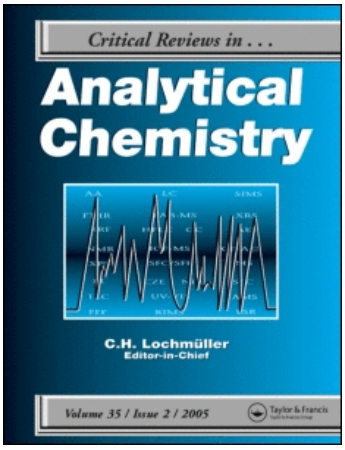

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Pesticide Analysis of Bee and Bee Product Samples

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Bee products possess therapeutic properties and are the source of many essential trace elements, which is why they are regarded as valuable food products. Honey bees may bring to the hive numerous contaminants deposited on the plants they visit, including pesticide without xenobiotics. The large-scale application of pesticides in agriculture and horticulture can lead to mass mortality among bees, and the chemicals find their way into bee products. The presence of xenobiotics in these products can lower their quality and cause their properties to deteriorate, which in consequence may endanger human health. All this means that the analysis of bees, honey and other bee products is becoming a matter of increasing urgency. In this context new analytical methodologies are needed, enabling a wide spectrum of analytes to be assayed in a single sample and during one analytical run. Attention is focused on new techniques of sample preparation and extract cleanup prior to the final determination step. Besides characterizing the honey bee and bee products, the article analyzes the literature data on the latest methodological developments for determining levels of a wide range of pesticides.

Keywords honey bee, bee products, pesticides, sample preparation, multiresidue methods, analytical procedures

INTRODUCTION

Pesticides are a large group of substances applied in agriculture. Because of their persistence and longevity in the environment, they can accumulate in its different compartments and may even turn up in areas where they have never been used. In addition, they are bioaccumulated in plant and animal tissues.

The application of pesticides, particularly in the spring and summer, can lead to mass mortality among bees, and the chemicals find their way into bee products. The presence of xenobiotics in these products may lower their quality and devalue their properties, and also endanger human health. It is therefore of the utmost importance to monitor pesticide levels in these unique food products.

Considered to be good bioindicators of environmental contamination with toxic substances, honey bees (and consequently bee products) send two signals regarding the state of pollution of the environment they inhabit: large-scale mortality and the presence in their bodies or products of contaminants like heavy metals, radioactive elements and Persistent organic pollutants (e.g., pesticides). The degree of contamination of bee products is therefore monitored not just to assess their quality but also the extent to which the insects' environment is contaminated.

The matrix composition of honey and the other bee products is complex. Sample preparation is therefore a key aspect of every analytical procedure used in the determination of pesticides in bee products. It is also the most time-consuming and labor-intensive step in such a procedure. The research carried out in numerous scientific institutions is generally conducted along two lines:

- The improvement of existing analytical methods;
- The development of new methodologies making use of *solvent-free (solventless) sample preparation techniques*; this is a consequence of the sustainable development concept being introduced to the analytical laboratory.

GENERAL CHARACTERISTICS OF THE HONEY BEE (*APIS MELLIFERA)*

The honey bee (*Apis mellifera)* is one of the social insects. A bee colony is a basic biological unit, without which no individual bee can exist independently. The focus of the colony is the queen, whose task it is to ensure the continuity of the species. The queen lays eggs, from which three kinds of bee can develop: queens, workers and drones. Fertilized eggs laid in the cells

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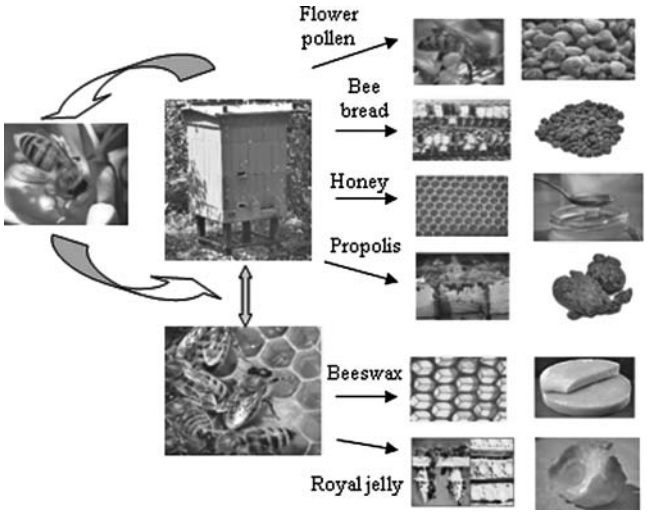

FIG. 1. Classification of bee products according to their origin.

of the honeycomb give rise to workers, whereas young queens develop from larvae fed solely on royal jelly. The worker bees are the smallest members of the colony and they perform all the jobs essential to its existence: building comb cells and guarding the hive, gathering food, bringing up the young bees. The males—drones—are only needed to fertilize the queen, after which they are driven out of the hive to die of hunger (1).

Honey bees gather nectar and pollen—their food—and in doing so fertilize (pollinate) entomophilous plants, e.g., fruit trees and rape. From the nectar, pollen and also resinous substances they make honey and the other products (1).

BEE PRODUCTS

While gathering nectar and pollen and the other substances they need for the production of honey and other products, bees can bring contaminants to the hive (1). As mentioned earlier, the presence of xenobiotics in bee products may lower their quality and devalue their properties, and also endanger human health.

Bee products can be divided into two groups. One contains the products of vegetable origin that are gathered by bees, i.e., pollen, honey and propolis. The other contains the secretions of bees, like royal jelly and beeswax.

Figure 1 illustrates the division of bee products according to their origin. Table 1 lists the characteristics of the above-mentioned bee products.

PATHWAYS TO CONTAMINATION OF BEE PRODUCTS

Being typical natural products, bee products contain substances that are beneficial to human health. Honey is formed from the nectar of flowering plants, upon which various contaminants may have been deposited. An increasingly large battery of pesticides is being used in agriculture to enhance crop yields and to combat plant diseases and pests. These compounds can accumulate in plants, and subsequently in bee products, thereby degrading their therapeutic properties. Contaminants to a greater or lesser extent affect the health of both humans and the bees themselves (2, 17). Figure 2 shows in diagrammatic form the pathways by which contaminants get into bee products (2, 14).

The presence of pesticides in bee products has prompted the implementation of a program for monitoring pesticide residues in order to assess the dangers to humans from exposure to these substances. According to the European Union's (EU) executory provisions, natural honey must be free from chemicals. That is why a range of legal regulations has come into force with respect to the maximum permissible concentrations (MPC) of pesticide residues in honey and other bee products (18–20).

The monitoring of chemical residues in food serves not just to protect the health of consumers; it is also carried out to meet obligations binding in the international food trade. EU member states are bound by new, unified principles of organizing and performing the monitoring of chemical residues in animal tissues, in food of animal origin, in water and in animal feeds, which are covered by Council Directive (96/23/WE), dated April 29, 1996. The need to carry out such monitoring is also stated in a Regulation of the European Parliament and Council (2004/882/WE) (21). Table 2 lists the medicinal products that can be used for veterinary purposes and other contaminants covered by the program for monitoring chemical residues in honey and other bee products.

Analysis of the transport pathways of the most important groups of contaminants, shown diagrammatically in Figure 2, and information gathered by the European Food Safety Authority provide the basis for stating that pesticides are xenobiotics frequently encountered in bee products. In the literature more and more information is appearing on new methodological and instrumental approaches that can be applied in this type of monitoring.

Table 3 lists information on the groups of pesticides with which bees most often come into contact while gathering pollen.

BEES AND BEE PRODUCTS – INDICATORS OF ENVIRONMENTAL POLLUTION

When honey bees visit flowers for their pollen and nectar they are in constant contact with the pesticides applied to protect crops and gardens. Being good bioindicators, honey bees and bee products can be used to assess the degree of environmental contamination with toxic substances, like pesticides, heavy metals and radioactive elements. (25, 26).

There are two scenarios for the mortality of honey bees ensuing from their direct contact with pesticides (27):

- While gathering nectar or pollen a honey bee comes into direct contact with the pesticides covering the plant (in this case, the flowers). Having been exposed to the action of the pesticides, the bee does not return to the hive, although the colony as a whole survives;
- The transport of xenobiotics together with pollen and/or nectar, and also on the body of the bee, may spell mortal danger to the entire colony, and is a source of contamination in bee products.

TABLE 1
Bee products—Basic information

Bee product	Method of production	Principal components	Additional information	References
Honey	Produced by bees from flower nectar, honeydew, or both these substances	Simple carbohydrates, water, polysaccharides, acids, proteins, mineral substances, pigments, aromatic substances, enzymes, hormones and vitamins	The composition depends largely on the species of plant from which the nectar/honeydew was gathered, the environmental and climatic conditions, and the actions of the beekeeper. Honey has antiseptic, bacteriostatic and even bactericidal properties. It is not only a highly nutritious and easily assimilable food (a natural sweetener) but is also an excellent cosmetic.	(2–4)
Pollen	Worker bees carry pollen to the hive in the form of multi-colored grains (pellets, moistened with a little nectar or honey)	Proteins, sugars, starch, cellulose, fats, mineral salts, aminoacids	The hive bees take the pollen from the workers and pack it into the cells of the honeycomb where they drench it with honey.	(1, 5–7)
Bee bread	Formed during the natural fermentation of the flower pollen packed into the honeycomb cells	Protein	The composition and properties differ, depending on the plant species and region from where the pollen was gathered.	(1, 5).
Beeswax	A secretion of the wax glands, which are situated on the ventral side of the abdomen of a young worker bee	A mixture of acids, hydroxy acids, alcohols, esters and hydrocarbons	Bees use wax mainly for building the honeycombs in which the next generation of bees is raised, for storing honey and pollen. Beeswax is a raw material for the pharmaceutical, cosmetics and food industries.	(1, 8-10)
Propolis	Formed from viscous resins and balsams gathered by bees from the buds of trees like birch, poplar, pine, alder and willow, and herbaceous plants, and enriched with their glandular secretions	Resins, essential oils, plant waxes, organic acids, alcohols, pollen	The quality of propolis depends on the botanical and geographical features of the plants, from which the substrates for its production were gathered. Bees use it as a sealant, for disinfecting comb cells, e.g. prior to egg laying by the queen. It happens that bees are unable to remove the body of a stung intruder such as a mouse; then, the body of the intruder is coated with propolis to prevent its decomposition (mummification). Propolis also acts bactericidally on *Varroa* mites. Because of its biological and therapeutic properties, propolis is used in medicine, in the production of cosmetics and by the food industry.	(11–14)
Royal jelly	A secretion of the hypopharyngeal glands of worker-feeder bees	Proteins and aminoacids	This is fed to all bee larvae in the first 3–4 days of their life and to the queen bee throughout her larval development and for as long as she lays eggs. Royal jelly has both nutritional and medicinal properties. For its production bees must have access to pollen.	(1 ,15, 16)

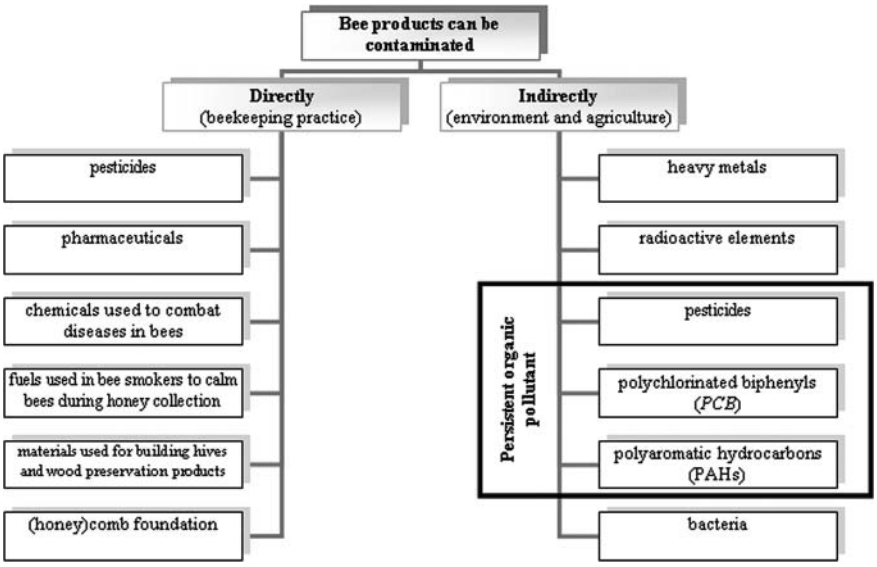

FIG. 2. The pathways by which the most important groups of contaminants are transported to bee products.

Bees possess a number of features predisposing them to be utilized as indicator organisms for assessing the extent of environmental contamination by pesticides (28):

TABLE 2
Medicinal products that can be used for veterinary purposes and other contaminants, covered by the program for monitoring chemical residues in honey and other bee products (22)

Group of compounds	Analyte
Antibacterial substances (antibiotics, sulfonamides, quinolines)	Amoxicycline, ampicillin, sulfadiazine, sulfamethoxazole, sulfamethoxypyridazine, sulfathiazol, sulfacetamide, chlorotetracycline, doxycycline, oxytetracycline, tetracycline, enrofloxacin, ciprofloxacin, etc.
Carbamates and pyrethroids	Carbaryl, propoxur, bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, permethrin, fenvalerate, fluvalinate
Acaricides	Amitraz
Organochlorine pesticides and polychlorinated biphenyls (PCBs)	DDT and its metabolites, α, β, γ – HCH, HCB, aldrin, dieldrin, endrin, heptachlor, heptepoxide, chlordane, bromopropylate, PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180
Organophosphorus pesticides	Chlorvinphos, chlorpiryfos, methyl chlorpiryfos, diazinon, dichlorvos, fenchlorphos, coumaphos, parathion, methyl parathion, trichlorfon

- They are ubiquitous and easily identified;
- Their populations are stable and exhibit quite a broad tolerance to pesticides, but they also display distinct external signs of shock when a critical level of contamination in their environment has been reached;
- A simple relationship exists between the level of contamination in the environment and the bioindicator parameter that serves to identify that contamination, e.g., impairment or intensification of the honey bee's physiological processes;
- They occur in a variety of habitats, including those contaminated by human activities;
- They are resistant to the weather, which means they can be utilized practically the whole year round;
- They are capable of trapping and accumulating contaminants.

The following evidence is offered in support of the above:

- Along with the raw materials (nectar, pollen, propolis, water), a bee brings to the hive the contaminants contained in them as well as those with which it comes into contact on the flowers and leaves of the plants it visits. Substances the bee inhales from the air are deposited within its body (20, 26, 29, 30);
- Bees collect nectar from plants growing in different types of soil and in different climates (1, 30);
- Bees cover large areas of crops. It has been estimated that they search an area within a radius of 3 km from

TABLE 3
Groups of pesticides with which worker bees may come into contact while gathering pollen

Pesticide group	Application	Additional information	Ref.
Organochlorine pesticides (OCPs)	Bactericides, fungicides, herbicides, insecticides; also as compounds stimulating crop growth	May be transported by the wind, may reach the ground elsewhere as a result of wet or dry atmospheric deposition, or may decompose. Some *OCPs* like aldrin, dieldrin, heptachlor, *DDT*, *HCH*, may still be detectable in the environment, even though they have been withdrawn, and even in regions where they were never applied. Depending on their persistence in the environment, they can be transported for tens, hundreds or thousands of kilometers.	(12)
Organophosphorus pesticides (OPPs)	Insecticides, fungicides, herbicides	May be carried to honey by bees gathering pollen from plants contaminated with these substances during their flowering period	(23)
Acaricides	Treatment of mites	Commonly applied to treat the mite species *Varroa jacobsoni* and *V. destructor*, responsible for the bee disease varroatosis	(24)

the hive, so that a single one is capable of covering some 7 km^2(1, 20, 31);
- Obtaining samples of bees and/or their products is quick and inexpensive (30);
- Worker-gatherer bees return to the hive for the night, so samples can be taken daily (29);
- Bees live for only a short time and reproduce rapidly, so that the population is quickly renewed (1, 32);
- Bees can adapt to unfavorable habitat conditions (1).

Assays of the degree of contamination of bee products are used not only to evaluate their quality, but also to determine the degree of contamination of the environment in which the bees from a given hive operate (29, 32). The best samples for analysis are the bees themselves—these can be sampled dead or alive (26, 33). In order to collect samples of bees and their products, monitoring stations are set up containing hives and their bee colony. Dead bees are collected in special traps placed just in front of the hive entrance (26).

Pesticide levels in bees, in honey, bee bread and other products are not always the same. They depend on the localization of the hives, the types of crops being cultivated in the vicinity, and the time of year when the monitoring is being carried out. In warm, humid years, when flowers produce larger amounts of nectar, pesticide contents in bees and their products are higher (34–38).

METHODOLOGIES FOR DETERMINING PESTICIDES IN BEES AND/OR BEE PRODUCTS

In view of the highly diverse matrix composition of biological samples, a wide range of sample preparation techniques as well as efficient means of extracting and cleaning up the compounds of interest prior to the final determination are required (39).

The procedures for determining pesticides in bee products are highly complex and consist of several important steps (see Fig. 3) (34).

The diversity of bee products, which is a consequence of their different physical states, matrix compositions and the types, quantities and interrelations of interferents, not to mention analyte concentrations, means that sample preparation is complicated and time-consuming. It should be borne in mind that the risk of analyte loss or destruction is inherent in sample preparation. Proper preparation of the sample is therefore essential if the final analytical results are to be reliable. In addition, collecting a representative sample is often difficult because of the inhomogeneity of biological samples (39, 40).

The next, very important step in sample preparation is isolation and/or enrichment, during which the analytes must be transferred to a secondary matrix of simple composition and their concentration raised to such a level that quantitative determination becomes possible. At this stage in the procedure the following operations are carried out:

- Analyte extraction;
- Matrix exchange of the analytical sample;
- Raising the analyte concentration in the sample.

The following extraction techniques are used at this stage:

- Gel chromatography (*GPC*) (41);
- Liquid-liquid extraction (42);
- Solid-phase extraction (*SPE*) (27, 42, 43).

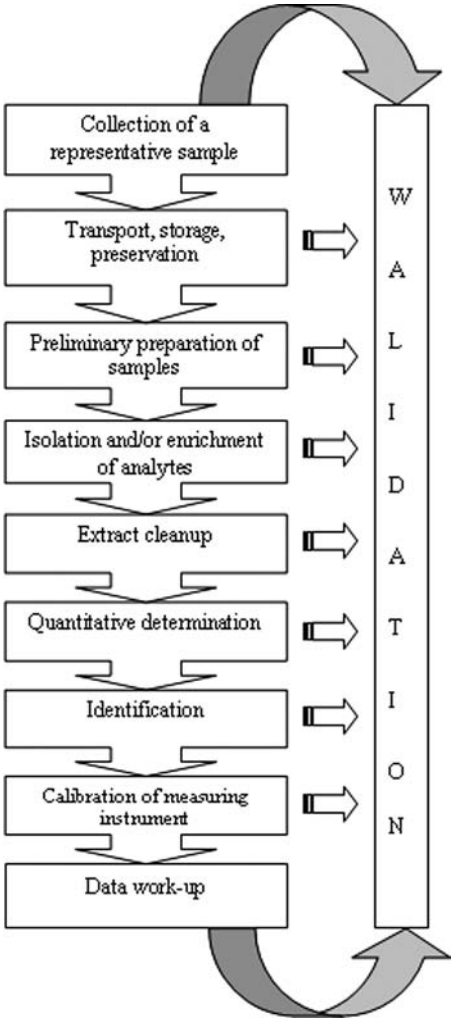

FIG. 3. General scheme of the methodologies for determining the xenobiotics present at low levels in samples of the contents of the materials characterized by a complex matrix composition.

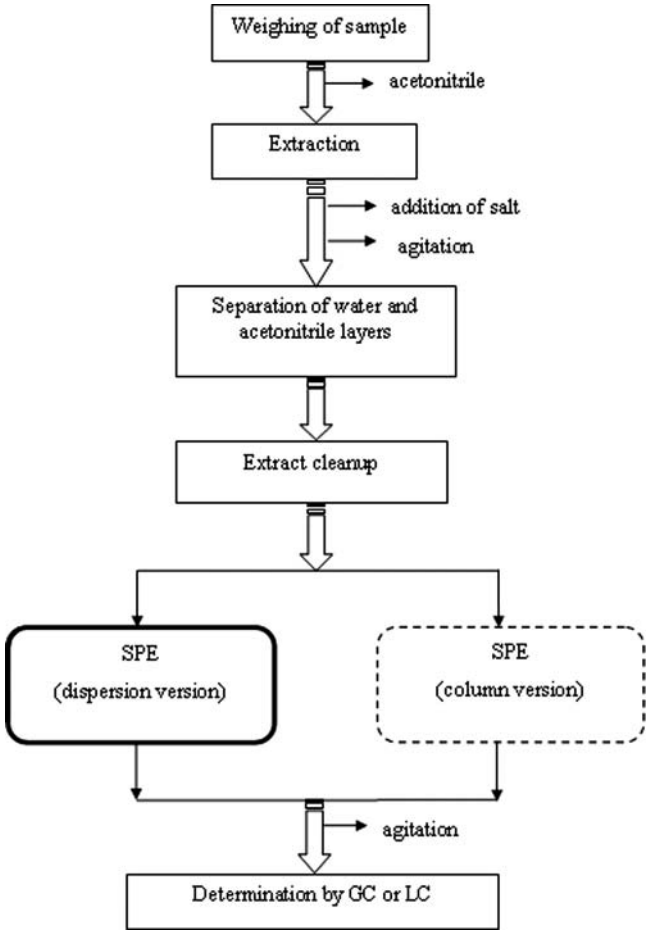

FIG. 4. General scheme of the QuEChERS procedure.

Table 4 sets out the metrological parameters of the techniques already used to extract pesticides from samples of honey.

The most serious interferents in the final determination of bee products are fats, pigments and carbohydrates. Samples are therefore cleaned up to lower the level of detection (LOD) of the analytical technique and/or to reduce the effect of matrix interferents. Efficient cleanup may, however, cause the partial loss of certain analytes. Insufficient cleanup, on the other hand, may give rise to errors in the quantitative determination (results may be overestimated as a result of contaminant peaks overlapping analyte peaks) (45, 46).

Table 5 lists the metrological parameters for the cleanup of honey samples during the determination of pesticides using SPE.

The choice of the final determination technique depends above all on the properties of the components to be determined. The determination of pesticides in biological samples depends not only on the matrix composition, but also on the MPC of these contaminants in food samples (56).

The majority of known methodologies for determining pesticides in bee products provide information on the presence and/or content in them of only certain classes of pesticides, such as:

- Organochlorine pesticides (57),
- Organonitrogen pesticides (58),
- Acaricides and insecticides (59, 60),

that is, the ones that are most frequently used by beekeepers. The reader can find out how the assays are actually carried out from review papers (61–63). The first reviews have also appeared in which known methodologies are compared in the context of their range of application in analytical practice and the relevant metrological parameters (25, 33, 41, 64–66).

Table 6 lists information gleaned from the literature regarding the analytical methodologies applied in the assay of pesticides in bees and bee products.

TABLE 4
Metrological parameters of standard techniques for extracting pesticides from samples of honey.

Analytes	Extraction technique	Sample size	Additional information	Reference
11 OCP	LLE	10 g	Sample heated at 35°C for15 min. Dissolved in 50 mL water. Extracted with 3 × 30 mL mixture of petroleum ether/ethyl acetate (80:20, v/v).	(44)
48 pesticides		10 g	Sample dissolved in 5 mL water and stirred. Extracted with 2 × (50 and 40 ml) mixture of acetonitrile/acetone/ethyl acetate /dichloromethane.	(45)
24 pesticides		4 g	Sample dissolved in 25 mL water. Extracted with 3 × 15 mL petroleum ether.	(46)
21 pesticides		50 g	Sample dissolved in 100 mL of a water/methanol mixture. Stirred mechanically for 3 h. Extracted with 2 × 100 mL *n*-hexane.	(47)
450 pesticides		15 g	Sample dissolved in 30 mL water and agitated for 15 min at 40°C. Extracted with a mixture of acetone and dichloromethane (10:40).	(48)
10 insecticides		10 g	Sample dissolved in 30 mL water and 20 mL aq. NaCl (5 min at 50°C). Extracted with 2 × 40 mL methylene chloride.	(49)
25 OCP 7 PCB 8 PBDE	*SPE*	5 g	Sample mixed with anhydrous sodium sulfate. *Florisil* column; conditioning: hexane; elution of analytes: 3 × 15mL hexane/acetone (2:1 v/v).	(4)
15 OCP 7 OPP 6 PCB		12 g	Sample dissolved in 40 mL methanol/water (70:30 v/v). Isolute ENV+ column; conditioning: 2 mL ethyl acetate, 2 mL acetone, 5 mL methanol/water (70:30 v/v); elution of analytes: 3 mL ethyl acetate.	(42)
42 OCP		5 g	Sample dissolved in 50 mL water and stirred (10 min). C_{18} column; conditioning: 10 mL methanol/water (1:1 v/v); elution of analytes: 10 mL methyl acetate, 4 mL methanol and 1 mL dichloromethane.	(43)
51 pesticides		10 g	Sample dissolved in 10 mL methanol/water (30:70 v/v). Column packed with C_{18}; conditioning: 5 mL methanol/water (30:70 v/v); elution of analytes: 10 mL hexane/ ethyl acetate (50:50 v/v).	(50)
22 OPP	*SPE*	5 g	Sample dissolved in 50 mL water. C_{18} column; conditioning: 10 mL methanol and 10 mL water; elution of analytes: 10 mL methyl acetate, 4 mL methanol and 1 mL dichloromethane.	(51)
15 OPP		1 g	Sample dissolved in 2 mL methanol. *Florisil* column; conditioning: 50 mL n-hexane/dichloromethane (1:1 v/v); elution of analytes: 30 mL *n*−hexane/dichloromethane (1:1 v/v).	(52)
17 pesticides	*OC LLE*	1 g	Sample dissolved in a mixture of water and acetone. Then aq. NaCl added. Cleanup with OCLLE (Chem Elut column). Gravitational elution of analytes on the column with ethyl acetate.	(53)
23 pesticides	*UE*	1 g	Sample dissolved in 1 mL methanol. Extracted with 2× (10 and 5 ml) ethyl acetate in an ultrasonic bath for 10 min.	(54)
16 pesticides	*SPME*	15 g	Sample dissolved in phosphate buffer; homogenization at 75°C using a magnetic stirrer (20 min, 700 rpm).	(55)
29 pesticides	*SFE*	5 g	Sample dissolved in 3 mL water and heated to 40°C; 2 g powdered cellulose added, then deep-frozen and lyophilized. Extracted with $CO_2$with the addition of 10% acetone (200 bar, 60°C, 20 min, gas flow rate 1.5 ml/min).	(27)

TABLE 5
Metrological parameters of the honey sample cleanup stage of pesticide determination

Analytes	Sample size	Additional information	Reference
29 pesticides	5 g	*Florisil* column. Conditioning: 5 mL ethyl acetate/*n*-hexane (1:1 v/v); elution: 2×5mL methylene chloride /*n*-hexane (80:20 v/v) and *n*-hexane/acetone (60:40 v/v).	(27)
11 OCP	10 g	*Florisil* capillary column. Conditioning: 10 mL *n*-hexane; elution: 25mL 20% (v/v) diethyl ether in 4 mL *n*-hexane.	(44)
48 pesticides	10 g	*Florisil* column. Conditioning: 5 mL acetone; elution: 2 × 10 mL hexane/ethyl acetate mixture in different proportions (80:20, 70:30, 60:40; 50:50 v/v).	(45)
24 pesticides	4 g	*Florisil* column. Conditioning: 10 mL petroleum ether; elution: 25 mL 5% diethyl ether in petroleum ether.	(46)
21 pesticides	50 g	*Florisil* column: silica gel (1:1); elution: *n*-hexane.	(47)
450 pesticides	15 g	Graphite soot column. Conditioning: 4 mL acetonitrile + toluene (3 + 1); elution: 25 mL acetonitrile + toluene (3 + 1).	(48)
23 pesticides	1 g	*Florisil* column.. Conditioning: 10 mL *n*-hexane; elution: 15 mL 30% ethyl acetate in *n*-hexane.	(54)

DEVELOPMENTAL TRENDS IN THE ANALYSIS OF PESTICIDES IN FOOD SAMPLES

Efforts to obtain reliable information on the presence and/or levels of the broadest possible spectrum of xenobiotics in food samples, if possible, in one single analytical run, are the driving force behind research and development in the analysis of contaminants in food, and hence, in bee products as well.

The most important of these trends are multi-residue methods and combined techniques.

Multi-residue Methods are analytical methodologies for the simultaneous analysis of trace amounts of a large number of analytes.

These methodologies should satisfy the following criteria (66), that is, they should:

- Enable the simultaneous detection of a large number of substances belonging to different groups of chemical compounds;
- Be characterized by a high yield of analytes, good precision and high sensitivity;
- Ensure that maximum amounts of interferents are removed during extract cleanup prior to the final determination;
- Be inexpensive, be easily implemented, provide the required information in a short space of time, and be environmentally benign.

These criteria are fulfilled by the ***QuEChERS*** methodology (*Quick, Easy, Cheap, Effective, Rugged and Safe*), developed by Anastassiades et al. (73). In view of its rapidity and cheapness, and the fact that it enables a wide spectrum of compounds to be determined, this approach has become very popular among scientists for determining pesticide residues, especially in food samples. As QuEChERS ensures excellent extract cleanup and high analyte yields (74), its use is increasing worldwide. Work is now in progress to optimize this methodology for analyzing food products and other matrices for their contents of particular groups of compounds (75–78). Figure 4 outlines the QuEChERS procedure in diagrammatic form (79).

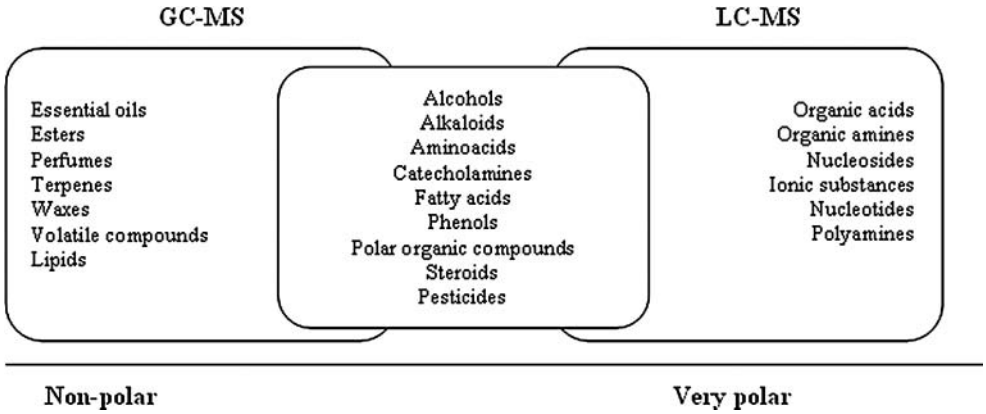

FIG. 5. Chemical compounds that can be determined by GC-MS and LC-MS.

TABLE 6
Parameters of analytical methodologies used to determine pesticide levels in bee products and in bees as research organisms.

Analytes	Sample type	Sample size	Preliminary preparation	Extraction technique	Cleanup	Final determination technique	Ref.
25 OCP	Honey	5 g	—	SPE	—	GC-MS	(4)
7 PCB							
8 PBDE						GC-ECD	
17 OCP	Propolis	1 g	Pulverization by combustion	LLE	SPE	GC-ECD	(12)
22 OPP	Honey	5 g	Dissolution	SPE	-	LC-APCI-MS	(19)
12 insecticides	Bees	0.5 g	Homogenization	MSPD	-	GC-NPD	(25)
29 pesticides	Honey	5 g	Dissolution	SFE	SPE	GC-ECD	(27)
37 pesticides	Bee	3 g	Lyophilization, homogenization	LLE	Filtration	GC-NPD	(30)
						LC-APCI-MS	
41 pesticides	Pollen	23 g	Deep-freezing	LLE	SPE	LC-MS/MS	(32)
18 OCP	Bee	13 g	Lyophilization	SPME	-	GC-NPD	(33)
29 pesticides	Bee	3 g	Lyophilization	SPE	GPC	GC-NPD/ECD/FID	(41)
15 OCP	Honey	12 g	Dissolution	SPE	—	GC-ECD/NPD	(42)
7 OPP							
6 PCB							
42 OCP	Honey	5 g	Dissolution	SPE	-	GC-MS LC-APCI-MS	(43)
11 OCP	Honey	10 g	Dissolution	LLE	SPE	GC-MS (SCAN)	(44)
48 pesticides	Honey	10 g	-	LLE	SPE	GC-MS (SIM)	(45)
24 pesticides	Honey	4 g	Dissolution	LLE	SPE	GC-ECD	(46)
21 pesticides	Honey	50 g	Dissolution	LLE	SPE	GC-ECD/NPD	(47)
450 pesticides	Honey	15 g	Dissolution	LLE	SPE	GC-MS	(48)
						LC-MS/MS	
10 insecticides	Honey	10 g	Dissolution	LLE	—	GC-NPD	(49)
51 pesticides	Honey	10 g	Dissolution	SPE	—	GC-MS(SIM)	(49)
22 OPP	Honey	5 g	Dissolution	SPE	—	LC-MS	(51)
	Bees	0.5 g	—	MSPD			
15 OPP	Honey	1 g	Dissolution	SPE	—	GC-ECD	(52)
17 pesticides	Honey	1 g	Dissolution	OC LLE	—	LC-MS/MS	(53)
	Bees	0.5 g	Deep-freezing and pulverization	OC LLE			
	Beeswax	0.5 g	Deep-freezing and pulverization	LLE			
23 pesticides	Honey	1 g	Homogenization	UE	SPE	GC-EI-MS	(54)
16 pesticides	Honey	1.5 g	Homogenization	SPME	—	GC–AED	(55)
150 pesticides	Bee	2 g	Homogenization	QuEChERS	dispersion SPE	GC-MS/MS	(67)
9 pesticides	Royal jelly	0.5 g	Homogenization	SPE	-	GC–micro-ECD	(68)
13 pesticides	Beeswax	0.1 g	Dissolution	LLE	SPE	GC-ECD	(69)
18 pesticides	Beeswax	2 g	Dissolution	LLE	SPE	GC-MS/MS	(70)
11 OPP	Honey	—	Dissolution	SPME	-	GC-FPD	(71)
15 OPP	Bees	0.5 g	Deep-freezing	MSPD	—	GC-MS	(72]

Combined Techniques

Combining chromatographic techniques with mass spectroscopy has made the rapid identification of separated compounds possible. Sample analysis by MS-MS is very quick and highly selective, it is excellent for analyzing groups of compounds with similar structures, and it efficiently removes the matrix interferents generally present in biological samples (80).

Gas chromatography coupled with mass spectrometry and electron ionization (GC-EI-MS) and liquid chromatography coupled with electrospray ionization and tandem mass

TABLE 7
Comparison of GC-EI-MS and LC-ESI-MS/MS with respect to the means of sample ionization [81-83].

	Typical concentration range of standard solutions	Mass range of analyzed sample	The analytes most commonly determined	Features of the ionization technique
GC-EI-MS	1–10, 000 ng/mL	10–1,000 Da	Low-molecular weight, inorganic, organic in solid form, volatile substances.	• "hard" ionization, during which the molecule fragments, • Universally applicable, • Individual substances can be identified by comparison of mass spectra with spectrum libraries..
LC-ESI-MS/MS	0.1–100 ng/mL	50—80, 000 Da	Medium- and high-molecular weight organic substances, peptides, proteins, polymers, nucleic acids in liquid form, non-volatile substances.	• "soft" ionization, during which only a molecular ion is formed, without the molecule fragmenting, • High sensitivity of determinations, • Low concentrations of analytes can be determined, • Costly, • Problems with defining analyte structure.

spectrometry (LC-ESI-MS/MS) are the most important techniques used for determining pesticides at the present time. Both have different selectivities depending on the compounds to be determined (81). Figure 5 lists the chemical substances that can be determined using GC-MS and LC-MS (82). Table 7 compares GC-EI-MS and LC-ESI-MS/MS with respect to the means of sample ionization.

SUMMARY

The presence of xenobiotics in food constitutes a serious threat to human health; hence, the monitoring and analysis of their residues in food is a matter of urgency.

Analysis of bee products and the bees themselves yields information on:

- The state of contamination of the environment in which a given bee colony operates;
- The state of contamination of bee products, renowned for their therapeutic properties, and the contaminants found in them that may endanger consumer health.

Hence the need to develop new analytical methodologies ensuring the concomitant determination of a broad spectrum of analytes from a single sample and in a single analytical run. Much attention is being given to novel methods, based on the concept of sustainable development, of sample preparation and extract cleanup prior to the final determination.

ABBREVIATIONS

Abbr./acronym	
AED	*Atomic emission detector*
APCI	*Atmospheric pressure chemical ionization*
DDT	*1,1,1 – trichloro – 2,2 – bis-(4'chlorophenyl) ethane*
ECD	*Electron capture detector*
EI	*Electron impact ionization*
ESI	*Electrospray ionization*
FID	*Flame ionization detector*
GPC	*Gel permeation chromatography*
GC	*Gas chromatography*
GC-MS	*Gas chromatography– mass spectrometry*
HCH	*Hexachlorocyclohexane*
LC	*Liquid chromatography*
LC-MS	*Liquid chromatograph y– mass spectrometry*
LC-MS/MS	*Liquid chromatography – tandem mass spectrometry*
LLE	*Liquid-liquid extraction*
MAC	*Maximum allowable concentration*
MRM	*Multiresidue method*
MS	*Mass spectrometry*
MSPD	*Matrix solid-phase dispersion*
NPD	*Nitrogen – phosphorus detection*
NPD	*Nitrogen – phosphorus detection*
OCLLE	*On-column liquid-liquid extraction*
OCP	*Organochlorine pesticides*
OPP	*Organophosphorus pesticides*
PAH	*Polycyclic aromatic hydrocarbons*

SFE *Supercritical fluid extraction*
SPE *Solid-phase extraction*
SPME *Solid-phase microextraction*
SIM *Selected ion monitoring*
SCAN *Scan monitoring*
UE *Ultrasonic extraction*

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