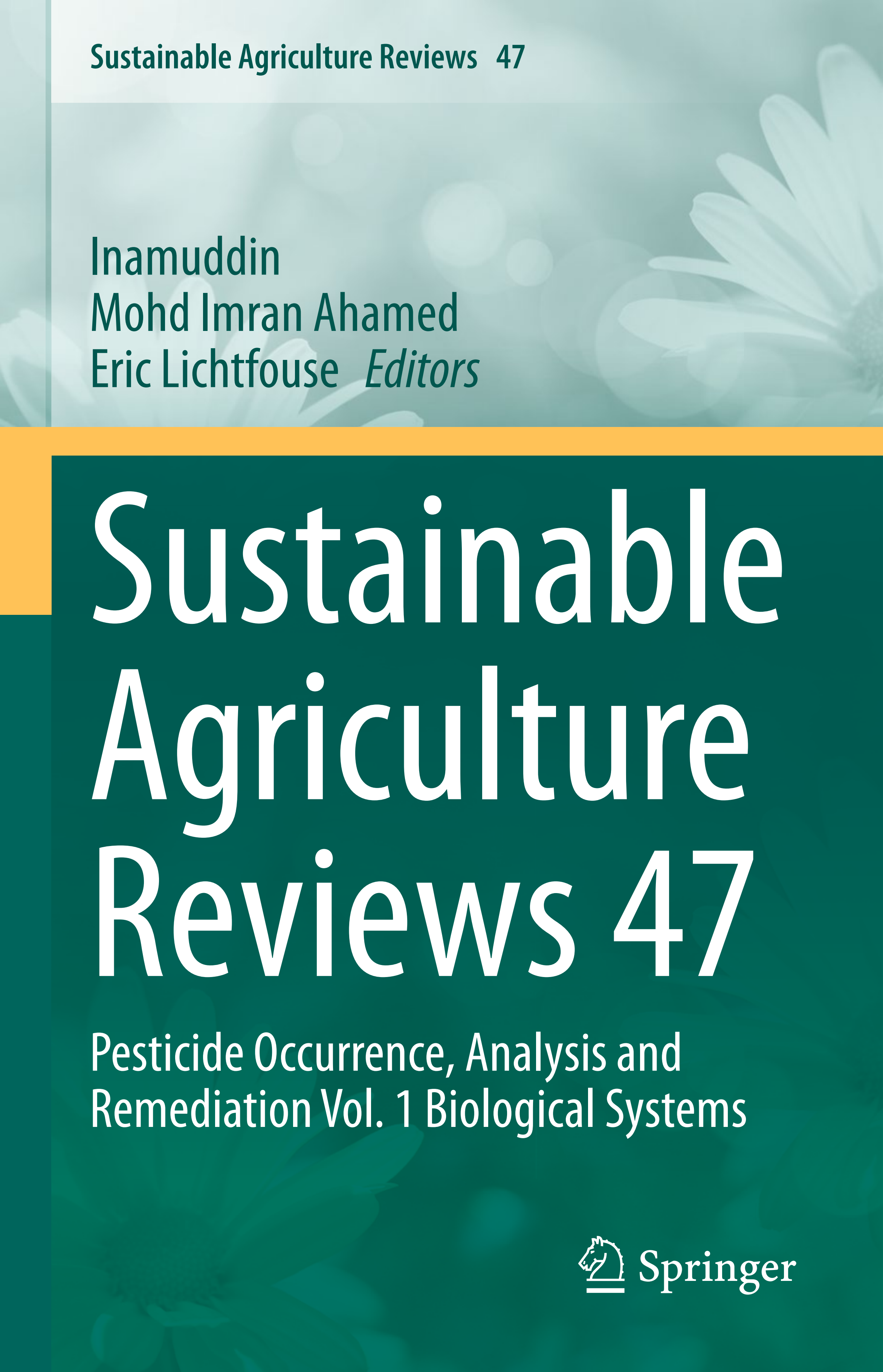

Sustainable Agriculture Reviews 47

Inamuddin
Mohd Imran Ahamed
Eric Lichtfouse *Editors*

Sustainable Agriculture Reviews 47

Pesticide Occurrence, Analysis and Remediation Vol. 1 Biological Systems

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Pesticide Occurrence, Analysis and Remediation Vol. 1 Biological Systems

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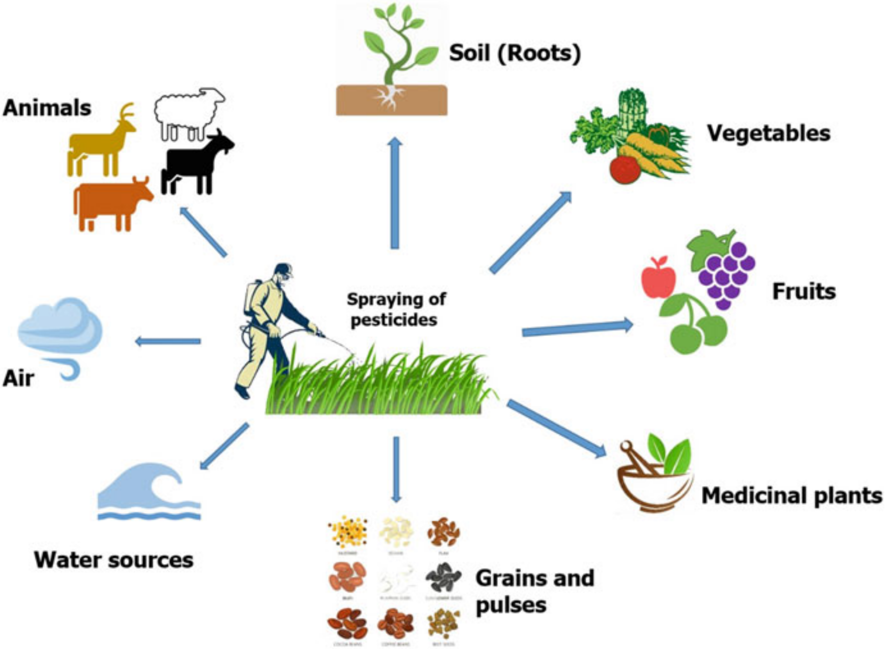
Preface

Pesticides play an important role in agriculture by protecting crops against pest attacks, thus improving the production yield. However, the use of pesticides causes toxicity to human beings because of their existence as pesticide residues. The main causes for pesticide residues in food are lack of knowledge regarding pesticide use and non-strategic agricultural practices to improve crop production. This book reviews the occurrence of pesticide residues in biotic and abiotic environments. The topics include pesticide residues present in aquatic animals, food, agriculture, horticulture, pollen and dairy products. This book will help researchers in academia and industry to solve critical issues related to pesticide residues.



Chapter 1 by Pareja et al. highlights trends in sample preparation approaches and instrumental techniques for pesticide residues determination in fish. Additionally, the occurrence of pesticides in fish is addressed. Perspectives in the field of analytical applications, food safety and environmental surveillance of pesticides in fish are described. Chapter 2 by González-Curbelo and González-Sálamo provides a critical vision of the analytical methodologies that have been and are also being currently applied for the extraction of multiclass pesticides from milk, dairy products and pollen with particular emphasis on the sample pre-treatment step, which is crucial for a suitable analytical determination. Chapter 3 by Mondal and Kole deals with various processes involved in pesticide transportation to the hydro-system. Methods for determination of multiple pesticides and their residual status in the aquatic system are discussed. The reason for the occurrence of banned organochlorine pesticides is explained. Deterministic approaches for risk assessment to aquatic health and options for management are highlighted. Chapter 4 by Soares at al. discusses the use of pyrethroid pesticides to control ectoparasites in poultry that, with an indiscriminate application, can leave residues in the environment and animal tissues and harm the health of poultry farmers. It also presents safe and clean new technologies as strategies to replace pesticide use. Chapter 5 by Fernandes et al. gives a summary of analytical methods for the analysis of pesticide residues in strawberries. Several sample preparation and chromatographic techniques are discussed in detail. The focus of the chapter is to show the importance of the analytical data for food safety and quality. Chapter 6 by Karasali and Pavlidis discusses the mechanism of formation and the environmental significance of 'Non-Extractable Residues' in soil. The term 'Bound Residues' and the new term 'Non-Extractable Residues' are defined and interpreted in different ways. The major focus is given to their types and the methods used for their identification and quantification. Chapter 7 by Sousa et al. addresses the relevance of human biomonitoring of pyrethroids. 3-phenoxybenzoic acid is a major urinary biomarker for numerous pyrethroids and most frequently assessed. Studies from different countries and continents that assess exposure in diverse populations are addressed, and finally, the different applied analytical methodologies are also discussed. Chapter 8 by Nazir et al. highlights the different techniques of pesticide contaminants removal from water. These include carbon nanotubes, graphene, nano-crystalline metal oxides, hydrogen peroxides, ultrasonic wave, hybrid process, bioremediation, photocatalytic degradation, adsorption, membrane separation, biopurification systems, composite material and ion exchange resins. The emphasis is on inexpensive detection, remediation and removal methods. Chapter 9 by Rano and Singh discusses different methods of pesticide contamination in various food residues and its related negative health issues. It further describes various chemical methods for the analysis of pesticide content in food. Different diagnostic methods have also been highlighted to quantify pesticide ingested via food and its bio-magnification in the body. Chapter 10 by Puthur et al. discusses the deleterious effects of various pesticides on the avian fauna inhabiting the wetland ecosystems. Pesticides, specifically organochlorines, organophosphates and carbamates, induce various hormonal imbalances and inactivation of enzymes, leading to altered or defective growth responses in birds, which is summarized in this paper. Chapter 11 by Clasen et al. addresses the problem of disorderly pesticide use and the risks it may pose to nature and humans. The methods of pesticide evaluation currently used as well as the occurrence and effects of different pesticides on aquatic and terrestrial organisms and humans are presented.

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Mohd Imran Ahamed received his Ph.D. degree on the topic "Synthesis and characterization of inorganic-organic composite heavy metals selective cation-exchangers and their analytical applications" from Aligarh Muslim University, Aligarh, India, in 2019. He has published several research and review articles in internationally acclaimed journals of. Dr. Ahamed has also edited various books published by Springer, CRC Press Taylor & Francis Asia Pacific and Materials Science Forum LLC, U.S.A. He has completed his B.Sc. (Hons) Chemistry at Aligarh Muslim University, Aligarh, India, and M.Sc. (Organic Chemistry) at Dr. Bhimrao Ambedkar University, Agra, India. His research work includes ion-exchange chromatography, wastewater treatment and analysis, bending actuator, and electrospinning.

Eric Lichtfouse is a Biogeochemist at Aix Marseille University who has invented carbon-13 dating, a molecular-level method allowing to study the dynamics of organic compounds in temporal pools of complex environmental media. He is Chief Editor of the journal *Environmental Chemistry Letters* and the book series Sustainable Agriculture Reviews and Environmental Chemistry for a Sustainable World. Dr. Lichtfouse is the author of the book *Scientific Writing for Impact Factor Journals*, which includes an innovative writing tool: the Micro-Article.

Chapter 1
Determination of Pesticide Residues in Fish

Lucía Pareja, Horacio Heinzen, María Verónica Cesio, Marcos Colazzo, and Andrés Pérez-Parada

Abstract Pesticide residues occurring in fish of marine and freshwater media is a continuous issue for food safety and environmental monitoring. In the last 40 years the analysis of organohalogen pesticides in marine fish has been conducted seeking to trace the bioaccumulation and biomagnification in food webs. The analysis of organochlorine, highly lipophilic compounds, was performed using sample preparation methods with a large solvent consumption followed by instrumental determination using gas chromatography with electron capture detectors. Fish as an analytical matrix presents particular challenges for residue analysis because of the large amount of different edible species, the variable proportion of fatty tissues and the they requirements for sample preservation. Moreover, different non-traditional tissues such as gills or the brain are of recent interest in environmental monitoring and pesticide exposure studies. In the last decades, new concepts for the sample preparation of trace contaminants in fish have been developed, based on sample

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miniaturization and minimal reagent consumption capable of analyzing a large palette of active principles, from non-polar to semi-polar ones.

The focus of this chapter is to present the complexity of the possible analytical matrices and the different strategies used for pesticide residues determination. The advent of liquid and gas chromatography coupled to tandem mass spectrometry, expanded the scope of target compounds monitored in fish, particularly for freshwater agroecosystems. The application of new mass spectrometry techniques for pesticide residue analysis in fish, along with the matrix effects observed with the applied instrumentation are deeply discussed. The evidence about different pesticides currently used worldwide and their occurrence in the tissues of freshwater fish at trace levels is presented. The bioaccumulation of banned persistent organic pollutants in edible fish is still a matter of interest for human exposure. Nevertheless, the regulation of maximum residue limits of currently used pesticides seeking a safe fish consumption is scarce.

Keywords Fish · Marine and freshwater · Currently used pesticides · Pesticide residues · Sample preparation · Gas chromatography · Liquid chromatography · Hyphenated mass spectrometry · Environmental monitoring · Food safety

1.1 Introduction

Different fish species, from marine and freshwater habitats are a key source of food for humans at global scale.

Synthetic organic contaminants have been monitored in edible fish from marine origin for more than 40 years. However, the compounds, named as legacy contaminants or persistent organic pollutants still play a role on health risks to aquatic ecosystems and human consumers (Boitsov et al. 2019; Pheiffer et al. 2018; Sun et al. 2018; Wu et al. 2018). The monitoring of organochlorine pesticides in fish muscle tissue initially started as a concern of bioaccumulation and biomagnification research. Highly persistent and non-polar compounds with log octanol-water coefficient higher than 5 such as hexachlorocyclohexane congeners, aldrin, and dichlorodiphenyltrichloroethane were found to occur in fish muscle tissues particularly associated to the high fat content (Pheiffer et al. 2018; Rios et al. 2019).

Widespread expansion of agricultural techniques based on application of pesticides led to an increasing use and disposal of pesticide residues in aquatic environments worldwide. Chemical diversity of pesticides has changed over the last decades and the currently used pesticides are, in general, more polar and less persistent. Nevertheless, significant exposure is evidenced in agricultural basins for freshwater fish species (Abalos et al. 2019; Ernst et al. 2018; Penland et al. 2018; Pheiffer et al. 2018; Picó et al. 2019; Vieira et al. 2019). The biological effect of the findings at sublethal levels is not well understood. The presence of pesticide residues in fish as multiple stressors in the aquatic environment can help to rationalize the effects of contaminants on non-target aquatic biota (Vieira et al. 2019). A multidisciplinary approach would be helpful to integrate ecological, ecotoxicological and chemical observations that will give, through pesticide residues in fish, a holistic vision of the situation of a given environment.

Pesticide residues occurrence in fish is not only relevant from a toxicological point of view. Fish exposed to different environments are useful sources of information about the contamination level of the surrounding agroecosystems even at basin scale (Ernst et al. 2018; Ríos et al. 2019; Vieira et al. 2019). Pesticides can be incorporated onto fish tissues through the fish gills, either via water exposure or by the ingestion of contaminated feed. The route by which the pesticide integrates the fish body is relevant to assess contamination at muscle level. In the first case, no biotransformation occurs; the pesticide could be incorporated as such. In the latter case, the pesticide could be not only metabolized in the gastrointestinal tract but also stored in the viscera, normally, the non-edible portion of fish. Other situations, crucial to evaluate bioaccumulation or biomagnifications, are the habits of the animals, either migratory or not, and the existing trophic interactions (Ernst et al. 2018; Pérez-Parada et al. 2018; Picó et al. 2019). From a physicochemical point of view, when a compound shows a solubility in water lower than 0.0002 g/L, could be adsorbed to the suspended organic matter in freshwater environments and distributed within the fish tissues, no matter the route of exposure. Moreover, the amount of lipids plays a significant role in the storage capability of fish species. Lipid amount varies significantly seasonally, and has to be considered during pesticide residue analysis (Crane et al. 2007; Pérez-Parada et al. 2018; Ríos et al. 2019; Wickham et al. 1997).

Exposure to different currently used pesticide families led to recent reports showing pesticide occurrence ranging from pyrethroids and organophosphate insecticides to fungicides. Bioaccumulation of strobilurins, triazoles, triazines and chloroacetamide herbicides at µg/kg levels have been proved (Pérez-Parada et al. 2018). Additionally, some environmental organic contaminants like pharmaceuticals and semi-polar pesticides with log octanol-water coefficient higher than 2, has been detected in fish (Ernst et al. 2018; Picó et al. 2019; Rojo et al. 2019; Vieira et al. 2019). Considering the levels of pesticides residues found in fish it is important to pay attention to the safety of fish consumption. Agricultural pesticides in fish are assorted as extraneous chemicals. For that reason, extraneous maximum residue levels (E-MRLs) is matter of current interest at *Codex Alimentarius* (Pérez-Parada et al. 2018). Few pesticides are currently regulated and monitored in commercial trade from those existing based on specific national requirements for both persistent organic pollutants and currently used pesticides. Existing regulation deserves special attention on a few specific substances handling maximum tolerance levels and action levels (Food and Drug Administration 2011).

Summarizing, fish is one of the main aquatic organisms where an immediate impact of contamination because of agricultural activities can be observed. Furthermore, is considered a fundamental component of a balanced diet. Since fish contribution in nutrients is indisputable, is necessary to ensure the quality of fish to preserve human health. For this reason, the evaluation of the level of pesticide residues in fish is important to ensure food safety (Molina Ruiz et al. 2015; Kalachova et al. 2013).

The interest in pesticide residues determination in seafood and particularly fish is actually not new. Interestingly, some well-established sample preparation methods for pesticide residues determination such as matrix solid phase dispersion were developed for organochlorine pesticides determination in fish (Long et al. 1991). New high throughput sample preparation methodologies, such as the variations of QuEChERS coupled to new and highly sensitive and selective instrumental techniques based on hyphenated mass spectrometry, has enabled a fast expansion of the scope to multi-class organic contaminants in fish at trace levels (Barbieri et al. 2019; Colazzo et al. 2019; Picó et al. 2019).

In this chapter, the determination of pesticides in fish, focused on contemporary sample preparation approaches will be discussed. The advantages and disadvantages as well as the coupling to modern tandem mass spectroscopic techniques will be addressed. Moreover, potential features of recent analytical tools aiming lower detectability of multi-class contaminants enabling an increase in knowledge of pesticide dynamics in the aquatic environment and food safety will be shown.

1.2 Fish Matrix Considerations

Fish and fish body parts are considered complex and variable matrices. As fish does not have a fixed chemical composition an approximate global composition, is generally accepted (Table 1.1). Furthermore, other minor components such as sugars, nucleotides and vitamins are also present. A thumb rule states that water and fat are inversely correlated, adding 80% of fish composition.

From an environmental point of view, the presence of compounds with high n-octanol-water partition coefficient such as persistent organic contaminants, pyrethroids and strobilurins in liver and viscera of fish give relevant information. Pesticide residues in the digestive apparatus of fish are indicative that the way of entry, is through food and water consumption. Therefore, the contaminants can be incorporated either from smaller fish, in a typical example of biomagnification or from sediments, catch tissue, as well as suspended organic matter. A higher frequency of dichlorodiphenyldichloroethylene findings in *Cnesterodon decemmaculatus*, a sediment feeding fish, caught in lagoons surrounded by farms where agricultural activities have been performed in the last hundred years was reported (Pareja et al. 2013).

Table 1.1 General proximate chemical composition of fish tissues

Chemical composition	Amount (%)
Water	65–90
Protein	10–22
Fat	1–20
Mineral	0.5–5

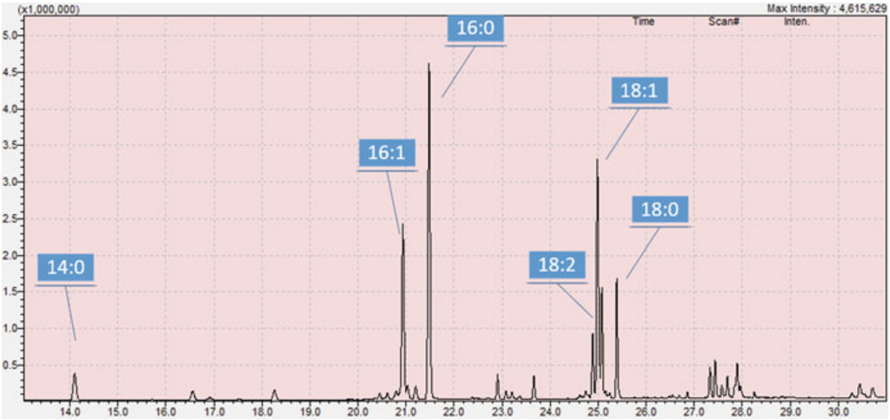

Fig. 1.1 Shows a typical fatty acid composition in fish fillet of *Prochilodus lineatus* captured in Uruguay River. The chromatogram was obtained by gas chromatography mass spectrometry in full scan mode. The main fatty acids are highlighted in blue; C14:0 myristic acid, C16:0 palmitic acid; C-18:0 stearic acid, C-18:1 oleic acid, C-18:2 linoleic acid, C-18:3 linolenic acid

Pesticide findings in fish fillets are a relevant issue from the food safety point of view. The possibility for the occurrence of residues in fish matrix is a combination of physicochemical properties and body compartments that could allow the partition between blood and tissues. Fat deposits are frequent in fish muscle, being the depositories for the most lipophilic compounds. As described above, the characteristics of the matrix varies not only with the fish species but also with the part of the fish to be studied. Moreover, matrix composition changes dramatically within species seasonally. The lipid amount increases when food is available but drops down when fish are in the spawning season. In addition, different amounts and types of lipophilic compounds such as vitamins, triglycerides, phospholipids, glycolipids, sterols as well as long-chain polyunsaturated fatty acids, among others could be present depending on the age and the fish species (Fig. 1.1). The composition of fish matrix should be taken into account when designing the sample preparation and instrumental determination technique because the described compounds can affect the determination of the target residues in different ways.

The size of the animal is also of paramount importance. Little fish, smaller than 5 cm like *Pomoxis spp, crappies,* or *Cnesterodon decemmaculatus,* madrecitas, are analyzed completes (Fig. 1.2). The aforementioned species are useful specimens for caged experiments in water contamination research studies where the animal exposure to the aquatic environment can be monitored in a given period of time. The information gathered has relevance when evaluating the environmental status of a given stream or catchments (Pareja et al. 2013).

In order to select a “fit for purpose” sample preparation method; the lipid content in the tissues should be calculated before pesticide residues determination, (Choi et al. 2016; Ernst et al. 2018; Molina Ruiz et al. 2015; Morrison et al. 2016; Yao et al. 2016). Different procedures are used for lipid determination: ISO 734-1:2008, Blight

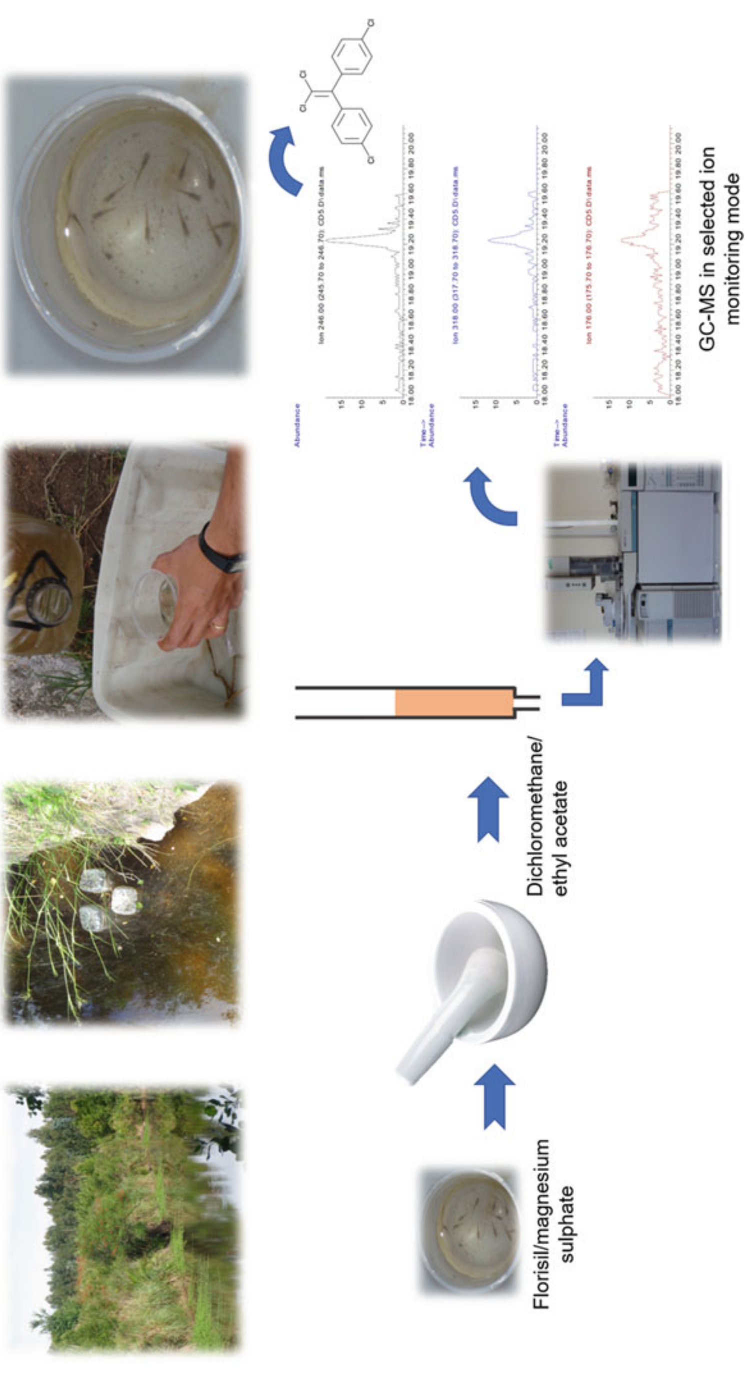

Fig. 1.2 Experimental design of a study performed at Cañada del Dragón, in Montevideo, Uruguay, for the analysis of pesticides in *Cnesterodon decemmaculatus*. The sample is extracted by matrix solid phase dispersion with gas chromatography mass spectrometry analysis in selected ion monitoring mode

& Dyer and Folch methods, among others (Ramalhosa et al. 2012). Moreover, in some cases moisture content is also evaluated (Sapozhnikova et al. 2015).

1.3 Importance of Fish Analysis

Pesticides have been considered as one of the most relevant environmental pollutants due to the persistence, mobility and long-term health effects on living organisms (Hernández et al. 2019). In the last decades, the interest was concentrated on persistent organic substances such as organochlorine and organophosphate pesticides because of the wide use, persistence and ability to bioaccumulate through trophic chains (Greco et al. 2010; Robinson et al. 2016). However, in recent years new types of widespread use organic contaminants were developed, and should be included in the analytical scope.

1.4 Analytical Methodologies for Pesticide Residues Determination in Fish Samples

Plenty of different analytical methods are reported in the literature to determine the presence of pesticide residues in fish. The targeted methodologies employed for these determinations must ensure true and precise results at low concentration levels for all the selected analytes, in accordance to the international guidelines. Moreover, straightforward, cheap and environmentally friendly methodologies should be preferred to carry out the task (Wilkowska and Briziuk 2011).

Generally, pesticide determination involves several steps; sampling, sample preparation including homogenization, extraction, clean-up, separation, detection, identification, quantification and data analysis (Samsidar et al. 2018).

1.4.1 Sampling, Transport, Traceability and Storage of Laboratory Samples

Fish tissue suffers very rapid spoilage, in terms of a few hours after harvest or catching, with a consequent potential change in matrix conditions over a reduced time. Therefore, fast storage under refrigerated or freezer conditions of the samples or performing the analysis immediately after sampling is recommended. All the reports stated that, once the sampling is performed, the samples should be transported refrigerated upon arrival at the laboratory and stored at −20 °C until analysis.

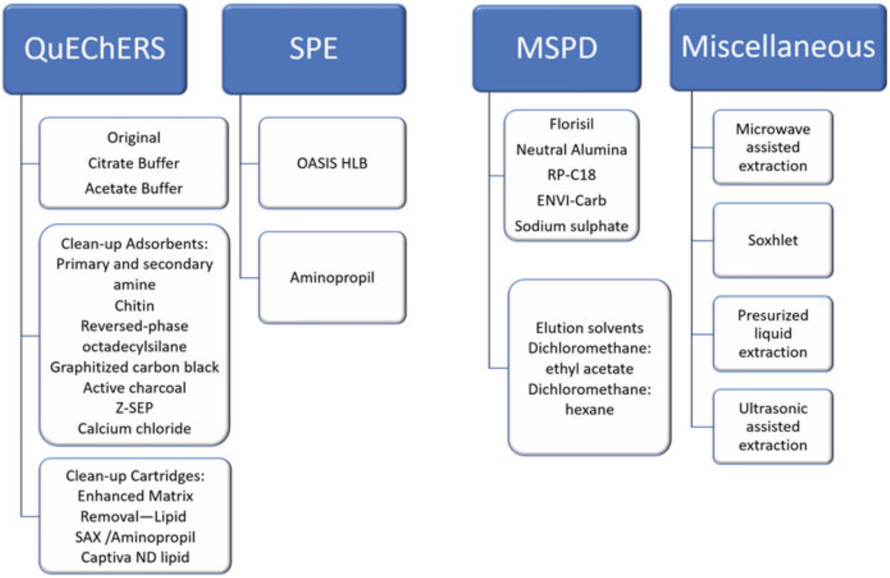

Fig. 1.3 Main extraction and clean up methodologies reported for the sample treatment of fish during pesticide residue analysis. *ASE* accelerated solvent extraction, *MSPD* matrix solid phase dispersion, *SPE* solid phase extraction, *UAE* ultrasonic assisted extraction, *MAE* microwave assisted extraction, *PLE* pressurized liquid extraction

Several authors reported the use of a lyophilized fish sample (Choi et al. 2016; He et al. 2017; Masiá et al. 2013; Mijangos et al. 2019; Picó et al. 2019; Zhao et al. 2019), while others prefer to use fresh or refrigerated samples. Regarding the homogenization of the samples, different approaches are described. Baduel et al. (2015) and Zhou et al. (2008), processed the samples with a kitchen blender, whereas Choi et al. (2016), removed the skin and used an ultra-dispenser to homogenize the fish muscle. Other protocols grinded the entire fish, including muscles, skin and bones (Picó et al. 2019). The main workflows reported for fish matrix are summarized in Fig. 1.3.

The most common tissues to be sampled are muscle fillet with or without the skin and liver tissue. According to the working document on the nature of pesticide residues in fish by SANCO, tissue samples should be dissected and the weight of each tissue recorded in the raw data. If the samples cannot be analyzed immediately, the sample should be stored frozen at or below −18 °C (European Commission 2013). Several reports advised to separate fish meat from bones and skin before homogenization (Chatterjee et al. 2016). Lipid peroxidation is one of the most problematic points when preparing the sample for analysis. Sample comminuting at room temperature, even of frozen fish causes heating of the sample, air absorption and lipid deterioration. The present peroxides can degrade the pesticides under study. Because of the possible degradation, assisted milling with dry ice or liquid nitrogen is advised. The sample obtained using the described methodologies is a homogenized fine powder that allows an easy and representative extraction of the studied compounds (Sapozhnikova et al. 2015).

1.4.2 Extraction and Clean-Up Methods for Pesticide Residues Analysis in Fish

The selection of a suitable solvent for the extraction step is one of the most important aspects to obtain acceptable results when developing pesticide residue analysis in complex matrices. Pesticide extraction from tissues depends not only on the physicochemical properties but also in the matrix composition.

Generally, the sample treatment comprises two main steps: the extraction and the clean-up step. The first one consists of the homogenization of the sample with an organic solvent or solution water: organic solvent in different proportions with or without pH adjustment. The extraction could be assisted using different shaking devices; namely an ultrasonic bath, a blender or a homogenizer. The clean-up step seeks to separate the target compounds from any other matrix components that may interfere during the instrumental analysis, either causing detection or quantitation problems (Bennet et al. 1997). The clean-up is performed using particular adsorbents and salts in a suitable combination for the removing of the specific compounds from each matrix, sometimes the procedure could be also done in two steps, for example the typical dispersive one and a freeze out procedure in order to better precipitate the fatty compounds (Anastassiades et al. 2003).

According to Lazartigueset al. (2011a, b) the speed of use of an Ultra-turrax during homogenization is of crucial importance; while a low speed might not be useful. Nevertheless, a high speed could potentially induce pesticide losses. A middle speed was the best option when maintaining the sample in a water bath. Ideally, the exchange area between the comminuted sample and the extraction solvent should be maximized (Lazartigues et al. 2011a, b).

Several protocols have been reported for pesticide determination in fish (Fig. 1.4). However, the most used for fish matrix are the QuECHERS template, matrix solid phase dispersion and solid phase extraction. However, other approaches, like Soxhlet extraction, pressurized liquid extraction or ultrasonic assisted extraction are reported especially for organochlorine residues determination in fish are described in Sect. 1.4.2.4.

1.4.2.1 Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) Extraction

In recent years, the scope of the analytical methodologies has been broadened from a single-family class of pesticides to several different chemical classes as well as other anthropogenic contaminants. QuEChERS provides a versatile template of several

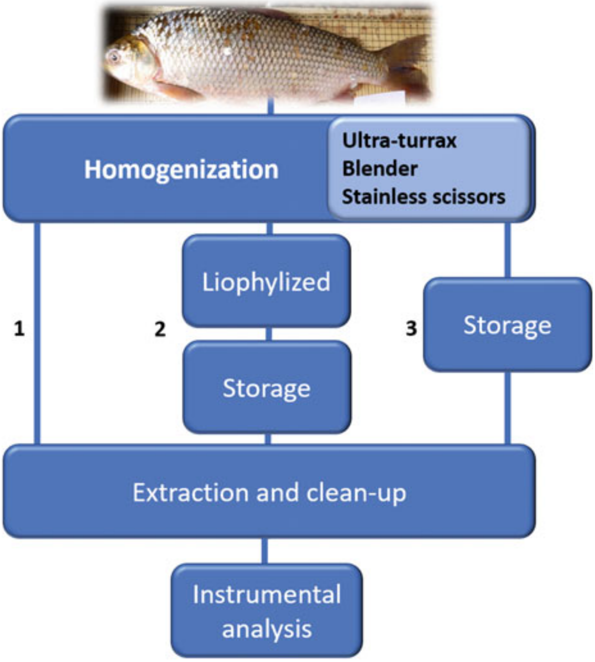

Fig. 1.4 Summary of three main workflows used for the sample treatment during pesticide residues analysis in fish. The general process consists of the homogenization of the sample followed in some cases by sample storage. Then, an extraction and clean-up step and finally the Instrumental analysis

protocols depending on the type of pesticide, matrix, instrument used or aim of the analysis. QuEChERS is a nice alternative with few steps, which provides in general, good analytical results.

The extraction is carried out with acetonitrile or acidified acetonitrile with citrate or acetate salts to buffer the extraction media. Phase separation is induced through a salting–out with sodium chloride followed by the addition of anhydrous magnesium sulfate as drying agent (Anastassiades et al. 2003; Anastassiades 2007; Lehotay et al. 2005). The formic acid as well as acetic acid is recommended to improve the stability of pesticides aforementioned methiocarb, chlorothalonil, or alachlor (Kaczyński et al. 2017).

The typical clean-up step of QuEChERS consists in a dispersive solid phase extraction, d-SPE, based on different sorbents and salts. The ideal sorbent is one capable of retaining co-extractives without interfering with the selected pesticides turning into a procedure as simple and fast as possible. The degree of purification using the QuEChERS template ranges from none to significant removing of the metabolites from the sample.

Several authors report the implementation of QuEChERS for fish samples. Table 1.2 summarizes the different sample preparation methods developed for the analysis of pesticide residues based on the QuEChERS template in fish. Most of the reports used the traditional clean-up with; primary and secondary amine, reversed-phase octadecylsilane, graphitized carbon black and magnesium sulfate (Barbieri

Table 1.2 Summary of the QuEChERS based methods reported for the determination of pesticides in fish matrix

Number of pesticides	Matrix	Extraction conditions	Clean-up conditions	% Rec (% RSD)	References
24	*C. carpio, A. sturio*	2.5 g sample + 5 mL H_2O + 10 mL MeCN. Shaken by hand 1 min + 0.5 g $Na_2HCit.1.5H_2O$ + 1 g $Na_3Cit.2H_2O$ + 4 g $MgSO_4$ + 1 g NaCl. Shaken by hand 1 min. 1 mL $CHCl_3$ was added and shaken for 1 min. Centrifugation for 15 min at 8700 rcf	6 mL aliquot + 1 g $MgSO_4$ + 150 mg PSA + 50 mg SAX + 50 mg NH_2. Shaken by hand for 1 min + centrifugation for 5 min at 5000 rcf. 4 mL aliquot + 1 mL $CHCl_3$ and 100 mg RP-C18. Shaken by hand for 1 min + Centrifugation for 5 min at 5000 rcf. 4 mL extracts + 40 μL 5% formic acid in MeCN, and evaporated under N_2 at 40 °C to dryness. Re-dissolved in 1 mL hexane. Freezed-out at −26 °C overnight. Separation of the precipitates by decantation.	61–122 (1–13)	Molina-Ruiz et al. (2015)
15	*Specie: not specified*	5 g sample + 5 mL H_2O vortexed for 1 min + 15 mL 1% acetic acid in MeCN + 2 mL hexane. Shaken by hand 1 min. 6 g $MgSO_4$ + 1.5 NaAc + vortex for 2 min. Centrifugation at 5000 r.p.m. for 5 min.	A portion of the middle layer freezed-out for 20 min at −20 °C + 100 mg $CaCl_2$ + 150 mg $MgSO_4$. 1 mL extract purified with 50 mg PSA + 50 mg Florisil + 150 mg RP-C18 + 150 mg $MgSO_4$, vortexed for 1 min and centrifuged for 5 min at 10,000 r.p.m.	60–127 (1–11)	Chatterjee et al. (2016)
5	*Specie: not specified* (Liver and muscle)	2 g muscle/0.5 g liver + 5 mL H_2O vortexed for 1 min + 10 mL 1% acetic acid in MeCN + 6 g	7 mL aliquot + 900 mg $MgSO_4$ + 150 mg RP-C18 + 150 mg PSA, shaken and centrifugation for	Muscle: 66–195 (0.09–13)	Han et al. (2016)

(continued)

Table 1.2 (continued)

Number of pesticides	Matrix	Extraction conditions	Clean-up conditions	% Rec (% RSD)	References
		$MgSO_4$ + 1.5 g NaAc. Shaken by hand 1 min. Centrifugation for 2 min at 2364 *x g*.	2 min at 2364 x*g*. 5 mL extract evaporated to dryness. Re-dissolved in 200 μL MeOH, separate into two aliquots which were dried again. One reconstituted with 100 μL MeOH: H_2O. The other in dichloromethane for analysis	Liver: 56–286 (0.02–9)	
52	*T. thymallus*, *L. cephalus*, *C. gobio*, *S. trutta marmoratus*, *S. trutta fario*	10 g sample + 5 mL H_2O + 10 mL MeCN. Shaken by vortex for 1 min at 2500 r.p.m. Centrifugation for 5 min at 4000 r.p.m.	7 mL aliquot +400 mg PSA + 400 mg RP-C18 + 1200 mg $MgSO_4$. Vortex 1 min at 2500 r.p.m. Centrifugation 5 min at 4000 r.p.m.	21–106 (3.3–20.6)	Barbieri et al. (2019)
5	*O. niloticus*	0.5 g + 9.2 mL acetone: hexane (1:1) shaken by hand 1 min + 2 g Na_2SO_4. Shaken manually 1 min + 4 $MgSO_4$ + 1 g NaCl, vortexed 1 min + sonication for 10 min. Centrifugation at 4 °C, for 10 min at 4000 r.p.m.	Supernatant evaporated to dryness, resuspended in 2 mL acetone: hexane (1:1, v/v) + 100 mg PSA + 23 mg RP-C18 mg + 400 mg $MgSO_4$, vortexed 1 min + Centrifugation at 4 °C, for 10 min at 5000 r.p.m. Filtration through 0.2 μm nylon filter for analysis	77.3–110.8 (3.4–12.6)	Stremel et al. (2018)
340	*E. Lucius*, *B. bjoerkna*, *S. cephalus*, *P. fluviatilis*, *L. aspius*, *T. tinca*, *S. lucioperca*, *A. brama*, *C. carassius*, *R. rutilus*, *L. lota* (Muscle and liver)	5 g liver or 10 g muscle + 10 mL 1% formic acid in MeCN. Shaken manually 1 min + 0.5 g $Na_2HCit.1.5H_2O$ + 1 g $Na_3Cit.2H_2O$ + 4 g $MgSO_4$ + 1 g	Extract + 1 g chitin (muscle) + 1 g chitin + 50 mg GCB (liver). Shaken by hand 1 min. Centrifugation for 10 min at 4000 r.p.m. Filter 1 mL supernatant through a 0.2 μm PTFE filter for analysis	70–120 (≤20)	Kaczynski et al. (2017)

		NaCl. Shaken 1 min. Centrifugation for 10 min at 4000 r.p.m.			
67	*M. obtusidens* (Muscle)	10 grams frozen sample + 10 mL MeCN. Shake vigorously by hand during 1 min + 1.5 g NaCl + 4 g $MgSO_4$. Shake vigorously by hand during 4 min. Centrifugation at 2260 *xg* during 5 min.	7 mL aliquot + 350 mg PSA + 180 mg RP-C18 + 1 g $MgSO_4$. Vortex for 30 s. centrifugation at 2260 x g for 5 min. Filter 1 mL supernatant through a 0.22 μm PTFE filter. Collect into a 2 mL screw-cap vial for analysis. Evaporate to dryness 4 mL solution under nitrogen stream. Re-dissolve in EtOAc for analysi-s.	36–123 (3- $\geq$ 20)	Colazzo et al. (2019)
42	*L. barbussclater, C. carpus*	1 g lyophilized sample + 10 mL MeCN. Shake vigorously by hand during 1 min + 1.5 g NaCl + 6 g $MgSO_4$. Shake vigorously by hand	50 mg PSA, 15 mg active coal, 50 mg RP-C18 and 150 mg anhydrous $MgSO_4$. Vortexed for 30 s. Centrifugation 5 min at 5000 r.p.m.	68–140 (5–22)	Masiá et al. (2013)
50	*B. guiraonis, M. salmoides, C. carpus*	2 g sample + 8 mL H_2O + 15 mL MeCN. Shake vigorously by hand during 1 min + 1.5 g NaCl + 6 g $MgSO_4$ + vortex 30 s. Centrifugation at 3000 r.p.m. at 20 °C for 5 min.	50 mg PSA, 15 mg active coal, 50 mg RP-C18 and 150 mg anhydrous $MgSO_4$. Vortexed for 30 s. centrifugation at 3000 r.p.m. at 20 °C for 5 min. Filter 1 mL supernatant through a 0.22 μm PTFE filter.	58–140 (5–22)	Masiá et al. (2015)
72	*Hoplias malabaricus Rhamdia quelen Pimelodus maculatus Paraloricaria vetula Hypostomus commersonni* *Salminus brasiliensis Megaleporinus obtusidens Prochilodus lineatus* (muscle)	10 g frozen sample + 10 mL MeCN. Shake vigorously by hand during 1 min + 1.5 g NaCl + 4 g $MgSO_4$. Shake vigorously by hand 4 min. Centrifugation at 2260 *x g*, 5 min.	7 mL aliquot + 350 mg PSA, 180 mg RP-C18 + 1 g $MgSO_4$. Vortexed for 30 s. centrifugation at 2260 x g, 5 min. Filter 1 mL supernatant through a 0.22 μm PTFE filter for analysis. 4 mL extract to dryness under a	36–123 (3– $\geq$ 20)	Ernst et al. (2018)

(continued)

Table 1.2 (continued)

Number of pesticides	Matrix	Extraction conditions	Clean-up conditions	% Rec (% RSD)	References
			nitrogen stream. Re-dissolve in EtOAc analysis.		
37	*Catfish, tilapia*	10 g lyophilized sample + 10 mL 1% acetic acid in MeCN. Shake vigorously by hand during 1 min + 1 g NaCl. Shake vigorously by hand during 1 min + 1.7 g NaAc + 0.3 g $MgSO_4$. Shake vigorously by hand during 1 min. Centrifugation 8 min at 3400 r.p.m.	3 mL aliquot + 75 mg PSA + 375 mg RP-C18 + 450 mg $MgSO_4$. Shake 1 min. Centrifugation 8 min at 3400 r.p.m. Filtration through 0.2 μm nylon filter for analysis	34.3–120.0 (2.1–20.0)	Munaretto et al. (2013)
27	*Specie: not specified*	10 g sample + 10 mL MeCN + Ultra-turrax for 1 min + 1 g NaCl + 4 g $MgSO_4$ + ceramic homogenizer, vortex 30 s, shake 1 min. Centrifugation 10 min x 3500 r.p.m.	Extract + freeze out −20 °C min 4 h, Extract 1 Non polar analytes: Extract 1 + 50 mg Z-sep, 150 mg $MgSO_4$, Vortex 1 min, shake 1 min. Centrifugation 10 min × 10.000 r.p.m., Vortex + Filtration through 0.2 μm PTFE filter for analysis Polar compounds: 500 μL xtract 1 + 1% Formic acid in MeCN, filtration through Captiva ND lipid cartridge. Evaporation to dryness ad reconstitute MeOH:H_2O (2:8, *v/v*) for analysis.	76–128 (2.2–27.5)	Baduel et al. (2015)
3	*Prawns, salmon, shark, tilapia, trout, tuna, marlin, arctic char*	5 g sample + 5 mL 1% Acetic acid in MeCN, sonication for 10 min + 0.5 g NaAc + 2 g $MgSO_4$.	1 mL extract +150 mg $MgSO_4$ + 50 mg PSA + 50 mg RP-C18, Vortex 1 min, shake	70–115 (2.9–14)	Rawn et al. (2010)

		Shake 2 min. Centrifugation 5 min at 3000 r.p.m.	1 min, Centrifugation 5 min at 3000 r.p.m. 500 µL aliquot concentrated to dryness + internal standard. The extract diluted with isooctane + Na_2SO_4, vortexed for analysis.		
50	*Specie: not specified*	2 g lyophilized fish + 8 mL H_2O + 15 mL MeCN + 6 g $MgSO_4$ + 1.5 g NaCl	2 mL extract + 300 mg $MgSO_4$, 100 mg PSA, 100 mg RP-C18 + 15 mg GCB.	58–140 (5–22)	Ccanccapa et al. (2016)
153	*R. quelen*	10 g sample + 10 mL MeCN shaken 1 min + 4 g Na_2SO_4 + 1 g NaCl. Manual shaking + centrifugation.	1 mL extract + 150 mg Na_2SO_4, 25 mg PSA + 25 mg RP-C18.	20–120 (1–22)	Munaretto et al. (2016)
40	*Iberian gudgeon, brown trout, European eel, bleak, pumpkinseed, Eastern iberian barbel, largemouth bass*	2 g lyophilized sample + 8 mL H_2O + 15 mL MeCN, shaken 30 s + 6 g $MgSO_4$ + 1.5 g NaCl, shaken for 1 min. Centrifugation 4 min at 4000 r.p.m.	2 mL extract + 300 mg $MgSO_4$, 100 mg PSA, 100 mg RP-C18 + 15 mg activated charcoal. Vortex for 30 s. Centrifugation 4 min at 4000 r.p.m. Filtration through 0.22 µm PTFE filter for analysis	<20 – >120 (< 20)	Belenguer et al. (2014)
113	*P. hypophthalmus*	5 g sample + 5 mL H_2O + vortex 1 min + 10 mL 1% Acetic acid in MeCN, vortexed 1 min + 6 g $MgSO_4$ + 1.5 g NaAc, vortex 2 min. Centrifugation 5 min at 1699 x *g*.	2 mL extract 20 min at −20 °C. 1.5 mL extract + 150 mg $MgSO_4$ + 100 mg $CaCl_2$. The supernatant + 50 mg PSA + 50 mg RP-C18 + 50 mg Florisil + 150 mg $MgSO_4$. Centrifugation 5 min at 10621 x *g*. Filtration through PTFE filter for analysis	62–119 (1–17)	Chatterjee et al. (2015)
6	*White sturgeon* (liver and gonad)	5 g sample + 5 mL MeCN + Ultra-turrax.	1.5 mL extract + 0.5 mL MeCN + 150 mg $MgSO_4$ + 50 mg		Morrison et al. (2016)

(continued)

Table 1.2 (continued)

Number of pesticides	Matrix	Extraction conditions	Clean-up conditions	% Rec (% RSD)	References
		The tubes were allowed to sit at room temperature for 30 min + sonication 15 min + 2 g $MgSO_4$ + 0.5 g NaCl, vortex 2 min. Centrifugation 5 min at 3000 *x g*.	PSA + 50 mg RP-C18. Centrifugation 5 min at 10621 x *g*. Repeat twice. Vortex 1 min + Centrifugation 5 min at 7200 *x g*.	60.8–108 (5.4–28.2)	
103	*Specie: not specified*	5 g sample + 5 mL H_2O + 10 mL MeCN. Shaken manually 1 min + 0.5 g $Na_2HCit.1.5H_2O$ + 1 g $Na_3Cit.2H_2O$ + 4 g $MgSO_4$ + 1 g NaCl. Shaken by hand 1 min + shaker 20 min. Centrifugation for 5 min at 8000 r.p.m. (10,560 *x g*).	6 mL extract + 900 mg $MgSO_4$ + 150 mg GCB + 150 mg RP-C18 + vortex 1 min. Centrifugation 5 min at 8000 r.p.m. (10,560 x *g*). 200 µL extract + 200 µL H_2O for analysis.	42–116 (1–11)	Zhang et al. (2017)
13	*Carp, catfish, Saury*	5 g sample + internal standards + room temperature for 30 min + 10 mL MeCN + vortex 30 s + 4 g $MgSO_4$ + 1 g NaCl + vortex 30 s + ultrasonic bath (40 kHz frequency and 200 W, 10 min). Centrifugation for 5 min at 5000 r.p.m.	1 mL extract + 50 mg PSA + 6 mL H_2O + 40 µL 1-undecanol + vortex 30 s. Centrifugation 5 min at 9000 r.p.m. The tube maintained in ice bath to solidify the organic extraction phase. The solidified portion transferred into a vial and melted at room temperature. Centrifugation 1 min at 5000 r.p.m. Separate the organic phase for analysis.	71.4–109.8 (≤ 15)	Wang et al. (2017)
18	Catfish	10 g sample + 10 mL MeCN, shaken by hand 30 s + 4 g	1 mL aliquot + 150 g $MgSO_4$ + 50 mg Z-SEP Plus.	71–119 (3–19)	

		$MgSO_4$ + 1 g NaCl. Shaken by hand 1 min. Centrifugation for 2 min at 3250 rcf.	Shaken + centrifugation for 2 min at 3250 rcf. 0.5 mL extract transferred to a vial an injection + standard addition for analysis.		Sapozhnikova and Lehotay (2013)
2	Croaker, salmon	4 g sample + 4 mL MeCN. Shaken for 10 min on a pulsed-vortexer at 80% in maximum pulsing. 2 g ammonium formate were added. Shaken for 1 min + centrifugation for 2 min at 3700 rcf.	Filtration of 0.5 mL extract through 0.2 µm PVDF filter + 75 mg $MgSO_4$ + 25 mg PSA + 25 mg Z-Sep + 25 mg RP-C18. Shaken by hand and transferred to an autosampler vial.	–	Sapozhnikova et al. (2015)

$CaCl_2$ calcium chloride, H_2O water, $CHCl_3$ Chloroform, *EtOAc* ethyl acetate, *GCB* graphitized carbon black, *MeCN* acetonitrile, *MeOH* methanol, $MgSO_4$ magnesium sulphate, *NaAc* sodium acetate, $Na_2HCit.1.5H_2O$ disodium hydrogencitrate sesquihydrate, $Na_3Cit.2H_2O$ trisodium citrate dihydrate, Na_2SO_4 sodium sulphate, N_2 nitrogen, NH_2 aminopropyl, *NaCl* sodium chloride, *PSA* primary and secondary amine, *PTFE* polytetrafluoroethylene, *PVDF* polyvinylidene fluoride, *RP-C18* reversed-phase octadecylsilane, *SAX* strong anion exchange adsorbent et al. 2019; Colazzo et al. 2019; Ernst et al. 2018; Munaretto et al. 2013; Stremel et al. 2018).

Different combinations of other adsorbents have also been assayed. Kaczysnki et al. (2017), reports a novel step extraction-clean-up strategy using chitin for the determination of 340 pesticides in fatty fish and liver matrix. Some others, highlight the use of a purification step with primary and secondary amine, reversed-phase octadecylsilane, magnesium sulphate and Z-sep® sorbent (Sapozhnikova and Lehotay 2015; Sapozhnikova et al. 2015). The authors also studied the efficiency of the afford mention dispersive solid phase extraction method and observed that Z-sep sorbent combination removed 83–95% of co-extracted matrix in incurred salmon and cracker (Sapozhnikova et al. 2015).

Each one of the mentioned sorbents interacts with different types of compounds from the matrix. The primary and secondary amine, removes polar pigments, acids and sugars, the graphitized carbon black interacts with color pigments, reversed phase octadecyl, is used for the removal of fats and non-polar components, whereas Z-sep® or Zep+® sorbent are for pigments and lipids.

Molina-Ruiz et al. (2015) evaluated a dual dispersive solid phase extraction method clean-up, consisting of the addition of chloroform with an octadecyl reversed phase, after the dispersive solid phase extraction combination of primary and secondary amine, strong anion exchange and aminopropyl resins. The strong anion exchange sorbent is ideally suited for the removal of carboxylic acids whereas; the amino group is a weak anion exchanger capable to extract fatty acids and sugars, among other carboxylates. The authors reported that strong anion exchange sorbent for the clean-up removes a high amount of fat and waxes with good recoveries and high sensitivity in samples with high lipid content.

Chitin, calcium chloride are also salts used in some cases; the first one for the removal of lipophilic compounds and other biomolecules while calcium chloride precipitate fatty acids and removes water providing better clean-up efficiency in some cases. Thus, ionic interactions with the sorbent or H-binding ionic become stronger providing better clean-up results. For example, Chatterjee and co-workers reported an exhaustive purification step based on cooling the samples at −20 °C for 20 min, followed by a dispersive solid phase extraction step with calcium chloride and magnesium sulfate, finally the obtained extract is again purified using primary and secondary amine, Florisil, reversed phase octadecyl, with magnesium sulfate as dissecant. Apparently, the simultaneous use of Florisil and primary and secondary amine removes co-extractives more efficiently than each one individually (Chatterjee et al. 2016).

More specific approaches to remove lipids are also depicted in the literature. For example, Lazartigues et al. (2011a, b), report the addition of 3 mL of hexane after the extraction and homogenization step but before the addition of the citrate salts. This approach generates less viscous extract that improves the liquid chromatography tandem mass spectrometry analysis. Baduel et al. (2015), optimized a new clean-up protocol for the determination of polar pesticides with Captiva ND lipid cartridges that removes proteins, lipids, phospholipids and other impurities providing lower matrix effects and avoiding low recoveries for some compounds.

Regarding the effect of the pH on the extraction efficiency, Baduel et al. (2015), evaluated the use of formic acid to acetonitrile with the citrate buffer during the salting out step. As known, buffered procedures provide satisfactory recoveries for acid-sensitive compounds. Different concentrations of formic acid at different amounts: 0%, 0.1%, 1% and 2% were tested. Two compositions of salts were also evaluated; the citrate buffer salts and the original salts of QuEChERS. No significant differences on the most lipophilic compounds with the citrate buffering or the addition of formic acid were found. However, for polar compounds the extraction is more pH dependent, mostly with those compounds, which present a carboxylic group in the molecule. Moreover, Rawn et al. (2010) used the acetate buffered version for the analysis of pyrethroids in several types of fish species with very good recoveries and coefficient of variation (Table 1.2).

The use of new absorbent for QuEChERS was also evaluated for pesticide residues analysis of fish. The Enhanced Matrix Removal of Lipids sorbent, was tested in salmon, a fish with a high lipid content. The lipid removal was around 80%, for 65 studied pesticides and the recoveries were between 70% and 120%. Nevertheless, recoveries of more lipophilic contaminants such polybrominated diphenyl ethers, polycyclic aromatic hydrocarbons and polychlorinated biphenyls were below 70% and adjustments using internal standards were needed for a proper determination of the above-mentioned compounds (Han et al. 2016).

1.4.2.2 Matrix Solid Phase Dispersion

Matrix solid phase dispersion methodology involves the extraction and clean-up in one single step. Is a rapid and easy procedure with few sample and solvent requirements in which the sample is blended with proper sorbents for example: Florisil, reversed-phase octadecylsilane, alumina, among others, until the homogenization is completed.

The main drawbacks are the great number of parameters to optimize like amounts of sample and sorbent, sorbent materials, clean-up characteristics and elution steps, as well as the automation (Long et al. 1991; Samsidar et al. 2018). Furthermore, the use of co-columns, consisting of other solid phase or chromatographic supports, incorporated at the bottom of the solid phase extraction column assists in analyte isolation or further clean-up, thus improving the matrix effects. Another way to eliminate co-extractives is to apply a sequential series of eluting solvents trying to remove the contents of the entire sample, including macromolecular components of the matrix. Besides the fact that the use of a solvent gradient is quite easy and cheap, the sequential elution had been applied to a limited number of compounds.

Barriada-Pereira et al. (2010), developed a protocol for the analysis of 20 organochlorine pesticides in muscle and liver fish. The adsorbent used was ENVI-Carb™ and sodium sulphate, after the matrix solid phase dispersion, the obtained extract is purified in a solid phase extraction cartridge containing ENVI-Florisil. A simple matrix solid phase dispersion procedure, mentioned above, was developed by Pareja et al. (2013). Small fishes are comminuted and blended with Florisil and magnesium sulfate. Then the analytes are eluted with a mixture of ethyl acetate and dichloromethane. The small lipid content of the selected class of fish allowed the direct injection by gas chromatography coupled to electron capture detection, for the determination of dichlorodiphenyldichloroethylene at trace level.

Another approach was reported by Li et al. (2017), for the determination of 21 organochlorine pesticides in several fish species. One gram of sample is blended with 6 g neutral alumina. The mixture is placed in an empty solid phase extraction column containing a polypropylene frit and 1 g of neutral alumina as a co-column adsorbent. The selected pesticides were eluted with hexane:dichloromethane (50:50 *v/v*) and 10 mL eluate were collected. The extract was concentrated to dryness and dissolved in isooctane for analysis.

Hela and Papadopoulos (2013), estimated the uncertainty associated for the analysis of chlorinated compounds in 4 different fish species. The method was based on the extraction of 1 g of sample which was blended with reversed-phase octadecylsilane:Florisil (80:20) at a ratio dispersant to sample equal to 2:1. The clean-up mixture is then placed into a 20 mL-syringe barrel-column containing a portion of glass wool and 2 g of a mixture reversed-phase octadecylsilane:Florisil (40:60). The elution solvent was dichloromethane:hexane (50:50, *v/v*). The eluate was concentrated and dissolved in hexane for analysis. The methodology allowed the determination of 19 compounds with recovery percentages between 68% and 115% and relative standard deviations below 10%.

1.4.2.3 Solid Phase Extraction

Solid phase extraction technique is combined in general with liquid-liquid extraction or solid-liquid extraction for better enrichment and purification. Solid phase extraction has been reported to be fast, and efficient for pesticide monitoring in various matrices. The main advantages of the method are low solvent consumption, short analysis time, and high efficiency in removing co-extractives (Samsidar et al. 2018).

Regarding fish matrix, Gan et al. (2016), performed a solid-liquid extraction combined with solid phase extraction for the analysis of 6 organofluorine pesticides. The methodology consists of the homogenization of 5 g of fish sample with 10 mL acetonitrile, using a vortex for 60 s and centrifugation at 5000 r.p.m. for 10 min. The previous step is repeated twice and then 10 mL hexane are added to the extract, the mixture is vortexed 30 s and centrifuged with the previous conditions. The supernatant is discarded and the addition of hexane is repeated. Finally, the remaining extract is subjected to SPE clean-up. The solid phase extraction was optimized by the comparison of the efficiency of different types of cartridges: reversed-phase octadecylsilane, Florisil, aminopropyl, OASIS®-HLB, and neutral alumina. The recoveries of the analytes using reversed-phase octadecylsilane, Florisil and neutral alumina were in the range from 30% to 86% whereas the use of aminopropyl and OASIS®-HLB resulted in better recoveries. Therefore, aminopropyl cartridges were used due to the high cost of the other ones.

1.4.2.4 Miscellaneous Techniques

Soxhlet method has been applied for the analysis of organochlorine pesticides in fish. However, is time-consuming, expensive regarding amounts of solvent, analyst time and energy (Le Doux 2011). Moreover, high-moisture samples need a desiccation step to ensure the penetration of the solvent into the samples (Muralidharan et al. 2009; Suchan et al. 2004).

Muralidharan et al. (2009), determined 10 organochlorine pesticides in 10 fish species using a Soxhlet extraction with hexane for 6 h, from a 10 g portion of fish muscle followed by an acid treatment with sulfuric acid, neutralization with saturated sodium hydroxide solution after a clean-up with silica packed in a glass column was performed eluting the analytes with 250 mL hexane.

Zhou et al. (2008) optimized a Soxhlet method based on the extraction of 10 g of sample grounded with 30 mg sodium sulfate and 200 mL of hexane:acetone (3:1, *v:v*) for 8 h. After the concentration of the extract, the lipid percentage was determined gravimetrically in an aliquot of the sample. The clean-up depended on the type of compound; for acid stable pesticides 3 g of activated ash, sulfuric acid impregnated and hexane washed silica was placed in an empty cartridge and the fat residue was loaded. The elution was performed with 2 × 5 mL hexane:dichloromethane (3:1, *v/v*). The eluates were concentrated and dissolved in hexane for analysis. For the non-acid stable pesticides, the fat extract was purified in a cartridge containing 2 g of alumina, silica gel and Florisil, impregnated with a 15% potassium hydroxide methanolic solution (50%, *v/v*). The elution was done with 2 × 5 mL hexane: dichloromethane (3:1, *v/v*). The eluates were concentrated and dissolved in hexane for analysis. The recovery percentages varied from 78% to 89% and the relative standard deviations were in the range 5–10%.

Matrix solid phase dispersion was used coupled to a Soxhlet procedure avoiding the use of sulfuric acid in sample dehydration step (Miglioranza et al. 2013). The protocol has been applied to the systematic study of persistent organic pollutants occurrence in different aquatic environments. The samples were homogenized with sodium or magnesium sulfate, Soxhlet extracted with hexane: dichloromethane (50:50, *v/v*), purified through silica gel and microgel permeation using Biobeads X300. The extract thus obtained was clean enough to perform large monitoring programs of persistent organic pollutants including all the legacy organochlorine pesticides with gas chromatography electron capture detection.

Pressurized liquid extraction or accelerated solvent extraction has been used for the extraction of organochlorine pesticides from fish liver and muscle. The method involves little solvent consumption and short times of extraction step. One of the disadvantages is that samples with high water content needs a desiccation step before the extraction. The methodology uses conventional solvents at elevated pressures, 10–15 MPa, and temperatures in the range 50–200 °C for short periods of time, from 5 to 10 min.

Suchan et al. (2004), optimized the extraction conditions of pressurized liquid extraction and compared the performance in terms of repeatability of the developed method with Soxhlet extraction. The relative standard deviations obtained were in the range from 3% to 14%, which were comparable to conventional Soxhlet. Usually, the worst repeatability was obtained for hexachlorohexane isomers for both methods. Regarding the comparison of the time needed for both methods, the authors concluded that pressurized liquid extraction requires less time per analysis. In addition, the cost per sample was calculated being also higher using Soxhlet extraction.

Choi et al. (2016) developed an extraction and clean-up method using pressurized liquid extraction for the analysis of 14 organochlorine pesticides in fish. The extraction solvent was hexane:dichloromethane (7:3, *v/v*) and the samples were extracted using a modification of the method 3545A of the US Environmental Protection Agency. The selection between alumina, Florisil, acid-silica, and different combinations, for lipid removal was performed using a type of fish with around 23% fat, being alumina and acid silica the adsorbents which removed the highest amount of lipids. Method validation was performed with certified reference materials and spiked fish. The recoveries were from 79% to 108% with relative standard deviations below 10%.

The pressurized liquid extraction method was also reported for the analysis of 18 organochlorine pesticides in a lyophilized fish sample. Two grams of sample were mixed with 10 g sodium sulphate and transferred to 33 mL sample cell and kept in an equilibrium state for at least 12 h before extraction. The sample was extracted with dichloromethane:hexane (1:1, *v/v*) at 10.3 MPa and 100 °C, 3 × 5 min cycle. The extracts were evaporated until constant weight and then reconstituted in hexane for the clean-up. Lipids removal was achieved with an acidic silica gel column of 300 mm length and 15 mm of internal diameter, packed with 1 g activated silica gel, 8 g 44% acidic silica gel, 8 g 22% acidic silica gel, 1 g activated silica gel and 4 g sodium sulphate. After conditioning with hexane, the extract was loaded and eluted with 100 mL dichloromethane:hexane (1:1, *v/v*). The eluate was evaporated to 1 mL and purified on a multi-layer silica gel basic alumina oxide and Florisil column. The recoveries of the method ranged from 54% to 115% with relative standard deviations between 7% and 20% (Zhao et al. 2019).

Microwave-assisted extraction has also been tested for both organochlorine and organophosphates pesticides extraction from fatty fish tissue.

He et al. (2017) reported the use of microwave-assisted extraction methodology for the determination of 25 organochlorine pesticides in fish tissues. The method was based on the extraction of 2 g of sample with 25 mL solvent with the following instrument conditions: working power 1200 W, and gradient temperature program increased to 100 °C within 10 min constant for 10 min and then to ambient temperature within 30 min. The filtered extract was concentrated to 1 mL and 10 mL ethyl acetate:cyclohexane (1:1, *v/v*) were added. The solvent was again concentrated up to 2 mL and centrifuged at 4 °C for 15 min at 12,000 r.p.m. The supernatant was filtered and purified by gel permeation chromatography, graphitized carbon black, using a mobile phase of ethylacetate:cyclohexane (1:1, *v/v*) with a flow rate of 5 mL/min and a Biobeads SX-3 (300 × 200 mm) column. The fraction was collected from minute 10 to 39 and purified in a multi-layer chromatography column with a 10 mm internal diameter containing; 12 cm wet of neutral alumina, 12 cm wet of neutral silica gel and 1 cm sodium sulfate. The elution was made with 10 mL hexane which were discarded and then 50 mL of dichloromethane:hexane (1:1, *v/v*). The extract was concentrated to dryness and dissolved in hexane for analysis.

In 2011, an extraction of 8 g of sample with 5 g of sodium sulphate and 15 mL acetone by ultrasonication for organophosphate pesticides analysis was reported. Two times extracts were combined and concentrated under reduced pressure vacuum and the residue was dissolved in 8 mL methanol:water (5:95, *v/v*). The extract was cleaned-up using a hollow fiber liquid-phase microextraction device. Several parameters influence the efficiency of hollow fiber liquid-phase microextraction technique, including an organic solvent, speed of agitation, pore size, porosity internal diameter and wall thickness. The recovery percentage differed between the fish species evaluated, but in general were in the range of 71.8–95.5% with relative standard deviations below 18.9% (Sun et al. 2011).

Another multi-class methodology based on the use of ultrasonic extraction of fish matrix was described by Kong et al. (2018) using 2 g of sample that were extracted with 10 mL acetonitrile and 200 μL 0.1 M ethylene diamine tetraacetic acid disodium solution with a grinder for 30 s. The mixture is agitated by vortex for 5 min, placed in an ultrasonic bath at 40 °C for 10 min and again agitated for another 5 min. The extract was centrifuged at 10000 × *g* for 10 min and the supernatant transferred to a glass tube. Afterwards 10 mL ethyl acetate were added to the sample and the extraction procedure is repeated. The extracts were combined, dried under a nitrogen flow at 40 °C and the residue was dissolved in 1 mL of 0.1% formic acid in acetonitrile:water (2:3, *v/v*).

Liu et al. (2017), reported a straightforward methodology for the analysis of 10 pesticides in fish. In this method, 2 g of sample were treated in a sonicator for 10 min with 10 mL ethyl acetate. After 5 min centrifugation at 10,000 r.p.m. at 4 °C the supernatant was passed through an OASIS PRIME HLB cartridge and collected. The eluate was concentrated to dryness, dissolved in 70% methanol and filtered through 0.22 μm filter for analysis.

Mijangos et al. (2019), applied a pulsed time of 0.8 s, 10% amplitude, at 0 °C in an ice-water bath to sonicate 0.5 g freeze dried liver or muscle sample or 0.1 g gills and brain with 7 mL methanol: water (95:5, *v/v*) for 30 s. The extract was filtered through 0.45 μm, evaporated to 1 mL approx. and diluted with 6 mL water adjusted at pH 2 with formic acid and an appropriate volume of ethylene diamine tetraacetic acid disodium solution. The extract was loaded in a 200 mg-OASIS HLB previously conditioned cartridge and eluted with 6 mL water for cleaning purposes and finally eluted with 6 mL methanol. For liver matrix the extract was concentrated to approx. 1 mL and poured into a 30 mL vessel where 6 mL water with 30% sodium chloride were incorporated twice; first adjusted to pH 2 with formic acid and then adjusted with ammonium hydroxide to pH 10. The final concentration was achieved by adding ethylene diamine tetraacetic acid disodium. Afterwards, pre-cleaned 50 mg-portions of polyethersulfone were added. The vessels were closed and extracted at room temperature overnight at 800 r.p.m. The polymer was removed, washed with milli-Q water, dried and desorbed in 1 mL methanol by soaking for

32 min in an ultrasonic bath. The method was validated for 41 multi-class compounds in which four of them were pesticides. The recoveries ranged from 69% to 145% with relative standard deviation in the range 2–33%.

Shin (2006), developed a method for the determination of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid in frog and fish tissues. The developed method was based on the ultrasonic extraction of 5 g sample with 20 mL 1 M sodium hydroxide:acetone (1:9, *v/v*) for 5 min. Then the extract was concentrated to 0.2 mL, dissolved in 9 mL methanol-1 N sodium hydroxide (1:2, *v/v*) and heated for 15 min at 70 °C. The solution was extracted with 6 mL methyl, ter-butyl ether and the organic phase discarded. The aqueous solution was adjusted to pH 2 with 20% Hydrochloric acid and extracted with 8 mL of petroleum ether. Afterwards, 2 mL 10% sulfuric acid in methanol were incorporated, the mixture was heated for 30 min at 80 °C, concentrated to 0.2 mL for analysis. The 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid presented recovery percentages of 96.8% and 93.8% with relative standard deviations below 6% at 2 μg/kg.

A single solid-liquid extraction method was developed by Lazartigues et al. (2011a, b). An Ultra-turrax was used for the extraction of 3 g of sample maintained at 20 °C in a water bath with 10 mL acetonitrile:water (50:50 *v/v*), and 4 g of magnesium sulphate. The tube was shaken 10 min and centrifuged at 12,000 g for 10 min at 25 °C. Then another solid-liquid extraction was performed with ethyl acetate:cyclohexane (75:25, *v/v*) in the same conditions. After evaporation of the extract the residue was reconstituted with 0.9 mL acetonitrile:water (10:90, *v/v*) and stored in the dark at −20 °C for analysis. Recovery rates were from 36% to 115%, with relative standard deviations below 22% for all the evaluated compounds.

1.5 Separation and Determination

As previously stated, the analysis of fish samples is a challenging task which involves working with a complex matrix, low concentration of target compounds and analytes with a broad spectrum of physico-chemical properties. In this review, we focused on the most important and used chromatographic methods, which represent the vast majority of the studies in the literature, related to fish analysis. Gas chromatography was the most widespread method for the separation and determination of pesticides. However, the use of liquid chromatography has gained grown since new pesticides with more polar characteristics were released.

1.5.1 Gas Chromatography

Gas chromatography is the most common technique and the preferable instrumentation when dealing with less polar, highly volatile or easily vaporized pesticides.

Different detectors are available, such as electron capture detector, flame photometric detector, nitrogen phosphorus detector and flame ionization detector. Nowadays for pesticide residues analysis involving food safety and environmental monitoring the most frequent coupling implies the use of a mass spectrometer in single mode or in tandem configuration.

Conventional detectors such as electron capture detector or flame photometric detector have been largely applied for the determination of pesticide residues in fish. These detectors are intended for the analysis of halogenated compounds like organochlorine and organophosphate residues can be combined with the determination of other contaminants like polychlorinated biphenyl compounds (Zhou et al. 2008; Sun and Chen 2008; Malhat and Nasr 2011; Stremel et al. 2018).

The coupling of a mass detector is widely reported and several configurations are used. In gas chromatography coupled to mass spectrometry different options can be used, from low-resolution single quadrupole to a high-resolution magnetic sector, either working with electron impact ionization or negative chemical ionization (Colazzo et al. 2019; Zhao et al. 2019; Li et al. 2017; Ernst et al. 2018; Molina-Ruiz et al. 2015).

In gas chromatography tandem mass spectrometry better sensitivity and selectivity can be achieved with a triple quad or hybrid quadrupole-time of flight. Therefore, is implemented in the analysis of multi-class pesticides, such as organochlorine. organophosphate, pyrethroids, diverse fungicides and herbicides. To fulfill the current requirements from the guidelines for pesticides residues analysis about identification and confirmation of the studied analytes, the tandem mass spectrometer, in particular the triple quad is the default detector of choice (SANTE 2017a). As can be seen in Table 1.3, several examples of pesticide residue analysis in fish by gas chromatography coupled to different detectors have been reported.

Not only pesticides are included but also others contaminants of relevance like polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers and polychlorinated biphenyl compounds (Chatterjee et al. 2015, 2016; Sapozhnikova and Lehotay 2015; Baduel et al. 2015; Nácher-Mestre et al. 2014).

1.5.2 Liquid Chromatography

Liquid chromatography is frequently used for high polarity, low volatility and thermally labile compounds. UV/vis detector can be used for trace analysis in fish, but a lot of work must be done in relation to the sample treatment as it is not a specific detector. The application of liquid chromatography for pesticide residue analysis has been reported worldwide. Jabeen et al. (2015) worked with liquid chromatography for the determination of nine pesticides including pyrethroids, carbamates and neonicotinoids.

Liquid chromatography hyphenated with triple quad, quadrupole time of flight coupled and linear trap quadrupole-Orbitrap mass spectrometry has been adopted for multi-residue pesticide analysis in fish. This alternative enhances sensitivity and

Table 1.3 Instrumental conditions for determination of contaminants in fish samples

Number of pesticides	Extraction method	LOQ (µg/kg)	Instrumentation			References
			Chromatography column	Mobile phase	Detector	
52	QuEChERS	0.04–87.1	Purospher STAR RP-18e column (150 × 2.1 mm, 2 µm)	MeCN/Water	LC-MS/MS (ESI-QqQ)	Barbieri et al. (2019)
51	Ultrasound and SPE; QuEChERS	0.0015–15	N/R	N/R	LC-MS/MS	Pico et al. (2019)
44	QuEChERS	1–10	Zorbax Eclipse XDB C-18 (150 × 24.6 mm, 5 µm)	MeCN/0.1% formic acid in water	LC-MS/MS (ESI-QqQ)	Colazzo et al. (2019)
23		15–50	TRACET TR-5MS (30 m × 0.25 mm, 0.25 µm)	Helium	GC-MS (EI-Q)	
18	ASE	0.5	DB-5-MS (60 m × 0.25 mm, 0.25 µm)	N/R	GC-MS (EI/NCI-Magnetic Sector)	Zhao et al. (2019)
44	QuEChERS	0.6–9.6	Zorbax Eclipse XDB C-18 (150 × 24.6 mm, 5 µm)	MeCN/0.1% formic acid in water	LC-MS/MS (ESI-QqQ)	Ernst et al. (2018)
28		11–85	TRACET TR-5MS (30 m × 0.25 mm, 0.25 µm)	Helium	GC-MS (EI-Q)	
7	MeCN/AcOEt extraction		Accucore RP-MS C-18 (100 × 2.1 mm, 2.6 µm)	MeCN/Water (both containing 0.1% formic acid)	LC-MS/MS (ESI-Q-Orbitrap)	Kong et al. (2018)
4	FUSLE	0.2–0.5	Kinetex F5 100 Å core-shell (100 × 2.1 mm, 2.6 µm)	MeOH:water (95:5)/water:MeOH (95:5)	LC-MS/MS (ESI-QqQ)	Mijangos et al. (2019)
340	QuEChERS	5–50	KINETEX C-18 (100 × 2.1 mm, 2.6 µm)	MeOH/Water (both containing 0.5% of formic acid and 5 mM of ammonium formate)	LC-MS/MS (ESI-QqQ)	Kaczyński et al. (2017)

103	QuEChERS	1–10	Poroshell 120 EC-C-18 (100 × 3.0 mm, 2.7 μm)	MeOH/0.1% formic acid 5 mM ammonium acetate in water	LC-MS/MS (ESI-QqQ)	Zhang et al. (2017)
21	MSPD	0.037–0.100	HP-5MS (30 m × 0.25 mm, 0.25 μm)	Helium	GC-MS (NCI-Q)	Li et al. (2017)
10	AcOEt extraction followed by SPE	0.03–2.50	ZORBAX SB-Aq column (100 × 3.0 mm, 1.8 μm)	MeCN/0.1% formic acid in water	LC-MS/MS (ESI-QqQ)	Liu et al. (2017)
5	QuEChERS + ultrasound	0.8–9.6	DB-5-MS (30 m × 0.32 mm, 0.25 μm)	Nitrogen	GC-ECD	Stremel et al. (2018)
82	MeCN extraction	2–13	VF-5MS (30 m × 0.25 mm, 0.25 μm)	Helium	GC-MS/MS (EI-QToF)	Chatterjee et al. (2016)
153	QuEChERS	5–25	Zorbax Eclipse Plus C-18 (50 × 2.1 mm, 1.8 μm)	MeOH/Water:MeOH (92:2) (both containing 0.1% (v/v) of formic acid and 5 mmoL/L of ammonium formate)	LC-MS/MS (ESI-QToF)	Munaretto et al. (2016)
6	MeCN extraction	0.3–0.5	Atlantis T3 C-18 (150 × 2.1 mm, 3 μm)	MeOH/0.1% formic acid, 5 mM ammonium acetate buffer (pH = 5)	LC-MS/MS (ESI-QqQ)	Gan et al. (2016)
150	QuEChERS with Pulsed-vortexing shaker	N/R	Rti-5MS (15 m × 0.53 mm, 1 μm)	Helium	LPGC-MS/MS (QqQ)	Sapozhnikova et al. (2015)
105	MeCN (1% acetic acid) extraction	1–11	HP-5MS (30 m × 0.25 mm, 0.25 μm)	Helium	GC-MS/MS (EI-QToF)	Chatterjee et al. (2015)
50	QuEChERS	0.23–11.25	Luna 18 (150 × 2.0 mm, 3 μm)	MeOH/Water (both containing 10 mM ammonium formate)	LC-MS/MS (ESI-QqQ)	Masiá et al. (2015)
24	QuEChERS	4–9	HP-5MS (30 m × 0.25 mm, 0.25 μm)	Helium	GC-MS (EI-Q)	Molina-Ruiz et al. (2015)
18	QuEChERS			N/R		

(continued)

Table 1.3 (continued)

Number of pesticides	Extraction method	LOQ (µg/kg)	Instrumentation			References
			Chromatography column	Mobile phase	Detector	
		0.08–2.0	DB-5-MS (30 m × 0.25 mm, 0.25 µm)		GC-MS/MS (EI-QqQ)	Baduel et al. (2015)
9		0.4–3.0	XDB-C-18 (100 × 2.1 mm, 1.8 µm)	MeOH/Water (both containing 0.1% formic acid)	LC-MS/MS (ESI-QToF)	
9	AcOEt extraction	N/R	Supelcosil TM LC-18 (250 × 4.5 mm, 5 µm)	MeCN/Water	HPLC-UV/Vis	Jabeen et al. (2015)
6	QuEChERS	N/R	Rti-5MS (15 m × 0.53 mm, 1 µm)	Helium	LPGC-MS/MS (QqQ)	Sapozhnikova et al. (2015)
54	QuEChERS	N/R	Kinetex XB C-18 (50 × 2.1 mm, 1.7 µm)	MeOH/Water (both containing 0.1% formic acid)	LC-MS/MS (ESI-LTQ-Orbitrap)	Farré et al. (2014)
133	QuEChERS	N/R	DB-5MS (30 m × 0.25 mm, 0.25 µm)	Helium	GC-MS (APCI-QToF)	Nácher-Mestre et al. (2014)
40	QuEChERS	0.03–11.25	Luna 18 (150 × 2.0 mm, 3 µm)	MeOH/Water (both containing 10 mM formic acid)	LC-MS/MS (ESI-QqQ)	Belenguer et al. (2014)
50	QuEChERS	0.9–11.25	Luna 18 (150 × 2.0 mm, 3 µm)	MeOH/Water (both containing 10 mM ammonium formate)	LC-MS/MS (ESI-QqQ)	Masiá et al. (2013)
37	QuEChERS	1–10	VF-5-MS (30 m × 0.25 mm, 0.25 µm)	Helium	GC-MS/MS (EI-QqQ)	Munaretto et al. (2013)
18	QuEChERS	N/R	Rti-5-MS (15 m × 0.53 mm, 1 µm)	Helium	LPGC-MS/MS (QqQ)	Sapozhnikova and Lehotay (2013)
14		N/R		Nitrogen	GC-FPD	

	Chloroform/acetone extraction		PAS-1701 (30 m × 0.32 mm, 0.25 μm)			Malhat and Nasr (2011)
13	MeCN/water extraction	0.1–49	Zorbax XDB-C-18 (50 × 2.1 mm, 1.8 μm)	MeCN/0.0125% (v/v, pH = 4.04) acetic acid	LC-MS/MS	Lazartigues et al. (2011a, b)
8	QuEChERS	N/R	HP-5 (30 m × 0.32 mm, 0.25 μm)	Helium	GC-MS (NCI-Q)	Rawn et al. (2010)
1	MeCN extraction	N/R	DB-608 (30 m × 0.53 mm, 0.83 μm)	Nitrogen	GC-FPD	Sun and Chen (2008)
13	Acetone/hexane extraction	N/R	DB-5 (30 m × 0.32 mm, 0.25 μm)	Nitrogen	GC-ECD	Zhou et al. (2008)

APCI atmospheric-pressure chemical ionization, *ASE* accelerated solvent extraction, *AcOEt* ethyl acetate, *EI* electron impact ionization, *ESI* electrospray ionization, *FUSLE* focused ultrasonic solid-liquid extraction, *GC-ECD* gas chromatography electron capture detection, *GC-FPD* gas chromatography flame photometric detector, *GC-MS* gas chromatography mass spectrometry, *GC-MS/MS* gas chromatography tandem mass spectrometry, *HPLC-UV/Vis* High Performance Liquid Chromatography ultraviolet-visible detector, *LC-MS/MS* liquid chromatography tandem mass spectrometry, *LPGC-MS/MS* low pressure gas chromatography tandem mass spectrometry, *LTQ* linear trap quadrupole, *MeCN* acetonitrile, *MeOH* methanol, *MSPD* matrix solid phase dispersion, *NCI* negative chemical ionization, *Q* quadrupole, *QqQ* triple quadrupole, *QToF* quadrupole time-of-flight, *SPE* solid phase extraction selectivity, improving the quantification limit of the method at sub part per billion levels and, depending on the configuration, allowing the identification of non-target compounds.

One of the most frequently used mass spectrometric detectors is the triple quad, which has the multiple reaction monitoring mode of data acquisition, that allows the determination of a larger number of targeted compounds in a single run with the highest sensibility. Several authors reported the use of triple quadrupole tandem mass spectrometry for the analysis of fish samples.

Barbieri et al. (2019) validated a methodology for the determination of 52 medium to highly polar pesticides in fish muscle with low quantification limits. Ernst et al. (2018) and Colazzo et al. (2019) reported the analysis of 44 multi-class pesticides residues in fish muscle with electrospray ionization in multiple reaction monitoring mode. Kaczyński et al. (2017) determined 340 pesticides residues in fatty fish with detection limits between 0.05 and 1.2 μg/kg.

High resolution mass spectrometry was also introduced to pesticide residue analysis in fish. time-of-flight detector is also available to be coupled with liquid chromatography and has the advantage of allowing high resolution and high sensitivity when analyzing non targeted compounds in the full scan mode.

Munaretto et al. (2016) evaluated two scan modes: full scan and all ions mass spectrometry, to assess the best option for screening analysis in spiked fish fillet samples. The study included the analysis of 153 pesticides residues among other organic contaminants from different chemical classes such as veterinary drugs and personal care products. The authors found that full scan acquisition was more reliable as allowed to detect around 84% of the compounds in the automatic identification and quantification mode whereas “all ions” mass spectrometry detected 72%. Most of the compounds presented a screening detection limit value of 10 μg/kg in both full scan acquisition modes.

In 2015, Baduel et al. (2015) presented a straightforward multi-residue method to analyze polar and non-polar compounds in biological matrices such as fish. Baduel and co-workers combined targeted multi-residue analysis using gas chromatography–triple quadrupole mass spectrometry and multi-targeted analysis complemented with non-target screening using liquid chromatography quadrupole time-of-flight mass spectrometry with limits of quantification between 0.2 and 9 μg/kg.

The Orbitrap™ mass analyzer, implements the principles of Fourier transform through an electrostatic axially harmonic orbital trapping technique to yield high-resolution mass spectra. It provides high mass resolution, higher than 15,000 full widths at half maximum, and high mass accuracy, below 2 ppm, but without mass selection (Farré et al. 2014). This detector in combination with a single quadrupole or a linear ion trap quadrupole has been used in the analysis of pesticides and pharmaceutical residues in fish and aquatic food (Kong et al. 2018; Farré et al. 2014).

1.6 Matrix Effect

Matrix effect is defined as the influence on the changes in the intensity of the detector response due to compounds present in the matrix, which generally co-elute with the analyte. Matrix effect depends therefore on the matrix compounds present in the extracts to be analyzed, the chromatographic behavior and the differential sensibility towards the detectors employed for the determination. The effect could be noticed as an increase of the signal named: signal enhancement or decrease, signal suppression. The comparison of these effects with the neat signal yielded by the compound of interest in the conditions of the analysis are classified as positive in the former case and negative in the latter. The evaluation can be performed by comparing the slopes values of the matrix match calibration curve and the solvent using the formula

$$\text{Matrix effect\%} = [(\text{Slope matrix}/\text{Slope solvent}) - 1] \times 100$$

The signal enhancement gives positive matrix effects whereas negative values correspond to signal suppression. Matrix effects are supposed to be compensated using matrix matched calibration curves. European Commission SANTE guidelines establish that the potential for matrix effects to occur should be assessed during method validation. In general, matrix effect is notoriously variable in occurrence and intensity, but some techniques are particularly prone to them. When the techniques used are not inherently free from such effects, quantification should be performed by matrix-matched calibration. Extracts or samples of blank matrix preferably of the same type as sample may be used for calibration purposes (SANTE 2017a).

The matrix effect can be observed in any type of detector. In specific detectors, such as flame photometric detector in gas chromatography or fluorescence in high performance liquid chromatography which are related to a relatively narrow specific chemical property or composition of the molecule, these effects can be minimal, but matrix effect should be evaluated in any case. Very dirty samples can cause signal enhancement or mask completely the analyte signal, for example in flame photometric detector when looking for trace organophosphate compounds as the emission due to the C-H bond of organic matter can be noticeable in the detector.

Matrix effects are classified as negligible when the value is less than 20%, medium between 20% and 50% and high when the value is above 50% (Kmellar et al. 2008). Sometimes matrix effect could be higher than 100%. The 20% of matrix effect can be assigned to the inherent uncertainty of pesticide residue analysis, and therefore, have no biased influence in the determination. As the matrix effects grows, the uncertainty of the identity of the detected analyte could also increase.

Residue identification in trace analysis using chromatography coupled to mass spectrometry is performed by the similarity (± 0.1 s) of the retention time with respect to a given standard. Matrix matched calibrations can compensate for these effects, as for high load of co-extractives pesticide solutions, changes in retention times of the compounds are commonly observed.

The other identification criteria in trace analysis are that the ratio between qualifier and quantifier ions should not differ more than 30% when compared with the same relation determined in solvent. Matrix components can alter the ratio and therefore identification can be hampered. In some cases, matrix effect can be overcome by using exact mass measurements. The probability of wrongly assign an ion using an error mass of 5 ppm is below 5%.

Exact mass measurements also prevent in a high extent misleading identification due to co-elution of matrix components. The SANTE document, proposed that mass resolutions higher than 50,000 identify unambiguously any compound with a molecular weight below 600 Da, the common situation for pesticide molecules (SANTE 2015).

1.6.1 Matrix Effect in Gas Chromatography Analysis

Matrix effects can have distinctly sources in pesticide residue analysis. The most accepted one is the signal enhancement observed when an extract containing pesticide residues is injected in the gas chromatography. Signal enhancement is due to the minimization of analyte degradation when going through the inlet of the chromatograph by the extract components. The liner is usually not completely deactivated, although the glass surface has been silanized. The Si-OH groups that remained free react with the analytes at the hot temperature of the inlet, degrading them.

The extracts for pesticide residue analysis usually contain co-extractives of different chemical origin that reacts with silanol groups hampering pesticide degradation, acting as analyte protectants. Analyte protectants having a polyol structure have been proposed to be added to the working solutions, allowing to overcome the “liner degradation effect”. Nevertheless, the compounds proposed do not achieve a proper protection of the late eluting compounds, such as the temperature sensitive pyrethroids. Thus, the protective effect of extracts can be rapidly lost, as the liner accumulates carbonized organic matter. When enough amount of “dirty” extracts has been injected, the degraded organic matter, react with the analytes giving poor defective peaks e.g. tailing, bad shape or variable area.

Fish extracts from QuEChERS procedures are very dirty. Although, in gas chromatography analysis, most of the analysts do not inject the acetonitrile extract as such and prefer to change the solvent prior injection to a less expansible in the vapor phase and polar ones, like ethyl acetate or isooctane, the co-extracted poly unsaturated fish lipids deposit and degrade quickly in the injector and the liner. The highly reactive peroxides formed, degrade more quickly the analytes.

The most commonly employed conventional detector in the determination of organochlorine and halogenated contaminants in fish is the electron capture detector due to the high sensibility and specificity of the electron capture detector (Ballesteros et al. 2014). One of the main drawbacks of electron capture detector is that can be easily inactivated by lipidic compounds in the matrix. Lipophilic compounds not necessarily co elute with the matrix co-extractives but are of paramount importance as tend to deposit and passivate the detector. To keep the detector functioning at adequate levels of sensibility an exhaustive clean-up of the sample is required.

The traditional sample treatments include gel permeation chromatography procedures that have been actually miniaturized. Nowadays, combinations of absorbents such as reversed-phase octadecylsilane, Z-sep or the enhanced matrix removal of lipid adsorbent contribute to eliminate the triglycerides of the sample extract, allowing a better and longer detection performance.

As pointed out above, actually the routinely applied detectors in pesticide trace analysis, are the mass spectrometer, used either alone or in tandem. The most employed configurations are low resolution quadrupoles. High resolution configurations such as time of flight and Orbitrap instruments have up to now been scarcely applied to the analysis of pesticide residues in fish.

In gas chromatography mass spectrometry, the most common ionization mode is electronic impact at 70 eV. When using electronic impact ionization technique, isobaric matrix interferences could hamper the determination of some pesticides. Matrix effects in gas chromatography mass spectrometry have been described as low to medium ones in a recent publication (Colazzo et al. 2019). In this report 25 gas chromatography-amenable analytes showed matrix effect below 20%, in agreement with a previous study of pesticides residues in fish, performed using low pressure gas chromatography tandem mass spectrometry that showed negligible matrix effects for most of the analyzed compounds (Colazzo et al. 2019; Sapozhnikova and Lehotay 2013).

1.6.2 Matrix Effect in Liquid Chromatography Analysis

On the other hand, the matrix effects in pesticide residue analysis during liquid chromatography electrospray ionization tandem mass spectrometric determination arise in the ionization step. The matrix co-extractives compete with the analytes to reach the droplet surface. As a consequence, less analyte molecules are capable of ionize and the signal is lowered. The overall effect is called “signal suppression”. When lipophilic compounds are present tend to occupy the droplet surface lowering even more the possibility to the analytes of reaching the surface.

According to the literature, although the complexity of the fish matrix, and despite the difficulties at the injection port in gas chromatography that fish extracts showed, the matrix effects in liquid chromatography tandem mass spectrometry were negligible for more than 90% of the analytes when modification of the unbuffered QuEChERS version was employed to determine 65 currently used pesticides in freshwater fish (Colazzo et al. 2019).

Matrix effects in fish depend on the type of extract and the fish species. Extracts with high lipid content cause the most common problems during the determination. In order to avoid interference during the analysis, the use of specific absorbents such as reversed-phase octadecylsilane, zirconium dioxide and Z-sep, or freeze out techniques is desirable. From that point of view, the use acetonitrile as a solvent is useful. On the other hand, acetonitrile has a relatively high polarity and cannot compete effectively with triglycerides, composed by highly unsaturated fatty acids, where the less polar compounds such as the organochlorine pesticides are trapped.

Although time consuming, the freeze out is particularly useful to eliminate the triglycerides. In order to be effective, lower temperatures than the usual −20 °C have to be reached. Dry ice or cryo-cooler systems that reach up to −70 °C, are advisable to be employed. Freeze out time is dramatically lowered when very low temperatures are reached, from 10 h or overnight to 15–30 min.

The type of extract and the whole sample preparation procedure are the clue to perform a proper determination of pesticide residues in fish. Some of the inherent limitations of the instrumental systems are not easy to overcome once the extract is injected into the chromatograph. Matrix effects are complex and needs a deeply evaluation on a case by case basis.

1.7 Occurrence of Pesticides in Fish

Monitoring studies on the occurrence of pesticides and other contaminants in fish have two distinctive goals. On one side, the general food safety concern on the widespread occurrence of toxic pollutants in the environment and the well-known capability of fish to bioaccumulate or biomagnified non-polar compounds. On the other hand, the use of aquatic wildlife for biomonitoring strategies at both, temporal and spatial scales (Pérez-Parada et al. 2018).

As stated above, major focus on the occurrence of persistent organic pollutants has been evidenced in seawater and freshwater species (Penland et al. 2018; Rose et al. 2015). Recent findings report pesticides in fish usually at μg/kg levels.

Environmental quality standards in biota have been defined for a bunch of persistent organic pollutant compounds. The standards were defined as the concentration below which no harmful effects are expected to wildlife or humans and applied as an approach for persistent organic pollutants monitoring (European Commission 2013).

Biota monitoring is particularly important in the case of hydrophobic substances that tend to accumulate in sediments or in the fat tissues of living organisms. Outcomes in large-scale monitoring of pollutants in fish evidenced the co-occurrence of organochlorine compounds, polychlorinated dibenzo p-dioxins and polychlorinated dibenzofurans, polychlorinated biphenyl compounds, polybrominated diphenyl ethers as well as perfluorinated compounds in European freshwater basins (Abalos et al. 2019; Picó et al. 2019).

Growing evidence in Spanish river basins showed a plethora of chemicals being accumulated in fish. However, an increasing relevance for perfluorinated compounds and ultraviolet filters occurring in fish state the significance of emerging freshwater pollution (Picó et al. 2019). Several persistent organic pollutants including dichlorodiphenyltrichloroethane and hexachlorocyclohexane congeners were found to occur in remote areas such as in Antarctic or Arctic areas. Organochlorine pesticides were found in Antarctic notothenioid fish species (Lana et al. 2014). Distribution of these persistent organic pollutants among different tissues such as muscle, liver, gonads, and gills, has evidenced the role of lipid content on the pattern of persistent organic pollutants distribution in tissues. Authors showed that while gonads showed higher levels of dichlorodiphenyltrichloroethane and hexachlorohexane, the most significant polybrominated diphenyl ethers flame retardant concentrations were recorded in gills (Lana et al. 2014).

Similarly, recent reports showed levels of dichlorodiphenyltrichloroethane, hexachlorohexane and hexachlorobenzene in Arctic fish with economic importance such as *Gadus morhua* and *Melanogrammus aeglefinus* in Barens Sea, Norway. The authors showed an apparent decrease from 1992 to 2015 period over the persistent organic pollutant levels found in these fishes where the concentrations decrease from higher than 100 μg/kg in 90's to 20 μg/kg in 2015. However, the authors questioned the possibility of removing the pollutants from the marine environment in the foreseeable future, considering the permanent leakage from continents and the fact that are still being produced in some parts of the world (Boitsov et al. 2019).

Concerning freshwater resources, organochlorine levels were recently studied in muscle and liver tissues from fish at central Andes streams in Argentina. *Oncorhynchus mykiss* and *Odontesthes bonariensis* fish species were found having different persistent organic pollutant but mainly dichloro-diphenyl-trichloroethane congeners rounding 500 μg/kg levels at lipid weight. Hexachlorohexane, chlordane and hexachlorobenzene were also found but in a lower extent (Ríos et al. 2019). The authors positively correlated the lipid content of target tissues to the uptake levels of organochlorine pesticides highlighting the relevance of fat content in tissue (Ríos et al. 2019).

As seen, most knowledge is currently evidenced for compounds with proven bioaccumulation ability exposed by larger n-octanol-water partition coefficient values for most organochlorine compounds. Recent findings underline the relevance of new families of chemicals n-octanol-water partition coefficient lower around 3; such as S-metolachlor an herbicide, the insecticide chlorpyrifos or trifloxystrobin fungicide (Ernst et al. 2018; Picó et al. 2019). However, other pesticides such as carbendazim having a n-octanol-water partition coefficient close to 1 were found in freshwater fish.

Table 1.4 shows relevant reports of currently used pesticides and persistent organic pollutant in freshwater fish. As stated, the advent of liquid chromatography tandem mass spectrometry monitoring enabled an increase in the scope of analytes among different pesticides occurring in different fish tissues. Many reports state the occurrence of organic pollutants at trace levels with very low n-octanol-water partition coefficient (Ernst et al. 2018; Picó et al. 2019; Rojo et al. 2019). In addition, currently used pesticides have been reported in freshwater fish with different feeding and migratory habits over a wide range of concentrations worldwide (see Table 1.4). The Olmstead-Tukey diagram indicates an easy way for contaminant classification among levels found and frequency of detection for a set of samples. The diagram enables to categorize among dominant, frequent, occasional and rare compounds between the reported analytes, Fig. 1.5 (Ernst et al. 2018).

Table 1.4 Summary of recent findings of pesticide residues in freshwater fish species

Water course	Fish specie	Detected pesticides	Concentration range in fish muscle (μg/kg) wet basis	References
Argentina. Mendoza River and Yaucha River at Andes region	*Oncorhynchus mykiss* *Odontesthes bonariensis*	OCs (mainly DDT, HCH, and HCB)	<LOQ to 614	Ríos et al. (2019)
Argentina: Pergamino River	*Jenynsia multidentata*	17 CUPs (mainly fenvalerate and bifenthrin)	<LOQ to 1500	Brodeur et al. (2017)
Argentina: La Peregrina Lake	*Odontesthes bonariensis* *Oligosarcus jenynsii* *Cyphocharax voga*	OCs (mainly endosulfan)	<LOQ to 26	Barni et al. (2016)
Australia: Rivers at Southeast Queensland	*Gambusia holbrooki*	5 CUPs (mainly chlorpyrifos, atrazine and linuron)	<LOQ to 54	Scott et al. (2018)
India: Rivers at Andhra Pradesh and Kerala regions	*Pangasianodon hypophthalmus*	6 CUPs (parathion-methyl, malathion, chlorpyrifos, fipronil, quinalphos, etofenprox) and 2 OCs (p,p′-DDE, p,p′-DDT)	<LOQ to 473	Chatterjee et al. (2015)
China: Pearl River and Dongjiang River	*Cirrhinus molitorella* *Tilapia nilotica* *Hypostomus plecostomus* *Channa argus* *Channa asiática* *Monopterus albus*	OCs (mainly β-HCH and p,p′ DDE)	14–5560	Sun et al. (2018)
Poland: Biebrza River and Narew River	*Perca fluviatilis* *Leuciscus aspius* *Tinca tinca* *Sander lucioperca* *Abramis brama* *Carassius carassius* *Rutilus rutilus* *Blicca bjoerkna* *Lota lota*	S-metolachlor and OCs (DDTs and HCHs)	<LOQ to 14	Kaczynski et al. (2017)
Spain: Guadalquivir, Júcar, Ebro and Llobregat Rivers	*Luciobarbus sclateri* *Cyprinus carpio* *Salmo trutta* *Gobio lozanoi* *Pseudochondrostoma polylepis* *Micropterus*	25 CUPs (mainly chlorpyrifos)	<LOQ to 840[a]	Picó et al. (2019)

(continued)

Table 1.4 (continued)

Water course	Fish specie	Detected pesticides	Concentration range in fish muscle (μg/kg) wet basis	References
	salmoides *Barbus guiraonis* *Lepomis gibbosus* *Alburnus alburnus* *Anguilla anguilla* *Esox lucius* *Barbus graellsii* *Silurus glanis*			
Uruguay: Uruguay River and Negro River	*Hoplias malabaricus* *Rhamdia quelen* *Pimelodus maculatus* *Paraloricaria vetula* *Hypostomus commersonni* *Salminus brasiliensis* *Megaleporinus obtusiden,* *Prochilodus lineatus*	30 CUPs (mainly metolachlor, pyraclostrobin, trifloxystrobin)	<LOQ to 194	Ernst et al. (2018)
United States: Yadkin-Pee Dee River	*Anguilla rostrata* *Ictalurus furcatus* *Lepomis macrochirus* *Ictalurus punctatus* *Cyprinus carpio* *Micropterus salmoides* *Moxostoma collapsum* *Moxostoma macrolepidotum* *Ictiobus bubalus* *Cyprinella nivea*	OCs (β-HCH and heptachlor epoxide)	<LOQ to 49	Penland et al. (2018)

LOQ limit of quantification, *CUPs* currently used pesticides, *DDT* dichloro-diphenyl-trichloroethane, *HCH* hexachlorocyclohexane, *HCB* hexachlorobenzene, *OCs* organochlorine pesticides, *p,p′-DDE*: dichlorodiphenyldichloroethylene

[a]Report on dry basis (dry weight, dw)

Active biomonitoring approaches using caged fish has been reported as strategy for freshwater monitoring in agricultural areas. Pesticides identified in fish tissues, particularly liver, has been reported and correlated with different biological markers of endosulfan exposure (Vieira et al. 2019). Perspectives in monitoring approaches are focusing the development of non-target screening in different fish tissues that will lead to an increase in evidence of detailed exposure and metabolism of these pollutants.

Some analytical issues should be considered in large scale analysis. Due to the high n-octanol-water partition coefficient of persistent organic pollutant, partition

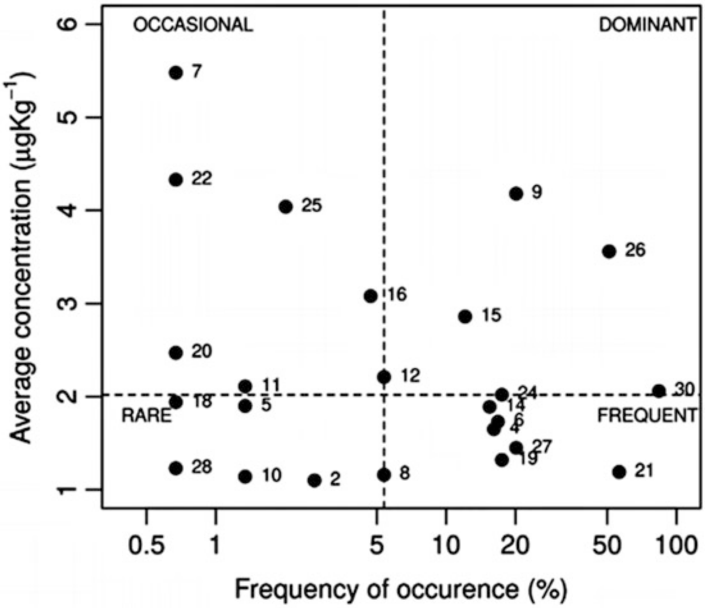

Fig. 1.5 Olmstead-Tukey diagram for the pesticides found at sampling sites. Frequency of occurrence in percentage vs the average concentration in µg/kg of the quantified pesticides for the whole data set. (Reprinted with permission of (Science of the Total Environment, Elsevier B.V) from Ernst et al. 2018)

with acetonitrile is sometimes not enough to extract them properly from the fatty portions of the samples. Some approaches using acetone or toluene as extraction solvent followed by gel permeation chromatography to clean-up the lipidic fraction have been used for monitoring programs seeking the good maintenance of the chromatographic equipment (Ballesteros et al. 2014; Miglioranza et al. 2013).

An interesting study of contaminant occurrence in fish in a salty lake showed that after the toxicological evaluation of organochlorine pesticides levels found the acceptable daily intake for endosulfan and polychlorinated biphenyl compounds were largely overpassed in fish from “La Chiquita” Lake in Argentina (Ballesteros et al. 2014).

Other possible aim of monitoring studies regarding fish is to evaluate the aquatic environment and the effect of the surrounding land. Different approaches for monitoring studies of aquatic environments using fish as target organisms should be performed. If the interest lies on the effect of contaminants on biota, field studies provide a richer information on the effect of combined stressors that otherwise cannot be properly evaluated under laboratory conditions.

Water quality can be assessed performing cage trials with small fishes, like “madrecitas” *Cnesterodon decemmaculatus*, that act as samplers of different chemicals present in the water streams. The strategy has the advantage of performing a selective water “sampling” for weeks. Figure 1.2 shows the experimental design of

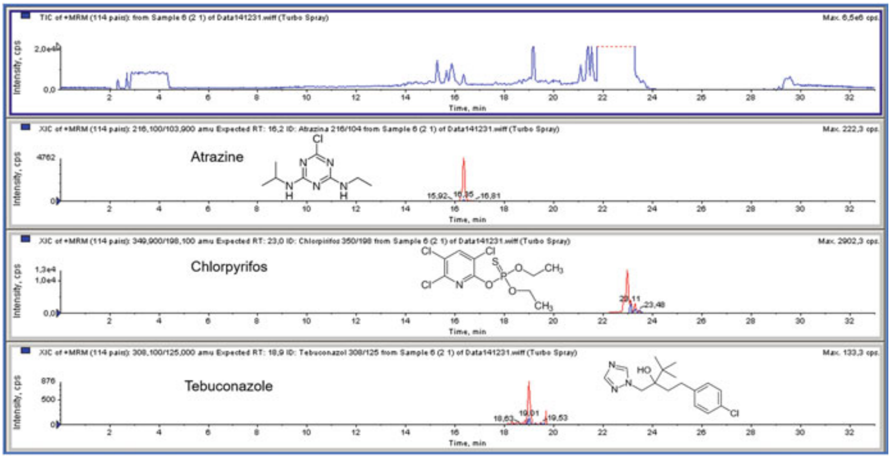

Fig. 1.6 Extracted ion chromatogram obtained by liquid chromatography tandem mass spectrometry in multi reaction monitoring mode of a *Luciopimelodus pati* sample from Uruguay River. The chromatogram indicates the presence of three pesticides, atrazine, chlorpyrifos and tebuconazole in a concentration range 0.1–30 µg/kg

such a study, performed at the Cañada del Dragón, in Montevideo, Uruguay, in a traditional farming region. After analyzing the whole *C. decemmaculatus* exposed to the muddy waters of the stream during 3 weeks of experiment for persistent organic pollutants determination, dichloro-diphenyl-dichloroethylene was detected (Pareja et al. 2013). The study of fish from different agroecosystems in Uruguay showed the presence of pesticide residues directly related to the agrochemicals used in different crops (Pareja et al. 2013) evidencing the suitability of fish as environmental biomonitors of aquatic systems surrounding farm activities. Moreover, a study of 50 fish samples from the Uruguay River reported that atrazine, chlorpyrifos and tebuconazole were the most frequently found pesticides, in a concentration range between 0.1 and 30 µg/kg. Figure 1.6 shows the extracted ion chromatograms of a positive sample in which atrazine, chlorpyrifos and tebuconazole were detected. Interestingly, some pesticides supposed having rather short half-life in water and soil accumulate in fish bodies. Despite trifloxystrobin is not one of the most employed fungicides in Uruguay and that the soil half-life is less than 2 days, the fungicide was found in 84% of the analyzed samples (Pérez-Parada et al. 2018).

1.8 Conclusions

Currently, monitoring of environmental pollutants such as pesticide residues in fish is of great concern for ensuring food safety and evidence environmental fate of semi-polar and non-polar pesticides in aquatic media.

Fish has shown a high potential as a biomonitor of the aquatic environment even at basin level. Compounds that are not supposed to be found in freshwater have been detected accumulated in fish. The relevance of the findings to fish health is still unknown.

Despite the fact that there are several multi-residue methodologies for pesticide residues, multi-class or multi-residue methods for highly polar compounds are needed for broadening exposure surveillance. The trend for the multi-class analysis is to develop environmentally friendly and cheap techniques capable to determine in one-sample preparation step different types of contaminants at the lowest possible concentrations.

Although state-of-the-art for pesticide residues determination in fish is based on target screening, metabolites are commonly present in the environmental compartments and therefore the determination of the transformed products in fish should also be considered. Therefore, non-target analysis particularly dedicated to organic multi-contaminants, metabolites and possible degradation products of pesticides with toxicological relevance using high-resolution mass spectrometry is expected to be incorporated soon in order to bring a better knowledge on the chemical pollution of the aquatic environment.

Further research is needed to spread analytical outcomes aiming potential hazards and ecotoxicological effects characterization.

The challenge is to face the analysis of pollutants whose relevance for the environment health is unknown. Pesticide watch lists elaborated by regulatory authorities are needed to preserve water bodies and implement regulations that enable environmental and public health protection as well as studies looking for the implementation of maximum residue levels for extraneous substances in fish.

Non-target analysis is a trend in environmental chemistry but is also of enormous importance in food security and nutritional capacity evaluation, as fish is one of the natural resources with relevance for the world population. So far, high resolution mass spectrometry techniques have been implemented in several environmental matrices such as wastewater and surface water. The use of similar protocols to analyze marine and freshwater fish will open new and exciting fields for future research.

Finally, the global maximum residue limits settlement for fish consumption to ensure food safety is missing. Maximum residue limits are associated with good agriculture practice accomplishment, but the findings of residues in fish from agricultural pesticides cannot be linked to any possible good agriculture practices. However, the risk for consumers health cannot be avoided and is desirable to expand the concept of extraneous-maximum residue limits suggested by the *Codex Alimentarius* to current used pesticides, within realistic risk assessment studies. The new regulatory frameworks will surely foster the development and expansion of pesticide residue analysis in fish, reaching new analytical advances, better understanding of the pesticide uptake and effect on fish and the discovering new horizons to broad the scope food safety science.

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Chapter 2
Analysis of Pesticide Residues in Pollen and Dairy Products

Miguel Ángel González-Curbelo, Javier González-Sálamo, Diana Angélica Varela-Martínez, and Javier Hernández-Borges

Abstract Pesticides are probably one of the chemical groups of compounds most widely studied and regulated worldwide. Despite the advances in agricultural practices that have taken place especially during the last years, the truth is that they are still being used in high amounts nearly in every country around the globe for different purposes. Their effects on human health have brought up the need to strictly monitor their presence in a wide variety of samples, especially in foods, to assess the potential hazards for humans and the environment. Of importance is their occurrence in milk or dairy products, as a consequence of their extremely high consumption, but also in pollen, in which it is clear nowadays that they are affecting bee's presence.

This book chapter pretends to provide a critical vision of the analytical methodologies that have been and that are also being currently applied for the extraction of multiclass pesticides from such commodities, i.e. milk, dairy products and pollen, with particular emphasis on the sample pre-treatment step, which is crucial for a suitable analytical determination. In the case of milk and dairy products, it is of

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special importance the removal of fats and proteins, while the analysis of pollen should face the extremely low amounts of sample available. In both cases, the *Quick, Easy, Cheap, Effective, Rugged* and *Safe* (QuEChERS) method has been the most commonly applied.

Keywords Pesticides · Sample preparation · QuEChERS method · Extraction · Chromatography · Mass spectrometry · Pollen · Milk · Dairy products · Analysis

2.1 Introduction

Since their introduction, pesticides have played an important role in agriculture by protecting cultivars against pest attacks and, therefore, improving the production yield. Their positive benefit is more than clear, though several drawbacks of their use have also been found, among which there could be cited their toxicity or endocrine disrupting effects, among others (Ewence et al. 2015). As a negative effect of a pesticide is found or demonstrated, suitable actions are taken, and it is being replaced by others less persistent or toxic, though their toxicity may not be as low as desirable.

Despite the fact that this group of compounds is used worldwide at high amounts and in a wide variety of cultivars and situations, they are probably the group of chemical substances most controlled and legislated in the whole world. In this sense, many countries have strict legislations in which they have stablished maximum residue limits (MRLs) (The European Comission 2019) or banned certain substances, as well as monitoring programs to effectively control their presence in the market. In some cases, specific legislations are also applied for food to be consumed by infants or children (European Regulation 609/2013 2013), as a result of their vulnerability. The main reason for this has probably been the fact that they are a group of compounds of important concern, as a result of the first negative and evident effects that were observed since their initial use.

Milk and its derivatives are essential food products widely consumed around the world due to their nutritive qualities, including their contribution as a main source of calcium. Like any other food commodity, pesticides have also been found in them at different concentrations (Herrera-Herrera et al. 2019; Ingenbleek et al. 2019; Montiel-León et al. 2019), so the knowledge of such occurrence levels is important to develop actions that contribute to the management of the production, as well as to minimize these residues. Moreover, many pesticides tend to bioaccumulate through the food chain due to their fat solubility, but both lipophilic and hydrophilic pesticide residues can persist at detectable levels. In fact, their introduction in milk samples and, as a result, in dairy products, begins with the consumption of contaminated water, feed, grass or corn silage, though they can also appear as a result of their direct application on dairy cattle for ectoparasitic control or the use of insecticides in stables or dairy factories (Bates 2004). Consequently, the occurrence of pesticide residues in these products has been a matter of increasing concern caused by potential consumers' risks, particularly for vulnerable groups within the population, such as infants and children. Then, several regulatory organizations such as the European Union and the Food and Agricultural Organization have set maximum residue limits of pesticides in milk and its derivatives as well as stringent controls in order to minimize such problems. For example, maximum residue limits stablished by the European Union Regulation No 396/2005 and its subsequent amendments, range between 0.8 and 2000 μg/kg (The European Commission 2019), while the maximum residue limits stablished by the Codex Alimentarius fall within the range 0.4–1000 μg/kg (Food and Agriculture Organization of the United Nations and World Health Organization 2018). Thus, reliable analytical methods are required to quantify pesticide residues in these animal origin products. However, these matrixes contain important amounts of fats, proteins and carbohydrates, which undoubtedly interfere in the analytical determination, and even small amounts of them can cause deterioration of the analytical system. At the same time, lipophilic pesticides can be retained in fat-rich samples and can be poorly recovered from them. Therefore, it becomes necessary the development and application of suitable sample preparation methods that allow the removal of such high molecular-mass ingredients while providing unambiguous evidence to confirm the identity and the quantity of the analytes of interest at very low levels. These methods include extraction and clean-up steps based on the precipitation of fats and proteins or, more recently, the use of new highly selective sorbents to achieve high throughput analysis.

Also concerning the agri-food sector, and, particularly, agricultural practises, it should be highlighted the frequent unnoticed presence of pesticides in pollen which has been reported in a relatively low number of occasions and which is causing an important mortality of many insects, including bees. Though frequently underestimated, bees highly contribute to the preservation of the ecosystems as well as human well-being by pollinating plants, especially wild ones, and increasing the productivity yields. Since they are present in agroecosystems it is not strange that they transport pesticides back to the colony. Therefore, the presence of pesticide residues in the environment where bees live has become an important problem as a result of the so-called colony collapse disorder and its corresponding negative effects on their ecosystems (Palmer et al. 2013; Thompson et al. 2014). Therefore, the presence of pesticides in pollen is also an important issue, though not frequently studied or considered. Concerning the analytical methods developed up to now, the most important problem that has to be overcome is the low amount of sample that is frequently available. For example, typical amounts of pollen collected from wildflowers are lower than 0.5 g. Therefore, if larger amounts are necessary for analysis, a composite sample should be collected from several field margins and less information on exposure variability would be obtained (David et al. 2015).

The aim of this book chapter is to review the existing analytical methods for pesticide residues analysis in dairy products and pollen. In both matrices, pesticide analysis is an area in constant evolution and improvement. In the particular case of pollen, their presence may have negative effects over honeybees or other pollinators and, therefore, they can be present in wax, bee bread, propolis, royal jelly and honey, while when found in dairy products, they may have more impact on humans' health as a result of their extremely high consumption (Food and Agriculture Organization of the United Nations 2019).

2.2 Pesticide Analysis in Pollen

Despite its importance, a relatively low number of methods has been reported for pesticide residues analysis in pollen, at least, compared to other related matrices such as honey (Souza Tette et al. 2016). The first works that dealt with this topic were based fundamentally on single residue analysis (i.e. methyl-parathion (Ross and Harvey 1981), 2-chloroethanol (Bruns and Currie 1983), carbaryl (Spittler et al. 1986), imidacloprid (Bonmatin et al. 2003; Rossi et al. 2005), vinclozolin (Dwuznik and Kubik 2003), fipronil and its metabolites (Jiménez et al. 2007; Kadar and Faucon 2006) or imidacloprid and its metabolites (Rossi et al. 2005; Schöning and Schmuck 2003)) using ethyl acetate, dichloromethane or acetonitrile as extracting solvents and subsequent clean-up steps through liquid–liquid partitioning or solid-phase extraction. However, bees are exposed to a multitude of pesticides that can vary depending on the types of surrounding crops. Among them, neonicotinoid insecticides have traditionally been the most studied in pollen, fundamentally using liquid chromatography because of their thermolability and high polarity (Chen et al. 2013; López-Fernández et al. 2015; Sánchez-Hernández et al. 2016; Valverde et al. 2016; Yáñez et al. 2014a), but other chemical families must be considered, being necessary to develop multiresidue methods. In this sense, since chromatographic techniques are the most used for multiresidue analysis of pesticides in food, including bee products, and they are subject to strong matrix effects which can lead to wrong determinations, an additional consideration must be done: pollen is a highly complex matrix that contains considerable amounts of compounds such as lipids (10–20%) and proteins (30–40%), but also aminophenols, vitamins and polar pigments. Consequently, the analysis of pesticide residues in pollen is a great challenge as a result of the presence and diversity of more and more new analytes at ultra-trace levels. Therefore, sample preparation methods should consider additional strategies to minimize the interferences with the signals from the analytes and the damage of the very expensive chromatographic systems, as well as to simultaneously allow the detection and quantification of the analytes of interest, without neglecting the importance of speed and low cost.

More recently, other works have also been based on solid-liquid extraction (Li et al. 2015; Sánchez-Hernández et al. 2016; Yáñez et al. 2014a, b; Zhang et al. 2013) or even on matrix solid-phase dispersion (Sánchez-Brunete et al. 2008; Torres-Perea et al. 2013; Vazquez-Quintal et al. 2012); some of them have also simultaneously analysed a larger amount of pesticide residues in pollen (see Table 2.1). As an example, Zhang et al. (2013) analysed a set of 26 organochlorine, organophosphorus and pyrethroid pesticides using 50 mL of petroleum ether as extraction solvent and subsequent solid-phase extraction clean-up step with activated carbon and C_{18} prior to gas chromatography with micro electron capture

Table 2.1 Recent examples of the application of traditional analytical methods to the analysis of pesticide residues in pollen

Pesticides	Sample amount	Solvents and sorbents	Separation technique	Limit of quantification	Recovery study	Residues found	Comments	References
Solid-liquid extraction								
26 multiclass pesticides	5 g	50 mL petroleum ether and solid-phase extraction clean-up with activated carbon and C_{18}	Gas chromatography-micro electron capture detector	0.89–65.64 μg/kg	86.8–123.1% at 30, 90 and 150 μg/kg	8 collected samples were analysed and residues of α- and γ-HCH were detected at low levels in 2 of them.	Activated carbon plus C_{18} showed higher extraction efficiency compared with C_{18}, Florisil® and activated carbon and activated carbon plus Florisil® as sorbents in solid-phase extraction.	Zhang et al. (2013)
Spinosad (spinosyns A and D)	2 g	15 mL acetone	High-performance liquid chromatography-mass spectrometry	0.4–0.7 μg/kg	90–105% at 0.4, 0.7, 150 and 400 μg/kg	20 collected samples were analysed, and no residues were detected.	Acetone showed higher extraction efficiency than MeOH, ethyl acetate, and acetonitrile as extraction solvent.	Yáñez et al. (2014b)
7 neonicotinoid insecticides	2 g	10 mL dichloromethane	High-performance liquid chromatography-mass spectrometry	1.2–9.1 μg/kg	86–106% at 2, 5, 10, 250 and 1000 μg/kg	27 collected samples were analysed, 2 different samples contained acetamiprid and imidacloprid	Dichloromethane was more effective than water, MeOH, acetone, ethyl acetate, acetonitrile and a mixture of ethyl acetate and dichloromethane	Yáñez et al. (2014a)

(continued)

Table 2.1 (continued)

Pesticides	Sample amount	Solvents and sorbents	Separation technique	Limit of quantification	Recovery study	Residues found	Comments	References
						residues at 5.8 and 5.2 μg/kg, respectively.	(50:50, v/v) as extraction solvent.	
11 multiclass pesticides	1 g	10 mL ethyl acetate:hexane (7:3 v/v) and clean-up with dispersive solid-phase extraction using 500 mg Z-Sep	Gas chromatography-mass spectrometry	1.4–17.9 μg/kg	81–111% at three different levels	4 collected samples were analysed and at least 4 pesticide at levels from 4.36 to 89.95 μg/kg were detected.	Dispersive solid-phase extraction showed lower matrix effects compared with gel permeation chromatography as clean-up step.	Li et al. (2015)
3 neonicotinoid insecticides	2 g	10 mL dichloromethane	Ultra-high-performance liquid chromatography-tandem mass spectrometry	2.0–4.0 μg/kg	88–101% at three different levels	No samples were analysed.	–	Sánchez-Hernández et al. (2016)
Matrix solid-phase dispersion								
Fipronil	4 g	4 g Florisil® and 10 mL acetonitrile	Gas chromatography-electron capture detector, gas chromatography-mass spectrometry	3.3 μg/kg for gas chromatography-electron capture detector and 0.7 μg/kg for gas chromatography-mass spectrometry	91–104% at 2.5, 5, 10, 25 and 50 μg/kg	10 commercial samples were analysed, and no residues were detected.	Florisil® provided cleaner extracts than alumina and C_{18}. Acetonitrile showed higher extraction efficiency compared with ethyl acetate	Sánchez-Brunete et al. (2008)

							and hexane. Freezing-out clean-up (overnight in the freezer at −20 °C) was used.	
8 organochlorine pesticides	0.5 g	0.5 g C_{18} and 8 mL acetonitrile	Gas chromatography-mass spectrometry	1.3–249.1 µg/kg	42–101% at 0.1, 0.5 and 0.9 µg/kg	No samples were analysed.	Solid-phase extraction clean-up with 0.5 g primary-secondary amine gave cleaner extracts than with graphitized carbon black. Freezing-out clean-up (12 h in the freezer at −10 °C) was used.	Vazquez-Quintal et al. (2012)

detection. Authors evidenced that activated carbon plus C_{18} gave better performance compared with C_{18}, Florisil®, activated carbon and activated carbon plus Florisil® in terms of peaks of impurities and recovery values. In another interesting example, Yañez et al. (2014a) developed a new method to determine dinotefuran, nitenpyram, thiamethoxam, clothianidin, imidacloprid, acetamiprid, and thiacloprid by solid-liquid extraction with 10 mL of dichloromethane followed only by evaporation and reconstitution and subsequent high-performance liquid chromatography-mass spectrometry analysis. Different solvents were tested to extract the neonicotinoid pesticides and the best results in terms of recovery were obtained with dichloromethane, i.e. mean recovery ~95%. However, the authors did not consider its higher toxicity, and acetonitrile could be a possible alternative because it also gave acceptable recovery percentages (>70%). Moreover, the use of acetonitrile has also the advantage of being able to precipitate proteins and limit lipid solubility.

Regarding the use of matrix solid-phase dispersion, Vázquez-Quintal et al. (2012) showed that good recovery percentages were obtained when a mixture of eight organochlorine pesticides were extracted from only 0.5 g of pollen samples using 0.5 g of C_{18} as solid support, 0.5 g of primary-secondary amine for clean-up step and 8 mL of acetonitrile as eluent followed by a final freezing-out step. In the latter, it should be noted that the low solubility of lipids in acetonitrile allowed their precipitation with low temperature and subsequent filtration, which did not require extra reagents, but involved an additional operation and materials. Thus, the freezing-out step is only recommended when further clean-up is necessary (Sánchez-Brunete et al. 2008; Torres-Perea et al. 2013; Valverde et al. 2016; Vazquez-Quintal et al. 2012; Vázquez et al. 2015). Moreover, authors compared the use of primary-secondary amine and graphitized carbon black as sorbents for the solid-phase extraction clean-up step due to their strong affinities for fatty acids, sugars and some ionic lipids, and pigments such as chlorophyll and carotenoids, respectively. The use of primary-secondary amine provided the cleanest chromatograms (lower background and lower intensity of the co-extracted material peaks).

In the work by Torres-Perea et al. (2013), authors chose a smaller amount of sample to analyse five organophosphorus pesticides. Specifically, they used 0.4 g of Florisil® and 1 mL of acetonitrile to extract dichlorvos, diazinon, methyl parathion, malathion and coumaphos from 0.1 g of pollen. A final freezing-out clean-up step was also applied. However, dichlorvos and methyl parathion were not detected at a level as consistent as 100 μg/kg and recovery values for malathion were lower than 11%.

Although in some works relatively low amounts of sample, solvents and sorbents are used, traditional methods are not always efficient because they have a lot of steps and they also do not have enough analytical performance. In this sense, and as it is well known, current trends in sample treatments are focused on the simplification of all steps to reduce the amounts of reagents and time spent while a large number of pesticides are analysed.

Among the most recent methods applied for the large multiresidue analysis of pesticides, the *Quick*, *Easy*, *Cheap*, *Effective*, *Rugged* and *Safe* method (QuEChERS) stands out, which has become the first option for this purpose over the years (González-Curbelo et al. 2015; Varela-Martínez et al. 2019). In fact, its excellence has been recognized internationally through two official methods, namely AOAC 2007.1 (Lehotay et al. 2007) and the European Standard EN 15662 (European Committee for Standarization 2008), which use buffered conditions (acetate and citrate buffers, respectively) to increase extraction efficiency of base-sensitive pesticides. Briefly, the original QuEChERS approach consists in two steps: (1) A solvent partitioning using acetonitrile as extraction solvent and NaCl and $MgSO_4$ to induce salting-out, and (2) a dispersive solid-phase extraction procedure based on $MgSO_4$ to remove residual water and primary-secondary amine as principal clean-up sorbent. Although traditional methods were used with more or less performance in sample preparation for the determination of pesticides in pollen, the truth is that the QuEChERS method is clearly simpler, cheaper and increases sample throughput.

The initial version of the QuEChERS method was created for the analysis of such residues in fruits and vegetables, but its applications have extended to almost any type of sample due to its easy modification depending on the properties of the analytes and the matrix composition. In 2010, Mullin et al. (2010) reduced the usual 10–15 g of sample to 3 g to successfully apply this method to pollen, but coupling a dual layer cartridge with primary-secondary amine and graphitized carbon black. Since then, other authors have developed different applications to analyse pesticides in pollen (see Table 2.2). As another example, the citrate buffered QuEChERS approach resulted in a higher extraction from pollen samples of the vast majority of the 26 multiclass pesticides analysed by de Oliveira et al. (2016) compared with traditional solid-liquid extraction (see Fig. 2.1). Moreover, they also reported that the QuEChERS method significantly decreased matrix effects. Both results were probably caused by the salting-out effect that, on the one hand, increases the transfer of analytes from the aqueous phase to the organic phase, but on the other hand, limits the amount of co-extracted material.

Beyond the two official methods, its application to pollen samples requires other modifications for the elimination of undesired compounds given the high complexity of this matrix. On the one hand, the addition of hexane together with acetonitrile and water in the first stage removes lipids that can interfere with the mass spectrometer (Chen et al. 2013; López-Fernández et al. 2015; Wiest et al. 2011). This is because hexane is a non-polar solvent with a density lower than that of acetonitrile, in which the lipids preferably dissolve, and which can be subsequently removed from the acetonitrile phase by simple centrifugation. However, a decrease in recovery can be noticed for very non-polar compounds (Wiest et al. 2011). Even though, cleaner extracts, lower background signals and improved method sensitivity were obtained, and also good recovery values for the majority of pesticides since they have at least some polarity (Chen et al. 2013). On the other hand, in the second stage, the use of different sorbents in the dispersive solid-phase extraction, as well as the already mentioned freezing-out step just before the injection (Valverde et al. 2016; Vázquez et al. 2015), ensures that residual lipids are not introduced into the chromatographic system. Therefore, it reduces the negative consequences of the matrix effect on the analytical robustness. Regarding dispersive solid-phase extraction, C_{18} and

Table 2.2 Examples of the application of the QuEChERS method to the analysis of pesticide residues in pollen

		Extraction		Dispersive solid-phase extraction (per mL extract)									
Pesticides	Sample amount	Solvents	Salts	$MgSO_4$ (mg)	Primary-secondary amine (mg)	Graphitized carbon black (mg)	C_{18} (mg)	Separation technique	Limit of quantifications	Recovery study	Residues found	Comments	References
80 multiclass pesticides	2 g	8 mL water, 3 mL hexane, 10 mL acetonitrile	4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydrogencitrate sesquihydrate	150	25	–	25	High-performance liquid chromatography-tandem mass spectrometry, gas chromatography-tandem mass spectrometry	0.5–230.7 µg/kg	60–120% for most pesticides at 25, 75 and 150 µg/kg	130 collected samples were analysed and at least 21 pesticide at levels from 2 to 3674 µg/kg were detected.	–	Wiest et al. (2011)
8 neonicotinoid insecticides	2 g	8 mL water, 3 mL hexane, 10 mL acetonitrile	4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydrogencitrate sesquihydrate	150	50	–	50	High-performance liquid chromatography-tandem mass spectrometry	0.1–0.5 µg/kg	90–105% at 5 and 50 µg/kg	13 collected samples were analysed and residues of imidacloprid and thiamethoxam were detected at 0.2 and 2.2 µg/kg.	The addition or not of graphitized carbon black were compared, and no significant differences were found.	Chen et al. (2013)
253 multiclass pesticides	2 g	2 mL water, 4 mL acetonitrile	0.8 g $MgSO_4$, 0.2 g NaCl, 0.2 g trisodium citrate dihydrate, 0.1 g disodium hydrogencitrate sesquihydrate	150	25	–	25	High-performance liquid chromatography-tandem mass spectrometry, gas chromatography-tandem mass spectrometry	<5 µg/kg	70–120% at 5 and 50 µg/kg	41 collected samples were analysed, and all samples contained at least two pesticides at levels from 0.2 to 1832.5 µg/kg. The total pesticide	Five different clean-up methods were evaluated and freezing-out clean-up (3 min with dry ice) followed by dispersive solid-phase extraction and	Vázquez et al. (2015)

											residue load ranged from 3.7 to 1856.2 µg/kg.	solid-phase extraction (40 mg Z-Sep) was selected	
20 multiclass pesticides	0.1 g	0.4 mL water, 0.5 mL acetonitrile	0.2 g $MgSO_4$, 0.05 g sodium acetate	–	8.3	8.3	8.3	Ultra-high-performance liquid chromatography-tandem mass spectrometry	0.02–2.5 µg/kg	71–102% at 5 and 40 µg/kg	An undetermined number of collected samples were analysed and at least 13 pesticide were detected at levels from 0.10 to 67 µg/kg.	125 or 50 mg of Supel^{TM} QuE primary-secondary amine/C_{18}/graphitized carbon black (1/1/1) per 0.5 mL extract were compared. An additional extraction of the sorbent with 150 µL acetonitrile/toluene (3:1) was included.	David et al. (2015)
7 neonicotinoid insecticides	5 g	10 mL water, 10 mL hexane, 10 mL acetonitrile	6 g $MgSO_4$, 3 g sodium acetate	–	–	–	–	High-performance liquid chromatography-tandem mass spectrometry	0.4–4.3 µg/kg	81–99% at 12.5 µg/kg	An undetermined number of commercial samples were analysed and no residues were detected, except in one sample containing residues of acetamiprid and	Supelclean™ ENVI-Carb II/primary-secondary amine cartridge was used as clean-up.	López-Fernández et al. (2015)

(continued)

Table 2.2 (continued)

Pesticides	Sample amount	Extraction		Dispersive solid-phase extraction (per mL extract)				Separation technique	Limit of quantifications	Recovery study	Residues found	Comments	References
		Solvents	Salts	$MgSO_4$ (mg)	Primary-secondary amine (mg)	Graphitized carbon black (mg)	C_{18} (mg)						
											imidacloprid at 9 and 13 µg/kg.		
26 multiclass pesticides	2 g	15 mL acetonitrile	4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydrogencitrate sesquihydrate	750	95	–	–	Gas chromatography-tandem mass spectrometry	10–100 µg/kg	70–120% for most pesticides at 25, 100 and 300 µg/kg	21 commercial samples were analysed and 7 samples contained at least one pesticide at levels from 5.0 to 432 µg/kg.	The QuEChERS extraction efficiency was higher than solid-liquid extraction ones.	de Oliveira et al. (2016)
7 neonicotinoid insecticides	2 g	2 mL water, 6 mL acetonitrile (acetic acid 1%)	1 g $MgSO_4$, 0.5 g NaCl, 0.8 g trisodium citrate dihydrate	75	12.5	–	12.5	Ultra-high-performance liquid chromatography-tandem mass spectrometry, gas chromatography-mass spectrometry	2.1–4.0 µg/kg	91–105% at 5, 63 and 300 µg/kg	20 commercial samples were analysed and no residues were detected.	Freezing-out clean-up (2 min with dry ice) was carried out before dispersive solid-phase extraction.	Valverde et al. (2016)
54 multiclass pesticides	1 g	10 mL water, 10 mL acetonitrile (acetic acid 1%)	0.5 g $MgSO_4$, 2 g sodium acetate	150	50	3.75	50	Ultra-high-performance liquid chromatography-tandem mass spectrometry, gas	0.0025–1.59 µg/kg	70–120% for most pesticides at 5, 50 and 500 µg/kg	48 collected samples were analysed and at least 19 pesticide were detected at	Different combinations varying the amount of graphitized carbon black (0, 3.75, 7.5	Tong et al. (2016)

								chromatography-mass spectrometry			levels from 3.6 to 4516.4 µg/kg.	and 15 mg) were evaluated.	
66 multiclass pesticides	2 g	3 mL water, 10 mL acetonitrile (acetic acid 1%)	0.5 g $MgSO_4$, 2 g sodium acetate	150	50	3.75	50	Ultra-high-performance liquid chromatography-tandem mass spectrometry, gas chromatography-mass spectrometry	0.0025–7.76 µg/kg	70–120% for most pesticides at 5, 50 and 500 µg/kg	189 collected samples were analysed and residues of 32 pesticides were detected at levels from 6.5 to 12475.5 µg/kg. Only 7 samples had no pesticides at all.	–	Tong et al. (2018)

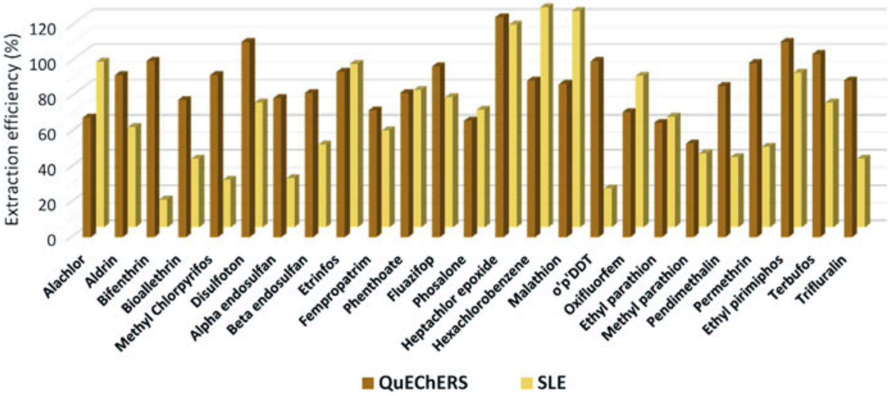

Fig. 2.1 Extraction efficiency using the EN 15662 *Quick, Easy, Cheap, Effective, Rugged* and *Safe* (QuEChERS) method and solid-liquid extraction (SLE) with acetonitrile. As can be seen a higher extraction of the vast majority of the 26 multiclass pesticides analysed is achieved with QuEChERS compared with traditional SLE. (Reprinted with permission of Elsevier from de Oliveira et al. (2016))

graphitized carbon black have been examined in combination with primary-secondary amine to improve the removal of long chain fatty compounds and sterols, and pigments, respectively (David et al. 2015; Tong et al. 2016, 2018). This is the case of the work developed by Chen et al. (2013), in which they studied the use of graphitized carbon black for cleaning the extracts previously obtained from the application of the European Norm 15,662 QuEChERS method to extract 8 neonicotinoid insecticides. In this sense, it must be taken into account that the authors chose the weaker citrate buffer instead of acetate buffer to improve the performance of the extraction since this family of pesticides would be protonated under acidic pH. The results showed that when combinations of primary-secondary amine + C_{18} and primary-secondary amine + C_{18} + graphitized carbon black were used, no significant differences were found between them. In other words, similar clean extracts and good recovery values were reached. However, it should be noted that the authors performed the initial extraction including the use of hexane. Instead, Tong et al. (2016) evaluated the addition of graphitized carbon black without adding hexane in the initial extraction (they used the AOAC 2007.1 QuEChERS method with acetate buffer). In this case, the results showed that the addition of 3.75 mg per mL of extract provided better results in terms of recovery and matrix effect for the determination of 54 multiclass pesticide residues compared with no addition or with the addition of 7.5 or 15 mg of graphitized carbon black.

The so-called zirconia-based family of sorbents, such as Z-Sep and Z-Sep+, have been recently introduced as sorbents in the dispersive solid-phase extraction step for fatty matrices (Rajski et al. 2013; Sapozhnikova and Lehotay 2013), and pollen has not been the exception. Concretely, Vázquez et al. (2015) evaluated five different clean-up methods: (a) dispersive solid-phase extraction with 50 mg primary-secondary amine + 50 mg C_{18} + 300 mg $MgSO_4$, (b) freezing-out followed by

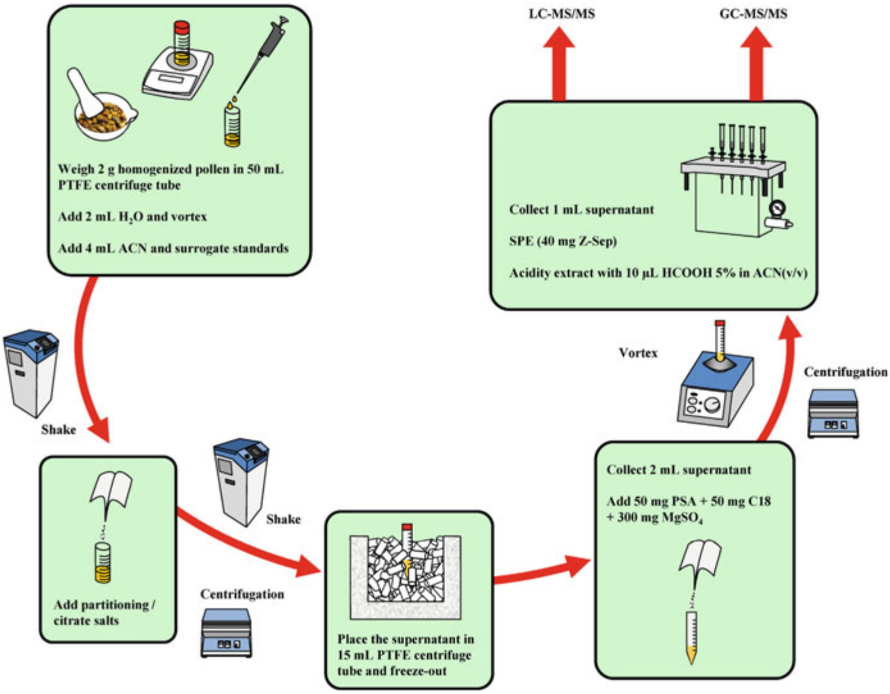

Fig. 2.2 Pesticide extraction and clean-up steps from pollen samples. (Reprinted with permission of Elsevier from Vázquez et al. (2015))

dispersive solid-phase extraction with 50 mg primary-secondary amine + 50 mg C_{18} + 300 mg $MgSO_4$, (c) freezing-out followed by dispersive solid-phase extraction with 40 mg Z-Sep + 50 mg primary-secondary amine + 50 mg C_{18} + 300 mg $MgSO_4$ and (d) freezing-out followed by dispersive solid-phase extraction with 50 mg primary-secondary amine + 50 mg C_{18} + 300 mg $MgSO_4$ and solid-phase extraction with Z-Sep (40 and 50 mg) for the determination of 253 multiclass pesticides by liquid chromatography–tandem mass spectrometry and gas chromatography–tandem mass spectrometry for the determination of 253 multiclass pesticides. The last combination provided the best recovery values and lowest material co-extraction. In this sense, a high-performance liquid chromatography-quadrupole time of flight-mass spectrometry was used to compare the amount of co-extracted material in each extract from the different clean-up alternatives. Figure 2.2 show the different steps of the final global method.

All the works published so far in which the QuEChERS method has been applied for the analysis of pesticides in pollen have used composite samples ranging between 1 and 5 g. Although these methods have shown a good general performance, they have not provided information on single bee pollen samples contamination or if the sample amount available was not enough to carry out a suitable analysis. Therefore, the final challenge is to obtain good recovery values and adequate sensitivity for individual pollen samples of a few milligrams. An example is the work developed by David et al. (David et al. 2015) in which a modified AOAC 2007.1 QuEChERS method in combination with ultra-high-performance liquid chromatography-tandem mass spectrometry analysis was successfully applied to 20 multiclass pesticides in 0.1 g individual pollen samples. Recovery values were in the range 71–102% with relative standard deviation values below 20% at 5 and 40 μg/kg. To achieve these results, a dispersive solid-phase extraction clean-up step composed by 50 mg of primary-secondary amine/C_{18}/graphitized carbon black (1/1/1, w/w/w) per 0.5 mL of extract was applied, as well as an additional dispersive solid-phase extraction step for extraction purposes using 150 μL of acetonitrile/toluene (3/1, v/v) to desorb the more hydrophobic analytes from the graphitized carbon black and C_{18} sorbents. The final acetonitrile/toluene extraction step improved the recovery dramatically.

2.3 Pesticide Analysis in Dairy Products

Up to now, many extraction methods have been used for the analysis of pesticide residues in dairy products using different separation and detection techniques, although most of them have focused mainly on milk analysis (see Tables 2.3 and 2.4). Among these methods, liquid-liquid extraction using different solvents has been one of the most popular in the last years. However, the selection of a suitable solvent or combination of solvents that allows the simultaneous isolation of lipophilic and hydrophilic pesticides is a very difficult task, even more in the presence of considerable amounts of fats and proteins which must be removed or at least minimized. Regarding this last aspect, several clean-up methods have been used in combination with liquid-liquid extraction, highlighting gel permeation chromatography and solid-phase extraction. In the first case, gel permeation chromatography can be considered as a universal and even automated technique to separate high molecular weight molecules as fats and proteins from the compounds of interest. In fact, gel permeation chromatography clean-up is used in official methods due to its wide range of scope (United States Food and Drug Administration 1994). On the contrary, solid-phase extraction with a variety of sorbents allows removing the co-extracted material of low molecular weight that remains in the organic extract. For this reason, it is common to find the combination of both clean-up techniques after the initial extraction. As an example, Zheng et al. (2014) successfully applied a method based on an initial extraction with hexane/acetone (1/1, v/v) and subsequent clean-up steps combining gel permeation chromatography and solid-phase extraction with Florisil® for the determination of organochlorine pesticides in milk and milk powder. In this last matrix, water was previously added for homogenization as the supplier recommended. The mixture of a non-polar solvent as hexane with a polar solvent as acetone provided better extraction efficiency compared with these solvents separately and with methanol, dichloromethane, ethyl acetate and acetonitrile. Kiljanek et al. (2013) also used a combination of gel permeation chromatography and solid-phase extraction achieving an excellent performance, but using a

Table 2.3 Recent examples of the application of other analytical methods to the analysis of pesticide residues in dairy products

Pesticides	Sample amount	Solvents and sorbents	Separation technique	Limit of quantification	Recovery study	Residues found	Comments	References
Solid-liquid extraction/liquid-liquid extraction								
7 multiclass pesticides	5 mL milk	5 mL acetonitrile and oasis solid-phase extraction clean-up with 60 mg	Capillary electrophoresis-tandem mass spectrometry	3–25 μg/kg	78–102% at 0.5 maximum residue limit, maximum residue limit and 2× maximum residue limit	100 commercial samples were analysed and 8 samples contained at least one pesticide at levels from 10 to 89 μg/kg.	–	Blasco et al. (2009)
Cyfluthrin, cyhalothrin, cypermethrin, deltamethrin	10 g milk	20 mL hexane, 20 mL acetonitrile	Gas chromatography-electron capture detector, gas chromatography-mass spectrometry	9–24.6 μg/kg	82.9–109% at 0.5 maximum residue limit, 2× maximum residue limit and 4× maximum residue limit	12 commercial samples were analysed and no residues were detected.	Freezing-out clean-up (30 min at −72 °C, and 30 min more at −24 °C) was used. The use of solid-phase extraction with 1 g of Florisil® showed poorer results than without it.	Khay et al. (2009)
44 multiclass pesticides	5 mL milk	5 mL acetonitrile (formic acid 1%) and dispersive solid-phase extraction clean-up with 6 g discovery DSC-18, 1 g $MgSO_4$ and 1 g primary-secondary amine	High-performance liquid chromatography-tandem mass spectrometry	0.2–10.1 μg/kg	60–113% at 2 and 4 μg/L	35 collected samples were analysed and no pesticides residues were detected.	Bovine milk was studied. Acetonitrile provided higher extraction efficiency than methanol. Discovery DSC-18 showed higher recovery values than graphitized carbon black both in combination with $MgSO_4$ and primary-secondary amine.	Dagnac et al. (2009)
34 multiclass pesticides	20 g milk or 1.5 g cream	18.5 mL water only for cream. 20 mL acetone/cyclohexane/ethyl acetate (2:1:1, v/v/v), 8 g $MgSO_4$, 1.5 g NaCl and gel permeation chromatography and solid-phase extraction with	Bidimensional gas chromatography-mass spectrometry	0.2–0.4 μg/kg	70–120% for most pesticides at 0.2, 0.4, 1, 2, 10 and 50 μg/kg	A very old cream sample was analysed and residues of heptachlor epoxide and DDE-p,p′ were detected at	Bovine milk was studied.	Hayward et al. (2010)

(continued)

Table 2.3 (continued)

Pesticides	Sample amount	Solvents and sorbents	Separation technique	Limit of quantification	Recovery study	Residues found	Comments	References
		250 mg graphitized carbon black and 500 mg primary-secondary amine clean-up				380 and 68 μg/kg.		
49 organophosphorous pesticides	5 g milk	40 mL acetone and methylene chloride (1:1, v/v) and gel permeation chromatography clean-up	Gas chromatography-flame photometric detection	3.3–25 μg/kg	50.3–139.4% at three different levels	20 commercial samples were analysed and 7 samples contained at least one pesticide at levels from 6 to 71 μg/kg.	–	Yang et al. (2012)
13 organophosphorous pesticides	20 g milk	40 mL acetone, 60 mL petroleum ether and gel permeation chromatography and solid-phase extraction with 1 g silica clean-up	Gas chromatography-flame photometric detection	10 μg/kg	73.1–106.2% at 10, 20 and 30 μg/kg	No samples were analysed.	Bovine milk was studied.	Kiljanek et al. (2013)
Diazinon, alachlor, chlorpyrifos, butachlor	0.5 mL milk	1 mL methanol and miniaturized homogeneous liquid–liquid extraction clean-up with 0.5 mL butyl acetate and 7 mL water	Gas chromatography-thermoionic specific detection	1–5.3 μg/L	80–114% at 25, 50 and 100 μg/L	No samples were analysed.	Bovine milk was studied.	Hassan (2014)
30 organochlorine pesticides	5 g milk or milk powder	10 mL water for milk powder and 20 mL hexane and acetone (1:1, v/v) and 5 g NaCl. Gel permeation chromatography and solid-phase extraction with 1 g Florisil® as clean-up	Gas chromatography-tandem mass spectrometry	8 μg/kg	70.1–114.7% at 0.8, 2 and 10 μg/kg	50 commercial samples were analysed and no residues were detected.	Bovine milk was studied. Hexane and acetone (1:1, v/v) provided higher recovery values than methanol, dichloromethane, ethyl acetate and acetonitrile	Zheng et al. (2014)
Carbofuran, chlordimeform	5 g milk, yogurt, milk powder or cheese	40 mL acetonitrile-ethyl acetate-acetic acid (49.5:49.5:1, v/v/v) and Oasis hydrophilic lipophilic balance solid-phase extraction clean-up	High-performance liquid chromatography-tandem mass spectrometry	0.02–0.1 μg/kg	74.3–104.5% at 0.02 and 4 μg/kg for carbofuran and 0.1, 0.2 and 0.5 μg/kg for chlordimeform	50 collected samples of each matrix were analysed and no pesticides residues were detected.	Freezing-out clean-up (in the freezer at −80 °C) was used.	Xie et al. (2015)

Fenpropathrin, cyfluthrin, cyhalothrin, fenvalerate, deltamethrin	5 mL milk	3 mL acetonitrile, 1 g NaCl in the initial extraction and dispersive solid-phase extraction clean-up with 50 mg multi-walled carbon nanotubes and 100 mg $MgSO_4$ in dispersive solid-phase extraction	Gas chromatography-electron capture detector	8.73–16.2 μg/kg	81.8–112.1% at 20, 50 and 100 μg/kg	A commercial sample was analysed and no pesticides residues were detected.	Acetonitrile provided higher extraction efficiency than methanol and acetone. Multi-walled carbon nanotubes showed higher recovery values than C_{18}, but similar to primary-secondary amine which is more expensive.	Gao and Sun (2018)
Matrix solid-phase dispersion								
6 carbamate pesticides	3 mL milk	12 g sand and 5 mL heated water (90 °C)	High-performance liquid chromatography-tandem mass spectrometry	3–8 μg/kg	76–104% at 0.5 maximum residue limit, maximum residue limit and 2× maximum residue limit	No samples were analysed.	Bovine milk was studied. Heated water at 90 °C provided higher recovery values than water at 50, 70 and 110 °C.	Bogialli et al. (2004)
Solid-phase extraction								
42 multiclass pesticides	10 mL milk	10 mL water. 200 mg C_{18} and 5 mL methanol	Ultra-high-performance liquid chromatography-tandem mass spectrometry	0.20–0.67 μg/kg	60–120% at 0.5, 10 and 50 μg/kg	15 commercial samples of each matrix were analysed and all samples contained at least 2 pesticides at levels from 0.1 to 28.1 μg/kg.	Solid-phase extraction showed higher extraction efficiency compared with liquid-liquid extraction methods and EN 15662 and AOAC 2007.1 QuEChERS methods. C_{18} cartridges showed higher recovery values than Oasis hydrophilic lipophilic balance.	Aguilera-Luiz et al. (2011)
Phosphamidon, dimethoate, diazinon, chlorpyrifos	120 mL milk	Dilution with 12 mL water, 100 mg magnetic nanoparticles Fe_3O_4@graphene-cyanopropyltriethoxysilane and 1 mL acetonitrile as desorption solvent	Gas chromatography-micro electron capture detector	0.05–1.9 μg/L	82–94% at 1 μg/L for phosphamidon and dimethoate, and 20 μg/L for diazinon and chlorpyrifos	An undetermined number of collected samples were analysed and no pesticides residues were detected.	Bovine milk was studied. The studied Fe_3O_4@graphene-cyanopropyltriethoxysilane magnetic nanoparticles provided higher recovery values than cyanopropyl and C_{18}-solid-phase extraction.	Nodeh et al. (2016)

(continued)

Table 2.3 (continued)

Pesticides	Sample amount	Solvents and sorbents	Separation technique	Limit of quantification	Recovery study	Residues found	Comments	References
15 organochlorine pesticides	10 mL milk	15 mg ZnO nanoparticles-carbon foam in polypropylene membrane protected μSPE and 300 μL toluene as desorption solvent	Gas chromatography-mass spectrometry	0.6–5.5 μg/L	85.1–100.7% at 1, 25 and 50 μg/L	An undetermined number of collected samples were analysed and no pesticides residues were detected.	ZnO nanoparticles-carbon foam showed higher extraction efficiency than annealed carbon foam, multi-walled carbon nanotubes, ZnO-carbon nanotubes and C_{18}.	Sajid et al. (2016)
Solid-phase microextraction								
41 multiclass pesticides	1 mL milk	500 μL formic acid, 2490 μL water and polydimethylsiloxane-divinylbenzene fibre in direct immersion-solid-phase microextraction	Low pressure-gas chromatography-tandem mass spectrometry	0.02–1.00 μg/L	81–110% at 10, 25 and 50 μg/L	15 commercial milk, 3 human and 17 goat milk samples were analysed and residues of p,p′-DDE were detected in human and got samples at levels <20 μg/L.	Whole, skimmed, powdered, unprocessed goat and human milk samples were studied. Polydimethylsiloxane-divinylbenzene fiber showed better sensitivity than the polydimethylsiloxane fiber. Direct immersion-solid-phase microextraction diluted in acidified milk samples provided better sensitivity results than without dilution and acidification, and head-space mode.	González-Rodríguez et al. (2005)
35 multiclass pesticides	1 mL milk	10 mL water and polydimethylsiloxane-divinylbenzene fibre in direct immersion-solid-phase microextraction	Gas chromatography-micro electron capture detector	0.010–1.9 μg/L	69–139% at 10, 15 and 60 μg/L	35 commercial and collected samples were analysed and only 1 sample contained residues of chlordane at 1 μg/L.	Bovine milk was studied. Polydimethylsiloxane-divinylbenzene fiber showed better sensitivity than polydimethylsiloxane, polyacrilate, carboxen-polydimethylsiloxane and carbowax-divinylbenzene fibers.	Fernández-Álvarez et al. (2008)

10 organophosphorous pesticides	12 mL milk	Polydimethylsiloxane-divinylbenzene fibre in head-space-solid-phase microextraction	Gas chromatography-mass spectrometry	6.5–32.9 μg/L	0.05–0.70% (absolute) at 500 μg/L	8 collected milk samples were analysed and residues of chlorpyrifos and ethion were detected.	Bovine milk was studied. Polydimethylsiloxane-divinylbenzene fiber shower higher extraction efficiency than polyacrylateand polydimethylsiloxane fibers.	Rodrigues et al. (2011)
Lindane, heptachlor, aldrin, dieldrin, endrin	0.5 mL milk	1.5 mL saturated NaCl solution and divinylbenzene -carboxen-polydimethylsiloxane fibre in head-space-solid-phase microextraction	Gas chromatography-electron capture detector	0.5–1.2 μg/L	76–120% at 2 and 40 μg/L for Lindane and heptachlor, 4 and 40 μg/L for aldrin, 3 and 20 μg/L for dieldrin, and 5 and 100 μg/L for endrin	3 commercial milk samples were analysed, and no pesticides residues were detected.	Divinylbenzene -carboxen-polydimethylsiloxane fiber showed higher extraction efficiency than polydimethylsiloxane-divinylbenzene, polyacrylateand polydimethylsiloxane.	Merib et al. (2014)
Dispersive liquid-liquid microextraction								
Penconazole, hexaconazole, tebuconazole, triticonazole, difenoconazole	5 mL milk	1 g NaCl and 3 mL acetonitrile in the initial extraction, and 1 mL acetonitrile extract, 5 mL water and 40 μL chloroform in dispersive liquid-liquid microextraction	Gas chromatography-flame ionization detector, gas chromatography-mass spectrometry	13–180 μg/L	45–96% at 500, 4000 and 10,000 μg/L	No samples were analysed.	Bovine milk was studied. Acetonitrile formed a two-phase system added to milk sample in the initial extraction in contrast with acetone, tetrahydrofuran and methanol. Chloroform showed higher extraction efficiency compared with carbon tetrachloride and 1,1,2,2-tetrachloroethane in dispersive liquid-liquid microextraction.	Farajzadeh et al. (2011)
7 pyrethroid pesticides	5 mL milk	1 g NaCl and 3 mL acetonitrile in the initial extraction, and 2 mL acetonitrile extract, 5 mL water, 30 μL hexadecane and 800 μL acetone in dispersive liquid-liquid microextraction	Gas chromatography-electron capture detector	0.24–1.47 μg/L	90.2–108.4% at 5, 10 and 20 μg/L	A commercial milk sample was analysed and no pesticides residues were detected.	Hexadecane showed higher extraction efficiency compared with 1-dodecanol and 1-undecanol as extraction solvent, as well as, acetone compared with methanol and ethanol as dispersant in dispersive liquid-liquid microextraction.	Gao et al. (2018)

Table 2.4 Examples of the application of the QuEChERS method to the analysis of pesticide residues in dairy products

		Extraction		Dispersive solid-phase extraction (per mL extract)									
Pesticides	Sample amount	Solvents	Salts	$MgSO_4$ (mg)	Primary-secondary amine (mg)	Graphitized carbon black (mg)	C_{18} (mg)	Separation technique	Limit of quantification	Recovery study	Residues found	Comments	References
32 multiclass pesticides	15 g milk	15 mL acetonitrile (acetic acid 1%)	6 g $MgSO_4$, 1.5 g sodium acetate	150	50	–	50	Gas chromatography-mass spectrometry, high-performance liquid chromatography-tandem mass spectrometry	<10 µg/kg	70–120% for most pesticides at 50 and 500 µg/kg	No samples were analysed.	The QuEChERS method and a matrix solid-phase dispersion method (0.5 g sample, 2 g C_{18}, 2 g Na_2SO_4, 2 g Florisil®, 10 mL acetonitrile) were compared and similar results were obtained. Dispersive solid-phase extraction and conventional solid-phase extraction (500 mg primary-secondary amine, 500 mg C_{18}) were compared; higher and more consistent recovery values were obtained for some acid/base pesticides with dispersive solid-phase extraction mode.	Lehotay et al. (2005)

98 organophosphorous pesticides and carbamate pesticides	10 g cheese	15 mL acetonitrile (acetic acid 1%)	6 g $MgSO_4$, 1.5 g sodium acetate, 4 g NaCl	150	25	–	25	High-performance liquid chromatography-tandem mass spectrometry	10 μg/kg	70–120% for all pesticides at 10 and 200 μg/kg	No samples were analysed.	–	Chung and Chan (2010)
14 multiclass pesticides	15 g milk	15 mL acetonitrile (acetic acid 1%)	6 g $MgSO_4$, 1.7 g sodium acetate	240	120	–	98	Gas chromatography-electron capture detector, gas chromatography-mass spectrometry	1.72–12.22 μg/kg	72–99% at 500 and 5000 μg/kg	No samples were analysed.	A response surface methodology was used to optimize the amounts of sodium acetate (0.5–2.5 g), primary-secondary amine (200–600 mg per 5 mL extract) and C_{18} (200–600 mg per 5 mL extract) for each pesticide.	Jeong et al. (2012)
12 organochlorine pesticides	10 g milk	20 mL acetonitrile	4 g $MgSO_4$, 1 g NaCl	100	16.7	–	–	Gas chromatography-electron capture detector, gas chromatography-mass spectrometry	10 μg/kg	72–108% at 10, 50 and 100 μg/kg	An undetermined number of collected samples were analysed and no residues were detected.	–	Selvi et al. (2012)
20 organochlorine pesticides	7.5 mL of milk	15 mL acetonitrile (acetic acid 1%), 10 mL water	6 g $MgSO_4$, 1 g NaCl	150	50	–	83	Gas chromatography-electron capture detector	1–3 μg/L	70–120% for most pesticides at 0.01, 0.05 and 0.1 μg/L	No samples were analysed.	Freezing-out clean-up (20 min in the freezer) was used after initial addition of 7.5 mL water, but before acetonitrile extraction.	Singh et al. (2012)

(continued)

Table 2.4 (continued)

Pesticides	Sample amount	Extraction		Dispersive solid-phase extraction (per mL extract)				Separation technique	Limit of quantification	Recovery study	Residues found	Comments	References
		Solvents	Salts	$MgSO_4$ (mg)	Primary-secondary amine (mg)	Graphitized carbon black (mg)	C_{18} (mg)						
7 carbamates	10 g milk	30 mL acetonitrile	5 g NaCl	–	–	–	–	Ultra-high-performance liquid chromatography-tandem mass spectrometry	0.033–0.23 μg/kg	85.4–110.9% at 10, 50 and 100 μg/kg	20 commercial samples were analysed and 6 samples contained at least one pesticide at levels from 1.3 to 3.2 μg/kg.	A solid-phase extraction clean-up (500 mg NH_2, 4 mL methanol-dichloromethane (5:95, v/v)) was applied.	Liu et al. (2013)
20 multiclass pesticides	10 mL milk	10 mL acetonitrile	4 g $MgSO_4$, 1 g NaCl	–	–	–	–	Ultra-fast liquid chromatography-tandem mass spectrometry	Not reported	70–120% for most pesticides at 10 and 50 μg/kg	No samples were analysed.	Bovine milk was studied. Freezing-out clean-up (2 h in the freezer at −20 °C) was used instead dispersive solid-phase extraction. The QuEChERS method showed higher extraction efficiency than other four different methods, which used methanol or ethyl acetate as extraction solvents.	Oliveira et al. (2014)

5 organophosphorus pesticides	5 mL milk	2 mL acetonitrile	1 g NaCl	–	–	–	–	Gas chromatography-flame photometric detection	0.3–1.0 µg/L	80.5–106.5% at 10, 50 and 100 µg/L	3 commercial samples were analysed and no residues were detected.	Dispersive liquid-liquid microextraction (15 µL 1-dodecanol as extractant, 300 µL methanol as dispersant) with solidification of floating organic droplets technique was used after initial extraction.	Miao et al. (2015)
187 multiclass pesticides	5 mL milk	10 mL acetonitrile (acetic acid 1%)	2 g $MgSO_4$, 1 g sodium acetate	200	50	–	–	High-performance liquid chromatography-tandem mass spectrometry	8–12 µg/kg	75–120% for most pesticides at 10, 25 and 50 µg/kg	220 collected samples were analysed and 2 samples contained carbaryl at 0.005 and 0.025 µg/L.	The QuEChERS method was compared with solid-liquid extraction with ethyl acetate (10 mL, acetic acid 2%) and acetonitrile (10 mL, acetic acid 2%) and no differences in recovery values were found.	Imamoglu and Oktem Olgun (2016)
28 carbamate pesticides	3 g cheese	7 mL water, 10 mL acetonitrile (acetic acid 1%)	4 g $MgSO_4$, 1 g NaCl	–	–	–	–	Ultra-high-performance liquid chromatography-tandem mass spectrometry	0.5–4.7 µg/kg	70–115% at 5 and 10 µg/kg	No samples were analysed.	Gorgonzola, Roquefort, and Camembert cheeses were studied. Z-Sep + showed higher recovery values and lower matrix effects than primary-	Hamed et al. (2017)

(continued)

Table 2.4 (continued)

Pesticides	Sample amount	Extraction		Dispersive solid-phase extraction (per mL extract)				Separation technique	Limit of quantification	Recovery study	Residues found	Comments	References
		Solvents	Salts	$MgSO_4$ (mg)	Primary-secondary amine (mg)	Graphitized carbon black (mg)	C_{18} (mg)						
												secondary amine and C_{18} in dispersive solid-phase extraction. The amount of Z-Sep + was tested and 33.3 mg per mL of extract was selected.	
30 multiclass pesticides	20 mL milk	16 mL acetonitrile	8 g $MgSO_4$, 2 g NaCl	–	10.4	–	–	High-performance liquid chromatography-diode array detector	5–50 μg/L	70–120% for most pesticides at 5, 10 and 50 μg/L	10 commercial samples were analysed and at least 7 pesticide at levels from 0.3 to 18.7 μg/L were detected.	Bovine milk was studied. 25 mg Z-Sep and 5 mg Z-Sep + per 12 mL extract for clean-up were used.	Rejczak and Tuzimski (2017)
167 multiclass pesticides	15 mL milk and 7.5 g cheese or cream	7.5 mL water, 15 mL acetonitrile (acetic acid 1%)	6 g $MgSO_4$, 1.5 g sodium acetate	112.5	18.8	–	50	High-performance liquid chromatography-tandem mass spectrometry	1.1–13.1 μg/kg	80.4–117.3% at 10 and 50 μg/kg	92 commercial samples (56 of milk, 28 Feta cheese, 8 cream) were analysed and no residues were detected.	–	Golge et al. (2018)
238 multiclass pesticides	5 g milk	15 mL acetonitrile	2 g $MgSO_4$, 0.5 g NaCl	150	–	–	50	Ultra-fast liquid chromatography-tandem mass spectrometry, gas chromatography-	0.02–10 μg/kg for ultra-fast liquid chromatography-tandem mass spectrometry and 0.1–10 μg/kg for	70–120% for most pesticides at 2, 5 and 10 μg/kg for ultra-fast liquid chromatography-tandem mass	20 commercial samples were analysed and no residues were detected.	Bovine milk was studied.	Jadhav et al. (2019)

								tandem mass spectrometry	gas chromatography-tandem mass spectrometry	spectrometry and 10, 25 and 100 μg/kg for gas chromatography-tandem mass spectrometry			
41 multiclass pesticides	7.5 mL milk	15 mL acetonitrile	2 g NaCl, 7 g Na_2SO_4	112.5	37.5	–	–	Gas chromatography-electron capture detector, gas chromatography-mass spectrometry	2–50 μg/L	91.4–117.6% at limit of quantification, 2× limit of quantification, 5× limit of quantification and 10× limit of quantification	60 commercial samples were analysed and no residues were detected.	Freezing-out clean-up (10 min in the freezer at −8 °C showed higher recovery values than 4 and 15 °C) was used after initial addition of 7.5 mL water, but before acetonitrile extraction. Primary-secondary amine showed optimum clean-up efficiency in the dispersive solid-phase extraction compared with C_{18}, graphitized carbon black and Florisil®.	Tripathy et al. (2019)

mixture of petroleum ether and acetone for the extraction of organophosphorus pesticides from milk and silica as solid-phase extraction sorbent.

Despite the good analytical results, these techniques are still tedious and time consuming since it consists of many steps. Moreover, when a sample is relatively viscous or dense as in the case of milk, it does not always percolate through the cartridges commonly used in conventional solid-phase extraction. Contrary, in dispersive solid-phase extraction the sorbent is added directly into the bulk extract to develop a clean-up without any packaging or conditioning. Therefore, the procedure is greatly simple, and the total analysis time is reduced. Dagnac et al. (2009) used a dispersive solid-phase extraction clean-up step for the extraction of 44 multiclass pesticides from raw bovine milk. Graphitized carbon black and Discovery® DSC-18 were tested both in combination with $MgSO_4$ and primary-secondary amine using 5 mL of acetonitrile (formic acid 1%) per 5 mL of milk sample in the initial extraction. As can be seen in Fig. 2.3, the mixture consisting of $MgSO_4$/DSC-18/primary-secondary amine (6/1/1, w/w/w) provided higher recovery compared to $MgSO_4$/graphitized carbon black/primary-secondary amine (6/0.1/1, w/w/w), except for carbetamide, chloridazon, dimefuron, diuron and ioxynil. In both

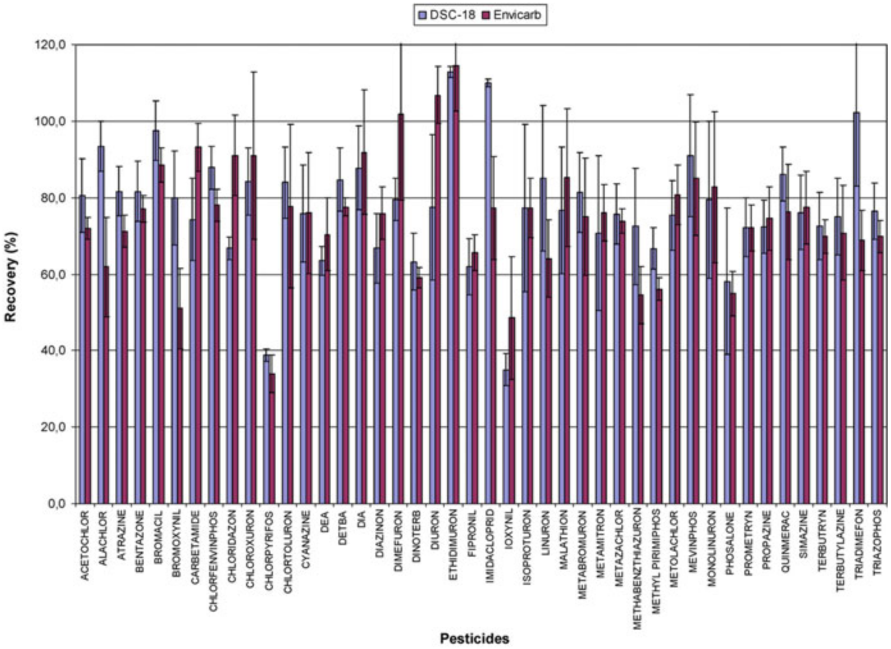

Fig. 2.3 Compared pesticide recovery on Discovery® DSC-18 and Supelclean™ Envicarb™ graphitized carbon black materials both with $MgSO_4$ and primary-secondary amine in dispersive solid-phase extraction for extraction of raw milk sample. As can be seen, the mixture consisting of $MgSO_4$/DSC-18/primary-secondary amine provided higher recovery compared to $MgSO_4$/graphitized carbon black/primary-secondary amine, except for carbetamide, chloridazon, dimefuron, diuron and ioxynil. (Reprinted with permission of Elsevier from Dagnac et al. (2009))

cases, only chlorpyrifos, ioxynil and bromoxynil showed recovery values lower than 50%. Gao et al. (2018) also compared different sorbents for purification purposes by dispersive solid-phase extraction for the determination of five pyrethroid pesticides in milk. Initial extraction was carried out with the addition of 3 mL of acetonitrile and 1 g of NaCl under acidic conditions. On the one hand, the addition of NaCl promotes that protein molecules cluster and settle and, on the other hand, pH adjustment at 4–5 with 0.1 mol/L NaH_2PO_4 solution causes the denaturation of proteins and fats. Both multi-walled carbon nanotubes and primary-secondary amine removed co-extracted material achieving high recovery percentages, but the clean-up effect of C_{18} was not effective. However, the price of primary-secondary amine is much higher than that of multi-walled carbon nanotubes and the latter were selected for validation and analysis purposes.

As previously commented, freezing-out step is also a classical but easier and cheaper alternative as clean-up step, which offers enough fat removal from samples, particularly when non-polar solvents are used. Such is the case of the work developed by Khay and co-workers (2009) in which they extracted pyrethroid pesticides from milk samples using only hexane and subsequent three freezing-out steps at different temperatures and times (70 min in total). They observed that the highly complex initial mixture was separated completely into two phases: one of them containing the solvent-hexane and the pesticide residues, and the other phase mainly composed of fats. This procedure was so effective, that when an additional solid-phase extraction step using Florisil® was incorporated, the results in terms of recovery or matrix effect did not improve. In other words, the use of multiple freezing-out steps did not require additional stages for purification purposes. It must be taken into account that the same does not happen when matrices with a higher fat content are considered. Xie et al. (2015) exemplified this fact by analysing a broad set of veterinary drugs, mycotoxins and two pesticides, i.e. carbofuran and chlordimeform, in milk, yogurt, milk powder and cheese -milk powder and cheese have a fat content of around 20% compared to 5% of milk and yogurt. In this work, they used a mixture of acetonitrile/ethyl acetate/acetic acid (49.5/49.5/1, v/v/v) in the initial extraction, a subsequent freezing-out clean-up step to remove most of lipids and finally an Oasis® hydrophilic lipophilic balance solid-phase extraction clean-up step. Although different temperatures, i.e. −20, −40 and −80 °C, and frozen times, i.e. 10, 20, 30, 40, 50 and 60 min, were tested for the low-temperature clean-up, a further solid-phase extraction clean-up procedure was necessary because matrix interferences remained in the extract.

Solid-phase extraction has also been used for pesticide extraction purposes from dairy products through new strategies beyond the simple use of conventional sorbents. Such is the case of the use of magnetic dispersive solid-phase extraction in which magnetic nanoparticles are applied. Compared to conventional solid-phase extraction, magnetic dispersive solid-phase extraction is faster, easier and less solvent consuming since it is based on the separation of the adsorbent from the sample by using an external magnet. As a successful application, the combination of Fe_3O_4 magnetic nanoparticles for ease of separation, graphene for enhanced adsorption and polar cyanopropyltriethoxysilane for selectivity purposes resulted in the synthesis of a highly efficient sorbent for the preconcentration, i.e. high enrichment factors of 2400, of polar and non-polar organophosphorus pesticides from milk samples (Nodeh et al. 2016). Specifically, this method involves only the dilution of 120 mL of milk with 12 mL of water, direct adsorption during 2 min with 100 mg of the new sorbent and a desorption process during 1 min using 1 mL of acetonitrile without any other treatment prior to the chromatographic analysis (see Fig. 2.4). Moreover, the reusability of the new sorbent was studied, and the results showed that 100 mg per 120 mL of milk sample give satisfactory and unchanged recovery even after ten extractions.

Another strategy derived from conventional solid-phase extraction is called membrane protected micro-solid-phase extraction, in which the sorbent is packed inside a porous polymeric bag (normally polypropylene) through heat-sealing. Therefore, the sorbent does not come into direct contact with the extract, avoiding the high molecular co-extracted material from the matrix as fats and proteins, but the analytes pass through the porous sheet to the sorbent. Subsequently, the bag is removed from the extract and the analytes are desorbed using a suitable solvent under ultrasound. As in conventional solid-phase extraction, the sorbent selection is a key point to guarantee the full extraction of the analytes. In this case, given the clean-up step that occurs in situ, high surface area sorbents but not so selective can be used. Among these new sorbents, on the one hand, novel carbon-based materials have gained prominence over the last decade due to their extremely high surface area and excellent chemical and mechanical stability. On the other hand, as already seen,

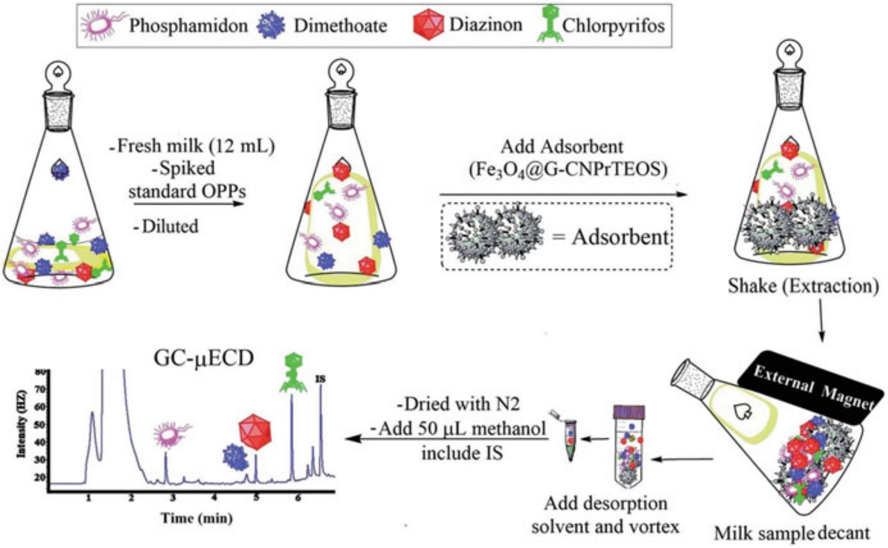

Fig. 2.4 Schematic process of the magnetic solid-phase extraction of organophosphorus pesticides (OPPs) from milk. *IS* internal standard, *GC* gas chromatography, *µECD* microelectron capture detector, *CNPrTEOS* cyanopropyltriethoxysilane. (Reprinted with permission of The Royal Society of Chemistry from Nodeh et al. (2016))

zirconia-based sorbents have also shown their excellent characteristics for this purpose. Combining these features, Sajid et al. (2016) employed ZnO nanoparticles incorporated carbon foam as sorbent and toluene as desorption solvent in membrane protected micro-solid-phase extraction for the determination of organochlorine pesticides in milk. The amounts of sorbent and desorption solvent were only 15 mg and 300 μL, respectively, for 10 mL of milk sample. The influence of different sorbents on the extraction efficiency of the selected analytes by the micro-solid-phase extraction method is shown in Fig. 2.5. As can be seen, the developed sorbent (ZnO-carbon foam) provided a higher extraction efficiency compared with ZnO nanoparticles incorporated carbon nanotubes, multi-walled carbon nanotubes, annealed carbon foam and C_{18}. This better performance was attributed, on the one hand, to π-π electrostatic interactions between carbon foam and the organochlorine pesticides and, on the other hand, to the high uniformity provided by the nanoparticles, which maximize such interactions.

The above-mentioned conventional methods have been gradually replaced by miniaturized methods such as solid-phase microextraction and liquid-phase microextraction techniques, particularly dispersive liquid-liquid microextraction, to significantly reduce the amount of solvents, stages and cost. As it is well-known, solid-phase microextraction is a technique which requires little or no organic solvents and integrates sampling, extraction, preconcentration and introduction of analytes into a single uninterrupted step. There are two different modes: direct immersion-solid-phase microextraction in which a coated fibre is introduced into the sample to extract non-volatile or semi-volatile analytes; or headspace-solid-phase microextraction, which extracts volatile analytes from the headspace of the

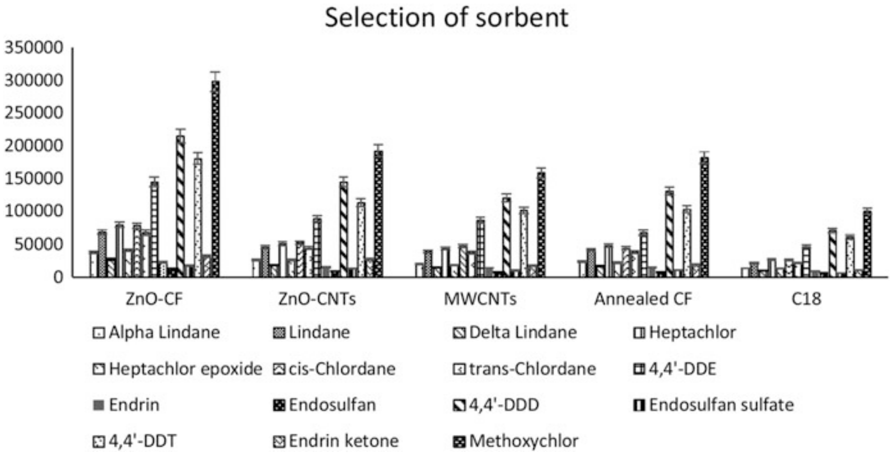

Fig. 2.5 Influence of different sorbents on the extraction efficiency of organochlorine pesticides by micro-solid-phase extraction. As can be seen, ZnO carbon foam (CF) as sorbent provided a higher extraction efficiency compared with ZnO nanoparticles incorporated carbon nanotubes (CNTs), multi-walled carbon nanotubes (MWCNTs), annealed carbon foam (CF) and C_{18}. (Reprinted with permission of Elsevier from Sajid et al. (2016))

samples. In this last case, the sample is heated, the analytes pass into the vapor phase and they are adsorbed by the fibre without contact with the matrix. Independently of the solid-phase microextraction mode used, fibre composition is a factor that must be considered since various types of polymeric fibres are commercially available. Among them, polydimethylsiloxane-divinylbenzene fibre has proven to be the most suitable coating to extract both high and low volatile pesticides from milk samples over other compositions such as polydimethylsiloxane (González-Rodríguez et al. 2005; Rodrigues et al. 2011), polyacrylate (Fernández-Álvarez et al. 2008), carboxen-polydimethylsiloxane (Fernández-Álvarez et al. 2008) and carbowax- divinylbenzene fibres (Fernández-Álvarez et al. 2008).

Although both modes provide adequate performance, some considerations must be taken into account when analysing matrices as complex as dairy products. For example, if direct immersion-solid-phase microextraction is used, the sample must be diluted with water to minimize the adsorption of fats and other high molecular weight components on the active sites of the fibre. Otherwise, high matrix effect on the calibration and poor repeatability will be suffered, including a considerable reduction of the fibre life. Such is the case of the procedures developed by González-Rodríguez et al. (2005) and Fernández-Álvarez et al. (2008). In the first work, 1 mL of milk sample was diluted with water at various dilution levels (1/2, 1/3, 1/4 and 1/5, v/v) being the dilution 1/4 (v/v) the one that was selected. This dilution reduced the sensitivity but increased the precision and the fibre lifetime. In the second work, 1 mL of milk was diluted up to 10 mL with water and the pesticides were extracted satisfactorily. It should be noted that Fernández-Álvarez et al. (2008) also compared the effect of sample dilution using the head-space-solid-phase microextraction mode for 10 mL of milk sample. As can be seen in Fig. 2.6, no important differences in terms of sensitivity from both types of experiments (with

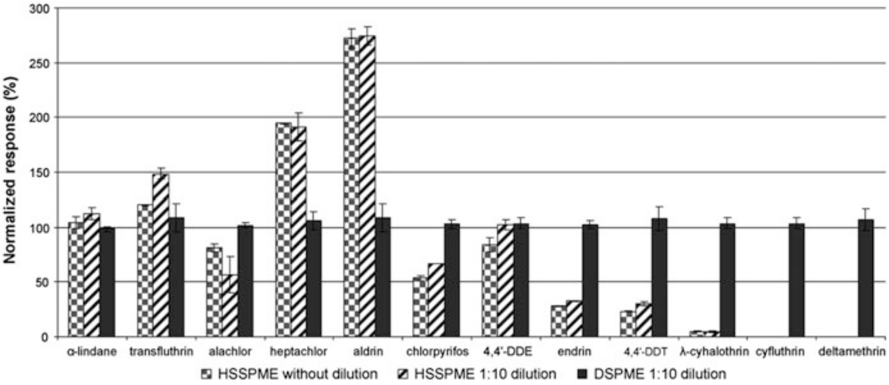

Fig. 2.6 Influence of sampling mode and dilution on the solid-phase microextraction (SPME) of some selected pesticides from milk samples. Responses were normalized to direct immersion-solid-phase microextraction 1:10 dilution responses. As can be seen, no important differences in terms of sensitivity were found for headspace SPME (HSSPME) and direct immersion SPME (DSPME). (Reprinted with permission of Elsevier from Fernández-Álvarez et al. (2008))

and without dilution) were found. On the one hand, it would have been expected that sensitivity decreases proportionally to the dilution, but, on the other, this is offset by the lower matrix effect that favours the transfer of the analytes to the fibre.

Since the dilution strategy is not so useful for head-space-solid-phase microextraction, Merib et al. (2014) studied it along combined with the modification of the ionic strength by adding a saturated solution of NaCl. The variation in ionic strength changes the liquid/vapor equilibrium of the system and with it could change the chromatographic response of the analytes. In this regard, for a total volume of 2 mL in all cases, three different combinations of milk sample and NaCl saturated aqueous solution, i.e. 3/1, 1/1 and 1/3, v/v, were tested. The results showed that dilution increases ionic strength significantly, which improved the transfer of the analytes to the headspace and, therefore, the chromatographic peak areas for all pesticides studied, so the best results were obtained using 0.5 mL of milk and 1.5 mL of NaCl solution (see Fig. 2.7).

Dispersive liquid-liquid microextraction is another miniaturized procedure that can evade the inherent drawbacks of solid-phase microextraction such as the fragility and limited lifetime of the fibre as well as sample carryover. It is based on a ternary system composed of a water immiscible organic solvent, a water miscible dispersion

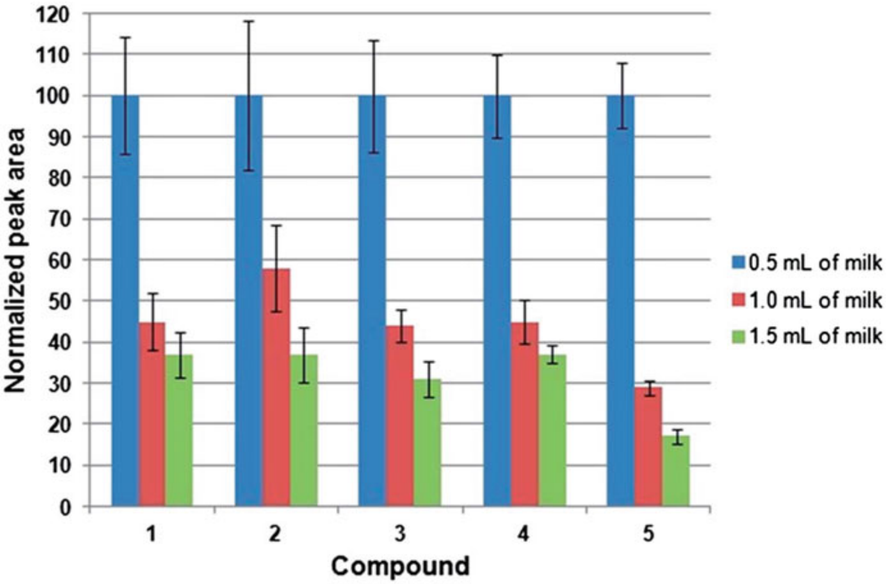

Fig. 2.7 Normalized peak areas of the target compounds: (1) lindane, (2) heptachlor, (3) aldrin, (4) dieldrin and (5) endrin using a total sample volume of 2 mL combining milk and a saturated aqueous solution of NaCl. The results show that dilution with the saturated aqueous solution of NaCl improved the transfer of the analytes to the headspace and, therefore, the chromatographic peak areas for all pesticides studied (the best results were obtained using 0.5 mL of milk and 1.5 mL of NaCl solution). (Reprinted with permission of The Royal Society of Chemistry from Merib et al. (2014))

solvent and the aqueous extract containing the analytes of interest. The mixture of both solvents is introduced into the aqueous donor phase, producing a dispersion, and after centrifugation, the drop of solvent containing the analytes can be collected, either at the bottom of the tube or at the surface when lower density solvents than water are used. This extraction method provides high enrichment factors very easily at a very low cost, in addition to be an environmentally friendly technique since it requires low amounts of organic solvents. As an example of the use of this method for pesticides extraction from dairy products, Farajzadeh et al. (2011) initially extracted a group of five triazole pesticides employing acetonitrile and NaCl as salting-out agent. After agitation and centrifugation, an upper organic phase containing the analytes and an aqueous lower phase with the precipitated co-extracted material were obtained. Then, a portion of the acetonitrile phase was mixed with only 40 μL of chloroform and water. After that, triazole pesticides were collected into the fine droplets of chloroform formed at the bottom of the tube and directly injected in the chromatographic system. As expected, the type of extraction solvent used plays a fundamental role in the extraction efficiency. In this sense, three organic solvents, i.e. chloroform, carbon tetrachloride, and 1,1,2,2-tetrachloroethane, were studied as extraction solvents because they have suitable properties including higher density than water, high extraction efficiency for pesticides, low solubility in water and good chromatographic performance. Chloroform gave the highest recovery values. Contrary, Gao et al. (2018) used 30 μL of hexadecane as extraction solvent for the extraction of 7 pyrethroid pesticides from milk, which has lower density than water, so the resulting droplet was floating on the top of the sample and could be easily collected by solidifying it at low temperature. This new version is called dispersive liquid-liquid microextraction based on the solidification of a floating organic droplet, which is even simpler, faster, cheaper and less toxic (Yamini et al. 2019).

Only 2 years after the appearance of the QuEChERS method, its first application to the determination of pesticide residues in fatty food matrices was developed including milk together with eggs and avocado (Lehotay et al. 2005). In this first study, the AOAC 2007.1 QuEChERS method proved to be a good option again to effectively extract 32 pesticides of a wide polarity range from these low fatty foods, i.e. 2–20% fats, while co-extraction of fats was minimized as well as highly polar proteins, salts and sugars. In this sense, it should be noted that for other high fatty dairy products such as butter, >20% lipids, it would be necessary to use non-polar solvents to extract highly lipophilic analytes that remain into the fats (Chiu et al. 2018). However, if the fat percentage is relatively low as it occurs in milk, high recovery values even for highly lipophilic pesticides will be obtained using acetonitrile.

Despite the above, a certain amount of residual fats remained in the acetonitrile extract, so it is necessary to apply clean-up steps prior to chromatographic analysis. Lehotay et al. (2005) focused on this issue by comparing dispersive solid-phase extraction and conventional solid-phase extraction as clean-up procedures. In the dispersive solid-phase extraction mode, 50 mg of primary-secondary amine and 50 mg of C_{18} were used together with 150 mg $MgSO_4$. In contrast, the solid-phase extraction experiment consisted in a stack of 500 mg of C_{18} and 500 mg of primary-secondary amine cartridges in the solid-phase extraction manifold, adding a 1 cm layer of $MgSO_4$ on the top (5 mL of acetonitrile were added for cartridge conditioning). The results in terms of clean-up were slightly better for the solid-phase extraction mode, but recovery percentages were similar for the majority of pesticides and even higher and more consistent for pesticides with acid/base properties, i.e. acephate, carbendazim, imazalil, methamidophos, pymetrozine, and thiabendazole, in the dispersive solid-phase extraction mode. In any case, it is clear that the dispersive solid-phase extraction mode is much easier, faster and even cheaper than the solid-phase extraction mode, while providing satisfactory analytical performance and good clean-up. However, the authors evidenced that there is an important dependence between recovery of pesticides and the fat content of the sample.

After this first work, an increasing number of papers regarding the determination of pesticides in milk and its derivatives employing the QuEChERS method have been published (see Table 2.4). In fact, it has been the method most used worldwide due to its simplicity that greatly saves time and costs.

The majority of these papers are based on the easy adaptation of the use of sorbents in the dispersive solid-phase extraction step according to the pesticide/dairy product combination under study to ensure its robustness. As an example, Jeong et al. (2012) optimized the amounts of primary-secondary amine and C_{18} in the clean-up step by a response surface methodology. However, the optimized quantities, i.e. 600.00 mg primary-secondary amine, and 489.96 mg C_{18}, per 5 mL of extract, besides being somewhat impractical, removed the most lipophilic pesticides with the other fatty compounds because of the relatively high amount of C_{18} used. To overcome these problems, other sorbents such as amino propyl and diatomaceous earth have also been used for cleaning the extracts from dairy products containing pesticides. In the first case, Liu et al. (2013) incorporated a solid-phase extraction clean-up step with 500 mg of amino propyl for the simultaneous quantification of seven carbamates in milk. Although amino propyl cartridges provided a wide range of clean-up by having both polar and ion exchange properties, previous steps of column conditioning as well as the corresponding slow elution were necessary. In the second case, Manav et al. (2019) used for the first time 500 mg of diatomaceous earth together with 300 mg of $MgSO_4$ and 150 mg of C_{18} per 7 mL of extract in the dispersive solid-phase extraction mode for the determination of 25 pesticide residues in milk, cheese, yogurt and cream. The use of diatomaceous earth provided a good cleaning of the extracts without impairment in recovery, so it could be a sorbent whose application is extended in the future due to its good performance and very low price.

As already mentioned, zirconia-based family of sorbents have been used for fat-rich samples because they adsorb the majority of fatty non-polar interferences and their application for dairy products is not the exception. In the study developed by Rejczak et al. (2017), Z-Sep and Z-Sep + demonstrated ability to remove more fat than C_{18} and Florisil® as well as higher recovery values and better reproducibility. In particular, the best results were achieved using a mixture of 125 mg primary-secondary amine, 25 mg Z-Sep and 5 mg Z-Sep + per mL of 12 mL of milk extract previously evaporated to dryness and reconstituted in 1.2 mL of acetonitrile, obtaining recovery percentages in the range 70–100% for the majority of the pesticides with relative standard deviation values lower than 20%. Basically, conventional sorbents attract fats through hydrophobic interaction, while ZrO binds these components with electron donating groups because it acts as a Lewis acid. Therefore, effective and highly selective cleaning is achieved, without removing the analytes of interest. Analogous results were obtained by Hamed et al. (2017) when they compared Z-Sep+, primary-secondary amine and C_{18} using 100 mg in all cases for dispersive solid-phase extraction clean-up of 3 mL of extract of Gorgonzola cheese (50% fat content). The average recovery for the extraction of 28 carbamate pesticides was 86%, 84% and 75% for Z-Sep+, primary-secondary amine and C_{18} with values of matrix effect of 13%, >25% and >20%, respectively. Subsequently, the proposed method using Z-Sep + was also applied to Roquefort (32% fat content) and Camembert (45% fat content) cheeses showing enough clean-up efficiency to remove fatty components and satisfactory recovery values in all three cheeses.

Finally, the freezing-out step has also been incorporated into the QuEChERS method to determine pesticides from dairy products, either by applying it before the dispersive solid-phase extraction step (Singh et al. 2012; Tripathy et al. 2019) or even replacing it (Oliveira et al. 2014). In the first approach, 7.5 mL of milk sample were diluted with water and kept at low temperature to perform the freezing-out step. Then, the salting-out acetonitrile extraction and the dispersive solid-phase extraction with 900 mg of $MgSO_4$, 300 mg of primary-secondary amine and 500 mg of C_{18} per 6 mL of extract (Singh et al. 2012) or with 900 mg of $MgSO_4$ and 300 mg of primary-secondary amine per 8 mL of extract (Tripathy et al. 2019) were performed. In this last work, effect of temperature in the freezing-out step on recovery was studied at 4, 8, and 15 °C. The recovery values ranged from 82.6% to 102.4%, 91.8% to 113.4% and 74.8% to 96.7% with relative standard deviation values of 1.24–2.26%, 1.05–2.36%, and 1.63–3.50% at 4, 8, and 15 °C, respectively. Moreover, the maximum average recovery was obtained at 8 °C, resulting 105.4% with a relative standard deviation of 1.6%. Regarding the second approach, 10 mL of milk samples was directly extracted with acetonitrile and salt assisted, and then the supernatant was transferred to another tube containing Na_2SO_4 (1.5 g) and stored at -20 °C for 2 h without subsequent dispersive solid-phase extraction step. This easy method showed higher extraction efficiency than other methods which included dispersive solid-phase extraction with $MgSO_4$, primary-secondary amine and graphitized carbon black and solid-phase extraction with C_{18}. The recovery values were in the range of 70–120% for the vast majority of the 20 multiclass with relative standard deviation values lower than 20%. As authors said, the procedure does not have additional steps, provides a significant reduction of the extraction time and cost, as a result of the removal of one step from the overall procedure in which very expensive sorbents were used.

2.4 Conclusions

The development, validation and the correct application of analytical methods is an important issue in many disciplines in order to guarantee the obtention of feasible results. Such importance is even higher if the target analytes have a negative or harmful effect on humans or on the environment, and this is what happens in the analysis of pesticides.

Concerning the presence of pesticides in foods, in particular, in milk or dairy products, a wide variety of methods have been used for their analysis. In all cases, an important challenge must be faced, that is, the removal of fats and proteins, especially for lipophilic pesticides. Among the different method that have been proposed, sorbent-based sample pre-treatment procedure highlight, in particular solid-phase extraction and also the QuEChERS method, though miniaturized extraction techniques (solvent or sorbent-based) have also been applied including the application of nanomaterials though in few cases.

The application of pesticides may also have a negative effect on the environment. A clear example is their presence in pollen which results in negative effects over bees. As previously shown, the analysis of pesticides in this type of samples must face an important issue: the low amount of sample and the low concentrations at which these compounds appear. Even though, successful methods (though not as many as expected) have been developed, especially for the analysis of neonicotinoid insecticides, which have traditionally been the most studied in this type of samples. Among them, the QuEChERS method stands out as a result of the advantages previously mentioned.

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Chapter 3
Monitoring and Evaluation of Pesticide Residues in Aquatic Systems

Rahul Mondal and Ramen Kumar Kole

Abstract Pesticides are primarily used for crop protection in agriculture with global consumption of about two million tons per annum. Herbicides, insecticides, and fungicides are the major three classes of pesticides covering about 95% of the total pesticides use. Besides controlling the target pests, a large number of these pesticides adversely affect humans and other non-target organisms with acute and chronic toxic effects. Therefore, people who are professionally engaged in the process from pesticide production to application are most susceptible to various adverse health effects due to acute and chronic exposure. In addition, pesticides following application are circulated in various biotic and abiotic components of the environment including water. Moreover, a number of pesticides are persistent in the applied crops leading to residual toxicity in food. Consequently, the general population is also exposed to trace levels of pesticide residues through food and water. Therefore, regular monitoring of pesticide residues is indispensable for assessment of food and environmental safety.

This chapter reviewed: (1) The processes facilitating distribution of pesticides from agro-ecosystem to hydro-system; (2) Analytical methods for monitoring of pesticide residue in water; (3) Status of pesticide residues occurring in aquatic system; (4) Reasons for frequent occurrence of organochlorine pesticides in water bodies; and (5) Methods for assessment of aquatic ecological risk due to pesticides. Following application in field, pesticides are transported to hydro-system through runoff, leaching and preferential water flow in dissolved and particulate phase. The multi residue methods based on chromatography with tandem mass spectrometry have been developed and validated for trace analysis of organic pollutants such as pesticides of diverse chemical classes in aquatic system. Over the past few decades, the residual level of various pesticides up to 5.7 μg/g level have been detected in

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aquatic system of different countries across the world. Most of the organochlorine pesticides, such as dichlorodiphenyltrichloroethane, hexachlorocyclohexane, and endosulfan failed to meet the guideline values, have been banned. The reasons for occurrence of banned organochlorine pesticides are attributed to: (i) the old sources and their transformation into stable isomeric products, (ii) sedimentation succeeded by re-suspension to release organochlorine pesticides; and also due to (iii) the continued use of the banned organochlorine pesticides to combat vector borne diseases. Potential risks to aquatic life are also evaluated by computing risk quotient, toxic unit, and various risk models for hazard analyses. Leaching potentiality of pesticides to pollute groundwater may also be evaluated using groundwater ubiquity score index. Social awareness, safe and judicious use of toxic pesticides, use of bio-pesticides, designing strict policies and effective remediation technologies are recommended to minimize the pesticide pollution in aquatic system.

Keywords Pesticides · Toxicity · Environmental transportation · Multiresidue methods · Monitoring · Aquatic health · Hazards · Risk assessment

3.1 Introduction

To begin this chapter, the authors wish to quote a context addressed by a famous biologist Miss Rachel Carson who first ever documented the adverse environmental effects of pesticides in her renowned book entitled "Silent Spring".

> "A Who's Who of pesticides is therefore of concern to us all. If we are going to live so intimately with these chemicals eating and drinking them, taking them into the very marrow of our bones - we had better know something about their nature and their power. . ."
>
> — Rachel Carson (1962), Silent Spring

3.1.1 General

To ensure food security agriculture has heavily relied on application of synthetic pesticides such as insecticides, acaricides, fungicides, rodenticides, herbicides, and antibiotics for crop protection. Globally pesticides are used in only 25% of the agricultural lands with the annual consumption of about two million tons per annum. The maximum share of pesticides consumption is in Europe (45%) followed by United States of America (25%) and the rest of the world (25%). Among the different classes of pesticides, the use of herbicides is maximum (about 47.5%) followed by insecticides (29.5%), fungicides (17.5%) and only 5.5% is shared by other classes (Agarwal et al. 2015). The use of the synthetic pesticides has certainly helped to maintain, and in many instances, increased food production around the world. Besides controlling pests in agriculture, pesticides are also used to control human and livestock disease vectors and also to prevent or control organisms affecting other human activities.

The continual application of these pesticides over the last few decades has led to pest resistance which also resulted in pest resurgence. A number of pesticides are persistent in the crop field leading to residual toxicity in food and environment. Besides, the farmers who work with pesticides application are also suffering from chronic toxicity. A survey reported annual deaths of 240,000 by various chemicals through acute poisonings and 186,000 annual deaths through self-poisonings using pesticides (Prüss-Ustün et al. 2011). According to World Health Organization about 23% of global death is caused by modifiable environmental factors, including chemical water pollution (WHO 2016). In addition, the general population is chronically exposed to relatively low doses of chemical substances that do not naturally occur in the environment. One of the groups, particularly noteworthy due to their inherent toxicity are biocides such as pesticides. Most of these pesticides are potent neurotoxins in high doses, but they may cause diverse toxic effects due to chronic exposure to low doses. In fact, a large number of these pesticides may adversely affect human and environmental health.

Therefore, the occurrence of pesticides residues are monitored in many countries for assessment of food and environmental safety. For instance, during a three year period (2013–2015) many participating countries in the frame of the European Union coordinated programmes monitored conventional (28,912 nos.) and organic (1940 nos.) food samples for 213 pesticides. Overall, the conventional foods were detected with one or more pesticide residues in 44% of the samples analyzed while the occurrence of pesticide residues in organic samples was much lower (6.5%). About 1.2% of the conventional and 0.2% of organic food samples were above the residual safety limit known as maximum residue limit of the respective pesticides (EFSA 2018). In India, different food commodities have been analyzed for the occurrence of pesticide residues during the last decade: 2008–2018. About 2.1% out of 1,81,656 samples analyzed were found to contain pesticide residues above the MRL prescribed by Food Safety and Standard Authority of India (MPRNL 2018).

Besides, the pesticides following application in the fields are distributed in the environment causing ecological and environmental toxicity. Therefore, despite having many benefits, pesticides may impart toxicity hazards to the environment (Golfinopoulos et al. 2003). In fact, pollution caused by pesticides appeared twice: first due to agricultural production and secondly for pesticide manufacturing and storage in the world's top ten worst toxic pollution (Harris and McCartor 2011). Consequently, ecotoxicological effect of pesticide residues on aquatic environment has also become a matter of concern. Such effects at the organism or ecological level may also include potential impacts on human health, if the suspected water is used for public consumption (Singh et al. 2012). Therefore, regular monitoring and assessment of pesticide residues occurring in water is indispensable for management of toxic pesticide residues.

3.1.2 Toxicity of Pesticides in Human

Pesticides are potentially toxic chemicals to humans with acute and chronic health effects. Acute toxicity is the ability of a pesticide to cause harmful health disorders following a single and rapid exposure through oral, dermal or respiratory routes. The symptoms are produced immediately after exposure or within 48 hours which may include irritation in eye, skin and respiratory tract, allergic sensitisation, diarrhoea, vomiting, extreme weakness, seizures and/or death. Therefore, people who are directly exposed to pesticides during pesticides manufacturing, transport, distribution, preparation of spray solution and application in various fields are at high-risk. Such exposure of pesticides through skin and respiratory routes has been reported to produce non-Hodgkins lymphoma (Margni et al. 2002). A number of projects relating people suffering from various health problems like anemia, diarrhea, goiter, and thyroid were correlated with pesticides exposure (Lu 2009).

Adverse health effects due to chronic toxicity are produced after a long-term (few months to years) exposure of a pesticides. Long term pesticide exposure has been correlated to various diseases like asthma, attention deficit and hyperactivity disorder, cancer, depression, non-Hodgkin lymphoma and Parkinson's disease (Rauh et al. 2006; Maurya and Kumar 2013; Okoya et al. 2013). The general population who are not professionally engaged with pesticides may also be exposed to trace levels of pesticide residues through food and water. Therefore, various national and international organizations reviews evidence and develop maximum residue limit of pesticides in food and water to ensure safety to human health.

Some of the pesticides having reproductive and endocrine-disrupting effects are organochlorines like dichlorodiphenyltrichloroethane and its analogues, hexachlorocyclohexane and its isomers, dicofol, and endosulfan; organophosphates like malathion, parathion; carbamates like methomyl, carbaryl; and synthetic pyrethroids (CSE 2003). Endocrine disruptors are the substances that interfere with hormones and hormonal balance either by imitating or blocking hormones like oestrogen, testosterone and thyroid hormone (Li et al. 2008; Uchida et al. 2010). Adverse reproductive effects of persistent organic pollutants like dichlorodiphenyltrichloroethane have been documented (UNEP 2003; De Jager et al. 2009; Rocheleau et al. 2009; Bornman et al. 2010; Giordano et al. 2010). Incidence of breast cancer in women was reported to promote infection of mammary glands by atrazine, a triazine herbicide (Kettles et al. 1997) and organochlorines (Payne et al. 2001) detected in local drinking water bodies.

3.1.3 Effect of Pesticides on Aquatic Health

Pesticides may contaminate soil and surface water through spray-drift which acts as a point source pollution. Later on, horizontal and vertical spreading of the same through runoff and leaching transformed the point source to a non-point one. This caused terrestrial and aquatic ecological complications and imposing risk to human health (Kruawal et al. 2005). The ecological effects of these pollutants in aquatic ecosystems are broad-ranging owing to their diversified chemical nature (Li and Kole 2004). The declining population of marine mammals has been correlated with the pesticides exposure especially due to organochlorines and sensitive coral reefs due to synthetic pyrethroids (Tanabe et al. 1994; Elfman et al. 2011). The likelihood of chronic exposure to low concentrations of various pesticides like azoxystrobin, synthetic pyrethroids, organophosphates, and pesticide mixtures with impact on different fish species, salmon, trout and other macro-invertebrate communities have been documented (Moore and Waring 2001; Sandahl et al. 2004; Tierney et al. 2008; Olsvik et al. 2010; Schäfer et al. 2011).

The residues of pesticides in aquatic environments have also been reported to cause adverse toxicological impact on different aquatic organisms like arthropods, dolphin, fish, macro-crustaceans, rotifers and snails (Osti et al. 2007; Ansara-Ross et al. 2008; Bakry et al. 2011; Alonso et al. 2012). Sub-lethal effects of some herbicides have direct impact on growth and cell density of phytoplankton in water due to the structural similarity of the herbicides with the cellular chemicals of target plant species (De Lorenzo et al. 2011). The herbicide glyphosate has been reported to exhibit genetic toxicity, teratogenicity and endocrine disrupting effects in different aquatic organisms, amphibians and birds (Benachour and S'eralini 2009; Paganelli et al. 2010; Jones et al. 2011). **Some research also reported the effects of atrazine on aquatic frogs by** altering its genetic characteristics by which frogs could develop female organs at low doses of atrazine and the feminized males may reproduce with male frogs (SDWF 2019).

3.1.4 Environmental Fate of Applied Pesticides

Pesticides after application are circulated in various environmental compartments like air, water, soil and may also enter into the plant and animal systems. Environmental fate of the applied pesticides depend on its physical and chemical properties like persistence, solubility in water, sorption coefficient in soil, and vapor pressure as well as the environmental conditions (Tiryaki and Temur 2010). Pesticides following exposure into the environment undergo various transformations reactions like hydrolysis, photolysis, oxidation, and reduction under biotic and abiotic conditions. In the next section, special attention has been given to exposure of the applied pesticides to water bodies.

3.2 Transportation of Pesticides from Agro-Ecosystem to Hydro-System

Pesticides after application are primarily distributed to other compartments of the environment through water (Masiá et al. 2013). The adjacent water bodies receive pesticides through diffusion or directly from the point sources. Diffusion of pesticides is mainly mediated by transportation through lateral movement like surface or sub-surface runoff, vertical movement like leaching, and preferential flow of pesticide fluxes through soil matrices (Reichenberger et al. 2007; Sangchan et al. 2012; Tang et al. 2012). In addition, wind and soil erosion, spray drift, and volatilization followed by regional atmospheric deposition through precipitation also play a significant role on pesticides circulation into the atmosphere (Wu et al. 2004; Felsot et al. 2010; Messing et al. 2013). The pathway of pesticide transportation in the aquatic environment has been well described by Gilliom et al. (2006) and schematically presented in Fig. 3.1. However, following factors should be

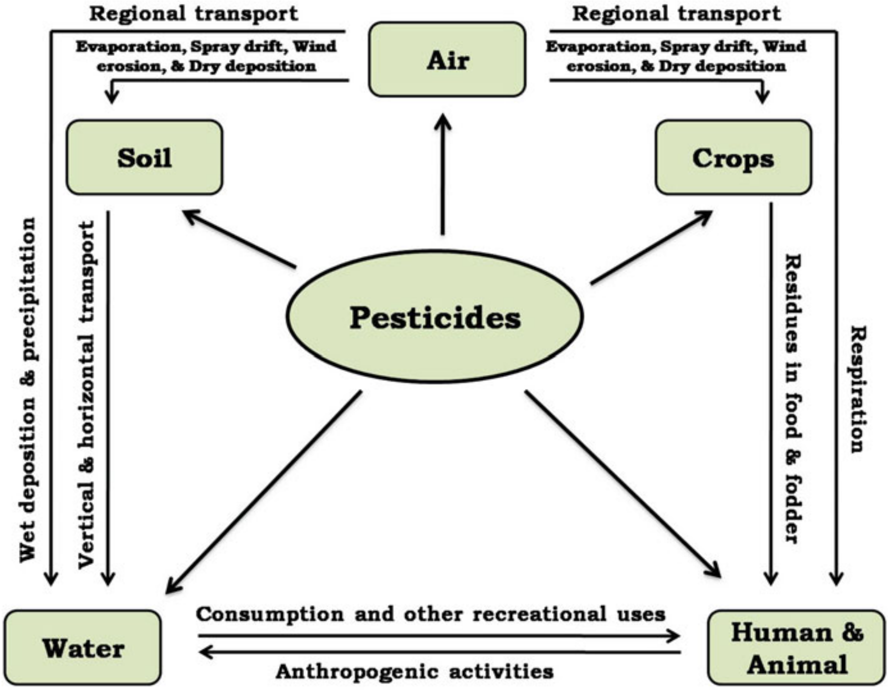

Fig. 3.1 Pesticide transport in and around aquatic environment. (Modified after Fenik et al. 2011): Pesticides after application are mostly circulated to other compartments of the environment through waterways polluting surface and ground water bodies. Circulation is mainly mediated by various diffusive environmental processes such as surface or sub-surface runoff, seepage, leaching, and preferential drain flow. Other native factors like spray drift, volatilization followed by precipitation, wind and soil erosion also play significant role on pesticide transportation in the environment

considered for understanding the translocation of pesticides from agro-ecosystem to hydro-system.

3.2.1 Rainfall

Rainfall is one of the important factors which trigger leaching of pesticides through porous soil matrices (Tiktak et al. 2004). Pesticide applications during dry periods succeeded by overland flow and seasonal first flush usually causes the highest pesticide losses from the application site and significantly intensifies the pollutant concentration into the nearby aquatic bodies (Riise et al. 2004; Tiefenthaler et al. 2008; Wittmer et al. 2010). Transportation of soil applied chemicals directly to ground and surface water is due to preferential water flow through macro-pores, co-transport with colloidal matter or may be due to both the processes (Williams et al. 2000; Hesketh et al. 2001; Roulier and Jarvis 2003; Tang et al. 2012; Brown and van Beinum 2009).

Loss of pesticides by runoff from agricultural fields is facilitated by irrigation after application and also during the wet months, while the loss is reduced with decrease in rainfall (Kennedy et al. 2001; Southwick et al. 2009; Santhi and Mustafa 2013; Ikpesu 2015). However, higher load of pesticides in dry season in river aquatic system was attributed to less rainfall during dry season leading to reduction of water volume which was diluted with rain water during wet months (Mondal et al. 2018). Heavy rainfall shortly after application of pesticides in wet soils may induce rapid transport to groundwater. Thus, the timing, intensity and duration of rainfall in respect of pesticide application are the key factors influencing pesticide losses through runoff.

3.2.2 Soil Type

Pesticides residues contaminating ground and surface water bodies are transported through dynamic soil-water micro-environment and mostly influenced by the soils profile, structure and texture. Soils of recent origin characterized by immature and weak profiles, catalyzes pesticide transportation in dissolved phase through downward movement. Soil composed of sand, silt, and clay in equal proportion known as loamy soil shows good porosity and texture providing sufficient drainage when other conditions are favorable for agriculture. These loamy soils are continuously replenished by recurrent floods. Soil having similar properties, named as alluvial soil, have been mostly found in India and become the possible cause of pesticide contamination of ground and surface water bodies in the plains of Indus-Ganga-Brahmaputra and Narmada-Tapi (Singh et al. 2005, 2007; Siddiqui and Fatima 2017).

Short-term pesticides loading was also observed in a river under agricultural watershed having clay loam and clay loam to sandy clay loam soil types with well organized soil porosity in Thailand (Schuler 2008; Sangchan et al. 2012). The extraordinary high concentration of dieldrin (0.02–19.41 ng/mL) in surface water near lake Vela, Portugal was attributed to weak retention capacity of the adjoining sandy loam soils with low organic matter content (Abrantes et al. 2008, 2010). Soils with high clay particles and organic matter content enhance pesticides movement via surface runoff, while leaching is favored in lighter sandy soils with low clay and organic matter content (Wu et al. 2003; Kerle et al. 2007; Wightwick and Allinson 2007). These factors also determine the sorption affinity, volatilization, hydrolysis, photolysis, and biotic or abiotic degradation of the applied pesticides (Kookana et al. 1998).

3.2.3 Transport Phases

Movement of pesticides from agricultural fields to water bodies may occur in solution phase or may be transported by adsorbed phase on suspended, or colloidal particles, or on dissolved organic carbon along the surface of sloping agricultural land. Pesticide movement is influenced by water solubility and hydrophobicity. Hydrophobicity is measured by octanol-water partition coefficient which indicates the ability of the pesticides for adsorption on particulate matter.

3.2.3.1 Dissolve Phase

Pesticides with high water solubility are assumed to be most susceptible to leaching through vertical transport to reach ground water and mostly found in dissolved phase (Go’mez-Gutie’rrez et al. 2006; Reichenberger et al. 2007). Due to higher water solubility organophosphate and carbamate pesticides have mostly been detected in dissolved phase (Wang et al. 2009; Oliver et al. 2012). In contrast, the hydrophobic pesticides with low water solubility like organochlorines and pyrethroids are preferentially adsorbed on suspended or colloidal particles and aquatic organisms and create long term pollution (Elfman et al. 2011; Bhadouria et al. 2012; Mondal et al. 2018). Interestingly, the absence of α-isomer of hexachlorocyclohexane and prevalence of δ-isomer of hexachlorocyclohexane in sediment sample was also attributed to relative solubility between the isomers (Prakash et al. 2004).

However, pH has a direct effect on pesticide solubility in water. At low soil pH, solubility of dichlorodiphenyltrichloroethane increases proportionally with humic acid concentration (Singh et al. 2012). Among the organochlorines, the relative difference between the physico-chemical properties favored dichlorodiphenyltrichloroethanes to retain on the particulate phase compared to hexachlorocyclohexanes (Yang et al. 2005).

3.2.3.2 Particulate Phase

Pesticides fate and transportation through suspended particulate matter is governed by particle sorption capacity. Pesticide loss through particulate phase is inversely related to sorption strength *i.e.,* octanol-water partition coefficient (K_{oc}) but other factors like rainfall and irrigation triggers colloid-associated transport with maximum discharge (Tang et al. 2012). Three different pesticide runoff patterns were identified in the Mae Sa watershed in northern Thailand (Sangchan et al. 2012).

The first pattern is rainfall facilitated colloidal surface runoff of pesticides with low K_{oc} value like atrazine and dimethoate leading to higher pesticides concentration in surface water bodies. The second pattern deals with sub-surface or preferential flow of pesticides like chlorpyrifos and cypermethrin with moderate to high log K_{oc} values resulting intermittent high concentrations in ground water bodies. The third pattern is related to long-term deposition of pesticides like persistent organochlorines which would act as old source of pollution that contributes random concentrations in water sources (Sangchan et al. 2012).

Nevertheless, clay mineralogy and soil organic matter content also influences the sorption capacity of cationic pesticides like glyphosate and paraquat and non-ionic pesticides like chlorpyrifos and diuron. Moreover, the size and surface area of the colloidal particles regulate the settling velocity, lateral transport, and deposition pattern of pesticides that adsorbed on particles (Walker 2001; Erkmen et al. 2013).

In general thumb rule is hydrophobic pesticides with high K_{oc} are more susceptible to move via particulate surface runoff compared to the hydrophilic pesticides with low K_{oc} which are susceptible to leaching through the soil profile in dissolve phase.

3.2.4 Application Method

The pesticides applied as fine sprays may be moved offsite by the prevailing wind speed and pollute local water bodies as spray drifts (Stephenson et al. 2006). Pesticide drift resulting from foliar application is also significantly contributing towards pesticide pollution in the environment, especially in the intensive cultivation areas (Schulz 2001; FOCUS 2007; Bereswill et al. 2012). The extent of pesticide losses due to spray drift have been assessed in some review articles (Ucar and Hall 2001; Gill and Sinfort 2005; Felsot et al. 2010). Pesticides with high vapor pressure may also enter into the atmosphere directly as a result of quick volatilization from the application site and precipitate through rain, thereby contaminating the aquatic environment (Gevao et al. 2000).

However, pesticides applied using dry formulations are lost as windblown sediment particles with detrimental implication for water quality (Glotfelty et al. 1989; Clay et al. 2001). Pesticide losses through soil erosion may be reduced by soil incorporation of herbicides (Tiryaki and Temur 2010). Moreover, pesticides applied in water-soluble form are also vulnerable to losses through runoff, while water immiscible forms like emulsified formulations are more susceptible to move via particulate surface runoff (Gish et al. 2011).

3.2.5 *Ground Water Table*

Depth of ground water table shows a positive correlation of pesticide pollution. Preferential flow is the principal mechanism controlling movement of pesticides through soil-water dynamics to the nearest groundwater table regardless of soil texture (Tang et al. 2012). This might be catastrophized by first rainfall event after pesticide application reaching ground water table (Wittmer et al. 2010). The presence of some herbicides like atrazine, simazine, and diuron in bore holes water was due to high groundwater levels in United Kingdom (Lapworth et al. 2006).

In addition, pesticide concentration in aquatic system is also related to agricultural management practices in the catchment area (Zablotowicz et al. 2006; Anderson et al. 2013). Various other related factors like local meteorological conditions; type of pesticides including its physico-chemical properties, application doses, frequency of application and equipment used; edaphic factors like pH, temperature, moisture, organic matter, topography, vegetation, and microbial activity; and drainage system (Kerle et al. 2007; Tiryaki and Temur 2010).

3.3 Methods for Residue Analysis of Pesticides in Water

Reliable analytical method is required to be developed for estimation of chemical pollutants like pesticides in water bodies. Residue analysis of pesticide in aqueous system is quite challenging task due to their occurrence at trace level (Brondi et al. 2011). A single analytical method may not be suitable for estimation of all the pesticides occurring in water (Kouzayha et al. 2012; Tsochatzis et al. 2012). Therefore, in the field of pesticide residue monitoring, multi residue methods (MRMs) are developed for identification and quantification of pesticides belonging to different classes like organochlorines, organophosphates, synthetic pyrethroids, carbamates, chloroacetamides, phenoxy carboxylic acids, triazines, and triazoles simultaneously using a single analytical technique involving sample processing, extraction, clean up and selective detection. Decades of research works have been carried out for development of MRMs by modifying the extraction procedure and the instrument used for pesticide residue analysis in environmental matrices.

3.3.1 Extraction

There are many innovative attempts made for extracting organic constituents from aqueous substrates like water which reduces the time of analysis, reagents amount, simplifies the analytical steps, and efficient enough in terms of recovery percentage. Several methods have been developed all over the world to isolate pesticides from water samples, including liquid-liquid extraction (Lari et al. 2014; Mondal et al. 2018); solid-phase extraction (Tsochatzis et al. 2012; Maurya and Kumar 2013); solid-phase micro-extraction (Cerejeira et al. 2003); accelerated solvent extraction (Carabias-Martinez et al. 2005); microwave-assisted extraction (Papadakis et al. 2006); matrix solid-phase dispersion (Valsamaki et al. 2006); membrane extraction techniques (Lambropoulou and Albanis 2007); super critical fluid extraction (Abbas et al. 2008); column based or diversified solid-phase extraction (Rajendran et al. 2005; Li et al. 2016) and more recently the application of enzyme-linked immuno sorbent assays (Székács et al. 2015) and Quick, easy, cheap, effective, rugged and safe, QuEChERS (Yurtkuran and Saygı 2013; Trivedi et al. 2016) methods followed by analysis in various chromatographic instruments.

A survey of literatures indicates that large volume (250–1000 mL) of matrices has been taken for extraction of environmental water samples considering detection of analytes at trace level like ng/mL or less. It has also been suggested that water samples should be extracted using diverse combination of solvent mixtures with wide range of polarity to extract all the target analytes having different physico-chemical properties and polarities (Ukpebor 2015; Mondal et al. 2018). Therefore, traditional methods of extraction were found to be suitable for water samples and it is widely confined to liquid-liquid extraction and solid-phase extraction procedures using versatile combinations of solvent system.

In liquid-liquid extraction (LLE) method, extraction of analytes has been done by partitioning water sample with water immiscible solvent with formation of two layers whereas in solid-phase extraction (SPE) method water samples are filtered through a reverse phase cartridge column containing a solid stationary phase usually silica coated with organic polymer followed by elution with solvents as the mobile phase. The stepwise extraction of diversified classes of analytes from water sample using LLE and SPE has been illustrated by Majzik-Solymos et al. (2001) as follows.

Before going into any extraction processes, it is very necessary to filter water sample by passing through glass fiber filter paper (0.45 μm) that eliminates particulate and other suspended solid matter which may present in sample. Neutral and basic analytes can easily be removed from a clean aqueous media but special attention is given for extraction of acidic analytes where pH of the medium is adjusted within 1.8–2.0 by using buffering agents such as sulphuric acid solution or, 20% hydrochloric acid or, ascorbic acid solution. In liquid-liquid extraction (LLE), filtrated water sample is taken into a glass-separatory funnel containing saturated sodium chloride solution that produces a salt out effect, or emulsion. Then it is extracted with the solvent of interest (e.g., dichloromethane, ethyl acetate, hexane, acetone, diethyl ether, and methanol) or, combination of solvent mixtures (e.g., ethyl acetate: dichloromethane, dichloromethane: hexane, dichloromethane: methanol). The separated organic solvent phase is then collected through anhydrous sodium sulfate. Subsequently, the solvent is concentrated and sometimes exchanged into the other solvent of interest or, instrument amenable solvent at this stage. After concentrating of organic phases, clean-up is carried out with florisil or, silica or, alumina followed by derivatization with methyl or, pentafluoro benzyl bromide. Finally, the aliquot is filtered through membrane filter paper (0.2 μm) and transferred into vial for analysis in various chromatographic and spectrometric instruments. In case of solid-phase extraction (SPE), nonpolar reverse phase C_{18} (octadecylsilyl) cartridge column have been used for extraction. To avoid dryness of the cartridge column, it has firstly been solvated with methanol succeeded by sample aliquot. Afterward, the filtrated water sample has been loaded into the column with a definite flow rate. For complete extraction of acidic pesticides, the cartridge is seldom cleaned up with buffered solution (pH 2). The target analytes which are adsorbed on the cartridge column during sample loading, have been eluted later with wide polarity ranges of mobile phases starting from polar to semi-polar solvents (e.g., acetonitrile, dichloromethane, ethyl acetate, acetone, and methanol) or, combination of solvent mixtures (e.g., acetone: ethyl acetate, acetonitrile: methanol, and dichloromethane: methanol). Finally, the extracts are cleaned-up, derivatized, and filtered followed by analysis in instruments.

Overall, the advantage of SPE over LLE is that it requires less organic solvents to isolate a wide range of analytes from liquid matrices (Olsen et al. 1998). However, instrumental interferences have been reported due to the occurrence of phthalate esters in the commercial SPE cartridge containers which make SPE less apparently reliable than LLE (Awofolu and Fatoki 2003).

3.3.2 Instruments

In the early 70s, thin layer chromatography was the method of choice for qualitative detection due to the simplicity of development in two dimensions, specificity and the possibility of reaching low limits of detection for an acceptable budget often using fluorescence detection. After that, the only alternative with acceptable limits of detection was gas chromatography with various detecting devices such as electron capture detector (Erkmen et al. 2013; Trivedi et al. 2016); micro-cell electron capture detector (Yang et al. 2005; Raghuvanshi et al. 2014); flame photo-metric detector (Reddy and Reddy 2010; Modoodi et al. 2011); pulsated flame photo-metric detector (Allinson et al. 2015); nitrogen phosphorus detector (Allinson et al. 2015; Singare 2016); mass spectrometry, MS (Leong et al. 2007; Santhi and Mustafa 2013); ion-trap mass spectrometry (Smalling et al. 2013); and double mass spectrometry, MS/MS (Yuan et al. 2015; Mondal et al. 2018). A main advantage of MS detection is that identification of the analytes is based on molecular information. Therefore, the application of MS detection in combination with chromatographic system has spread rapidly (Székács et al. 2015).

Traditionally, non-polar to middle polar, volatile, and thermally stable pesticides were analyzed with gas chromatography whereas more polar, low volatile, and thermally labile pesticide classes are in most of the cases appraised with high-performance liquid chromatography with ultra-violate-visible (UV-VIS) detection (Chowdhury et al. 2012; Singh et al. 2015); diode-array detection (Cerejeira et al. 2003; Tsochatzis et al. 2012); mass spectrometry, MS (Ccanccapa et al. 2016) and double mass spectrometry, MS/MS (Meyer et al. 2011; Allinson et al. 2015) detection system. However, in terms of detection specificity and sensitivity, high resolution gas chromatography and high resolution mass spectrometry are found to be superior detection systems (Rajendran et al. 2005; Kim et al. 2007).

3.3.3 Standard Method

Various methods for analysis of pesticide residues in water have been standardized by different national and international organisations like Association of Official Analytical Chemists (AOAC), American Public Health Association (APHA), Food Safety and Standard Authority of India (FSSAI), and United States Environmental Protection Agency (USEPA). The origin of the standard method was promulgated by USEPA in 1984 for analysis of organochlorine pesticides and polychlorinated biphenyls in industrial and municipal wastewater as method 608. According to the method, 1 L of sample is extracted with dichloromethane using separatory funnel followed by analysis in gas chromatography coupled with electron capture detector (USEPA 1984).

USEPA provides general guidance for quantitative extraction or dilution of aqueous samples for analysis by one of the semi-volatile or non-volatile determinative methods like separatory funnel liquid-liquid extraction in method 3510, organic contaminants in water by microextraction in method 3511, continuous liquid-liquid extraction in method 3520, and solid-phase extraction in method 3535. The procedures for clean-up and chromatographic analysis of the resultant extracts are described in method 3600 and method 8000, respectively (USEPA 2019a). These test methods were widely accepted and practiced all over world (Abrantes et al. 2010; Singh et al. 2012; Ogbeide et al. 2015).

Other international and national agencies like AOAC, APHA and FSSAI have also adopted the USEPA method and modified accordingly (AOAC 1998; APHA 2012; FSSAI 2016). However, depending upon the target analytes and suitability, the necessary revision of the USEPA test methods have been made from time to time (USEPA 2019b). Based on these standard and published methods, a number of self-modified methods have been widely practiced and validated in most of the research laboratories for routine analysis of multiple pesticides in water sample.

3.3.4 Method Validation

Analytical procedures followed in different research laboratories should be validated for its intended use. The quality of an analytical method has been judged by the outcomes from method validation parameters. As suggested by Directorate-General for Health and Food Safety (DG-SANTE 2018), the parameters considered for method validation for residue analysis of multiclass pesticides in environmental matrices like water sample, includes the following criteria as described below.

3.3.4.1 Specificity

It is the study on the retention time and area of individual analyte or, mixture of analytes by injecting their standard solution into chromatographic-spectrometric instrument with six times at one concentration. Relative standard deviation of retention time and area for each analyte is calculated, the values of which should not exceed 2% and 5%, respectively.

3.3.4.2 Linearity

It is also known as calibration range within which the analyte response for a given concentration or amount is linear in nature in both pure solvent and matrix. Thus, calibration curves are prepared from the areas under the peak against each calibration standards. Therefore, a linear instrumental response will be obtained within that range when correlation coefficient (r^2) of the curve is at least 0.99.

3.3.4.3 Sensitivity

A method must be sensitive enough to detect analytes at lower level. It could be justified by determining limit of detection (LOD) and limit of quantification (LOQ). The LOD signifies the lowest level of detection of an analyte with confidence, but that may not be quantified. Whereas, LOQ is the lowest concentration in a sample that can be determined with acceptable accuracy and precision. LOD and LOQ have been determined as follows:

(i) $\mathrm{LOD} = 3.3\ (\alpha/S)$ and
(ii) $\mathrm{LOQ} = 10\ (\alpha/S)$

Where, α denotes for standard deviation of the response and S denotes the average slope of the calibration curve.

Currently it has been suggested that analysis of pesticide residue in environmental samples requires the method LOQ must be lower than the maximum concentration level or, maximum residue level established for a specific pesticide in a specific substrate. In case of water, method LOQ must be lower than the maximum acceptable concentration of 100 ng/L for individual pesticide and their transformation products, and 500 ng/L for total pesticides (EC 2016).

3.3.4.4 Accuracy

It is measured by performing recovery study in which the measured value of analyte in a sample corresponds to the true value. The recovery experiment is conducted by spiking water samples at three levels *i.e.,* limit of quantification (LOQ); LOQ × 5; and LOQ × 10 each with 6 replicates along with a non-spiked blank sample as control. The accepted limit of recovery percentage for multi residue method should be within 70–130% and relative standard deviation should not exceed 20%.

3.3.4.5 Precision

It is the closeness of the measurements obtained from multiple sampling from a homogeneous sample under a defined condition. The principal precision measures include repeatability relative standard deviation (RSD_r) and reproducibility relative standard deviation (RSD_R). Repeatability of a method is estimated within a laboratory in single day experiment by an individual analyst. It is performed by spiking water sample at limit of quantification (LOQ) × 5 in 3 replicates. Recovery percentage and relative standard deviation (RSD) is calculated on the same day, while, reproducibility is judged by the same experiment conducted by different analysts on different days. The recovery percentage should be within 70–130% and RSD should not exceed 15%.

3.3.4.6 Matrix Effect

Interference due to co-extractives from sample matrix was evaluated by extracting different volumes of water sample following solid-phase extraction procedure (Kuster et al. 2008). The variable behavior observed was termed as "interference factor" (*fi*) which was estimated for each pesticide in accordance with the following equations:

$$f_1 = \left[\left\{\mathrm{C}_{\mathrm{sample(spiked)}} - \mathrm{C}_{\mathrm{sample(non\text{-}spiked)}}\right\}/\mathrm{RC}_{\mathrm{aqueous(spiked)}}\right]$$

Where, $\mathrm{C}_{\mathrm{sample\ (spiked)}}$ is calculated concentration obtained from the analysis of water sample spiked with the analytes at certain concentration level like 500 ng/mL; $\mathrm{C}_{\mathrm{sample\ (non\text{-}spiked)}}$ is the calculated concentration in sample, and $\mathrm{RC}_{\mathrm{aqueous\ (spiked)}}$ is real calculated concentration obtained from the analysis of an aqueous standard solution with same spiking concentration level as in $\mathrm{C}_{\mathrm{sample\ (spiked)}}$ *i.e.,* 500 ng/mL.

Thus, a $fi = 1$ indicates no matrix interferences; fi more than one means enhanced response; and fi less than one indicates decreased response.

Therefore, the procedures selected by a laboratory for analysis of pesticide residues in water samples must be verified in terms of the parameters as described under Sect. 3.3.4.1, 3.3.4.2, 3.3.4.3, 3.3.4.5 and 3.3.4.6 for adoption.

3.4 Status of Pesticide Residues in Aquatic System

Occurrence of pesticide residues in aquatic system including surface and ground water, sediment, aquatic biota, and rain water of different countries across the world has been documented over the past few decades. The global scenario on pesticide contamination in aquatic environment is depicted in Table 3.1.

3.4.1 In Surface Water

Surface water comprising oceans, estuaries, rivers, canals, lakes, ponds, and other open water bodies covers about 71% of the Earth's surface. Till then 785 million people do not have the ensured drinking water supply including 144 million people who entirely depends on surface water (WHO 2019). The catchment areas of water reservoirs have been spread with growing agricultural crops due to suitability of agricultural activities like irrigation and drainage. The farmers are using pesticides for protection of crops from insects and other pests. This may become the point source of pesticides pollution in the adjacent surface water bodies. High concentration of pesticides malathion (2.610 ng/mL) and dieldrin (1.671 ng/mL) was detected in Ganga River water in Kanpur, India due to surface runoff from the intensive agricultural araes around the river banks (Sankararamakrishnan et al. 2005).

Anticipating similar reason, some others investigation have been conducted all over the world where a significant amount of agricultural pesticide residues were detected into the nearby open water bodies (Kuster et al. 2008; Pujeri et al. 2010; Meyer et al. 2011; Elfman et al. 2011; Oliver et al. 2012; Chowdhury et al. 2012). Apart from the detection of agricultural pesticides into surface water bodies, the presence of non-agricultural pesticides like organochlorines along with other agricultural pesticides was also observed due to the non-point sources of pollution (Cerejeira et al. 2003; Reddy and Reddy 2010; Mishra and Sharma 2011; Santhi and Mustafa 2013; Lari et al. 2014; Trivedi et al. 2016). The surface water samples collected from Hooghly River basin in India has also been reported to be contaminated with non-agricultural organochlorines and agricultural pesticides like atrazine, butachlor, chlorpyrifos, metalaxyl, methyl parathion, monocrotophos, and phorate in the range of 1.100–5.190 ng/mL and 0.003–0.168 ng/mL, respectively (Mondal et al. 2018). Thus, the co-occurrence of agricultural and non-agricultural

Table 3.1 Global scenario on pesticide contamination in aquatic system

Sl.	Aquatic samples–type and source	Pesticides detected	Concentration (ng/mL or, ng/g)	References
1.	Drinking water from hand pumps, tap and supply water, Haryana, India	T-HCHs, T-DDXs, and T-endosulfans	0.03–0.85	Kaushik et al. (2012)
2.	Drinking water from dug wells, Cocoa farms, Ghana	HCHs, 4,4′-DDT, and dieldrin	ND-0.05	Fosu-Mensah et al. (2016)
3.	River and municipal drinking water; San Francisco, Philippines	λ-Cyhalothrin, cypermethrin, deltamethrin	0.0005–0.094	Elfman et al. (2011)
4.	Surface and ground water, Kanpur, India	HCHs, dieldrin, and malathion	ND-2.61	Sankararamakrishnan et al. (2005)
5.	Surface and ground water, Karnataka, India	Endosulfan, chlorpyrifos, malathion, monocrotophos, and metalaxyl	ND-210	Pujeri et al. (2010)
6.	Surface and ground water, Assam, India	T-HCHs and T-DDXs	4.40–6.90	Mishra and Sharma (2011)
7.	Ground water, Lebanon	DDXs, HCB, aldrin, diazinon, and metalaxyl	$\leq$ 0.014	Kouzayha et al. (2012)
8.	Surface and ground water, Maharashtra, India	HCHs, endosulfans, dichlorovos, chlorpyrifos, phorate, and methyl parathion	ND-0.78	Lari et al. (2014)
9.	Surface and ground water, Portugal	Lindane, α-endosulfan, atrazine, chlorfenvinphos, molinate, and simazine	BDL-0.63	Cerejeira et al. (2003)
10.	Surface and ground water; Hungary	OCPs, OPPs and many other fungicides and herbicides	ND-56	Székács et al. (2015)
11.	Surface water, Hyderabad, India	HCHs, DDXs, endosulfans, heptachlors, aldrin, dicofol, chlorpyriphos, malathion, methyl parathion, ethion, and atrazine	ND-2.03	Reddy and Reddy (2010)
12.	Sharda River, Uttar Pradesh, India	HCHs, DDXs, heptachlor epoxide, and dieldrin	ND-0.28	Maurya and Kumar (2013)
13.	Surface water, Mt. LoftyRanges, Australia	Carbaryl, fenarimol, chlorpyrifos, azinphos methyl, bupirimate, penconazole, procymidone,	0.01–5.46	Oliver et al. (2012)

(continued)

Table 3.1 (continued)

Sl.	Aquatic samples–type and source	Pesticides detected	Concentration (ng/mL or, ng/g)	References
		propiconazole, and pirimicarb		
14.	Surface and irrigated water, Bangladesh	DDXs and heptachlor	ND-8.29	Chowdhury et al. (2013)
15.	River and waste water, Luxembourg	Herbicides	0.001–7.41	Meyer et al. (2011)
16.	River Kuano, Uttar Pradesh, India	HCHs and DDXs	ND-0.02	Singh and Mishra (2009)
17.	River Ghaggar, Haryana, India	T-HCHs and T-DDXs,	0.021–1	Kaushik et al. (2010)
18.	River Gangotri, Uttarakhand, India	T-HCHs, T-DDXs, T-endosulfans, T-aldrins, and T-heptachlors	ND-0.08	Mutiyar and Mittal, (2013)
19.	River Gomati, Lucknow, India	α-HCH, 4,4′-DDE, α-endosullfan,, heptachlor, alachlor, and dicofol	ND-135	Trivedi et al. (2016)
20.	River Selangor, Malaysia	Lindane, DDXs, endosulfans, heptachlor, dieldrin, chlorpyrifos, and diazinon	ND-1.85	Leong et al. (2007)
21.	Ebro River, Spain	Insecticides and herbicides of different chemical groups	ND-114.90	Kuster et al. (2008)
22.	River water, Mae Sa watershed, Thailand	Endosulfans, atrazine, dimethoate, chlorothalonil, chlorpyrifos, and cypermethrin	ND-9.70	Sangchan et al. (2012)
23.	Thailand Selangor River, Malyasia	Lindane, DDXs, HCB, chlordanes, mirex, and dieldrin	0.006–0.025	Santhi and Mustafa (2013)
24.	River Yamuna and canal water, Haryana-Delhi, India	T-HCHs, T-DDXs, and T-endosulfans	0.012–1.57	Kaushik et al. (2008)
25.	Lake and river water, Yunnan Plateau, China	4,4′-DDE, HCB and HCHs	ND-37.56	Yang et al. (2007)
26.	Vasai Creek water, Mumbai, India	HCBs, DDXs, endosulfans, aldrin, dieldrin, endrin, chlorpyrifos, and ethion	0.001–0.294	Singare (2016)

(continued)

Table 3.1 (continued)

Sl.	Aquatic samples–type and source	Pesticides detected	Concentration (ng/mL or, ng/g)	References
27.	Rain water, Haryana, India	HCHs, DDXs, and some other agricultural insecticides	0.02–7.06	Kumari et al. (2007)
28.	Agricultural water, Mysore, India	HCHs, DDXs, α-endosulfan, heptalchlor, aldrin, and dieldrin	ND-0.05	Modoodi et al. (2011)
29.	Agricultural water, Iran	4,4′-DDE and α-endosulfan	ND-0.0002	Modoodi (2013)
30.	Lakes and paddy field water, Rangpur, Bangladesh	Chlorpyrifos, carbofuran, and carbaryl	ND-3.395	Chowdhury et al. (2012)
31.	Paddy field and drainage canal water, Axios River, Greece	Penoxsulam, tricyclazole, 3,4-dichloroaniline, propanil, azoxystrobin, molinate, and profoxydim	BDL-24.30	Tsochatzis et al. (2012)
32.	Well water, Hyderabad, India	Lindane, DDXs, and endosulfans	0.15–10.96	Shukla et al. (2006)
33.	Shallow boreholes water, Triassic Sherwood Sandstone Aquifer, UK	Herbicides	0.03–0.58	Lapworth et al. (2006)
34.	Bore and dug well water, Gangetic plains, Unnao, India	T-HCHs, T-DDXs, T-endosulfans, T-heptachlors, T-chlordanes, aldrin, dieldrin, endrin, and methoxychlor	BDL-0.21	Singh et al. (2005)
35.	Open and bore well water, Tamil Nadu, India	HCHs, DDXs, and endosulfans	ND-19.20	Jayashree and Vasudevan (2007)
36.	Ganga river water and sediment, Bhagalpur, India	Lindane, DDXs, endosulfans, and methyl parathion	ND-3329.30	Singh et al. (2012)
37.	Ganga river water and sediment, Allahabad, India	HCHs, DDXs, and aldrin	ND-24.50	Raghuvanshi et al. (2014)
38.	Hooghly river water, sediment, pond, tube well water, West Bengal, India	T-HCHs, T-DDXs, T-endosulfans, chlorpyrifos, methyl parathion, monocrotophos, phorate, atrazine, butachlor, and metalaxyl	ND-4.13	Mondal et al. (2018)

(continued)

Table 3.1 (continued)

Sl.	Aquatic samples–type and source	Pesticides detected	Concentration (ng/mL or, ng/g)	References
39.	Ebro river water and sediment, Spain	Lindane, DDXs, PCBs, HCBs, atrazine, simazine, and molinate	ND- 0.17	Go'mez-Gutie'rrez et al. (2006)
40.	Jukskei river water and sediment, South Africa	HCHs, DDXs, endosulfans, heptachlor, and endrin	0.27–22,914	Sibali et al. (2008)
41.	Jinjiang river water and sediment, China	HCHs and DDXs	ND-25.95	Yang et al. (2013)
42.	Guadalquivir river water, sediment and biota, Spain	OPPs, imazalil, carbendazim, pyriproxyfen, imidacloprid, buprofezin, hexythiazox, triazines, and diuron	0.0002–13.2 (mean)	Masiá et al. (2013)
43.	Songhua river water, sediment and fish, China	T-HCHs and T-DDXs	0.039–0.34	Cai et al. (2014)
44.	Owan river water, sediment and fish, Nigeria	HCHs, DDXs, endosulfans, heptachlors, aldrin, dieldrin, endrin, phosphor-methyl-glycine, atrazine, and carbofuran	0.01–2.34 (mean)	Ogbeide et al. (2015)
45.	Warri river water, sediment and fish, Nigeria	Paraquat dichloride	0.02–1087	Ikpesu (2015)
46.	Wetland water and fish, Kolkata, India	OCPs	0.02–8.90	Nag et al. (2016)
47.	Ebro river water, sediment and biota, Spain	OPPs, carbamates, triazines, azol, benzimidazole, ureas, hexythiazox, buprofezin, tebuconazole, and imidacloprid	ND-0.41	Ccanccapa et al. (2016)
48.	Streams, ponds, canals water and sediment, Northern Indo-Gangetic plains, Unnao, India	T-HCHs, T-DDXs, T-endosulfans, T-chlordanes, T-heptachlors, aldrin, dieldrin, endrin, methoxychlor, and HCB	BDL-74.06	Singh et al. (2007)
49.	Urban wetlands water and sediment, Melbourne, Australia	Insecticides, herbicides, and fungicides	0.001–59	Allinson et al. (2015)

(continued)

Table 3.1 (continued)

Sl.	Aquatic samples–type and source	Pesticides detected	Concentration (ng/mL or, ng/g)	References
50.	Zha Long wetland sediment, China	T-HCHs, T-DDXs, endosulfans, aldrin, dieldrin, endrins, and methoxychlor	ND-483.04	Liu et al. (2012)
51.	Inland wetland fish, Karnataka, India	T-HCHs, T-DDXs, T-endosulfans, and dieldrin	BDL-51.7	Dhananjayan and Muralidharan, (2010)
52.	Anzali wetland water and sediment, Iran	T-HCHs, T-DDXs, and T-PCBs	ND-180.81	Javedankherad et al. (2013)
53.	Surface and ground water, sediment, and fish, Lake Vela, Portugal	HCB, HCHs, DDXs, endosulfans, heptachlor, aldrin, dieldrin, endrin, methoxychlor, alachlor, and glyphosate	00.02–20.40	Abrantes et al. (2010)
54.	Agboyi creek water and sediment, Lagos	HCHs, heptachlors, chlordanes, endosulfans, aldrin, dieldrin, endrin, and methoxychlor	ND-691	Williams (2013)
55.	Karabogˇaz Lake water and sediment, Turkey	Malathion, etofenprox, molinate, oxamyl, propamocarb, tebufenozide, linuron, and piperonyl butoxide	ND-385	Yurtkuran and Saygı (2013)
56.	Manyas lake water and sediment, Turkey	T-HCHs, T-DDXs, T-heptachlors, aldrin, dieldrin, and endrin	ND-17.17	Erkmen et al. (2013)
57.	Lake sediment and aquatic species, Nokoué and Cotonou Lagoon, Republic of Bénin	γ-HCH, DDXs, α-endosulfan, aldrin, and dieldrin	BDL-289	Pazou et al. (2014)
58.	Suspended particulate material, Lake Chaohu, China	HCHs, DDXs, α-endosulfan, HCB, heptachlor, aldrin, and isodrin	0.11–20.37	He et al. (2015)
59.	Seawater and sediment, Bay of Bengal, India	T-HCHs, T-DDXs, and T-PCBs	0.251–6.570	Rajendran et al. (2005)
60.	Estuary water, sediment, fish and sand crabs, Central California, USA	DDXs and many other agricultural pesticides	0.002–14	Smalling et al. (2013)

(continued)

Table 3.1 (continued)

Sl.	Aquatic samples–type and source	Pesticides detected	Concentration (ng/mL or, ng/g)	References
61.	Water and suspended particulate matter, Hangzhou Bay, East China Sea, China	T-HCHs and T-DDXs	0.001–0.029	Li et al. (2016)
62.	Sediment and fish, East and West coasts, India	HCHs and DDXs	BDL-721.88	Pandit et al. (2001)
63.	Sediment, fish and aquatic sp., coastal marine environment, Mumbai, India	T-HCHs and T-DDXs	ND- 34.10	Pandit et al. (2006)
64.	Sediment, Keoladeo National Park, Rajasthan, India	HCHs, DDXs, endosulfans, aldrin, dieldrin, endrin, and heptachlors	ND-5.558 (mean)	Bhadouria et al. (2012)
65.	Sediment, Bohai and Yellow Sea, China	PCBs, PAHs, and DDXs	ND-5734.20	Ma et al. (2001)
66.	Sediment, Black Sea, Danube delta, Europe	PCBs, HCHs, DDXs, endosulfans, aldrin, dieldrin, and endrin	0.06–72	Fillmann et al. (2002)
67.	Sediment, East China Sea, China	T-HCHs and T-DDXs	0.05–10.94	Yang et al. (2005)
68.	Sediment, Ariake Bay, Japan	T-HCHs, T-DDXs, T-heptachlors, T-Chlordanes, HCB, aldrin, dieldrin, endrin, and mirex	ND-0.95	Kim et al. (2007)
69.	Sediment, Daya Bay, South China	Ethoxychlor	0.01–34.34	Wang et al. (2008)
70.	Sediment, East Coastal Sea, China	HCHs, DDXs, and HCB,	0.14–4.96	Lin et al. (2012)
71.	Sediment, Shuangtaizi Estuary, China	DDXs and HCHs, and PCBs	0.02–36.68	Yuan et al. (2015)

Notation: BDL below detection limit, *ND* not detected, *DDXs* (4,4′ + 2,4′)-isomers of dichlorodiphenyltrichloroethane (DDT) + its metabolites, *HCHs* (α + β + γ + δ)-isomers of hexachlorocyclohexane (HCH), *HCBs* hexachlorobenzene analogues, *Chlordanes* (*cis* + *trans*)-chlordane, *Endosulfans* (α + β)-isomer of endosulfan + its metabolites, *Endrins* endrin + its metabolites, *Heptachlors* heptachlor + heptachlor epoxide, *OCPs* Organochlorine pesticides, *OPPs* Organophosphorous pesticides, *PAHs* polycyclic aromatic hydrocarbon analogues, *PCBs* polychlorinated biphenyl analogues, *T* Total of detected isomers + metabolites

pesticides from point and diffused sources appears to have major toxicological impact on aquatic organisms, recreational uses, and on human health, if considered for drinking.

3.4.2 In Ground Water

Surface water contamination by pesticides is generally predominant during agricultural seasons on short-term basis, while for ground water bodies it is a prolonged environmental process that continuously impedes the drinking water quality (Cerejeira et al. 2003). Soils may act as pesticides reservoir from which they can easily move to the ground water and the situation is especially facilitated in well-drained loamy soil during heavy rains. Consequently, many pesticides like lindane (0.900 ng/mL), malathion (29.835 ng/mL), and dieldrin (16.227 ng/mL) have been reported to occur in some of the ground water samples in Kanpur, India (Sankararamakrishnan et al. 2005). Furthermore, higher levels of organochlorine pesticides contamination in ground water was attributed to the non-turbulent flows resulting inadequate dilutions and degradation as compared to surface water (Kaushik et al. 2012). Frequent detection of α-isomer of hexachlorocyclohexane (0.39 ng/mL), α-endosulphan (0.78 ng/mL), chlorpyrifos (0.25 ng/mL), and methyl parathion (0.09 ng/mL) were noticed in groundwater from eastern regions of Maharashtra, India (Lari et al. 2014).

Gooddy et al. (2005) identified five potential pesticide leachers *viz.,* atrazine, bentazone, clopyralid, isoproturon, and mecoprop which were frequently detected in ground water. As systemic pesticides are directly applied on the soil surface, or sub-surface zone, they have higher tendency to leach and contaminate ground water bodies. For this reason, chlorpyrifos, a systemic insecticide, was detected in ground water samples including tube well (0.247–35 ng/mL) in India and in drinking water sources (0.02–0.06 ng/mL) in Ghana (Pujeri et al. 2010; Fosu-Mensah et al. 2016; Mondal et al. 2018). Likewise, metalaxyl, a systemic fungicide (0.014 ng/mL) and butachlor, a systemic herbicide (0.060 ng/mL) was also found in the ground water samples from Terbol, Lebanon (Kouzayha et al. 2012) and Hooghly River basin, India (Mondal et al. 2018), respectively.

Therefore, soil-aquifer dynamic system mostly influenced by rainfall induced groundwater recharge appears to control the mobility of pesticides through soil profile, contaminating ground water bodies and thereby threatening drinking water quality (Lapworth et al. 2006).

3.4.3 In Sediment

The sediments can act as a main sink for pesticides in rivers and lakes. The adsorbed pollutants like pesticides on the sediment particles might be diluted by erosion of river bed sediments, runoff and re-suspension. Sediment associated microbial activity is adversely affected by the deposition of pesticide residues in sediment over time leading to generation of partially decomposed organic matter accompanied by some secondary toxic compounds (such as carbon dioxide, hydrogen sulphide, and methane) which affect both aquatic biota and environment (Amaraneni 2002). Hence, several researchers have declared sediment samples for analysis along with the water samples (Go'mez-Gutie'rrez et al. 2006; Sibali et al. 2008; Singh et al. 2012; Raghuvanshi et al. 2014).

The existence of organochlorines and some other agricultural pesticides in sediment samples was recorded in the range of 0.66–2.14 ng/g in Owan River, Nigeria (Ogbeide et al. 2015). Ccanccapa et al. (2016) evidenced most frequent contamination of sediment samples of Ebro River, Spain with chlorpyrifos, diazinon, and diclofenthion. Moreover, a recent inspection by Mondal et al. (2018) also stipulated the occurrences of total pesticides (mainly by organochlorines with 1.25 ng/g) in Hoogly River, India. Consequently, the normal functioning of aquatic ecology is adversely affected by pesticide polluted sediment particles followed by long range transport.

3.4.4 In Aquatic Biota

Pesticides may directly enter the fresh water resources from point source of application like spray drift, or indirectly through surface runoff, or sub-surface drain flow. Pesticides are basically biocides with toxicological impact on aquatic flora and fauna. Currently, some of the agricultural lands are being converted into aquaculture farm or integrated with fish farming. The intensive use of pesticides in aquaculture farm to control pests, diseases, and ecto-parasites appears to be the major reason of contamination in water and sediment. These pesticides are finally getting accumulated into aquatic organisms, hampering aquatic life.

Among the organochlorine group of pesticides, hexachlorocyclohexane and dichlorodiphenyltrichloroethane shows negative temperature co-efficient action with degree of poisoning but positive relationship with degradation. Therefore, at higher temperature the pesticides produce lower toxicity due to faster degradation, and vice versa. This relates to the lower accumulation of organochlorine pesticides in fish from tropical countries like India rather than those in temperate countries due to their quick volatilization and transformation in the tropical environment (Pandit et al. 2001). Additionally, the half-lives of the semi volatile pesticides in the biological system are short at high temperature which accelerates the rate of elimination of pesticides in fish around tropic regions.

A systemic study by Nag et al. (2016) documented the accumulation of organochlorine pesticides (1.23–8.9 ng/g) in fish (*Cyprinus carpio*) from East Kolkata Wetland, India. Although, organochlorine pesticides are hydrophobic in nature but shows relatively lower lipophilicity (depending upon temperature co-efficient) to the aquatic organisms, whereas pyrethroids and other chlorinated group of pesticides showed higher toxic effects to aquatic species. Pyrethroids have higher hydrophobicity as well as lipophilicity that facilitate their entry into the biological system of aquatic biota (Elfman et al. 2011). Wijngaarden et al. (2005) also concluded that pyrethroids have greater magnitude of toxicity to aquatic organisms than organophosphorus insecticides. Due to this reason bifenthrin and cyfluthrin were detected upto 218 ng/g level in 25–100% samples of fish and sandcrabs in a central California estuary (Smalling et al. 2013). Further bio-magnification of these higher levels of pesticide residues in the food chain may be a great concern for bio-safety.

3.4.5 In Rain Water

Rainfall is an efficient mechanism for cleansing the atmosphere through the processes of rainout and washout. The hydrologic cycle in the atmosphere facilitates the circulation of pesticides into different components of the environment during and after their application. Pesticides can become airborne from the applied surface through volatilization and wind erosion followed by deposition in unintended areas by wet (e.g., rain and snow) and dry (e.g., particle settling) depositional processes (Bidleman et al. 2006). Transportation of pesticides through rainwater directly affects the surface water quality particularly during surface runoff events (Majewski et al. 2014).

Pesticides with higher Henry's law constant and vapor pressure are likely to be dissipated rapidly into the atmosphere in tropical climate with lower residues in soil and water (Kaushik et al. 2008). Consequently, the presence of organochlorines (e.g., hexachlorocyclohexanes and dichlorodiphenyltrichloroethanes, endosulfans, and heptachlor), organophosphorus (e.g., monocrotophos, chlorpyriphos, methyl parathion, malathion, and triazophos), and synthetic pyrethroids (e.g., cypermethrin, fenvalerate, and deltamethrin) pesticides in rain water was detected in Hisar, Haryana, India (Kumari et al. 2007). The pesticides were vaporized after spray application into the cotton field followed by their dissolution in rain water. The occurrence of pesticide in the rain water has been regularly monitored and reviewed by United State Geological Survey (Vogel et al. 2008).

Therefore, the issue regarding the occurrence of pesticide residues in aquatic system needs continuous monitoring on long term basis for assessment of eco-toxicological implication and to suggest options for management.

3.5 Reasons for Occurrence of Organochlorine Pesticides in Water Bodies

Although worldwide restriction was imposed on the use of persistent dirty-dozen organochlorine pesticides *viz.,* 6 isomers of dichlorodiphenyltrichloroethane, 4 isomers of hexachlorocyclohexane, and 2 isomers of endosulfan, the trend of residues in water samples of developing countries indicated that maximum pollution in water was contributed mainly by organochlorines rather than other group of pesticides (Table 3.1). To ensure food and environmental safety, the use of dichlorodiphenyltrichloroethane in agricultural sectors and hexachlorocyclohexane in both agricultural and for vector control programme have been restricted since 1993 and 1997, respectively (UNEP 2003; NVBDCP 2016; DPPQS 2019). Afterwards, the production, use, and sale of endosulfan have also been forbidden in India since 2011 (DPPQS 2019). Despite the strict regulation, the usages of these pesticides have still been witnessed in different parts of the country which plays a pivotal role on distribution of persistent organic pollutants across the world (UNEP 2003; Yadav et al. 2015). The occurrence of organochlorine pesticides in aquatic system could be justified by the following reasons.

3.5.1 Old Sources

Until the official ban on agricultural uses of hexachlorocyclohexane, its two forms *viz.,* technical hexachlorocyclohexane which is a mixture of α, β, γ, and δ- isomers and lindane which contain the γ-isomer only were popularly used for almost forty years. Insecticidal importance was given to commercial dichlorodiphenyltrichloroethane which contains 77% of 4,4′-dichlorodiphenyltrichloroethane as active ingredient and 15% of dichlorodiphenyldichloroethylene and dichlorodiphenyldichloroethane as make up the balance impurities. Similarly, commercial form of endosulfan is composed of two stereoisomers *viz.,* α and β in 70:30 ratio. The longer persistence of high concentration of α and β isomeric forms of endosulfan in river water up to 0.739 ng/mL and sediment up to 0.050 ng/mL was explained by the nature of the conformational isomers α- and β-endosulfan interconnected with inseparable bonds (Singh et al. 2012). The residues of parent molecules might also be present in soil due to historical uses and became an old source of pesticide pollution by degrading naturally into other stable isomeric products with due course of time, manifested with agricultural or seasonal runoff to hydro-system (Kaushik et al. 2008).

3.5.2 Isomeric Stability

If there is no fresh input, the composition of the parent pesticidal compound will be progressively decreased, while the formation of the transformed products will be slowly increased due to environmental breakdown with time. The predominance of β- and γ-hexachlorocyclohexane and 4,4′-dichlorodiphenyltrichloroethane in relatively higher proportions in comparison to other isomers of the respective pesticides has been documented (Kumari et al. 2007; Modoodi et al. 2011; Maurya and Kumar 2013; Raghuvanshi et al. 2014; Mondal et al. 2018). It may be due to the fact that these isomers may be recalcitrant in nature showing resistance towards microbial transformation (Erkmen et al. 2013). In case of dichlorodiphenyltrichloroethanes, 4,4′-dichlorodiphenyltrichloroethane undergoes slow degradation or metabolite conversion through oxidative dehydrochlorination and reductive dechlorination to produce stable and toxic metabolites *viz.,* 4,4′-dichlorodiphenyldichloroethylene and 4,4′-dichlorodiphenyldichloroethane under aerobic and anaerobic conditions, accordingly, with half-life of 2–15 years (Kim et al. 2007).

The transformation of commercial hexachlorocyclohexane (*i.e.,* γ-hexachlorocyclohexane) to most stable isomer *i.e.,* β-hexachlorocyclohexane could have been caused by ultra-radiation in water (Kumari et al. 2007). Wang et al. (2007) considered β-hexachlorocyclohexane as the least reactive and most stable isomer among hexachlorocyclohexanes. β-isomer of endosulfan has longer half-life of about 150 days under neutral conditions compared to α-isomer with a half-life of 35–50 days in soil and water (Singh et al. 2012). Aldrin may be transformed by various environmental processes like photolysis and microbial degradation to its isomeric product dieldrin which is more persistent in soil and water (Chowdhury et al. 2013).

3.5.3 Sediment as Sink

Unlike other pesticide molecules, the organochlorine pesticides due to their hydrophobic characteristics have higher affinity for suspended particulate matters present in aquatic system. It has been hypothesized that the organochlorine pesticides in the aquatic system preferably bind to the particulate phase *i.e.,* sediment via sedimentation processes and then re-suspended into aqueous phase through adsorption and desorption processes (He et al. 2015). Due to poor water solubility and slow degradation of dichlorodiphenyltrichloroethane in sediment and soil (75–100% in 4–30 years), the level of occurrence of total dichlorodiphenyltrichloroethane was much higher in aquatic sediment (up to 4203.4 ng/g) than water (up to 489 ng/L) samples of river Ganga in India (Singh et al. 2012). The measured concentration of organochlorine pesticides in water samples (1.43–8.60 ng/mL) was also lower than that in sediment samples (17.05–39.14 ng/g) in lake Manyas, Turkey (Erkmen et al. 2013).

3.5.4 Production and Disposal

Organizations still engaged in production of these persistent molecules are usually under direct Government supervision for non-agricultural use as well as for export purposes and dispose the generated wastes in unprotected area. As per the Stockholm Convention, a maximum amount of 10,000 tonnes of dichlorodiphenyltrichloroethane each year is permitted until a suitable alternative is prescribed to control the vector borne diseases usually predominant during monsoonal periods (Chakarborty et al. 2010). The amount of contaminant discharges into the Western Mediterranean Sea as Ebro River inputs was estimated to be 167 kg/

year of organochlorine compounds and 1258 kg/year of polar pesticides and became the main source of pesticide pollution (Go'mez-Gutie'rrez et al. 2006).

Production of lindane *i.e.,* γ-isomer of hexachlorocyclohexane also generates its toxic isomers α, β and δ as by-product and the wastes are discarded in the environment (Prakash et al. 2004; Kaushik et al. 2008). Such exposure into the environment may also originate from waste disposal sites, landfills and contaminated sites as well (Lari et al. 2014). For instance, the fungicide hexachlorobenzene which has never been registered in Malaysia was frequently detected at low levels in aquatic samples around Selangor River basin, Malaysia (Santhi and Mustafa 2013). In fact, it has been generated as a by-product resulting from production of chlorinated solvents required for manufacturing various chlorinated pesticides and also from improper waste treatment technologies followed by their direct discharge into the open stream.

3.5.5 Public Health Programme

Due to lack of suitable alternatives, dichlorodiphenyltrichloroethane is still used for public health and hygiene purposes like National Malaria Eradication Programme in India (NVBDCP 2016) and many other countries with high rainfall (2000–3000 mm), humidity (60–90%) and warmer climates (22–33 °C). The use of dichlorodiphenyltrichloroethane in disease vector control programme is continued in approximately 14 countries including India, and many other countries are preparing to reintroduce dichlorodiphenyltrichloroethane to combat malaria mainly because of their cheapness and effectiveness (Van den Berg 2009). Consequently, the increased concentration of dichlorodiphenyltrichloroethane was evidenced in river Ganga, India due to discharge of local hospital wastes into the open water stream. These hospitals are using dichlorodiphenyltrichloroethane for public health activities (Singh et al. 2012). Another study reported, a significant amount of total dichlorodiphenyltrichloroethanes (5.402–6.904 ng/mL) and hexachlorocyclohexane (4.403–5.574 ng/mL) residues in various water resources of Assam, India due to the continued use of dichlorodiphenyltrichloroethane in malaria eradication programme for more than 30 years (Mishra and Sharma 2011).

In this aspect, the degradate fraction can be used as an indicator of increasing or decreasing inputs of persistent organochlorines into the environment relative to their parent molecules. It also gives an idea on the nature and types as well as sources of the metabolites presence in the study area. This can be computed as follows:

$$\text{Degradate fraction } (\mathrm{D_f}) = \frac{\sum(\text{All degradates})}{\text{Parent compound}}$$

In the situation where data are available for a parent pesticide and its degradates and assuming parent pesticide as unity, the degradate fraction, $\mathrm{D_f}$ more than 1 indicates long-term degradation from historical accumulation as seen in lake Manyas, Turkey (Erkmen et al. 2013). Conversely, D_f less than 1 indicates short-term degradation which may results from current uses like National Malaria Eradication Programme as observed in the river basin of Hooghly in India (Mondal et al. 2018). For instance, dichlorodiphenyltrichloroethane can be degraded to its biological metabolites 4,4′-dichlorodiphenyldichloroethylene and 4,4′-dichlorodiphenyldichloroethane. The ratio of (4,4′-dichlorodiphenyldichloroethylene +4,4′-dichlorodiphenyldichloroethane)/ 4,4′-dichlorodiphenyltrichloroethane could be considered as an indicative index of the extent of dichlorodiphenyltrichloroethane release into the environment (Kaushik et al. 2010; Erkmen et al. 2013; Mondal et al. 2018). Likewise, the ratio of β-isomer of hexachlorocyclohexane to total hexachlorocyclohexanes and α-hexachlorocyclohexane to γ-hexachlorocyclohexane were considered as an indicative index of hexachlorocyclohexane pollution in aquatic system (Lin et al. 2012; Yuan et al. 2015; Li et al. 2016).

An attempt has, therefore, been made to replace these persistent organochlorine pesticides by organophosphorous pesticides for crop protection (Leong et al. 2007; Lari et al. 2014) and recently substituted by eco-friendly new molecules such as neonicotinoids (Reddy and Reddy 2010; Saha et al. 2017).

3.6 Aquatic Ecological Risk Evaluation

Residues of pesticides following their application end up into food and environmental components including water bodies. Contaminated surface and ground water directly affects non-target and beneficial organisms in aquatic and terrestrial environment. While the share of pollution from point sources appears to be decreasing due to several years of control measures, but pollution from non-point sources originating from agriculture and urban discharges is a matter of great concern for deteriorating water quality (Agrawal et al. 2010). Moreover, pesticides reach to the aquatic environment in different magnitude and in combination with various other aquatic components which makes the toxicological assessment more complicated (Cruzeiro et al. 2015). The potential risk on aquatic health is assessed through two approaches: (a) Establishment of standard water quality guideline, and (b) Prediction of risk to aquatic biota. The detail of aquatic ecological risk evaluation process is discussed below.

3.6.1 Standard Water Quality Guidelines

The degree of ecological risk associated with aquatic health containing pesticide residue levels at or below the referred guideline limits in water for drinking is uncertain. Water quality guidelines for pesticide residues also exist for the intended uses and the values should be specific for the purpose. The primary purpose of theses guideline values is not only the protection of user safety but also to protect at least 95% of aquatic species (Guy et al. 2011).

3.6.1.1 International Standards

The World Health Organization internationally publishes guidelines value for safe limits for pesticides in water. Quality guidelines are also established by other regulatory authorities of different countries *viz.,* Australia, Canada, European Union, Japan, New Zealand, Taiwan, and United States. The regional standard limits are often termed as health value, maximum acceptable value, and maximum acceptable concentration which are also almost same as guidelines value of World Health Organization. The values are revised and updated time to time. The method for fixation and the limits differ considerably from one zone to another. Some examples of those limits are provided in Table 3.2. Most commonly developed limits are for drinking water, but the standards values for ambient water, agricultural irrigation, animal husbandry, and effluent waters have also been proposed.

3.6.1.2 Indian Standards

The first version of Indian Standard *viz.,* IS 10500:1991 was formulated for pesticides residues, dissolved solids and nitrate in drinking water based on the internationally available specifications for drinking water (BIS 2003). As per the European Economic Commission (EEC), the standard guideline limit for sum of pesticides in surface water is 1.0 ng/mL. The concentration of total pesticides in drinking water should be within 0.5 ng/mL and the individual pesticide should be within 0.1 ng/mL. Bureau of Indian Standards (BIS) independently accepted EEC guidelines of pesticide in India in respect of drinking water (Sankararamakrishnan et al. 2005; Raghuvanshi et al. 2014; Mondal et al. 2018). Later on, the former version IS 10500:1991 was revised to IS 10500:2012 with the assistances of European Union Directives (80/778/EEC and 98/83/EEC), United States Environmental Protection Agency standard, World Health Organization guideline, and the Manual on Water Supply and Treatment by Government of India (BIS 2012).

In view of the above, various researchers across the world (Cerejeira et al. 2003; Go'mez-Gutie'rrez et al. 2006; Yang et al. 2007; Meyer et al. 2011; Chowdhury et al. 2012; Székács et al. 2015) including India (Prakash et al. 2004; Singh et al. 2007; Reddy and Reddy 2010; Bhadouria et al. 2012; Mutiyar and Mittal 2013; Singare 2016) assessed the risk due to the detected pesticides in the aquatic system in respect of their adopted standard water quality guidelines. Consequently, some of the pesticidal chemicals which failed to comply with the guidelines have been banned, restricted or kept on watch list in some countries. In a head-to-head comparison between different countries, Brazil ranks first on banning of the suspected 81 numbers of toxic active ingredients of pesticides followed by Saudi Arabia (71 no.), European Union (60 no.), United Kingdom (60 no.), Cambodia (58 no.) and India

Table 3.2 Different standard guideline limits (in ng/mL) for some pesticide residues in water

Sl.	Pesticides	WHO (GV)	USEPA (MCL)			New Zealand (MAV)	Japan		Australia		Canada (MAC)			
			Drinking water	Acute	Chronic		For public use	Effluent from paddy field	GV	HV	Drinking water	Irrigation	Livestock	Fresh water
1.	Acephate	–	–	–	–	–	–	800.00	3.00	10.00	90.00	–	–	–
2.	Atrazine	2.00	3.00	–	–	2.00	–	–	0.50	20.00	5.00	10.00	5.00	1.80
3.	Buprofezin	–	–	–	–	–	10.00	100.00	–	–	–	–	–	–
4.	Butachlor	–	–	–	–	–	–	300.00	–	–	–	–	–	–
5.	Carbaryl	–	–	–	–	–	4.00	–	5.00	30.00	–	–	1100.00	0.20
6.	Carbofuran	7.00	40.00	–	–	8.00	–	–	5.00	10.00	90.00	–	–	–
7.	Chlorpyrifos	30.00	–	0.083	0.041	70.00	30.00	8.00	–	–	90.00	–	24.00	0.0035
8.	Chromafenozide	–	–	–	–	–	–	7000.00	–	–	–	–	–	–
9.	DDXs	2.00	–	1.10	0.001	2.00	–	–	0.06	20.00	–	–	–	–
10.	Dicofol	–	–	–	–	–	–	–	–	3.00	–	–	–	–
11.	Dimethoate	6.00	–	–	–	–	–	–	–	50.00	20.00	–	3.00	6.20
12.	Endosulfans	–	–	0.22	0.56	–	–	–	0.05	30.00	–	–	–	0.02
13.	Imidacloprid	–	–	–	–	–	200.00	2000.00	–	–	–	–	–	–
14.	Isoprothiolane	–	–	–	–	–	40.00	400.00	–	–	–	–	–	–
15.	Lindane	2.00	0.20	2.00	0.08	–	–	–	0.05	20.00	–	–	4.00	0.01
16.	Malathion	–	–	–	0.10	–	10.00	100.00	–	50.00	190.00	–	–	–
17.	Metalaxyl	–	–	–	–	100.00	–	500.00	–	–	–	–	–	–
18.	Methomyl	–	–	–	–	–	–		5.00	30.00	–	–	–	–
19.	Monocrotophos	–	–	–	–	–	–	20.00	–	1.00	–	–	–	–
20.	Methyl parathion	–	–	0.065	0.013	–	–	–	0.30	100.00	50.00	–	–	–
21.	Pendimethalin	20.00	–	–	–	20.00	100.00	–	–	300.00	–	–	–	–

(continued)

Table 3.2 (continued)

Sl.	Pesticides	WHO (GV)	USEPA (MCL)			New Zealand (MAV)	Japan		Australia		Canada (MAC)			
			Drinking water	Acute	Chronic		For public use	Effluent from paddy field	GV	HV	Drinking water	Irrigation	Livestock	Fresh water
22.	Phorate	–	–	–	–	–	–	–	–	–	2.00	–	–	–
23.	Pretilachlor	–	–	–	–	–	40.00	400.00	–	–	–	–	–	–
24.	Propargite	–	–	–	–	–	–	–	–	50.00	–	–	–	–

Notation: GV guideline value, *MAV* maximum acceptable value, *MCL* maximum concentration level, *HV* health value, *MAC* maximum acceptable concentration, ‘-’ guideline values have not yet been fixed
Source: Hamilton et al. (2003)

(53 no.), while Swaziland and United States of Americal (both 1 no.) ranks last in this banning hierarchy (PAN 2019). Maximum banning was imposed on organochlorine group of pesticides, especially chlordane by 141 countries, dichlorodiphenyltrichloroethanes by 135 countries and hexachlorocyclohexanes by 132 countries (PAN 2019).

3.6.2 Presumption of Ecological Risk and Hazards

Ecological risk assessment due to pesticides is important for assessment of the potential ecological impact on non-target aquatic organisms such as fish, invertebrates, flora and fauna. This may indirectly act as an indicator of potential impacts on human health (Qian et al. 2006). Ecological risk due to pesticides may be assessed using risk assessment models and also using multiple realistic approaches (Ogbeide et al. 2015; Mansano et al. 2016; Thomaidi et al. 2017; Mondal et al. 2018) which are usually based upon the data from comprehensive monitoring, pesticide use pattern, fate and transport, toxicological value and exposure (USEPA, 2019c). Risk imposed by the detected pesticides in the monitored water samples can be calculated through various approaches as described below.

3.6.2.1 Water, Sediment and Biota

Abrantes et al. (2010) recommended a simplest deterministic ecological risk assessment to assess the risk imposed by diffuse pesticide pollution. Risk quotient (RQ) for each toxic chemical present in surface water and sediment was computed using the following equation.

$$\mathrm{RQ} = (\mathrm{MC}/\mathrm{LC}_{50}) \times \mathrm{AF}$$

Where, MC = measured (or estimated) concentration (ng/mL or, ng/g) indicating the highest exposure level of the chemical; LC_{50} = the lowest lethal concentration (ng/mL) of the chemical (to kill 50% of the aquatic organisms) reported in published literature or data base; and AF = assessment factor to compensate the experimental uncertainty that arises during extrapolation of laboratory derived toxicological data (obtained in limited number of aquatic test organisms) applied to the actual environmental conditions (Sangchan et al. 2012). At least one such short term toxicological evaluation from each of the three aquatic trophic levels *viz.,* algae, daphnids, and fish should be considered. AF value of 1000 may be considered as per European Commission (EC 2003).

The risk quotient (RQ) for the detected pesticides in the aquatic system of Karaboğaz Lake, Turkey was also assessed using the following equation proposed by Yurtkuran and Saygı (2013).

$$\mathrm{RQ} = \mathrm{PEC}/\mathrm{PNEC}$$

Where, PEC = predicted environmental concentration (ng/mL); and PNEC = predicted no effect concentration (ng/mL). The highest experimental value on pesticide residue obtained in water was used as PEC. Whereas, no observed effect concentration values for the most sensitive species were taken as PNEC. The comprehensive database ‘FOOTPRINT’ on pesticide was used for fish, algae and particularly invertebrates. Toxicological data for each trophic level (algae, crustaceans and fish) could also be obtained from Pub Chem Chemistry databases and Pesticide Properties Data Base (Cruzeiro et al. 2015).

Ogbeide et al. (2015) adopted the combination of the above two approaches proposed by Abrantes et al. (2010) and Yurtkuran and Saygı (2013) for risk assessment due to pesticides in the aquatic system of Owan River, Nigeria. According to this approach:

(i) $\mathrm{RQ} = \mathrm{PEC/PNEC}$
(ii) $\mathrm{PNEC} = \mathrm{LC}_{50} \times \mathrm{AF}$(In this case 100 was taken as AF)

This approach was further extended to evaluate the magnitude of exposure to the Owan River fish by means of hazard quotient (HQ). Hazards quotient was obtained as follows.

(i) $\mathrm{HQ} = \mathrm{EADI/RfD}$
(ii) $\mathrm{EADI}(\mu\mathrm{g/kg/day}) = \mathrm{C(biota)} \times \mathrm{CR/BW}$

Where, EADI = estimated average daily intake derived in consonance with World Health Organization (WHO 1997) and Fianko et al. (2011); RfD = reference dose indicating the likely exposure without any appreciable risk; C = mean concentration of pesticide residues in biota (μg/g); CR = consumption rate (kg/capita/day), and BW = average body weight (kg).

A more recent work by Mondal et al. (2018) estimated the potential risk due to pesticides in aquatic system of Hooghly River basin in India by computation of risk quotient for aquatic health in respect of Environmental Protection Agency’s level of concern for aquatic and semi-aquatic animals and plants (USEPA 2019c). The quotient categorizes high- or low-risk in respect of acute and chronic toxicities to four representative aquatic organisms such as algae and one aquatic plant, fish and one invertebrate. The risk quotient (RQ) was computed using the equation as depicted below.

$$\mathrm{RQ} = \mathrm{EEC}/(\mathrm{LC}_{50}\ \mathrm{or}, \mathrm{EC}_{50}\ \mathrm{or}, \mathrm{NOAEC})$$

Where, EEC = estimated environmental concentration (ng/mL); LC_{50} and EC_{50} = median lethal and effective concentration (ng/mL), respectively; and NOAEC = no observed adverse effect concentration (ng/mL). The toxicological data like LC_{50}, EC_{50}, and NOAEC were obtained from Pesticide Properties Data Base (PPDB 2017).

3.6.2.2 Sediment

A considerable amount of pesticides entering the aquatic system are adsorbed onto the sediments. Therefore, risk assessment due to pesticide residues in aquatic environment should also include sediment as suggested by Water Framework Directive and also by the Environmental Quality Standards (EC 2000; 2008; EU 2013). Different methods *viz.,* toxic unit summation, hazard index, and toxic equivalent factor approach for such assessment have also been proposed but rarely applied for risk assessment in sediments (Booij et al. 2015; De Castro-Catalá et al. 2016). Evaluation of sediment toxicity is usually based on dissolved phase pesticides present in pore water. The concentration of the pesticides in sediments could be transformed to pore-water concentrations (C_{pw}) in accordance with the equilibrium-partitioning method as follows:

(i) $C_{pw} = C_s/K_d$
(ii) $K_d = K_{oc} \times f_{oc}$
(iii) $\log K_{oc} = a \times \log K_{ow} \times b$

Where, C_s = concentration of pesticide in sediment; K_d = partitioning coefficient; K_{oc} and K_{ow} = organic carbon-water and octanol-water partitioning coefficient for the target pesticide, respectively; f_{oc} = total organic carbon fraction in sediment; a and b are constants which were standardized as 0.72 and 0.49, respectively (Schwarzenbach and Westall 1981).

3.6.2.3 Risk of a Site

Allinson et al. (2015) and Ccanccapa et al. (2016) applied toxic unit as a risk indicator for ecotoxicological risk assessment in an aquatic site due to the detected level of toxicants. Evaluation was based on acute toxicity values of the pesticide on aquatic biota representing three trophic levels *viz.,* algae, daphnia, and fish of a unit or, area or, site. It was computed as follows.

$$\mathrm{TU_i} = \mathrm{C_i}/\mathrm{EC_{50i}}$$

Where, TU_i = toxic unit of a compound i; C_i = measured concentration (ng/L) in water; EC_{50i} = effective concentration (ng/L) of compound i for lethal effect to 50% of the test organisms. Site specific toxic stress (TU_{site}) could also be presumed by adding all the individual TU_i at the respective site:

$$\mathrm{TU_{site}} = \sum\nolimits_{i=1}^{n} \mathrm{TU_i}$$

Harmful effect due to pesticides present in the aquatic environment could be expected in the cases where risk quotient (RQ) or, toxic unit is more than 1. The values of RQ in between 0.1 to 1 indicate medium risk and less than 0.1 indicates low risk.

In the situation where the measured concentration was less than the limit of quantification (LOQ) *i.e.,* not detectable, 1/2 of LOQ could be considered for assessing risk for aquatic health (USEPA 2000; Biswas et al. 2019).

3.6.2.4 Risk Models

Two tiered models of hazard analyses *viz.,* concentration addition (CA) and independent action (IA) have been proposed by Backhaus and Faust (2012) which was further redefined and simplified by Cruzeiro et al. (2015) for risk assessment due to pesticides in the aquatic environment of Mondego River Estuary, Portugal. Risk quotient (RQ) was calculated separately following the first and second approach of the classical CA model (first tiered). The first tiered first approach is represented as the sum of the measured environmental concentrations (MEC) or, predicted environmental concentration (PEC) divided by predicted no effect concentration (PNEC) ratio. The first tiered second approach is the Risk quotient of sum of the toxic units, RQ_{STU}. PNEC is computed by dividing the minimum median lethal and effective concentration (EC_{50}) value at one trophic level with assessment factor (AF). For assessment of short term effects, the value of AF is considered as 100 (EC 2011).

When both the approaches fail *i.e.,* $RQ_{MEC\text{ or, }PEC/\ PNEC}$ and RQ_{STU} are more than 1, a supplementary IA *i.e.,* second tiered should be considered as recommended by Backhaus and Faust's (2012) model. The ratio of RQ_{STU}/ TU_{max} could be used by which higher toxicity may be predicted from CA than IA (Junghans et al. 2006; Silva and Cerejeira 2014). The two tiered approach for prediction of environmental risk due to pesticides is schematically presented in Fig. 3.2.

In this perspective, Ccanccapa et al. (2016) assessed the ecotoxicological risk due to pesticides occurring in Ebro River, Spain through risk quotient (RQ) and/or toxic unit (TU) estimation. RQ for each pesticide was calculated (*i.e.,* RQ = EC/ PNEC) following the European guidelines (EC 2003) for the representative aquatic species at three trophic levels as discussed earlier (Abrantes et al. 2010; Yurtkuran and Saygı 2013; Ogbeide et al. 2015). Here, estimated mean or maximum concentration of detected pesticides is taken as environmental concentration (EC) (Abrantes et al. 2010) whereas, median and maximum measured environmental concentration (MEC) was considered for calculation of RQ_{median} and $RQ_{maximum}$, respectively, for aquatic samples of ponds and wetlands in Melbourne, Australia (Allinson et al. 2015). Predicted no effect concentration (PNEC) indicating acute or chronic toxicity can be derived as follows:

(i) $PNEC_{acute}$ = Lowest short-term EC_{50}/AF
(ii) $PNEC_{chronic}$ = Lowest long-term NOEC/AF

In this case the assessment factor (AF) was taken as 1000 and all other ecotoxicological data such as median lethal and effective concentration (EC_{50}) and no observed adverse effect concentration (NOAEC) were obtained from Pesticide

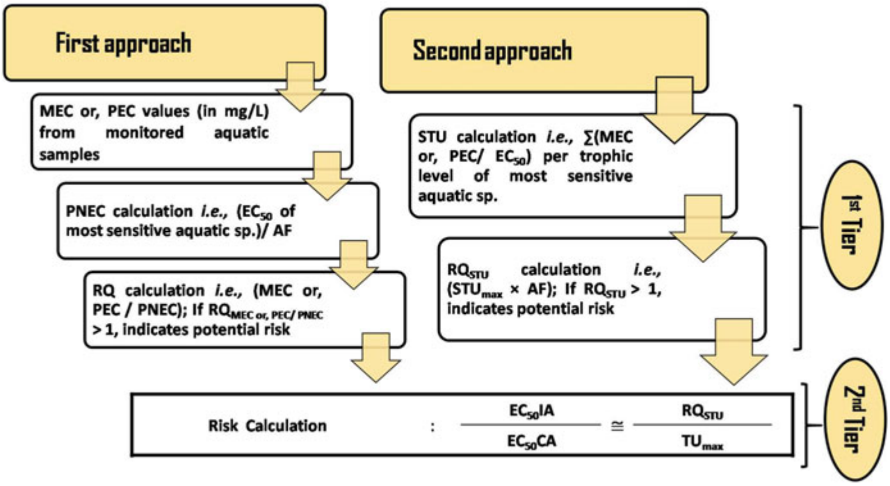

Fig. 3.2 Two tiered approach for prediction of environmental risk due to pesticides. (Modified after Silva and Cerejeira 2014): Two tiered models *viz.,* concentration addition (CA) and independent action (IA) has been followed for estimation of risk and hazard in aquatic ambience. First tiered model has been subdivided into first and second approaches of risk prediction. When both the approaches fail, the second tiered model is considered for estimating risk in the aquatic environment. *Notation: MEC* measured environmental concentration, *PEC* predicted environmental concentration, *PNEC* predicted no effect concentration, EC_{50} median lethal and effective concentration, *AF* assessment factor, *RQ* risk quotient, *STU* sum of toxic units, *IA* independent action, *CA* concentration addition

Properties Data Base (PPDB). The values absent in PPDB may also be computed from the ecological structure activity relationship software ECOSAR™ v.1.11 by which the least toxicity could be predicted for each species (Ccanccapa et al. 2016).

3.6.2.5 Ground Water

On the other hand, Masiá et al. (2013) applied groundwater ubiquity score (GUS) index to indicate the potential risk of ground water pollution by pesticides through leaching around the Guadalquivir River basin in Spain. GUS index was derived by combining pesticides persistence and mobility data in soil contaminating ground water bodies (Gustafson 1989). Two values *i.e.,* 1.8 and 2.8 were generated from the following function:

$$\mathrm{GUS} = \log_{10}\left(\mathrm{t_{1/2}}^{\mathrm{soil}}\right) \times [4 - \log_{10}(\mathrm{K_{oc}})]$$

The above equation allows for categorizing pesticides into three classes, *viz.,* (i) potential leachers with GUS index more than 2.8; (ii) non-leachers with GUS index less than 1.8; and (iii) transient leachers with GUS index in between 1.8–2.8. In relation to this, pesticides with low GUS value *viz.,* imazalil, chlorpyriphos, ethion, and tebuconazole were prevalent in sediment while pesticides with high GUS value like diazinon are considered as potential leachers to pollute the groundwater.

Therefore, evaluation of ecological risk in the aquatic system by using above stated options provides a valuable facts and figure on aquatic risk and hazards of pesticide chemicals.

3.7 Conclusion

Plant protection products known as pesticides, are the accepted ecological weapons in modern agriculture. However, many of these pesticides are persistent in the crop field leading to residual toxicity in food and environment posing risk to human and ecological health. Thus, the occurrences of pesticide residues are monitored in many countries for assessment of food and environmental safety.

Water is considered as the principal mode for pesticides circulation from the point of application to various environmental components. Movement of pesticides from farmland toward water bodies through colloidal phases (dissolve and particulate) is facilitated by various environmental diffusive processes such as runoff and leaching. Pesticides with high water solubility are mostly found in dissolve phase and contaminate the ground water through vertical transport whereas hydrophobic pesticides are preferentially adsorbed on colloidal particles and persist through long range transport. Pesticide concentration in aquatic system is also related to various other agricultural practices around the catchment area and meteorological conditions. Hence, the issue regarding the occurrence of pesticide residues in aquatic system needs continuous monitoring.

Monitoring of pesticides residues demands a steady and reliable multi residue method which could analyze chemical compounds of diverse physico-chemical properties in a single analytical procedure. Various combinations of solvent system are practiced widely for extraction of water samples by liquid-liquid extraction or solid phase extraction techniques followed by estimation using different chromatographic instruments coupled with selective detection system. Recently, mass spectrometric (MS) and double mass spectrometric (MS/MS) system is used for unequivocal detection with confirmation of identity of the pesticides at trace level. Standard method of analysis is highly suggested for estimation of pesticide residues in water samples. However, any analytical procedure selected by a laboratory should be validated for its intended parameters.

Contamination of aquatic system by pesticides residues has been reported in different countries across the world. The co-occurrence of agricultural and non-agricultural pesticides has been reported in various water resources. Ground water contamination is of particular concern for the areas where it serves as the source for drinking water. The sediments considered as a main sink for pesticides in rivers and lakes and are adversely affecting the ecological functioning of aquatic system. Pesticide residues in the aquatic system can further be bio-magnified through the food chain. Movement of pesticides through raindrop may affect the surface water quality particularly during surface runoff events. This necessitates periodical monitoring of pesticide residues in the aquatic system on long term basis for assessment of eco-toxicological implication and to suggest options for ecological management.

The trend of residues in water samples of different countries indicated that maximum pollution in water was contributed mainly by non-agricultural persistent dirty-dozen organochlorine pesticides rather than other groups of pesticides. The residues of these parent molecules present in the soil from historical uses followed by their transformation into stable isomeric products over time, manifested with agricultural or seasonal runoff to hydro-system. Sedimentation succeeded by re-suspension also administers the occurrences of organochlorine pesticides in the aquatic system. Moreover, dichlorodiphenyltrichloroethane is permitted in some countries in public health programme to control vector borne diseases.

The potential risk due to pesticides on aquatic health is evaluated through establishment of standard water quality guideline limits to predict risk and to protect at least 95% of the aquatic biota. Various international governmental and non-governmental agencies such as United States Environmental Protection Agency, World Health Organizaton, and European Union prescribed guidelines for drinking water quality in respect of pesticide residues which have been modified or, adopted further by other countries. Global harmonization of standard guidelines needs to be expedited more. Usually, the standards for drinking water are applied for assessment of ground water quality. Pesticidal chemicals which failed to comply with the guidelines have been banned, restricted or kept under surveillance in some countries. Maximum banning was imposed on organochlorine group of pesticides rather than other groups of chemical.

There are multiple approaches for ecological risk assessment through computation of risk quotient for each pesticidal chemical to evaluate potential risks to aquatic life at each trophic level. Toxic unit could be used as a risk indicator for estimating risk of a site. Hazard analyses models with two tier system *viz.,* concentration addition and independent action have been employed for prediction of environmental hazard and for risk assessment due to pesticides in aquatic system. On the other hand, groundwater ubiquity score could have been used to indicate the potentiality of pesticides to pollute ground water.

In view of above, it is recommended that education and training programmes should be implemented to raise general awareness regarding the importance and impacts of pesticides pollution due to agricultural and other anthropogenic activities. Farmers should be encouraged to adopt production systems with reduced use of pesticides through good agricultural practices, organic cultivation, integrated pest management, and integrated vector management. Indiscriminate use of pesticide chemicals including misuse and over use of pesticides, use of non-recommended application technology should be avoided. Advanced and relatively less-toxic stable formulations such as controlled release formulations e.g., gel, encapsulated, and polymer coated formulations may be emphasized to minimize leaching potentiality compared to the older formulations such as wettable powder, dust, granules, and pellets. Furthermore, alternative effective strategies of vector control are to be explored to reduce reliance on dichlorodiphenyltrichloroethane. Use of bio-pesticides of microbial and botanical origin could be a potential alternative to synthetic pesticides due to their novel mode of action, lesser persistency, and thereby reduce the risk to human and the eco-system.

An effective legal, technical, and administrative framework could be developed at regional level with respect to protocols concerning management of pesticides pollution in water bodies. Laws, regulations, and policies should be strict enough on the production, emission, distribution, storage, usage and disposal of persistent organic pollutants like pesticides. Ratification of international conventions relevant to persistent toxic chemicals needs to be expedited further. To avoid the complicacy and multiplicity of different guideline limits, initiatives should be taken for harmonization of legislation among the developed and developing countries through sharing of knowledge and exchange of information, as well as technical cooperation.

However, in the situation where it's obvious that the pesticide residues are being inevitable in the aquatic system, decontamination of toxic pesticides from the water bodies could be accomplished by employing several popular techniques which includes adsorption on charcoal, activated carbon and modified polymer; chemical coagulation and oxidation; photochemical, electrochemical and biological treatment; filtration through bio-filter and nano-filtration; membrane bio-reactor; and phytoremediation. It is significant to say that currently an utmost importance are being stressed to design and find out some inexpensive and simple technologies which could treat bulk amount of domestic and agricultural wastewater contaminated with wider chemical ranges of pesticide pollutants.

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Chapter 4
Pyrethroid and Residues in Chickens and Poultry Litter

Carlos Eduardo Carlos da Silva Soares, Vildes Maria Vildes Scussel, and Fabiano Fabiano Dahlke

Abstract Poultry litter has been utilized to accommodate the birds, providing comfort, depriving the animals of direct contact with excretions and with the floor, also to avoid temperature variation inside the poultry house. However, it provides shelter, hiding place and food for some insects and mites, especially ectoparasites, the principal pests present in the poultry house, and the main reason for pesticide applications. The most widely used pesticides and repellents worldwide to control the proliferation of arthropods in litter and poultry facilities belong to the pyrethroid, cypermethrin, deltamethrin and phthalates such as dimethyl phthalate, respectively. Their application is part of the routine of several poultry farmers from chick housing to the final stage of the poultry production. Depending on the infestation, the application procedure may be carried out more than once during the poultry production cycle both in broiler and in commercial laying hens. Remediation strategies related to the environment, food and human exposure to these pesticides are developed in research. That also includes utilization of alternative/green decontamination methods for controlling pests with ozone gas/cold plasma/nanoparticles which can increase food safety, replacing pesticide use in poultry litter and avoid whole poultry environment exposure to those toxic compounds.

Keywords *Alphitobius diaperinus* · Arthropods · Cypermethrin · Dimethyl phthalate · Ozone · Pesticide · Poultry litter · Pyrethroids · Residue

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4.1 Introduction

Several poultry companies developed technical biosecurity standards in which Pyrethroid Group insecticides has been used to control ectoparasites in poultry raising. However, lot of research report the low susceptibility of insects and mites to these active molecules, especially cypermethrin (Silva et al. 2008; Leffer et al. 2011), quite a product marketed for control of ectoparasites in poultry production. Many products contain the active ingredient cypermethrin in its formulations as one of the main ingredients and in various concentrations (Athanassiou et al. 2015; Saillenfait et al. 2015; Sakka et al. 2018).

Pesticides, when systematically applied to the environment without control and supervision are capable of contaminating the farmworker, environment and animal feed. Therefore, its presence both in the form of residues in meat and eggs, as well as in the environment and wildlife, might further compromises the health of workers and consumers (Brown et al. 1996; Costa et al. 2013; Singleton et al. 2014; Del Prado-Lu 2015). As ectoparasites control in poultry currently depends on the use of pesticides, there are lot of information on the application of green methods and proper management of pesticides, which eliminate sources of contamination, especially within poultry sheds.

4.2 Characteristics of Pesticides Used in Poultry Farming

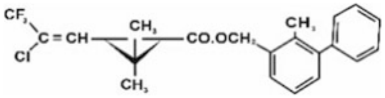
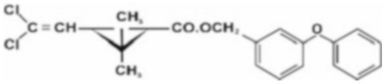
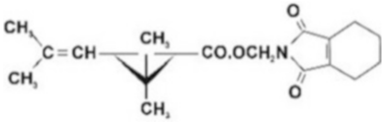
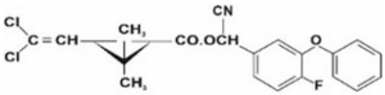
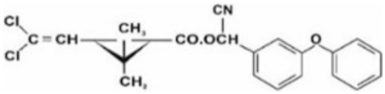
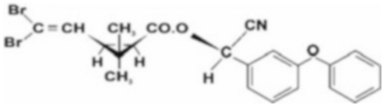
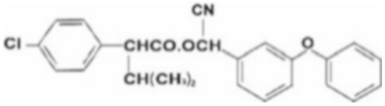
The Pyrethroid Group has two divisions according to its structure, Type I and Type II which contains an alpha-cyan group in its molecule and they are classified as II – moderately hazardous) and III – slightly hazardous (Soares et al. 2018b; WHO 2010). The Table 4.1 presents some pyrethroid group pesticides that have been established for use in both ornamental and production poultry.

4.2.1 Dimethyl Phthalates

Dimethyl phthalate, with molecular formula ($C_{10}H_{10}O_4$), is a low molecular weight member of a group of chemical compounds, commonly known as phthalates. These compounds are commonly used in a wide range of consumer goods, as plasticizers to add flexibility to plastic consumer products. Dimethyl phthalate is also used as an insect repellent for mosquitoes, flies and for control of ectoparasites.

Dimethyl phthalates have been used for the control of arthropods in poultry production, added as one of the main ingredients in pesticide and repellent formulations. Some companies do not present in their sites the package leaflet with pesticide composition, described only on the packaging. Was one of the first synthetic repellents used during World War II (1939–1945) to repel flies, disease-

Table 4.1 Classification, application, class toxicological and chemical structures of some pesticides used in poultry farming

Active ingredient	Application	Class toxicological	Chemical structure
Type I			
Bifenthrin	Agricultural Domestic	II	
Permethrin	Agricultural Domestic	II	
Tetramethrin	Agricultural Domestic	III	
Type II			
Cyfluthrin	Agricultural Domestic	II	
Cypermethrin	Agricultural Domestic	II	
Deltamethrin	Agricultural Domestic	II	
Fenvalerate	Agricultural Domestic	II	

Soares et al. (2018b) and WHO (2010)

causing arthropods (Harris et al. 1997). Reported as persistent organic pollutants and endocrine disrupters, phthalates are widely investigated due to their effects on the environment and human health (Zhang et al. 2017; Bono-Blay et al. 2012; Filazi et al. 2016).

Kuzukiran et al. (2018) evaluated the presence of Phthalic Acid Esters in eggs production from *cages*, *free-range* and *organic* systems, and reported that dimethyl phthalate has being fond more abundant in eggs produced by cages system. The frequency of active ingredient in eggs collected in cages was 76, while in free range and organic were 40% and 52%, respectively. Dimethyl phthalate is used in a wide range of consumer products. It has been the subject of great public concern in recent years.

4.3 Pyrethroid Group Used in Poultry Farming

Cypermethrin and deltamethrin are highly active synthetic pyrethroid used as an insecticide in large-scale commercial agricultural applications, due to the fast-action neurotoxin in insects. In poultry production, cypermethrin has been applied for the control of ectoparasites, flies, beetles and mites (Marangi et al. 2012). The high-density confined housing systems used in poultry production create conditions that favor the development of this insects and parasites. Despite the current poultry management practices recommended for the control of these pests, little progress has been made in the search of alternatives to the use of chemical insecticides, particularly pyrethroids (Soares 2018).

The use of pyrethrum powders as an insecticide began around 1855 in the US and 1886 in Japan. Pesticide coils, for example were developed in 1890 in Japan, and pyrethrum extract-containing oil formulations were available in 1919 in the US (Katsuda et al. 2011). However, by their use over the years, it is vital to estimate the amount of pesticides ingested via food so that the actual risk to the population can be assessed. Human studies have shown positive results regarding the presence of pyrethroids (Sassine et al. 2004; Azmi et al. 2006; Corcellas et al. 2012).

In humans, when applied to the earlobe to test the effects, fenvalerate, cypermethrin and permethrin induced paraesthesia (Flannigan et al. 1985). It is applied to agricultural crops as well as domestic environments and plays a vital role in pest control agriculture (Hebeish et al. 2010). The applications of pyrethroids are recommended by the World Health Organization for the control of plenty of types of pest. However, they might present moderate acute toxicity to public health (WHO 2015).

As adults, birds are less susceptible to pyrethroids than mammals. However, in the embryonic phase, their sensitivity to these active ingredients may possibly cause malformations depending on the type and concentration administered (Vijverberg and vandenBercken 1990; Bhaskar et al. 2016; Chaphekar and Kamble 2015).

4.4 Rearing of Birds and Poultry Litter

Broilers are reared in intensive systems and use the poultry litter often composed of shavings or rice husks for accommodation during their development period. At each breeding stage, its structure and physicochemical characteristics are modified as it is turned over by birds, machinery and when excreta are deposited (Fig. 4.1) (Avila et al. 2007).

Fig. 4.1. Poultry litter used to accommodate birds: structure composed of wood shavings after housing the birds. (**a**) 1st day – structure intact (**b**) 21st day – slightly degraded and (**c**) 45th day – fragmented structure and dark. (Authors, 2019)

Fig. 4.2 Insect disease vectors: presence of (**a**) *A. diaperinus* on wooden pillars of poultry facilities (**b**) chick with enteric disease caused by bacteria (**c**) flies are common insects on chicken carcasses (Soares et al. 2018b; Authors, 2019)

In order to maintain quality, several precautions are needed to combat the ectoparasites that are present in the poultry shed (insects) and bird's tegument (mites), responsible for economic and health losses to the birds (Fig. 4.2). These living organisms can re-infestation due to their rapid life cycle and their resistance to various types of pesticides.

The presence of cypermethrin degradation and half-live rates in the poultry environment depend on the physical–chemical conditions of the poultry litter as, water activity may range from (0.74–0.95), moisture content (mc) (11% – 40%), pH (7.35–8.51) on the 3rd and 45th day, respectively (Soares 2018). In poultry litter, half-live averages 3.75 days with average degradation rates of (0.21 ± 0.08) ppm/day (Ong et al. 2016).

Systematically applied to poultry farms, the cypermethrin is also capable of contaminating animal feed. The use of pyrethroids without control and surveillance has increased the risks of their presence in the form of residues in meat, as well as in the environment and wildlife such as birds, insects and mammals (Alonso et al. 2012; Łozowicka 2013). Due to its ingestion/inhalation, it causes acute and subacute poisoning in various degrees in mammals and birds (Spinosa et al. 2008).

4.5 Possible Sources of Pesticide Contamination

Insects can cause grain damage, both in storage and in the field, and to minimize this damage, chemical pesticides are used during crop development or silage preloading in grain storage units (Savi et al. 2014a, b; Moore 2017).

4.5.1 *Rice Paddy*

The use of rice paddy that is residual rice production as a substrate for poultry litter may increase the risk of pesticide contamination of the Pyrethroid Group, making litter possible (Costa et al. 2014). In many rice producing countries, pesticide application, especially cypermethrin, is used in grain cultivation (Palm 2007; Mushtaq et al. 2019). Figure 4.3 shows the stored rice rusk for use as poultry litter stray chicks on rice paddy.

4.5.2 *Feeding of Birds*

In many commodities that are the staple feed of poultry, especially corn, cypermethrin is one of the most commonly used active ingredients in pest control, and when investigated, levels of residues above regulated Maximum Residue Levels (MRLs) are often found by each country (Rita et al. 2014; Lozowicka et al. 2014). Figure 4.4 shows (a) corn grain stored in bags, ready for processing poultry feed production and (b) feed already prepared for poultry consumption. In addition to corn, poultry feed includes soybean meal and oil in its formulation to adjust its energy value (da Silva Costa et al. 2015). During soybean planting, poultry litter is

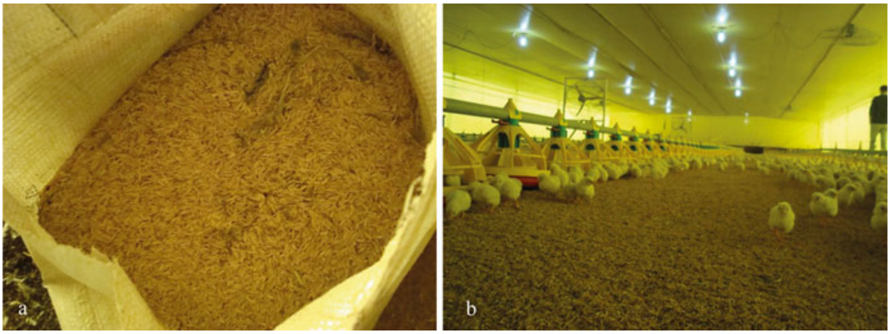


Fig. 4.3 Other substrates used in poultry litter: bags with (**a**) stored rice rusk for use as poultry litter (**b**) chicks scattered in the poultry litter of rice husk showing an ideal environment of thermal comfort for the age of birds at this age. (Authors, 2019)

Fig. 4.4 Grain storage and processing unit: (**a**) whole corn in bags prepared for processing without previous treatment of decontamination by pesticide residues (**b**) pelleted foods based on corn and soybeans. (Authors, 2019)

used for fertilization, and many agrochemicals used in the planting area for oil production can contaminate the final product (Patthanaissaranukool and Polprasert 2016). In this context, pesticides thrown on agricultural land contaminate and leave residues in shallow layers of soil only 30 cm deep (Jabbar et al. 1993).

Another ingredient used in the diet of poultry is wheat, which also has cypermethrin as a chemical compound used in crops for the control of insects of the order Lepidoptera and Hemiptera (Pasini et al. 2018). In a study by Westcott and Reichle (1987) applying an aqueous emulsion of cypermethrin with concentrations of 28 g /hectare in wheat crops, they reported that in 27 days the residues decreased considerably to the point that cypermethrin and deltamethrin were not found in the grains. In another situation, after investigating 45 wheat samples obtained from farmers in two areas in Kazakhstan, Lozowicka et al. (2014) reported the presence of deltamethrin (pyrethroid) residues in 1 of the samples. To investigate the occurrence of pesticide residues in stored and imported wheat grains, Uddin et al. (2011) listed three pyrethroid pesticides, with cypermethrin concentrations ranging from 0.14 to 0.20 μg/kg in two contaminated samples.

Chicken breeding has selected from increasingly voracious animals; when there is a period of fasting due to a feed supply problem, they begin their natural scratching and digging behavior for food, and ingest larvae and beetles possibly contaminated by the applied pesticide (Stringhini et al. 2006).

4.5.3 Water and Soil

Contamination of surface waters and rainfall by pesticides caused by intensive use of pesticides in agriculture points to the degradation of water quality (Moreira et al. 2012; Del Prado-Lu 2015).

When drinking water is distributed by companies or natural sources, there may be residues of pyrethroid pesticides (Sereda et al. 2009). Structures intended for composting, when poorly designed, contribute to the contamination of the region's

Fig. 4.5 Poorly constructed structures that compromise the environment close to the farms (**a**) unsecured compost for animal entrances and deteriorated and (**b**) poultry construction close to cisterns and rivers. (Authors, 2019)

soil and rivers (Fig. 4.5), as the pesticide applied in this environment may not degrade (Fenoll et al. 2011). When the practice of managing grain producers during planting is not sustainable, indiscriminately applied pesticide concentrations can pollute fauna, flora, soil, rivers and oceans (Hoai et al. 2011; Alonso et al. 2012).

4.6 Residues in Poultry Products

Contamination of food occurs primarily in grain crops, where there is an increase in the use of synthetic pesticides, raising the risk of contamination in water and feed provided to poultry and consequently in meat and eggs. In poultry production, the control of mites and insects often depends on pyrethroid group acaricides. As there is resistance to this chemical compound, repetition in its application and out of the recommended dosages are frequent, possibly accumulating in the eggs and tissues of birds (Marangi et al. 2012; Aulakh et al. 2006).

4.6.1 Meat

Contamination of grains by pesticides is of concern and draws attention to their monitoring before their destination for feed production, as they pose a risk to human and animal health. Pyrethroid residues and other such Groups (organochlorines and organophosphates) have been described above the limits established in diets, meat tissues and water in broiler farms (Mishra et al. 2018).

After investigation of meat products purchased from supermarkets and slaughterhouses, no pyrethroid pesticide residues were found in the samples collected. However, groups such as organochlorines and organophosphates were present in the collected samples (Azmi et al. 2006). Poultry meat products are commonly free of residues due to their rapid breeding process (6 weeks), their short half-live in poultry feed and litter, and when the time of active ingredient administration and slaughter is respected (Marzouk et al. 2016; Ong et al. 2016).

When evaluating cypermethrin and chlorpyrifos residues in carcasses of broilers at 7, 14 and 21 days old, Silva et al. (2008) applied g the pesticide to the environment and have immersed larvae and beetles of *A. diaperinus* in solution with pesticides and forcibly feeding the birds with 10 larvae and beetles for 21 days. They had found residual levels (mg/kg) lower than the limit established by the country's legislation in the samples of thigh and thigh muscle, liver and fat.

4.6.2 Fresh Eggs

Currently, there is a need to discuss the bioaccumulation and biotransformation of insecticides in animals, as residues are found in various animal tissues and products, including eggs. At levels above the recommended, the cypermethrin pyrethroid showed 66 times higher concentrations than the maximum residue levels (MRLs) in fresh eggs when the commercial pesticide was applied to control ectoparasites. Residues of Active ingredients were present in one third of the samples collected (Parente et al. 2017). On the other hand, low levels of pesticide residues in egg reported in some research may mask a risk to human and animal health as several studies show the bioaccumulation of lipophilic pyrethroid pesticides in both mammalian and poultry organisms (Alonso et al. 2012; Dallegrave et al. 2018).

Eggs are important foods in the human diet, and pesticide residues are concentrated in the yolk that is the lipid fraction. If pesticides are applied during their formation, they may be contaminated (Dallegrave 2017). Investigating eggs and chicken meat sold in supermarkets, Ahmad et al. (2010) reported the presence of organochlorine pesticide residues, and Dichlorodiphenyltrichloroethane was detected with high incidence, even banned in some countries for use in agriculture.

4.6.3 Fertile Eggs

Fertile eggs exposed to pesticides and fungicides applied to poultry farms and crops production can be contaminated and trigger a teratogenesis process in chicks, preventing them from reaching maturity, as they have no beak, undeveloped limbs and diminished brain (Budai and Lehel 2012). After immersing fertile eggs for 1 h on the 7th day of incubation at 3 different concentrations of a commercial pesticide, the embryos showed growth retardation, high mortality, skeletal malformations in all embryos at the tested concentrations (Taparia et al. 2014).

Concentrations of 100, 200 and 400 ppm cypermethrin applied to incubated eggs are capable of triggering teratological changes at 11th day of incubation, preventing eye and beak formation, reduction of body size, head and eye, showing some

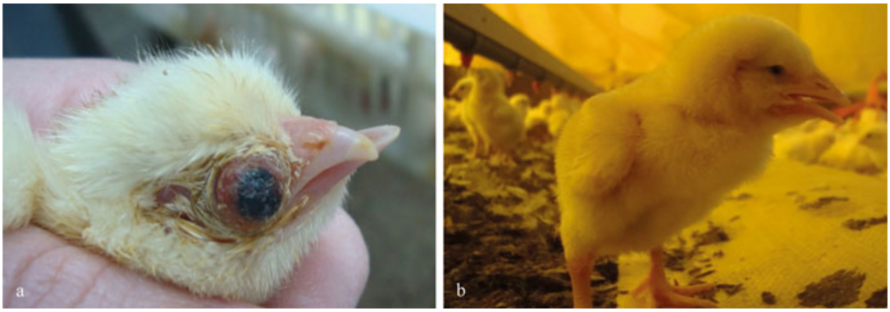


Fig. 4.6 Eye malformations and disturbed body development in newly hatched chicks (**a**) ocular deformation and curved beaks seconds after hatching (**b**) apparently healthy bird with disproportionate morphological structures in broiler chicks. (Authors, 2019)

pyrethroid toxicity used in poultry farming (Anwar 2003). Figure 4.6 shows chickens breeding hens with different eye and morphological malformations.

4.7 Methodology of Analysis for Detection and Quantification of Pesticides in Poultry Meat and Eggs

Determination of pesticide residues in food plays an important role in assessing and estimating their environmental exposure in both rural and urban environments. The release of some synthetic pest control compounds needs to be monitored, especially in developing countries, as they will increase the number of contaminated foods and new contaminants present in them.

4.7.1 Analytical Methods for Meat Products and Poultry Eggs

There are currently several fast and accurate analytical methods for the determination of pesticide residues in animal and plant foods, some specific for high lipid matrices. However, chemical groups of these contaminants present difficulties for their simultaneous extraction, cleaning and analytical separation due to their different physicochemical properties. Several chromatographic and detection techniques allow the evaluation of agricultural production compliance with Good Agricultural Practices, enabling new regulatory decisions to ensure food safety (Izquierdo et al. 2007).

Gas and liquid chromatography techniques are applied to pesticide residue analyzes. However, gas chromatography is most commonly utilized in pesticide research and is combined with a range of detectors such as mass selective detector, electron capture detector, mass spectrometry, photo-diode array, ion Trap-Mass Spectrometric and electrospray ionization. The molecules analyzed define the detectors to be used for identification and quantification of compounds.

There are advantages and disadvantages in detection techniques, so that their use needs to be evaluated, as some have greater affinity for a certain type of pesticide, difficulty in its use, high costs for acquisition and maintenance, low selectivity, limited linear range and matrix can saturate the detection system. Currently, Liquid Chromatography Mass Spectrometry -LC/MS or Liquid Chromatography Ultraviolet -LC/UV Detection is preferred when analytes are transparent due to ease of sample preparation, various available column combinations and possible mobile phases when compared technique of Gas Chromatography Mass Spectrometry -GC/MS. However, pyrethroid structures and physicochemical properties have better analytical results when analyzed by Gas Chromatography Electron Capture Detector -ECD or Mass Spectrometry -MS (Frenich et al. 2008; Dallegrave 2017). Table 4.2 shows the chromatographic equipment and detectors in animal matrices.

4.7.2 Preparation of Poultry Litter for Chromatographic Analysis

Step in which it is necessary to know the matrix well, its centesimal composition is important, because it is a very complex matter. Poultry litter is made up of various foreign matter such as soil, fungi and mycotoxins, feces, pesticide and antibiotic residues, high humidity – depending on the rearing phase, dead insects, carcass residues and various other pigments that may interfere with test results. Because chromatographic techniques coupled with mass spectrometry are very sensitive and selective, there is this concern with sample preparation. Today, research and commercial laboratories around the world have implemented in their protocols the QuEChERS method (*Quick, Easy, Cheap, Effective, Robust and Safe*). Figure 4.7 shows micrographs made by scanning electron Microscopy of different structures of the chicken litter on day 0 and 35 days of housing. It is observed that on day zero, the structures of the parenchymal cells are slightly degraded, while on the 35th day the poultry litter has its surface totally dominated by the colony of toxigenic fungi.

4.8 Remediation Strategies

Pyrethroids have long been used to repel and eliminate insects and are ideal for home use because of their low toxicity in warm-blooded animals. However, the overuse of the product contaminates the environment and food, causing problems to human health. Some physical and chemical strategies to limit the use of pesticides and

Table 4.2 Matrices and analysis of pyrethroid pesticide residues, occurrence and presence in poultry products

Samples			Analysis methodology			
Country	Year	Matrices	Pesticides	Concentrations – range (ug.g^{-1})	Instrumental analysis	References
Egypt	1986	Chicken Meat Eggs	Deltamethrin Cypermethrin Fenvalerate	0.03–3.9	GC^1/ ECD^2	Saleh and Ibrahim (1986)
Brazil	2007	Broiler Meat	Cypermethrin	NI^a	GC/MSD^b	Silva et al. (2008)
Polland	2010	Poultry Muscle	Deltamethrin Permethrin Cypermethrin Fenvalerate	NI	GC/ECD	Niewiadowska et al. (2010)
Hong Kong	2012	Chicken Meat Eggs	Deltamethrin Permethrin Cypermethrin Fenvalerate	NI	LC^3 ESI^c MS^5/MS	Chung and Lam (2012)
Italy	2012	Chicken Meat	Permethrin Carbaryl	0.006–16	LC/MS/MS	Marangi et al. (2012)
	2014	Chicken Eggs	Deltamethrin Cypermethrin Fenvalerate	NI	GC/MS/MS	Dell'Oro et al. (2014)
Germany	2015	Poultry Meat Eggs	Deltamethrin Cypermethrin Fenvalerate	NI	GC and LC/MS/MS	Hildmann et al. (2015)

Malaysia	2016	Poultry (Manure)	Cypermethrin Chlorpyrifos	0.03–56.27	LC/PDA[6]	Ong et al. (2016)
Brazil	2017	Chicken Eggs	Cypermethrin	0.0003–6.48	GC/MS	Parente et al. (2017)
China	2019	Chicken Eggs	Acetamiprid Pyrimethanil Fipronil sulfone	0.00068–0.0075	UHPLC[4]-MS/MS	Zhang et al. (2019)

[a] NI- not informed; [1] GC- Gas chromatography; [3] LC- Liquid chromatography; [4] UHPLC- Ultra high-performance liquid chromatography; [5] MS – mass spectrometry

[b] MSD – mass selective detector; [2] ECD – electron capture detector

[c] ESI – electrospray ionization; [6] PDA – photo-diode array

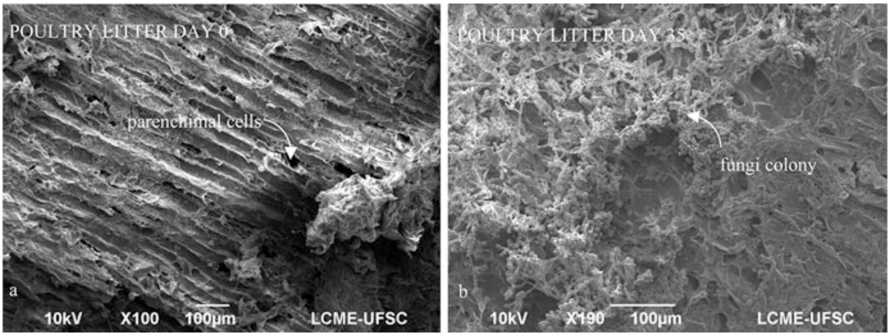


Fig. 4.7 Scanning Electron Microscopy Micrograph of brood of birds collected at various ages and parenchymal cell structures (**a**) zero day – plant tissue little degraded by fungi [100×] (**b**) and colonies of fungi occupying all plant tissue 35 days after housing [190×]. (Authors, 2019)

consequently minimize the presence of residues in foods are applied in several research works.

4.8.1 Ozone

Ozone gas (O_3) which is considered a generally recognized as safe gas has a half-life of approximately 30 min in atmospheric conditions and reduces with high temperatures and low pressures (Khadre et al. 2001). It is used as an effective antimicrobial in both liquid and gaseous form leaves no residue and is suitable for decontaminating food, equipment and environments (Christ et al. 2016).

The use of O_3 gas to degrade pesticides such as deltamethrin in wheat grains was effective after treatments with concentrations of (60 μmol/mol) and exposure times (120 and 180 min). The pesticide was significantly reduced under these conditions. However, there was no reduction when exposed for 60 min (Savi et al. 2014a, b).

The elimination of larvae and beetles from *A.diaperinus*, one of those responsible for applying pesticides to poultry farms, was achieved by exposing O_3 at different concentrations and times under low airflow, resulting in the elimination of 100% of larvae and beetles. New field application studies are being conducted to try to reduce pesticide application, prioritizing workers' health and improving food safety by minimizing the danger of residues in meat and eggs (Soares et al. 2018a).

4.8.2 Thermal and No Thermal Treatment

Heat treatment as a decontaminant to reduce pesticide content in meat is effective, and chicken meat had a low initial concentration and became minimal after cooking, being the safest according to (Sengupta et al. 2010). Cooking may be an agent capable of degrading some types of pesticides and other food contaminants in meat. However, further discussion is needed as these micro-pollutants can be released in cooking juice, broken by heat or evaporated (Planche et al. 2017).

The use of cold plasma has been used in several areas of agriculture, tested as a decontaminant in pathogenic microorganisms in meat and other types of food. However, chicken eggs showed changes in yolk taste and texture, changes in breast color as well. Have been reported (Dirks et al. 2011; Lee et al. 2012; Zhuang et al. 2019). In this context, further research should be conducted to decontaminate meat and eggs with pesticide residues without changing the organoleptic characteristics of products, as this new technology is promising for food safety.

4.8.3 Composting

Poultry litter composting can be used as a remediation strategy, as it is a recycling process made by living organisms that use the substrate (organic matter) as an energy source. Ideal environment for pesticide degradation, as it has characteristics such as the temperature that allows biochemical reactions favoring an action of microorganisms (Hwang et al. 2001; Petric et al. 2009; Farah Nadia et al. 2015).

For this bioremediation strategy, knowledge about the activity and behavior of microorganisms in the composting stages, especially thermophilic, is essential, given the conflicts of some researchers over the function of the thermophilic stage of contaminant bioremediation (Boudh and Singh 2018).

4.8.4 Protective Measures

The handling of synthetic pesticides in agriculture has caused some problems for human health in many developing countries. Often the farmer has information about the risks inherent in the misuse of chemicals. However, it is not fully aware of the toxicity of the pesticide used (Dasgupta et al. 2007; Oduwaiye et al. 2017).

In family farms poultry is not the only source of income. The production of diversified crops makes it urgent to complete the activities of each production, and this situation often causes the farmer not to use the necessary personal protective equipment (PPE) increasing the damage caused by occupational exposure. Research reports that the employer, in many cases, even though required by law, does not provide personal protective equipment to employees, so the worker himself has to purchase his equipment (Underhill and Rimmer 2015). In tropical countries, humid and hot weather conditions, especially in the summer season, discourage applicators from using appropriate equipment.

In this context of contamination, it is necessary to implement poultry prevention regulations, monitoring and standardize the dumping of poultry waste (Moyce and Schenker 2018). The grace period and the safe amount of pesticides applied to both grain and poultry farms for arthropod control must be respected, so that food waste and are minimized occupational poisoning.

4.8.5 Nanoparticles for Removal of Pesticides

A different organophosphate removal method was tested to remove these compounds from agricultural effluents using microfiltration, based on a bio purification system using coconut fiber, soil and the oxytetracycline antibiotic. (Huete-Soto et al. 2017). Nanotechnology was utilized by Yang et al. (2014) to test vitamin D supplementation in poultry feed and is currently used as a tool for the degradation of different pesticide groups in several matrices. (Makkliang et al. 2016; Dehghani et al. 2017). In poultry production, there is a vast field for new research and applications involving nanotechnology in the poultry environment, mainly for the decontamination of chicken litter, eggs and animal feed.

4.9 Conclusion

The presence of insecticides from several groups of pesticides applied at high concentrations applied and by not considering the grace time of chemical compounds can contaminate meat and eggs with residues.

These applications take place during planting, grain storage and poultry litter for the control of harmful living organisms. In this context, new control and/or decontamination strategies aim at producing safe food, avoid resistance from harmful living organisms and contributing to the welfare of birds and rural workers.

Currently, there are efficient established methods for degradation of these chemical compounds. However, there is a need to develop equipment to control these pests in the poultry environment, especially poultry litter, which would reduce pesticide use and concern with residues in meat and eggs.

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Chapter 5
Extraction Procedures and Chromatography of Pesticides Residues in Strawberries

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Abstract The importance of food quality has become a crucial issue due to the widespread use of pesticides in agriculture. The frequent use of pesticides has resulted in contamination of the environmental, fruit crops and also caused potential risk to human health. Strawberries are at the top of the list of fruits with the highest concentration of pesticides. To date, several analytical techniques have been used for the extraction and detection of pesticides in strawberries, from classic to advanced analysis methodologies.

In this chapter, the studies published by the scientific community performed from 2000 to the present, concerning the extraction and analysis of pesticide residues in strawberries, were discussed. The most broadly used detection technique for the analyse of pesticides in strawberries is mass spectrometry combined with gas and/or liquid chromatography. In general, multi-residue methods with selective sample treatment methodologies have been developed for this purpose. Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS), liquid-liquid, solid-liquid, and solid-phase extraction and microextractions are the selected sample preparation techniques. The analytical parameters of the methodology and the findings on pesticide residues are described. The presented chapter shows the importance of these data in terms of food safety on the situation regarding pesticide residue in strawberries and human health regarding the risk of the ingestion of pesticides contaminated strawberries.

Keywords Chromatography · Strawberries · Pesticides

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5.1 Introduction

Pesticides can prevent large crop losses and will, therefore, continue to play a role in agriculture. As they are intrinsically toxic and deliberately spread in the environment, the production, distribution, and use of pesticides require strict regulation and control. Consumers are looking for the assurance that the food they eat is safe. Chemical diversity of these pesticides also presents a considerable challenge to food safety chemists, who require a range of analytical techniques to extract pesticides from fruit samples and then to accurately determine their identity and concentration at trace levels. The classic and innovative sample preparation and chromatography techniques provide food safety chemists tools that can meet the analytical challenges. Therefore, the sections below aim to discuss the available pre-treatment, extraction, and detection methods that are used for the determination of pesticide residues in strawberries. The methodologies will be quite valuable to food analysts and regulatory authorities for monitoring the quality and safety of strawberry samples.

5.1.1 Food Safety and Pesticides

Analysis of contaminants in food remains one of the most important determinations in nowadays. Pesticides are commonly used in agriculture to improve the yield and quality and extend the storage life of food harvests (Raina 2011; Anna Stachniuk and Fornal 2016). Pesticides must undertake extensive efficacy, toxicological, and environmental testing to be registered by governments for legal practice in certain applications. The applied pesticides and their degradation products may persist as residues in the agricultural products, which becomes a concern for consumer exposure. Therefore, in order to ensure rigorous food safety standards, maximum residue levels that limit the quantities and types of chemical residue that can be legally present in foodstuffs, are set by regulatory bodies worldwide United States Department of Agriculture and European Union (Regulation (EC) No. 396/2005; USDA database). Pesticides can be grouped according to the types of pests which they kill or inhibit, namely, insecticides, herbicides, fungicides, rodenticides, fumigants, among others. They can be also grouped according to the chemical families. Most pesticide active ingredients are either inorganic or organic carbon-based. Inorganic pesticides do not contain carbon and are often derived from mineral ores (e.g. copper, sulphur) or their salts. Organic carbon-based pesticides are grouped into families with similar molecular structures. Pesticides within the same chemical family often have similar properties. The most common pesticide groups used for pest control are insecticides (e.g. organophosphates, organochlorine, carbamates, pyrethroids among others), herbicides (e.g. phenoxy, benzoic acid, bipyridylium, ureas, among others) and fungicides (e.g. dithiocarbamates, dicarboximides, benzimidazoles, among others). Over a quarter of the food consumed in Europe contains two or more pesticides, according to the annual report published by the European Food Safety Authority. The last European Food Safety Authority report shows that over 60% of cherries, strawberries, lettuce, and bananas were found to have two or more pesticide residues (EFSA report 2019). For example, chlorpyrifos is one of the pesticides most often found in European Union food and most often exceeding the maximum residue limits. Chemical mixtures detections are a concern in terms of consumers safety. Furthermore, persistent and highly toxic pesticides and other persistent organic pollutants that have been banned, continue to be detected in our food. Moreover, some of these pesticides found in European Union food presented endocrine-disrupting properties. Food contamination with pesticides has been considered as an important source of several serious diseases such as cancer, birth defects, and damage in the endocrine, nervous, and immune systems (Anna Stachniuk and Fornal 2016). In order to ensure the conformity of food products with food safety, reliable, effective and sensitive analytical methods for the analysis of pesticide residues are essential. Thus, the methods used in chromatography are documented as the technique highly specific and sensitive for the analysis of pesticides (Raina 2011).

5.1.2 Strawberries

Strawberries are packed with vitamins and minerals such as vitamins, carbon, folate, potassium, and manganese. They are low-calorie, fiber-rich and abundant in antioxidants and polyphenols (Hidalgo and Almajano 2017). Although, strawberries are on the list of the most contaminated foods and they are eaten unpeeled, which increases the risk of contaminant intake (Fernandes et al. 2014). In 2017, the world production of strawberries was 9.22 million tons, led by China with 40% of the total, and the United States with 16%. The strawberry market plays an imperative role in world fruit consumption. In strawberry production, pesticides are applied to control diseases and pests in the field to increase crop yield.

5.1.3 Analytical Methods for Pesticides Quantification in Strawberries

According to the guidelines assumed by the European Commission's Directorate for Health and Food Safety, strawberries are included in the fruit group with 'high acid content and high water content' (SANTE/11813/2017). The analytical procedures used for the pesticide determination in strawberries often follow different approaches, with a specific analytical procedure for the extraction and cleanup steps followed by chromatographic detection (Fig. 5.1). Due to many pesticides on the market and the low concentrations reported, accurate, highly selective, fast and

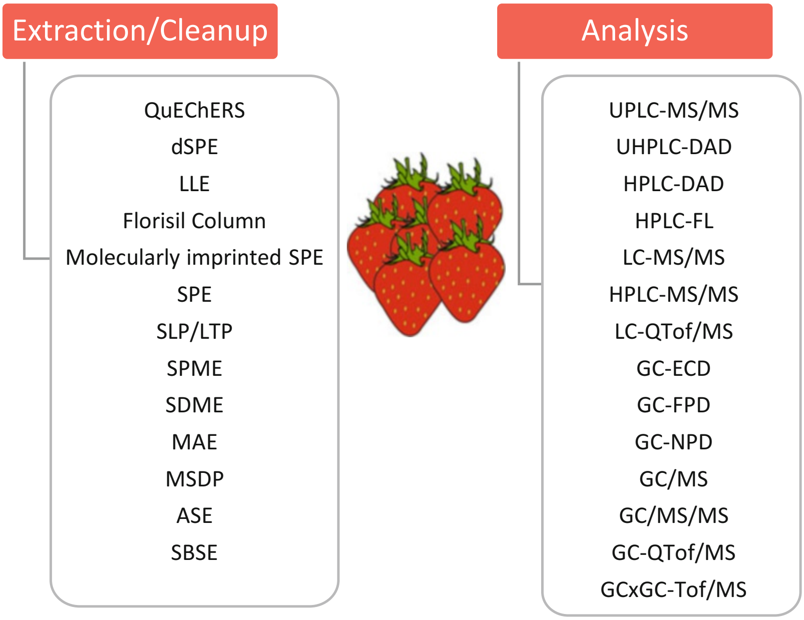

Fig. 5.1 Sample preparation methods examples and analytical methods used in pesticide analysis of strawberry samples. *ASE* accelerated solvent extraction, *DAD* Diode array, *d-SPE* Dispersive solid phase extraction, *ECD* Electron capture detector, *FL* Fluorescence, *FPD* Flame photometric detector, *GC* Gas Chromatography, *HPLC* High performance liquid chromatography, *LC* Liquid chromatography, *LLE* Liquid-liquid extraction, *MAE* Microwave-assisted extraction, *MS* Mass spectrometry, *MS/MS* tandem Mass spectrometry, *MSPD* matrix solid-phase dispersion, *NPD* Nitrogen and phosphorus detector, *Q* Single quadrupole, *QuEChERS* Quick Easy Cheap Effective Rugged Safe, *SBSE* Stir bar sorptive extraction, *SDME* Single drop microextraction, *SLE/LTP* Solid-liquid extraction with low temperature partition, SPE Solid phase extraction, *SPME* Solid phase microextraction, *ToF* Time of flight, *UHPLC* Ultra high performance liquid chromatography, *UPLC* Ultra performance liquid chromatography

sensitive analytical methods are desired. Nowadays, the multi-residue methods, able for the analysis of large numbers of pesticides in a single run, is one the most common efficient approach (Susana Grimalt and Dehouck 2016). There are several studies focus on the extraction, analysis, and quantification of pesticide residue on agricultural products, notably strawberry. Some of these studies are present in Table 5.1. Many extraction methods have been developed for pesticide analysis (Fig. 5.1). The majority of these studies use Quick Easy Cheap Effective Rugged Safe (QuEChERS) as the extraction procedure (Abad-Fuentes et al. 2015; Anagnostopoulos et al. 2013; Christia et al. 2015; Evard et al. 2015; Virgínia et al. 2011; Frenich et al. 2008; He et al. 2015; Huskova et al. 2009, 2010; Koesukwiwat et al. 2010; Lawal et al. 2019; Lehotay et al. 2010, 2011; Lucini and Molinari 2011; Malhat et al. 2014; Martinez-Pierna et al. 2018; Oshita and Jardim 2014, 2015a, b; Qi et al. 2016; Romero-Gonzalez et al. 2014; Stachniuk and Fornal 2013; Sun et al. 2015; Taibon et al. 2015; Vera et al. 2013; Wang et al. 2015; You et al. 2017; Zhang

Table 5.1 Analytical methods for pesticides analysis in strawberries

Number of pesticides	Sample preparation	Detection	Recoveries	LOD/LOQ	References
2	**Extraction:** QuEChERS (4 g anhydrous $MgSO_4$ and 1 g anhydrous NaOAc) **Solvent:** MeCN **Cleanup:** d-SPE (50 mg PSA, 150 mg anhydrous $MgSO_4$, and 150 mg C18)	GC–QToF/MS	70–120%	– / 0.01 mg/kg	Cervera et al. (2014)
13	**Extraction:** QuEChERS (4 g anh$MgSO_4$, 1 g NaCl, 1 g NaCit.H_2O and 0.5 g NaHCit.H_2O **Solvent**: MeCN **Cleanup:** d-SPE (150 mg anh$MgSO_4$ and 25 mg PSA	LC–MS/MS	70–120%		Oshita and Jardim (2014)
7	**Extraction:** QuEChERS (6 g anh$MgSO_4$, 1.5 g NaOAc) **Solvent:** 1% (v/v) acetic acid in MeCN **Cleanup:** d-SPE (150 mg anh$MgSO_4$, 50 mg PSA, 50 mg C18 and 50 mgGCB)	UPLC–MS/MS	84–136%	0.8–2.0 / – μg/L	Abad-Fuentes et al. (2015)
19	**Extraction:** QuEChERS ($MgSO_4$ and NaOAc). **Solvent:** 0.1% (v/v) acetic acid in MeCN **Cleanup:** d-SPE ($MgSO_4$ and SiO_2)	UPLC-MS/MS	65–138%	0.1–2.5 / 0.3–7.5 ng/L	Christia et al. (2015)
11	**Extraction:** QuEChERS (4 g $MgSO_4$, 1 g NaCl, 1 g NaCit.$H_2$0 and 0.5 g NaHCit.$H_2$0 NaHCit. H20 **Solvent:** MeCN **Cleanup:** d-SPE (150 mg $MgSO_4$, 25 mg PSA)	UHPLC-DAD	70–120% except chlorpyrifos		Oshita and Jardim (2015b)
10	**Extraction:** QuEChERS (4 g $MgSO_4$, 1 g NaCl, 1 g NaCit.$H_2$0 and 0.5 g NaHCit.$H_2$0 **Solvent:** MeCN **Cleanup:** d-SPE (900 mg anh$MgSO_4$ and 150 mg PSA)	UHPLC-QqLIT	77–115%	0.01–0.2 / 0.05–1 μg/Kg	He et al. (2015)

(continued)

Table 5.1 (continued)

11	**Extraction:** QuEChERS (4 g $MgSO_4$, 1 g NaCl, 1 g NaCit.H_2O and 0.5 g NaHCit.H_20 **Solvent**: MeCN **Cleanup:** d-SPE (150 mg $MgSO_4$ and 25 mg PSA)	LC-MS/MS	72–108%		Oshita and Jardim (2015a)
		UHPLC-MS/MS	72–108%		
		UHPLC-DAD	79–119%		
3	**Extraction:** QuEChERS (6 g anh$MgSO_4$ and 1.5 g NaOAc) **Solvent:** MeCN **Cleanup:** d-SPE (150 mg $MgSO_4$, 50 mg PSA and 50 mg C18)	HPLC-MS/MS	81–105%	0.6 / 2 μg/kg	Sun et al. (2015)
3	**Extraction:** QuEChERS (4 g $MgSO_4$, 1 g NaCl, 1 g NaCit.H_20 and 0.5 g NaHCit.H_20 NaHCit.H_20 **Solvent:** MeCN **Cleanup:** d-SPE (855 mg $MgSO_4$, 150 mg PSA, 45 mg GCB)	UHPLC-QToF/MS	82–105%	⍰1 / 1.7–2 ng/mL	Taibon et al. (2015)
4	**Extraction:** QuEChERS (6 g anh$MgSO_4$ and 1.5 g NaOAc) **Solvent**: MeCN **Cleanup:** d-SPE (150 mg $MgSO_4$, 50 mg PSA, 50 mg C18)	HPLC-MS/MS	86–105%	– / 3.3 μg/kg	Wang et al. (2015)
		GC-MS/MS		– / 1.0–6.0 μg/kg	
1	**Extraction**: QuEChERS (6 g $MgSO_4$ and 1.5 g NaOAc) **Solvent:** 1% (v/v) acetic acid in MeCN **Cleanup:** d-SPE (150 mg $MgSO_4$, 25 mg PSA, and 25 mg C18)	GC-ECD GC-MS			Covaciu et al. (2016)
1	**Extraction:** QuEChERS (6 g $MgSO_4$ and 1.5 g NaOAc) **Solvent**: MeCN **Cleanup:** d-SPE (150 mg anh$MgSO_4$ and 50 mg PSA)	LC-QToF/MS	77–100%	0.4–1 /–μg/L	Qi et al. (2016)

1	**Extraction:** LLE **Solvents:** MeOH, aqueous NaCl and hexane **Cleanup:** SPE (10 g florisil, 0.5 g charcoal and 2 g anh$MgSO_4$) **Solvent:** conditioner and elution (DCM/acetone mixture (85:15 v/v))	HPLC-DAD	85–93%	0.002 / 0.01 mg/kg	Saber et al. (2016)
20	**Extraction:** d-SPE (PANI-coated zeolite NaY sorbent) **Solvent:** MeCN and NaOH	HPLC	70–113%	0.05–1 / 0.20–7 mg/L	Arnnok et al. (2017)
2	**Extraction:** MIP-SPE **Solvents:** conditioner (6 mL MeOH and double-distilled water); elution (2 mL MeOH/double-distilled water/acetic acid (95:5:2, v/v/v))	HPLC	87–92%	– / 0.011–0.015 mg/kg	Li et al. (2017a, b)
1	**Extraction:** QuEChERS (2 g NaCl) **Solvent:** MeCN **Cleanup:** d-SPE (150 mg $MgSO_4$ and 15 mg hydroxylated multi-walled carbon nanotubes)	LC-MS/MS	92–111%	– / 5 ng/g	Li et al. (2017a, b)
12	**Extraction:** QuEChERS (6 g $MgSO_4$ and 1.5 g NaOAc) **Solvent:** Ethyl acetate **Cleanup:** d-SPE (150 mg $MgSO_4$ and 50 mg PSA) **Solvent:** Ethyl acetate	GC–ECD	73–120%	– / 0.001–0.01 mg/kg	Vilca et al. (2017)
1	**Extraction:** QuEChERS (4 g $MgSO_4$ and 1 g NaCl) **Solvent:** MeCN **Cleanup:** d-SPE (150 mg $MgSO_4$ and 30 mg PSA)	HPLC–MS/MS	89–106%	– / 10 μg/kg	You et al. (2017)

(continued)

Table 5.1 (continued)

7	**Extraction:** QuEChERS (4 g $MgSO_4$ and 1 g NaCl) **Solvent:** MeCN **Cleanup:** d-SPE (10 mg sorbent (Fe_3O_4@TEOS@MPS) and 10 mg PSA)	GC-FPD GC-MS/MS	72–115%	LODs and LOQs values were in range 3.64–10.38 μg/kg	Fernandes et al. (2018)
5	**Extraction:** QuEChERS (6 g anh$MgSO_4$ and 1.5 g NaOAc) **Solvent**: 1% (v/v) acetic acid in MeCN **Cleanup:** d-SPE (750 mg anh$MgSO_4$, 125 mg C18 and 125 mg PSA)	LC–MS/MS	89–114%	MDL 0.01–0.05 ng/g / MQL 0.02–0.1 ng/g	Martinez-Pierna et al. (2018)
41	**Extraction:** QuEChERS (4 g $MgSO_4$ and 1 g NaCl) **Solvent:** MeCN **Cleanup:** d-SPE (150 mg $MgSO_4$, 5 mg multi-walled carbon nanotubes and 25 mg PSA)	GC-MS	60–123%	– / 1–20 μg/kg	Zhao et al. (2018)
8	**Extraction:** QuEChERS (6 g $MgSO_4$ and 1.5 g NaOAc) **Solvent:** MeCN **Cleanup:** d-SPE (150 mg $MgSO_4$ and 50 mg PSA)	HPLC-MS	89–120%	0.02–0.54 / 0.07–1.79 μg/kg	Lawal et al. (2019)
5	**Extraction:** QuEChERS (4 g anh$MgSO_4$ and 1 g NaAc) **Solvent:** MeCN **Cleanup:** d-SPE (175 mg PSA and 1 g anh$MgSO_4$)	GC-FPD	82–100%	0.02–0.05 / 0.07–0.1 μg/g	Souza et al. (2014)
15	**Extraction:** QuEChERS (4 g anh$MgSO_4$ and 1 g NaAc) **Solvent:** MeCN	UPLC/QqQ-MS/MS	71–112%	0.2–3 / 0.5–10 μg/kg	Romero-Gonzalez et al. (2014)

1	**Extraction:** QuEChERS (4 g anh$MgSO_4$ and 1 g NaCl) **Solvent:** MeCN **Cleanup:** d-SPE (100 mg PSA, 20 mg GCB and 600 mg anh$MgSO_4$)	HPLC-DAD	99–110%	0.003 / 0.01 μg/g	Malhat et al. (2014)
7	**Extraction:** QuEChERS (4 g anh$MgSO_4$ and 1 g NaCl) **Solvent:** MeCN (w/ 1% acetic acid) **Cleanup:** d-SPE (50 mg PSA and 300 mg anh$MgSO_4$)	GC-ECD	72–115%	0.49–2.27 / 1.64–7.57 μg/kg	Lorenz et al. (2014)
1	**Extraction:** SPE (Extrelut) **Solvent**: Elution with DCM	HPLC/QqQ-MS/MS	75–87%	0.5 / 1.0 μg/kg	Lemes et al. (2014)
1	**Extraction:** SLE/LTP **Solvent:** MeCN	GC-ECD	98–109%	9 / 31 μg/kg	Heleno et al. (2014)
11	**Extraction:** SLE/LTP **Solvent:** MeCN	GC-ECD	79–111%	0.004–0.016 / 0.011–0.047 mg/kg	Guedes et al. (2014)
56	**Extraction:** QuEChERS (4 g anh$MgSO_4$, 1 g NaCit.$H_2$0, 0.5 g NaHCit.$H_2$0 and 1 g NaCl) **Solvent:** MeCN **Cleanup:** d-SPE (25 mg PSA, 2.5 mg GCB and 150 mg anh$MgSO_4$)	LC-MS/MS	–	0.5–22.7 /– μg/kg	Stachniuk and Fornal (2013)
10	**Extraction:** SPME (PDMS/DVB fiber)	GC–ToF/MS	89–117%	0.5–5 μg/kg	Souza-Silva et al. (2013)
6	**Extraction:** LLE **Solvent:** 40 mL acetone and 60 mL dichlorometane:hexane (1:1 v/v)	GC-ECD	77–115%	0.003–0.007 / 0.005–0.013 mg/kg	Soares et al. (2013)
1	**Extraction:** SPE (HLB) **Solvents:** conditioner: acetone; wash: water; Elution: ethyl acetate	GC-MS/MS	77–82%	0.001 / 0.003 mg/kg	Peruga et al. (2013a, b)
6	**Extraction:** LLE **Solvent:** 40 mL acetone:water (70:30)	LC–(ESI)-MS/MS	82–110%	0.1–0.7 / 0.3–2.3 μg/kg	Peruga et al. (2013a, b)

(continued)

Table 5.1 (continued)

1	**Extraction:** QuEChERS (5 g NaCl) **Solvent**: MeCN **Cleanup:** SPE (absorbent cotton,1 cm anh$MgSO_4$, 1 g charcoalcelite chromatographic mixture (1:20, w/w), and 1 cm anh$MgSO_4$) **Solvents:** conditioner: hexane:acetone (97:3 v/v); Elution:hexane: acetone (97:3 v/v)	HPLC-DAD	93–100%	– / 0.01 mg/kg	Malhat et al. (2013)
1	**Extraction:** QuEChERS (4 g $MgSO_4$ and 1 g NaCl) **Solvent:** MeCN **Cleanup:** d-SPE (25 mg PSA and 150 mg $MgSO_4$)	GC–NCI–MS	94–114%	23 ng/kg / 1 μg/kg	Hrouzkova et al. (2013)
		GC–EI–MS	95–103%	46 ng/kg / 1 μg/kg	
27	**Extraction:** QuEChERS (6 g anh$MgSO_4$, 1.5 g NaCit.H_2O, 0.75 g NaHCit.H_2O and 1.5 g NaCl) **Solvent:** MeCN and water **Cleanup:** d-SPE (150 mg PSA, 50 mg C18 and 150 mg anh$MgSO_4$) **Solvent:** MeCN	LC-MS/MS	–	–	Fernandes et al. (2014)
171		LP-GC-MS/MS GC × GC-ToF/MS	–	–	
14	**Extraction:** QuEChERS (4 g anh$MgSO_4$, 1 g NaCit.H_2O, 0.5 g NaHCit.H_2O and 1 g NaCl) **Solvent:** MeCN	LC-MS/MS LC-ToF	33–109%	– / 0.02 mg/kg	Anagnostopoulos et al. (2013)
4	**Extraction:** molecularly imprinted matrix solid-phase dispersion **Solvent:** DCM and acetic ester	Micellar electrokinetic chromatography with DAD	58–98%	20.9–24.4 / 69.7–81.3 μg/kg	Wen et al. (2012)
1	**Extraction**: QuEChERS (100 g Na_2SO_4, 3.5 g $NaHCO_3$ and 0.3 g EDTA) **Solvent:** MeCN	LC–MS/MS	80–91%	0.0012 / 0.01 mg/kg	Peruga et al. (2012)
3	**Extraction:** QuEChERS (4 g anh$MgSO_4$ and 1 g NaCl) **Solvent:** MeCN **Cleanup:** SPE (silica cartridge) **Solvent:** conditioner: and Elution: MeCN	HPLC–DAD	90–115%	0.11–0.54 mg/kg	Lopez-Fernandez et al. (2012)

3	**Extraction:** LLE **Solvent:** 80 mL acetone; 25 mL saturated NaCl solution; 250 mL distilled water and 100 mL DCM	GC-MS	85–98%	0.005 / 0.01 μg/kg	Galhiane et al. (2012)
1	**Extraction**: Headspace **Solvent:** 1.5% stannous (II) chloride in 5 M HCl	GC-FPD	82–87%	0.004 / 0.01 μg/kg	
1	**Extraction:** SPE (C18) **Solvent:** conditioner: MeCN/water 80:20 (v/v); Elution: MeCN	HPLC-fluorescence	83–89%	0.001 / 0.01 μg/kg	
14	**Extraction:** SDME **Solvent:** hexane	GC-MS/MS	70–97%	0.002–0.11 / – μg/kg	Fernandes et al. (2012a, b, c)
25	**Extraction:** QuEChERS (6 g anh$MgSO_4$, 1.5 g NaCit.H_2O, 0.75 g NaHCit.H_2O and 1.5 g NaCl) **Solvent:** MeCN **Cleanup:** d-SPE (150 mg PSA, 50 mg C18 and 150 mg anh$MgSO_4$)	GC-MS/MS	60–154%	0.1–8.1 / 0.3–26.9 μg/kg	Fernandes et al. (2012a, b, c)
49	**Extraction:** QuEChERS (6 g anh$MgSO_4$, 1.5 g NaCit.H_2O, 0.75 g NaHCit.H_2O and 1.5 g NaCl) **Solvent:** MeCN **Cleanup:** d-SPE (150 mg PSA, 50 mg C18 and 150 mg anh$MgSO_4$)	GC-MS/MS	59–154%	<12 / – μg/kg	Fernandes et al. (2012a, b, c)
5	**Extraction**: MIP-SPE **Solvent:** conditioner: MeOH and Britton–Robinson buffer; Elution: MeOH	GC-FPD	65–88%	0.02–0.05 / – μg/g	Baldim et al. (2012)
2	**Extraction:** QuEChERS (6 g anh$MgSO_4$ and 1.5 g NaAc) **Solvent:** MeCN Cleanup: d-SPE (50 mg PSA, 50 mg C18 and 150 mg anh$MgSO_4$)	HPLC-MS/MS	81–93%	0.6 / 2 μg/kg	Zhang et al. (2011)

(continued)

Table 5.1 (continued)

12	**Extraction:** Pressurized liquid extraction **Solvent:** ethyl acetate	LC-QqQ-MS/MS LC-QIT-MS/MS LC-QqTF-MS/MS	> 70%	– / 0.0005–0.4 mg/kg	Soler et al. (2007)
8	**Extraction:** HS-SPME (PDMS fiber) Other conditions: MeOH/acetone (1:1), and topped up with distilled water containing 10% NaCl to 5.0 g	GC-ECD	> 80%	0.01–1 μg/L / –	Chai et al. (2008)
53	**Extraction:** QuEChERS (4 g anh$MgSO_4$, 1 g ammonium acetate) **Solvent:** MeCN with 1% acetic acid	UPLC-MS/MS	72–110%	0.2–3.0 / 0.05–10 μg/kg	Frenich et al. (2008)
100	**Extraction:** Sample was homogenized with sodium sulphate and Sodium hydrogen carbonate **Solvent:** ethyl acetate	UPLC-ToF/MS	62–153%	0.01–0.02 mg/kg / –	Taylor et al. (2008)
25	**Extraction:** QuEChERS (4 g anh$MgSO_4$ and 1 g NaCl) **Solvent**:10 mL MeCN	GC–MS (EI and NCI)	70–110%	0.1–5 g/kg / –	Huskova et al. (2009)
8	**Extraction:** HS-SDME **Solvent:** 1.5 μL toluene drop to the headspace a 5 mL aqueous solution	GC-ECD	70–104%	2–1000 / 6–3000 μg/L	Kin and Huat (2009)
13	**Extraction:** LLME and MMSPE procedure **Solvents**: 1-octanol, toluene:hexane (60:40 v/v)	GC-ECD	83–123%	0.5–1.10 / 1.69–3.85 μg/kg	Bedendo and Carasek (2010)
31	**Extraction:** QuEChERS (4 g anh$MgSO_4$ and 1 g NaCl) **Cleanup:** d-SPE (25 mg PSA and 150 mg $MgSO_4$) **Solvent:** 10 mL MeCN	GC-MS (EI)	–	– / < 5 mg/kg	Huskova et al. (2010)

13	**Extraction:** SPE (C18 Conditions: preconditioned with MeCN followed by H_2O into another flask containing 10 g NaCl	UPLC-MS/MS	majority 2-120%;	0.001–0.043 ng/g / –	Kamel et al. (2010)
14	**Extraction:** QuEChERS (4 g anh$MgSO_4$, 2.5 g $NaOAc{\cdot}3H_2O$) **Cleanup:** d-SPE (50 mg PSA, 50 mg C18 and 150 mg anh$MgSO_4$) **Solvent:**1% acetic acid in MeCN	HPLC-MS/MS	41–107%	–	Lehotay et al. (2010)
1	**Extraction:** LLE **Solvent:** DCM	HPLC-PAD	89–94.4%	10 μg/kg / –	Chen and Zhang (2010)
3	**Extraction:** HS-SPME (sol–gel hybrid PDMS fiber) **Solvent:** DCM	GC	86–111%	0.005 / 0.015–0.016 μg/kg	Ibrahim et al. (2010)
150	**Extraction:** Buffered and unbuffered versions **Cleanup**: d-SPE/disposable: 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, and 7.5 mg GCB. **Solvent:** MeCN	LP-GC-ToF/MS	>126 analytes 70–120%	10 ng/g / –	Koesukwiwat et al. (2010)
8	**Extraction:** HS-SPME (PDMS fiber) Conditions: optimum dilution was made with distilled water containing 10% NaCl	GC-ECD	79–90%	0.01–1 / 0.05–5 μg/L	Chai and Tan (2010)
1	**Extraction**: LLE **Solvent:** petroleum ether and methylene chloride	GC-ECD	–	–	Yarpuz-Bozdogan et al. (2011)
88	**Cleanup:** Envi-Carb cartridge coupled with NH_2-LC. **Solvent:** MeCN	GC–MS	63–137%	0.006–0.05 / 0.02–0.15 mg/kg	Yang et al. (2011)
14	**Extraction:** QuEChERS (6 g anh$MgSO_4$, 1.5 g NaCl, 1.5 g NaCit.H_2O, and 0.75 g NaHCit.H_2O) **Cleanup:** 150 mg PSA, 150 mg $MgSO_4$, and 50 mg C18 **Solvent:** MeCN	GC- MS/MS	46–128%	3.43–9.90 μg/kg / –	Fernandes et al. (2011b)
151	**Extraction:** QuEChERS (6 g anh $MgSO_4$ and 1.5 g NaOAc)	LP-GC-ToF	–	–	Lehotay et al. (2011)

(continued)

Table 5.1 (continued)

	Cleanup: d-SPE (75 g anh$MgSO_4$, 1.25 g PSA, 1.25 g C18 and 0.188 g ENVI-Carb GCB) **Solvent:** MeCN 1% glacial acetic acid				
5	**Extraction**: QuEChERS (4 g anh$MgSO_4$ and 1 g NaCl, followed by 1 g NaCit.H_2O and 0.5 g NaHCit.H_2O) **Solvent**:10 mL MeCN **Cleanup:** d-SPE (0.85 g $MgSO_4$ and 0.125 g PSA)	LC-MS/MS	87–96%	0.003–0.04 mg/kg / –	Lucini and Molinari (2011)
2	**Extraction:** LLE **Solvent:** Acetone	LC-QTOF	76–122%	–	Grimalt et al. (2007)
151	**Extraction:** QuEChERS (4 g $MgSO_4$, 1 g NaCl); **Solvent:** MeCN **Cleanup:** d-SPE (25 mg PSA, 150 mg $MgSO_4$)	GC-MS/MS	71%–110%	4 µg/kg / 10 µg/kg	Bolanos et al. (2007)
6	**Extraction:** LLE **Solvent:** MeCN with added NaCl	LC-MS	>78%	– / 0.01–0.5 mg/kg	Caboni et al. (2006)
4	**Extraction:** MAE and SPME (PDMS or PDMS–DVB fiber) **Solvent:** water co-solvent: MeCN	GC-MS	–	0.9–13.8 / 2.8–41.3 µg/kg	Sanusi et al. (2004)
7	**Extraction:** SPME (PDMS/DVB fiber) **Solvent:** water with NaCl	GC-MS	–	0.003–0.025 / – mg/kg	Beltran et al. (2003)
7	**Extraction:** HS-SPME (PDMS-coated fiber) **Solvent:** water with Na_2SO_4	GC-MS	76–94%	5.2–10.7/17.3–35.7 µg/kg	Lambropoulou and Albanis (2003)
5	**Method:** modified AOAC 985.22, 1995	GC	–	–	Hakala et al. (2003)
38	**Extraction:** LLE **Solvent:** Ethyl acetate with Na_2SO_4, $NaHCO_3$	HPLC-MS	70–81%	–	Taylor et al. (2002)

2	**Extraction:** HS-SPME (polyacrilate fiber) **Solvent:** Buffer Britton-Robin son 0.2 M with NaCl	GC-MS	91.6–106%	2.0–3.0 / 6.1–9.0 ng/g	Navalon et al. (2002)
5	**Extraction:** SPME (polyacrilate fiber) **Solvent:** water with NaCl	GC-MS	–	30.0–100.0 ng/kg / –	Zambonin et al. (2002)
92	**Extraction:** LLE **Cleanup**: GPC **Solvent:** MeCN	GC-NPD, GC-FPD	70–121% (86 pesticides) 22–54% (5 pesticides)	–0.3–20 ng/g / –	Ueno et al. (2001)
17	**Extraction:** Liquid **Solvent:** acetone, petroleum ether, and methylene chloride	GC-FPD GC-ECD GC-MS	–	–	Safi et al. (2002)
14	**Extraction:** MSPD (Florisil or silica) and anh. $MgSO_4$ **Solvent:** elution with hexane-ether ethyl (2:1, v/v), DCM-acetone (9:1, v/v) and hexane	GC-ECD/GC-NPD	83–103%	– / 0.01–0.40 mg/kg	Morzycka (2002)
5	**Extraction:** MAE **Solvent:** water	HPLC–DAD	–	0.013–0.022 / 0.067–0.074 mg/kg	Cao et al. (2001)
	Extraction: SPME (PDMS/DVB fiber)				
5	**Extraction:** MSPD (C8 adsorbent)	LC–APCI–MS	62–89%	0.005–0.05 / 0.02–0.2 mg/kg	Pous et al. (2001)
	Solvent: DCM				
	and MeOH				
11	**Extraction:** ASE with SPME (PDMS/DVB fiber)	GC-MS	0.4–20%	1– 40 / – µg/g (ASE-SPME) 0.5–5 / – µg/g (ASE-SBSE)	Wennrich et al. (2001a, b)
	Solvent: water–acetone (9:1) and water				
	Extraction: ASE with SBSE				
	Solvent: water–acetone (9:1)				

(continued)

Table 5.1 (continued)

11	**Extraction:** ASE with SPME and ASE with SBSE **Solvent:** water-acetone, and water	GC-MS	–	<0.5–5 μg/g (ASE-SBSE) and 1–40 μg/g (ASE-SPME)	Wennrich et al. (2001a, b)
76	**Extraction:** LLE and GPC **Solvent:** MeCN	GC-ECD	70–125%	0.1–5 ng/g / –	Ueno et al. (2000)

APCI atmospheric pressure chemical ionization, *ASE* accelerated solvent extraction, *C18* octadecylsilyl, *DAD* Diode array, *DCM* dichloromethane, *d-SPE* Dispersive solid phase extraction, *DVB* divinylbenzene, *ECD* Electron capture detector, *EDTA* Ethylenediamine tetraacetic acid, *EI* electron impact, *ESI* Electrospray ionization mode, *FPD* Flame photometric detector,*GC* Gas Chromatography, *GCB* graphitized carbon black, *GPC* Gel permeation chromatography, *HPLC* High performance liquid chromatography, *HS* Headspace, *IT* Ion trap, *LC* Liquid chromatography, *LLE* Liquid-liquid extraction, *LLME* Liquid-liquid microextraction, *LOD* limit of detection, *LOQ* limit of quantification, *LP* low-pressure, *MAE* Microwave-assisted extraction, *MDL* method detection limit, *MeCN* acetonitrile, *MeOH* methanol, $MgSO_4$ Sulphate magnesium, *MIP-SPE* molecularly imprinted solid-phase extraction, *MMSPE* microporous membrane solid-phase extraction, *MQL* method quantification limit, *MS/MS* tandem Mass spectrometry, *MS* Mass spectrometry, *MSPD* matrix solid-phase dispersion, Na_2SO_4 Sodium sulfate, $NaCit.H_2O$ Sodium citrate tribasic dihydrate, *NaCl* Sodium Chloride, $NaHCit.H_2O$ Sodium hydrogen citrate sesquihydrate, $NAHCO_3$ Sodium bicarbonate, *NaOAc* sodium acetate, *NaOH* Sodium hydroxide, *NCI* negative chemical ionization, *NPD* Nitrogen and phosphorus detector, *PAD* photodiode array detector, *PDMS* Polydimethylsiloxane, *PSA* Primary secondary amine, *Q* Single quadrupole, *QqLIT* triple quadrupole-linear ion trap hybrid, *QqQ* Triple quadrupole, *QuEChERS* Quick Easy Cheap Effective Rugged Safe, *SBSE* Stir bar sorptive extraction, *SDME* Single drop microextraction, SiO_2 Silicon dioxide, *SLE/LTP* Solid-liquid extraction with low temperature partition, *SPE* Solid phase extraction, *SPME* Solid phase microextraction, *ToF* Time of flight, *UHPLC* Ultra high performance liquid chromatography, *UPLC* Ultra performance liquid chromatography et al. 2011; Zhao et al. 2018). The remain extraction methodologies embrace dispersive solid-phase extraction (Arnnok et al. 2017; Zhao et al. 2018), molecularly imprinted solid-phase extraction (Li et al. 2017a, b; Wen et al. 2012), extraction using solvents (Chen and Zhang 2010; Grimalt et al. 2007; Peruga et al. 2013a, b; Sheu and Chen 2009; Taylor et al. 2008; Ucan et al. 2009; Walorczyk and Gnusowski 2009; Zhao et al. 2018), solid-phase extraction (Kin and Huat 2009; Lemes et al. 2014; Yang et al. 2011; Zhao et al. 2018), solid-liquid extraction with low temperature partition (Guedes et al. 2014), liquid-liquid extraction (Akiyama et al. 2002; Galhiane et al. 2012; Taylor et al. 2002; Zhang et al. 2011), pressurized liquid extraction (Soler et al. 2007), microwave-assisted extraction (Cao et al. 2001), matrix solid-phase dispersion (Pous et al. 2001), and solid-phase microextraction (Beltran et al. 2003; Cao et al. 2001; Chai et al. 2008; Chai and Tan 2010; Ibrahim et al. 2010; Kin and Huat 2009; Lambropoulou and Albanis 2003; Navalon et al. 2002; Sanusi et al. 2004), vapour phase-liquid phase microextraction (Gonzalvez et al. 2011), polypropylene microporous membrane solid phase extraction (Bedendo and Carasek 2010) and single-drop microextraction (Fernandes et al. 2012a, b, c; Kin and Huat 2009). For most of the studies, recoveries were higher than 70%. The concentration of the analytes in the samples was adjusted with the recoveries (Anagnostopoulos et al. 2013).

5.1.3.1 Sample Preparation

An accurate sample preparation procedure is required in order to obtain a representative and homogeneous sample. The sample preparation procedure is still the main step in pesticide quantification, taking higher than 80% of the time of analysis (Chen et al. 2008). The entire process of sample preparation can include sample homogenization, analytes extraction from the matrix, and extract purification in order to remove interfering substances. The reduction of the complexity of the sample treatment has been achieved with a simultaneous evolution in the extraction methods and an improvement in the accuracy and precision of the analytical detection techniques (Perestrelo et al. 2019).

5.1.3.2 Extraction and Cleanup

The first step of the strawberry sample treatment for an analytical protocol generally involves a grinding step with frozen or non-frozen samples. The next crucial step is liquid-solid extraction that consists of analytes extraction into a liquid phase (usually with an organic solvent), which can be used for additional cleanup and concentration steps. Several organic solvents have been used in pesticides extraction in strawberries: acetonitrile with/without 1% acetic acid (Abad-Fuentes et al. 2015; Christia et al. 2015; Covaciu et al. 2016), ethyl acetate (Soler et al. 2007; Taylor et al. 2002, 2008; Vilca et al. 2017), dichloromethane (Chen and Zhang 2010; Ibrahim et al. 2010; Pous et al. 2001; Wen et al. 2012), hexane (Fernandes et al. 2012a, b, c), methanol (Baldim et al. 2012; Huskova et al. 2009; Pous et al. 2001), toluene (Chai et al. 2008), petroleum ether (Gonzalvez et al. 2011; Safi et al. 2002), methylene chloride (Gonzalvez et al. 2011; Safi et al. 2002), acetone (Grimalt et al. 2007; Ueno et al. 2001), and water with Sodium Chloride (Chai et al. 2008; Chai and Tan 2010) (Beltran et al. 2003; Zambonin et al. 2002). There are also some studies that mentioned the use of different mixtures: methanol:acetone (1:1) (Chai et al. 2008), hexane:acetone (1:1) (Akiyama et al. 2002), hexane:ether ethyl (2:1) (Morzycka 2002), dichloromethane:acetone (9:1) (Morzycka 2002) and water:acetone (9:1)) (Wennrich et al. 2001a, b).

5.1.3.3 Quick, Easy, Cheap, Effective, Rugged, Safe Method

In 2003, Anastassiades et al. (2003) presented a new approach based on organic solvent (with acetonitrile) extraction followed by a cleanup using dispersive solid phase extraction with different sorbents (primary secondary amine and octadecylsilyl) for the extraction of pesticides in fruit and vegetables. The present sample treatment procedure was called Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS). The advantages demonstrated by the method allowed to became popular namely because of its minimal use glassware, solvents and laborious analytical steps (Susana Grimalt and Dehouck 2016). Out of a total of 88 scientific papers between 2000 and 2018, around 50% are based on the QuEChERS using acetonitrile with or without 1% acetic acid as an extraction solvent.

This technique is the most reported for the analysis of pesticides in strawberries. Table 5.1 shows the number of research papers with QuEChERS during the last decade. The results obtained with this technique are consistent and the obtained recoveries reach values between 70% and 120% for most of the studied compounds.

In general, three reference methods are widely described, the original unbuffered method (original QuEChERS) consists of 4 g sulphate magnesium and 1 g Sodium Chloride, the European Committee for Standardisation (CEN-15662) (Method EN 15662 2007-10-24) which use acetonitrile with a citrate buffer during the extraction; and the AOAC "Method 2007.01", using for the extraction, acetonitrile and acetate buffer (AOAC Official Method 2007.01). With the addition of Sodium Chloride salt, the solubility of the analyte in the aqueous phase reduces and allows migration to the organic solvent (salting-out effect). Sulphate magnesium is added to remove the excess of water. Sulphate magnesium is hygroscopic and will react with water to form $MgSO_4{\cdot}7H_2O$ (solid). The buffer salts are used preferentially for pH labile compounds.

The QuEChERS method allows the simultaneous extraction of non-polar and polar compounds with acceptable recoveries, making it suitable for the extraction of several compounds. The final extract, being solved in acetonitrile, gives full flexibility in the choice of the determination technique. This technique also uses smaller amounts of solvent, compared to solid phase microextraction, solid-phase extraction, and liquid-liquid extraction, making it cheaper, faster, and better for the environment. Co-extracted compounds tend to adsorb in chromatographic systems such as injection port and column, resulting in matrix effects and poor chromatographic performance. To decrease the matrix effects and increase the chromatographic performance, an efficient cleanup step is coupled to the QuEChERS extraction to remove co-extractives. The cleanup step removes organic acids, lipids, proteins, excess water, anthocyanins, and sugars. The composition of this cleanup step consists of sulphate magnesium to remove the remaining water, octadecylsilyl sorbent to remove fat and lipids and primary secondary amine sorbent to remove organic (fatty) acids and sugars. Weak anion exchange sorbent, namely primary secondary amine, is used to remove co-extractives as fats and sugars that can form hydrogen bonds. Graphitized carbon black is used to retain planar molecules, such as chlorophylls, carotenoids, and sterols (Fumes et al. 2015; Perestrelo et al. 2019). The salt and the sorbents ensure that the co-extractives end up in the solid phase, and only the pesticides remain in the organic layer. Another promising sorbent is Supel QuE Z-Sep (zirconia on silica) (Madej et al. 2018). Zirconium will react with hydroxyl groups following Lewis Acid-Lewis base interactions. An advantage of the Supel QuE Z-step sorbent is that this significantly diminishes fatty matrix interferences and various pigments (better than traditional octadecylsilyl sorbent). It can replace the traditional octadecylsilyl and primary secondary amine sorbents in current methods without additional method development (Masiá et al. 2016).

5.1.3.4 Other Sample Preparation Techniques

Classical extraction techniques based on Liquid-liquid extraction is yet one of the chosen methods for the extraction of pesticides from strawberries. Due to the simplicity of the techniques and the possibility of customization, Liquid-liquid extraction became the basis of several purification techniques. In general, in terms of recoveries, the Liquid-liquid extraction showed also a good performance. For example, florisil columns (Morzycka 2002; Saber et al. 2016) and gel permeation chromatography (Ueno et al. 2000, 2001) have been reported as a cleanup step combined with Liquid-liquid extraction (Caboni et al. 2006; Chen and Zhang 2010; Galhiane et al. 2012; Grimalt et al. 2007; Peruga et al. 2013a, b; Saber et al. 2016; Soares et al. 2013; Ueno et al. 2001; Yarpuz-Bozdogan et al. 2011). Solid-phase extraction is reported in three papers, octadecylsilyl (Galhiane et al. 2012), Extrelut (Lemes et al. 2014), and HLB (Peruga et al. 2013a, b) are the report cartridges. In these 3 studies with solid-phase extraction, only one pesticide was extracted and octadecylsilyl cartridge (Galhiane et al. 2012) presented a better recovery value. Other approaches were described based on microextraction techniques, namely, solid phase microextraction (Beltran et al. 2003; Cao et al. 2001; Chai et al. 2008; Chai and Tan 2010; Ibrahim et al. 2010; Lambropoulou and Albanis 2003; Navalon et al. 2002; Sanusi et al. 2004; Souza-Silva et al.2013; Zambonin et al. 2002), stir bar sorptive extraction (Wennrich et al. 2001a, b) and Single drop microextraction (Fernandes et al. 2012a, b, c; Kin and Huat 2009), which consume a reduce volumes of solvents and can even be done in solvent-less and faster way. The solid phase microextraction is described in 9 studies using three different fibers

(Polydimethylsiloxane, Polydimethylsiloxane/ divinylbenzene, and polyacrylate) (Beltran et al. 2003; Cao et al. 2001; Chai et al. 2008; Chai and Tan 2010; Ibrahim et al. 2010; Lambropoulou and Albanis 2003; Navalon et al. 2002; Sanusi et al. 2004; Souza-Silva et al. 2013; Zambonin et al. 2002). Other studies reported different approaches of solid phase microextraction with combination with microwave-assisted extraction and accelerated solvent extraction (Wennrich et al. 2001a, b). In addition, further applications such as matrix solid-phase dispersion, with C8 adsorbent, florisil and silica and also with a molecularly imprinted material, have been reported as a sample preparation approach.

5.1.4 Analysis

The analysis of the purified sample is the last step of the analytical process. For pesticide analysis, chromatographic techniques have long been the preferred methods for analysis of pesticides. Gas chromatography and liquid chromatography with mass spectrometry provide the sensitivity, accuracy, and separation required for the quantification of compounds present in strawberries.

5.1.4.1 Gas Chromatography

Gas chromatography is one of the classical methods of pesticide quantification. Gas chromatography technique is suitable for the separation and quantitative determination of compounds that are volatile or semi-volatile and thermally stable at the temperature of the measurement. Gas chromatography amenable pesticide classes that do not need derivatization include; chloroacetanilides, organochlorines, organophosphorus, pyrethroids, and triazines. Moreover, some products of triazines, organochlorines, and phenylureas are gas chromatography amenable and when derivatized some transformation products of organophosphorus, pyrethroids, and phenoxyacid herbicides are also gas chromatography amenable. Derivatization procedure is applied for some pesticides more polar such as phenoxy acid herbicides and carbamates(Raina 2011). The analytical approach for pesticides residue analysis used gas chromatography with 4 different detectors: electron capture detector (Covaciu et al. 2016), nitrogen and phosphorus detector (Morzycka 2002; Ueno et al. 2001), flame photometric detector (Morzycka 2002; Ueno et al. 2001) and mass spectrometry (Zhao et al. 2018). The first three detectors presented high sensitivity and selectivity for pesticides of interest:

For organochlorine pesticides by the presence of halogens (chlorine), the electron capture detector shows the best sensitivity and selectivity. The organophosphorus and nitrogenated pesticides are preferably analysed by nitrogen and phosphorus detector. The flame photometric detector because of the filter for sulfur and phosphorus is a specific analyser for pesticides composed of these elements. Particularly for these detectors, specific methods are performed, usually with one or two classes of pesticides. Nowadays, the labs prefer multi-residue analysis with mass spectrometry due to its versatility, higher sensitivity, and selectivity for several pesticides. The mass spectrometry presents different performances such as tandem mass spectrometry (Oshita and Jardim 2015a; Wang et al. 2015) and high-resolution mass spectrometry. The most common mass spectrometry analyzers used in the strawberry analysis are single quadrupole, triple quadrupole (Soler et al. 2007), ion trap, hybrid quadrupole ion trap, time of flight (Souza-Silva et al. 2013) and orbitrap.

5.1.4.2 Levels of Pesticides Found in Strawberries by Gas Chromatography

A total of 45 papers reporting the presence of pesticides in strawberry samples (Table 5.2). The organochlorine pesticides were found in several studies (Fernandes et al. 2011b, 2012a, b, c; Hakala et al. 2003; Vilca et al. 2017; Wennrich et al. 2001a, b). Two of them reported concentrations above the maximum residue levels (Wennrich et al. 2001a, b, c). In 2012, Fernandes et al. (2012a, b, c) reported the presence of 7 different organochlorine pesticides in strawberries. Pyrethroids (Akiyama et al. 2002; Beltran et al. 2003; Fernandes et al. 2012a, b, c; Sanusi et al. 2004) and organophosphorus pesticides (Akiyama et al. 2002; Fernandes et al. 2018; Lambropoulou and Albanis 2003; Lorenz et al. 2014; Safi et al. 2002; Ueno et al. 2001; Zhao et al. 2018) were also detected by gas chromatography analysis. Several works described the presence of different pesticides (e.g. iprodione, cyprodinil, myclobutanil, among others (Cervera et al. 2014; Guedes et al. 2014; Morzycka 2002; Souza-Silva et al. 2013; Zambonin et al. 2002). The highest values found by Gas chromatography analysis were reported for iprodione (1.05 mg/kg) and mepanipyrim (0.32 mg/kg) (Fernandes et al. 2012a, b, c) in Portugal, and myclobutanil (24–119 mg/kg) and propiconazole (54–500 mg/kg) (Souza-Silva et al. 2013) in samples from Canada.

5.1.5 Liquid Chromatography

Liquid chromatography is a useful tool in the detection of pesticides that are thermolabile, non-volatile, do not need to be derivatized. For the analysis of metabolites, usually more polar and less volatile, liquid chromatography is also suitable (Anna Stachniuk and Fornal 2016). The liquid chromatography detectors include; diode array electrochemical, fluorescence, ultraviolet-visible, mass spectrometry, and refractive index. Mass spectrometry provides the most sensitivity and specificity detection and mass spectrometry data provides valuable information regarding the molecular weight, structure, identification, and quantification of the target analytes. The chemical classes of pesticides that have been analysed by liquid chromatography methods include, phenylureas, sulfonylureas, carbamates, dithiocarbamates, phenoxyacid, pyrethroids, and azoles pesticides (Raina 2011). Typically,

Table 5.2 Levels of pesticides found in strawberry samples

Pesticides	Levels mg/kg	Detection	References
Cyprodinil	0.15	GC–QTOF MS	Cervera et al. (2014)
Fenhexamid	0.11		
Boscalid	0.013–0.028	UPLC–MS/MS	Abad-Fuentes et al. (2015)
Aldicarb-sulfone	0.002 (dw)	UPLC-MS/MS	Christia et al. (2015)
Carbofuran-3-hydroxy	0.003 (dw)		
Dimethoate	0.004 (dw)		
Disulfoton sulfone	0.009 (dw)		
Phosalone	0.004 (dw)		
Phoxim	0.002 (dw)		
Pyrazophos	0.002 (dw)		
Cyazofamid	0.006 (dw)		
Propachlor	0.004(dw)		
Metalaxyl	0.00018–0.17	UHPLC-QqLIT	He et al. (2015)
Myclobutanil	0.00064–0.10		
Hexaconazole	0.0045–0.12		
Triadimefon	0.00054–0.0015		
Epoxiconazole	0.00034–0.00074		
Pyraclostrobin	0.043–0.052	HPLC-MS/MS	Sun et al. (2015)
Myclobutanil	0.031–0.131		
Difenoconazole	0.039–0.096		
Pyraclostrobin	0.028–0.043	HPLC-MS/MS, GC-MS/MS	Wang et al. (2015)
Iprodione	0.42–0.60		
Tebuconazole	0.044–0.17		
Cyprodinil	0.21–0.65		
Iprodione	0.0086	GC-ECD, GC-MS	Covaciu et al. (2016)
Pyrisoxazole enantiomers Day 0	0.205–0.454	LC-QToF/MS	Qi et al. (2016)
Pyrisoxazole enantiomers Day 7	0.124–0.277		
Pyrisoxazole enantiomers Day 21	0.043–0.070		
Hexythiazox Day 3	0.287–0.989	HPLC-DAD	Saber et al. (2016)
Hexythiazox Day 7	0.211–0.750		
Hexythiazox Day 14	0.014–0.418		
Thiamethoxam	0.031	HPLC	Arnnok et al. (2017)
Carbofuran	0.012		
Trichlorfon	1.4	HPLC	Li et al. (2017a, b)
Fluazinam	0.097–0.135	LC-MS/MS	Li et al. (2017a, b)
Endosulfan sulphate	0.003–0.026	GC–ECD	Vilca et al. (2017)
Chlorpyrifos	0.0038–0.0041	GC-FPD, GC-MS/MS	Fernandes et al. (2018)
Malathion	0.3	GC-MS	Zhao et al. (2018)

(continued)

Table 5.2 (continued)

Pesticides	Levels mg/kg	Detection	References
Dimethoate	0.010–0.12	GC-ECD	Lorenz et al. (2014)
Ethylenethiourea	0.001–0.0014	HPLC/QqQ-MS/MS	Lemes et al. (2014)
Chlorothalonil	0.10–0.91	GC-ECD	Guedes et al. (2014)
Iprodione	0.18–2.16		
Difenoconazole	0.09–0.43		
Azoxystrobin	0.09–0.87		
Myclobutanil	24–119	GC–ToF/MS	Souza-Silva et al. (2013)
Propiconazole	54–500		
Etoxazole	0.08	HPLC-DAD	Malhat et al. (2013)
Cyprodinil	0.002–0.004	LP-GC-MS/MS, GC × GC-ToF/MS	Fernandes et al. (2014)
Fenpropathrin	0.029–0.043		
Fluazifop-butyl	0.003–0.005		
Lindane	0.0032–0.027	GC-MS/MS	Fernandes et al. (2012a, b, c)
Aldrin	0.0007–0.0012		
Endrin	0.0002–0.0044		
β-endosulfan	0.0071		
p,p′-DDD	0.0001–0.0004		
o,p′-DDT	0.0001–0.0012		
Methoxychlor	0.0002–0.0019		
Bifentrin	0.054	GC-MS/MS	Fernandes et al. (2012a, b, c)
Cyprodinil	0.040–0.047		
Fludioxonil	0.12		
Iprodione	1.05		
Malathion	0.03		
Mepanipyrim	0.32		
Tetraconazole	0.034		
Tolylfluanid	0.04		
Lindane	0.027	GC-MS/MS	Fernandes et al. (2012a, b, c)
Iprodione	1.071		
Pymetrozine	0.06	UPLC-MS/MS	Frenich et al. (2008)
Oxamyl	0.01–0.06		
Nitenpyram	0.01–0.07		
Methomyl	0.04–0.15		
Carbendazim	0.01–0.10		
Thiamethoxam	0.02		
Imidacloprid	0.01–1.00		
Acetamiprid	0.01–0.25		
Thiacloprid	0.06–0.09		
Promecarb	0.06		
Spinosad	0.01–0.04		
Indoxacarb	0.03–0.04		
Azoxystrobin	0.045	LC – MS/MS	Taylor et al. (2008)
Pyrimethanil	0.199		
Myclobutanil	0.028		

(continued)

Table 5.2 (continued)

Pesticides	Levels mg/kg	Detection	References
Fenhexanid	0.084		
Bupirimate	0.034		
Boscalid	0.312		
Myclobutanil	0.067		
Cyprodinil	0.029		
Pyraclostrubin	0.053		
Fluodioxinil	0.023		
Iprodione	0.041	GC- MS	Huskova et al. (2009)
Dicofenol (diff application mode)	0.0002–0.269	GC-ECD	Yarpuz-Bozdogan et al. (2011)
Lindane	0.013–0.027	GC-ECD, GC-MS, GC-MS/MS	Fernandes et al. (2011b)
β-endosulfan	<LOD-0.007		
Azadirachtin A	0.03	LC-MS	Caboni et al. (2006)
Azadirachtin B	0.01		
Bifenthrin	<LOD–0.13	GC-MS	Sanusi et al. (2004)
λ-Cyhalothrin	0.014–0.07		
Acrinathrin	<LOD–0.03		
Deltamethrin	<LOD–0.1		
λ-Cyhalothrin	n.d.-0.21	GC-MS	Beltran et al. (2003)
Diazinon	0.17	GC-MS	Lambropoulou and Albanis (2003)
Fenitrothion	0.17–0.18		
Fenthion	0.17–18		
Parathion Ethyl	0.17		
Bromophos Methyl	0.16		
Bromophos Ethyl	0.15–0.16		
Ethion	0.15		
Tolylfluanid	0.01–0.12	GC-MS	Hakala et al. (2003)
Dichlofluanid	0.01–0.15		
Iprodione	0.03–0.4		
Endosulfane	0.004–0.007		
Azinfos-methyl	0.02		
Myclobutanil	0.50–0.53	HPLC-MS	Taylor et al. (2002)
Penconazole	0.05		
Pyrimethanil	0.18–0.19		
Triadimefon	0.011	GC-MS	Zambonin et al. (2002)
Penconazole	0.0056		
Myclobutanil	0.018		
Procymidone	<0.1	GC/MS, LC-FL	Akiyama et al. (2002)
Bifenthrin	<0.05		
Triflumizole	<0.5		
Bitertanol	<0.5		
Dichlorvos	<0.1		
Acetamiprid	<0.5		
Carbaryl	≤ 1		

(continued)

Table 5.2 (continued)

Pesticides	Levels mg/kg	Detection	References
Carbaryl	0.018	GC-FPD, GC-NPD	Ueno et al. (2001)
Myclobutanil	0.005		
Chlorpyrifos	0.019	GC-FPD, GC-ECD, and GC-MS	Safi et al. (2002)
Chlorthalonil	0.079		
Penconazole	0.129		
Iprodione	0.573		
Captafol metab	2.196		
Chlorfluazuron	0.234		
Dichlofluanid	0.040–0.500	GC-ECD, GC-NPD	Morzycka (2002)
Procymidone	0.040–0.550		
Vinchlozolin	0.008–0.350		
Malathion	0.018	GC – FPD, GC-NPD	Ueno et al. (2001)
Carbendazim	0.06–3.7	HPLC-DAD	Cao et al. (2001)
Diethofencarb	0.03–2.07		
Azoxystrobine	0.38–2.9		
Bupirimate	<LOD–0.21		
Metalaxyl	0.10–0.40	LC–APCI–MS	Pous et al. (2001)
Myclobutanil	0.8		
Thiabendazole	0.45–0.60		
1,2,4-TCB	0.0006–0.0009	CG-MS	Wennrich et al. (2001a, b)
1,2,4,5-TeCB	0.0005–0.0011		
PCB	0.0008–0.0022		
HCB	0.0005–0.0016		
Lindane	0.0008		
p,p′-DDE	0.0069–0.074		
p,p′-DDD	0.0069–0.066		
p,p′-DDT	0.0037–0.016		
1,2,4,5-TeCB	0.0005–0.016		
PCB	0.0005–0.016		
HCB	0.0005–0.016		
p,p′-DDE	0.0037–0.074		
p,p′-DDD	0.0037–0.074		
p,p′-DDT	0.0037–0.074		

APCI atmospheric pressure chemical ionization, *DAD* Diode array, *DDD* Dichlorodiphenyldichloroethane, *DDE* Dichlorodiphenyldichloroethylene, *DDT* diclorodifeniltricloroetano; *dw* dry weight, *ECD* Electron capture detector, *FL* Fluorescence, *FPD* Flame photometric detector, *GC* Gas Chromatography chromatography, *HCB* Hexachlorobenzene, *HPLC* High performance liquid chromatography, *LC* Liquid chromatography, *LP* low-pressure, *MS/MS* tandem Mass spectrometry, *MS* mass spectrometry, *NPD* Nitrogen and phosphorus detector, *PCB* polychlorinated biphenyl, *Q* Single quadrupole, *QqLIT* triple quadrupole-linear ion trap hybrid, *QqQ* Triple quadrupole, *TCB* Trichlorobenzene, *TeCB* tetrachlorobenzenes, *ToF* Time of flight, *UPLC* Ultra high performance liquid chromatography, *UPLC* Ultra performance liquid chromatography pyrethroids have been analyzed by gas chromatography, however, some chromatographic programs are described in the literature for pyrethroid analysis using liquid chromatography (Bariani and Rocha 2010; Silva et al. 2019; Hyland et al. 2019; McCoy et al. 2012; Shimadzu 2014; Tuck et al. 2018; Wu et al. 2012).

5.1.5.1 Levels of Pesticides Found in Strawberries by Liquid Chromatography

Most of the levels found in strawberries are in the range of μg/kg (Arnnok et al. 2017; Caboni et al. 2006; Cao et al. 2001; Christia et al. 2015; Frenich et al. 2008; Li et al. 2017a, b; Malhat et al. 2013; Pous et al. 2001; Saber et al. 2016; Sun et al. 2015; Taylor et al. 2002, 2008; Wang et al. 2015). The ng/kg levels were reported in the studies conducted by He et al. (180 ng/kg−0.17 mg/kg) (He et al. 2015) and in the work of Qi et al. (210–450 ng/kg) (Qi et al. 2016). He et al. (2015) reported the use of liquid chromatography triple quadrupole-linear ion trap hybrid mass spectrometry determination. It was stated that increasing confidence in the analytical results with unambiguous identification using triple quadrupole-linear ion trap hybrid technology was achieved. In the work of Qi et al. (2016) a liquid chromatography coupled to quadrupole time-of-flight mass spectrometry was used. A convenient, reliable, and sensitive liquid chromatography coupled to quadrupole time-of-flight mass spectrometry method for enantio-separation was developed. The method was then applied for the determination of pyrisoxazole enantiomers in different types of matrices (vegetables, fruit, and soils). The results showed an advantage of the highly efficient enantio-separation ability of liquid chromatography and the high-resolution detection of quadrupole time-of-flight mass spectrometry, providing an alternative way for ultra-sensitive analysis of chiral pesticides. Lemes et al. (2014) demonstrate that tandem mass spectrometry with electrospray ionization mode is a useful tool due to the high sensitivity and unequivocal identification in the determination of ethylenethiourea (mainly degradation and/or biotransformation product of ethylenebisdithiocarbamate fungicides), the authors reported concentration of ethylenethiourea in strawberries fruit between 1.0 and 1.4 μg/kg levels.

The highest concentration of pesticides found in strawberries samples were found in the study conducted by (Li et al. 2017). A different approach with a molecularly imprinted polymer and liquid chromatography with diode array detection was used for the detection and quantification of the trichlorfon and monocrotophos in fruits. Trichlorfon was found with a 1.4 mg/kg level in strawberries.

Liquid chromatography with diode array was used to study carbendazim, diethofencarb, azoxystrobine, and bupirimate (Cao et al. 2001) with concentrations found ranged from 0.06 to 3.7, 0.03 to 2.07, 0.38 to 2.9, and < limit of detection – 0.21, respectively. A different approach with microwave-assisted extraction and solid phase microextraction sample preparation technique followed by High-performance liquid chromatography analysis were also proposed (Cao et al. 2001). The authors stated that the developed method should be extended to other matrices and analytes that could be present at very low concentrations. Accelerated solvent extraction shows to be an effective methodology for extracting pesticides, reducing the matrix effects of the samples. The extracting step does not include the use of organic solvents and consequently is environmentally friendly. In terms of sensitivity, the method, with detection limits at low μg/kg levels, are in accordance with the requirements set by European and international regulations for the maximum residue level.

5.2 Conclusion

Pesticides have permitted farmers and handlers of foodstuffs to expand production into new geographical areas, extend shelf life, increase production volume, and enhance the appearance of many commonly grown foods. From the moment of its application, pesticides are distributed by the main ecosystems: atmosphere, water, soil, and sediments, as well as plants and animals. Therefore, low concentrations of these substances can be found in food, thus constituting a potential risk for human health considering their toxicity and the exposure to these compounds (Fernandes et al. 2011a). Several extraction procedures have been reported to determine pesticide residues in strawberries. It is notorious that the introduction of QuEChERS marked a new period in this thematic. New approaches described new extraction methods combined with chromatography analysis to provide better quantitative recoveries at low concentrations. The performance of the chromatography and mass spectrometry detectors is a crucial combination to solve many problems related to food safety. This chapter shows the progress in extraction methodologies and the modern detection equipment, and how this innovative progress has contributed to the best control of food, allowing for an increase in food safety and quality standards.

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Chapter 6
Non-extractable Pesticide Residues in Soils

Helen Karasali and George Pavlidis

Abstract Non-extractable or bound pesticide residues are defined as the residues that continue to bond in soil after extraction with methods which do not considerably modify their chemical nature. The formation of bound or non-extractable residues has been considered since the end of 1960's as a pathway for the removal of chemicals from the freely available into a tightly adsorbed state. Non-extractable residues are tightly bound to soil organic matter whilst the mechanism of their re-release, the time and their form still remain unknown. Their existence in environmental matrices is a key issue as bound or non-extractable residues are linked with the environmental protection and human health. Additionally, it is hard to distinguish the parent compound and metabolite fractions in the bound residues. In some cases the non-extractable residues are considered an environmental solution as toxic compounds are entrapped in soil organic matter and thus are not further released. Multiple applications of pesticides significantly increase the concentration of bound or non-extractable residues but their long-term significance is still not clear. Although the existence of bound residues is known since 1964 many issues remain a 'black box' and should be further addressed.

In order to quantify the amount of non-extractable or bound residues in soil, radiolabelled pesticides are typically used. In that way the amount of the non-extractable residues that is measured corresponds to the non-extractable radioactivity which is frequently determined by sample combustion and liquid scintillation counting. For accurately determination of non-extractable pesticide residues Nuclear Magnetic Resonance technique is commonly used due to its non-disruptive nature. Furthermore, other disruptive techniques are used such as alkaline or acidic extraction, pyrolysis which allows obtaining information on the chemical structure of bound residues. A question that still remains open is whether bound residues could be considered as an environmental solution for immobilizing toxic compounds

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or an unacceptable route for loading the environment with chemicals whose future fate could not be considered.

Keywords Bound residues · Non-extractable residues · Soil · Bioavailability · Soil organic matter · Binding · Pesticides · Definitions · Degradation · Risk assessment

6.1 Introduction

Soil is often called the 'Skin of Earth' and is a relatively complex matter compared to other environmental compartments. It is a highly heterogeneous material not only across Earth's land surface but also with depth (Bone et al. 2010) and is composed of inorganic differently sized mineral ingredients such as sand, silt and clay, organic matter and living entities (Fitzpatrick 2008). It is an important environmental medium as it is related to the agricultural productivity and the environmental quality. It is the main and first recipient of organic chemical pollutants, such as pesticides, which are intentionally or unintentionally released in the environment and may result in 'soil pollution'. 'Soil pollution' refers to the existence of chemicals in the soil with concentration that has adverse effects on any non-targeted organism. As it cannot be directly measured or visually detected, 'soil pollution' is considered as a hidden danger (Rodríguez-Eugenio et al. 2018).

Nowadays, soil contamination is concerned as an alarming problem and in Europe it has been recognized as the third most important warning issue to soil functions (Rodríguez-Eugenio et al. 2018). Potential environmental pollution from pesticides has also raised worries especially for regulatory authorities as they may constitute one of the most harmful classes of pollutants for human health and the environment (Liu et al. 2010; Vega et al. 2005). The lessons learned from the extensive pollution of the aquatic and soil ecosystems urged the authorities to adopt appropriate legislative measures in order to protect the environment against contamination (Vega et al. 2005). Recently, soil significance became a crucial subject in European political schedules (Louis et al. 2014).

Pesticides entering into the environment go through several abiotic and biotic ways, though leach to the groundwater and volatilization to the atmosphere is the most predominant. An unknown portion of pesticides remains in soil and it could be immobilized as bound or non-extractable residues or exist as 'free residues' (Fig. 6.1) (Fenner et al. 2013; Gavrilescu 2005; Benoit and Barriuso 1997). The term 'bound' residues as well as 'free' residues are used to point out that the latter can be readily extracted form soil without changing their chemical structure whereas, the former resist to such extraction (Gevao et al. 2000).

The dividing line between 'bound' and 'free' residues is not clear as even the 'free' residues are not completely free from binding with the soil and may in some extend exhibit, the properties that usually are ascribed to bound pesticides (Gevao et al. 2000).

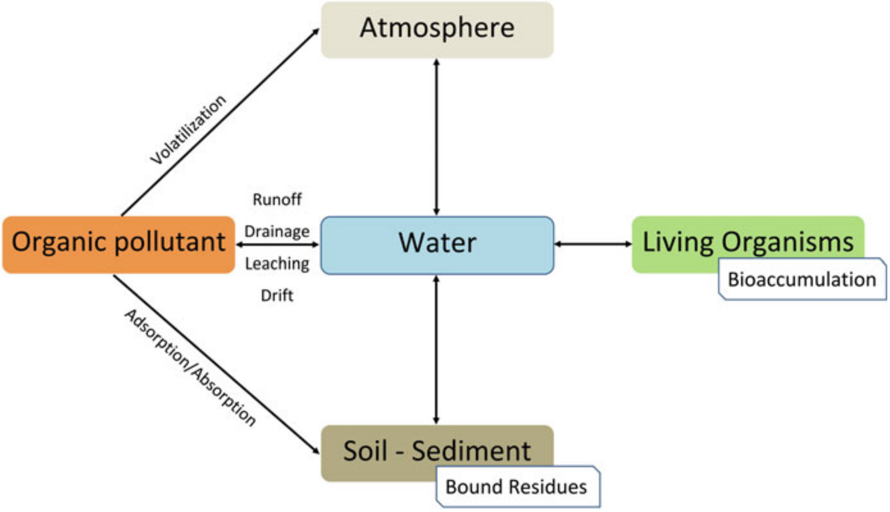

Fig. 6.1 Distribution of organic pollutants in the environment (Modified after European Centre for Ecotoxicology and Toxicology of Chemicals-ECETOC 2013). Organic pollutants may be transported between environmental compartments via different physicochemical processes

Research on non-extractable residues in soil was originated in the mid-1970s, however prior researches have been performed on plants (end of 1960's). Non-extractable residues formation is caused due to various physical and chemical interactions between the parent compound and its metabolites/transformation products with soil organic matter and clay minerals (Bollag et al. 1992, 1998; Piccolo et al. 2001). Non-extractable residues or bound residues extraction, determination and identification could be considered as a challenging and demanding work. Their presence can only be demonstrated with the use of radiolabeled pesticides to properly labeling atom (Bailey and White 1964). The need for standardization procedures has been recognized.

Decades of discussions about non-extractable or bound residues have been done over the years, and many definitions have been proposed by researchers, however, significant confusion still exists about the irreversibility of binding procedures, the conditions of their re-release, the extraction procedures, their identification and quantification and even about their definition. There is also debate with respect on how non-extractable residues should be treated in the environmental risk assessment.

Concerning regulatory evaluation of pesticides and specifically their fate and behavior in the environment many issues as regards non-extractable residues or bound residues should be addressed and guidance should be given to authorities as their existence in soil has a substantial effect on pesticides fate and behavior in the environment (Girardi et al. 2013).

The aim of the present chapter is to update and briefly summarize the existing knowledge on pesticide bound residues or non-extractable residues in soil.

6.2 Nature of Soil Colloids

Soil is an extremely variable and intricate material that covers the earth's land surface and offers an appropriate medium for plant growth. It is a mean of water storage, supply and purification, a modifier of the atmosphere; and an appropriate environment for living organisms. Soil is mainly composed of a) *organic matter* inherited from the decay of plant residues, animal leftovers and microbial tissues, b) *minerals* acquired by the disintegration of rocks, c) *gases* gained from the atmosphere and reactions of roots, d) *microorganisms* and chemicals, e) *liquids*; mainly water, acquired from the atmosphere as well as chemical, physical and microbial reactions in soil, and f) *living organisms* such as worms, insects and microbes, while weather conditions play a decisive role in creating a great variety of soil types concerning soil components content. Though, the variability in soil's composition affects its physical properties, its water and nutrient holding capacity and consequently the fate and behavior of chemicals reaching in soil e.g. adsorption, leaching or chemical decomposition (Calderbank 1989).

The soil mineral fraction is composed of crystalline clay minerals, quartz, amorphous silica, plus crystalline and amorphous oxides and hydroxides of iron and aluminum (Calderbank 1989).

Soil organic matter is a complex mixture of organic ingredients inherited from the (partly or totally) decomposed and non-decomposed organic materials of animal and plant origin (Lichtfouse et al. 1998) and exists in many different forms in soil. It is generally synonymous with humus although this term is usually referred to the well decomposed organic matter called humic substances (Lichtfouse et al. 1998; Rodríguez-Eugenio et al. 2018). Soil organic matter has been additionally separated into humic (e.g. proteins, fats, waxes etc) and non-humic constituents (humic and fulvic acid and humin) (Calderbank 1989).

Soil humic ingredients have been considered as a physically and chemically heterogeneous combination of high-molecular weight aliphatic and aromatic ingredients, produced by secondary reactions during the decay of dead organisms and the microbial activity (Senesi 1993). Though, humic materials composed of exclusive un-decayed plant and animal tissues, their partial decomposition products, and the soil biomass (Senesi 1993). However, the accurate formation of humic substances remains a mystery primarily due to the lack of structural identification at molecular level (Berthier et al. 2000). Humic substances constitute a pool of soil organic carbon encompassing 60–70% of its total quantity (Senesi 1993).

Soil is the medium that supports a wide-variety of living entities such as earthworms, insects, microorganisms as well as small mammals. Soil microorganisms play a pivotal role not only in decomposing organic matter and producing humic constituents in soil, but also in chemically altering the inorganic compounds entering the soil (Senesi 1993).

Soil colloids are the colloidal clusters of soils, consisting primarily of the smallest particles of clay and humus, which play a key role in ion exchange and soil fertility. They are the most active constituents of soil and control its physical and chemical properties. Soil colloids are vital because their surfaces ulcerate soil nutrients dissolved in soil, water as positively charged mineral ions and cations (Senesi 1993). Soil colloids are distinguished into two major categories, the organic and the mineral fraction. Among them the organic part (soil organic matter) is mainly responsible for the adsorption of chemicals (e.g. pesticides), however the mineral elements could also be involved in the adsorption phenomena but in a lesser extent. Nevertheless, their individual contribution is very difficult to be measured in real field conditions. This is quite reasonable as soil organic matter is intimately linked to clay creating a clay-organic complex in which the clay is coated with organic substances (Ahlrichs 1972).

Mineral and organic colloids retain acidic groups which are usually neutralized by cations and have been attracted to the clay and humin surfaces. These cations may be replaced by other cations existing in soil which are known as exchangeable cations. The amount of exchangeable cations retained by soil is referred to as its "cation exchange capacity" (Ahlrichs 1972).

Chemicals (e.g. pesticides) entering the soil could possibly be adsorbed due to their attractiveness in solution or vapor phase to the solid surface (Calderbank 1989). Though, soil colloids are generally liable for the adsorption phenomenon due to their small particle size and large surface area. Humic compounds have higher cation exchange capacity than mineral compounds and consequently organic matter is expected to play a significant role in the absorption of pesticides.

6.3 Types of Soil-Pesticide Interactions

Pesticides in soil comprise of parent compounds and their degradation products that feature a wide range of physical and chemical properties and belong to different chemical classes. Regarding their interactions with humic substances, they can generally be divided into two groups; i.e., those involving specific chemistry forces and the ones that encompass unspecific physical forces (Senesi 1993). Hydrophilic, ionic, ionizable, basic and acidic pesticides belong to the first category whereas hydrophobic, nonionic and non-polar pesticides fit to the second group.

Since 1964 many research activities have been published concerning the behavior of pesticides in soil systems. Adsorption, both chemical and physical one, has been evoked for the explanation of the differences in the behavior of a particular pesticide with different soil types. Adsorption in that case, is the phenomenon that occurs due to the attraction or abhorrence between a solid surface and a vapor solution, as a result of the interaction between the fields of force emanating from the absorbent surface and the absorbate (Bailey and White 1964). Pesticides functional groups, acidic or basic character, water solubility, polarity and charge distribution often play a vital role in the number of adsorption mechanisms that may operate in combination. Pesticides and their degradation products are initially sorbed by sites that offer the strongest binding and when these sites become occupied then sites offer weaker binding will be filled (Calderbank 1989).

Adsorption is undoubtedly the most important type of interactions between soil and pesticides which consequently controls their concentration in the soil liquid phase. Its extent solely depends on the properties of soil and the individual pesticide, which concerning the pesticide comprise its molecular structure, solubility and its acid-base nature (Gevao et al. 2000). Pesticides and their degradation products are sorbed by soil organic matter via physical-chemical binding by particular mechanisms and forces with variable degrees of strengths (Calderbank 1989). The chemical reactions between unaltered pesticides and/or their metabolites frequently preside over the formation of stable chemical bonds resulting in increasing persistence of the pesticide residue in soil and consequently in decreasing its availability to interact with biota or leaching and transporting to other environmental compartments (Calderbank 1989).

The nature of binding forces associated with the adsorption processes of pesticides onto the soil humic substances and the mechanisms includes ionic binding, hydrogen and covalent bonding, charge-transfer or electron donor-acceptor mechanisms, van der Waal forces, ligand exchange, hydrophobic bonding or partitioning and sequestration. These mechanisms do not take place simultaneously; however, two or more may occur concurrently depending on the functional groups of the pesticides and the acidity of the system (Gevao et al. 2000).The use of spectroscopic techniques such as Nuclear Magnetic Resonance, Fourier Transform Infrared Spectroscopy and Electron Spin Resonance in soil analysis have generated evidence on the mechanism by which non-extractable residues interact with soil organic matter. The type of interaction determines the probability and the release rate as well as the form of the molecule that has been immobilized (Kästner et al. 2018). The key mechanisms are presented below.

6.3.1 Ionic Binding

Ionizable pesticides existing in cationic or anionic form under environmental conditions could bind to corresponding opposite charged soil components, thus resulting in strong binding as in the case of covalent bonds. This binding type is considered as reversible (Kästner et al. 2018). Ionic binding involves interaction between the cationic organic groups of the pesticide, such as amines and heterocyclic nitrogen atoms (acceptors) with the carboxylic or phenolic groups (donors) at the organic matter surface (Senesi 1993).

Pesticides and/or their metabolites which exist in cationic form or can be protonated and become cationic are absorbed onto soil humic substances by ionic bonding or cation exchange. This is the dominant mechanism for cationic pesticides such as diquat and paraquat applied in soil in the form of their salts. Both of them exhibit high water solubility and low volatility though they bind on to soil humic substances by ion exchange through their cationic group and form significantly stable and unreactive bonds with the carboxyl groups of the humic substances. However, according to Senesi, possible binding sites on the humic complexes are not employed due to steric hindrance effects (Senesi 1993).

It has also been reported that less basic pesticides such as triazines, amitrole and dimefox, which are weak bases with low water solubility and relatively high volatility, may be protonated at the amino-group at a pH value close to their pKa in order to form ionic bonds with the carboxylate and phenolate group of humic substances (Gevao et al. 2000).

6.3.2 Hydrogen Bonding

Hydrogen bonds are favored when humic substances containing abundant oxygen and hydroxyl functional groups and are considered to play a dynamic role in the adsorption of non-ionic polar pesticides, including substituted ureas, alachlor, metolachlor, malathion, glyphosate and phenylcarbamates. Concerning acidic and ionic pesticides such as dicamba, asulam and others, they can interact with soil organic matter by hydrogen bonding at pH values below their pKa (Gevao et al. 2000).

6.3.3 Van der Waals and Dipole-Dipole Forces

Van der Waals force is a distance-dependent interaction between atoms or molecules. Unlike ionic or covalent bonds, they are comparatively weak and therefore more susceptible to disturbance (Senesi 1993). The van der Waals force quickly vanishes at longer distances between interacting molecules and for that reason their contribution would be greatest for the ions which are in close contact with the surface. Interactions between non-ionic and non-polar pesticides on suitable humic acid compounds are of particular relevance. Since these forces are additive (Senesi 1993), their involvement augments with the size of the interacting compound and its capacity to adjust to the adsorbate surface. Van der Waals forces have been observed for pesticides such as DDT, carbaryl, parathion and picloram (Ahlrichs 1972).

6.3.4 Covalent Binding

A covalent bond is a chemical bond that involves the sharing of electron pairs between atoms. It is well known that interactions between nucleophiles and electrophiles species play a significant role. A nucleophile is a reactant that provides a pair of electrons to form a new covalent bond while an electrophile is a species that accepts a pair of electrons to form a new covalent bond Calderbank 1989). Nucleophiles may also enhance covalent bond formation. Covalent bonds between pesticides and their degradation products with soil humic substances are frequently facilitated by chemical, photochemical and enzymatic catalysts leading to stable and in most cases irreversible incorporation into soil. Anilines and phenols which are released by several pesticides are subject to formation of covalent bonds with soil organic matter (Ahlrichs 1972). Therefore, pesticides and their degradation products with similar structure with phenolic compounds can form covalent bonds with humus.

6.3.5 Charge-Transfer (Electron Donor/Acceptor)

Interaction of an electron-rich π-system with an electron-deficient π -system leading to the transfer of an electron and formation of a π -anion and a π –cation (Kästner et al. 2018). Humic compounds contain in their molecules electron-deficient moieties such as quinones and elector-rich centers such as diphenols facilitating their electron donating and accepting properties (Ahlrichs 1972). Charge transfer complexes are created through electron donor and acceptor mechanisms with pesticides retaining alternatively electron donor or electron acceptor possessions (Gevao et al. 2000). It has been reported that infrared analysis provided evidence that paraquat, diquat, triazines, substituted ureas and amitrole form charge-transfer complexes with humic acids. In the cases of triazine, substituted ureas and amitrole, it should be noted that they act as electron donor with the electron deficient structures in humic acids (Ahlrichs 1972).

6.3.6 Ligand Exchange

Adsorption through ligand exchange implies the substitution of somewhat weak ligands, partially holding a polyvalent metal ion, associated to soil organic matter by a suitable part of pollutants molecule. In soil, ligands of silicates or metal oxides can be exchanged by ligands of chemicals like phosphonates, sulfonates etc. (Kästner et al. 2018). Trazines and other ionic pesticides are expected to bind humic substances by this mechanism (Gevao et al. 2000).

6.3.7 Hydrophobic Bonding and Partitioning

Hydrophobic bonding of humic compounds has been suggested as a significant mechanism for the retention of non-ionic and non-polar pesticides that interact weakly with water. This type of adsorption is considered significant mechanism for organochlorine pesticides and additional for some triazine and phenylureas pesticides.

In contrast to adsorption, partitioning designates a procedure in which the adsorbate dissolves into the organic phase. Though, partitioning is prominent from adsorption by the homogeneous, non-specific distribution of the sorbed material into the entire volume of the organic phase. Partitioning could be described as an equilibrium process equivalent to that between two immiscible solvents (Ahlrichs 1972).

6.3.8 Sequestration

The term ‘sequestration’ refers to the intact pesticide that no longer remains available in the matrix e.g. via physical entrapment in small pores and interstitial cavities which inhibit free movement of the molecule, unless the concerned matrix undergoes some structural change permitting release (European Centre for Ecotoxicology and Toxicology of Chemicals-ECETOC 2013).

6.4 Definition of Bound or Non-extractable Residues

The term ‘Bound Residues’ and the most newly term ‘Non-Extractable Residues’ has been defined and interpreted in many different ways over the past years (Kästner et al. 2014). Generally and independently of the binding process in soil, bound or non-extractable residues are considered the remaining pesticide residues after application until the next growing period or the planting of the succeeding crop (Craven 2000). The term ‘bound residues’ was initially mentioned in 1964 by Bailey and White, whereas has firstly been defined in 1975 by an American Institute of Biological Sciences, Environmental Task Force. Based on that definition bound pesticide residues are considered the non-extractable and chemically not identified compounds which remains in the fulvic and humic acids, as well as in humin fractions of *the soil* after comprehensive sequential extraction with both non-polar and polar organic solvents (Anonymous 1975). The same definition has been suggested in 1976 by Kaufman. Since then many other researchers have given different definitions or interpretations. Khan in 1982 defined as bound residues in soil and plants the chemical species which originate from the use of pesticides and cannot be extracted with extraction techniques commonly used in pesticide residue analyses. As chemical species Khan meant the parent compound or the compounds come from it and extraction had the meaning of the exhaustive removal of the chemical species from soil by the use of organic solvents (Khan 1982). According to that definition metabolites with no pesticidal action or chemical ionic species that are not extracted with organic solvents and require extraction at specific pH were not considered as bound residues.

The abovementioned definitions are based on the un-extractability of the bound residues when the extraction methods used, do not considerably alter the chemical nature of the residue; however, transformation products and/or metabolites were not included. Many other researchers defined bound residues in a very similar way to that of Khan.

In 1984 Roberts formulated a more accurate definition for Non-Extractable Residues that was adopted by the International Union of Pure and Applied Chemistry (IUPAC) and is the most cited one in the literature. Roberts defined the 'Non-extractable' pesticide residues, also stated as 'bound' or "non-extracted" residues, in plants and soil as the chemical entities generated from pesticides which cannot be extracted by methods that are not significantly alter the chemical structure of those residues and/or the matrix. Fragments recycled through metabolic pathways leading to natural products are excluded from this definition (Roberts 1984; Kearney 1982). The nature of the bond can be explained in part by matrix-altering extraction methods and sophisticated analytical techniques. Later, the scope of this definition expanded to include the metabolites of the parent compound (European Centre for Ecotoxicology and Toxicology of Chemicals-ECETOC 2013).

In 1989 Calderbank introduced the concept of the biological activity of bound residues and the possibility of the modification of soil structure; however, this idea still remains doubtful and is not frequently used in the literature. According to Calderbank, 'bound residues' in soils are defined the residues of a specific pesticide or its degradation products which are incapable to exert their original biological activity to any substantial range and/or cannot be extracted without degradation from the soil by methods which are able to destroy the soil structure without altering the chemical structure of the compound (Calderbank 1989).

In 1998, Führ et al., widened the definition of bound residues in order to include the metabolites of the parent compound. According to Führ 'Bound residues' are considered the pesticide residues in soil, plant or animal which still remain in the matrix in the form of the parent compound or its metabolite(s) after extraction. Bound residues are considerably less bioaccessible and bioavailable. The extraction technique must not substantially modify the pesticides themselves or the structure of the matrix. The nature of the bond can be explained by matrix-altering extraction methods and sophisticated analytical techniques (Führ et al. 1998).

In 2000 a simplified categorization of pesticide residues was introduced and pesticide residues in soil were distinguished in two categories the 'free' and the 'bound'. As 'free' were considered the residues that could be readily extracted from soil without modification of their chemical structures, whereas the 'bound' resist to such extraction (Gevao et al. 2000).

In 2002 a modified definition from the International Union of Pure and Applied Chemistry (IUPAC) has been provided in the Organization for Economic Co-operation and Development (OECD) guidelines (OECD 2002).

The Organization for Economic Co-operation and Development (OECD) characterizes as 'Bound residues' or 'non-extractable-residues' in soil, plant or animal, the pesticide residues which remain in the matrix after extraction as the original pesticide or its metabolite(s)/transformation products, lessening significantly their bioaccessibility and their bioavailability. The extraction method must not significantly modify the structure of the pesticide and/or of the matrix. The nature of the

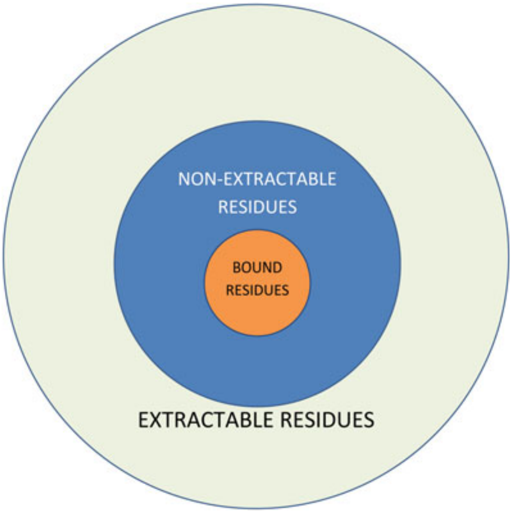

Fig. 6.2 The concept of extractable, non-extractable and bound residues (Modified after European Centre for Ecotoxicology and Toxicology of Chemicals-ECETOC 2013). Bound residues in fact do constitute the lesser fraction of Non-Extractable Residues in soil

bond can be explained in part by matrix-altering extraction methods and sophisticated analytical techniques (OECD 2002).

The definitions of non-extractable residues and bound residues are commonly used interchangeably, although they are not identical, leading to misunderstanding. Discrete definitions for those terms along with extractable residues have been provided later (European Centre for Ecotoxicology and Toxicology of Chemicals-ECETOC 2013; Kästner et al. 2018) (Fig. 6.2).

Extractable Residues (ER) are defined the pesticide residues which can be extracted from the soil using 'mild' extraction methods. 'Mild' extraction methods include aqueous and cold solvent extraction without excessive added energy. These residues are considered as bioavailable and must be taken into account in any impact/risk assessment as they are either freely available or only weekly adsorbed to soil matrix (European Centre for Ecotoxicology and Toxicology of Chemicals-ECETOC 2013; Kästner et al. 2018).

Non-extractable residues (NER) are defined the pesticide residues that cannot be extracted with 'mild' extraction methods; however, they can be extracted using harsher conditions. This type of residues are strongly linked to the matrix, however they may be potentially reversible, but the partitioning is very much in favor of 'binding' to components of the matrix' (European Centre for Ecotoxicology and Toxicology of Chemicals-ECETOC 2013; Kästner et al. 2018).

Bound residues (BR) are defined the pesticide residues that are strongly related with the matrix, often through covalent (or similar) bonds. They are normally indistinguishable from the soil organic material e.g. humus and they are not available either for degradation and/or for indigenous organisms. These residues should be not taken into account in any impact/risk assessment as they usually cannot be released from the matrix or can only be released under extreme conditions where the integrity of the matrix or the compound may be affected (European Centre for Ecotoxicology and Toxicology of Chemicals-ECETOC 2013; Kästner et al. 2018).

The definitions proposed by the European Centre for Ecotoxicology and Toxicology of Chemicals-ECETOC have also been accepted by the European Medicine Agency (EMA) for veterinary medicines (EMA 2016). Though, the non-extractable residues reflect the analytical procedure whereas bound residues reveal the chemical state of the compound. In practice, there is no method available to separate non-extractable residues from bound residues (EMA 2016).

Recently the theory of non-extractable residues 'NER' was reviewed and they were discriminated in three categories Type I, Type II and Type III, based on their process of formation. Consequently the total amount of Non-Extractable Residues (NER) is the sum of the three types (Kästner et al. 2014, 2018). In more detail:

'Type I' of Non-Extractable Residues (NER) are considered the non-extractable **sequestered and entrapped** residues encompassing the parent molecule, its transformation products or both and practically they can be released. 'Type II' of Non-Extractable Residues (NER) are considered the pesticide residues that are covalently bonded to soil or sediment organic matter or to biological tissues in organisms and are being tightly bonded with very low remobilization rates like that of humic matter degradation, derived either from the parent compound or the transformation products. 'Type III' of Non-Extractable Residues (NER) encompasses biogenic Non-Extractable Residues (bioNER) derives from the biotic degradation of the pesticides resulting in their conversion to different biomolecules such as amino *acids and phospholipids* (Kästner et al. 2018).

6.5 Bioaccessibility and Bioavailability of Pesticides

It is well recognized by the environmental scientists that the bioavailability of pesticide residues in environmental systems is a noteworthy issue, which controls not only their total fate and behavior in soil but also their biodegradability and toxicity for biota (Semple et al. 2004, 2007). The bioavailability of pesticide residues in soil is governed by numerous factors with the most significant being the physicochemical properties of the compound and the soil, the ageing time in the soil, the microorganisms of concern and their activity, as well as the climate of the area (Kästner et al. 2018), and all the above shall be assessed in order to identify the potential risk posed by the pesticide and its non-extractable residues.

The terms bioavailability and bioaccessibility should be plainly defined in order to avoid misinterpretation. The ***bioavailable*** fraction of a pesticide residue in soil is defined as the portion that is freely available to cross an organism's cellular pellicle from the medium the organism resides at a given time, whereas the ***bioaccessible*** part is defined as the portion which is available to cross an organism's cellular membrane from the environment, in case that the organism has access to the pesticide, though, the pesticide may be either physically removed from the organism or become only bioavailable after a period of interval (Kästner et al. 2018). Thus, bioaccessibility covers what is really bioavailable at a given time as well as what is theoretically bioavailable and comprises together the readily available pesticide residues present in the water-soluble fraction of soil and those which can become available after desorption from the soil matrix.

Pesticide residues that have been immobilized in soil organic matter could be bioaccessible when soil conditions are modified or the pesticide compound is degraded as in the case of manure application (Yee et al. 1985).

Although, an undoubtedly defined soil extraction procedure for the characterization and quantification of the formation of non-extractable pesticide residues in soil does not exist, their quantity is estimated in relation to the total amount of non-extractable pesticide residues in various matrices such as sludge, compost, soil, and sediments and depends on the extraction procedure, on the matrix itself, and on the isotope labeling position of the test substance (Kästner et al. 2018). The extraction regime is divided into the non-destructive and destructive part. The non-destructive extraction procedure is frequently a three-stage procedure. The first stage is the extraction with aqueous solutions for the determination of the bioavailable portion. It is followed by the extraction of water and organic solvent mixtures for the determination of the readily desorbable portion, which is the bioaccessible fraction. Exhaustive techniques such as accelerated solvent extraction, Soxhlet or microwave extraction are used as the third step of non-destructive procedures for the slowly desorbable pesticide residues. This sequence only leaves the non-extractable residues (Kästner et al. 2018).

Type I and II NER can be distinguished by using silylation (and subsequent analysis for parent substances and transformation products and comparison to the amounts of bioNER formed) of the organic matrix leading to dis-aggregation of humic matter and release of sequestered type I non-extractable pesticide residues, whereas the ones covalently bound to the organic matrix or organo-mineral complexes will not be released (Kästner et al. 2018).

Type III bio-non-extractable pesticide residues can be analysed by full hydrolysis of the matrix with subsequent amino acid analysis (Kästner et al. 2018).

The total non-extractable pesticide residues containing matrix may be additional examined with respect to the binding mechanisms using cleavage or matrix disaggregating methods through subsequent appropriate analytical techniques such as mass spectrometry or Nuclear Magnetic Resonance spectroscopy.

6.6 Agricultural Factors Affecting Bound Residue Formation

Environmental and agricultural factors impact on the type of binding and consequently the fate of pesticides and their degradation products in the soil. Among the most important are: the concentration of the individual pesticide, the number and the mode of applications, the effect of ‘ageing’ and the soil modifications with both organic and inorganic fertilizer (Gevao et al. 2000).

6.6.1 Concentration

The persistence of pesticides and their degradation products in soil has been proved to increase with increasing concentration, while the formation of bound residues diminishes at higher concentrations (Gevao et al. 2000). It has been reported that, the formation of bound residues diversifies irreversibly with increased application rates, whereas the soil micro-organisms play a vital role, as well. Microbial degradation of pesticides is controlled by the availability of the pesticide to soil microorganism. At high application rates, only a small fraction of the pesticide in soil would be in solution and thus available to micro-organisms. It should be mentioned that high concentrations of the pesticide are considered to be toxic for the micro-organisms.

6.6.2 Repeated Applications

Pesticide half-life in soil depends on a number of elements counting climate, soil type as well as the chemical nature of the pesticide. However, the number and interval of applications could also influence the pesticide half-life in soil. Multiple applications of a certain pesticide should be measured as a common agricultural practice in order to achieve desired results and for that reason a clear understanding of the fate and behavior of the applied pesticide in soil under repeated applications is crucial in emerging environmental conventional pesticide control strategies. Samuel and Pillai reported in 1991 that repeated applications result in increased formation of bound residues (Samuel and Pillai 1991). Generally, repeated applications have two effects on the fate of pesticides in soil, ‘accelerated dissipation’ and ‘decelerated’ binding, both of which are considered significant in transfer and retention processes of the applied pesticide (Samuel and Pillai 1991). Consequently, these two effects decline pesticide persistence in soil, thereby lessening potential environmental hazards.

6.6.3 Mode of Application

Cognate with incorporation of pesticides may considerably reduce their losses by volatilization and run-off and thus localize its most significant portion into soil deeper layers. Racke and Lichtenstein reported that the formation of bound residues differs based on the method of application, hence, a greater amount of bound residues formed during incorporation of a pesticide into the soil (Racke and Lichtenstein 1985).

6.6.4 'Ageing'

Ageing is thought to be the result of either a redistribution of the chemical from weaker to stronger adsorption sites, slow chemisorption, or covalent bond formation among the pesticide and the soil organic matter (Gevao et al. 2000). Chemisorption is considered responsible for a continual strengthening of adsorption links and described as an initial rapid sorption followed by a slow but constant rate of sorption (Gevao et al. 2000). Covalent bonds between pesticides and soil humic substances lead to the creation of very stable bonds. It has been observed that these types of interactions may occur with the chloroaniline degradation products of urea and aniline pesticides (Kästner et al. 2018).

It is supposed that the formation of unextractable–bound residues is a time-dependent procedure and thus increasing contact time between soil and pesticide or ageing leads to the formation of a larger proportion of complexes being engaged 'permanently' in soil. With longer residence times in the soil, bound pesticide residues tend to lose their biological activity and become even more resistant to degradation and extraction (Gevao et al. 2000).

6.6.5 Soil Modification with Organic and Inorganic Fertilizers

Soil modifications with organic constituents like manure have been proved to modify the fate of a pesticide in soil. Many researchers reported enhanced dissipation and formation of bound residues in soil after modifications with organic substances (Doyle et al. 1978).

6.7 Bound and Non-extractable Residues

6.7.1 Mechanism of Formation of Bound and Non-extractable Residues

Soil is a highly complex matter composed of living organisms, organic and inorganic materials; nevertheless climate conditions influence its formation (Food and Agriculture Organization-FAO 2006). There is a large variety of soils as regards their composition in clay, sand, silt and soil organic matter (SOM) content, soil texture as well as water and nutrient holding capacity (Calderbank 1989). Soil Organic Matter or humus is also a very complex material formed from microbial actions and chemical reactions containing a wide range of plant and animal remains while its composition is not fixed and primarily depends on the climate. It consists of non-humic substances such as proteins and fats as well as of humic substances which generally consist of humic and fulvic acid and humin (Barraclough et al. 2005).

Although, Soil Organic Matter is the minimum part of the soil; it is of primary significance, as it influences almost all soil physical, chemical and biological properties, yet is the leading element affecting the interactions between the organic pollutants within soil (Kästner et al. 2014). The ability of soil to hold on chemicals such as pesticides is attributed both to chemical reactions and adsorption phenomena taking place on the active surfaces of mineral particles and soil organic matter (Dec et al. 1997; Calderbank 1989; Scheunert and Reuter 2000; Mordaunt et al. 2005). When a pesticide is released in the environment the strength and the nature of the binding process with soil components mainly depends on its physicochemical properties, the soil clay content and mainly the soil organic matter content which plays a predominant role. It is difficult to be determined precisely the contribution of soil organic matter and clay content in this binding process. There are two main types of adsorption, the physical or Van der Waals adsorption which is of low binding strength and the chemical reaction due to coulombic forces (electrostatic bonding) which is of high strength binding (Bailey and White 1964). In the case of chemical reaction a stable bond is formed between the parent molecule and/or its degradation product with soil constituents and consequently the persistence of residue in soil is increased. Bound residues were considered the ones that are covalently bonded with soil components (Mordaunt et al. 2005). As regards the formation of Type I non-extractable residues, it has been proved that they are formed through non-covalent binding processes with different interaction types such as: electrostatic, ionic, ion-dipole, ligand exchange, hydrogen bonding, dipole-dipole, charge transfer, hydrophobic and Van der Waals. The formation of Type II non-extractable residues is based only on covalent-binding process (Gevao et al. 2000; Kästner et al. 2018).

The population of living micro-organisms affects the formation of bound residues (Nowak et al. 2013). Parameters such as water content have a contradictory effect on the formation of bound residues, as in some cases there is little or no influence, whereas in other cases there is a great influence (Anderson 1981; Cupples et al. 2000; Helweg 1987). Agricultural practices used in specific areas have a direct or indirect effect on soil constituents such as soil organic matter and consequently on binding processes.

The relation between the formation of bound residues and the properties of a pesticide has not been studied extensively and there are only few publications available. Nevertheless, based on the published data there is evidence that specific chemical classes have higher capacity than others to form bound residues. In 2002 it was reported that triazines have higher capacity to form BR in comparison with dinitroanilines (Laabs et al. 2002). In 1984, Roberts, reported that carbamates and s-triazines formate considerable amounts of bound residues. Organophosphates are the compounds that form the least quantity of bound residues whereas carbamates and specifically dithiocarbamates form the largest proportion of bound residues (Barriuso et al. 2008). Furthermore, other researchers proved that pesticides containing aniline or phenol groups in their molecule have high capacity to form bound residues (Katan and Lichtenstein 1977; Talebi and Walker 1993; Roberts 1984; Nowak et al. 2010; Kaufman and Blake 1973; Kaufman 1976). Additionally, pesticides containing a large number of halogens (electronegative atoms) in their molecule form reduced quantities of bound residues comparing with molecules with lower number of halogens (Scheunert and Korte 1985; Barriuso et al. 2008; Reddy and Locke 1994).

The formation of bound residues in soil decreases their bioavailability and thus it could be considered as a useful process for enhancing the natural reduction of toxic contaminants in soil.

6.7.2 Extraction – Identification – Quantification

As previously discussed, there are three types of non-extractable residues (Type I, Type II and Type III) which are formed by competing processes and specific extraction schemes have to be used for their determination (Kästner et al. 2018). The third type of non-extractable residues; the biogenic residues or bioNER (Type III) are of no environmental concern as they cannot be distinguished from other biogenic residues and from soil organic matter, however, they can be separated from Type I and Type II Non-Extractable Residues (NER). Therefore, the total amount of Non-Extractable Residues (NER) should be considered the sum of Type I and Type II after the subtraction of biogenic Non-Extractable Residues (bioNER). Extraction procedures for the determination of Type I and II Non-Extractable Residues are thought to be ‘exhaustive’ extractions. The first type of Non-Extractable Residues (Type I), is bioaccessible or becoming bioavailable after release (which is a slow process in the environment) whereas Non-Extractable Residues Type II is in principle bioaccessible only after degradation of soil organic matter to which the residues are covalently bound. The latter process is extremely slow with degradation rates ranging from years to decades (Kästner et al. 2014).

The determined amount of bound or non-extractable residues highly depends on the experimental methodology used for their extraction form soil and for that reason a sequence of different extraction steps have been proposed (Barriuso et al. 2008).

In the first extraction step is often used aqueous solutions such as solutions of salts like calcium or sodium nitrate and ammonium acetate or nitrate, for releasing the rapidly desorbing fraction which are bioavailable for soil microorganisms. Further extraction of available residues with appropriate combination of water and organic solvents at room temperature is performed. However, the selection of solvents is a crucial step along with the analyte physical properties such as its solubility in water and organic solvents. The first extraction step is followed by the extraction of soil matrix in organic solvents such as methanol, acetone or acetonitrile (European Centre for Ecotoxicology and Toxicology of Chemicals-ECETOC 2013; Kästner et al. 2018).

Finally, the strongly adsorbed compounds remaining in soil after the above described procedure should be extracted. At this stage, harsh extraction procedures such as soxhlet, accelerated solvent extraction, supercritical fluid extraction and microwave assisted extraction should be applied. However, it should be kept in mind that these conditions will lead to matrix structural changes (European Centre for Ecotoxicology and Toxicology of Chemicals-ECETOC 2013). The stability of the parent compound and/ or its transformation products should be tested in advance. The extracted residues can be determined by mass spectroscopic of Nuclear Magnetic Resonance (NMR) techniques.

The abovementioned sequence of extractions could leave only Non-Extractable Residues (Type I, Type II and possibly Type III) in the soil matrix and it could be further possible to differentiate the different types of Non-Extractable Residues.

The different types of Non-Extractable Residues could be considered with destructive methods such as silylation which is thought to be a gentle derivatisation method and consequent analysis of the released compounds.

Silylation could break hydrogen bonds between polar functional groups and consequently changes the hydrophylicity of the organic matter, resulting in the release of Non-Extractable Residues Type I that are entrapped in the matrix, however pesticide residues covalent bonded to soil organic matter such as Non-Extractable Residues Type II are not released. Though the two fractions of Non-Extractable Residues, Type I and Type II, can be easily then quantified when radioactively labeled chemical are used and when the biogenic Non-Extractable Residues or Type III have been analyzed. Biogenic Non-Extractable Residues are determined by full hydrolysis of the matrix with subsequent analysis of amino acids and phospholipids when labeling with radioactive and stable isotopes are used. The determination of their amount is crucial as it has to be subtracted from the total amount of non-extractable residues (Kästner et al. 2014).

In practice the whole above-mentioned procedure is used for the quantification of non-extractable residues, however, it could not provide any information on their chemical identity.

In almost all definitions of bound or non-extractable residues in soil it was indicated that ‘the method used for the extraction of the pesticides must not significantly change the chemical structure of the compounds themselves or of the matrix. However, even by ‘mild’ extraction method used could lead to soil structural changes.

It must be taken into account that all the above-mentioned procedures have limitations and for that reason have not been included in any guidance document yet. Standardized and tested methods to be used in routine analysis are absolutely necessary.

As regards the structural identification of non-extractable residues, specific spectroscopic techniques such as Nuclear Magnetic Resonance (NMR), Electron Spin Resonance (ESR) and Fourier Transform Infrared Spectroscopy (FTIR) have been used (Barraclough et al. 2005; Berns et al. 2005; Riefer et al. 2013). Although, Nuclear Magnetic Resonance is a technique more frequently used for the determination of bound residues, its low sensitivity along with the high natural background should be taken into account (Berns et al. 2005).

6.7.3 Bound Residues –Release and Environmental Significance

When a pesticide is released in soil environment it forms a significant amount of non-extractable or bound residues with consequently reduction of its bioavailability and thus decreasing its amount that interacts with the biota. The bioavailability of contaminants in soil controls their degradation rate and hence their fate in soil. It has generally been believed that under certain conditions bound residues are released in the environment but with unknown chemical structure due to inherent difficulties in their identification. The stability of bound residues in soil systems has been studied many years ago and has been proved that bound residues are not always immobilized irreversibly in soil. The parent compound and its metabolites could be released under certain conditions with long term environmental impact. For that reason, bound residues are taken into consideration in the environmental risk assessment (Roberts 1984).

Many researchers wonder if the formation of bound residues is an environmental solution or a problem to be solved. When a pesticide enters in soil then a number of microbial and chemical transformations are taken place and bound residues will become indistinguishable from soil organic matter. Therefore, the environmental significance of bound residues link to the extent that they cannot be distinguished from soil organic matter. In case that soil organic matter releases pesticides that are environmental harmless then the formation of bound residues could be considered as a solution (Barraclough et al. 2005). On the contrary, when soil organic matter releases the parent compound and/or transformation–degradation products then it could be a serious problem that should be taken into account. It is generally believed that chemicals strongly bonded in soil, cannot be easily moved from the binding sites and consequently could not be considered as bioavailable and they have reduced toxicity as well as low possibility to contaminate water bodies. However, it is still not known if chemicals could be continually bonded in soil or released in the environment and poses a threat for it (Khan 1982).

Parent compounds their degradation products and/or metabolites, may interact with soil organic matter and consequently be absorbed resulting in a process called “ageing”, meaning that the compound gradually be more strongly bonded to, or entrapped in, the soil organic matter and respectively become less bioavailable.

The amounts of non-extractable residues generated in soil depend not only on their physicochemical properties but also on soil properties such as clay content, soil organic matter, Cation Exchange Capacity (CEC), soil pH and its living organisms (Guimarães et al. 2018).

Regarding the fate and behavior of an individual pesticide in the environment there are physicochemical parameters that provide strong indication about the molecule mobility or persistence and will be discussed below.

The **octanol/water partition coefficient** (P_{ow} or K_{ow}) is a critical physicochemical parameter that is used for the prediction of the adsorption and the risk of bioaccumulation of pesticides and their degradation products on soil and sediment compartments. Pesticides with logarithmic octanol/water partition coefficient (P_{ow}) value higher than 3 are considered as more hydrophobic (lipophilic) and have a higher possibility to partition into the soil organic phase, thus adsorb onto the soil organic matter and consequently to bioaccumulate. In case that logarithmic octanol/water partition coefficient (P_{ow}) is lower than 1 or negative, the pesticide is considered as hydrophilic, has a greater affinity for water and thus less possibility to adsorb onto soil organic phase (European Centre for Ecotoxicology and Toxicology of Chemicals-ECETOC 2013). The logarithmic value of the octanol/water partition coefficient is solely correctly describing the partition coefficient of uncharged (neutral) compounds, however in the case of ionisable compounds the octanol/water partition coefficient should be corrected according to pH value of the soil (European Centre for Ecotoxicology and Toxicology of Chemicals-ECETOC 2013). Although, soil pH has not a noteworthy effect on the non-ionic molecules sorption, for ionic compounds the sorption could be quite sensitive to soil pH.

Soil adsorption coefficient (K_d) measures the concentration of a pesticide adsorbed onto soil solid matrix per its concentration adsorbed in the aqueous phase at adsorption equilibrium. Soil adsorption coefficient values can differ significantly for an individual pesticide due to differences in the soil organic matter, which are not considered.

Soil organic carbon-water partition coefficient (K_{oc}) is defined as the ratio of the soil adsorption coefficient (K_d) versus the fraction of organic carbon in soil. For non-polar pesticides, the soil organic carbon-water partition coefficient provides a strong indication on its adsorption aptitude onto soil.

Both, soil adsorption coefficient (K_d) and soil organic carbon-water coefficient (K_{oc}) measures the mobility of the pesticide in soil. When the logarithmic value of the soil organic carbon-water partition coefficient (K_{oc}) is lesser than 1.5, then the pesticide presents negligible adsorption to soil and is likely to leach through the soil to groundwater, however for values higher than 4.5 then the pesticide adsorbed very strongly to soil thus its migration to groundwater could be considered negligible (European Centre for Ecotoxicology and Toxicology of Chemicals-ECETOC 2013).

Water solubility is another important parameter indicating the likelihood of the bioavailability of a pesticide and the possibility to form Non-Extractable Residues. A pesticide that is insoluble in water has the tendency to be adsorbed strongly in soil. When the water solubility of a pesticide is lower than 0,1 mg/L then it is considered insoluble in water, while for values higher than 10.000 mg/L it is considered very soluble (European Centre for Ecotoxicology and Toxicology of Chemicals-ECETOC 2013).

Finally, **Henry's law constant** provides a measure of the capacity of a pesticide to undertake exchange from water into the atmosphere in the interface of air-water.

Henry's Law Constant values higher than 10.133 Pa-m^3/mole indicate that the molecule is very volatile from water whereas values lower than 0,010133 Pa-m^3/mole specify a non-volatile molecule (European Centre for Ecotoxicology and Toxicology of Chemicals-ECETOC 2013).

Regarding the biogenic formation of Non-Extractable Residues there is lack of information as few studies are available in the literature. A recent study has been focused on the formation and the fate of biogenic Non-Extractable Residues (Nowak et al. 2013) and the results showed that the potential risk of bound residues from metabolized compounds in soil is highly overestimated. Based on their results the authors proposed that Non-Extractable Residues should be considered as a single component in the environmental risk assessment.

6.8 Conclusions

The reason of this brief review is to make available a summary of the existing knowledge regarding 'non-extractable residues' or 'bound residues' in soil. This area is quite challenging and there are issues that have to be addressed. It seems that although their definition is not specific and clarifications needed, the most important issue raised is their biological ability and the degree of their irreversibility, as soil pollution is directly correlated with the availability of pesticides.

However, our understanding of bound or non-extractable residues has improved meaningfully over the past years due to the developments of new extraction methodologies along with the analytical techniques engaged. A number of binding mechanisms have been recognized and parent molecules as well as metabolites have been determined. Based on the current knowledge bound or non-extractable residues could be considered as an environmental solution as entrapped molecules cannot be released easily. However, as many issues still need clarifications further research on this issue is deemed absolutely necessary in the near future.

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Chapter 7
Pyrethroids Metabolites in Human Urine Samples

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Abstract Pyrethroids are a class of extensively used insecticides for human and animal health protection. Concern has been growing about the possible human health effects from environmental exposure, e.g. chronic exposure to low levels, to pyrethroids. Several metabolites, specific or common to different pyrethroids, can be used for the assessment of pyrethroids exposure. 3-Phenoxybenzoic acid is a major urinary biomarker for numerous pyrethroids used nowadays and has been extensively analyzed and reported. Biomonitoring pyrethroids in human samples is undoubtedly necessary in order to support the clarification of potential correlations between exposure and adverse health effects.

The present chapter is an overview of pyrethroids' metabolitesanalyses in human urine samples, including analytical methods and reported values. Data from several studies from 1990 until March 2019 were collected. Studies from different countries and continents that assess exposure in diverse populations are addressed and different applied analytical methodologies are also discussed. Additionally, human biomonitoring was performed based on highly sensitive analytical methods and reports values for several metabolites, showing the relevance of human biomonitoring of pyrethroids. Some metabolites were below the human biomonitoring assessment values of 2 μg/L for the general population. However, we must consider that exceeds in the upper exposure percentiles of the general population, e.g. children.

Keywords Pyrethroids · Metabolites · Urine · Human biomonitoring · Health · Population · Chromatography · Exposure assessment · Toxicity · Metabolism

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7.1 Introduction

Pyrethroids are a class of extensively used insecticides for human and animal health protection. They are produced from naturally occurring pyrethrins that can be found in pyrethrum extract of the *Tanacetum cineraraefolium.* Their chemical structure is based in an acid and alcohol moiety with an ester bond (Saillenfait et al. 2015). The main active of compounds of the extract are pyrethrin I and II, with pyrethrin I having a monocarboxylic acid moiety while pyrethrin II has dicarboxylic acid (Wakeling et al. 2012).

Even though the study of the chemical structures of the natural pyrethrins started in the 1920s, only five decades later pyrethrins stereochemistry was completed and elucidated (Kaneko 2010). Since then more than 20 analogs molecules are registered and used commercially worldwide. Pyrethroids annual production exceeds a value of $3 billion dollars, these group of insecticides are considered the second-most used in the world (Han et al. 2017). Pyrethroids gained this significant market share by replacing the more toxic or persistent pesticides as the organophosphate or the organochlorines (Klimowska and Wielgomas 2018). Nowadays, pyrethroids are one of the most frequently insecticides detected in a variety of food items (Dalsager et al. 2018).

Synthetic pyrethroids can be classified in two categories, first and second generation. The first-generation pyrethroids, are based on the chrysanthemic acid present pyrethrin I and are highly sensitive to environmental conditions namely to temperature, light and air. As a result, these insecticides have been used mostly in indoor environment. The second-generation pyrethroids, generally have 3-phenoxybenzyl alcohol derivatives. Therefore, they have a great insecticidal activity and additionally a satisfactory stability for application in outdoor. Consequently, the second-generation pyrethroids have been used for agricultural pests control worldwide (Kaneko 2010). Although the potency increased, first and second generation pyrethroids still presented a significant sensitivity to light (Wakeling et al. 2012). The first photostable pyrethroid being developed was permethrin in 1973, which is still one of the most extensively used in agricultural practices (Wakeling et al. 2012). Pyrethroids can be grouped in two classes namely type I and type II, considering toxicological and physical properties. The main difference between groups is the lack, type I or presence of a cyano group, type II. With type II, pyrethroids being more stable due to the presence of the cyano group (Kaneko 2010).

Although considered relatively safe, pyrethroids seem to have a significant impact in human health, being associated with adverse effects namely in the neuro system (Barr 2008; Klimowska and Wielgomas 2018). Several metabolites, specific or common to several pyrethroids, can be used for the assessment of pyrethroids exposure. 3-Phenoxybenzoic acid is considered the main urinary biomarker for several pyrethroids currently used (Morgan et al. 2016a). Therefore, 3-phenoxybenzoic acid has been included as a urinary biomarker in the U.S. national health and nutrition examination survey and Canadian health measures survey biomonitoring programs, as well as other biomonitoring studies worldwide (Aylward et al. 2018).

7.2 PyrethroidsMetabolism and Toxicity

7.2.1 Metabolism

The major exposurepathways to pyrethroids by workers are through respiratory tract, mostly aerosols and dermal contact. For general population is ingestion, i.e. dietary sources (Ratelle et al. 2015). In a review, which included 30 pyrethroids, was concluded that the major metabolic reactions for the studied compounds were commonly oxidation, ester hydrolysis, and conjugation (Kaneko 2010).

After absorption pyrethroids undergo phase I reactions, occurring in the liver, which are oxidation and ester hydrolysis. Oxidation occurs by action of cytochrome P450, in particular human cytochrome P450 2C8, the cytochrome P450 2C9, and cytochrome P450 3A4, and hydrolysis mostly by human carboxylesterase 1 and 2. Then metabolites are conjugated in phase II reactions to generate hydrophilic and lipophilic forms. Mainly glucuronides, sulfates, or amino acid conjugates with elimination half-lives less than 12 h. These hydrophilic conjugates are frequently found and are readily excreted into urine due to higher water solubility (Kaneko 2011; Ratelle et al. 2015; Scollon et al. 2009).

As seen in Table 7.1, some metabolites are specific such as 4-fluoro-3-phenoxybenzoic acid for cyfluthrin or can be common to several parent compounds as 3-phenoxybenzoic acid indicating exposure to cypermethrin, cyhalotrin, ethofenprox, permethrin, deltamethrin, phenothrin, fenpropathrin or esfenvalerate (Morgan et al. 2016a; Ueyama et al. 2010). The most often measured metabolite in several biomonitoring studies and programs is 3-phenoxybenzoic acid (Ueyama et al. 2010). 3-Phenoxybenzoic acid and 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid are essentially excreted in the free and glucuronide form. Until now, only a few studies were performed regarding human toxicokinetics of pyrethroids

Table 7.1 Pyrethroids and some of metabolites. (Adapted from Ueyama et al. 2010)

Metabolites	Parent compounds
4-fluoro-3-phenoxybenzoic acid	cyfluthrin
3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid	cyfluthrin, cypermethrin and permethrin
3-phenoxybenzoic acid	cypermethrin, cyhalotrin, ethofenprox, permethrin, deltamethrin, phenothrin, fenpropathrin, esfenvalerate
cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid	deltamethrin
chrysanthemum-dicarboxylic acid	allethrin, prallethrin, imiprothrin, resmethrin, tetramethrin

(Leng et al. 1997a; Ratelle et al. 2015; Scollon et al. 2009; Woollen et al. 1992), therefore data on this subject is still scarce for many of these compounds. In section exposure assessment the several human studies are presented and discussed in more detail.

Owing pyrethroids short half-lives some limitations should be considered when performing human biomonitoring studies. It is well known that the levels of these less persistent insecticides tend to change in the course of the day and in between days (Barr 2008; LaKind et al. 2019). Thus, one spot urine sample presents limitations, and some authors suggest pooled or even 24 h samples to overcome this constraint. Being this aspect one of the most critical in the assessment of the daily pyrethroid exposure levels (Ueyama et al. 2010). Furthermore, is fundamental to have a deep knowledge on metabolism for a proper biomarker selection.

7.2.2 Toxicity

The pyrethroids insecticidal potential relays on their powerful potential to disrupt the brain and nervous system of insects. Unfortunately, the neurotoxic effect presented in insects is not selective and pyrethroids can affect other non-target species namely humans (Barr 2008). Pyrethroids act on the voltage-gated sodium channels in insect neuronal membranes. However, human sodium channels were considered more resistant. Although this seems to apply in general, in mammals some more sensitive ion channels exist. Additionally, pyrethroids may also target sensitive calcium and chloride channels (van Thriel et al. 2012).

On the other side, children seem to be more susceptible than adults, with the effects of pyrethroids on sodium channels being comparable to the mode of action of some anticonvulsants that are associated with neurodevelopment disorder in fetus occurring in epileptic women using these drugs during pregnancy (van Thriel et al. 2012). In a preliminary study Domingues et al., concluded that the levels 3-phenoxybenzoic acid in urine of autistic children presented differences compare to control ones (Domingues et al. 2016).

Pyrethroids were also included in the list of possible endocrine-disrupting compounds by the United States environmental protection agency (López-Gálvez et al. 2018). Concern has been increasing regarding the possible human health effects from environmental exposure, e.g. chronic exposure to low levels, to pyrethroids. Several epidemiological studies have associated pyrethroids urinary levels to detrimental effects on male reproductive system (Han et al. 2008; Saillenfait et al. 2015), diabetes (Park et al. 2019), cancer (Chen et al. 2016; Ding et al. 2012) or even thyroid hormone levels (Hu et al. 2019). Other studies have focused in exposure during pregnancy particularly in the effect on infants (Furlong et al. 2017; Saillenfait et al. 2015), however the results are inconsistent. Recent reviews have analyzed the data gather from these studies and have considered that the associations were inconsistent or contradictory and thus further research is needed to fully characterize the possible adverse outcomes (Boffetta and Desai 2018; Burns and Pastoor 2018; Saillenfait et al. 2015).

7.3 Methods

To determine analyte concentrations accurately in urine samples, several sample handling steps are necessary prior to the analysis. The handling steps usually start with enzymatic hydrolysis of the conjugated analytes, using enzymes such as beta-glucuronidase, to the native analyte (Dereumeaux et al. 2018; Ratelle et al. 2015; Trunnelle et al. 2014b). In some cases, prior to the extraction, an acid is added to the samples since allows the determination of free and conjugated pesticides (Radwan et al. 2014; Thiphom et al. 2014; Trunnelle et al. 2014a). The traditional extraction techniques based on liquid-liquid extraction and solid-phase extraction continues to be used currently for insecticide analysis in human specimens, being the solid-phase extraction technique the most used for pyrethroidmetabolites analysis in urine samples, Table 7.2.

7.3.1 Liquid-Liquid Extraction

Liquid-liquid extraction is based on the partition of an analyte between two immiscible phases, usually, organic solvent, i.e. organic phase and aqueous phase, e.g. urine. Liquid-liquid extraction is a low-cost technique that is easy to implement and widely used in sample preparation for cleanup and enrichment, which results in signal enhancement. However, this technique has some inconveniences, such as: time-consuming; difficult to automate; frequently uses toxic solvents; and less efficient for highly polar compounds. The solvent choice is based on its polarity; generally, polar solvents extract polar analytes more efficiently than do nonpolar analytes. Therefore, liquid-liquid extraction solvent can extract all molecules with similar physicochemical properties, endogenous and exogenous, i.e. xenobiotics, from the matrix, making this extraction technique not specific for an analyte. Solvents such as n-hexane (Fortes et al. 2013; Kim et al. 2019; Lu et al. 2013; Toshima et al. 2012; Wielgomas and Piskunowicz 2013; Wu et al. 2013; Ye et al. 2017a,b), dichloromethane (Han et al. 2017), toluene (Yoshida 2017), tert-butyl methyl ether (Leng et al. 2006; Ueda et al. 2018) have been selected to perform liquid-liquid extraction by several authors for the pyrethroidmetabolites analysis in urine samples. Table 7.2 shows the sample preparation and analytical parameters details for metabolites analysis in urine samples. In general, n-hexane was the preferred solvent used for the liquid-liquid extraction procedures for gas chromatography analysis.

Table 7.2 Described methodologies for pyrethroids metabolites determination in urine samples

Extraction method	Detection method	Unit	LOD/LOQ	Recoveries (%)	References
Gas chromatography					
N.A.	N.A.	–	–	–	Chen et al. (1991)
	MS	µg/L	0.01–0.05/–	–	Leng et al. (2006)
			0.1/–	–	Ding et al. (2014)
			0.0025–0.005/ 0.0082–0.016	76–86	Coker et al. (2018) and Eskenazi et al. (2018)
		nM	0.5/–	–	Bevan et al. (2013)
HF-LPME	ECD	µg/L	1.6–17/–	15.4–34.1	Lin et al. (2011)
LLE	MS		–/0.5	98.60	Fortes et al. (2013)
			0.02/–	98	Ueyama et al. (2009)
			0.0053–0.013/ 0.018–0.043	37–96	Couture et al. (2009) and Fortin et al. (2008)
			0.04/–	–	Wang et al. (2007)
			0.5–1/–	–	Leng et al. (1997b)
			0.5/–	–	Leng et al. (1997a)
			0.2/–	–	Leng et al. (2003)
LLE w/ n-hexane	ECD		4.9–38/–	76.4–155.8	Lin et al. (2011)
	MS		0.010–0.014/–	–	Kim et al. (2019)
			0.05–0.1/–	–	Wielgomas et al. (2013)
			0.1/–	–	Wielgomas (2013)
			0.1/–	91–108	Lu et al. (2013)
			0.08/–	89.1–103	Wei et al. (2012)

(continued)

Table 7.2 (continued)

Extraction method	Detection method	Unit	LOD/LOQ	Recoveries (%)	References
		–	–	94.32	Han et al. (2008) and Xia et al. (2008)
		µg/L	0.05/–	76.6–125.1	Schettgen et al. (2002b)
			0.10/–	96.2–105.8	Wu et al. (2013)
Hydrolysis with hydrochloric acid. LLE w/hexane	MS		0.1/–	–	Jurewicz et al. (2015)
			0.1/–	–	Radwan et al. (2015)
			0.1/–	–	Radwan et al. (2014)
			0.1/–	–	Wielgomas and Piskunowicz (2013)
	MS/MS		0.01/–	90–104	Schettgen et al. (2016)
Hydrolysis w/ β-glucuronidase. LLE- acidification and extracted w/ hexane. Derivatization and extracted a 2nd time w/ isooctane/ hexane.	MS		0.006–0.01/–	–	Oulhote and Bouchard (2013)
Hydrolysis w/ β-glucuronidase. LLE- extraction w/ hexane. Derivatization w/ hexafluoro-2- propanol plus diisopropylcarbodiimide			0.006–0.01/–	74–82	Dewailly et al. (2014)
LLE w/ butylmethylether	MS/MS		0.03/0.1	–	Göen et al. (2017)
LLE w/ toluene	MS		0.01–0.12/ 0.04–0.41	62–95	Yoshida (2017)
LLE w/ tert-butyl methyl ether	MS/MS		0.01–0.06/–	32–116	Ueda et al. (2018)
Hydrolysis with hydrochloric acid. LLE- w/ tert-butyl-methyl-ether	MS	µg/L	0.02/–	–	Osaka et al. (2016)
			0.054–0.22/–	≥ 93%	Singleton et al. (2014)
LLE w/ dichloromethane			0.1/–	9.07–104.29	Han et al. (2017)

(continued)

Table 7.2 (continued)

Extraction method	Detection method	Unit	LOD/LOQ	Recoveries (%)	References
LLE w/ hexane/acetone		µg/mL	0.15/0.5	–	Boumba et al. (2017)
SPE		µg/L	0.3–0.5/–	90–98	Angerer and Ritter (1997)
SPE (Waters oasis® MAX)			0.05/–	92–104	Elflein et al. (2003)
SPE (C18)	ECD		2/–	75–95	Tuomainen et al. (1996)
	MS		0.1–0.5/–	–	Hardt and Angerer (2003)
			0.1–0.2/–	–	Heudorf and Angerer (2001)
	MS/MS	pg/mL	0.002–0.019/–	87–121	Arrebola et al. (1999)
	MS	µg/L	–/0.1 0.2	–	Schettgen et al. (2002a)
Microextraction by packed sorbent			–/0.06–0.08	92–124	Klimowska and Wielgomas (2018)
Acid extraction and β-glucuronidase hydrolysis			0.004–0.006/ 0.011–0.019	–	Dereumeaux et al. (2018)
Salting-out assisted liquid–liquid extraction			1.5–60.6/–	74–110	Niu et al. (2017)
Liquid chromatography					
N.A	MS	µg/L	0.1–0.4/–	–	Lu et al. (2006)
			0.1/–	–	Migneron-Foisy et al. (2017)
	MS/MS		0.1–0.4/–	–	Panuwet et al. (2018)
			0.03/–	–	Dalsager et al. (2018)
SPE semi-automated	MS		0.1–0.6/–	–	López-Gálvez et al. (2018)
SPE	MS/MS		0.1–0.6/–	–	Wylie et al. (2017)
	N.A	µg/mL	0.05/0.15	85–106	Gammuk et al. (2017)

(continued)

Table 7.2 (continued)

Extraction method	Detection method	Unit	LOD/LOQ	Recoveries (%)	References
	UPLC-MS/MS	µg/L	0.003–0.067/–	80–120	Glorennec et al. (2017)
	MS/MS		–/0.3–5	–	Arcury et al. (2018)
			0.22–1.39/–	–	McKelvey et al. (2013)
			0.1–0.35/–	–	Young et al. (2013)
SPE (C18 cartridges)	UHPLC/Q-ToF MS	fmol	10/–	–	Khemiri et al. (2017) and Khemiri et al. (2018)
	N.A	µg/L	2.5/–	–	Smith et al. (2002)
SPE (Waters oasis® HLB)	MS		0.05–0.10/–	100–110	Khemiri et al. (2017)
	MS	µg/L	–/0.25	–	Morgan et al. (2016a)
	UPLC-MS/MS	pg/mL	18–19/– 0.05–0.31/–	94–100%	Garí et al. (2018)
	MS/MS	µg/L	0.1–0.5/–	72–93%	Baker et al. (2004)
			0.03–0.4/–	94.4–95.5%	Davis et al. (2013)
			0.04/–	90–112%	Toshima et al. (2012)
			0.1–0.5/–	–	Panuwet et al. (2009)
Enzyme hydrolysis. SPE SPE (waters oasis® HLB)			0.25/–	–	Watkins et al. (2016)
	UPLC-MS/MS	ng/µL	0.15–0.30/–	–	Raymer et al. (2014)
Hydrolysis w/ β-glucuronidase/sulfatase.	MS	µg/L	0.16–2.54/–	–	Trunnelle et al. (2014b)
SPE (waters oasis® HLB)	MS/MS		0.25/–	–	Morgan et al. (2016b)
Hydrolysis with β-glucuronidase/arylsulfatase. SPE (C18)	UHPLC/Q-ToF MS		0.1–0.3/–	–	Ferland et al. (2015)
			0.1–0.3/–	–	Ratelle et al. (2016)
LLE w/ hexane	MS/MS		0.015/0.020–0.030	61–90%	Roca et al. (2014)

(continued)

Table 7.2 (continued)

Extraction method	Detection method	Unit	LOD/LOQ	Recoveries (%)	References
	UPLC-MS/MS		0.005–0.007/–	76–101%	Ye et al. (2017a) and Ye et al. (2017b)
LLE w/ diethyl ether-methylene chloride (4:1)	MS/MS		0.5/–	46–98%	Baker et al. (2000)
Hydrolyzed w/ β-glucuronidase aryl sulfatase. QuEChERS (4 g $MgSO_4$; 1 g NaCl; 1 g NaCitrate; 0.5 g disodium citrate sesquihydrate) extraction	UHPLC–HRMS		–/10	–	Roca et al. (2014)
ELISA					
Immunoassays	ELISA	–	–	>86%	Kim et al. (2007)
SPE		μg/L	0.1/2	67–111%	Trunnelle et al. (2014a)
			1.94/2.5	87.3–98.0%	Thiphom et al. (2014)

N.A not available, *LOD* limit of detection, *LOQ* limit of quantification, *ECD* electron capture detector, *MS* mass spectrometry, *MS/MS* tandem mass spectrometry, *UPLC* ultra performance liquid chromatography, *UHPLC* ultra-high performance liquid chromatography, *Q-ToF* quadrupole time-of-flight mass spectrometry, *HRMS* high resolution mass spectrometry, *HF-LPME* hollow fiber liquid-phase microextraction, *SPE* solid phase extraction, *LLE* liquid-liquid extraction, *QuEChERS* Quick, Easy, Cheap, Effective, Rugged, and Safe, *ELISA* enzyme linked immunosorbent assay

7.3.2 Solid-Phase Extraction

Solid-phase extraction is commonly used as an alternative extraction/clean-up method to liquid-liquid extraction for extraction of contaminants in liquid samples (Lambropoulou and Albanis 2007). A typical solid-phase extraction cartridge is made of a short column, usually an open syringe barrel, including a sorbent with a nominal particle size, packed between porous metal or plastic frits. Solid-phase extraction is based on the partition of a solute among the solid stationary phase and the mobile liquid phase, i.e. the biological matrix and numerous solvents. Several sorbents that are used nowadays correspond to the need for general purpose, class-specific and even compound-specific extractions. The improved stationary phases allow the extraction of compounds that were challenging to extract by organic phases. Particularly for pyrethroidmetabolites analysis in urine samples, Oasis HLB (Morgan et al. 2016a, b; Panuwet et al. 2009; Toshima et al. 2012) was the mostly used as well as C18 cartridges (Arrebola et al. 1999; Ferland et al.

2015; Hardt and Angerer 2003; Heudorf and Angerer 2001; Khemiri et al. 2018; Ratelle et al. 2015, 2016; Schettgen et al. 2002a). The solid-phase extraction procedure is frequently divided in four steps: conditioning, retention, selective washing and elution. One of the studies regarding pyrethroidmetabolites in urine samples reported the use of QuEChERS, i.e. Quick Easy Cheap Effective Rugged Safe (Roca et al. 2014) with recoveries values $\geq$93%. The microextraction procedures were also performed using micro extraction by packed sorbent (Klimowska and Wielgomas 2018) and hollow fiber liquid-phase microextraction for pyrethroidmetabolites (Lin et al. 2011).

7.3.3 *Analysis*

The potentially toxic nature of pesticide residues and the consequent implementation of strict regulatory guidelines has led the search for fast, sensitive and reliable analytical methods to determine the target compounds at trace levels (Lehotay et al. 2016). The use of gas chromatography coupled to mass spectrometry (Dereumeaux et al. 2018; Fortes et al. 2013; Jurewicz et al. 2015; Kim et al. 2019; Klimowska and Wielgomas 2018; Leng et al. 1997a; Lu et al. 2013; Radwan et al. 2015; Wielgomas and Piskunowicz 2013; Yoshida 2017) and tandem mass spectrometry (Göen et al. 2017; Ueda et al. 2018) are frequently chosen for the analysis of volatile and nonpolar pesticidemetabolites such as pyrethroid (Tao et al. 2013). In addition, traditional detectors such as electron capture detector is also used (Lin et al. 2011; Tuomainen et al. 1996). However, because pyrethroidmetabolites have lower volatility and thermal stability and are more polar comparing to parent compounds, the analytical method selected is liquid chromatography coupled to mass spectrometry. Moreover, ultra-high-performance liquid chromatography coupled to with quadrupole time-of-flight mass spectrometry showed to be an interesting tool (Khemiri et al. 2018; Lin et al. 2011). More recently, a bioanalytical method using an enzyme-linked immunosorbent assay, that is a lower-cost technique has been established and shown to be a suitable alternative approach for the analysis of pyrethroid metabolite in urine samples for exposuremonitoring (Kim et al. 2007; Thiphom et al. 2014; Trunnelle et al. 2014a). The Table 7.2 summarizes the analytical techniques used for the pyrethroidmetabolites analysis.

7.4 Exposure Assessment

7.4.1 *Reported Levels in Non-occupational Exposed Population*

Since the turn of the millennium an increasing amount of studies have evaluated the internal pyrethroids exposure in the general population and in specific subpopulations, such as children or pregnant women, by measuring urinary concentrations of pyrethroidsmetabolites. These studies are reported in annex Table 7.3. 3-Phenoxybenzoic acid has been the most extensively reported metabolite due to its high detection ratio, which would be expected considering that this metaboliteindicates exposure to several parent compounds, see metabolism section. However, other pyrethroidsmetabolites have been measured, less often, such as 4-methyl-2,3,5,6-tetrafluorobenzyl alcohol, 2,3,5,6-tetrafluorobenzyl alcohol, 4-methoxymethyl-2,3,5,6-tetrafluorobenzyl alcohol, 2,3,5,6-tetrafluoro-1,4-benzenedimethanol, 3-(2-carboxyprop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid, trans-chrysanthemum dicarboxylic acid, 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid, 2-methyl-3-phenylbenzoic acid, 2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropanecarboxylic acid, 2,2,3,3-tetramethylcyclopropanecarboxylic acid, and 2,3,5,6-tetrafluorobenzoic acid (Ueda et al. 2018; Yoshida 2017). These pyrethroidsmetabolites have been analyzed in Japanese children (Ueda et al. 2018) and adults (Yoshida 2017), being these two of the most comprehensive studies with 9 and 11 metabolites, respectively.

Generally, the levels of 3-phenoxybenzoic acid reported for non-occupational exposed population are lower than 2 μg/L, annex Table 7.3, which is below the human biomonitoring assessment values from the German human biomonitoring commission. The same is true for cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (1 μg/L) and trans 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (2 μg/L) (Apel et al. 2017). However, in some studies, annex Table 7.3values higher than these human biomonitoring assessment values were achieved. These higher values are probably due to indoor insecticide or residential application (Baker et al. 2004; Leng et al. 2003).

An important aspect in human biomonitoring studies is the selection of the matrix and time of the sampling. By collecting urine samples in several occasions particularly in the morning, first morning void and 24-h, authors found that the lowest values for 3-phenoxybenzoic acid were achieved in the first morning void and the highest in the 24-h samples. Additionally, was concluded that single/spot samples may not reflect the real exposure (Morgan et al. 2016b).

Attempting a correlation between 3-phenoxybenzoic acid concentrations in plasma and in urine, authors found no correlation, probably because plasma 3-phenoxybenzoic acid concentrations reveals cumulative exposures and urinary 3-phenoxybenzoic acid reveals acute exposures (Thiphom et al. 2014). Other important aspect for human biomonitoring assessment is the profound knowledge of pyrethroidsmetabolism, and some studies have help clarify this matter. Namely the urinary excretion of chrysanthemum dicarboxylic acid was studied in three volunteers after oral intake of 0.3 mg pyrethrin I. Chrysanthemum dicarboxylic acid was detected through the first 36 h following the intake and during the first 4 h authors found that the elimination was most rapid with mean elimination half-life of 4.2 h (Leng et al. 2006).

Diet is thought to be an important route of exposure to pyrethroids. Using a multivariate model, adjusted for sex, age, body mass index, smoking habits and household insecticide exposure authors showed that a high intake of fresh, uncooked vegetables (odds ratio: 5.31; 95% confidence interval: 1.32–21.3) and elevated intake of cooked vegetables, namely cruciferous (odds ratio: 4.67; 95% confidence interval: 1.07–20.5) and leafy vegetables (odds ratio: 6.88; 95% confidence interval:

1.50–31.7), were related with high urine 3-phenoxybenzoic acid levels. This study data, suggest vegetable intake plays an important role in human pyrethroids exposure (Fortes et al. 2013). Additionally, studies with an organic diet intervention found that lower urinary levels could be found during the dietary intervention in an adult population (Göen et al. 2017). Similar associations were observed for children with organic diet intervention. Still, the major input for children exposure was thought to be residential use of pyrethroids. Additionally, older children, 8–11 years old, seemed to be exposed to higher levels which was likely attributed to the use of pyrethroids around the premises where older children engaged in the outdoor activities. Still, authors emphasized that young children behaviors such as hand-to-mouth behaviors and close proximity to the floor, can put them at higher risk of pesticide exposure (Lu et al. 2006). Pyrethroid floor wipe concentrations were found to be significant predictors of child creatinine-adjusted urinary metabolite levels and that indoor residential exposure to pyrethroid insecticides is also an important route for this particular population (Trunnelle et al. 2014b).

In a study comparing urban and rural population, higher urinary values were observed for the children living in a rural area. However, correlations between urinary levels of children and their parents were only achieved for the ones living in an urban area. Additionally, in both areas, higher concentrations of 3-phenoxybenzoic acid were seen in children compared to adults. The increased 3-phenoxybenzoic acid levels were linked with the use of pesticide formulations on pets in the previous 6 months (Wielgomas and Piskunowicz 2013). Likewise, mother and children exposure levels were positively linked with the housing conditions (Trunnelle et al. 2014a). In the rural population of Quebec Canada, summer was considered a time of greater exposure (Couture et al. 2009). Also for a Canadian population the amounts of pyrethroids, essentially permethrin and cypermethrin, absorbed were in the same range in adults and children (Fortin et al. 2008).

A comparison of the levels of exposure to pyrethroids in pregnant populations showed that pregnant French women have a tendency to be less exposed to these compounds than Chinese and Caribbean mothers, more exposed than American ones, (Dewailly et al. 2014), and have comparable levels of exposure of Japanese mothers. Furthermore, urinary levels of pyrethroidmetabolites during pregnancy were positively linked to smoking habit, consume of fish and alcohol, the use of domestic pesticides and living near crops. Which highlights the importance of non-dietary routes when evaluating exposure to pyrethroids (Dereumeaux et al. 2018).

In French children, metabolites of cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, 3-phenoxybenzoic acid, and 4-fluoro-3-phenoxybenzoic acid were detected in 84, 95, 64, 63, and 16% of the urine samples, respectively. In this study predictors of pyrethroids were evaluated and was concluded that children with parents occupationally exposed to pesticides were around 3-times more probable to have higher 3-phenoxybenzoic acid urinary concentrations (odds ratio: 2.8, 95% confidence interval: 1.2–6.5) (Glorennec et al. 2017). The same conclusion was drawn for children, in Northern Thailand, of farmers or with agricultural families (Panuwet et al. 2009).

Concerning temporal trends for pyrethroids a rise particularly for 3-phenoxybenzoic acid has been observed in the recent years for children and adults.

Analyzing two cycles of the human biomonitoring program in Canada four, i.e. cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid and 3-phenoxybenzoic acid, of the five pyrethroidmetabolites measured in urine were detected in ≥60% of samples. Comparing the two cycles an increase of 72% was observed for 3-phenoxybenzoic acid. However, authors think is still premature to draw temporal trends (Haines et al. 2017). An increase in the levels of 3-phenoxybenzoic acid was also observed in the United States population (Jain 2016). Which may be linked to the increased residential use of pyrethroids following the phase-out of organophosphatepesticides (Trunnelle et al. 2014b).

Finally, correlations between health outcomes and pyrethroids were explored. In a study with Chinese girls, authors concluded that pyrethroids exposure may increase the risk of delayed pubertal onset (Ye et al. 2017b). Also pyrethroids exposure was linked with gonadotropin levels and pubertal development in Chinese boys (Ye et al. 2017a). In a study performed in a pregnant population, an increase in the total amount of pyrethroidsmetabolites, i.e. the sum of cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, and 3-phenoxybenzoic acid, was associated with a decrease in birth weight (adjusted $\beta = -96.76$ g per log10 unit increase, 95% confidence interval:–173.15 to −20.37) while the individual urinary metabolite levels were not associated. Thus, an adverse association of birth weight with prenatal exposure to pyrethroids was observed (Ding et al. 2014). For the elderly population, the community-level exposure to pyrethroids was associated with pulmonary function reduced indicating that a more strict control of these insecticides is necessary to protect the elderly (Kim et al. 2019). Many studies have addressed the impact of pyrethroids in male fertility founding a positive connection between exposure and male fertility abnormalities (Jurewicz et al. 2015; Radwan et al. 2014, 2015; Toshima et al. 2012; Young et al. 2013).

7.4.2 *Reported Levels for Occupational Exposed Population*

Studies that have reported levels for occupational exposed subjects are described in annex Table 7.3. These studies have analyzed urine samples from exposed workers particularly farmers, pest control operators, airplane crews after disinfection, greenhouse workers or even textile workers. The reported median/geometric urinary levels are several orders of magnitude higher than for the general public, and usually the exposures occur through dermal route or inhalation.

In one particular study urinary pyrethroidsmetabolites levels were assessed in textile workers with different age range, job responsibilities and time since application, i.e. samples collected 1 day and 7 days after application. The results showed high levels of pyrethroids exposure in textile workers. With cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid and 3-phenoxybenzoic acid being the dominant metabolites. Levels of three metabolites, cis-3-(2,2-dichlorovinyl)-2,2-

dimethylcyclopropane-1-carboxylic acid, 3-phenoxybenzoic acid, and trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, were associated with responsibilities and ages of the workers. And the ratios of trans- to cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid could indicate that textile workers exposure was through dermal absorption and inhalation (Lu et al. 2013), as accordingly to previous studies different ratios are present depending on the route, e.g. dermal administration of cypermethrin, trans- to cis- ratio is approximately 1:1.

Other authors reported the kinetic time courses of λ-cyhalothrin metabolites after dermal application in volunteers and concluded that the metabolites were almost totally excreted over 84-h period post-application. On average, 0.12% and 0.08% of the applied λ-cyhalothrin dose was presented in urine as cis-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropane carboxylic acid and 3-phenoxybenzoic acid, respectively, which suggests a low dermal absorption fraction of this pyrethroid. The study showed the evaluation of dermal exposure to the pyrethroid, λ-cyhalothrin, using cis-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropane carboxylic acid and 3-phenoxybenzoic acid as potential biomarkers (Khemiri et al. 2018).

7.5 Conclusions

There is widespread exposure to a number of pyrethroids among the general population. Children and pregnant women are groups of concern due to neurotoxic potential of pyrethroids compounds. Food is a relevant source of pyrethroids exposure, but other sources such as indoor environment were also considered pertinent.

Values reported for the general population concerning 3-phenoxybenzoic acid are lower than 2 μg/L, which is below the human biomonitoring assessment values. The same is true for cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (1 μg/L) and trans 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (2 μg/L). Nonetheless we must assume that exceedances occurred in the upper exposure percentiles of the general population, including children. As discussed in the present chapter population living in rural areas, or with activities involving pesticide application such as farming presented higher urinary levels. The same is true for summertime. The relevance of these findings must be evaluated in more detail, but steps to reduce exposure to pyrethroids are most likely to be required.

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Appendix

Table 7.3 Exposure assessment of pyrethroids (median or geometric mean, other cases are indicated)

Country	year	Sample description		FB-Al	MTFBL	MMTFBL	$HOCH_2$-FB-Al	CH_3-FB-Ac	CxCA	cis-CDCA	trans-CDCA	ClF_3CA	CTFCA	MPBA	MCPA	TMCA	TFBA	DBCA	FPBA	trans-DCCA	cis-DCCA	3-PBA	α-cypermethrin	deltamethrin	2-ClBA	CPBA	Unit	References
USA	2009-2011	24 h samples from adults 19 to 50 years old		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.68	-	-	-	-	µg/L	(Morgan et al. 2016a)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.74	-	-	-	-	µg/g creatinine	
	October 2010 and February 2011	Firefighters		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	-	n.d	0.54	-	-	-	-	µg/L	(Behniwal and She 2017)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	-	n.d	0.49	-	-	-	-	µg/g creatinine	
	June-October 2012	Latino males	Farm workers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.24	1.04	-	-	-	-	µg/g creatinine	(Arcury et al. 2018)
			Non-farm workers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23.0	1.71	-	-	-	-		
	June-October 2013		Farm workers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.69	1.03	-	-	-	-		
			Non-farm workers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.91	1.25	-	-	-	-		
	1999-2008	Spot samples from population 12–40 years old		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-	-	-	µg/L	(Migneron-Foisy et al. 2017)

USA	2012	all	From adults w/no known occupational exposure	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	0.83	6.34	3.41	1.52	-	-	-	-	µg/L	(Davis et al. 2013) [a]
	June to October 2010	Male farm workers		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.81	-	-	1.90	-	-	-	-	µg/L for ≥ LOQ	(Raymer et al. 2014)
	June and December of 2009	End-of-day single spot void from farm worker families, living in Mendota	mothers 23–51 years of age	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.17	-	-	-	-	µg/L	(Trunnelle et al. 2014a)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.10	-	-	-	-	ug/g creatinine	
			children 2–8 years of age	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.11	-	-	-	-	µg/L	
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.97	-	-	-	-	ug/g creatinine	
	2009-2010	Working on disinfected aircrafts	From flight attendants 18-68 years range	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	n.d	2.94	0.68	7.06	-	-	-	-	µg/L	(Wei et al. 2012)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	n.d	3.92	0.98	9.01	-	-	-	-	µg/g creatinine	
		Working on non-disinfected aircrafts		-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	n.d	0.49	0.21	0.96	-	-	-	-	µg/L	
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	n.d	0.58	0.23	1.12	-	-	-	-	µg/g creatinine	

(continued)

Table 7.3 (continued)

Country	year	Sample description		FB-Al	MTFBL	MMTFBL	$HOCH_2$-FB-Al	CH_3-FB-Ac	CxCA	cis-CDCA	trans-CDCA	ClF_3CA	CTFCA	MPBA	MCPA	TMCA	TFBA	DBCA	FPBA	trans-DCCA	cis-DCCA	3-PBA	α-cypermethrin	deltamethrin	2-CIBA	CPBA	Unit	References
USA	July 2003-May 2004	all	1st morning and last void samples of 15 consecutive days from children 3–11 years range	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	0.08	0.54	0.40	0.58	-	-	-	-	µg/L	(Lu et al. 2006)
	n.a	From subjects suspected to have residential exposure		-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.39	0.95	2.9	1.8	2.1	-	-	-	-	µg/L	(Baker et al. 2004)
	2009-2010	Spot urine		-	-	-	-	-	-	-	-	-	-	-	-	-	-	< LOD	< LOD	< LOD	-	0.76	-	-	-	-	µg/L	(McKelvey et al. 2013)
	January 2000 and May 2003	Males aged 20–54		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.18	0.12	0.18	-	-	-	-	µg/L	(Young et al. 2013)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.14	0.10	0.15	-	-	-	-	µg/g creatinine	

USA	2001–2002	Age	6–11 years	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.423	-	-	-	-	µg/L	(Jain 2016)
			12–19 years	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.274	-	-	-	-		
			20–64 years	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.316	-	-	-	-		
			65+ years	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.359	-	-	-	-		
		Gender	Male	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.269	-	-	-	-		
			Female	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.389	-	-	-	-		
		Race/ ethnicity	Non-Hispanic white	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.323	-	-	-	-		
			Non-Hispanic black	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.377	-	-	-	-		
			Mexican American	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.272	-	-	-	-		
			Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.319	-	-	-	-		
		Smoking status	Non-smoker	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.356	-	-	-	-		
			Smoker	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.315	-	-	-	-		
	2009–2010	Age	6–11 years	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.744	-	-	-	-		
			12–19 years	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.347	-	-	-	-		
			20–64 years	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.429	-	-	-	-		
			65+ years	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.42	-	-	-	-		
		Gender	Male	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.377	-	-	-	-		
			Female	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.507	-	-	-	-		
		Race/ ethnicity Smoking status	Non-Hispanic white	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.451	-	-	-	-		
			Non-Hispanic black	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.354	-	-	-	-		
			Mexican American	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.39	-	-	-	-		
			Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.511	-	-	-	-		
			Non-smoker	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.438	-	-	-	-		
			Smoker	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.433	-	-	-	-		

(continued)

Table 7.3 (continued)

Country	year	Sample description		FB-Al	MTFBL	MMTFBL	HOCH$_2$-FB-Al	CH$_3$-FB-Ac	CxCA	cis-CDCA	trans-CDCA	ClF$_3$CA	CTFCA	MPBA	MCPA	TMCA	TFBA	DBCA	FPBA	trans-DCCA	cis-DCCA	3-PBA	α-cypermethrin	deltamethrin	2-ClBA	CPBA	Unit	References
USA	October 2009 and May 2011	From healthy, non-pregnant adult ages 19–50 years old	Bedtime	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.96	-	-	-	-	µg/L	(Morgan et al. 2016b)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.94	-	-	-	-	µg/g creatinine	
			First morning void	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.83	-	-	-	-	µg/L	
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.74	-	-	-	-	µg/g creatinine	
			24 h	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.98	-	-	-	-	µg/L	
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.30	-	-	-	-	µg/g creatinine	
	December 2007–November 2009	End-of-day spot urine sample	From adults 18–57 years of age	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d - 1.32	0.43 - 22.4	4.99 - 121	-	0.82	-	-	-	-	µg/L	(Trunnelle et al. 2014b) [b]
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d - 1.03	0.13 - 22.1	2.79 - 119	-	0.61	-	-	-	-	µg/g creatinine	
			From children 2–8 years of age	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.09 - 1.48	0.49 - 0.77	10 - 111	-	0.50	-	-	-	-	µg/L	
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.60 - 0.87	0.77 - 0.99	26.2 - 77.2	-	0.86	-	-	-	-	µg/g creatinine	
	n.a	From adults and children with no known exposure		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	≤ 30	-	-	-	-	µg/g creatinine	(Baker et al. 2000)

Canada	2007-2009	Spot sample from children between 6–11 years of age		-	-	-	-	-	-	-	-	-	-	-	-	-	-	< LOD	< LOD	0.15	0.05	0.20	-	-	-	-	µg/L	(Oulhote and Bouchard 2013)
	n.a	Agricultural workers	Inspections, weeding, or harvesting	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< LOD - 0.321	-	0.073 - 0.826	-	-	-		µmol/mol creatinine	(Ratelle et al. 2016)[c]
			Applicators	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< LOD - 0.870	-	0.073 - 1.28	-	-	-	-		
	2009–2011	3–79 years of age		-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.a	< LOD	n.a	n.a	0.29	-	-	-	-	µg/L	(Haines et al. 2017)[c]
	2007-2009	6-79 years of age		-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.a	< LOD	0.20	0.085	0.25	-	-	-	-		
	2009–2011	3–79 years of age		-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.012	< LOD	0.12	n.a	0.43	-	-	-	-		

(continued)

Table 7.3 (continued)

Country	year	Sample description	FB-Al	MTFBL	MMTFBL	$HOCH_2$-FB-Al	CH_3-FB-Ac	CxCA	cis-CDCA	trans-CDCA	ClF_3CA	CTFCA	MPBA	MCPA	TMCA	TFBA	DBCA	FPBA	trans-DCCA	cis-DCCA	3-PBA	α-cypermethrin	deltamethrin	2-ClBA	CPBA	Unit	References
Canada	From adults dermally exposed to λ-cyhalothrin	3 h after exposure	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	45	-	-	-	-	µmol/mol creatinine	(Khemiri et al. 2018)
		6 h after exposure	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	59	-	-	-	-		
		9 h after exposure	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	66	-	-	-	-		
		12 h after exposure	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	79	-	-	-	-		
		24 h after exposure	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	62	-	-	-	-		
		36 h after exposure	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	63	-	-	-	-		
		48 h after exposure	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	47	-	-	-	-		
		60 h after exposure	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	44	-	-	-	-		
		72 h after exposure	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28	-	-	-	-		
	June-August 2006	Children 6-10 years 12 h urine samples	-	-	-	-	-	-	-	0.163-31.8	-	-	-	-	-	-	0.044-71.5	0.065-6.75	2.66-303	0.118-97.4	0.174-250	-	-	-	-	pmol/kg body weight/12 h	(Couture et al. 2009) [b]
		Adults 18-70 years 12 h urine samples	-	-	-	-	-	-	-	0.099-550	-	-	-	-	-	-	0.037-16.9	0.042-23	0.165-438	0.129-122	0.179-491	-	-	-	-		

Canada	2005	Children 6-12 years	Night-time 12 h urine	-	-	-	-	-	-	0.009-1.5	-	-	-	-	-	-	0.006 -0.65	0.005-0.23	0.01-18	0.007-19	0.01-53	-	-	-		µg/L	(Fortin et al. 2008)[b]
		Adults 18-64 years		-	-	-	-	-	-	0.009-0.68	-	-	-	-	-	-	0.006 -0.27	0.005-0.10	0.01-25	0.07-8.2	0.01-24	-	-	-			
		all	24 h urine	-	-	-	-	-	-	0.009-0.44	-	-	-	-	-	-	0.006 -0.15	0.005-0.05	0.01-11.1	0.007-4.3	0.01-15.5	-	-	-			
			Daytime 12 h urine	-	-	-	-	-	-	0.009-0.62	-	-	-	-	-	-	0.006 -0.17	0.005-0.06	0.01-11	0.007-6.7	0.01-9.6	-	-	-			
Canada	Summer of 2013	Workers from a sweet corn farm-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.038 - 0.605	-	0.032 - 2.56	-	-	-	-	µmol/mol creatinine	(Ferland et al. 2015)
France	2011	Spot sample from pregnant women-		-	-	-	-	-	-	-	-	-	-	-	-	-	0.23	n.d	0.27	0.16	0.36	-	-	-	-	µg/L	(Dereumeaux et al. 2018)
				-	-	-	-	-	-	-	-	-	-	-	-	-	0.33	n.d	0.38	0.22	0.5	-	-	-	-	µg/g creatinine	
	2009 to 2012	1st morning void samples from six years old children-		-	-	-	-	-	-	-	-	-	-	-	-	-	0.20	<0.003	0.22-	0.09	0.02	-	-	-	-	µg/L	(Glorennec et al. 2017)

(continued)

Table 7.3 (continued)

Country	year	Sample description		FB-Al	MTFBL	MMTFBL	HOCH$_2$-FB-Al	CH$_3$-FB-Ac	CxCA	cis-CDCA	trans-CDCA	ClF$_3$CA	CTFCA	MPBA	MCPA	TMCA	TFBA	DBCA	FPBA	trans-DCCA	cis-DCCA	3-PBA	α-cypermethrin	deltamethrin	2-ClBA	CPBA	Unit	References
France	n.a	From adults 24-62 years range		-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.183	Not report	0.331	0.187	0.628	-	-	-	-	µg/L	(Le Grand et al. 2012)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.221	Not report	0.315	0.200	0.551	-	-	-	-	µg/g creatinine	
UK	n.a	From adults		-	-	-	-	-	-	-	-	3.2	-	-	-	-	-	1.6	-	1.6	0.8	6.1	-	-	-	-	µg/L	(Bevan et al. 2013)
				-	-	-	-	-	-	-	-	1.8	-	-	-	-	-	1.3	-	1.8	0.7	4.3	-	-	-	-	µg/g creatinine	
Switzerland	2015	Conventional diet	From adults	-	-	-	-	-	-	-	-	-	0.08	-	-	-	-	0.09-0.11	n.d	0.75-0.98	0.10-0.22	0.19-0.33	-	-	-	-	µg/L	(Göen et al. 2017)[d]
		Organic food diet		-	-	-	-	-	-	-	-	-	n.d	-	-	-	-	n.d	n.d	0.38-0.50	n.d	<0.01-0.07	-	-	-	-		
Netherlands	n.a	all	From male adults	-	-	-	-	-	-	-	< 0.05	-	-	-	-			0.04	<0.01	0.17	0.05	0.26					µg/L	(Leng et al. 2006)
Spain	n.a	farmworkers	From adults	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	-	-	2.4	-	-	-	-	µg/L	(Garí et al. 2018)
		general population		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.79	-	-	1.1	-	-	-	-		
	n.a	From 6 pest control operators		-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d - 69	-	205 - 823	156 - 519	785 - 1404	-	-	n.d - 58	-	pg/mL	(Arrebola et al. 1999)[b]
		From non-occupationally exposed		-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	-	n.d - 123	n.d - 54	269 - 320	-	-	n.d	-		

Spain		From 6 pest control operators		-	-	-	-	-	-	-	-	-	-	-	-	-	-	nd - 69	-	205 - 823	156 - 519	785 - 1404	-	-	nd - 58	-	pg/mL	(Arrebola et al. 1999)[b]
		From non-occupationally exposed		-	-	-	-	-	-	-	-	-	-	-	-	-	-	nd	-	nd - 123	nd - 54	269 - 320	-	-	nd	-		
		From children of Valencia Region		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d - 29.12	-	-	-	-	µg/L	(Roca et al. 2014)[b]
Greece	n.a	From 52-year-old poisoning suicide male		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.41	0.46	-	-	mg/L	(Boumba et al. 2017)
Denmark	January 2010 to December 2012	From newly pregnants		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.20	-	-	-	-	µg/L	(Dalsager et al. 2018)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.23	-	-	-	-	µg/g creatinine	
Italy	January to May	all	From Caucasians adults	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.66	-	-	-	-	µg/g creatinine	(Fortes et al. 2013)[a]
		female		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.74	-	-	-	-		
		male		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.52	-	-	-	-		
Finland	n.a	From greenhouse workers		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.4-51.7	-	-	-	-	µg/L	(Tuomainen et al. 1996)[b]

(continued)

Table 7.3 (continued)

Country	year	Sample description		FB-Al	MTFBL	MMTFBL	HOCH$_2$-FB-Al	CH$_3$-FB-Ac	CxCA	cis-CDCA	trans-CDCA	ClF$_3$CA	CTFCA	MPBA	MCPA	TMCA	TFBA	DBCA	FPBA	trans-DCCA	cis-DCCA	3-PBA	α-cypermethrin	deltamethrin	2-ClBA	CPBA	Unit	References
Germany	n.a	all	From children 3-14 years range	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.25	0.12	0.43	-	-	-	-	µg/L	(Schulz et al. 2009)
		female		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	0.15	0.49	-	-	-	-		
		male		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.22	0.11	0.39	-	-	-	-		
	n.a	Spot samples workers in indoor pest control		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.7 - 70.3	1 - 13.9	2.5 - 42.5	-	-	-	-	µg/g creatinine	(Angerer and Ritter 1997)[b]
	n.a	24 h urine from a healthy person not occupationally exposed to pyrethroids		-	-	-	-	-	-	-	-	-	-	-	-	-	-	< 0.03	0.1	0.4	0.5	0.5	-	-	-	-	µg/L	(Leng et al. 1997b)
	n.a	24 h urine of pest control operators		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<0.03 - 277				-	-	-	-	µg/L	(Leng et al. 1996)[b]
	n.a	24 h urine of pest control operators		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5 - 277				-	-	-	-	µg/L	(Leng et al. 1997a)[b]
	March and August 1998	Samples from 1177 residents of the former American Forces housing estates in Frankfurt		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< LOD–7.18	< LOD–5.74	< LOD–17.13	< LOD–9.06	-	-	-	-	µg/g creatinine	(Heudorf and Angerer 2001)[b]

Germany	n.a	24 h samples of 46 persons from the general population	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< 0.05	< 0.05	0.11	0.06	0.16	-	-	-	-	µg/L	(Schettgen et al. 2002b)
	n.a	Spot samples from 1177 inhabitants of a residential area	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.2	0.4	0.2	-	-	-	-	-	µg/L	(Schettgen et al. 2002a)
	n.a	24 h samples from persons after using vaporizer plates containing δ-allethrin in a household	-	-	-	-	-	-	n.d	2.3 - 6.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	µg/L	(Elflein et al. 2003)
	1998	Spot samples from children/ adolescents that had never used pyrethroids in home or for medical reasons	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10	0.05	0.37	0.09	-	-	-	-	-	µg/L	(Heudorf et al. 2004)[a]
	n.a	30 samples from subjects exposed to pyrethrum	-	-	-	-	-	-	-	0.23	-	-	-	-	-	-	-	-	0.29	0.15	0.49	-	-	-	-	µg/L	(Leng and Gries 2005)
		15 samples from not-exposed subjects	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	0.04	0.01	0.08	0.05	0.13	-	-	-	-		
	summer/autumn 2012	Spot samples of 38 persons, between 26 to 58 years old	-	-	-	-	-	-	-	-	0.04	-	<0.01	-	-	-	0.04	<0.01	0.17	0.08	0.22	-	-	-	<0.01	µg/L	(Schettgen et al. 2016)
			-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	0.04	-	0.14	0.09	0.26	-	-	-	-	µg/g creatinine	

(continued)

Table 7.3 (continued)

Country	year	Sample description		FB-Al	MTFBL	MMTFBL	HOCH$_2$-FB-Al	CH$_3$-FB-Ac	CxCA	cis-CDCA	trans-CDCA	ClF$_3$CA	CTFCA	MPBA	MCPA	TMCA	TFBA	DBCA	FPBA	trans-DCCA	cis-DCCA	3-PBA	α-cypermethrin	deltamethrin	2-ClBA	CPBA	Unit	References
Germany	n.a	24 h samples after exposure	19 agriculture workers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< LOD	< LOD	< LOD	0.6	-	-	-	-	µg/g creatinine	(Hardt and Angerer 2003)
			15 pest control workers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< LOD	< LOD	1.8	1.4	-	-	-	-		
			2 greenhouse workers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	< LOD	2.9	2.9	-	-	-	-		
		Controls 24 h urine samples			-	-	-	-	-	-	-	-	-	-	-	-	-	-	< LOD	< LOD	0.5	n.a	-	-	-	-		
	1996-1999	From participants exposed at home and at the work place, e.g. bakery, restaurant	Before pest control operation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.1	0.1	0.1	0.1	-	-	-	-	µg/L	(Leng et al. 2003)
			1 day following a pest control operation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.1	0.1	0.1	0.1	-	-	-	-		
			3 days following a pest control operation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.1	0.1	0.1	0.1	-	-	-	-		
			4-6 months following a pest control operation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.1	0.1	0.1	0.1	-	-	-	-		
			10-12 months following a pest control operation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.1	0.1	0.1	0.1	-	-	-	-		
Poland	2008 and 2011	Single spot sample men aged under 45 years of age		-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-	0.15	0.13	0.16	-	-	-	-	µg/L	(Radwan et al. 2014)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	0.14	0.11	0.13	-	-	-	-	µg/g creatinine	
		Spot sample from men under 45 years of age		-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-	0.16	0.12	0.16	-	-	-	-	µg/L	(Radwan et al. 2015)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	0.15	0.11	0.14	-	-	-	-	µg/g creatinine	

Poland	2010 and 2011	all	From subjects 5-77 years range	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	-	n.d	n.d	0.26	-	-	-	-	µg/L	(Wielgomas et al. 2013)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	-	n.d	n.d	0.22	-	-	-	-	µg/g creatinine	
	June–October 2011	all	Spot samples from adults 24-71 years range	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	-	n.d	n.d	0.278	-	-	-	-	µg/L	(Wielgomas 2013)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	-	n.d	n.d	0.271	-	-	-	-	µg/g creatinine	
			1st morning voids samples from adults 24-71 years range	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	-	n.d	n.d	0.317	-	-	-	-	µg/L	
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	-	n.d	n.d	0.3	-	-	-	-	µg/g creatinine	
			24 h urine samples from adults 24-71 years range	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	-	n.d	n.d	0.305	-	-	-	-	µg/L	
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	-	n.d	n.d	0.299	-	-	-	-	µg/g creatinine	
	May–June 2012	Preschool and school age children and their parents	Rural	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<LOD - 22.170	-	<LOD - 29.322	<LOD - 8.665	<LOD - 13.290	-	-	-	-	µg/L	(Wielgomas and Piskunowicz 2013)[b]
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	<LOD - 11.402	-	<LOD - 56.456	<LOD - 24.015	<LOD - 35.199	-	-	-	-	µg/g creatinine	
			Urban	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<LOD - 13.026	-	<LOD - 4.482	<LOD - 1.864	<LOD - 9.001	-	-	-	-	µg/L	
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	<LOD - 8.383	-	<LOD - 2.573	<LOD 3.844	<LOD - 5.945	-	-	-	-	µg/g creatinine	

(continued)

Table 7.3 (continued)

Country	year	Sample description		FB-Al	MTFBL	MMTFBL	HOCH$_2$-FB-Al	CH$_3$-FB-Ac	CxCA	cis-CDCA	trans-CDCA	ClF$_3$CA	CTFCA	MPBA	MCPA	TMCA	TFBA	DBCA	FPBA	trans-DCCA	cis-DCCA	3-PBA	α-cypermethrin	deltamethrin	2-ClBA	CPBA	Unit	References
Poland	January 2008 and April 2011	Spot sample from men under 45 years old		-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.31	-	0.33	0.25	0.30	-	-	-	-	µg/L	(Jurewicz et al. 2015)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.28	-	0.35	0.22	0.29	-	-	-	-	µg/g creatinine	
China	2013 to 2014	Healthy	1st morning urine voids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.42	1.03	0.74	-	-	-	-	µg/L	(Han et al. 2017)
		w/coronary heart disease		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.07	1.93	1.09	-	-	-	-		
	May 2014 to December 2015	Spot samples from girls 9-15 years range		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	-	-	1.11	-	-	-	-		(Ye et al. 2017b)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	-	-	1.42	-	-	-	-	µg/g creatinine	
		Spot samples from boys 9–16 years range		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<0.35	-	-	1.12	-	-	-	-	µg/L	(Ye et al. 2017a)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<0.33	-	-	0.92	-	-	-	-	µg/g creatinine	
	June 2010 to	all	Spot samples from infant at 12 months of age	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.2	0.44	-	-	-	-	µg/L	(Wu et al. 2013)
		female		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.04	0.2	0.41	-	-	-	-		
		male		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.97	0.21	0.47	-	-	-	-		
	1987-88	n.a		-	-	-	-	-	-	-	-	-	-	-	-	-	-	9 - 140	-	-	-	-	-	-	-	-	µg/L	(Chen et al. 1991)

China	2010	day 1	From textile workers 20-52 years range	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.8	19	16	-	-	-	-	µg/L	(Lu et al. 2013)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.4	13	12	-	-	-	-	µg/g creatinine	
		day 7		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.83	21	16	-	-	-	-	µg/L	
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.72	16	12	-	-	-	-	µg/g creatinine	
		day 1	From textile workers 18-44 years range	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.4	19	12	-	-	-	-	µg/L	
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.8	30	19	-	-	-	-	µg/g creatinine	
		day 7		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.7	43	26	-	-	-	-	µg/L	
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6.8	49	31	-	-	-	-	µg/g creatinine	
	July–September 2004	all	From workers of pesticide formulation plants	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	n.d	0.26-1.75	n.d-2.88	n.d	-	-	-	-	µg/L	(Lin et al. 2011)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	n.d	36-130	n.d-209	n.d	-	-	-	-	µg/g creatinine	
	2004-2006	all	From infertile men	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.487	-	-	-	-	µg/L	(Xia et al. 2008)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.019	-	-	-	-	µg/g creatinine	
		all		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.398	-	-	-	-	µg/L	(Han et al. 2008)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.925	-	-	-	-	µg/g creatinine	
	September 2010 - 2012	Spot urine from pregnant women from rural regions		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.47	0.37	0.54	-	-	-	-	µg/L	(Ding et al. 2014)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.49	0.39	0.57	-	-	-	-	µg/g creatinine	

(continued)

Table 7.3 (continued)

Country	year	Sample description		FB-Al	MTFBL	MMTFBL	$HOCH_2$-FB-Al	CH_3-FB-Ac	CxCA	cis-CDCA	trans-CDCA	ClF_3CA	CTFCA	MPBA	MCPA	TMCA	TFBA	DBCA	FPBA	trans-DCCA	cis-DCCA	3-PBA	α-cypermethrin	deltamethrin	2-ClBA	CPBA	Unit	References
Republic of Korea	December 2012 April 2015	female	Samples from elderly aged ≥ 60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	1.26	-	-	-	-	µg/L	(Kim et al. 2019)
		male		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-		
		female		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.6	-	-	-	-	µg/g creatinine	
		male		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.12	-	-	-	-		
Thailand	Samples from vector control operators 18-60 years range			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-		(Gammuk et al. 2017) [a]
	2006	Pong Yaeng	1st morning voids from male farmers 20-65 years range	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	n.d	0.04-49.2	n.d	0.1-21	-	-	-	-		(Panuwet et al. 2018)
		Inthakhin		-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	n.d	0.04-11.0	n.d	0.1-9.1	-	-	-	-		
	n.a	all	1st morning void from students 12–13 years range	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.48	0.26	0.55	-	-	-	-	µg/L	(Panuwet et al. 2009)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.55	0.29	0.48	-	-	-	-	µg/g creatinine	
		female		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.55	0.29	0.55	-	-	-	-		
		male		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.56	0.30	0.25	-	-	-	-		
		Agricultural family		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.67	0.29	0.38	-	-	-	-		
		Non-agricultural family		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.46	0.30	0.15	-	-	-	-		
	n.a	Consumers	Morning samples	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.86	-	-	-	-		(Thiphom et al. 2014)
		Farmers		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16.1	-	-	-	-		

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Japan	n.a	Spot samples from non-smoking 20-52 years range		-	0.04-0.09	n.d	-	-	0.45	-	-	-	0.36	0.51	0.30-0.76	n.d	0.20-1.10	-	n.d	-	0.44-5.39	0.38-1.45	-	-	-	-	µg/L	(Yoshida 2017)[b]
	2012	Samples from 3-year-old children		0.08	n.d	n.d	0.05	-	-	-	0.66	-	-	-	-	-	-	-	-	0.14	0.08	0.58	-	-	-	-		(Ueda et al. 2018)
	n.a	Samples from men 29-58 years range		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.547	-	-	-	-		(Toshima et al. 2012)
	August-September of 2012 and in February of 2013		3-year-old children		-	-	-	-	-	-	0.15	-	-	-	-	-	-	-	-	0.76		1.16	-	-	-	-	µg/L	(Osaka et al. 2016)
				-	-	-	-	-	-	-	0.19	-	-	-	-	-	-	-	-	1.13		1.71	-	-	-	-	µg/g creatinine	
	August 2005	all	Samples from non-farming 39–85 years range	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.29	-	-	-	-	µg/L	(Ueyama et al. 2009)
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.40	-	-	-	-	µg/g creatinine	
		female		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.28	-	-	-	-	µg/L	
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.45	-	-	-	-	µg/g creatinine	
		male		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.32	-	-	-	-	µg/L	
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.32	-	-	-	-	µg/g creatinine	
		all	Samples from farming workers 40–85 years range	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.38	-	-	-	-	µg/L	
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.46	-	-	-	-	µg/g creatinine	
		female		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.31	-	-	-	-	µg/L	
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.50	-	-	-	-	µg/g creatinine	
		male		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.46	-	-	-	-	µg/L	
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.40	-	-	-	-	µg/g creatinine	

(continued)

Table 7.3 (continued)

Country	**year**	**Sample description**		**FB-Al**	**MTFBL**	**MMTFBL**	**HOCH$_2$-FB-Al**	**CH$_3$-FB-Ac**	**CxCA**	**cis-CDCA**	**trans-CDCA**	**ClF$_3$CA**	**CTFCA**	**MPBA**	**MCPA**	**TMCA**	**TFBA**	**DBCA**	**FPBA**	**trans-DCCA**	**cis-DCCA**	**3-PBA**	**α-cypermethrin**	**deltamethrin**	**2-ClBA**	**CPBA**	**Unit**	**References**
Japan	December 2004	Worker that sprayed pesticides within 2 days before analysis	First morning void samples	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.4	-	-	-	-	µg/g creatinine	(Wang et al. 2007)
	August 2005			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.3	-	-	-	-		
	December 2004	Worker that didn't sprayed pesticides within 2 days before analysis		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.9	-	-	-	-		
	August 2005			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.7		-	-	-		
Mexico	2016	Farm workers	Age between 19-46 years old	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.94	0.88	-	1.83	-	-	-	-	µg/g creatinine	(López-Gálvez et al. 2018)
	1997 and 2001	Pregnant women	1st Trimester	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.27	-	-	-	-	µg/L	(Watkins et al. 2016)
			2nd Trimester	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.26	-	-	-	-		
			3rd Trimester	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.23	-	-	-	-		

10 Caribbean countries	August 2008 and April 2011	Pregnant or delivering women	Antigua and Barbuda	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.11	0.03	1.28	0.50	1.77	-	-	-	-	µg/L	(Dewailly et al. 2014)
			Belize	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	n.c	0.14	0.07	0.21	-	-	-	-		
			Bermuda	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	0.02	0.63	0.24	0.56	-	-	-	-		
			Dominica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.c	0.02	0.23	0.10	0.45	-	-	-	-		
			Grenada	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	0.05	0.65	0.22	0.81	-	-	-	-		
			Jamaica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.07	n.c	0.22	0.11	0.32	-	-	-	-		
			Montserrat	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.c	n.c	0.31	0.14	0.36	-	-	-	-		
			St. Lucia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	0.02	0.42	0.17	0.58	-	-	-	-		
			St. Kitts and Nevis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02	0.02	0.58	0.24	0.64	-	-	-	-		
			St. Vincent and the Grenadines	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02	0.01	0.46	0.20	0.54	-	-	-	-		
			Featured Caribbean countries	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	0.02	0.40	0.17	0.54	-	-	-	-		
Egypt	2008	Agriculture Applicator	Baseline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<LOD - 0.15	0.85 - 2.85	-	-	-	-	µg/g creatinine	(Singleton et al. 2014) [c]
			Peak	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.86 - 17.32	4.69 - 15.78	-	-	-	-		
			Post application	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.48 - 2.09	2.93 - 5.68	-	-	-	-		
		Agriculture Engineer	Baseline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.07 - 0.19	0.47 - 1.43	-	-	-	-		
			Peak	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.84 - 6.05	2.26 - 4.32	-	-	-	-		
			Post application	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.71 - 1.09	1.35 - 2.92	-	-	-	-		
		Agriculture Technician	Baseline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<LOD - 0.06	<LOD - 0.78	-	-	-	-		
			Peak	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.65 - 2.00	1.44 - 2.26	-	-	-	-		
			Post application	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.19 - 1.15	1.53 - 3.25	-	-	-	-		

(continued)

Table 7.3 (continued)

Country	year	Sample description	FB-Al	MTFBL	MMTFBL	$HOCH_2$-FB-Al	CH_3-FB-Ac	CxCA	cis-CDCA	trans-CDCA	ClF_3CA	CTFCA	MPBA	MCPA	TMCA	TFBA	DBCA	FPBA	trans-DCCA	cis-DCCA	3-PBA	α-cypermethrin	deltamethrin	2-CIBA	CPBA	Unit	References
Ghana	September to October	1st morning urine voids from pregnant women,2nd or 3rd trimester	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	n.d	-	0.17-0.32	-	-	-	-	µg/L	(Wylie et al. 2017)
			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n.d	n.d	-	0.43-0.77	-	-	-	-	µg/g creatinine	
South Africa	2012 -2015	Spot sample from women prior and post delivery	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.223	n.d	0.357	0.306	0.712	-	-	-	-	µg/L	(Coker et al. 2018; Eskenazi et al. 2018)/
			-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.181	n.d	0.248	0.289	0.576	-	-	-	-	µg/L adjust. creatinine	
Australia	n.a	From 9 pest control workers that use bifenthrin		-	-	-	-	-	-	-	-	-	n.d - 31.9	-	-	-	-	-	-	-	n.d - 30.0	-	-	-	-	µg/g creatinine	(Smith et al. 2002)
		From 4 subjects that use other pyrethroids,i.e. permethrin deltamethrin, but not bifenthrin		-	-	-	-	-	-	-	-	-	n.d - 1.4	-	-	-	-	-	-	-	n.d - 61.1	-	-	-	-		

a) mean; b) min-max; c) medians range; d) means range; n.a- not available; n.c.- not calculated; n.d- not detected; LOD- limit of detection; LOQ- limit of quantification; USA – United States of America; h- hours; FB-Al: 2,3,5,6-tetrafluorobenzyl alcohol **; MTFBL also known as CH_3-FB-A1:**4-methyl-2,3,5,6-tetrafluorobenzyl alcohol **; MMTFBL also known as CH_3OCH_2-FB-Al:** 4-methoxymethyl-2,3,5,6-tetrafluorobenzyl alcohol; **$HOCH_2$-FB-Al:** 2,3,5,6-tetrafluoro-1,4-benzenedimethanol**; CH_3-FB-Ac:**4-methyl-2,3,5,6-tetrafluorobenzoic acid **; CxCA** : 3 -(2-carboxy prop -1-enyl)-2,2-dimethylcyclopropanecarboxylic acid**; cis-CDCA:** cis-chrysanthemum dicarboxylic acid **; trans-CDCA:** trans-chrysanthemum dicarboxylic acid **; ClF_3CA:** chlorotrifluorovinylcyclopropanecarboxylic acid **; CTFCA:** 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid **; MPBA also known as MPB** :2-methyl-3-phenylbenzoic acid **; MCPA:** 2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylic acid **; TMCA:** 2,2,3,3-tetramethylcyclopropanecarboxylic acid; **TFBA:**2,3,5,6-tetrafluorobenzoic acid; **FPBA:** 4-fluoro-3-phenoxybenzoic acid; **DBCA also known as Br_2CA:** cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid; **trans-DCCA also known as trans Cl_2CA:** trans 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid**; cis-DCCA also known as cis-Cl_2CA**:cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid; **3-PBA:** 3-phenoxybenzoic acid**; 2-CIBA**: 2-(4-chlorophenyl)-3-methylbutyric acid; **CPBA:** 4-chloro-α-isopropyl benzene acetic acid

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Chapter 8
Remediation of Pesticide in Water

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Abstract Pesticides, widely used in agriculture to kill pests, have serious adverse effects on the environment and human health. Different types of pesticides such as insecticide, fungicide, herbicide, and biochemical pesticide can cause serious illness upon short-term exposure such as skin diseases, eye irritation, and nausea, and upon long-term exposure may cause cancer and Parkinson's disease. These pesticides are also normally drained in water bodies, further contaminating the water system with their residues. Water contamination from the pesticides residue has been increasing due to increased agricultural demands for the use of pesticides, which in turn becoming a major threat to the environment and eco-system. Various techniques have been employed for the removal of pesticide contaminants from water which include hydrogen peroxides, ultrasonic wave, hybrid process, bioremediation, photocatalytic degradation, adsorption, membrane separation, bio-purification systems, composite material, ion exchange resins, carbon nanotubes, graphene, and nanocrystalline metal oxides. This chapter discusses the low-cost detection, remediation and removal techniques of the pesticide contaminants present in water. The coating and slow-release method of pesticides are also elaborated to ensure long

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lasting effects of the pesticides to kill the pests, whilst retaining the fertility of the soil and the plant growth, with minimal impacts on the environment.

Keywords Pesticides · Types · Production · Consumption · Contamination · Removal technologies · Detection · Environmental hazards · Health-effects · Remediation

8.1 Introduction

In the past years, with the expanding agricultural sector, chemicals such as fertilizers and pesticides have enjoyed exponential market growth and consumption. Fertilizer use enhances the soil fertility which directly impacts the metabolism of the crops. These improved metabolism has direct impact on the productivity and agricultural produce output. The side-effect is that the immune active process in the crops may have been altered to meet the high ratio of productivity to the time and space. As a consequence, the pathogens or pests become active and are attracted to the crops for food. To get rid of these pests, pesticides in the form of chemical or mixture of chemicals or biological agents, are introduced, to tempt, attract and kill the pest (Lewis et al. 1997). The main purpose of pesticides is therefore to protect the plants from the pests (David Pimentel 2009), which can be the unwanted plants, internally or externally grown pathogen, microbes, insects, germs or organisms which include weeds, cockroaches, bed bugs, termites, ants, flies, rodents and mosquitoes. These pests can destroy the plants and reduce the growth (Altieri and Nicholls 2003). An estimated 70,000 species of pests exist which include 9000 species of insects and mites, 50,000 plant pathogens and 8000 weeds. Out of these, less than 10% organisms are actually pests which could actually harm the crops. Yet, about $40 billion are invested on pesticides per year to save crops worldwide. Despite this huge investment, 35–42% of crops are still harmed by the pests, where 14% losses are caused by insects, and 13% by plant pathogens and weeds, globally (David Pimentel 2009).

Pesticides work through the prevention, repulsion and destruction of pests and weeds. Pesticides can be classified into different categories on the basis of the purpose such as Insecticides, Herbicides, Fungicides, or Wood-preservatives; or on the basis of chemical structure such as Organochlorines, Carbamates, Chlorophenols, Synthetic pyrethroids. The first phase of pesticide application, in the era before 1870s, involves the use of natural substances such as sulfur. Between 1870 and 1945, in the second phase, natural materials and inorganic substances are used to prepare the synthetic pesticides of inorganic nature. Since 1945, in the third phase, the synthetic pesticides of organic nature such as dichlorodiphenyltrichloroethane and dehydroalanylethylamide are introduced. At the beginning, there are only three types of organic synthetic pesticides, which include carbamate, organo-phosphorous and organochlorinated insecticides. Later, fungicides and herbicides are introduced. From 1960–2005, the use of insecticides has reduced from 36.5% in 1960 to 25% in 2005, and the consumption of herbicides has increased from 20% in 1960 to 48% in 2005 (Zhang et al. 2011).

Pesticides contain both inert and active components (Cox and Surgan 2006), but may be non-specific (Relyea 2004), and can also harm or destroy other organisms. However, some pesticides available in the market are manufactured to kill the specific kind of pests. Most pesticides may also be listed as a plant growth regulator to facilitate the ripening of food crops or increase the woody character of the plant. The unregulated use of pesticide has become a major problem in Asia, and many developed countries have opted for the use of eco-friendly or easily degradable chemicals. In less developed countries, the poor farmer may only concern about getting the productivity increased, with less concern on the health and environmental impact. Pollutants move into the environment directly such as through leaking during transportation, or exposure from storage, disposal and industrial sites. Pesticides from agriculture may seep into the soil through rain, wind and irrigation water. Consequently, the pesticides move into the watercourses and groundwater from the soil by runoff, penetration and wastewater treatment plant. The compounds remain in the soil and sludge as they have less bioavailability. While pesticides are beneficial and save plants from harmful insects, the chemicals are toxic not only to the pests, but also human beings (Edwards 1993). The toxicity is governed by the dose-time relationship (Banerjee 1999), which means the amount of pesticide and the exposure must be known, before its application. The toxicity increases the risk of many diseases if comes in direct contact with the skin. Pesticide poisoning affect human health, and can cause vomiting, headache, weakness, dizziness, nausea, muscle cramp and many other diseases (Richter 2002), like respiratory tract irritation, eye and skin irritation, and even loss of consciousness. Over a long period of exposure, excessive pesticide can lead to the development of cancer, asthma, anxiety and Parkinsons diseases.

The aim of this review is to address this concern by understanding the different types of pesticides and highlighting the issues on low-cost detection, remediation and removal protocols of the pesticide contaminants in water.

8.2 Types of Pesticides

8.2.1 Insecticides

Pesticides, natural or man-made, are poisonous substances to prevent crops from pests (Zhang et al. 2011). The natural pesticides are produced via biochemical, microbial, botanical and mineral routes. Chlorpyrifos is an example of biochemical pesticide having biomolecular link to the plant family, and therefore with a significant role in the agriculture field. Nicotine is another example of natural pesticide in the leaves of tobacco. Microbial pesticide such as acetochlor and butachlor, are the micro-organisms which confer the self-defence property to the plants. These are also more eco-friendly. Man-made pesticides is a synthetic application of chemicals for plant conservation from external environmental effects at industrial level (Tang et al. 2019). Most of the pesticides used are insecticides as insects have caused major disturbance to the crop production. In the selection of the pesticide, the nature of the insect such as the habitat, its way of living, the type of food it consumes and its impact on the crop, has often been overlooked. The characteristic of the pesticide whether in solid, liquid, granules or aerosol form, must be suitable to the type of the pests. Pesticides are classified based on their toxicity, and listed in different ordinary colour band to indicate the toxicity level. Red band indicates an extremely toxic effect, yellow band indicates highly toxic, and blue and green band show the moderate and low toxicity level, respectively. Pesticides may include rodenticides which are specific to kill mice, rats and moles. Fumigants, which include cyanides, aluminium phosphate and methyl bromide, kill any living organism including insects, fungi or herbs. At room temperature, fumigants are found in gaseous or vapour forms, and are highly poisonous. Mosquitoes and other flying insects are best attacked by the aerosol spray in which the droplet of the pesticide is suspended in the air while the insects move in and inhale these droplets. For crawling insects or larvae on water surface, the surface powder may be most suitable. The insecticide mostly kills by getting into the inner body system of the insects or pest and act like the poison which destroys the whole-body system internally. The droplet of the aerosol pesticide sticks to the body wall of the insect and enters the body wall via dermal entry.

Table 8.1 shows the different classes and types of insecticides. The major ones include organochlorines, organo-phosphates and carbamates, and others include diethyltoluamide (DEET) and citronella (World Health Organization 2009). Organochlorines have been developed in the 1940s and are effective on many insects. The use however has been prohibited by the US Environmental Protection Agency (EPA) due to its effects on the environment and human health. Many of the organochlorines are banned in the US although many are also still being used in other countries. Organochlorines are volatile and fat-soluble, and therefore found in higher concentration in fatty acids. By ingesting these fatty acids, humans may be exposed to organochlorines (Taiwo 2019). Aldrin and dieldrin are organochlorines commonly applied to soil and seeds to protect from insects. Dieldrin is also used to protect clothes and carpets from moth, and has been detected in various dairy products and in crops. In tropical areas, dieldrin is used to control vector-borne disease like malaria. Both are banned in the US in 1970 due to their persistence in the environment and their bioaccumulation in foods and as vapours which eventually affect humans and animals (IARC Working Group 2016). Chlordane and heptachlor are organo-chlorines, used between 1950–1980, to kill fire ants, termites and other insects. Both are also banned in the US (Metcalf and Horowitz 2014). The effects of small doses on humans are unknown, but at large doses, they may block inhibitory neurotransmitters of the central nervous system leading to symptoms like headache, muscle cramp, nausea and vomiting. Animals exposed to these chemicals may get liver enlargement or tumours on the liver.

Dichlorodiphenyltrichloroethane is a broad-spectrum insecticide, produced and consumed until 1972, and banned thereafter for all types of use. In some countries, it

Table 8.1 Different classes and types of insecticides, fungicides and herbicides

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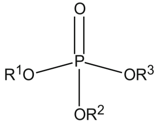
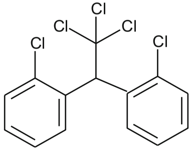
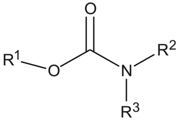
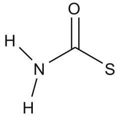
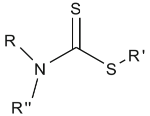
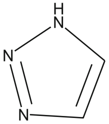
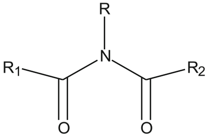
 Organophosphates	 Organochlorines	 Carbamates	
2. Fungicides			
 Thiocarbamates	 Dithiocarbamates	 Triazoles	 Dicarboximides
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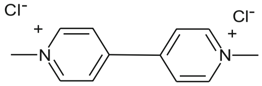
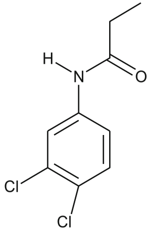
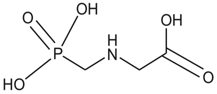
 Paraquat	 Propanil	 Glyphosate	

is still used for endemic and malaria control. It may be converted into its other stable forms which are persistent in the environment, and exposed to animal and plant tissue, primarily the dairy products, meat and fish. Endrin, an isomer of dieldrin, has been used as an insecticide, rodenticide or avicide, and also banned in the US (Pellegrino-Peard 1995). Hexachlorocyclohexane and its beta and gamma isomers, are used to kill the insects affecting plants and soil-dwelling insects (Calvelo-Pereira et al. 2006). Myrex, an organochlorine used since 1977, to kill fire ants, is a highly persistent chemical in the environment, and can be found in aquatic animals and foods. Animals exposed to Myrex in the lab have developed enlarged liver and liver tumours, and also showed reproductive abnormalities leading to decreased fertility. Hexachlorobenzene, an organochlorine used between 1930–1970, and later banned in 1984, is also a popular fungicide. 2,4,5-Trichlorophenol and 2,4,6-Trichlorophenol are the two organochlorines used as intermediates in the synthesis of various pesticides, but are no longer produced on a commercial scale (Faroon et al. 1995). Humans may be exposed to these chemicals by eating infected food or by inhaling the exposed air. At high-level exposure, animals may get increased rates of hepatic tumours, leukaemias and lymphomas (Nhanes 2009). The major reasons for the banning of most of these organochlorine insecticides are their persistence in the environment and their bioaccumulation in aquatic and terrestrial food chains. Pesticide effectiveness largely depends on their life cycle. Because of their short life cycle, the population of insects and pests rapidly develop resistance. This at the end defeats the purpose of its production and intended application, and at the same time causes major hazards to the environment and living-beings (Nauen et al. 2012).

8.2.2 Fungicide

Table 8.1 shows the different types of fungicides which include thiocarbamates, dithiocarbamates, triazoles, dicarboximides and also others such as cupric salts. Fungicide is applied to control the moulds or fungi which affect the crop stability or life cycle. During plugging, handling and packing of fruits such as citrus, there may be wounds to the fruits, and the long time storage and humidity are the ripe conditions for fungus to grow. Cytosporone B is a fungicide used to preserve citrus from fungal attack (Yin et al. 2019). The triazole fungicide has gained much importance in the US which are under the threat of Asian soybean rust which is a fungal pathogen. Triazoles, developed in the 1980s, are a large family of chemicals comprising of members including cyproconazole, difenoconazole, fenbuconazole, flutrifol, or ipconazole, and are among the widely used pesticides (Livingston et al. 2004). In Florida, vegetables, citrus or field crops are treated with triazoles, to combat the turfgrasses diseases. The triazoles are applied when fungal infection has just started, and not when the fungus has developed. The formulations may be dry or liquids, wet table or water-soluble powders. Triazoles move through xylem, reach the leaves and their mode of action is very specific that is by inhibiting the synthesis of sterol, an important component of fungal membranes. Minor interaction with triazoles may cause irritation and redness to the skin. It has low toxic effects when exposed orally, and if inhaled, may irritate the nose, lungs and throat (Souders et al. 2019). The triazole product labelled with 'Caution' or 'Warning' sign suggests that it may cause injuries to the eyes though temporarily. The handler must therefore be equipped with personal protective equipment to protect the eyes during its application. Micobutanil which is labelled as 'Danger' could cause severe eye damage and may be irreversible. Animals exposed to micobutanil have the organs including testes, kidneys, adrenal glands and thyroid, affected. At high doses of propiconazole and tebuconazole, increased cases of liver tumours are reported in the lab animal studies, though no reproductive, developmental or chronic effects, are observed (Ward et al. 2006). Tebuconazole is classified by the USEPA as a possible human carcinogen (Choi 2014). Difenoconazole is highly hazardous to fish, but not toxic to birds and bees (Fishel 2005).

8.2.3 Herbicides

Herbicides such as paraquat, glyphosate and propanil (Table 8.1) fight the extra or unwanted plantae which grow in the crop field. Glyphosate is a broad-spectrum systemic herbicide, introduced by Monsanto (USA) in the 1970s, followed by isopropyl ammonium, ammonium and sodium salts of glyphosate in the late 1970s (Baylis 2000). Trimethyl sulfonium salt of glyphosate is introduced in 1989 by Zeneca (Belgium). Other companies that produce glyphosate are Cheminova (Denmark), Alkaloida (Hungary), AIMCO (India), Shin Dar, Shinung, Comelets (Taiwan) and Nortox (Brazil). By the end of 1996, with more than 40 units manufacturing glyphosate, China has become the major producer, resulting in an oversupply of glyphosate and at reduced price. To meet the supply and demand economics, the number of manufacturing units in China is halved by the end of 1997. Monsanto uses iminodiacetic acid and phosphorous trichloride as intermediates to produce glyphosate. The hydrochloride salt of iminodiacetic acid is prepared first, followed by the treatment with phosphorous acid and formaldehyde to produce N-phosphonomethyl iminodiacetic acid. Then the isopropylamine salt of N-phosphonomethyl imino-diacetic acid is formed, and its oxidation leads to glyphosate isopropylamine salt (Woodburn 2000). In 1996, Monsanto has started the production of glyphosate via different processes. Aminoacetonitrile is prepared from the reaction of methanol and hydrogen cyanide, while iminodiacetic acid is preferably prepared via diethanolamine hydrogenation in the presence of a copper catalyst. The produced disodium salt of iminodiacetic acid needs no purification or the removal of wastes, thus is more cost-effective. The number of crops in which glyphosate can be applied has significantly increased. The total area which glyphosate is applied in 1994 is 52 million hectares throughout the world, including in non-crop areas such as homes, gardens, and across the railroads. The total usage of glyphosate is around 67,100 tons worldwide in 1995, of which 16,000 tons are used in non-agricultural areas especially in North America, West Europe and Japan. In

1996, the consumption of glyphosate has increased to approximately 70 million hectares, and an estimated 42,500 tons of glyphosate are applied globally in both agriculture and non-agriculture sectors. The worldwide market of glyphosate will continue to increase in the future as genetically modified glyphosate crops have been introduced, and at the lower price, especially in the US market (Woodburn 2000).

8.3 Production and Consumption

The Food and Agriculture Organization (FAO) 2017 report concludes that the world-wide production of pesticide increases at the rate of 11% per year. The production in the 1950s is at 0.2 million tons, and reaches five million tons in the year 2000s (Fig. 8.1). China, USA, France and Brazil are the major producer of pesticides. The production of pesticide can be directly linked to the population growth. Table 8.2 shows a country-wide statistics of pesticide consumption. In the 1990s, the sale of pesticides is almost constant between $270–300 billion throughout the world, of which insecticides are sold the most and fungicides the least. During 2007–8, herbicides consumption is the highest especially in the developed countries as fruits and vegetables mostly require the use of pesticides (Carvalho 2017). Many of the pesticides being produced globally are in the two categories – organophosphate and organochlorine. China produces high volume of pesticides annually due to the high consumption, estimated at 400,000 tons, and organophosphate is among those being produced that exhibits high toxicity ratio (Tang et al. 2019).

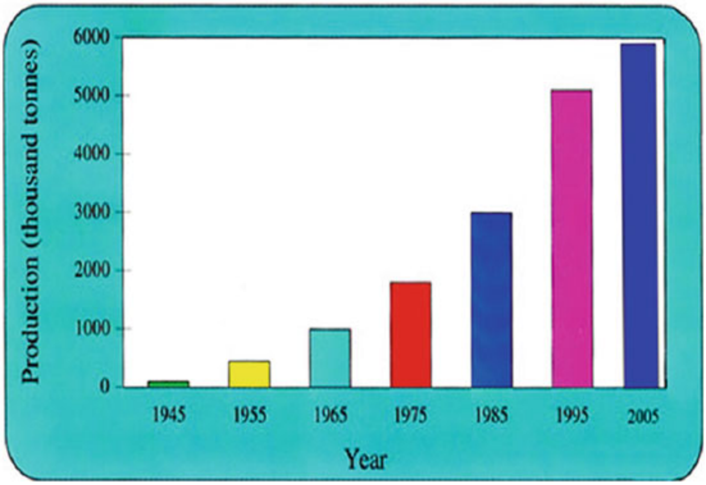


Fig. 8.1 Pesticides production per decade. Maximum production of pesticides has been achieved in 2005 at almost 6000 tons annually. (Reprinted with the permission of Pesticides, environment, and food safety, F. P. Carvalho, creative common attribution (CC BY 4.0) from (Carvalho 2017))

Table 8.2 Top pesticide consuming countries of the world

Rank	Country	Annual pesticide consumption (millions of kilograms)
1	China	1806
2	United States	386
3	Argentina	265
4	Thailand	87
5	Brazil	76
6	Italy	63
7	France	62
8	Canada	54

Carvalho (2017)

USA exports more pesticides than it imports. The pesticides used on maize and soybean dominated the market in 2007. Maize consumes two times more pesticides than the soybean, and which is mostly herbicides, and include atrazine, glyphosate, tefluthrin, cyfluthrin, tebupirimfos s-metolachlor, acetochlor and mesotrione. Atrazine consumption for maize in 2005 is almost double the consumption of glyphosate (Zhang et al. 2011). In France, the consumption of pesticides is the highest, followed by Germany, although Germany is the major producer. In 2007, 36,919, 2101 and 26,808 tons of fungicides, insecticides and herbicides respectively are used in France. China has the 25% world production of crops and feeds the 20% world population with food (Xu et al. 2017). The production of pesticides in China is 2.3 million metric tons in 2015, and increases 7.1 percent every year. In China, hexachlorocyclohexane, dichlorodiphenyltrichloroethane and other organochlorinated pesticides have been banned in 1983 (Li et al. 2006), and in 2007, organophosphorus pesticides, parathion methyl, parathion, methamidophos are also banned (Zhang et al. 2011). In 2008, China imports 44,000 tons of pesticides, but also exports 55,000 tons of fungicides, 136,000 tons of insecticides and 277,000 tons of herbicides.

In developing country such as Pakistan, the import of pesticides is estimated at USD106.5 million in 2014, and worth USD127.6 million in 2023. Around 25% of the pesticides are imported in 2016 to meet domestic demand. The agrochemicals market, with increasing importance of biopesticides in place of chemical control, is expected to reach USD320 million by 2023 at a Compound Annual Growth Rate (CAGR) of 7.9% for the estimated period between 2018–2023. The pesticides are produced by different companies, as shown in Table 8.3, such as the Economy pesticide (DG Khan), Sygenta (Karachi), Agrow Limited (Lahore), Umm Al Quwain (UAQ) Chemicals (Multan) and Prime Brothers (Lahore) (AgReport 2018). Brazil has also been listed as among the country producing the most pesticides. Brazil spends USD12 billion on pesticide production to meet the requirement of 238 million tons per year in the agricultural sector. This poses great challenges for environmental protection and conservation due to the high amount of pesticides introduced into the environment (Della-Flora et al. 2019). South Africa consumes about 3% of the world pesticide production. With the development of agriculture for food and for

Table 8.3 Locally used pesticide for cotton

Sr#	Pesticide	Manufacturer	Concentration	Frequency/ Dosage	Quantity	Price (Rs)	To Kill	Pesticide image
1	Bifuthrine	Economy pesticides (D.G Khan)	100 g/l	200–250 ml/ acre	500 ml	500–600	Jacid, White bee, caterpillar	
2	Prfeunofas + Cypermethrine	Sygenta Pakistan (Karachi)	400 g/l + 40 g/ l	500–600 ml/ acre	1000 ml	800–900	Thrimps, White bee, caterpillar	
3	Imidacloprid	UAQ Chemical Insdustry (Multan)	25% w/w	75 g/acre	200 g	250	White bee, Jacid	
4	Imidacloprid + Acetamiprid	Agrow Limited (Lahore)	20% w/v + 0.2% w/v	240 ml/acre	240 ml	400	White bee, Jacid	

5	Thiamathocazam	Sygenta Pakistan (Karachi)	250 g/kg	24 g in 100 l/ acre	24 g	300	Caterpillar	
6	Emamectine Benzoate	UAQ Chemical Insdustry (Multan)	1.3% w/v	200 ml/acre	200 ml	200–250	Caterpillar	
7	Cypermethrine+ Dimethoate	Agrow Limited (Lahore)	48 g/l + 130 g/ l	800–1000 ml/acre	1000 ml	800	White bee, Jacid, Thrimps	
8	Acetamiprid	Scion Crop Sciences (Bahawalpur)	200 g/l	125 ml/acre	250 ml	350–400	White bee, Jacid, Aphid, Thrimps	

(continued)

Table 8.3 (continued)

Sr#	Pesticide	Manufacturer	Concentration	Frequency/ Dosage	Quantity	Price (Rs)	To Kill	Pesticide image
9	Lamda cyhalothrine	Scion Crop Sciences (Bahawalpur)	25 g/l	330 ml/acre	1000 ml	150–200	White bee, Jacid, Thrimps	
10	Lufenuron	Prime Brothers (Lahore)	50 g/l	250 ml/acre	400 ml	400	Jacid, White bee,	
11	Emamectine benzoate + Lufenuron	Agrowish Chemicals & Seeds (Lahore)	1% w/v + 2% w/v	300–400 ml/ acre	400 ml	500	Cotton pest	

12	Cabofuran	Cotton Quality International		8 kg/acre	8 kg	500	Cotton Pest, Rice Pest, Corn Pest	
13	Chlropyrifos	Scion Crop Sciences (Bahawalpur)	400 g/l	1000 ml/acre	1000 ml	800–900	Jacid, White bee, Jacid	

Modified from Khooharo (2008); Khooharo et al. (2008)

Table 8.4 Different pesticides used for different purposes (Characterization of the coated materials are performed using Fourier-transform infrared spectroscopy (FTIR), ultraviolet–visible spectroscopy (UV-VIS), X-ray diffraction (XRD) and scanning electron microscopy (SEM))

Sr#	Pesticide	Purpose	Dose/ frequency	Side effects	Singular/ combination	Preparation	Characterization	Coating	References
1	Avermectine	Kill weeds, insects	56.27% av./ loaded with pH of 2 for 80 h	High pH can affect release rate	Coating of Avermectine with hydrogel	Polyoligo (ethylene glycol) methyl ether methacrylate (POEGMA)	SEM, UV visible	Hydrogel based on water ethanol system	Sheng et al. (2015)
2	Paraquat	Green plant tissue	54.4952% in 3 days	Fatal for human if come in contact	Coating of Paraquat with Lignin-based polyacrylic acid (LBPAA) hydrogel	Equilibrated by using bath incubator for 20 h	SEM, FTIR	LBPAA hydrogel	Sun et al. (2016)
3	Cyfluthrine	Kill insects, invertebrates	53.7373% in 3 days	Not dangerous	Coating of Cyfluthrine with LBPAA hydrogel	Equilibrated by using bath incubator for 20 h	SEM, FTIR	LBPAA hydrogel	Sun et al. (2016)
4	Amitrole (AMT)	Feed for plants	66% in 10 h	Toxic to human, plants and animals	AMT coated with methoxy modified kaonilite	Soaking method	XRD, IR, DRIFT SPECTRUM	Methoxy modified kaonilite	Tan et al. (2015)
5	Cyhalofop-butyl	Kill Grass weeds	31.35173% in 3 days	Direct exposure can be adverse	Coating of with Cyhalofop-butyl LBPAA hydrogel	Equilibrated by using bath incubator for 20 h	SEM, FTIR	LBPAA hydrogel	Sun et al. (2016)
6	1,2 Dibromo-3-chloropropane (DBCP)	Control nematodes	10% In 10 days	No side effect	Coating of starch xanthate	Oxidative Crosslinking	SEM, FTIR, XRD	Starch xanthate	Shasha et al. (1976)

livelihood of the poor segments of the society, there will be increased consumption of pesticides in the future (Zhang et al. 2011).

There is a variety of pesticides for different crops (Table 8.4). Locally used pesticides for cotton and rice are shown in Table 8.3. The detail regarding the price, usage for specific killing, concentration, dosage/frequency, manufacturer and quantity suggest that the most effective and inexpensive pesticide is carbofuran, lufenuron and emamectine benzoate. The major concern with these pesticide usage is its toxicity especially in the spray form. The toxicity of pesticides is concerned with the dose-time relationship, which necessitates the understanding of how much pesticide is used on the crops and for how much time it is active (Achiri et al. 2020). Bifuthrine for example comes in the concentration of 100 g/L and can be applied on 200–250 acres at a quantity of 500 mL. Pesticides in spray form will come in direct contact with the atmosphere (van der Werf 1996) and human skin, can cause air pollution (Pimentel 1995) and affect living-beings and the soil fertility (Altieri and Nicholls 2003). Table 8.4 shows that there are different coating materials applied on different pesticides using different methods of preparations. However, both the non-biodegradable polymer coating materials such as tetra butyl ammonium hydroxide solution and the biodegradable materials such as methoxy modified kaolinite, are used.

8.4 Impact of Pesticides on Environment and Health

Pesticides are composed of many toxic and carcinogenic compounds and poisonous substances (Gilden et al. 2010). They may be harmful to the people who are in contact with them directly or indirectly or even through the air (Sarwar 2015). In developing countries, the major problem is to achieve the best management of wastewater coming out from industries. Industrial wastewaters are sometimes indiscriminately discharged directly into the natural surfaces like natural drains, or nearby fields. The quantities and characteristics of these industrial wastewater sewage discharge depend on the amount of water used, and their average daily production and are therefore different from one industry to another. Tables 8.5 and 8.6 show the sources and different hazardous contaminants commonly found in wastewater such as the suspended solids, pathogens, heavy metals, nutrients and refractory organic materials and biodegradable organics. Some of these heavy metals are among the most toxic elemental pollutants, belonging to the transition metals. These include mercury (Hg) and cadmium (Cd), while the dominant one among these is iron (Fe). The metals of the representative elements are tin (Sn) and lead (Pb), while arsenic (As) and selenium (Se) are also causing major water pollution. Both pesticides and persistent organic pollutants fall in the same categories as the polycyclic hydrocarbon and some halogenated hydrocarbon, which exhibit remarkable resistance to environmental factors, and may take years and decades to degrade (Ullah et al. 2019). Endocrine disrupting pesticides is an example of major group of persistent organic pollutants. Chlordecone (Fig. 8.2), an insecticide which is resistant to

Table 8.5 Major contaminants found in wastewater

Sources	Contaminants	Effects on environment
Industrial and domestic use	Biodegradable organic materials	Cause degradation of biological materials that uses the oxygen of receiving water and results in unwanted conditions.
Different domestic waste	Different diseases causing agents or pathogens	Transmits different communicable diseases.
Mining or industrial waste	Mainly heavy metals	Toxic and may interfere with discharge and reuse.
Industrial and domestic wastes	Heavy nutrients and refractory organic materials	Cause odor and taste problems, eutrophication, may be toxic or carcinogenic.

Ilyas et al. (2019)

Table 8.6 Major unessential metals found in industrial wastewater

Metals	Sources	Effects and significance
Cr	Metallic platting	Harmful/toxic as essential Cr(III) and Cr(VI)
Mn	Acid mine water, Industrial wastes	Toxic to plants, Damages pigments in excessive amount
Fe	Corrosion, Industrial wastes, Microbial action	Necessary nutrient, Damages pigments in excessive amount
Hg	Industrial wastes, coal and mining	Transferrable by anaerobic bacteria, Hazardous
Se	Natural sources such as coal	At high level toxic, at low level necessary
Zn	Metallic platting, plumbing, Industrial wastes	Necessary metal, at higher level toxic to plants
Cu	Mining, metallic planting, Industrial wastes	Harmful to plants and algae at high level
Pb	Industrial wastes, mining	Hazardous to wildlife, highly toxic
As	Products mining, chemicals of Industries wastes	Toxic, possible to cause cancer

Ilyas et al. (2019)

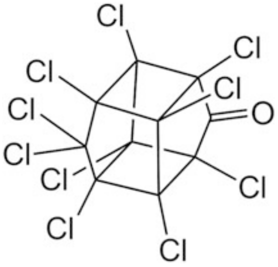


Fig. 8.2 Structure of Chlordecone (an Organochlorine)

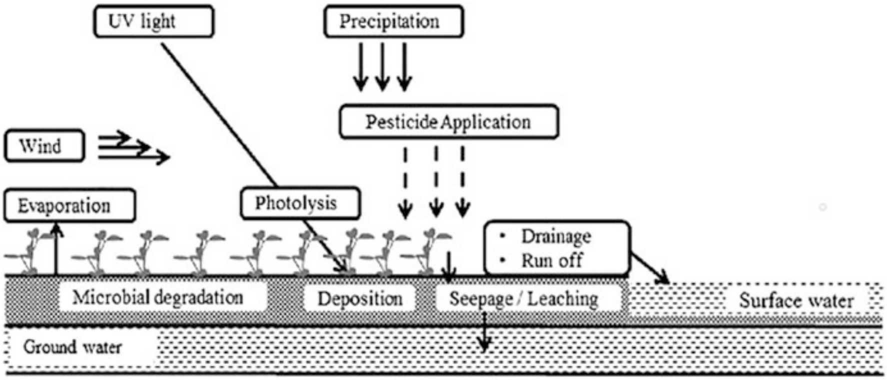


Fig. 8.3 Pathways of the pesticide losses and degradation. Pesticides leach into the soil and groundwater thereby having harmful effects. (Reprinted with the permission of Nanoencapsulation, Nano-guard for Pesticides: A New Window for Safe Application, Nuruzzaman et al., ACS license from (Nuruzzaman et al. 2016))

photolytic, biological and chemical degradation, degrades very slowly, with poor solubility in water. It may not involve in any chemical reaction while present in water but exhibits its availability as long as it lasts. This is hazardous to the marine ecosystem and may disturb the aquatic life (Budzinski and Couderchet 2018). Figure 8.3 shows the pesticide pathway from when it is applied on the crop, and the ways it may reach the marine or aquatic life and cause the pollution. When they are sprayed, some of the pesticide molecules go into the air, mix and create air droplet. With the rainwater, the droplets fall into the water reservoir. As a part of water resource, the pesticide component soon reach the river, lakes or ocean from the flood or rainwater and affect the aquatic life (Pena et al. 2019). Pesticides therefore eventually not only kill the pests but also are harmful to human. The World Health Organization (WHO) report estimates almost 3,000,000 cases and 220,000 death associated with the exposure to pesticides (Dixit et al. 2019).

The exposure to pesticides could lead to many diseases (Table 8.7) like cancer, hormonal disorders, asthma allergies and increased sensitivity (Van Maele-Fabry et al. 2010). The short term exposure of pesticides in 48 hours may cause respiratory tract irritation, sore throat and/or cough, allergic, eye sensitization and skin irritation, nausea, vomiting and diarrhoea. Long term exposure may cause Parkinson disease, asthma, depression and anxiety, and cancer in human. The absorption rate of the pesticide into human body also differ based on the body parts (Fig. 8.4). European Food Safety Authority (EFSA) has classified tomatoes as among the green vegetables that have been most infected with pesticides. The pesticide residues detected in the fields with tomato crops have acute toxicity at low level, but the presence could lead to side effects such as abdominal pain, nausea, skin rash, headache, vomiting, itchiness, eye irritation, nose bleeding and diarrhoea. International Agency for Research on Cancer (IARC) has classified “chlorothalonil”, a member of group 2B-the legate, as the pesticide exceeding the Maximum Residue Levels (MRLs) with

Table 8.7 Human health effects with exposure to pesticides

Type of disease	Health effects due to pesticides (%)
Leukemia	5
Diabetes	8
Parkinson's disease	8
Cancer	15
Asthma	5

Kim et al. (2017)

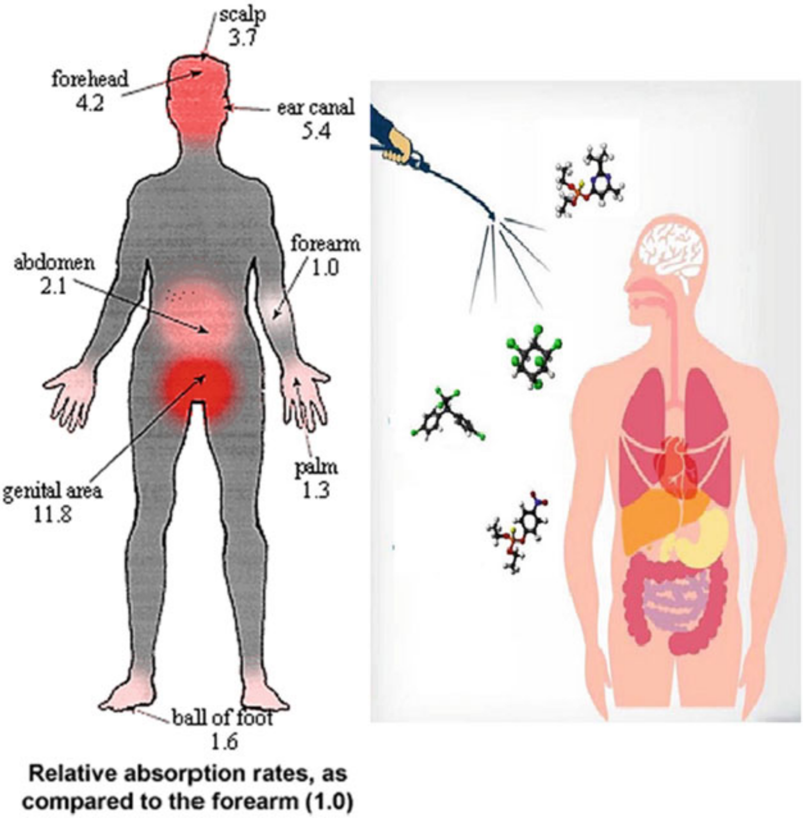


Fig. 8.4 Absorption of pesticides in human body. (Reprinted with the permission of Exposure to pesticides and the associated human health effects, Kim et al., Elsevier license from (Kim et al. 2017))

the possibility to cause cancer in humans, and can cause tumour and kidney damage in animals over long term exposure. The other two members of the group, captan and deltamethrin, are however classified as not cancer-causing (Hlihor et al. 2019). Comprehensive research on soil sample and different vegetable samples collected from Khyber Pakhtunkhwa, Pakistan has shown that the samples contain varied concentrations of 30 endocrine disrupting pesticides. Of these, 12 are hazardous organochlorine pesticides listed by United Nation Environmental Program (UNEP) – dichlorodiphenyltrichloroethane, aldrin, heptachlor, endrin, alfa hexachlorocyclohexane and beta hexachlorocyclohexane, toxaphene, endosulfan, dieldrin, chlordane, mirex and lindane, and graded by the Stockholm agreement as the persistent organic pollutants. Heptachlor epoxide possesses no potential cancer risk but may be hazardous to children through vegetable consumption (Ali et al. 2019). Enzymes of the nervous system and hepatic enzymes are active biomarkers for neurotoxicity and hepatotoxicity originating from pesticides (Hayat et al. 2019).

8.5 Detection of Pesticides in Water

There is a strong connection between life on earth and environmental parameters, that any setback that happens to one, will inevitably affects the other. Water being the most abundant component of this nexus is now seriously contaminated. Getting the amount of pesticides polluting the water system detected, quantified (Della-Flora et al. 2019) and remediated, is of paramount importance. Among the commonly utilized pesticides, organophosphates, neonicotinoids, carbamates, diacyl hydrazine, benzimidazoles and phenyl urease have not been rigorously analysed in the water samples. Chromatographic techniques coupled to the detectors are generally utilized for pesticide analyses because of their effectiveness, with a more stable quality of analyses and efficiency. Liquid Chromatography–Electrospray–Tandem Mass Spectrometry (LC-MS) is one of the most important procedures to examine the polar and thermally unstable pesticides in natural samples with the ability to screen various classes of pesticides in complex matrices in a single run (Stachniuk and Fornal 2016). The method has high selectivity and specificity but may need pre-concentration and purification of the samples, such as the use of Liquid-Liquid Extraction (LLE) and Solid Phase Extraction (SPE), before the chromatographic analyses (Dujaković et al. 2010). Gas chromatography (GC), in combination with electron capture detection, nitrogen phosphorous detection, flame photometric detection and mass spectrometry, are suitable to analyse organophosphorus pesticides in watery samples. The main components of the GC are the chromatography section, the heater, and the detector. The chromatographic section holds the isolating media in a fixed position to a constant flow of a carrier gas. It is made from a long tight, metal, or glass tube loaded up with a pressed material comprising of a fluid stationary stage on a strong support. The stationary phase for the most part is a natural silicon (Geiger et al. 2012). Pesticides estimation can also be carried out using Gas Liquid Chromatography (GLC) where the stationary phase is fluid, and the carrier phase is gas, for the separation of compound to take place. The GLC is necessary when the compound is unstable at high working temperature.

Some of the common pesticides contaminating the surface and ground waters, such as organochlorine and Triazins, require pre-concentration techniques (Kuster et al. 2008). LLE is an established technique to pre-concentrate samples (He and Lee

1997) and routinely used in the analyses of pesticides (Fatoki and Awofolu 2003; Tahboub et al. 2005; Rezaee et al. 2006). Nonetheless, ordinary liquid–fluid extraction utilizes large amount of solvent, may be unsafe, and tedious to perform (Caldas et al. 2010; Sassolas et al. 2012). The SPE and the LC-MS analyses have been carried out to correlate the effects of normally utilized farming pesticides present in the above and underground water, on the crops (Nogueira et al. 2004; Rodrigues et al. 2007; Carvalho et al. 2008). The types of cartridge/dissolvable blends and pH may be important factors to optimize the SPE, where pH above 6 has not resulted in the best separation of a few selected pesticides (Su and Jen 2010). When utilizing hydrophilic-lipophilic balance (HLB) cartridge, the elution with methanol/acetonitrile gives lower, yet adequate recovery. For carbendazim, acetonitrile is not suitable for elution (Kumar and Puri 2012). Solid Phase Micro-Extraction (SPME) can be incorporated for the concentration and identification of pesticides in groundwater as it is a scaled-down version, utilizing lower amount of solvent with improved selectivity. Single drop microextraction, fluid stage microextraction or liquid–fluid microextraction are lesser known techniques, as compared to the conventional liquid-liquid extraction, but use solvents in microliters in the extraction stage (Plakas and Karabelas 2012).

Identification of pesticides, as established by the US Environmental Protection Agency, is a continuous journey of improvement. Chromatographic techniques, though effective, are tedious, complex, requiring costly equipments and accessories and specialized handling. Pesticides detection by biosensors and nanolayers may lead the way towards a more robust, real-time and on site analyses. The quality of contacts between the detector or film and the solute or analyte characteristics, and also the water sample pre-processing steps and conditions, must however be looked into and optimized. Water-repelling pesticides for example may not be much held by the nanofiltration layers if the expanded adsorption on the film encourages their consequent dispersion into the penetrating side (Poinsot et al. 2014). Capillary electrophoresis is suitable for fast subjective and quantitative investigation of particles with a wide range of polarities and sub-atomic loads including micro-particles and substantial macromolecules. Its flexibility and high partition efficiency have made capillary electrophoresis an alternative to the generally utilized High Performance Liquid Chromatography (HPLC) and GC for pesticide partitioning and analyses in various grids (Elbashir and Aboul-Enein 2015). Another viable option is a biosensor with an immobilized organic component to capture the analytes with the bio-receptor and turns the response into an electronic signal via a transducer (Kumar et al. 2010). The miniaturized size, portable and simple to use makes biosensor attractive especially since it is tunable for different biorecognition components, enzymes, or whole cells for specific pesticide analyses (Pinero et al. 2011). Many types of biosensors have been developed for the detection of pesticides. Among these, tri-enzymatic biosensor utilizes peroxidase added to the bi-catalyst framework to build up the biosensor. The Quartz Crystal Microbalance (QCM) sensor depends on three catalysts for the detection of organophosphorus and carbamates pesticides. The tyrosinase-based biosensors have been used for the detection of carbamates and atrazine pesticides; the alkaline phosphatase-based biosensors for paraoxon; organophosphorous hydrolase biosensor for organophosphorus; and the glutathione-s-transferase catalytic biosensor for atrazine detection (Rojano-Delgado and Luque de Castro 2014). An acetylcholinesterase biosensor based on 3-carboxyphenylboronic acid/reduced graphene oxide–gold nanocomposites modified electrode has been developed for amperometric detection of organophosphorus and carbamate pesticides (Liu et al. 2011).

8.6 Technologies for Pesticides Removal

Environmental pollution in urban and industrial areas are attributable to the noise problem, air pollution by vehicle exhaust and water pollution by detergent and chemicals from industries. In rural areas, the major source of pollutions is from agricultural activities (Ippolito and Fait 2019), mainly from agrowastes, manure wastes, fertilizer leaching and pesticide residues. Figure 8.5 shows the pesticide cycles which trace the origin and the flow of pesticides in the environment. Various

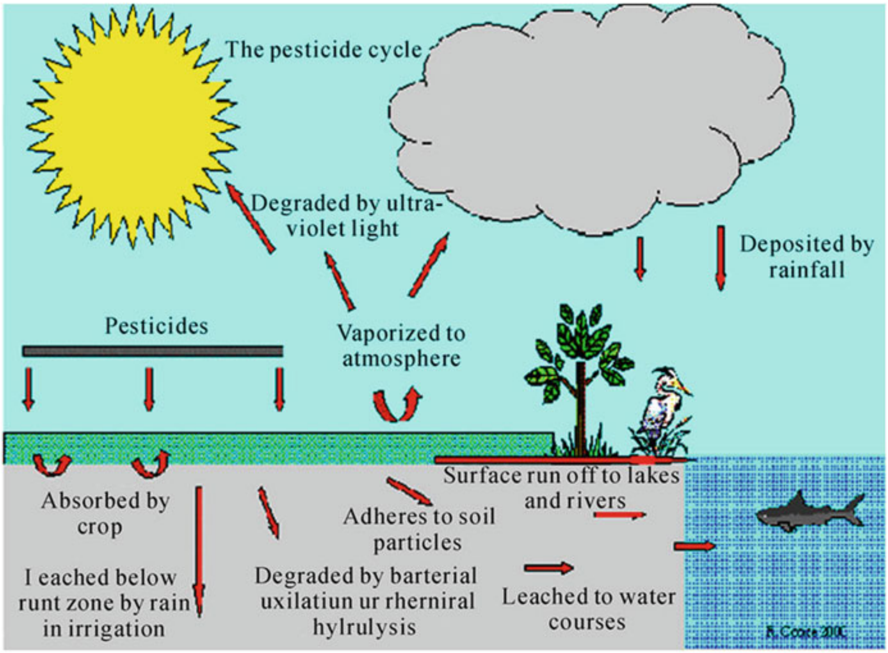


Fig. 8.5 Pesticide cycle. The pesticides are absorbed by the crops and some are degraded by bacteria/microorganisms present in the soil. Pesticides released into the soil may leach to the water courses. The pesticides may also vaporize into the atmosphere which are then degraded by ultra-violet light and also captured in the atmosphere and deposited as rainfall. (Reprinted with the permission of Water pollution with special reference to pesticide contamination in India, Agrawal et al., creative common attribution (CC BY 4.0) from (Agrawal et al. 2010))

chemical methods, particularly the carbon-14 (^{14}C) labelled compound, have been used to study the degradation rates of these pesticides in soils as well as the aquatic environment. The toxicity and degradation life cycle of organochlorine and organophosphate pesticides have been analysed. Organochlorine pesticide which is the first to be introduced is also the most toxic, and requires more time to degrade. Organochlorine contains highly toxic chemicals such as dichlorodiphenyltrichloroethane, hexachlorocyclohexane (HCH), heptachlor, aldrin, chlordane, hexachlorobenzene (HCB), endrin and toxaphene, while organophosphate pesticides are less toxic and also require less degradation time (Carvalho 2017). Organochlorine pesticides are widely used in agriculture in the last 30 years because of their chemical stability. They percolate into the natural environment through surface runoff from waste landfills and rural areas. Dichlorodiphenyltrichloroethane and HCH have been banned because of their presence in the sediments, and they accumulate in fishes. Organochlorine pesticides are endocrine disruptors even in minute quantity, and may cause infertility and cancer.

A number of methods for the removal of pesticides have been developed for the separation of pesticides from the mixtures of substances and fluid streams. There are three basic methods – chemical, biological and physical remediation methods. The extraction of pesticides depend primarily on factors such as pH, nature of pesticide, type of matrix and the cost of investment. Several technologies that have been developed are carbon nanotubes, graphene, nanocrystalline metal oxides, hydrogen peroxides, ultrasonic wave, hybrid process, bioremediation, photocatalytic degradation, adsorption, membrane separation, bio-purification systems, composite material and ion exchange resins (Marican and Durán-Lara 2017). Materials and composite materials have been tested for use in adsorption, membrane separation, biodegradation and photocatalysis. Nanomaterials with numerous chemical groups can enhance the efficiency for the removal of specific compounds and mainly used in adsorption, degradation and filtration processes.

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8.6.1 Hydrogen Peroxide and Ultrasonic Waves

The oxidation of pesticides by hydrogen peroxide and ultrasonic waves have been tested for pesticide removal. For specific pesticide sample solution, the concentration of hydrogen peroxide, the solution pH, the course of reaction, and the kinetics of degradation need to be evaluated and optimized, such as in a glass reactor. Hydrogen peroxide uses oxidant while ultrasonic waves achieve sono-degradation for effective pesticide removal. Ultrasonic processes can be performed in a reactor composed of an ultrasonic processor, water ice bath and reactor cell. The source of ultrasonic wave, the frequency, the power, pH and duration of treatment must be tested. Upon neutralization of the sample, the sonication can be terminated, and the Total Organic Carbon (TOC) of the sample assessed. Pesticides concentration in the solution can also be reduced by using a hybrid process of hydrogen peroxide and ultrasonic waves. Efficiency depends upon temperature, hydrogen peroxide concentration and pH, which are related to the formation of OH^- (Kida et al. 2018).

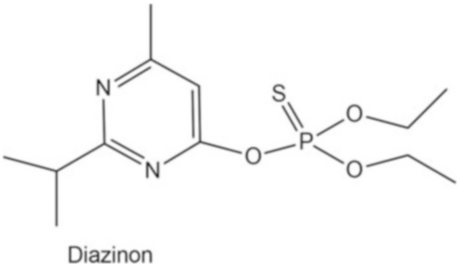
8.6.2 Photocatalysis

Diazinon (Fig. 8.6), an organophosphate insecticide, is widely used, and is categorized by the WHO as hazardous class II chemical to mammals, humans and aquatics. Extraction of diazinon from contaminated water by adsorption, membrane separation, chemical coagulation and filtration have resulted in high consumption of chemicals, incomplete removal, high cost and time-consuming. Photocatalytic degradation of diazinon has been evaluated using $FeTiO_2$ Bent-Fe photocatalyst at pH 5 (Phuong et al. 2019). The solution is first placed in the dark to attain adsorption equilibrium, before visible light from a 36 W compact bulb is used between 400–700 nm. The sorption of more than 30% pesticide onto the $FeTiO_2$ Bent-Fe photocatalyst is achieved in the dark, and the photocatalytic degradation starts upon visible light incidence. The photocatalytic material absorbs a specific quantity of incident light for the generation of many electrons and holes. These holes and electrons react with water and oxygen to generate oxidative radicals, superoxide and hydroxyl anion radicals, to produce harmless inorganic material from diazinon degradation. A total of nearly 60% diazinon removal is achieved by the adsorption and photocatalytic degradation process (Phuong et al. 2019).

8.6.3 Bioremediation

Bioremediation uses natural resources, more economical and can be carried out *in situ* without interrupting the activities around the contaminated zone. Natural vegetation plays an important role to reduce environmental pollution, by being a constant sink for the various chemicals. Furthermore, plants and microbes as key biological agents, work in synergy to degrade the waste materials. Nevertheless, bioremediation is a relatively slower technique as compared to the chemical and physical methods. The selection of microbes or plants for the remediation purposes

Fig. 8.6 Diazinon ion



depends upon the amount and nature of contamination. The plant-based remediation must take into account the soil type, amount of nutrients and water. To achieve the best results, phytoremediation may be combined with other remedial techniques. However, the fact that some recalcitrant chemicals can persist in the environment over a long period is indicative of the limitations of the natural degradation processes. When a pesticide reaches to the ground, it will be absorbed and later degraded. When the pest moves toward the root section, it may be exposed to the pesticide, gets anchored and then the process of degradation takes place (Chaudhry et al. 2005). Endosulfan in high concentration in natural soil are harmful to the fungi and the bacteria. The high pesticide concentration may however be tolerated by certain strains and degraded further without any toxic metabolites. Some new bacterial strains have been isolated that show high biodegradation potentials for endosulfan via hydrolytic pathway (Xie et al. 2011) such as *Stenotrophomonas maltophilia* OG2 for the degradation of alpha endosulfan. The rate is influenced by the concentration of endosulfan, pH and temperature, with more than 80% of alpha endosulfan is degraded within ten days (Ozdal et al. 2017). Biodenitrification is an efficient method for 70% removal efficiency of endosulfan with sand filtering. When wheat straw is used as a substrate, 90% removal efficiency is obtained. One-third removal is attributable to the adsorption onto the wheat straw, and the remaining is due to the biodegradation. Endosulfan can be degraded by *Bordetella petrii* I GV 34 and *Bordetella petrii* II GV 36, where *Bordetella petrii* I achieves 89% degradation of alpha endosulfan and 84% degradation of beta endosulfan. On the other hand, *Bordetella petrii* II can degrade both endosulfan isomers at 82% (Odukkathil and Vasudevan 2015). Innovative techniques for endosulfan degradation has been demonstrated under gamma irradiation in oxidation-reduction processes (Fig. 8.7). The dose of gamma irradiation and the electrons released in the aqueous system act as primary reacting components to enhance endosulfan removal. The removal however can be inhibited when the reaction contains nitrate, nitrite, carbonate, humic acid, bicarbonate and ferric ions. The endosulfan degradation pathways and the formation of metabolites suggest that the hydroxyl radicals could attack the bonds of sulphur-oxygen and the chlorine atom (Shah et al. 2014). Figure 8.8 shows the endosulfan degradation pathway by microorganisms where alpha-endosulfan is firstly degraded to endosulfan sulfur by oxidation carried out by monooxygenase, which is then further hydrolyzed by hydrolase to endosulfan diol. The final product is endosulfan ether (Kumar and Philip 2006; Weir et al. 2006; Kataoka and Takagi 2013).

8.6.4 Adsorption Technology

Adsorption is one of the most effective and low-cost methods for pesticide removal. There is no elaborate preparative work, and only involves accumulation of the target substance at the sorbent surface, making it simple to apply at the contaminated site. The more active surface area available for target ion sorption, the higher will be the adsorbent capacity to remove the contaminants. Adsorbent should have high

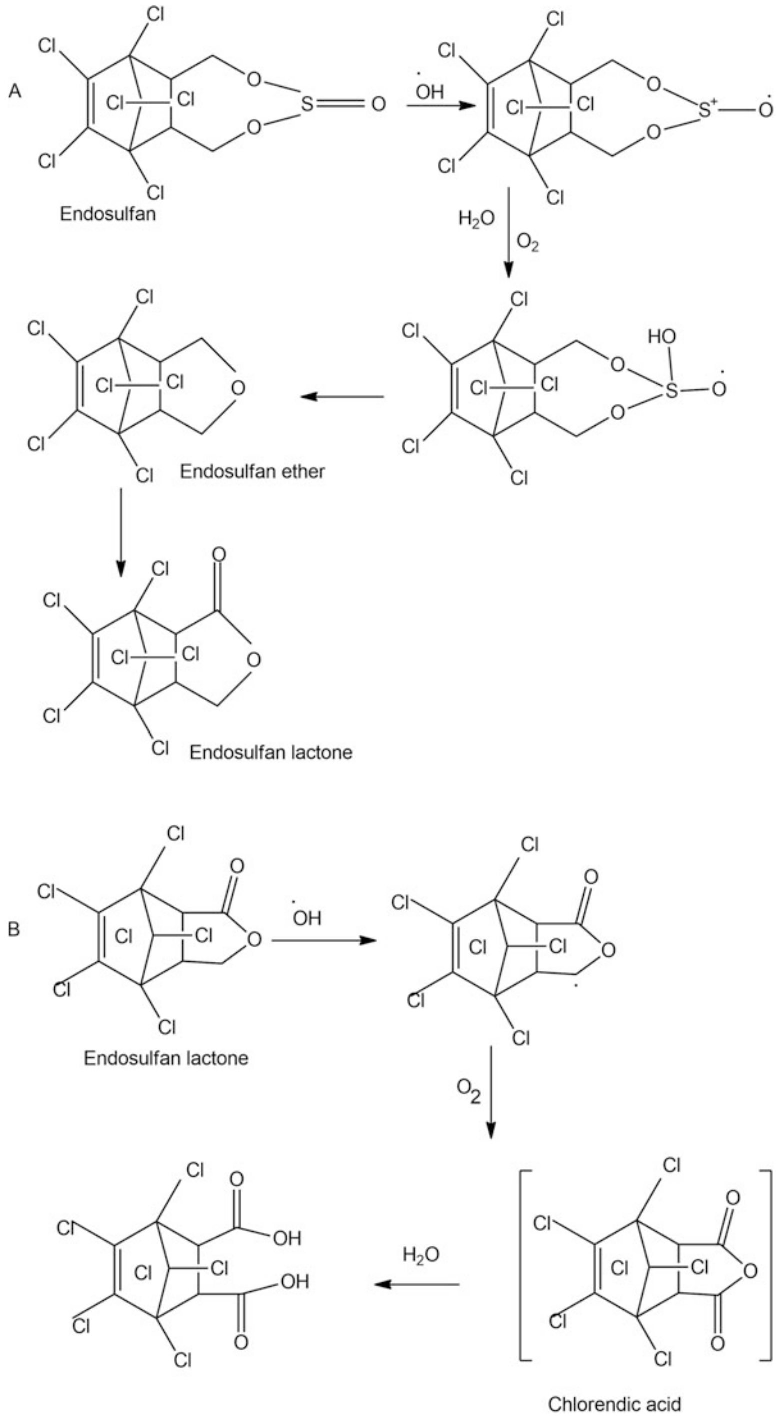


Fig. 8.7 Endosulfan degradation pathway by hydroxyl radicals generation under gamma irradiation in oxidation-reduction processes. In the presence of gamma radiations, endosulfan is first

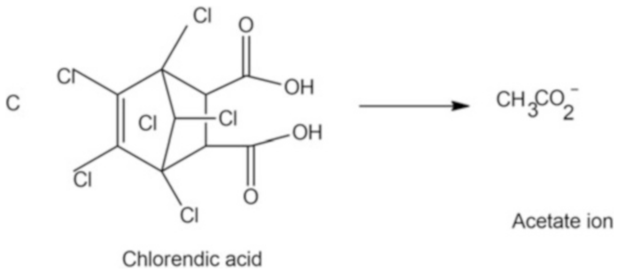


Fig. 8.7 (continued) converted to endosulfan ether, releasing water and oxygen. Endosulfan is further converted to endosulfan lactone, and then to chlorendic acid with the addition of oxygen and water

porosity and take less time for the pesticide uptake. Some of the most commonly used adsorbent is aluminum, bauxite, silica gel, zeolites, and activated carbon. The ion exchange material is also beneficial for the adsorption of pesticides like atrazine and isoproturon. Activated carbons based on natural materials such as apricot stone, rice straw, corn cob, rapeseed stalk, olive kernels and soy stalk, are attractive as they are highly adsorptive, effective to remove various pesticides and could be regenerated. The application depends on the size and shape of the carbon, whether granular or powder form, and the conditions of the sorption process for optimal sorbate removal such as pH, temperature, contact time and batch or continuous mode of operation (Ali et al. 2012; Sharma and Bhattacharya 2017). Biochar, a low cost and environmentally-friendly adsorbent, is a porous, carbonaceous material, formed from the biomass at elevated temperature under low amount of oxygen. It has low carbon content which maintains water limit, improves air circulation and helps to improve microbial growth. Biochar can enhance the soil fertility as well as the crop yield, and has also been used in carbon sequestration, greenhouse gas emission reduction, pollution control, and as catalyst, super capacitor, and in energy conversion and storage (Varjani et al. 2019). With large surface area and porous structure, biochar could reduce pesticide exposure to the environment, assist the biodegradation of pesticides, increase the activity of the microbes, and improve the soil fertility. Figures 8.9 and 8.10 show the morphology of carbon nanotube and the multi-walled and single-walled carbon nanotube used for pesticides removal (Dehghani et al. 2019). Complete removal of pesticide, can be achieved economically and effectively because of its higher surface area and capacity. This is shown by the high removal of diazinon using multi-walled carbon nanotube (Dehghani et al. 2019). Granular activated carbons, graphene and electrospun membranes have also been tested for the removal of bacteria, heavy metals, and pesticides in water and wastewater (Rajapaksha et al. 2018). The different types of grapheme-based sorbents include Reduced Graphene Oxide, Graphene Sand Composite, Graphene Coated Silica, Cellulose Graphene Composite, Magnetic Graphene Nano-composite, $CoFe_2O_4$@TiO_2/Reduced Graphene Oxide Photocatalyst and Graphene Coated

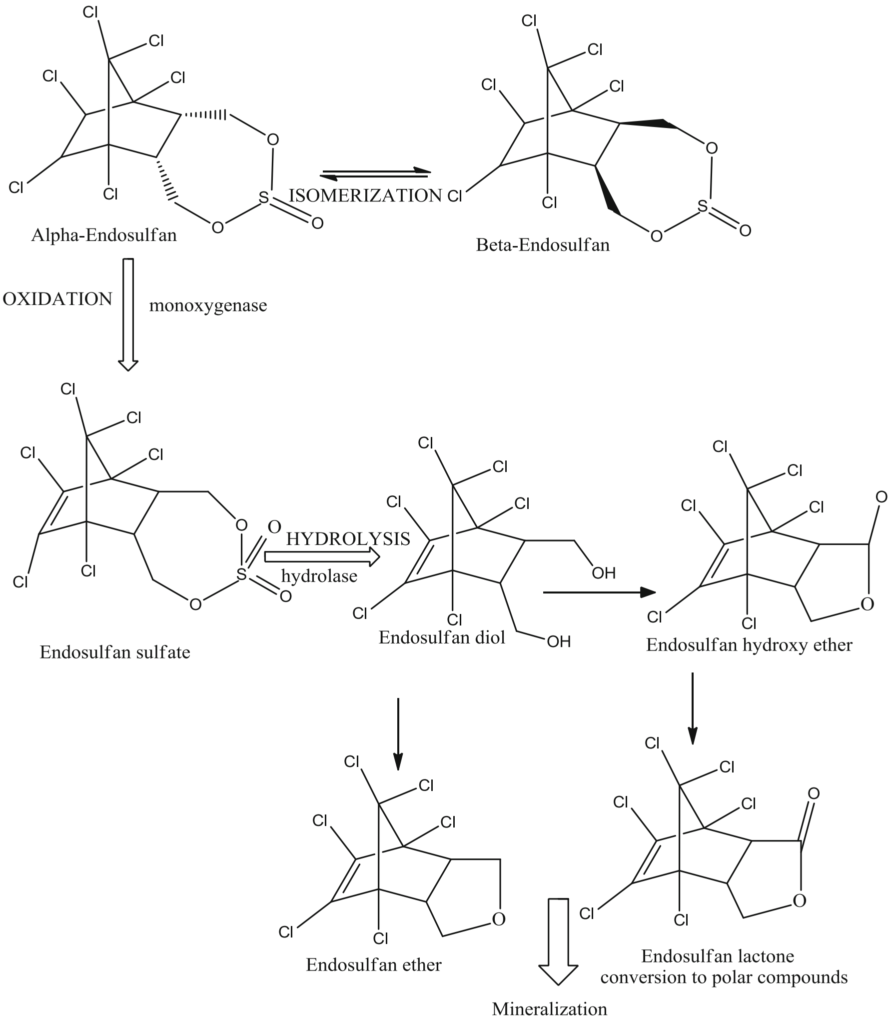


Fig. 8.8 Endosulfan degradation pathway by microorganisms. Alpha-endosulfan is firstly degraded to endosulfan sulfur by oxidation carried out by monooxygenase, which is then further hydrolyzed by hydrolase to endosulfan diol. The final product obtained is endosulfan ether

Fiber as Micro-extraction Medium. The effectiveness of graphene is highly dependant upon its preparation, its specific active site for specific type of pesticide, and if it could work in a wider range of pH as the change in pH may be required for the removal of some pesticides (Rajapaksha et al. 2018).

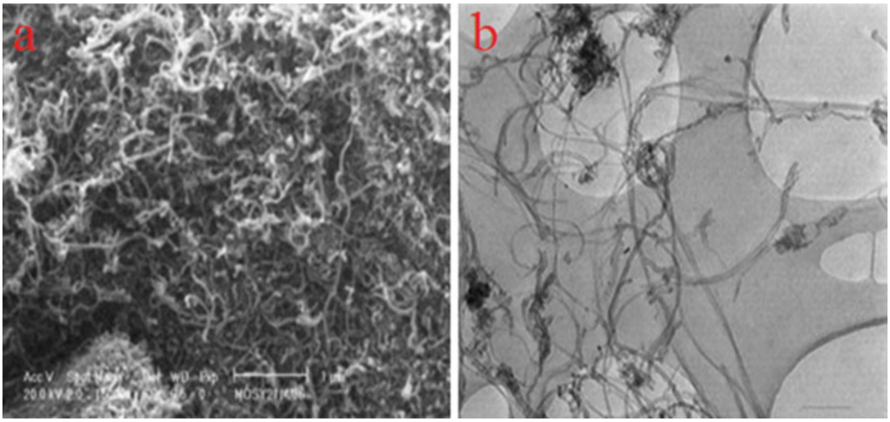


Fig. 8.9 The morphology of carbon nanotubes under (**a**) Scanning Electron Microscope, and (**b**) Transmission Electron Microscope.. (Reprinted with the permission of High-performance removal of diazinon pesticide from water using multi-walled carbon nanotubes, Dehghani et al., Elsevier license from (Dehghani et al. 2019))

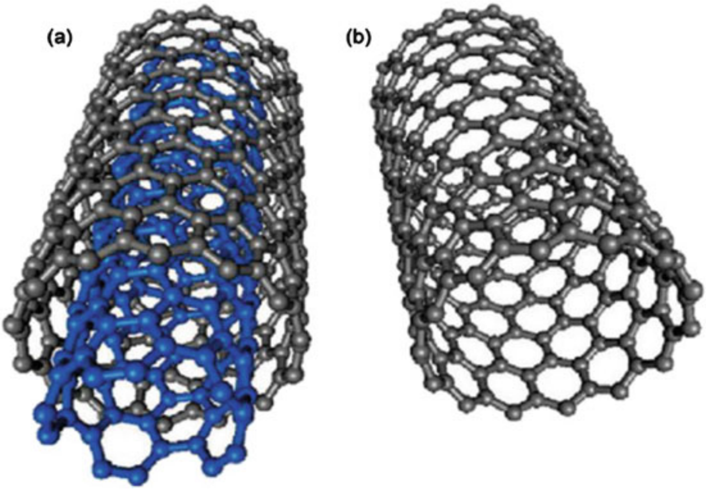


Fig. 8.10 Carbon nanotubes for pesticides removal (**a**) multiwalled carbon nanotube, (**b**) single walled carbon nanotube (Reprinted with the permission of high-performance removal of diazinon pesticide from water using multi-walled carbon nanotubes, Elsevier license from (Dehghani et al. 2019))

8.6.5 Coating and Controlled Release of Pesticides

Coating is a technique used to control the release of the pesticide from the encapsulating material or the surface, to deliver the pesticide or fertilizer in a controllable

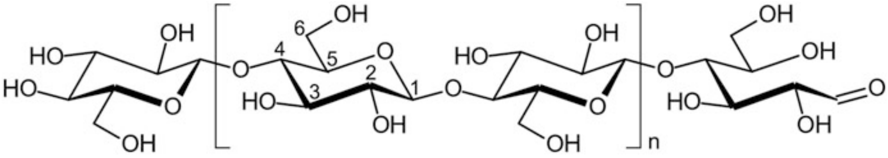


Fig. 8.11 Structure of cellulose. (Reprinted with the permission of Production of cellulose nanofibrils: A review of recent advances, Nechyporchuk et al., Elsevier license from (Nechyporchuk et al. 2016)).

environment to the growing crops (Qiu and Park 2001). Controlled release delivers active ingredients at a predetermined rate depending on the degradation, breaking and dissolution of the chemicals and ensures the delivery of the compounds in response to time (Ummadi et al. 2013). This could result in a prolonged action (Roberts et al. 1993), whilst attempting to maintain the concentration level (Goodson et al. 1983). The controlled release with coating may effectively deliver the pesticide at the level sufficient enough to kill the pest over a longer duration and at the same time reduce the pesticide usage. The controlled release system may also include a less active agent (Dey et al. 2008) to reduce the toxicity on the untargeted organisms (Mitra et al. 2001), and the risk to the environment (van Lenteren et al. 2006).

As shown in Table 8.4, different pesticides with hydrogel coating have been explored for the controlled release, with the most effective one being the hydrogel-coated carbofuran. Naturally occurring polymers such as cellulose are of great importance to be developed as coating material as they are biodegradable and renewable resources, Cellulose is the most abundant natural polymer, and an important component of a green plant (Graham 1992). It is a polysaccharide, composed of repetitive unit of glucose molecule ($C_6H_{10}O_5$), consisting of 1, 4-β anhydroglucose units which is the alcoholic hydroxyl groups (Fig. 8.11). The hydroxyl group within the structure forms an intermolecular and intramolecular hydrogen bond with the macromolecules. The structure of cellulose is the same from different sources but its mechanical properties and degree of polymerization differs (Mohanty et al. 2005). Cellulose however is not soluble in common solvent, and specific conditions are required such as the dissolution of cellulose in many intermediate and ionic solvents (Swatloski et al. 2002; Lindman et al. 2010). Cellulose is soluble in polar solvents like lithium chloride/dimethylacetamide (LiCl/DMAc) (Ishii et al. 2006; Yilmaz and Bengisu 2003), N-methylmorpholine oxide (NMMO) (Zhang et al. 2001), and alkali aqueous system (Cai and Zhang 2005). Cellulose can also be dissolved in alkali and urea solution when kept at lower temperature to dissolve the cellulosice pulp and cellulose acetate. Cellulose acetate, an ester of cellulose, is a classical membrane material and can be prepared from cotton and pulp celluloses. Cellulose can be converted into cellulose acetate by acetylation of cotton cellulose with acetic acid in the presence of a catalyst. Cellulose acetate can also be prepared by reacting cellulose with acetic anhydride (Wu et al. 2005). To prepare the cellulose and cellulose acetate-based hydrogel, the cellulose and cellulose acetate is dissolved in tetrabutyl ammonium hydroxide (TBAH) (Abe et al. 2012), acetone (Liu and Hsieh

2002), NaOH/Urea solution (Cai and Zhang 2005) and then the hydrogel can be made. Cellulose acetate is biodegradable, non-toxic, and with great capacity to absorb water more than the ordinary absorbing capacity of materials (Kabiri and Zohuriaan-Mehr 2003). This makes it widely used in agriculture, to help in water retention, reduce water consumption, and improve retention of fertilizer in the soil. It is also used in the fabrication of thin films on the surface of different materials, pharmaceuticals, biomedical and as a coating material. A chitosan-coated nitrogen, phosphorus and potassium compound-based fertilizer with controlled-release and water-retention properties has been reported. The biomaterial is degradable in soil and environment-friendly, and could be especially useful in agricultural and horticultural applications (Wu and Liu 2008). The cellulose-based hydrogel has been widely used and prepared as the coating material for agrochemicals (Krajewska 2005), but not extensively for the pesticides as highlighted in Table 8.4. The cellulose-based hydrogel has been made to control the release of emamectine benzoate, lufenuron, a combination of both and carbofuran. The pesticide control mechanism is of great interest to effectively make the pesticide usable for a long time and to facilitate reduced impact on the environment and living organisms (Howarth 1991). The release kinetics study of the pesticides from the composite structure has been investigated using the Fickian equation or zero-order (Rudzinski et al. 2002). The cellulose-based hydrogel, when used as a coating material for the pesticides, will increase the fertility of the plants (Han and He 2010) whilst controlling the virulent pests. The coating ability of the cellulose and cellulose acetate for the pesticides could defend the plants from the pest attack and increase the fertility of the crops by becoming a part of the plants, after releasing the pesticide from its porous surface (Novak et al. 2009).

8.7 Conclusion

Over-consumption of pesticides may pose deleterious effects on the environment and human health such as causing cancer and Parkinson’s disease. The contamination of pesticides in the water system is increasing due to increased agricultural activities to meet the global demand for food production especially in developing countries. Different techniques have been developed to remediate pesticide pollution in water system such as the use of hydrogen peroxide and ultrasonication, photocatalysis and bioremediation. The most effective and economical method is by adsorption using activated carbon and biochar. Smart and composite materials have also been developed based on carbon nanotubes, grapheme and ion-exchange resin. The future technology based on coating and slow-release of pesticides must be developed especially based on cellulose and cellulose acetate to ensure the virulent pests can be controlled, whilst improving the fertility of the plants, with minimal impact on the environment and living beings.

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Chapter 9
Strategy for the Inspection of Pesticide Residues in Food and Agriculture

Sujoy Rano and Mukesh Singh

Abstract Pesticides are the group of complex chemical compounds which are used to kill pests. They are widely used in agriculture to increase the crop yield, minimize post-harvest losses in addition to pest control. Apart from agriculture, pesticide has a wide application in poultry industry, dairy industry and also to increase the shelf life of fruits and vegetables. Due to its extensive use, pesticides are frequently found in the food residues. Being complex in nature they hinder with ecological and physiological functioning. They enter human body via food chain from food sources of both plant and animal origin like fruits vegetables, fishes, cereals and meats. High demand of food to feed the rapid growing population, lack of knowledge regarding pesticide complexity, non-strategic agricultural practices and being cheap in price have led to its overuse and misuse. Pesticide contamination embosses serious health issues to all age groups and may affect every possible body organs. The growing bio-magnification in successive tropic levels of food chain remains a life challenge.

Various methods to assess the residual pesticide content have been developed like gas chromatography, two single-residue methods, spectrophotometry, high performance liquid chromatography, spectrophotometry, and different extraction processes. Pesticide inspection is also done by diagnosing of different body fluids like blood, urine, serum, breast milk, semen and also toxicological studies on cell line with the development of different biomarkers. Reports of scientific studies by different international organisation have helped in assessment of traces of different pesticides, its mode of action in human body and further help in minimizing its effect. Immediate action such as shifting to organic farming, preventing the misuse of pesticides, through washing and soaking the vegetables in water for some time before cooking may help in minimising pesticide exposure to a great level.

Keywords Pesticides · Agriculture · Food residue · Gas chromatography · Fruits · Vegetables · Exposure · Health issues · Contamination · Toxicity · Biomarkers · Organochlorine · Organopophate

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9.1 Introduction

9.1.1 Pesticide and Its Wide Scale Use

The world population is growing at a very fast rate and the population count is expected to be 9.1 billion by 2050. To feed such a large population, global food production need to be accelerated (Rahaman et al. 2018). Food plants suffer from a great economic loss due to the interference of various species of pests and weeds worldwide and thus to curb this loss application of pesticides comes into play (Robson and DerMarderosian 2012). Pesticides are the group of complex chemical compounds which are used to kill pests. They mainly comprise of such compounds tagged as insecticides (e.g. organophosphates, aldrin, organochlorines, di-chlorodiphenyl-trichloroethane, carbamates), fungicides or antimycotics (e.g, thiophthalimides, substituted benzenes, ethylene bis-dithiocarbamates), rodenticides (e.g, 1,3-indandiones, Coumarins/4-hydroxycoumarins, chlorophenoxyacetic acid, 4-thiochromenones), and fumigants (e.g, ethylene di-bromide, methyl bromide, di-chloropropane, propylene oxide, di-bromochloropropane, chloropicrin) (Abdollahi et al. 2004). Pesticides are being extensively used in comprehensive ways during the production of different foods to control the growth of fungus, different weeds or to refrain from crop damage by various pests. The agricultural sectors mostly use pesticide to check the damage caused by pests and to boost the overall yield as well as quality of the produced crop and to increase the storage life when used postharvest (Neme and Satheesh 2016). The advantage accompanying to the use of pesticides are recognized from the dilution in crop damage due to pests, increased crop productivity, and other less obvious factors contribute to the reliability of modem agriculture practices on the use of pesticides. The mechanical methods in overcoming the hurdles of crop damage may demand more energy usage, increase in soil erosion and sedimentation in water, and require a willing and able labour force which is often not as efficacious (Ridgway et al. 1978). The rate of crop destruction and hence a great economic loss is found to me more prominent in developing and under developed countries. Due to this reason these countries account for one-fourth of the world's pesticide use (Robson and DerMarderosian 2012). Hence, pesticides turn out to be an indispensable part of modern agricultural science. However, one has to be cognizant of the detail impacts of pesticides on day to day life with increased demand of agricultural production (Table 9.1).

Table 9.1 Percentage of different pesticides under use for agricultural purpose in India

Pesticides	Percentage of use
Insecticides	60%
Fungicides	19%
Herbicides	16%
Bio-pesticides	3%
Others	2%

Devi et al. (2018)

9.1.2 Adverse Outcome of Extensive Pesticide Use

The wide scale use of pesticides in agriculture results into considerable benefits. At the same time, the possible adverse outcomes of pesticides must also be considered. While the efficacy of pesticides are normally quantified from the analysis of direct costs related to the use of pesticide and corresponding crop production, a quantitative estimation of the risks associated are much more troublesome to obtain (Pimentel et al. 1993). Developing countries with large population has a very high demand to increase the agricultural production. To meet this demand and further protection of crops, use of cheap chemical fertilizer and pesticide use are practised because either these are easy to synthesize or easily available in market at low price. Even countries like India which has an industrial capability but due to high demand of agricultural production, they mainly invest in the production of such chemicals (Carvalho 2006). Due to the different application techniques used, such as spraying along with a poor regulatory framework and treatment process, these chemicals ultimately leeches to the aquatic and terrestrial environment. The continuous use of these may cause contamination to the environment and ground water quality, interferes with ecological functions and its exposure to the public and food residues may turn out to cause adverse life risks (Chaukura et al. 2016). A high percentage of deaths all over the world are caused from agricultural pesticide poisoning killing around 250,000–370,000 people each year (Dawson et al. 2010). The cause of illness and deaths are mainly due to illiteracy, lack of complete knowledge of the use and its effects, inadequate safety standards which sometimes lead to its misuse or overuse (Pimentel et al. 1992; Atreya 2008).

9.2 Pesticide Traces in Human: Its Modes and Exposure

9.2.1 Different Modes of Pesticide Exposure to Humans

In a report by Franklin et al., said that the annual global consumption of pesticides is around two million tons (Franklin et al. 2017). Most pesticide exposure to humans is expected during its production and application. The general population can also get exposure to agricultural food residues via food chain or any cross contamination with fresh food items and drinking water. Thus food consumption constitutes a prime route for exposure to contaminants from wide sources, including pesticide. Due to its persistent exposure throughout, a low level of its metabolic residue in found in the body tissues of most of the people around of the world including all age groups (Blair et al. 2015; Kim et al. 2017). Pesticides include varieties of chemical groups with varying compositions, having different active ingredients and most of them are either known or suspected to cause serious health hazards (Cox and Surgan 2006). Large scale use of different pesticides in agriculture as well as for storage purpose is the common exposure for self-harm in various rural and sub urban households (Guo et al. 2018). Still research works in this field are not yet enough to illustrate the actual cause and mode of action of different health disorders due to ingestion of complex pesticide residues via food. The growing health concern among people have inclined them in the consumption of nutrient and fibre rich green vegetables, dairy products and poultry meats to be included in their diet in a balanced form (Ganry 2005). It is found worldwide that an annual death of 0.4 million is due to 600 million food borne illnesses and the main cause of it is poor practise of pesticide usage and negligence during its handling (Chekol et al. 2019; Tegegne and Phyo 2017). In a survey conducted among 1000 farmers in Cambodia, Laos and Vietnam, it was found that 100% of the sampled farmers in Vietnam, 73% in Cambodia and 59% in Laos were found to over use pesticides. Pesticide exposure were associated mostly due to their different job profiles like the one involved in pest management, one directly dealing with pesticide sellers or the one involved in pesticide spraying to the fields. The study also estimated that the overuse of these pesticides can be reduced by 68% if the agricultural practice is substituted by bio-pesticide, 74% if substituted with plant organic wastes, 34% if farmers practise hand picking of insects from the field (Schreinemachers et al. 2020). Different studies have previously highlighted the overuse of pesticides in different countries like in Thailand 79% (Grovermann et al. 2013), in Nepal 70% (Jha and Regmi 2009) and in France 42% (Lechenet et al. 2017) overuse for vegetable production. Studies have revealed that the occurrence of different toxic residues in vegetables, dairy products and poultry meats may cause acute health issues if on short exposure and it further leads to chronic conditions depending on exposure duration (Hayajneh 2015). Various works have also revealed that over 90% of pesticide contamination in human is via food obtained from animal origin (Tecles et al. 2013). A high pesticide contamination is reported in tropical countries like India, to be present in dairy milk (Nag and Raikwar 2008) and similar contaminations in the milk products were also reported in Jordan (Nida et al. 2009). Most of the pesticides as reported are lipophilic in nature and thus its presence in milk and milk products, which has adequate amounts of fats, is quite common (Nag et al. 2007; Kalantzi et al. 2001). Another source of pesticide exposure to dairy product is the direct spraying of the chemical near the cattle accommodation to check the pest incursion (Akhtar and Ahad 2017; Losada et al. 1996) (Fig. 9.1).

9.2.2 Pesticide Residues Traced in Food Items of Densely Populated Countries

In densely populated country like India, more than 51% of food items are found to be adulterated with pesticide residues and 20% of it has the residues above the maximum permitted level in food items accepted globally. Thus due to continuous exposures through food commodities it is observed that a large population suffers from health effects such as loss of immunity, hormonal misbalance, decreased

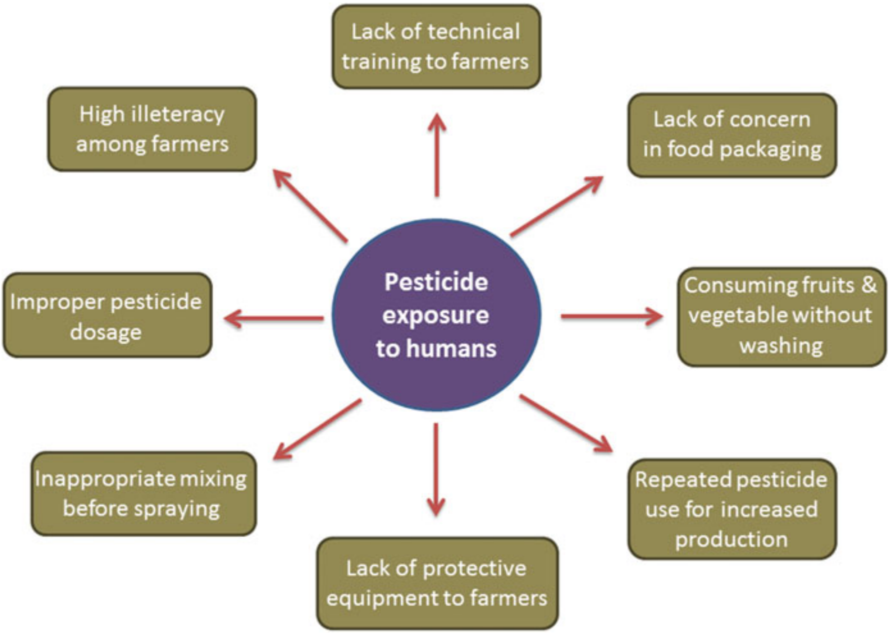

Fig. 9.1 It highlights different aspects and means of pesticide exposure to human which further may leads to health hazard

Intelligence Quotient, loss of fertility and cancer (Gupta 2004; Kesavachandran et al. 2009). In a study on 24 samples of cereals, pulses, vegetables, fruits, butter and edible oil, it was found that 85% of the samples had high level of organochlorine pesticides. Pesticide content was found to be more in cereal and edible oil samples than that of others (Kaphalia et al. 1990). China having 20% of the world population but only 10% arable land, has led to a surge in the pesticide usage to 1.8 million tons in 2016 to feed its population, making it the largest pesticide user worldwide (Matthews 2019). A very high percentage of hexachlorobenzene, dichloro-diphenyl-trichloroethane, chlordane compounds, hexachlorocyclohexane and heptachlor were diagnosed in majority of the food items consumed by most of China's population, major being aquatic foods, meats, and cereals (Zhou et al. 2012). Indonesia being the fourth largest populated country, practises intensive cropping to meet the hunger requirement of the country. This has led to intensive pesticide use and around 13 to 25 different pesticide residues are found in most of their agricultural food items (Sanchez et al. 2019) (Table 9.2).

Table 9.2 Highest annual consumption of total pesticides by the Asian Countries

Asian countries	Pesticide consumption (Ton product)
China	10,00,000
Malaysia	2,04,260
India	1,64,080
Thailand	1,32,509
Pakistan	1,29,589
Republic of Korea	1,00,000
Vietnam	50,000
Philippines	31,735
Bangladesh	22,100
Sri Lanka	6329
Myanmar	3030

Abhilash and Singh (2009)

9.2.3 Pesticide Exposure to Human in Undeveloped Countries

Exposure to pesticide among farmers and consumers in the developing or underdeveloped countries are a major threat to health. The occupational threat to pesticides is mainly due to improper handling of pesticides by workers and thus lays a drastic effect to the population consuming those foods. The misuse of the pesticide handling is mainly due to poor legislative framework and absent of technical knowledge among the workers and also due to lack of proper guidelines of usage. Lack of safety precautions, health hazard, and lack of information regarding first aid is just pushing the human life towards threat in these countries (Hashmi and Khan 2011). Uganda, mainly an agro based country with 85% of its economy relies mostly on agriculture. More than 35 million people here reside in rural areas and are involved as a labour for agriculture. Thus to have a greater production, endless use of chemical pesticides are practised. Human health concerns for these people due to lack of protective equipment and clothing and dosage control. To ensure greater productivity and maintain export compliance, health of the workers and other people are ruthlessly compromised (Karungi et al. 2011). Farmers in Bangladesh also use different chemical pesticides for increased agricultural production. Around 75% of them take only partial protection during pesticide handling but still suffers from disease of eyes, skin, gastro- intestine and urine. The study also reports that 67% of the marketed vegetables in Bangladesh have traces of pesticides which are greater than the acceptable daily intake level (Miah et al. 2014). In a study it was found that farmers of Egypt do not undergo safe pesticide handling. They also dispose the empty pesticide cans in the environment without any proper precaution and later found that most of them are completely unaware of the adverse effect of pesticide on health. The lack of knowledge is due to a void in the education system among them (Ibitayo 2006). Unfortunately the lack of agricultural resource and its malpractices have posed a greater health impacts on the population of these countries.

9.3 Traces of Pesticides in Different Food Items

9.3.1 Traces of Pesticide in Milk and Its Products

The prime source for the presence of pesticides in milk and milk products is the previously contaminated food given to livestock (Ray and Sen 2019). Organochlorine pesticide due to their bio-accumulative nature there remains a tendency to get accumulated in the fatty tissue like that of breast. The leaching of the contaminant from breast tissues to milk is a potent source of pesticide exposure to children. Reports have revealed that mothers' milk is also a prime source of pesticide exposure to children and its concentration is more in urban areas than rural areas. Percentage of pesticide exposure in mothers' milk is greater in Asian countries like China, India, Bangladesh, and Japan than that of European countries (Pirsaheb et al. 2015; Bergkvist et al. 2012). In a study by *Kaushik* et al. on fresh bovine milk post monsoon period from rural areas of Haryana (India), it was found that 43% of the samples were contaminated with hexa-chloro-cyclohexane, 53% by Dichloro-diphenyl-trichloroethane and 36% with endosulphan. The study revealed that 2% of the sample crossed the maximum residue limit recommended by World Health Organisation (Kaushik et al. 2014). A study conducted on 100 bovine milk samples in Brazil by *Avancini* et al., reported the presence of different types of organochlorine pesticides in 90% of the samples and 14% was above the maximum permitted level (2 ng/g) (Avancini et al. 2013). In North America different studies on commercial milk products took place in Mexico, and had revealed that most samples had frequent contamination of hexa-chloro-cyclohexane and heptachlor isomers, followed by Aldrin. It has also reported that the level of organochlorine compounds was below the maximum permitted level (Gutierrez et al. 2013; Tolentino et al. 2014). Different organochlorine pesticides were also detected in various milk samples in African countries and these were: aldrin, dieldrin, lindane, alachlor, hexachlorobenzene methoxychlor, endosulfan (α and β) and Dichloro-diphenyl-trichloroethane and their isomers (Kampire et al. 2011; Deti et al. 2014; Shaker and Elsharkawy 2015). Looking into the present rapid bio-magnification of different organochlorine pesticides due to its high concentration in bovine milk has gradually decreased due to a shift to organic pesticides but still the present level remains a matter of concern (Nag and Raikwar 2008).

9.3.2 Traces of Pesticide in the Products of Breweries

In brewery industries, during the process of making wine, rum, whisky, vodka; grapes or cereals that are used may be pesticide laden. During the processing, certain amount of pesticide may remain in the fermented beverage. There are many pesticide which are soluble in alcohol may have a high chance of retaining in the beverage and might be very difficult to remove. The trace of pesticide residues present in the

Table 9.3 Different number of pesticides required for different crops all across the world

Sl No.	Types of edible crop	Number of different pesticides used
1.	Cereals	642
2.	Fruits	589
3.	Vegetables	337
4.	Legume	223
5.	Tubers	224
6.	Sugar	170
7.	Tree Nuts	132
8.	Tobacco	74
9.	Chilly	6
10.	Cocoa	3

Madariaga-Mazón et al. (2019)

beverage actually do not interfere pre or post making process but cause harm when consumed. (Cabras and Angioni 2000). A scientific study on the presence of pesticides traces was performed on wines and beers from different brands like Budweiser, Coors, Miller Lite, Sam Adams, Samuel Smith Organic, New Belgium, Beringer, Barefoot and Sutter Home was performed. It was found that 19 of 20 samples had high glyphosate (pesticide) content. A surprising result obtained was that 3 out of 4 organic beers had glyphosate content but glyphosate is not allowed in organic farming. Contamination in beers and wines due to glyphosate is more common in United States (Cook and Fund 2019) (Tables 9.3).

9.3.3 Traces of Pesticides in Agricultural Food Items

Delhi (the capital of India) being one of the most populated cities in the world faces pesticide exposure in most of the vegetables they consume. Average organochlorine pesticide level in different vegetables was in the range 83–222 ng/g of vegetable sample. The hazard quotient and the lifetime cancer risk that was estimated was found to be above the acceptable limit (Chourasiya et al. 2015). Field research for the presence of different pesticides on different vegetables collected from the markets of Hyderabad and Secunderabad (India) was done. All sample fruits and vegetables collected had traces of some or the other pesticides in it. It was also deciphered that after washing the vegetables under luke warm water, a great reduction in the amount of pesticides was found (Dasika et al. 2012). The World Health Organization has recommended long before that the use of toxic pesticides must be restricted, but many developing countries failed to follow. As a result, organophosphate pesticide exposure to human on a daily basis has remained common in these countries (mostly the countries of Asia and Africa). Around 3,000,000 human beings all around the globe were affected by pesticide toxicity in between 2002 and 2005 and the prime source was agriculture. There are reports on maximum plant food contamination in

Morocco, Egypt, Iraq, Saudi Arabia, Sudan, Syria, Jordan, UAE, Pakistan and Yemen in the recent past (El-Nahhal 2004).

9.4 Health Issues from Pesticide Contamination via Different Food Sources

9.4.1 Pesticide Stimulates Oxidative Stress in Body

Intensive research works in the past 50 years over a large spectrum of population based studies led to effective understanding of the correlation between the pesticide and its ill effects on human health. Pesticides in the body upon metabolism, release active free radicles, increasing the oxidative stress of the body leading to cell damage. An imbalance between the production of different reactive oxygen species and body's capability to counteract its effect leads to an oxidative stress. The human body has got different mechanisms to curb the damage caused by these free radicals on entering the body. The first line of defence to prevent the damage is the enzymatic system that nullifies the oxidation but if the oxidative stress overtakes the maximum defence capacity, it leads to visible adverse health symptoms.(Abdollahi et al. 2004; Halliwell and Gutteridge 1990). In a study on farmers of age group ranging 25 to 56, superoxide dismutase and 8-hydroxy-2′-deoxyguanosine activity was analysed from blood samples pre- and post-pesticide application. A significant increase in superoxide dismutase was found post-pesticide application whereas no significant change was noticed for 8-hydroxy-2′-deoxyguanosine. Thus increased superoxide dismutase is a sign of oxidative damage caused due to pesticide toxicity (Ratana et al. 2019). A study was performed on 268 young children from Shanghai (China) residing in urban areas but near to agricultural land having a mean age of 2 years. It was found from their urine samples that most of them have elevated levels of 8-hydroxy-20-deoxyguanosine which is due to the exposure of organophosphate pesticides leading to oxidative cellular damage in these young children (Ding et al. 2012). A study over a group of people in Karnataka (India), were performed who were directly or indirectly exposed to pesticides. Blood samples of these people reported an increased level of serum malondialdehyde, erythrocyte superoxide dismutase, and catalase and glutathione peroxidase along with leucocytosis. Their increased level indicates body defence system to counteract the pesticide toxicity leading to oxidative damage (Hundekari et al. 2013).

9.4.2 Pesticide Exposure Leading to Multi Organ Malignancy

Based on the reports submitted by various scientific organizations and intense studies by 'International Agency for Research on Cancer' relation to pesticide exposure to human lifestyle, it is found that it is one of the paramount causes of malignancy. Health studies have outlined the formation of neoplasm which may result into different types of cancer such as cancer in breast, pulmonary passage, testicle, cervix, colon, pancreas, gastro-oesophageal passage, and many more (Baldi and Lebailly 2007; Dich et al. 1997; Penel and Vansteene 2007). In a case study on colorectal cancer, it was found that most patients had high level of organochlorine pesticides in their body. The presence of pesticides caused hyper-methylation of the p16 promoter in colorectal cancer patients which was estimated using specific methylation PCR assay (Abolhassani et al. 2019). Women suffering from malignant and benign breast cancer in Iran were subject to the presence of pesticides in a study. Pesticides have a tendency to get accumulated near the fat cell of the body and so the breast of a female is more prone to pesticide accumulation. The study revealed that a high concentration of 2,4-dichloro-diphenyl-trichloro-ethane and 4,4-dichloro-diphenyl-dichloro-ethylene was traced in those suffering from breast cancer compared to the one without breast cancer (Paydar et al. 2019). A similar result was also found in a study done on Indian women of age group ≤ 40 years. Apart from the previously mentioned two pesticides, traces of dieldrin, aldrin, methoxychlor, heptachlor, α-endosulfan, β-endosulfan and hexachlorocyclohexane was also found (Kaur et al. 2019). Cancer causing properties of pesticides can be controlled by various physiological and biochemical factors like age, gender, concentration of pesticide exposed, functional group of pesticide, duration of exposure, mode or source of exposure. On entering the body it mainly affects the soft tissues thereby affecting the genetic material and induces physiochemical damage to the cell organelles resulting in faulty gene expression (George and Shukla 2011).

9.4.3 Pesticide Intoxication Resulting Into Neonatal Abnormality

Various experimental reports have outlined inimical in-vitro developmental outcomes of some pesticides on animals leading to abortion, neoplasm of foetus, diminished foetal development, visceral and skeletal malformations. In many cases placental transfer of toxic chemical me lead to oxidative stress resulting into miscarriage and also mortality of both mother and child (Cavieres 2004). In a study using blood samples from umbilical cord of an infant whose parents had a history of pesticide exposure, it was reported that this pesticide exposure may be a causative factor for the development leukemia-associated chromosomal translocations in neonates (LaFiura et al. 2007). There are many strong evidences that have been reported that parental exposure to organophosphate pesticides affects a child's brain development and cause neurological disorders in different stages of growth, i.e. from neonatal stage to the age of 5 years (Sapbamrer and Hongsibsong 2019).

9.4.4 Neurodegenerative Disease as Result of Long Time Pesticide Exposure

Pesticides belong to the class of organophosphate have a profound neurological effect when exposed for moderate period of time and may give rise to symptoms like headache, dizziness, shaky hands, nausea and excess salivation, reflecting cognitive and psychomotor dysfunction (Kamel and Hoppin 2004). The prime target of pesticide toxicity to nervous system is the inhibition of acetylcholinesterase enzyme whose central role is to stimulate neurotransmitter activity. Inhibition of acetylcholinesterase leads to accumulation of acetylcholine near the synapse triggering excess nerve receptor stimulation (Heller and Hanahan 1972; Dvir et al. 2010; Hobbiger 1961; Kwong 2002). Mental health researchers have also revealed that long time exposure to pesticides in farmers have led to psychiatric disorder and increasing depression and a tendency to commit suicide (Meyer et al. 2010; Stallones and Beseler 2016; Mew et al. 2017). Presence of lipophilic pesticide in body may lead to systemic inhibition of complex-I creating a defect resulting into highly selective nigrostriatal dopaminergic degeneration leading to Parkinson's Disease (Betarbet et al. 2000; Ascherio et al. 2006; Semchuk et al. 1992). A list of pesticides belonging to different chemical groups pesticides (e.g., λ-cyhalothrin, fipronil, glufosinate, lufenuron and mepiquat) was suspected to produce chronic convulsive effects which may be a symptom of epilepsy. It is also found that epileptic seizures are mainly a result of long term exposure of pesticides at a very low concentration (Requena et al. 2018; Jett 2012).

9.4.5 Long Term Pesticide Exposure Leading to Chronic Renal Failure

Another serious matter of concern is that multiple high dose of pesticide exposures may lead to comprehensive and irreversible renal damage. The level of the contaminant in the blood of patients reveals persistent chronic exposure leading to dive effects on different organs of the body including kidney. Persistent pesticide levels in the blood gradually weakens the kidney cell leading to hypertension, proteinuria and may lead to end stage renal disease (Lebov et al. 2016; Ghosh et al. 2018). Nephrotoxicity arising due to the effect of different pesticide is well established in murine model. In a study on a bunch of people suffering from acute or chronic renal failure, traces of pesticides have been found in the blood. The pesticide retaining in the blood had decreased the glomerulus filtration rate in these patients (Siddharth et al. 2012). In a study on farmers who had a lifetime exposure to different herbicides, it was found that there are some pesticides which showed detrimental effect in the renal health. The actual mechanism of their action in affecting kidney is yet not clear. The herbicides pendimethalin, atrazine and dicamba had significantly lowered the glomerular filtration rate and increased blood creatinine of these people under study (Shearer et al. 2019).

9.5 Assessment and Inspection of Pesticide in Food Residues and in Humans

9.5.1 Various Means Attributed for Pesticide Assessment

Though the different pesticides have a wide application in different sectors, but agriculture remains a predominant source of this contamination. There are three main salient features that are taken into consideration while assessing the risks associated with pesticide exposure. Firstly, the amount or dosage of certain active ingredient presents, secondly, the physiochemical properties regulating the pesticide effect, and thirdly, the effect of toxicity on different organisms (Sattler et al. 2007). Different scientific regulatory bodies and organizations acknowledge the toxicological data which are obtained in humans under diagnosis should sustain as a record for further research as an effective health assessment (Orsine et al. 2012). The design of population studies of diverse geographical areas is one of the effective ways to assess and monitor the critical endpoints and pesticide exposure. Assessment of both external and internal exposure is equally important for a precise study and further regulation. External exposure can be estimated from the various modern analytical methods that have been developed. Relevancy in the internal manifestation is assessed by the diagnosis of body fluids such as blood, urine, saliva, semen, breast milk and also a detail study on the structure and function of genetic material, cells and its organelles (Greim 2001).

9.5.2 Analytical Methods to Assess Pesticide Residues in Food

Several analytical methods have been developed for the estimation and analysis of pesticide residues in fruits, vegetables, and other edible crops. Development of the ‘Multi Residue Method’ is strenuous because of the variation in physiochemical characteristics like polarity, solubility, pH, volatility. Several extraction methods have also come up like dispersing extraction, solid-phase extraction, microwave-assisted extraction and supercritical fluid extraction are also used (Łozowicka et al. 2012; Szpyrka et al. 2015; Bempah et al. 2012). Taking into consideration of all these factors scientifically upgraded detection methods like gas chromatography using different selective and sensitive detectors are applied. Gas chromatography remains the most widely used analytical procedure for pesticide analysis due to its high resolution magnitude (Chen et al. 2011). In a study on finding traces of pesticide in baby foods and adult duplicate diet sample, analytical means was applied. Fats contents in these foods is a real hindrance to the analysis since is requires multistep cleaning. Two methods were applied for the analysis; one is supercritical fluid extraction and secondly accelerated solvent extraction. But the supercritical fluid extraction-(gas chromatography/ mass spectroscopy) didn't stand up to the expectation, whereas accelerated solvent extraction based enzyme-linked immunosorbent assay and gas chromatography/ mass spectroscopy gave significant result. By the process of enzyme-linked immunosorbent assay chlorpyrifos was analysed and via gas chromatography/ mass spectroscopy malathion, chlorpyrifos, and 4,4′- Dichlorodiphenyltrichloroethane was analysed and their estimated range of pesticides lied between 0.3 and 110 parts per billion (Chuang et al. 2001). A modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) was found out for simultaneous enantioselective analysis of imidazole herbicides like imazethapyr, imazamox, and imazapic in different crops like soyabean, peanuts, wheat, maize and rice using a chiral reversed-phase ultra-performance liquid chromatography/ tandem mass spectrometry. When the system was run under optimum conditions, acceptable recoveries >64% were obtained. For all the three herbicides, it was found that the S-enantiomers eluted before the R-enantiomers (Li et al. 2019). Another quick and effective means of analysis was found in a study on fruits and vegetables. The pesticide was extracted from the fruit or vegetable juice prior to gas chromatography using acetonitrile mixed with an extraction solvent (1,2–dibromoethane). Pesticide extracted using this solvent is then injected into the separation system for its quantitative analysis (Farajzadeh et al. 2018). Glyphosate is one of the most widely used pesticides in agriculture and accounts for 60% of the global pesticide market. Most pesticides are analysed using chromatographic techniques but glyphosate being highly polar making it difficult to determine. Thus others methods like HPLC (High-performance liquid chromatography) combined with fluorescence detection, HPLC combined with detection by UV-vis, HPLC coupled with mass spectrometry, Gas Chromatography coupled to mass spectrometry, Gas Chromatography with detection by flame photometry have been studied. It was found that among all the above methods HPLC in combination with UV-vis and fluorescence detection had much improved sensitivity and selectivity (Melo et al. 2018) In tea plantation, around four classes of chemical contaminants are found and one among them is pesticides, this happens during its processing and packaging. A proposed method for analysis and quantification was hence studied by a group of scientist which uses liquid chromatography analysis using a silica bonded C18-pentafluorophenyl column coupled to high resolution mass spectrometry. In the study the recovery rate was found to be greater than 70% for about 80% of the contaminants; and 69% of the chemical contaminants were quantified and around 91% of the contaminants were well detected at its very low concentration (Cladiere et al. 2018). A study reported that cryopreserved samples of food leads to more precise and effective detection and hence quantification of pesticide residues in those foods (Roussev et al. 2019). Pesticides are also used to increase the shelf life of fruits like apple to make it usable for longer period. The fruits were analysed by various methods like two Single-Residue Methods, Spectrophotometry, High Performance

Liquid Chromatography, apart from the prior methods mentioned earlier (Lozowicka 2015; Ye et al. 2016).

9.5.3 *Clinical Diagnosis of Human Specimens for Pesticide Assessment and Its Toxicity*

Toxicity of pesticides in human have been diagnosed and estimated using embryonic, placental or hepatic human cell lines as models with respect to what is observed in fresh tissues or primary cells. Observation for apoptosis, cell necrosis on an exposure of 24 h against a certain minimal dose of pesticide confirmed the cytotoxicity (Mesnage et al. 2014; Mesnage et al. 2013). Beside all these clinical analysis, investigation on the ground of genotoxicity, endocrine misbalance, toxicity of specific organs must also be performed in the path of risk assessment due to complex pesticide exposure (Machado and Martins 2018). For the assessment of malignancy caused due to pesticide toxicity, the carcinogenic potency factor, can be estimated from the epidemiological studies in humans, which provides an indication and analysis of cancer cases due to certain dosage level of exposure (Barlow and Ellard 2006; Vermaes et al. 2012).

9.5.4 *Quantitative Assessment via Mathematical Model*

With the progressive chronic health risk because of exposure to pesticides, strict assessment can be validated using the 'Hazard Index' method which is formulated on the basis of dosage concentration of pesticide in the food residues (Boobis et al. 2008; Boon et al. 2008). A term, 'International Estimated Daily Intakes' was formulated which is calculated according to equation,

International Estimated Daily Intakes = $\sum$ [(Food chemical concentration × Food consumption)/Body weight],

which is then tallied with the 'Acceptable Daily Intakes' (Szpyrka et al. 2015). Potato is the third important single food crop being consumes globally and in India it is fourth important after rice, wheat and maize. Currently a diverse variety of potato plays an important role in daily diet. But besides being in great demand, a large amount of pesticide is required for its cultivation. Thus analyses of pesticide residues in potato have become a necessary act. On the basis of various studies performed in pesticide analysis for pesticide, this quantitative analysis was drawn to manage the hazard risk index. The estimated average daily intake (mg/kg/day) and acceptable daily intake (ADI) values (mg/kg/day) are the two criteria that are taken into account to calculate the hazard risk index. The reference data for the rate of food consumption, for calculation of ADI is taken from previously published literature as 42 kg/

person/year for potato, where 60 kg is considered as mean adult weight. Therefore, hazard risk index (HRI) is calculated using the following equation,

HRI = [EADI/ADI], (Juraske et al. 2011; Darko and Akoto 2008).

9.6 Biomarkers for Pesticide Inspection and Analysis in Human Body

9.6.1 What Are Biomarkers?

In everyday life humans are subjected to low dose of various chemical through food, drinking water, air, and drugs. These chemicals over a time get accumulated in the body and express a toxic effect leading to oxidative stress and tissue damage. The chemicals may be a combination of both known and unknown compounds. The prediction of these compounds and their level in the body can be determined from with the help of body samples (i.e body fluids or tissue) and a secondary known chemical that interact with the desired compound of interest. These analytes acts as an indicator or biomarker for the detection and estimation of our desired component (Hernández et al. 2019). Different pesticides affect the acetylcholinesterase activity and thus its activity status acts as a biomarker. Organophosphate pesticides, organochlorine insecticides are such chemicals that are estimated using blood because of their partition in the blood matrix. Urine samples also play a major role in the pesticide detection and its further assessment (Wang et al. 2010). Thus biomarkers are a cost effective and effective means to identify and analyse the exposure to a particular chemical hazard. It is indeed very difficult to recognise these biomarkers and its finding is a real matter of challenge. But once it is identified and the mechanism of the hazardous effect of the toxic chemical is known, it opens the door towards finding an effective solution to curb its exposure further (Fig. 9.2).

9.6.2 Biomarkers for Identification of Organophosphate Pesticide Exposure

Different liver enzymes have been used since 1970 as biomarkers to detect the presence organophosphate pesticides due to occupational exposure to factory workers or farmers. The enzyme levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are assessed for the pesticide exposure. Report says regular occupation exposure may increase these enzyme levels up to 10–50 times. The consistent increased level of liver enzymes may have a very adverse effect on the hepatic tissues (Awad et al. 2014). The evaluation of the level of butyrylcholinesterase (neurotransmitter) in the blood acts as an indication in the same way as acetylcholinesterase and is sometimes found to be strongly inhibited

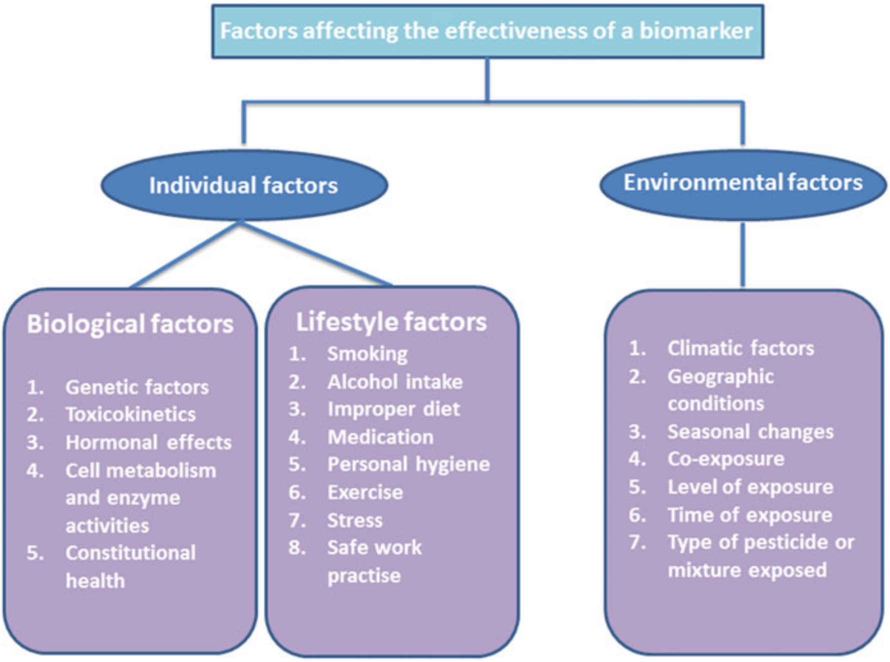

Fig. 9.2 There are many factors that govern the effectiveness of a biomarker under study and are hence divided into three sections, i.e. biological, lifestyle and environmental factors. (Kapka-Skrzypczak et al. 2011)

in case of occupational exposure (Bernal-Hernández et al. 2014; Singleton et al. 2015). Methyl chlorpyrifos metabolite (CP-me), 8-oxo-2′-deoxyguanosine and non-specific Dialkyl phosphate (DAP) metabolites have also been identified as an indicator since the metabolic route of these chemicals produces metabolites that are excreted via urine (Santos et al. 2013). Malathion monocarboxylic acid metabolite, 3,5,6-trichloro-2-pyridinol, and 4-bromo-2-chlorophenol are also among the specific biomarkers used for the identification organophophate pesticides exposure. These are also associated with the inhibition of acetylcholinesterase and butyrylcholinesterase, and thus help to draw a correlation among these two groups (Scher et al. 2008; Tuomainen et al. 2002; Albers et al. 2004). The potential use of paroxonase genotyping (PON-1), and the spotting of polymorphisms in PON-1 associated with arylesterase and cytidine monophosphatase activity, is an efficient biological marker for long term organophosphate pesticide exposure (Araoud 2011; Bernal-Hernández et al. 2014).

9.6.3 Biomarkers for Identification of Carbamate Exposure

Carbamates are a group of chemicals that is derived from carbamic acid and is a type of insecticide with structure similar to organophosphate insecticides. Two unique metabolites associate with this have been identified, that is 1-naphthol and 2-naphthol. These metabolites are found with increased concentration in the urine of the person who is exposed to carbamate poisoning and thus considered as its typical biomarker (Bouchard et al. 2008). In a cross section study conducted over a group of fishermen and farmers who had frequent exposure to carbamate for around 5 to 20 years. The pesticide exposure was investigated using nerve conductive velocity, current perception threshold (CPT). The investigation was performed using a Neurometer CPT/Eagle, on the index finger and on the first toe with three neuro-selective frequencies range that is 2000 Hz, 250 Hz, and 5 Hz. The result obtained was significant in all the three frequencies used and the current perception threshold values were found to be increased in both m medial and peroneal nerves. Elevated the current perception threshold was more prominent in farmers than fishermen. Thus measuring CPT values is used as a biomarker for person who suffers from carbamate toxicity (Lubis et al. 2008).

9.6.4 Biomarkers for Identification of Pyrethroid Exposure

Pyrethroid belongs to a class of synthetic pesticide (insecticides) with its chemical structure very similar to the natural pyrethrins (produces by the flower pyrethrums). Pyrethroid is one of the most widely used insecticides all across the world mainly for household purpose. It is less toxic compared to organochlorine and organophosphate pesticides but prolong exposure may have serious adverse effects on health (Housset and Dickmann 2009). The presence of this pesticide in the body is traced and assessed via urine analysis. Some non-specific biomarkers that have been found out for that analysis of pyrethroid, such as cis-2,2-(dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid, dicarboxylic acid, 4-fluoro-3-phenoxybenzoic acid and 3-phenoxybenzoic acid (Couture et al. 2009; Ratelle et al. 2016; Taneepanichskul et al. 2014). There are also many specific metabolites found in the urine that is considered as biomarkers such as 2,2-dichlorovinyl-3,3-dimethylcyclopropane-1-carboxylic acid and cis- and trans-2,2- (dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (Galea et al. 2015; Ferland et al. 2015).

9.7 Conclusion

Since it isn't manageable to alter the inherent toxicity of pesticides and check the complete exposure to human via water and food residues, necessary strategic action must be taken to minimize the human pesticide consumption to a great level by shifting to organic pesticides (Machekano et al. 2019). In case of raw fruits and vegetables, most of the pesticides reside on its surface since they cannot penetrate the thick skin. Only trace amount of pesticide concentration is found inside the fleshy region. Studies have found that household washing of such fruits and vegetables having thing skin can be easily removed by washing under cold or hot running water. Cooking is another means to nullify the effect of the pesticides. Thus washing well and cooking the vegetables have a very good synergetic effect in minimising pesticide contamination rather than eating it raw (Yang et al. 2012). In brewery industries treatment with activated charcoal is found to be an effective method of eliminating the traces of pesticide in the fermented beverages. Charcoal also is not found to interfere in the quality of processed product (Cabras and Angioni 2000).

Thus lack of knowledge and non-strategic treatment process actually leady to the high pesticide exposure and its adverse effect on health. Recent studies have shown a gradual decrease in the pesticide content in food residues as compared to previous reports. This may be due to the adoption of organic farming, approach of genetically modified crops which need minimal pesticide application, awareness regarding its ill effects.

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Chapter 10
Toxic Effects of Pesticides on Avifauna Inhabiting Wetlands

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Abstract Wetlands are considered as the interim zones, crammed between perpetually flooded deepwater environments and drained uplands, which support diverse range of biodiversity. Various chemical fertilizers and pesticides were used in the wetland for higher agriculture yield but the majority of them reach to non-target species especially on water birds. Studies have shown that there is a huge decrease in the population of water birds every year. Organophosphate, carbamates and organochlorines are the three major groups of pesticide widely used for agricultural purposes. Organochlorines are the potent blocker of the gamma-aminobutyric acid receptor, which is one of the major reasons for Raptor species depletion in the world. Organochlorines are banned in several countries due to its bio-accumulative nature but which still available in the markets under different brand names. The Organophosphates and carbamates have come as a replacement for organochlorines recently in wide variety of brand names.

The persistent use of pesticides interrupts the acetylcholine esterase enzyme resulting in altered posture in birds. Besides these changes on the feeding behavior, abnormal development, eggshell thinning, altered tones are the other major effects of these pesticides in birds. In majority of cases the effect is not direct, the applied pesticides taken up by fishes and worms which finally reach to birds through food web. Pesticide causes the mass extinction of local species in a particular area which abrupt the balance of natural ecosystem. Aves are one of the key factors help to improve the fertility of soil through their droppings, so huge decrease in wetland

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birds in turn influence the crop yield too. This review will help to improve the awareness among the different stakeholders leading to more healthy cultivation practices and to reduce the uncontrolled use of pesticides.

Keywords Pesticides · Avian fauna · Bioaccumulation · Toxicity · Wetlands · Carbamates · Organochlorines · Organophosphates · Herons · Cormorants · Pelicans

10.1 Introduction

Wetland ecosystems are vital to the maintenance of life on earth and provide essential services on which humankind depends. A wetland is an area that is saturated permanently or seasonally with water, such that it creates a distinct ecosystem as depicted in Fig. 10.1. The characteristic vegetation and unique soil conditions distinguish wetlands from other land forms and water bodies. According to the Ramsar International Wetland Conservation Treaty, wetlands are defined as naturally or artificially occurring marsh, peat land or water areas with static flowing water of low tide which does not exceed six meters (Gardner and Davidson 2011; Mitsch 2012).

Wetlands are considered as the transitional areas which support a diverse array of flora and fauna and are imperative to hominids in many ways. Wetlands are vital areas all through the world for nature defense, sedimentation control, and flood

Fig. 10.1 Wetland habitats a close look

prevention. Wetland plays a central part in the socio-economic firmness of local community activities such as fisheries (Kuzovkina and Quigley 2005). Wetlands prevent flooding by holding water much like a sponge, helps to counter balance the human effect on rivers by rejuvenating them and surrounding ecosystems. Because of its many cleansing benefits wetlands have been considered as the kidneys of our earth (Johnston et al. 1984).

Avian species are diverse and successful evolutionary groups widely distributed in tropical regions. Avian populations have a vital role in ecosystem balancing; they play a central role in pest control and pollination (Şekercioğlu et al. 2004). Wetland birds are important constituents of the wetland ecosystem and majority of the birds are water inhabitants, more than 400 species of water birds have been reported throughout the world. One of the major reasons in their depletion was agrochemical pollution. The Decline of bird populations in an ecosystem is a serious warning of pollution (Sivaperuman and Jayson 2000).

10.2 Avian Biodiversity of Wetlands

Wetlands provide a habitat for a vast array of organisms, including many endangered species as shown in Fig. 10.2. Almost 31 of the 57 endangered wetland avian fauna reside in China; thereby correlating the extensive habitat damage due to increased industrialization and development (Liu 2005). Wetlands of Panipat also have reported to inhabit near threatened avian species such as Painted Stork, Black-necked Stork, Oriental White and Alexandrine Parakeet (Kumar and Sahu 2020). Thus wetlands serve as delicate niches for various birds through out the world necessitating their preservation and prevention of pesticide pollution.

Some avian communities depend on wetlands entirely for breeding, nesting, feeding, rearing young ones or shelter during their life cycles. The vital birds exploiting the wetlands are water fowls, ducks, Herons, egrets, shorebirds, gulls, terns and Raptors. The birds of wetlands can be broadly distributed into three categories, residents, winter residents and transients based on their seasonal occurrence (Hunt Jr and Schneider 1987).

The avian diversity of wetlands in Kerala include 243 species of birds of which 130 (53.50%) are resident birds, 89 (36.33%) long distant migratory birds and 24 (9.87%) vagrants, in Indian scenario the total count is above 400 species (Praveen 2015). During their migration birds travel along the Central Asian- Flyway (CAF)- the flyway which extends from Central Siberia through the Himalayas to the Indian subcontinent). During peak annual migration periods, descend upon the coastal wetlands of India in search of refuge and food due to the unfavorable conditions in their native breeding grounds (Balachandran 2012).

Oriental darter - *Anhinga melanogaster* Spot-billed pelican- *Pelecanus philippens*

Asian openbill stork - *Anastomus oscitans* Common kingfisher- *Alcedo atthis*

Grey-headed swamphen - *Porphyrio poliocephalus* Lesser whistling duck - *Dendrocygna javanica*

Fig. 10.2 Wetland avian fauna diversity depciting some important aves

10.3 Classification of Pesticides

Pesticides are a class of chemical compounds intended for the destruction of pests, weeds and sometimes insects. Pesticides are commonly classified bactericides, insecticides, fungicides, herbicides, and rodenticides. Pesticides have the capacity to destroy a far-reaching range of pests, but some are progressive as target oriented. Wide variety of these compounds is designed in such a way as to disrupt the physiological mechanisms of the target organism, leading to metabolic abnormalities (Casida 2009; Mel′nikov 2012; Ortiz-Hernández et al. 2013). A study done by (Pimentel 1995) revealed that only 0.3% of administered pesticide reached to target organism the rest 99.7% goes into non-target organisms especially in the aquatic environment. Organochlorines, carbamates, and organophosphates are the commonly used classes of pesticides in the world for pest control programs. A classification of pesticides into a minimum (lower risk) pesticide list has been reported based on their level of toxicity to human and environmental health which could be used integrated pest management in Asian and African continents (Jepson et al. 2020).

10.3.1 Based on Chemical Structure

The structures of most of all the chemical compounds which are recognized as the principal constituents of pesticides are able to induce impediments in the normal activities of the target organisms; thereby leads to reducing vitality and dysfunction of the organism. A wide range of chemical compounds is known to produce toxic effects against various organisms (Bunch et al. 2003). Classification based on the structure of the compounds provides a basic idea regarding their chemical and physical characteristics (Fig. 10.3). Based on the structure, the pesticides are classified into organophosphorus, organochlorines, carbamates, pyrethroids, and pyrethrin.

10.3.2 Organochlorines and Organophosphorus

The organochlorinated compounds have been principally known to have significant uses in daily life as insecticides, fumigants as well as solvents (van der Hoff and van Zoonen 1999). They are lipophilic in nature and slow rates of environmental degradation along with low vapour pressures make them long-lasting and extremely effective pesticide candidates. Based on the structure of them, the pesticides can divide into the following groups such as (i) DDT and analogues (DDD, DDT, Methoxychlor and DDE), (ii) Hexachlorobenzene (Hexachlorobenzene) (iii) Mirex, Chlordecone and Kelevan (iv) Toxaphene (v) Hexachlorocyclohexane (α-

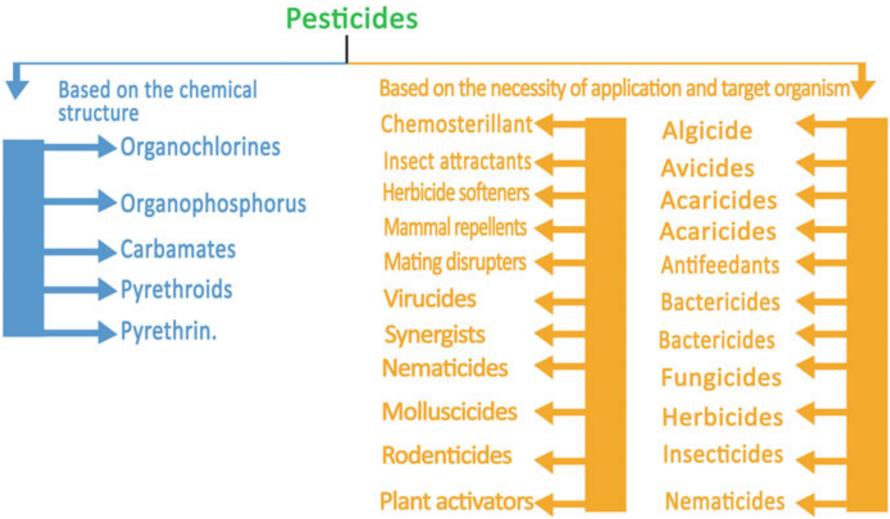

Fig. 10.3 Classification of pesticides based on composition and action

HCH, δ-HCH, β-HCH, and γ-HCH) vi) Cyclodiene (Heptachlor, Endosulfan I) (Genuis et al. 2016). The Organo phosphorous pesticides are recorded as the derivative form of the phosphoric acid (phosphates), dithio phosphoric acids (dithiophosphates), and thiophosphoric acid (thiophosphates). The DDVP, monocrotophos, fenitrothion, phosphamidon, dimethoate, Phosalone, methyl parathion, and phorate are included as the major Organo phosphorous pesticides those have significant applications in the day to day life (Kumar et al. 2016).

10.3.3 Carbamates, Pyrethroids, and Pyrethrin

Carbamates are a group of insecticides that posses resemblance to organophosphate (OP) insecticides in mechanical as well in structural ways. The carbamates are esters of thiocarbamic acids or carbamic acids. Carbaryl, carbofuran, cartap hydrochloride, Propoxur, and methomyl are included as the major pesticides come under this category (Zaahkouk et al. 2000). Pyrethrins are insecticides of botanical origin and they usually instigate impediments in the normal nerve function of the target insect, thereby cause paralysis and death. Pyrethroids (E.g., Furethrin, Cypermethrin, Tetramethrin, Allethrin, and Cyclethrin) are insecticides or synthetic origin whose structure is taken from the pyrethrins (Anadón et al. 2009).

10.3.4 Based on the Necessity of Application and Target Organisms

Approximately 1.8 billion people over the world are engaged in agriculture and most of them were use pesticides to protect the commercial products and crops that they harvest. Others use pesticides for public commercial applications and health programs; while significant groups of peoples use pesticides for garden and lawn applications in the home (Alavanja 2009). Therefore, the classification based on the use of insecticides can offer significant information regarding their toxicity against target organisms.

Algicides are the chemical products that are used to inhibit the proper development of algae (E.g. oxyfluorfen, simazine, isoproturon, isoproturon, copper sulfate, and diuron) (Ray and Mondal 2017). Fenthion and strychnine are usually used to kill various birds; hence they are called Avicides. The pesticides that are used to kill the ticks and mites or to impediment the normal growth of is usually called as Acaricides (E.g. carbofuran) (Lortz et al. 2010). Fentin, chlordimeforn, and azadirachtin are certain Antifeedants that primarily prevent the pests from feeding. As the name llustrates Bactericides (e.g. tetracycline, and streptomycin), Fungicides (triadimefon.), Bird repellents (E.g. thiram, ziram, diazinon), Insecticides (e.g. carbofuran, carbosulfan, triazophos), Mammal repellents, Insect repellents, and Molluscicides (e.g.thiodicarb,) produce toxic effects on their respective category of organisms (Kloos and McCullough 1982; Mason 1998). Instead of developing toxicity certain substances can alter the normal growth thereby increasing the flowering and reproduction rate of the target plant; such substances are referred to as Plant growth regulators (E.g. metoxuron, paclobutrazol, gibberellic acid). In some circumstances the farmers may use piperonyl butoxide like substances to augment the toxic effects of particular pesticide; such substances are referred to as synergists (Cedergreen 2014).

10.4 Persistence and Effect on Avian Biodiversity

The Asian continent is the highest pesticide polluted one with China being the predominantly pesticide consuming country of the continent (Sharma et al. 2019). A wide variety of pesticides are predominant in the wetlands of Australia with an increased occurrence of pesticides such as simazine and atrazine and insecticide bifenthrin was found at a concentration upto a maximum 59 μg/kg (Allinson et al. 2015). Mostly these pesticides are due to indiscriminate use of pesticides in drains outs from agricultural captured lands (Davis et al. 2008). Apart from these attempts to control insects such as mosquitoes, nonbiting midges as well as Argentine ants have contributed to insectide persistence of even banned chemical entities such as aldrin in wetlands of Perth even at non-permissible levels (Lettoof et al. 2020).

Studies indicate that pesticide free or lacking organic farming practices play a very promising approach to augment avian biodiversity (Kirk et al. 2020). As noted in wetlands of Panipat where insectivorous birds are predominant, the indiscriminate use of pesticides for agricultural as well as other industrial purposes would severely affect such insectivorous aves due to bioaccumulation of pesticides (Kumar and Sahu 2020). The bioaccumulation or organochlorine residues in the back feathers of raptors in Brazil are yet another evidence of pesticide accumulation in aves (Aver et al. 2020). Such declines in avian species diversity due to anthropogenic activities were also reported in different parts of the world for instance, a drastic decline of aves near thermal power plant of Panipat, Haryana (Hossain and Aditya 2016).

A striking decline in bearded reedling *Panurus biarmicus* was noted in the Spanish coastal wetlands compared to inland wetlands owing to the uncontrolled use of pesticides in the former for agriculture (Belenguer et al. 2016). The death of non-targeted organisms such as birds by pesticide abuse resulted in the death of 14 avian species in a 2 year duration alone along the sanctuaries of India (Jayakumar et al. 2020). A vast decline in various raptorial aves including peregrine falcon, the sparrow hawk and bald eagles has been reported predominantly in wetlands and grasslands (Mitra et al. 2011). Quite often it is difficult to estimate the pesticide toxicity in birds as they may perhaps decease away from the location of poisoning or it may be not cumulative enough to cause the mortality when little documentation is available in this aspect (Bormann et al. 2001).

10.5 Effect of Pesticides

Pesticides like organochlorines exhibit high persistence and it induces a cumulative effect on higher trophic level organisms like birds. Eggshell thinning, birth disorders, reproductive failures, and abnormal development are the major signs of pesticide poisoning in avians. In agricultural landscapes, key factors influencing the abundance of birds are agricultural rehearses such as agrochemical application, harvesting and tillage (O'connor and Shrubb 1990; Rodenhouse et al. 1995). Birds are very delicate to ecological fluctuations and they inhabit a high locus in the food chain so birds could be considered as bio indicators of pesticide pollution (Furness 1993).

10.5.1 Effect of Organochlorines

High environmental solubility and low aqueous solubility is the basic characteristic features of organochlorines. Organochlorine pesticides enter into the food chain as a residue of environmental application, factory discharge, and landfills. The repetitive usage of pesticides in farmlands leaches out into the water and taken up by fishes, which in turn taken up by the wetland birds results in poisoning. The acute toxicity of dichlorodiphenyltrichloroethane is by nerve impulse transmission impairment; the Dichlorodiphenyltrichloroethane induced toxicity will always enable the Na^+ channel leakage resulting disruption of action potential (Mitra et al. 2011). Dichlorodiphenyltrichloroethane metabolites also cause the eggshell thinning in birds; it inhibits the Ca^{2+} ATPase pump of the avian shell gland membrane and reduces the calcium carbonate transport from blood, which results in the thickness reduction. The cyclodiene derivate of the organochlorine family, endosulfan inhibit the Gamma aminobutyric acid (GABA) receptor and reduce the chlorine flow ultimately results in neurological disorders like convulsion, poorly developed claw in the higher class of birds like eagles (Hayes and Laws 1991; Krieger 2001; Walker 2003). Studies demonstrated that dieldrin has a cumulative effect on avian and induce hyperactivity, respiratory distress, and diarrhea in birds and which could be transferred to next generations, sometimes a single high dose exposure may be severe (Douthwaite 1995; Kidd and James 1991).

Chronic organochlorine exposure interferes with the breeding triumph of birds and induces variations in their coition behavior. The affected birds lost its territorial and home range barriers and exhibit less parental care to chicks (Fry 1995). Studies show that high Dichlorodiphenyltrichloroethane concentration for a longer duration alter the courtship attitude in ring doves (Haegele and Hudson 1977) and nocturnal activities in white-throated sparrows (Mahoney Jr 1975). Studies on mallard duck revealed that sub lethal amounts of dieldrin disturb the vicious behavior of mallard duck and induce total behavioral change (Peakall 1985). Endocrine system disrupting activity, embryonic deformity, immune suppression were also reported in herons (*Ardeidae*), cormorants (*Phalacrocoracidae*), Pelicans (*Pelecanidae*) and gulls (*Laridae*) around the world (Custer et al. 1998; Dirksen et al. 1995; Huertas et al. 2016).

Teratogenesis was another deformity of organochlorine exposure in birds malformed beak, skeleton problems are common after effect of chronic sub lethal exposure (Gilbertson and Fox 1977). Congenital aberrations and flaws in feather modeling of young terns (*Sternidae*) and scavengers were stated after organochlorine exposure in USA and Europe (Bourne et al. 1977; Wünschmann et al. 2018). Organochlorines are the prime reason for the population declination of fish-eating birds in different areas of India, Europe and report revealed that the bioaccumulation of organochlorines are increased in higher trophic levels (Naso et al. 2003; Seedikkoya and Shukkur 2004).

10.5.2 Effect of Organophosphates and Carbamates

Since 1980s organophosphates and carbamates are generally used pesticides across the globe due of their low bioaccumulation properties in contrast to organochlorines. Inhibition of acetylcholine esterase enzyme (AChE) of cholinergic synapses (Bishop 1998) in the central nervous system is the target site of action. Inhibition of acetyl choline esterase will cumulate neurotransmitter acetylcholine at the synaptic cleft in the neuromuscular junctions, thus disrupting transmission across synapses (Mitra and Maitra 2018; Samanta and Bandyopadhyay 2017). Birds are delicate to acute contact to organophosphates and carbamates pesticides due to persistent decreased activity of anticholinesterase detoxifying enzymes (Gupta et al. 2019; Parker and Goldstein 2000). Due to the increased action of acetyl choline esterase in the avian brains, the frequency of binding to organophosphate and carbamate is quicker than in birds compared to other vertebrates (Narváez et al. 2016; Sobhakumari et al. 2018).

As in the case of every toxin the mode of exposure of organophosphates and carbamates reaches to birds through diet. As per National Wildlife Health Center majority of water bird poisoning is through organophosphate exposure. Organophosphates and carbamates caused more than 335 bird species mortality in the Middle East between 1980 and 2002 especially on water fowls and mallard ducks (Fleischli et al. 2004).

Organophosphate and carbamates toxicity often results in anorexia and gastrointestinal abnormalities (Grue et al. 1991). Long term exposure of organophosphates even in small amounts will change feeding behavior and cognitive pattern in migrant birds (Nicolaus and Lee 1999). Studies on European Starlings shows that single minute dose of dicrotophos will cause lesions in the hypothalamus lateral region lead to food evasion and ultimately mortality (Angelova 2016; Kaur 2012).

Alteration in the male genitalia development in birds results in acute exposure to organophosphates and carbamates. Delayed development and degeneration of spermatogenic cells were also observed in lower order birds like prinias and sunbirds due to organophosphate exposure (Kuenzel 1994; Ottinger et al. 2009). Studies revealed that methyl parathion results in decreased testes cholinesterase activity in male white-throated munias (*Lonchura malabarica)* which is allied to the deteriorated germ cells in the seminiferous tubules (Maitra and Sarkar 1996). Altered low-level testosterone and male genitalia impairment were also reported in Rose-ringed parakeets (Maitra and Mitra 2008). Organophosphorus insecticides altered luteinizing hormone and progesterone level were also reported in cormorants and water fowls (Rattner et al. 1982; Scanes and McNabb 2003).

Sublethal exposure to organophosphates and carbamates will change the migratory and singing behavior in wetland birds especially in bird group which shows high social behavior like starlings and babblers (Mineau 2003). Abnormal incubation period and reduction in hatching success were also reported after organophosphate and carbamate exposure in storks, egrets, mallards, and shovelers (Grue et al. 1991; Mendelssohn and Paz 1977). Histo-pathological changes in immune tissues and organs were reported in exposure to high doses of organophosphates and carbamates which induce direct mutilation to the organs of the immune system (Ambali et al. 2010). Direct destruction to proteins and DNA were also reported in small birds like sparrows (Videira et al. 2001). Sub lethal exposure to chloropyriphos and methidathion will cause a drop in neutrophil count, WBC and lymphocyte count (Obaineh and Matthew 2009). Studies show that 5% carbofurangranule is adequate to eradicate a house sparrow (*Passer domesticus*) within minutes, while folks that consume one pellet of terbufos only have half survival chance (Mineau et al. 2005).

10.6 Conclusion

Synthetic pesticides bring serious effects during all developmental stages of birds; some of them are more susceptible some are least. Pesticide residue reaches to birds through food chain being a carnivores group and having mixed eating behavior the bioaccumulation is high in birds compared to other vertebrates. Toxicity in breeding time induces abnormal decrease clutch size, teratogenesis and increased rate of reproductive failure in avians. Pesticides cause extinction and population decline in several birds like the sparrow hawk (*Accipiter nisus*) peregrine falcon (*Falco peregrinus*), and bald eagle (*Haliaeetus leucocephalus*) (Mitra et al. 2011). The undiscerning use of pesticides must be controlled at least in biodiversity hotspots like wetlands, otherwise it will disturb the entire ecosystem since pesticide persist and travel long routes.

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Chapter 11
Analysis of Pesticide Residues in Biotic Matrices

Barbara Clasen, Tamiris Rosso Storck, Eloisa Lovison Sasso, and Tadeu Luis Tiecher

Abstract Many pesticides have been used in agriculture to increase the yield and quality of food. For this reason, regulations are developed to monitor the residues of these compounds in different matrices. The great challenge for the analysis of pesticide residues in biotic matrices is related to the great variety and quantity of chemical structures used to increase crop yields. In this sense, researchers are studying ways to broadly assess potential environmental risks, and it is necessary to develop and validate methods for analyzing high-yielding pesticides, wide coverage, sensitivity and low cost.

This chapter presents the main methods used to determine pesticide residues in aquatic, terrestrial and human ecosystems. as well as alternative methodologies, which help in the identification of polluted environments, such as the use of bioindicator organisms present in these environments and sensitive biomarkers, to evaluate the effects of these chemicals on the proposed matrices.

Keywords Environment · Contamination · Water · Soil · Human health · Pesticides · Metals · Toxicology · Biomarkers · Stress

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11.1 Introduction

Several classes of pesticides have been widely used in pest control, especially in agricultural areas. While reducing food production is prevented by the pesticides use, which are responsible for combating harmful organisms to cultivated plants. It is important to consider that their continued use can result in risks to human health and the environment.

The classification of pesticides follows two main criteria: chemical class and target organism. Regarding the chemical structure, they may belong to the class of organochlorines, organophosphates, carbamates, pyrethroids, organonitrogenates, triazines, benzimidazoles, etc. According to the target organism, pesticides are classified into insecticides, fungicides, herbicides, acaricides, among others. Globally, about 3 million tons of pesticides are used annually, which corresponds to US$ 40 billion in market values. Currently the number of active ingredients in commercial pesticide formulas is high. In the European Union there are approximately 500 substances approved as pesticides. The amount of pesticides employed varies annually and depends on economic and agronomic factors (Pimentel 2009; Karasali and Maragou 2016; Silva et al. 2019).

Despite the benefits of controlling pests that prevent decreased food production, pesticides are identified as environmental pollutants (Karasali and Maragou 2016). Estimates indicate that less than 0.1% of pesticides actually applied reach pests, that is, 99.9% have the potential to relocate to other environmental compartments, which may result in adverse effects on human health and the environment (Fig. 11.1). Therefore, since the 1960s, great attention has been paid by researchers, government regulators and public opinion to the impact of pesticides on human health and the environment (Silva et al. 2019).

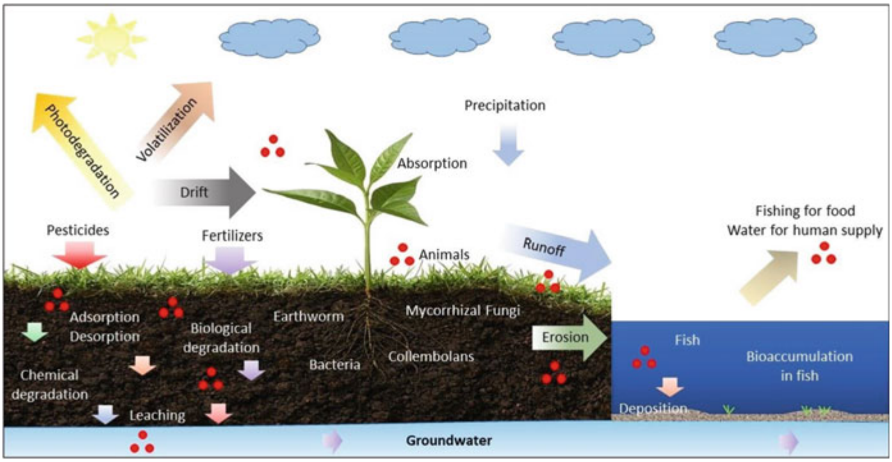

Fig. 11.1 Dynamics of pesticides in the environment

The pesticide residues effects on the environment depend on its ecotoxicity to terrestrial and aquatic organisms. Moreover, they depend on the concentrations reached in the different environmental compartments, such as, soil, water, plant and atmosphere. Which, in turn, depend on the mode and application conditions, the physicochemical properties, the amount or dose used, the environmental conditions and the behavior and fate of the pesticide in the environment.

11.2 Methods for Pesticide Evaluation

The advance in ecotoxicological research related to emerging pollutants, as well as treatment, occurrence, environmental dynamics, and the discovery of their ecological impacts, served as a basis for introduction and improvement of equipment necessary to determine these products in environment (Noguera-Oviedo and Aga 2016).

With new analytical instruments emergence, improved and refined, many contaminants could be identified as well as their metabolites (Farré et al. 2008). The emerging pollutants analysis, such as pesticides, is challenging, since most of these compounds are present in low concentrations in environment, which requires continuous updates related to the compounds to be detected as well as improvements in technologies for analysis (Teodosiu et al. 2018). Although advances in technologies and research on emerging pollutants have clarified many questions, many remain unanswered and new points have emerged (Noguera-Oviedo and Aga 2016). For example, the lack of information on contaminants metabolites, such as pesticides, in environment.

Due to problems related to pesticides overuses, efficient detection systems are increasingly required to protect organisms exposed to these compounds by contamination of water and food. Analytical methods capable of determining the presence of pesticides are normally carried out by thin layer chromatography, high performance thin-layer chromatography plates, gas chromatography, high performance liquid or mass spectrometry. However, there is a need for simple, inexpensive, fast response methods suitable for use in the field. International organizations such as the World Health Organization have shown interest in developing inexpensive and sensitive methodologies to determine pesticide residues in different environmental compartments.

Several methods for atrazine detection have been developed, based on ecotoxicological discovery problems caused by residues of this herbicide in water sources (Chuc et al. 2016). The analytical methods used are based on high performance liquid chromatography (HPLC), HPLC coupled to diode array detection (HPLC-DAD), HPLC coupled to mass spectrometry (LC-MS) and gas chromatography coupled to mass spectrometry (GC-MS) (Ma et al. 2003; Cai et al. 2004; Belkhamssa et al. 2016). Belkhamssa et al. (2016), emphasize the economic limitation of techniques such as GC-MS that require expensive equipment.

Other techniques related to enzyme linked immunoassays, such as ELISA, and other immunosensors for atrazine have been developed more recently (Byer et al. 2011; Barchanska et al. 2012). Electrochemical immunosensors (Jiang et al. 2008; Pardieu et al. 2009), surface plasma resonance biosensors (Dostálek et al. 2007) and optical immunosensors (Rodriguez-Mozaz et al. 2004) are also examples of equipment developed for herbicide detection. Chuc et al. (2016) developed a biosensor by assembling a layer-by-layer structure of polyaniline/graphene for electrochemical detection of atrazine, achieving success with its excellent sensitivity. While, Yangali-Quintanilla et al. (2010), used ELISA assay kits for atrazine detection in water, and found a concentration limit of 0.04 mg/L and an uncertainty of 15%.

The traditional methods limitation, such as HPLC-MS for pesticides detection, is time-consuming, expensive and requires qualified technicians for operation, although these methods present the best analytical performances (Chuc et al. 2016). High resolution mass spectrometry (HRMS) techniques have been developed to determine pesticides and their metabolites in water, however the limiting factor is also the high cost for routine analysis (Cai et al. 2004).

Therefore, strategies for making this measurement include optical systems such as spectrophotometry, fluorometry, voltammetry and, more often, potentiometry and amperometry (Alves and Oliveira-Silva 2003). The use of immunoassays and biomarkers as an alternative to traditional methodologies use has shown very interesting and promising results. For example, the use of analytical methods has been developed to detect anticholinesterase agents based on acetylcholinesterase inhibition. Molecular biomarkers use are important due to its ability to anticipate likely biological impacts and to provide a broad and integrated response to organism exposure to a large amount of xenobiotics.

11.3 Pesticide Residues in Aquatic Ecosystems

The pesticides use increased is commensurate with the need for food demand by growing world population (Liu et al. 2016). Pesticides are chemicals used to control plants and organisms that humans find undesirable (Chopra et al. 2011). However, these chemical compounds are responsible for serious problems related to environmental pollution in several countries (Arisekar et al. 2019).

Water contamination resources due to pesticide use is considered an important environmental problem (Heidar et al. 2017). Pesticides can occur in aquatic environments mainly through runoff, leaching, spray drift, erosion (Dabrowski et al. 2014), and also from municipal and industrial waste discharges (Jin et al. 2019). Surface water contamination (Sumon et al. 2018), groundwater (Estévez et al. 2012), as well as drinking water sources (Sjerps et al. 2019) by pesticides is well reported.

The pesticides physicochemical properties will influence their behavior in aquatic environment (Dabrowski et al. 2014), such as hydrophobic organic pesticides, which will avoid the aqueous phase, and may deposit in suspended particles and sediment, as well as accumulate in biological tissues (Gobas et al. 2018). Pesticides can cause damage to non-target organisms such as fish populations, even at sub-lethal concentrations, resulting in long-term adverse effects (Satyanarayan et al. 1999). Thus, resources water quality can be assessed through the living organisms that inhabit them (Grung et al. 2015), so fish are often used as bioindicators in ecotoxicological research (Van Der Oost et al. 2003). Among pesticides, organochlorine compounds are among the highlights in global concern due to their persistence, bioaccumulation and negative impacts on biota (Zhang et al. 2007). Organochlorine pesticides have low polarity, low aqueous solubility, high liposolubility (Khallaf et al. 2017) and resistance to biodegradation, because these characteristics, tend to bioaccumulate in organisms (Zhang et al. 2016; Ding et al. 2019; He et al. 2019).

Bioaccumulation refers to situations where organisms acquire higher xenobiotic compounds concentrations in their bodies than are present in environment in which they live (Walker 1990). Fish are exposed to pollutants in aquatic environment through gills, skin and through their diet (Clasen et al. 2018). As a result, pesticide residues can accumulate in aquatic organisms through direct absorption of water by gills or skin, contaminated food consumption and particulate matter ingestion (Van Der Oost et al. 2003).

Several studies on pesticide residues bioaccumulation in various fish species, both field studies (Yahia and Elsharkawy 2014; Clasen et al. 2018) and laboratory studies (Wang et al. 2015), are reported in many countries (Liu et al. 2016; Vieira et al. 2019). Arisekar et al. (2019) investigated the organochlorine and pyrethroids pesticides bioaccumulation in surface waters, sediments, fish tissues and aquatic plants in Thamirabarani River in southern India. Three freshwater fish species were collected in the first four points: *Catla catla*, *Labeo rohita* and *Oreochromis mossambicus* and in point five were collected marine fish that are under the influence of freshwater and marine: *Rastrelliger kanagurta*, *Lethrinus nebulosus*, *Sphyraena barracuda* and *Sardinella longicep*s. Residues of these compounds were detected in surface waters, sediments, gills, liver and muscle of fish and aquatic plants. It is noteworthy that endosulfan, aldrin, dieldrin, endrin and heptachlor exceeded the maximum residue levels established by World Health Organization for surface waters. The study observed that there was a positive correlation between fat content and organochlorines fish muscle bioaccumulation.

The pesticide residues concentration from tissue to tissue in fish, and is related to lipid content, exposure time and varies between species (Satyanarayan et al. 1999). Fish muscle tissues are being widely used for pesticide bioaccumulation evaluation (Sun et al. 2014, 2017; Clasen et al. 2018; Tran et al. 2019). Ernst et al. (2018), evaluated the pesticide residues presence in 149 fish muscles and found residues in 143. The authors relate the pesticides incidence on fish to environmental persistence, mobility and pesticide intensity use in local agriculture.

Most organochlorine pesticides, which have been widely used in past, have been banned from the Stockholm Convention on Persistent Organic Pollutants. These compounds were then replaced by organophosphate pesticides (Afful et al. 2010). However, it is known that organophosphate pesticide residues also tend to be concentrated in aquatic organisms, especially in lipid-rich tissues (Khallaf et al. 2017). Singh and Singh (2008) compared organochlorine and organophosphate insecticide residues bioaccumulation in female catfish (*Rita rita* and *Mystus tengara*) and carp (*Cyprinus carpio* and *Labeo rohita*) fish collected from polluted rivers, Gomti and Ganga, and pond unpolluted waterway considered as a landmark in India. The tissues analyzed were brain, liver and ovary. It can be verified that there was bioaccumulation of both pesticide residues in fish tissues, and that it was higher in catfish tissues than in carp. Singh et al. (2015) compared organochlorine – dicofol, and organophosphate – ethion and chlorpyriphos, pesticide residues presence in water, sediment and fish muscle (*Puntius* sp.) collected in Deomoni river near tea cultivation *Camellia sinensis*, in India. The results comparison revealed that the highest pesticides concentrations were present in fish muscles, followed by sediment and lastly in water.

Therefore, contamination by pesticides in water resources is a matter of concern due to the adverse effects on aquatic organisms, such as bioaccumulation, and mainly on human health through the consumption of contaminated water and fish. Thus, the use of fish as bioindicators serves to assess the contamination of aquatic environments and is widespread in ecotoxicological research. As a result, it is possible to obtain the necessary information to carry out actions to reduce negative impacts on the environment by pesticides.

11.4 Pesticide Residues in Terrestrial Ecosystems

When the molecule of a particular pesticide is deposited in the environment, in most cases it reaches the soil. The amount of pesticides applied may be precisely known, but their fate in the soil environment may be uncertain due to the possibilities of interactions in soil, climate and agricultural practices. In this environment, the molecule may have different behaviors, reaching different ecosystems and non-target organisms. The accumulation of pesticide residues from the soil occurs in superficial layers, which shelter a vast biodiversity of organisms responsible for nutrient cycling and from which plants extract nutrients for their growth and development. In addition, the non-detection of a pesticide in the soil may reflect its degradability or demonstrate its inability to detect its residues by currently available analytical methods. Soil and sediments are the major deposits of pesticides affecting the overall dispersion of pesticides to other environmental compartments. Such transport may impair the functioning of sink ecosystems and represent additional routes of exposure to soil contaminants to humans and other non-target organisms (Vryzas 2018; Neuwirthová et al. 2019).

The availability of the compound is dependent on its formulation, for example, granule, particle or droplet. The pesticides distribution and degradation in soil is related to the physicochemical properties of these substances – mainly in relation to solubility, vaporization pressure and chemical stability. In addition, environmental conditions such as temperature, solar radiation, winds and rainfall may affect the pesticide’s behavior in the environment.

Notwithstanding environmental interactions stimulate interest in soil pesticide monitoring, knowledge of their dynamics and their ecotoxicological effects. Nevertheless, in the European Union, for example, the pesticide residue supervision in soil is not required. Furthermore, studies on pesticides and their potential for soil contamination are scanty and restricted to one pesticide at a time. In addition, most research is conducted under laboratory conditions, with limited results about degradation, mineralization, fixation, binding and translocation processes that are dependent on climate parameters, soil type and microbial biomass.

Various studies have analyzed the distribution of pesticides (Qu et al. 2016; Chiaia-Hernandez et al. 2017; Hvězdová et al. 2018), but the lack of standardization due to use of different sampling methodologies, analysis, and sampling period for example, prevent a pesticide residues distribution understanding in world's soils (Silva et al. 2019). In a study by Silva et al. (2019), 76 pesticide residues were analyzed in 317 agricultural soil samples from across the European Union. The authors show that more than 80% of the analyzed soils contained pesticide, out of 166 pesticides mixtures. Compounds such as Glyphosate and its Aminomethylphosphonic acid metabolite, as well as Dichlorodiphenyltrichloroethane and broad-spectrum fungicides rarely surpassed predicted environmental concentrations in the soil and were beneath their toxic endpoints for standard soil organisms.

The most widely used organisms as soil bioindicators are earthworms, preferably of *Eisenia fetida* and *Eisenia Andrei* species, the colembolics *Folsomia candida* and *Folsomia fimetaria*, the *Hypoaspis aculeifer* mite and the microorganisms that participate in nitrogen cycle (Clasen and Lisbôa 2019). Survival, growth, reproduction and behavioral changes tests on earthworms are used to evaluate the effects of toxic chemicals, including pesticides. In addition, traditional toxicological tests based on physiological responses are used to assess sublethal and lethal toxicity. More recently, studies have documented the ecotoxicological effects on soil worms through the use of biochemical and molecular biomarkers, such as the determination of reactive oxygen species, enzymatic and non-enzymatic antioxidant activities and deoxyribonucleic acid (DNA) damage assessment. These analyzes provide new approaches to efficiently assess the effects of environmental pollution (Uwizeyimana et al. 2017).

Another source of contamination to soil organisms is metals from pesticides. Successive fungicides applications on annual and perennial crops are an important copper and zinc contamination source in soils. In this scenario stands out the viticulture, where traditionally are made frequent applications of copper-based fungicides, such as Bordeaux syrup [$Ca(OH)_2 + CuSO_4$] and copper oxychloride are made [$CuCl_2.3Cu(OH)_2$], to control leaf fungal diseases (Brunetto et al. 2017). The Bordeaux mixture also contributes to increase heavy metals contents in the soil, such as zinc (Mirlean et al. 2007). Among the annual crops, mancozebe fungicide and also copper oxychloride, zinc and copper sources, respectively, have been widely used in soybean (*Glycine max* (L.) Merr) crops in recent harvests.

Therefore, the copper and zinc levels increase in soils enhances the risk of environmental contamination, including groundwater contamination by heavy metals derived from fungicides (Brunetto et al. 2014, 2017). In addition, copper and zinc levels increase in forms with higher bioavailability may cause toxicity to plants grown in these environments, something that has been warned in several studies on phytotoxicity (Girotto et al. 2016; Tiecher et al. 2016a, b, c, 2017, 2018; De Conti et al. 2019). In plant species sensitive to high levels of heavy metals, leaf contents between 15–20 mg copper kg^{-1} and 150–200 mg zinc kg^{-1} cause reduced plant growth (Kloke et al. 1984; Kabata-Pendias 2011). Moreover, increase in these elements concentrations on tissues above the mentioned values causes several disturbances in plants (Tiecher et al. 2017, 2018), because copper participates in several physiological and biochemical processes (Yruela 2005). Zinc, on the other hand, can generate oxidative stress through interference with plants antioxidant defense system (Gratão et al. 2005). Thus, oxidative stress parameters, metallothioneins and antioxidant enzymes activity are excellent stress biomarkers caused by excess metals in plants.

In addition to triggering oxidative responses that cause damage to plant tissues. Excess copper in plants usually inhibits their growth due to interference with important cellular processes such as membrane permeability, chromatin structure, protein synthesis, enzymes activity in photosynthesis and respiration processes (Yruela 2005; Gratão et al. 2005). In addition, zinc toxicity can alter membrane permeability, electron transport chain (De Magalhães et al. 2004) and nutrient absorption and translocation (Wang et al. 2009).

11.5 Pesticide Residues in Humans

When questioned in food safety areas and public health, the use or contact with pesticides may pose risks due to individuals exposure nature, such as, either by contaminated food and water ingestion, accidental poisoning or attempted suicide, individuals exposure in non-target areas by pesticide drift by wind action or pesticide application on crops (Klaassen and Watkins 2012; Kim et al. 2017). In occupational exposure to pesticides, the majority of family farmers are admitted as a risk group. There is an acute poisoning risk, for example, specific reactions such as vomiting and headache, and chronic poisoning: onset of disease (Kim et al. 2017).

The herbicides entry into agricultural market was a milestone in extensive agriculture modernization by virtually eliminating the weeding stage. However, the herbicides stands out negatively for its relative solubility in water and for its remarkable persistence in soil. Such factors give these pesticides high hazard and possible carcinogenicity among humans (Baird 2002).

Among the effects observed in humans from other risk groups, prolonged exposure to certain chemical pesticides groups, for example, through the consumption of contaminated food, can also lead to reactive species accumulation, oxidative stress and immune system failures (Abdollahi et al. 2004; Dhouib et al. 2016). The insecticides and herbicides are correlated with various diseases emergence when there is long-term exposure (Koh et al. 2017).

The Paraquat® herbicide, is widely marketed and highly hazardous due to its high acute toxicity, is also known to induce oxidative stress by increasing the reactive oxygen species concentration, lipoperoxidation precursors and malondialdehyde increased levels, known to cause disorder in cellular metabolism (Zhu et al. 2017). In this intense oxidation and reduction process, cellular nicotinamide adenine dinucleotide phosphate (NADPH) is led to complete depletion (Klaassen and Watkins 2012). Also, a study conducted in 2017 proved that this herbicide tends to influence cytokine expression, altering immune responses (Veríssimo et al. 2017).

Insecticides have been used for pest control for decades and are still among the main strategies used to ensure agricultural productivity. The nonpolar binding nature of these chemicals makes them potentially stable to environmental degradation. Being fat soluble, they tend to accumulate in animals adipose tissue, promoting bioaccumulation and, in long term, biomagnification within food chain (Baird 2002).

Organophosphates, carbamates and pyrethroids, called "modern insecticides" and currently marketed in many countries, are less persistent in environment, but have a much more potent acute effect. This represents a serious occupational hazard, especially for rural workers who come into direct contact with these products. Organophosphates and carbamates inhibit enzymes acetylcholinesterase and butyrylcholinesterase functioning, preventing transmission between neural cells (Baird 2002), and may aggravate seizures (Jintana et al. 2009; Lionetto et al. 2013). Pyrethroids, in turn, activate sodium channels, which can cause neural dysfunction, such as tremors and convulsions (Klaassen and Watkins 2012). Other studies also point to synthetic pyrethroids and ethylene-bis-dithiocarbamates as endocrine thyroid disruptors (Klaassen and Watkins 2012; Piccoli et al. 2016).

Early pesticide exposures detection can significantly decrease these and other deleterious effects occurrence. Therefore, the biomarkers are a great research tool because they allow to correlate exposure, internal dose and damage to health of organisms (Table 11.1). Thus, blood tissue samples are widely used as biomarkers for their susceptibility to toxic effects from various exogenous chemicals, including pesticides, as they may interfere with hematopoietic process of figurative blood production (Klaassen and Watkins 2012) and also act in harmful way to the immune system (Luebke 2002).

11.6 Conclusions

Despite existing data on pesticide residues risks in the environment, further study on pesticide contamination in aquatic and terrestrial ecosystems is needed to understand what residues exposure levels under field conditions to non-target organisms are exposed and yet what is the potential for pesticide bioaccumulation in aquatic organisms, plants, soil fauna and the food chain. Given this scenario, supervision is essential to control pesticides use in agricultural crops. It is important that pesticides and their behavior in environment are monitored and that risks to organisms are taken into account.

Table 11.1 Studies using biomarkers to assess the exposure of organisms to pesticides

	Aquatic organisms	Terrestrial organisms	Humans
Application of biomarkers	Bioaccumulation and oxidative stress caused by pesticides in *Cyprinus carpio* reared in a rice-fish system. Clasen et al. (2018)	Presence of pesticides and toxicity assessment of agricultural soils in the Quintana Roo Mayan Zone, Mexico using biomarkers in earthworms (*Eisenia fetida*). Andrade-Herrera et al. (2019)	Biomarkers of oxidative stress in the blood of workers exposed to pesticides that do not inhibit cholinesterase Lozano-Paniagua et al. (2018)
	An integrated approach in subtropical agro-ecosystems: Active biomonitoring, environmental contaminants, bioaccumulation, and multiple biomarkers in fish. Vieira et al. (2019)	Biomarker responses in earthworm coelomocyte extract e noninvasively collected sample for pesticide effect assessment. Ecimovic et al. (2019)	Organochlorine pesticides induce inflammation, ROS production and DNA damage in human ovary epithelial cells: An in vitro study Shah et al. (2020)
	Fish inhabiting rice fields: Bioaccumulation, oxidative stress and neurotoxic effects after pesticides application. Rossi et al. (2020)	Biochemical responses and DNA damage induced by herbicide QYR301 in earthworm (*Eisenia fetida*). Wang et al. (2020)	New perspectives in the modulation of cytokine pathways by exposure to pesticides Costa et al. (2020)

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