentioned considerations the aim of this study was to optimise a fast and simple method that allows the multi-residue determination and quantification of 28 pesticides in honey in order to relate the typologies of contaminant source to their potential presence as residues in honey. The compounds investigated were selected as representative of pollutants arising from the environment (OCPs) as well as pesticides utilised for crop protection in IPM plans of apple orchards. Finally the pesticides were studied at a low concentration range (0.0005–0.074 mg/kg) with the aim of checking compliance with the MRLs set in Europe, reported in the Annexes of Regulation 396/2005 and its amendments (Regulation (EC) NO 396/2005).

2. Experimental procedure

2.1. Chemicals and reagents

Organochlorine pesticides (α-β-γ-δ HCH, Heptachlor, Heptachlor epoxide, Endosulfan I, Endosulfan II, Dieldrin, Aldrin, Endrin, Endrin Aldehyde, p-p′ DDE, p-p′ DDD, p-p′ DDT, Methoxychlor, >99% certified purity) were from Restek Corporation, Bellefonte, PA, USA. Pesticide standards of Chlorpyriphos, Penconazol, Captan, Bupiramate, Quinoxyfen, Fluazinam, Trifloxystrobin, Iprodion, Chlorantraniliprol, Spirodiclofen, Boscalid, Pyraclostrobin were purchased from Sigma–Aldrich, St Louis, Mo, USA. 4-nonylphenol, used as internal standard, was from Fluka and purchased from Sigma–Aldrich, St Louis, Mo, USA. Individual stock solutions of pesticides were prepared by dissolving 10 mg of each compound in 10 mL of acetone and stored in glass flasks at −20 °C. Mixed compound calibration solution, in acetone, was prepared from the stock solutions and used as spiking solutions as well. Matrix-matched standards were prepared by adding appropriate amounts of standards to the control matrix covering the range from 0.005 mg kg^{-1} to 0.1 mg kg^{-1}. Deionized water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Ethyl acetate, acetone and hexane of special grade for pesticide residue analysis (Pestanal) were purchased from Sigma–Aldrich, St Louis, Mo, USA.

2.2. Sample collection

A total of seventy-two honey samples from different areas were provided by the beekeeper procurers during 2012 in order to obtain representative sources of honey contaminants as summarized in Table 1.

All honey samples were stored at −20 °C until analysis to prevent any possible matrix alteration (fermentation phenomena).

2.3. Extraction and clean-up

In order to analyze a large number of pesticides from different classes, a simple method was optimised to expand range applicability.

A 10 g portion of honey sample was weighed in a 50 ml centrifuge tube then mixed with 10 ml water MilliQ and homogenized by vigorously shaking for 2 min, to reduce viscosity and facilitate handling. The sample was then mixed with 20 ml ethyl acetate, shaken for 2 min and placed in an ultrasound bath for 15 min. The resulting emulsion was quickly broken by centrifuging at 3000 *g* for 10 min. The supernatant was collected in round bottom flasks and the residue was re-extracted with 20 ml of ethyl acetate. The two portions were combined and the solvent was

Table 1
Origins of 72 honey samples from different production areas.

Sample no.	Origin information	Altitude (*m a.s.l*)	Area characteristic in relation to its potential pesticides sources
15	Market	–	Non-EU Produced (variable pesticides sources)
17	North Italy[a] (V.C.O.)	200	Industrialized area (OCPs source)
20	North Italy[b] (Trentino)	800	Intensive apple orchard (pesticides utilized in IPM* plan)
20	North Italy[c] (Valle Camonica)	1300	Organic production (no presence of industries or agricultural intensive systems; absence of pesticides according to LOD of method)

*IPM = integrated pest management; a = Verbano Cusio Ossola (north west); b = Trentino (north west); c = Valle Camonica (north east).