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Review

Analytical methods applied to the determination of pesticide residues in foods of animal origin. A review of the past two decades

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ABSTRACT

Pesticides are widely used in agriculture and can be transferred to animals in a number of ways. Consequently, reliable analytical methods are required to determine pesticide residues in foods of animal origin. The present review covers published methods and research articles (1990–2010) in which pesticide residues have been extracted from meat and meat products, milk and dairy products, fish and seafood, and eggs, then cleaned up, and isolated by chromatographic techniques to be identified and quantified by various detection methods. Recovery rates, quantification limits, the matrix effect and related parameters have all been considered. Lastly, future developments in this field are outlined.

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1. Introduction

Pest control in intensive agriculture involves treatment of crops (fruits, vegetables, cereals, etc.) pre- and post-harvests with a variety of synthetic chemicals generically known as pesticides [1]. Herbicides and insecticides are mainly used in the pre-harvest stages, rodenticides are employed in the post-harvest storage stages, and fungicides are applied at any stage of the process depending on the crop. These chemicals can be transferred from plants to animals *via* the food chain [2]. Furthermore, breeding animals and their accommodation can themselves be sprayed with pesticide solution to prevent pest infestations [3]. Consequently, both these contamination routes can lead to bioaccumulation of persistent pesticides in food products of animal origin such as meat, fish, fat, eggs, and milk.

Pollution by persistent chemicals is potentially harmful to the organisms at higher trophic levels in the food chain. Since diet is the main source of chronic exposure to low doses of these substances, humans are mainly exposed to these chemicals through ingestion [2,4,5]. The chronic effects of pesticides from food intake on human health are not well defined, but there is increasing evidence of carcinogenicity and genotoxicity, as well as disruption of hormonal functions [6,7].

To ensure that pesticide residues are not found in food or feed at levels presenting an unacceptable risk for human consumption, maximum residue levels (MRLs) have therefore been set by the European Commission. MRLs are the upper legal concentration limits for pesticide residues in or on food or feed. They are set for a wide range of food commodities of plant and animal origin, and they usually apply to the product as placed on the market. MRLs are not simply set as toxicological threshold levels, they are derived after a comprehensive assessment of the properties of the active substance and the residue behaviour on treated crops.

Both the periodic estimation of human exposure to persistent organic pollutants and the establishment by the EU authorities of MRLs in foods have required the development of analytical methods suitable for research purposes and inspection programmes [7–9].

Most pesticide residue detection methods for food samples comprise two key preparation steps prior to identification/quantification: extraction of target analytes from the bulk of the matrix, and partitioning of the residues in an immiscible solvent and/or clean-up of analytes from matrix co-extractives, especially fat which interferes with assays [10–13]. Complex samples such as meat products very often require a two-step clean-up which combines different chromatographic techniques in series [14]. When water-miscible extraction systems are used, such as with pesticide analysis in liquid milk for instance, it is also necessary to include a water removal or partitioning step [10].

However, most of these methods are time consuming and use large quantities of organic solvents to remove interference. Recent analytical developments have attempted to minimise the number of physical and chemical manipulations, the solvent volumes, the number of solvent evaporation steps, the use of toxic solvent, and have aimed to automate the extraction and clean-up procedures as far as possible [15].

Following the extraction/purification procedures, pesticide compounds are separated either on gas chromatography (GC) or liquid chromatography (LC), and then identified and quantified using different kinds of detection methods depending on the molecules to be analysed. Electron-capture detection (ECD), flame photometric detection (FPD), nitrogen–phosphorus detection (NPD), fluorescence detection, and diode-array detection (DAD) were mostly used for pesticide identification and quantification until recently. Many research papers on the analysis of pesticide residues in foods of animal origin report on the results achieved with these classical detection systems, even recently. But the expanding role of GC and LC coupled with mass spectrometry (MS) and tandem mass spectrometry (MS/MS) in pesticide residue analysis is clear in both monitoring and research applications.

The methodology for pesticide analysis in environmental and plant samples is very well documented and many examples are available in the literature. A number of recent reviews dealt with pesticide residue analysis in various foodstuffs [16–24]. However, none of these highlighted the problems, pitfalls and achievements in the foods of animal origin. Scientific documentation on analytical methods applied to pesticide determination in animal products is less abundant. This paper will aim to summarise the documentation published on the analysis of pesticide residues in meat and meat products, poultry and eggs, fish, and milk and dairy products over the past two decades.

2. Foods of animal origin

2.1. Meat and meat products

Breeding animals can accumulate persistent organic pollutants from contaminated feed and water, and/or from pesticide application in animal production areas (treatment of cowsheds, pigsties, sheepfolds, warrens and/or treatment of animals themselves) [25]. While pesticide compounds are mostly stored in the fat and muscle of animals, they can also reach other compartments such as the brain, liver and lungs [26]. Consequently, these chemical residues have been studied in the meat [14,27] and adipose tissue [14,28] of various species such as dairy cattle [29], beef cattle [3,12,15,30–34], pigs [25,26,31,35–37], sheep [34,37,38], rabbits [7], and camel [34]. Some papers have also reported on pesticide determination in offal [1,39–41] and in food containing meat products, such as infant [42] and processed [6,11,43] foods.

2.2. Poultry and eggs

Insecticides and acaricides are used in henhouses to control poultry ectoparasites such as red mite; chickens and laying hens can then be accidentally exposed to these chemicals [44]. Poultry can also be contaminated by feeding on plant materials that have been treated with pesticides during the growing and/or storage stages. Consequently, chickens and hens accumulate residues in muscles, fat, and liver [4,29,37]. Pesticide residues can also be detected in eggs [44–46], even long after the chemicals have been eliminated from the other tissues of the laying hens [47]. In some countries poultry and eggs can be a major source of human exposure to pesticide contamination [4].

2.3. Fish and shellfish

The uncontrolled agricultural discharge of pesticides has created significant environmental concern since these chemicals are prone to long-range transport. Through surface runoff, river inputs and atmospheric deposition, persistent pesticides have spread to every aquatic environment, entering the marine food chain [48–52]. Aquatic organisms such as fish and shellfish are able to accumulate pesticide residue concentrations several times higher than the surrounding water [5]. In aquaculture farms, fish feed, contaminated by pesticides, is a potential source of direct introduction into fish [53]. Consequently, fish are a major source of contamination for both top marine predators [54] and human consumers [48].

2.4. Milk and milk products

Like all foraging animals, lactating cows may be exposed to pesticides from contaminated feed; they can then accumulate residues

Table 1
Pesticides naturally detected in foods of animal origin during research or monitoring programmes (published results).

Matrix	Milk	Milk	Milk	Egg	Egg	Egg	Fish	Fish	Fish	Fish
References	[72]	[30]	[55]	[31]	[72]	[4]	[31]	[84]	[99]	[48]
N	7	1/156	8	29	4	24	24	14	3	72
Units	ng/g	μg/l	μg/l	ng/g	μg/g	ng/g	ng/g	ng/g	ng/g	ng/g
Σ-HCH				1.3–21.6		1.58	0.3–0.9	0.8–7.0		8.5–33.2
Σ-DDT		1.3		2.5–4.4		2.42	0.5–9.9	11.6–59.0	7.9	37.5–139.0
HCB				0.8–9.6			0.1–0.2	2.7–.3	<LOQ	2;7–.5
Aldrin										1;1 –13.3
Dieldrin								2.7 –4.4		
Endrin										
Heptachlor										
Hepta. epoxide										
Σ-Endosulphan				n.d.			n.d.			
Chlordane								5.2–9.0		
Simazine	n.d.–2.5				30.1–59.5					
Atrazine	n.d.–1.8				n.d.–52.2					
Deltamethrin			<LOD–1.45							
Cypermethrin			<LOD–3.68							

Matrix	Fish	Fish	Shellfish	Crab	Meat[4]	Pork meat	Beef meat	Chicken meat	Rabbit meat
References	[50]	[5]	[51]	[52]	[31]	[26]	[30]	[4]	[7]
N	14	389	18	9	77	4	8/10	48	?
Units	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g fat	ng/g	μg/g
Σ-HCH		225–660	3.3–25.8	2.0–25.7	0.2–21.4	1.2–3.4		0.05	
Σ-DDT	3.5–515.0	4–47	1.0–11.8	1;5–24;8	1.1–9.8	2.8–19.8	<3–6.23	0.12	
HCB					n.d.				
Aldrin			0.2–1.9						
Dieldrin		<LOD –6	0.2–1.9						
Endrin			0.6–5.6						
Heptachlor			0.7–5.9						
Hepta. epoxide		<LOD –6	0.3–7.2						
Σ-Endosulphan		5–22			n.d.				
Chlordane									
Simazine									0.9
Atrazine									n.d.
Deltamethrin									
Cypermethrin									

1. Σ-HCH, hexachlorocyclohexane (α, β, δ, and γ isomers).
2. Σ-DDT = DDT and its metabolites DDE & DDD, appear as both *o,p′*- and *p,p′*-isomers.
3. Σ-Endosulphan, α and β isomers;
4. Beef, pork, lamb, & chicken.
n.d., not detected.

in different compartments including milk. However, the contamination of milk by pesticides can also be caused by their application to the cow's body, in the cow barn, or even in the milk processing areas [55–57]. Milk contamination is of extreme concern since milk is an essential food for human health, widely consumed in the early stages of life [55]. This point is of particular interest since young children may be highly exposed to pesticides and pesticide residues, and they may be at higher risk of adverse health effects because of their physiological characteristics [58].

3. Pesticides

In the reported studies from the literature consulted for this review, products of animal origin were mostly analysed for five main groups of pesticides, namely organochlorine pesticides, organophosphorus pesticides, carbamates, pyrethroids, and triazines. Few of them have been detected in various animal products (Table 1). However, various other pesticides have been studied during validation methods for detecting residues in foods of animal origin (Table 2).

Organochlorine pesticides (OCPs), effective against a variety of insects, were widely used worldwide in agriculture and animal production until restrictions were introduced by developed countries in the late 1970s [59]. These pollutants have a highly stable, low volatile, non-polar, lipophilic nature, and consequently exhibit considerable environmental persistence with a tendency to bioaccumulate, leading to the contamination of foodstuffs, especially those with a high fat content [6,31]. Residue concentrations have decreased in monitored foods since these chemicals were banned in most countries, although trace levels are still detected in many foodstuffs [60]. European regulation establishing pesticide residue levels in food has prompted EU members to monitor OCPs [61–63].

Organophosphorus pesticides (OPPs), mainly used as insecticides, are esters of phosphoric acid with different substituents [25]. OPPs have widely varying physico-chemical properties such as polarity and water solubility [27]. Since these substances act through inhibition of acetyl-cholinesterase, they also represent a risk to human health [64]. Maximum residue limits (MRLs) have therefore been established for OPPs by the EU authorities [61–63].

Carbamate pesticides (CBs), or *N*-substituted carbamic acid esters (RO–C(O)–NR′R″), are used for broad-spectrum insect control around the world [65]. Exposure to carbamate pesticides, acting as acetyl-cholinesterase inhibitors, can lead to reversible neurologic disorders [64], and some are suspected carcinogens and mutagens [57]. CBs are thermally unstable compounds; their stability has been studied in foodstuffs such as beef and poultry liver [40]. The Canadian authorities recently initiated a re-evaluation of exposure to these substances [42]. In the EU, MRLs have been established for CBs [61–63].

Synthetic pyrethroid pesticides (PYRs) are effective broad-spectrum insecticides with low mammalian toxicity and short-term environmental persistence [3,56]. Pyrethroids

Table 2
Pesticides evaluated for analysis in foods of animal origin.

Class	Pesticide	References	Class	Pesticide	References
OCP	Aldrin	[3,6,9–11,30,31,34,36–39,48–51,60,75,78,80,90–94,96,99]	OPP	Acephate	[10,25,27,37,38,47,75,76,78]
				Azinphos-ethyl/-methyl	[1,10,43,47,75,76,78,97]
	Chlordane (*cis*-/*trans*-)	[3,6,9,11,17,28,30,34,36,39,78,84,90,94,96]		Carbophenothion	[10,43,47,78]
	Chlordene (*trans*-)	[11,30,31,84]		Chlorfenvinphos	[10,37,38,43,47,73,75,78,89]
	Chlorfenson	[1]		Chlorpyrifos methyl	[27,28,76,78,97]
	Chlorobenzilate	[1]		Chlorpyrifos	[10,25,27,32,37,38,43,47,73,76,96,97]
	Chlorthalonil	[37,38,78,96]		Dialifos	[43]
	DDT/DDD/DDE	[3–6,9–11,13–15,17,26,28,30,31,34,36–39,47–51,53,54,60,75,78,80,84,86,89–94,96,99]		Diazinon	[25,43,47,73,75,76,78,89,96,97]
				Dichlorvos	[10,37,38,43,73,75,78,96]
	Dichloran	[37,38,96]		Dicrotophos	[47]
	Dicofol	[1,9,75]		Dimethoate	[10,37,38,43,47,73,75,76,78,97]
	Dieldrin	[3,5,6,9–11,14,15,17,28,30,31,34,36–39,47,49–51,60,75,78,80,90,92–94,96]		Disulphoton	[43,73,75,78]
				Ethion	[10,28,37,38,43,47,73,75,78,97]
	Endosulphan (α-/β-)	[3,5,6,9–11,28,30,31,36–39,49,60,75,78,80,91,94,96,99]		Ethoprophos	[37,38,43,78]
				Etrimphos	[37,38,78]
	Endosulphan sulphate	[3,6,11,28,36,39,78,91,99]		Famfur	[37,38]
	Endrin	[3,6,9,11,14,17,30,31,34,36–39,49–51,60,78,80,89–94,96]		Fenamiphos	[10,37,38,47,76]
				Fenchlorphos	[43,47]
	Endrin aldehyde	[39]		Fenitrothion	[1,28,43,76,78]
	Endrin ketone	[39]		Fenthion	[10,37,38,43,73,76,78]
	Ethylan (Ethyl-DDD)	[11]		Fonofos	[43,78,96]
	HCB	[3,6,9–11,13,14,17,26,30,31,34,36–38,48–50,53,54,60,75,78,84,86,90,96,99]		Gardona	[47]
				Heptenophos	[37,38,78]
	HCH (α-/β-/γ-/δ-)	[3–6,9–11,13–15,26,28,30,31,34,36,38,39,47–49,53,60,75,78,80,84,89–94,96,99]		Iodenofos	[47]
				Isofenphos	[37,38,43,78,97]
	Heptachlor	[3,5,9,10,28,30,31,36–38,49–51,54,60,75,78,80,84,90–94,96,99]		Malaoxon	[43,78]
				Malathion	[10,27,32,37,38,43,47,73,75,78,89,96]
	Heptachlor epoxide	[3,5,6,9,10,14,15,17,28,30,31,36–39,47,50,51,60,75,78,80,84,89–94,99]		Methamidophos	[25,37,38,47,75,78]
				Methidathion	[10,25,43,47,75,78,89]
	Hexaconazole	[75,76]		Mevinphos	[43,73,75,78]
	Iprodione	[10,75,76,78]		Monocrotophos	[47]
	Isodrin	[49]		*O*-Methoate	[10,47,75,76,78]
	Methoxychlor	[37,38,49,75,78,84,90,91,94,99]		Paraoxon	[43,78]
	Mirex	[37,38,90,99]		Parathion	[25,37,38,43,47,73,75,76,78,89,96,97]
	Nonachlor (*cis*-/*trans*-)	[14,30,31,47,49,60,84,89,90]		Phorate	[10,25,32,43,73,76,78,96]
	Octachlorostyrene	[13,48]		Phosalone	[37,38,43,76,78]
	Oxychlordane	[3,6,9,17,36,84,90]		Phosmet	[42]
	Oxyfluorfen	[10]		Phoxim	[44,46]
	Pentachlorobenzene	[39]		Pirimiphos	[25,27,32,37,38,47,75,76,78]
	Pentachloronitrobenzene	[10]		Propetamphos	[29,78]
	Pentachlorophenol	[39]		Prothiofos	[27,32,78]
	Procymidone	[32,75,78]		Pyrazophos	[43,47,75,76,78,97]
	Quintozene	[37,38,75,78]		Pyrimifos-methyl	[43]
	TDE	[9,36,90,92,93]		Quinalphos	[37,38,43,47,75,97]
	Tecnazene	[3,78]		sulphotep	[37,38,43,75]
	Tetradifon	[1,75]		Sulprofos	[10]
	Vinclozolin	[32,37,38,75,78]		Tetrachlorvinphos	[10,43,78]
				Thionazin	[37,38,43]
Carbamate	Aldicarb	[10,38,40,41,57,76]	Thio-OPP	Bromophos	[43]
	Bufencarb	[40,41]		Coumaphos	[43,73]
	Carbaryl	[10,40–42,57,75,76,78]		Cyanophos	[47]
	Carbofuran	[10,32,40–42,57,75,76,78]		EPN	[43]
	Chlorpropham	[10,75,78]		Formothion	[43]
	Methiocarb	[40–42,78]		Mecarbam	[43,75,104]
	Methomyl	[40–42,57]	Pyrethroid	Alphamethrin	[29]
	Oxamyl	[42,76,104]		Bifenthrin	[3,12,15,33,75,78]
	Pirimicarb	[57,76,78]		Cyfluthrin	[9,33,35,56,75,78]
	Propham	[10,75,78]		Cyhalothrin	[3,9,12,35,56,75,78]
	Propoxur	[10,42,44,45,57,76]		Cypermethrin	[3,9,12,15,33,35,55,56,69,70,75,78]

Table 2 (*Continued*)

Class	Pesticide	References	Class	Pesticide	References
Triazine	Atrazine	[7,10,68,72,75,76,78,85,96]		Cyphenothrin	[33,56]
	Simazine	[7,10,68,72,75,76,78,85,96]		Deltamethrin	[3,9,15,29,33,35,55,56,75,78,95]
Amine	Diphenylamine	[10,78]		Fenvalerate	[3,9,10,33,75,78]
Fluoride	Ethalfluralin	[10]		Flucythrinate	[33,56,75,78]
Quinoxaline	Chinomethionate	[28]		Fluvalinate	[15,33,56,75,78]
Sulphured	Dimethipin	[28]		Permethrin (*cis-/trans-*)	[3,9,10,12,15,29,33,35,55,56,75,78,96]
Sulphite ester	Propargite	[10,78]		Tefluthrin	[33,56,78]
Benzoylurea	Chlorfluazuron	[83]	Benzoylurea	Hexaflumuron	[83]
	Diflubenzuton	[83]		Lufenuron	[83]
	Flucycloxuron	[83]		Teflubenzuron	[83]
	Flufenoxuron	[83]		Triflumuron	[83]
	Fluometuron	[83]	Others	(Multiresidues)	[10,76,78]

are non-polar to low-polarity lipophilic compounds [12,33]. Owing to their metabolism in animals, they tend to bioaccumulate in lipid compartments, becoming a potential source of human exposure through foodstuffs [3]. Maximum residue limits for PYRs have been set by several organisations, including the FAO/WHO [66] and the EU Council [61–63].

Triazines (TRZs) are among the most widely used herbicides in agriculture. Most of them are derived from *s*-triazine (1,3,5-triazine), but a few are based on 1,2,4-triazine [67]. The triazines are degraded by chemical and biological processes in their respective hydroxytriazines [65]. *s*-Triazines and their degradation products are weakly basic, poorly water-soluble compounds of low polarity, stable in the environment and therefore persistent. 1,2,4-Triazines have similar physico-chemical properties but are more polar [67]. These herbicides are suspected of causing cancers, birth defects, and disruption of hormone function [7]. MRLs for triazines in foodstuffs have also been established by the EU Council [61–63].

Other pesticides, such as benzoylureas, quinoxalines, amines, and fluorides, have been evaluated for analytical purposes in foods of animal origin (Table 2).

4. Extraction methods

In the past two decades, the most widely used pesticide extraction technique from foods of animal origin was direct solid–liquid extraction (SLE). This procedure consists in grinding chopped samples or extracted fats several times at high speed in selected organic solvents. This technical procedure has been applied to meat and meat products [3,7,11,12,15,33,35,42,43,68–70], animal fat [14,27,29,30,36], offal [25,29,41], eggs [44–47,71] and fish [31,36] for extracting different kinds of pesticides. Similarly, liquid–liquid extraction (LLE) is still the preferred method for extracting pesticide residues from liquid milk [10,25,29,30,35,55,57,69,70,72,73]. This procedure consists in shaking liquid milk samples several times in selected organic solvents for extracting pesticide residues from the bulk of the milk. Several SLE and LLE protocols have been standardised for extracting 23 OCP and 22 OPP residues from fatty foods of animal origin (milk and milk products, meat and meat products, fish and seafood, eggs) [74].

Most studies published on foods of animal origin have usually only dealt with one or two pesticide classes. The polarities of pesticide compounds are then more or less similar and the choice of solvent is generally not insuperable. Yet, in some cases, especially with multi-residue methods, solvent mixtures have to be able to extract pesticide residues with a wide range of polarities from the same matrix. However, it is difficult to extend a class-specific procedure to a wider range of analytes because the polarity of the extraction solvent mixture may not be suitable for efficient extraction of other classes of compounds [10]. Moreover, non-polar pesticides may have more polar compounds, as their metabolites or degradation products, whose extraction requires different solvent systems according to their polarity [69,70].

Overall, SLE and LLE are efficient methods in terms of recovery, except for a few pesticides such as hexachlorobenzene for instance which is extremely volatile [3]. Different recovery values have been reported when different solvent mixtures were used for extracting pesticide residues from the same matrix [7,15,68,75]. Different solvents and combinations thereof have been compared for extracting pesticides [55,76]. One of the EU standard SLE protocols was successfully used in an inter-laboratory study for determination of OCP and pyrethroid residues in milk, fish, eggs, and beef fat [9].

SLE and LLE mostly yielded recovery rates between 70 and 120% as recommended by the EU [77] (Table 3). Even multiresidue methods showed recoveries in this range for over 80% of the analytes [76,78]. However, low recovery rates were encountered for some pesticide/commodity pairs using these extraction methods (Table 3). On the other hand, such classical methods of analyte extraction using sample homogenisation and solid–liquid or liquid–liquid partitioning are time-consuming, labour-intensive, expensive in terms of materials and solvent volumes, and often cannot be completed before the materials in question have been placed on the market. Furthermore, evaporation of large solvent volumes is a source of analyte loss and atmospheric and environmental pollutions [60,79].

The traditional Soxhlet extraction method has also been applied to the extraction of organochlorine pesticides from meat [4,26,37,71,80], from eggs [4], and from fish [5,48,49,51,80]. Usually either n-hexane or an n-hexane–acetone mixture was used, however, other solvent systems such as ethyl acetate [37] or acetone: DCM [4] have been sporadically used. Furthermore, Soxhlet protocols have been integrated into the EU standard for extracting 23 OCP and 22 OPP residues from fatty foods of animal origin [74]. Soxhlet usually performs efficient extractions for a large range of pesticides; however, using this technique or performing it under unfavourable conditions can result in poor recovery rates. For instance, recoveries below 70% have been reported for a few pesticide residues (7 out of 46 OCPs and OPPs) extracted from animal muscles by Soxhlet using ethyl acetate [37]. In any case, Soxhlet extraction is time-consuming, and expensive in terms of energy (heating), analyst time (much handling), and solvent use (large volumes).

Pesticide supercritical fluid extraction (SFE) has been attempted for extracting contaminants from meat products [32,33,81,82]. SFE is usually an efficient extraction method, primarily applicable to solid samples. However, as well as its numerous advantages (efficacy, selectivity, short extraction times, low solvent volumes) it also has serious drawbacks (difficult optimisation, high apparatus and maintenance cost, high blank and noise levels) [67]. In the case of pesticide residue analysis, recoveries for several compounds were unacceptable [82]. Indeed, SFE techniques have not tended to be widely used for pesticide analysis in food from animal origin.

Table 3
Methods for determination of pesticide residues in foods of animal origin.

	Pesticides	Extraction procedure	Clean-up procedure	Separation technique	Recovery rate	LOQ	Ref.
Liver	4 OCPs 2 OPPs 1 CB	Solid:liquid extraction DCM:acetone [1:1] + Na_2SO_4	GPC SX-3 n-Hexane:chloroform:acetone [75:20:5] SPE silica n-Hexane:chloroform:acetone [75:20:5]	LC-DAD LiChrospher RP-18 5 μm gradient Water:acetonitrile:methanol	>90%	0.10–0.23 ng/g	[1][a]
				GC-ECD BP-5 25m	94–100%	0.10–0.23 μg/g Pirimicarb 0.46 μg/g Azinphos 2.03 μg/g	
Beef meat	19 OCPs 6 PYRs	Solid:liquid extraction Petroleum ether + Na_2SO_4 + sand	SPEs NT3/C18/Florisil Acetonitrile	GC–MS HP-5ms 30 m	70–110% but HCB 49-57%	5–125 ng/g	[3]
Poultry Meat & liver Egg	10 OCPs	Soxhlet Acetone:DCM [2:8]	Liquid partitioning SPE silica n-Hexane/n-hexane:DCM [3:2]	GC-ECD HP-5 30 m	n.s.	n.s.	[4]
Fish (muscle tissue)	10 OCPs	Soxhlet n-Hexane	SPE Silica n-Hexane	GC-ECD DB-608 30 m	94–103%	1 ng/g	[5]
Bovine milk	20 OCPs 4 PYRs 2 TRZs	Liquid:liquid extraction + Na_2SO_4	SPE Si-C18 Acetonitrile SPE Aminopropyl	GC-ELCD DB-608 30 m	69–128%	0.6–58.6 ng/g	[10]
	18 OPPs 6 CBs	Ethanol:ethyl acetate [9:95] freezing	Methanol: DCM [7:93]	GC-FPD DB-1701 30 m LC-Fluorimetry C18 5 μm gradient Water:acetonitrile λ_{ex}:340 nm λ_{em} 455 nm	54–136% 87–110%	0.3–1.8 ng/g 0.9 ng/g	
	Multiresidue			GC–MS HP-1 12 m	46–130%	9–141 ng/g	
Pork meat & meat products	24 OCPs	Solid:liquid extraction DCM + Na_2SO_4	GPC SX-3 DCM:c-hexane [15:85]	GC–MS HP MS-5 30 m	65–104%	2–25 ng/g fat	[11]
Beef meat	4 PYRs	Solid:liquid extraction Acetone + Na_2SO_4 Partitioning with petroleum Ether Na_2SO_4 column	SPEs Si-C18/Extrelut-NT3 Acetonitrile:n-hexane SPE Florisil n-Hexane:toluene	GC-ECD DB-608 30 m	87–110%	50–250 ng/g	[12]
Fish (muscle tissue)	8 OCPs	ASE with Na_2SO_4 n-Hexane:DCM [1:1] *vs.* [4:1] n-Hexane:Acetone [1:1] *vs.* [4:1] Soxhlet with Na_2SO_4 n-Hexane:DCM [1:1] *vs.* [4:1] n-Hexane:acetone [1:1] *vs.* [4:1]	GPC SX-3 c-Hexane:ethyl Acetate [1:1]	GC-ECD DB-5 or DB-7 60m	n.s.	n.s.	[13][a]
Animal fat	16 OCPs	Solid:liquid extraction Ethyl acetate:c-hexane [1:1]	GPC SX-3 c-Hexane:ethyl acetate [1:1] SPE Silica Toluene:acetone:n-hexane [10:2:88]	GC-ECD CPSil-8CB 60 m	77–90%	n.s.	[14]
Beef meat	5 PYRs	Solid:liquid extraction Iso-Octane + Na_2SO_4	Liquid partitioning SPE Florisil Acetonitrile	GC–ITMS DB-5 30 m	70–82%	2–18 ng/g	[15]
	5 OCPs				59–75%	2–24 ng/g	
Bovine milk	9 OPPs	Liquid:liquid extraction Acetone:acetonitrile [1:4]	Liquid partitioning SPE Silica C18 Acetonitrile then Iso-propanol (combined)	GC-NPD ZB-50 30 m	46–117%	5 ng/g	[25]
Boar muscle and liver	3 OPPs	Solid:liquid extraction Acetone:acetonitrile [1:4]	Liquid partitioning \SPE silica C18 Acetonitrile	GC-NPD ZB-50 30 m	65–77%	5 ng/g	[25]
Pork meat, fat and liver	6 OCPs	Tissues ground with Na_2SO_4 Soxhlet n-Hexane:acetone [3:1]	SPE acidified silica n-Hexane followed by DCM	GC-ECD HT-8 50 m	72–80%	0.2 ng/g	[26]

Table 3 (*Continued*)

	Pesticides	Extraction procedure	Clean-up procedure	Separation technique	Recovery rate	LOQ	Ref.
Meat	7 OPPs	Solid:liquid extraction Ethyl acetate + Na_2SO_4	2 SPEs Si-C18 in series Ethyl acetate:methanol [1:1] (fraction 1) Methanol (fraction 2)	GC-NPD DB-1701 30 m	32–102%	2–34 ng/g	[27]
Beef meat	18 OCPs 2 OPPs	Solid:liquid extraction Microwave assisted Acetonitrile	Freezing −70 °C/20min Centrifugation	GC-ECD SPB-608 30 m	OCP 45–90% OPP 74 & 95%	1.0–4.2 ng/g 1.3 and 2.4 ng/g	[28]
Pork, beef, lamb, chicken meat Fish, Egg	21 OCPs	Solid:liquid extraction Petroleum Ether + Na_2SO_4	GPC SX-3 n-Hexane:ethyl acetate [1:1]	GC-ECD Quadrex 007-2 50 m	80–110%	1–18 ng/g	[31]
Beef meat	OPPs	SFE CO_2	SPE Florisil Heptane then Acetone (2 fractions)	GC-NPD DB-1701 30m	78–95%	0.02–0.03 μg/g	[32]
Beef meat	10 PYRs	Solid:liquid extraction Acetonitrile	Filtration Freezing −29 °C/3H Liquid partitioning n-Hexane:acetonitrile	GC-ECD DB-17 30 m	29–100%	n.s.	[33]
Camel, bovine, sheep meat	9 OCPs	Solid:liquid extraction Petroleum ether + Na_2SO_4	Liquid:liquid partitioning (n-Hexane:acetonitrile) SPE Florisil Diethyl ether:n-Hexane:methylene chloride [1:29:70]	GC-ECD J&W 30 m	86–109%	n.s;	[34]
Bovine milk	4 PYRs	Liquid:liquid extraction n-Hexane	Freezing −72 °C/Liquid partitioning	GC-ECD HP-5 30 m	83–99%	9–25 ppm	[35]
Pork meat		Solid:liquid extraction n-Hexane	SPE Florisil		84–99%	10–27 ppm	
Fish oil	20 OCPs	Solid:liquid extraction Ethyl acetate:c-hexane [1:1]	GPC Envirosep-ABC c-Hexane:ethyl acetate [1:1]	GC–MS^2 VF-5ms 30 m	65–103%	6 ng/g	[36]
Pork fat					64–101%	6 ng/g	
Chicken meat	22 OCPs 23 OPPs	ASE Ethyl acetate Soxhlet Ethyl acetate Polytron Ethyl acetate	GPC Envirogel n-Hexane:ethyl acetate [1:1]	GC–MS^2 VF-5ms 30 m	62 93% 70–104% 59–93%	0.19–7.1 ng/g Acephate 14.6 ng/g	[37][a]
Lamb meat	22 OCPs 23 OPPs	Polytron Ethyl acetate	GPC Envirogel n-Hexane:ethyl Acetate [1:1]	GC–MS^2 VF-5 ms 30 m	72–91%	n.s.	[37]
Pork meat					71–102%		
Cattle adipose tissue	19 OCPs	ASE DCM:acetone [1:1] Concentration/Dissolution in n-Hexane: MTBE [1:1] Washing H_3PO_4/KCl	GPC SX-3 DCM:acetone [1:1] SPE silica DCM: n-Hexane (Fraction A non-polar OCP) Methanol:DCM (fraction B more polar OCP)	GC–MS DB-5 ms 30 m	41–111%	n.s.	[39]
Swine liver					24–88%		
Bovine, swine, duck livers	10 CBs	Solid:liquid extraction DCM + Na_2SO_4	GPC SX-3 DCM:c-hexane [1:1]				[40,41]
Poultry-based baby-food	10 CBs	Solid:liquid extraction Acetonitrile	Filtration SPE Oasis HLB MTBE: Methanol [9:1] SPE Aminopropyl DCM: Methanol [99:1]	LC-Fluorimetry NovaPak C18 4 μm gradient Water:methanol λ_{ex}:339 nm λ_{em} 445 nm	66–87%	2 ng/g but Methiocarb 4 ng/g	[42]
Pork meat & meat products	39 OPPs	Solid:liquid extraction DCM + Na_2SO_4	GPC SX-3 DCM:c-hexane [15:85]	GC-FPD DB-5 30 m OV-1701 25 m	40–100%	5–10 ng/g Malaoxon 20 ng/g	[43]
Egg	1 CB (Propoxur) 1 OPP (Phoxim)	Solid:liquid extraction Acetonitrile	Liquid partitioning SPE silica n-hexane/n-Hexane:DCM [4:6] (washing) DCM (Elution)	LC-DAD LiChrospher C18 5 μm Water:acetonitrile DAD 200–350 nm	85–90%	5 ng/g	[44,45]
Egg	OCP/OPPs	Solid:liquid extraction Acetonitrile	Liquid partitioning SPE Silica n-Hexane/n-hexane:DCM [1:1]	LC-DAD LiChrospher C18 5 μm Water:acetonitrile DAD 200–350 nm	89%	5 ng/g	[46]
Egg	6 OCPs	Solid:liquid extraction Acetonitrile	SPEs GNPC/Aminopropyl Acetone: Toluene [3:1] SPE Florisil Petroleum Ether	GC-ECD DB-225 30 m	86–108%	n.s.	[47]

Table 3 (*Continued*)

	Pesticides	Extraction procedure	Clean-up procedure	Separation technique	Recovery rate	LOQ	Ref.
Egg	28 OPPs	Solid:liquid extraction Acetonitrile	SPEs GNPC/Aminopropyl Acetone:toluene [3:1]	GC-FPD DB-225 30 m	60–148%	n.s.	[47]
Fish (muscle tissue)	10 OCPs	Soxhlet n-Hexane	SPE Florisil n-Hexane/n-hexane:DCM [42.5:7.5]	GC-ECD DB-5 30m	70–102%	0.12–3.10 ng/g	[48]
Shellfish	13 OCPs	Soxhlet n-Hexane:acetone [3:1] + Na_2SO_4	SPE H_2SO_4 impregnated Si (acid resistant OCP) SPE Alumina/Silica/Florisil (acid sensitive) n-Hexane:DCM [3:1]	GC-ECD DB-5 30 m	78–89%	0.1–0.6 ng/g	[51]
Mollusc, Crab	8 OCPs	Soxhlet n-Hexane:acetone [1:1]	SPE Na_2SO_4/Florisil n-Hexane	GC-ECD DB-1701 30 m	60–116%	0.1–0.6 ng/g	[52]
Fish feed	6 OCPs	Soxhlet Petroleum ether	GNPC (Envi-Carb) n-Hexane + H_2SO_4	GC-ECD AT-5 30 m	90–130%	3 ng/g	[53]
Lard	8 OCPs	ASE n-Hexane: DCM [1:1] + Na_2SO_4	SPE Acidified Silica n-Hexane SPE Silica/Activated Carbon n-Hexane: DCM [10:4]	GC–MS^2 ZB-1 60 m	45–86%	0.7–1.9 pg/g	[54]
Bovine milk	2 PYRs	Liquid:liquid extraction Acetonitrile	Freezing −20 °C/12H Filtration on Na_2SO_4	GC-ECD HP-5 30 m	n.s.	0.75 μg/l	[55]
Bovine milk	14 PYRs	Liquid:liquid Acetonitrile:Ethanol [5:1]	SPE ChemElut Light petroleum:acetonitrile:ethanol [100:25:5] GPC Envirosep-ABC Toluene	GC-FPD DB-5 30 m OV-1701 25 m	60–119%	n.s.	[56]
Bovine milk	6 CB	MSPD Crystobalite Hot Water (90 °C)	SPE (MSPD) Crystobalite Hot Water (90 °C) Filtration on regenerated cellulose filter (0.2μm)	LC–MS Alltima-C18 5 μM gradient Methanol:water ESI source	76–104%	3–8ng/g	[57]
Bovine milk	22 OCPs	MSPD Si-C18 n-Hexane	SPE Silica C18 Acetonitrile:water:n-hexane SPE neutral alumina n-Hexane	GC-ECD Quadrex 007-2 50 m	79–99%	0.02–0.62 μg/l	[60]
Bovine milk and cream	1 PYR Cypermethrin	Liquid:liquid extraction n-Hexane	Liquid:liquid partitioning (n-Hexane:acetonitrile) SPE Silica	GC-ECD DB-5 15 m	70–100%	10 ng/g	[69,70]
Muscle and liver (Vulture)	13 OCPs	Soxhlet n-Hexane	SPE Silica n-Hexane	GC-ECD DB-608 30 m	94–103%	1–5 ng/g	[71]
Egg (Vulture)	13 OCPs	Sample ground with Na_2SO_4/Celite/Alumina put in a glass column eluted with n-Hexane					
Bovine milk	2 TRZs	Liquid:liquid extraction n-Hexane:acetone [2:1]	SPE aromatic sulphonic acid Acetic Acid:water [1:99]/acetonitrile/water/buffer (washing) Acetonitrile:phosphate buffer [1:1] (elution)	LC-DAD C18 ODS 5 μm DAD 200–400 nm	n.s.	500 ng/g Atrazine 800 ng/g Simazine	[72]
Egg		Solid:liquid extraction Acetone	SPE Silica C18 Water (washing) then methanol				
Bovine milk	13 OPPs	Liquid:liquid extraction Ethyl acetate + Na_2SO_4	Evaporation/Liquid partitioning n-Hexane:acetonitrile [1:2.5]	GC-FPD HP-1 25 m	60–99% but Disulphoton 33% Ethion 43% Malathion 47%	5–19 ng/g	[73]
Minced meat Whole egg	136 multi-residues	Solid–liquid extraction Acetonitrile		LC-MS/MS BEH-C18 1.7 μm gradient Buffer:water/methanol MS Electrospray mode	70–120% for over 80% of the analytes	<0.01–0.05 mg/kg	[76]
Raw milk		Liquid:liquid extraction Acetone					
Meat-based baby-food	236 multi-residues	Solid:liquid extraction QuEChERS n-Hexane Acetonitrile:acetic acid [99:1]	centrifugation Hexane removed mixing with PSA + magnesium sulphate Centrifugation	GC–ITMS ZB-5 ms	70–121%	0.30–45.00 ng/g	[78]

Table 3 (*Continued*)

	Pesticides	Extraction procedure	Clean-up procedure	Separation technique	Recovery rate	LOQ	Ref.
Shellfish	14 OCPs	Soxhlet n-Hexane	SPEs Si-C18/Florisil Acetonitrile	GC-ECD PTE-5 30Acetonitrile	77–105% 82–98%	0.5–4.8 ng/g n.s.	[80]
Fish Bovine meat					69–125%		
Pork meat					79–110%		
Chicken meat					77–105%		
Bovine meat	9 BUs	ASE + Diatomaceous earth Ethyl acetate 80 °C 1500PSI	–	LC–ITMS/MS Luna-C18 5 μM gradient Methanol:water APCI source	28–106%	2–10 ng/g	[83]
Egg					83–99%		
Milk					70–106%		
Mackerel fillet Cod liver	12 OCPs	ASE + Na_2SO_4 Ethyl acetate:c-hexane [1:1]	GPC SX-3 c-Hexane:ethyl acetate [1:1] SPE silica n-Hexane	GC-ECD CPSil-2 50Acetonitrile	n.s.	n.s.	[84,86][a]
		Microwaves + Na_2SO_4 Ethyl acetate:c-hexane [1:1]	GPC SX-3 c-Hexane:ethyl acetate [1:1]				
Oysters	15 herbicides included 7 TRZs	ASE Acetonitrile	SPE Florisil Acetone SPE Oasis HLB Acetonitrile: Methanol [1:1]	LC-MS Zorbax SB-C18 5 μm gradient Acetonitrile:water MS Electrospray mode	n.s.	n.s.	[85]
Bovine milk	5 OCPs	MSPD Si-C18 Acetonitrile Acetonitrile/water (MSPD washing)	SPEs Si-C18(MSPD)/Florisil Acetonitrile SPE Florisil Petroleum ether:ethyl ether [94:6] Petroleum ether:ethyl ether [85:15] (Florisil–2 separated fractions)	GC-ECD DB-608 30Acetonitrile	76–98%	5 ng/g	[89]
Bovine milk	5 OPPs	MSPD Si-C18 Acetonitrile (extraction) Acetonitrile/water (washing)	SPEs C18 (MSPD) Florisil Acetonitrile	GC-FPD DB-1 30Acetonitrile	75–104%	10 ng/g	[89]
Crab	24 OCPs	Solid:liquid extraction Acetonitrile/Centrifugation	SPEs Si-C18/Na_2SO_4/Florisil 3% Toluene–Petroleum Ether (discard) then 2% Diethyl Ether–Petroleum Ether	GC-ECD DB-5 30Acetonitrile	48–102%	n.s.	[90]
Bovine milk		MSPD Si-C18 Acetonitrile	SPE Si C18 Acetonitrile SPE Florisil Petroleum ether:diethyl ether [98:2]		42–94%		
Shellfish	14 OCPs	MSPD Si-C18 Acetonitrile: Methanol [90:10]	SPEs SI-C18(MSPD)/Florisil Acetonitrile:methanol [9:1]	GC-ECD DB-5 25 m	66–84%	n.s.	[91]
Fish (muscle tissue)	9 OCPs	MSPD Si-C18 Acetonitrile	SPEs SI-C18(MSPD)/Florisil Acetonitrile	GC-ECD DB-5 25Acetonitrile	82–97%	n.s.	[92]
Bovine fat	9 OCPs	MSPD Si-C18 Acetonitrile	SPEs SI-C18(MSPD)/Florisil Acetonitrile	GC-ECD DB-5 25 m	71–110%		[93]
Fish (muscle tissue)	24 OCPs	Solid:liquid extraction n-Hexane:acetone [2:5] (Sonication)	Freezing −24 °C/filtration SPE Florisil *vs.* silica Acetone:n-hexane [1:9]	GC–MS DB-5ms 30 m	78–115%	0.5–2.5 ng/g	[94][a]
Composite diet samples	15 OCPs 9 OPPs 2 TRZs 2 PYRs	Soxhlet n-Hexane:acetone [1:1]	Liquid partitioning over diatomaceous earth Acetonitrile:n-hexane SPE alumina DCM:n-hexane [7:3]	GC–MS HP-5ms 30 m	65–135%	0.2–5.3 pg/g	[96]
Bovine milk	24 OPPs	Liquid:liquid extraction Acetonitrile:ethanol [5:1]	SPE ChemElut Light petroleum:acetonitrile:ethanol [100:25:5]	GC-FPD SPB-608 15 m	72–109% but Omethoate & Dimethoate 0%	3–66 ng/g	[97]
Fish (muscle & liver)	16 OCPs	Solid:liquid extraction n-Hexane (refluxing extraction)	HPLC Silica n-Hexane (mobile phase) Ethyl acetate (modifier)	GC–MS^2 DB-5ms 30 m	75–112%	0.3–4.5 ng/g	[99]
Bovine muscle	1 PYR Cypermethrin	Solid:liquid extraction n-Hexane + Na_2SO_4	Liquid:liquid partitioning (n-Hexane:acetonitrile) SPE Silica	GC-ECD DB-5 15 m	89–114%	50 ng/g	[102,103]

Table 3 (*Continued*)

	Pesticides	Extraction procedure	Clean-up procedure	Separation technique	Recovery rate	LOQ	Ref.
Bovine liver & kidney					71–100%	50 ng/g	
Bovine fat					83–103%	50 ng/g	

Abbreviations: APCI, atmospheric pressure chemical ionisation; ASE, accelerated solvent extraction; BU, benzoylurea; CB, carbamates; DCM, dichloromethane; DAD, diode array detector; ECD, electron-capture detector; ELCD, electrolytic conductivity detector; FPD, flame photometric detector; GC, gas chromatography; GPC, gel permeation chromatography; GNPC, graphitized non-porous carbon; HLB, hydrophilic–lipophilic balance (cartridge); HPLC, high performance liquid chromatography; ITMS, ion-trap mass spectrometry; LC, liquid chromatography; MS, mass spectrometry; MSPD, matrix solid-phase dispersion; MTBE, methyl *t*-butyl ether; NPD, nitrogen–phosphorus detector; n.s., not specified; OCP, organochlorine pesticide; OPP, organophosphorus pesticide; PYR, pyrethroid; SFE, supercritical fluid extraction; SPE, solid-phase extraction (cartridge); TRZ, triazine.

[a] Comparison of extraction procedures and/or purification steps (SPE cartridges) or detection techniques.

Recently, accelerated solvent extraction (ASE) using a pressurised liquid extraction system (Dionex®), was applied to organochlorine pesticide extraction from animal internal organs, muscles, adipose tissue, eggs and milk [37,39,54,83] as well as from fish liver and flesh [13,84] and shellfish [85]. The main advantages of ASE are low solvent consumption and the short time needed for performing the extraction step, but the purchase cost of the equipment is higher than that of the standard Soxhlet method [13]. A comparison of the ASE and Soxhlet extraction techniques showed that the efficacy of ASE was slightly higher compared to Soxhlet [37]. In the same study, both these methods were also compared to an extraction procedure using a Polytron® extractor apparatus (Kinematica, Luzern, CH) for mixing, dispersing, and homogenising ground meats in an extraction solvent system. ASE and Polytron® extraction gave higher recovery rates for several studied pesticides with the advantages of shorter extraction times and lower consumption of solvents compared to Soxhlet extraction. However, a few pesticides (7 out of 46 analysed) were poorly recovered (<70%) using ASE with ethyl acetate. On the other hand, Saito et al. [39] obtained quite consistent results with both ASE and conventional solvent extraction of organochlorine pollutants from swine internal and adipose tissues.

Microwave-assisted extraction (MAE) has also been tested for both OCP and OPP extractions from fatty fish tissue [84,86]. Application of microwave energy as a heat source causes selective heating of the matrix over the extractant. The highly localised temperature and pressure cause selective migration of target compounds from the material to the surrounding area more rapidly and with a similar recovery rate compared with conventional extraction [87]. MAE has been assayed and compared with the accelerated solvent extraction (ASE) method. The two analytical procedures gave similar results in terms of recovery [84].

Matrix solid-phase dispersion (MSPD) is a sample disruption/extraction method that combines the use of mechanical forces generated from the grinding of a sample matrix with irregular shaped particles (silica- or polymer-based solid supports) with the lipid solubilising capacity of a support-bound polymer (octadecylsilyl or others) to produce a sample/column material from which dispersed sample matrix components such as pesticide residues can be selectively isolated [88]. MSPD combines sample homogenisation, disruption, extraction, fractionation, and clean-up within a single process [19]. This methodology has been applied to the extraction of both OCP and OPP residues from whole milk [60,89,90], shellfish [91], fish [92], and beef fat [93]. The procedure is simple, rapid, and requires only small sample sizes and solvent volumes [91–93]. A similar technique using graphitized non-porous carbon (GNPC) material was proposed for extracting OCPs from fat; ENVI-carb® GNPC, which combines adsorption and anion-exchange retention mechanisms, has a strong affinity towards planar molecules and was used to remove pigments and sterols commonly found in foods and natural products [47,53]. Unfortunately, GNPC does not eliminate all kinds of co-extracted matrix compounds, whereas some pesticides such as thiabendazole and carbamates are strongly retained [78].

5. Clean-up methods

Matrix constituents can be co-extracted and later co-eluted with analysed components and can consequently interfere with analyte identification and quantification. Moreover, co-extracted compounds, especially lipids, tend to adsorb in GC systems such as injection port and column, resulting in poor chromatographic performance [94]. A thorough clean-up minimises such matrix issues, improves sensitivity, permits more consistent and repeatable results, and extends the capillary column lifetime [1,14]. Several approaches have been attempted to eliminate co-extracted interference from extracts, including freezing centrifugation, liquid–liquid partitioning, gel permeation chromatography (GPC), solid-phase extraction (SPE) and solid-phase micro-extraction (SPME).

Most of the published analytical methods involve the use of anhydrous sodium sulphate at one or more of the steps in order to remove water traces from the extraction solvent system, mostly during clean-up steps such as solvent partitioning or purification columns (as one of the layers of the column). However, some authors [1,3,8,11–13,15,26,27,29,31,37,41,43,54,71] utilised sodium sulphate from the grinding step during the extraction procedure to further disintegrate the sample. Sodium sulphate was also used at the early partitioning step of the analytical procedure for pesticide extraction from milk [10,73].

The significant differences in melting points have been used to separate lipids and pesticides after solvent extraction from meat, fish, and milk [28,33,35,55,94,95]. Lipids were precipitated in frozen form at very low temperature while pesticides remained dissolved in cold organic solvents. After eliminating the frozen lipids, most of the remaining interference was removed by a convenient clean-up procedure such as SPE [94].

Solvent partitioning has also been used, generally prior to additional clean-up procedures such as SPE, to eliminate co-extracted compounds from pesticide extracts. For instance, pyrethroid residues have been extracted from meat and milk using either iso-octane or n-hexane and the extract was then partitioned with acetonitrile [28,33,35,55,94,95]; Pagliuca et al. [25] extracted OPP residues from milk with an acetone–acetonitrile (1:4, v/v) system and then partitioned the extract with dichloromethane; Argauer et al. [15] analysed OCP residues in meat using the same procedure. Nonetheless, the partitioning phases often need subsequent clean-up through SPE [15,25,29,35]. Hamscher and his research group [44–46] thus extracted different classes of pesticides from eggs using acetonitrile and defatted the acetonitrile extract with an n-hexane liquid–liquid washing prior to SPE clean-up. However, solvent partitioning can lead to the loss of some analytes and thus leads to lower recovery rates, especially in multiresidue analysis.

Gel permeation chromatography (GPC) is considered a good technique for the separation of low molecular mass compounds (up to 400 μm) such as pesticides from high molecular mass compounds such as lipids (600–1500 μm) [1,14]. GPC has therefore been widely used for cleaning up extracts from foods of animal origin with a high fat content (Table 3) [1,6,11,13,14,31,36,37,39–41,43,84,86]. However, co-extracted compounds, including remaining trace amounts of lipids, can reach the GPC eluate and then interfere with the subsequent analysis. Complex samples such as fish, meat, and other fatty matrix extracts often require a two-step clean-up combining gel permeation chromatography and adsorption chromatography in series [1,14].

Solid-phase extraction (SPE) has also been used as a purification step to remove interfering compounds co-extracted with all classes of pesticides from a large selection of foods of animal origin. Adsorption chromatography applied to this field has mostly used adsorbents such as silica, C18-bonded silica, Florisil®, or alumina, but the use of aminopropyl-bonded silica and graphitized non-porous carbon (GNPC) packing materials has also been reported (Table 3). Doong and Lee [80] compared ready-to-use cartridges filled with three different adsorbents for cleaning up OCP extracts from shellfish. In the analytical conditions, only twelve OCPs out of the fourteen spiked were detected when a C18 cartridge was used. For alumina and Florisil, all of the fourteen OCPs were detected, with Florisil giving better results in term of recovery, repeatability, and efficiency in eliminating matrix components. Hong et al. [94] also compared Florisil and C18-bonded SPE adsorbents: for both SPE cartridges, several OCPs exhibited poor elution efficiency with hexane as the elution solvent; increasing the polarity of the solvent system using acetone overcame the poor elution of these compounds. However, Florisil was shown to be more efficient than C18 in removing fatty acids which interfered with the GC analysis of OCPs. Schenck et al. [90] also reported that slightly increasing the polarity of the elution solvent helped to improve to some extent the efficiency of SPE in terms of OCP recovery. Some authors used SPE directly after the extraction step, while others used it as an additional purification step after a GPC clean-up or liquid–liquid partition. Moreover, two or three columns of different adsorbents have also been used in series to achieve maximal analyte recovery with minimal matrix interference. The need for further clean-up following GPC could be overcome by the use of gas chromatography (GC) coupled with tandem mass spectrometry (MS/MS). However, up to three SPE columns, involving three different adsorbents (Extrelut NT3, C18-bonded silica, Florisil), have been used for cleaning up extracts even when GC–MS was the analytical method employed [3,12,51,96].

After MSPD extraction which also used adsorption properties, pesticide (especially OCP) residue extracts were mostly subject to additional clean-up through a single SPE (Florisil or neutral alumina) column for eliminating most of the residual co-extracted interference [60,91–93]. The C18 and Florisil combination columns allowed clean extracts to be obtained from milk, especially using acetonitrile as the elution solvent. Elution with either hexane or ethyl acetate, in the same analytical conditions, produced larger quantities of co-extracted fat [92,93]. A solid-matrix dispersion technique, using solid-matrix diatomaceous material (Chem Elut®) as the adsorbent, has also been developed for the analysis of OCPs, OPPs, and PYRs in milk [56,97,98]. The pesticide residues are then extracted by means of light petroleum saturated with acetonitrile and ethanol. This procedure minimises the formation of stable emulsions, a major drawback of the solvent extraction step [98]. A second clean-up step by adsorption (Florisil) or gel permeation chromatography (Envirosep-ABC) was added for OCP and PYR analyses by GC-ECD [30,56].

Several of the previously described techniques such as liquid–liquid partitioning, SPE and GPC, have been included in the European EN 1528 standard method for cleaning up OCP and OPP extracts from fatty foods of animal origin (milk and milk products, meat and meat products, fish and seafood, eggs) [74].

6. Separation and determination

At the present time, gas chromatography (GC) is the most widespread method for the separation and determination of most pesticides. However, liquid chromatography (LC) is also used for measuring levels of some pesticide residues such as carbamates and triazines, in foods of animal origin.

6.1. Gas chromatography

For GC separation, a great variety of stationary phases have been used in capillary columns of various geometries (Table 3). Usually each research group has only used one column for the same class of pesticides. However, some authors have reported the use of two columns with different polarities and different geometric parameters under the same or different chromatographic conditions [8,14,43,56,80,84,86,90,97]. Often the first column was used for analytical purposes, and the second column was used to confirm the peak identification [56,80]. Different columns have also been used when mass spectrometry (MS) detectors were compared to classical detectors for analysing residues from the same class of pesticides, even more so when compounds from different pesticide classes were analysed in one run in GC–MS [10,12,33,43,50,53].

GC is combined with different kinds of detection methods, mainly depending on the class of pesticides to be quantified. Electron capture detection (ECD) has usually been employed for OCP and PYR analyses. The quantification limits obtained by coupling GC with ECD have been reported to be mostly around 0.1–20 ng/g, depending on various parameters (Table 3). It has been reported that the use of ECD usually requires particular attention to be paid to the extract clean-up process [3]. Electrolytic conductivity detection (ELCD) after GC separation has also been proposed for the detection of several pesticide residues including OCP, pyrethroids, triazines, and a carbamate, from whole milk samples [10]. Limits of quantification ranged from 0.6 to 58.6 ng/g depending on the pesticide (Table 3). Both flame photometric detection (FPD) with phosphorus filter and nitrogen–phosphorus detection (NPD) have been used for OPP detection. Quantification limits have been reported as low as 2–66 ng/g (Table 3).

Besides these conventional element-specific detection methods, GC hyphenation with mass spectrometric detectors (MSDs) including single quadrupole, ion trap, and triple quadrupole mass spectrometers, has been adapted to the analysis of pesticide residues in foods of animal origin. At first, single quadrupole MSDs running in electron impact (EI) ionisation mode were used to confirm peak identification of pesticides previously determined and quantified by GC coupled with a specific detector [10,12,43,50,53]. However, GC equipped with a single quadrupole MSD has also been used for pesticide residue quantification [3,11,94,96]. Ion-trap mass spectrometry (IT-MS) in EI mode was also employed for determination and quantification of pesticides in foods of animal origin such as OCPs and PYRs in ground beef meat, as well as pesticide multiresidues in meat-based baby-food [15,33,78]. Pesticide residue detection has also been achieved by GC–MS in negative chemical ionisation (NCI) mode with methane as the reagent gas [39].

In GC coupled with mass spectrometry (MS), pesticides were identified by retention time and specific ions determined in selected ion monitoring (SIM) mode using both target and qualifier ions. The detection of one to six qualifier ions was required for

identification depending on various parameters including authors' choice, matrix, and pesticide. Although GC–MS in SIM mode provided adequate quantification at low levels as required for monitoring purposes, confidence in confirmation of identity is reduced if the selected ions are affected by matrix interference (see Section 7.2). Alternatively, MS/MS, by decreasing such matrix influence, may achieve a higher selectivity level and lower detection limit [18,36]. GC–MS/MS has been used for analysing pesticide residues on fatty food with either triple quadrupole [36–38] or ion trap mass spectrometers [54,99]. Both the multiple reaction monitoring (MRM) [36] and the selected reaction monitoring (SRM) [37] acquisition methods have been applied to the GC–MS/MS analysis of pesticide residues in foods of animal origin. The use of MS/MS may overcome the problems arising from chromatographic interference that occurred with GC-ECD [100] and consequently provide better sensitivity for the determination of OCPs [36,99]. Limits of quantification are shown in Table 3.

Using mass spectrometry has the obvious advantage compared to conventional element-specific detectors of being able to determine together pesticides from different classes and to identify their metabolites and degradation products in the same acquisition run [67]. Multiresidue methods including GC–MS have been developed for the routine analysis of pesticides from different classes, including OCPs, OPPs, PYRs, TRZs, and their degradation products, in composite foods and meat-based baby food [78,96].

6.2. Liquid chromatography

Liquid chromatography (LC) has been used for the analysis of polar and/or non-volatile and/or thermally labile pesticides for which GC conditions were not suitable, mainly carbamates and triazines, and their metabolites and degradation products.

Various stationary phases have been tested for the separation of pesticides [67], but generally reversed phase on octadecyl(C18)-bonded silica columns was preferred [1,7,10,18,42,44–46,65,72,85]. However, octyl(C8)-bonded columns have also been employed for separating carbamates extracted from beef and poultry meats [40,41].

LC has also been combined with conventional detectors such as fluorescence or UV detectors for identifying and quantifying pesticide residues. A derivatisation procedure using *o*-phthalaldehyde (OPA) has been introduced for the fluorescence detection of carbamates [101] which allowed low detection limits to be reached (Table 3) [10,40–42]. Most triazines exhibit absorption maxima in aqueous or methanolic solutions around 220–225 and/or 255 nm [67]. Liquid chromatography coupled with diode-array detector (LC-DAD) was thus used for analysing triazine contamination of rabbit meat and fat, and cow milk [7,72]. Moreover, propoxur and pirimicarb, two carbamate pesticides extracted from egg and liver respectively, have been successfully quantified at ppb level using a DAD after LC separation on an RP-18 column [1,44]. LC-DAD was also used for pesticide multiresidue determination in animal tissue [1].

Recently, LC has been coupled with different kinds of MS detectors, including single quadrupole, ion trap, tandem-MS, and time-of-flight-MS (TOF-MS) in order to determine pesticide residue levels and/or to elucidate their structures in aqueous and solid environmental samples as well as in foods of vegetable origin and baby food [16,18,20–22,102]. The enhanced selectivity afforded by MS/MS detection was able to discriminate between target pesticides that were marginally separated by LC [19]. A quite number of pesticides can be analysed by both GC–MS and LC–MS techniques. However, although a method based on LC–MS was considered to cover a wider scope than one based on GC–MS [76], some pesticide compounds are difficult to analyse by LC–MS. Sensitivity remains the point of concern with both techniques. However, electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) have improved the feasibility of the identification of pesticides of different chemical structures in food and vegetables, at concentrations comparable to those obtained by GC–MS [19]. Indeed, only two publications have dealt with pesticide residues in foods of animal origin using LC–MS [76,85]. Buisson et al. [85] used LC-MSD in electrospray mode for studying the contamination of oysters harvested from two estuary sites. The method was tested for fifteen herbicides and herbicide metabolites, including seven triazines; six pesticide residues were detected in seawater, but no pesticides were observed in oyster flesh. Unfortunately, the paper gave no information on the method's performance. LC–MS/MS was also used to detect 136 pesticide residues in spiked minced pork/beef meat, raw milk, and whole egg after a single extraction step [76]. However, this method was strictly validated only for honey and horse compound feed. Recently, mass spectrometry using time-of-flight (TOF) as the mass analyser was coupled with liquid chromatography (LC) for screening pesticide residues in honey and plant extracts [84,103,104]. With respect to comprehensive screening for residues, full scan techniques such as TOF-MS may be more appropriate than those using targeted acquisition such as MS/MS detection [76]. This high-resolution MS technology has also been described as an excellent tool for the identification and structural elucidation of metabolites and transformation products of pesticides in foodstuffs [103,104]. However, the narrow dynamic range of these instruments limits accurate quantification and restricts TOF-MS to identification and confirmatory analysis.

7. Recovery and matrix effects

7.1. Recovery

In an analytical method, various extraction and clean-up steps are mixed and matched to achieve maximal analyte recovery with minimal matrix interference at the final measurement step [10]. Recovery has been defined by the IUPAC as "the proportion of the amount of analyte present in or added to the analytical portion of the test material which is extracted and presented for measurement" (IUPAC 1996 Symposium on harmonisation of quality assurance systems for analytical laboratories, Orlando, 4–5 September 1996). In the pesticide analysis field, recovery rates in the range of 70–120% are considered to be acceptable and can be extended to routine analysis, as recommended by the Codex Alimentarius guidelines (Codex, updated at the 26th session of the Codex Alimentarius Commission, 30 June 2003) [35] as well as by the EU Commission guidelines [77].

Recovery studies are commonly performed on blank food samples spiked with known levels of studied pesticides. Unfortunately, it is not always possible to obtain totally residue-free matrices *e.g.* case of Mediterranean tuna [50]. Certified reference material can also be used if available [36]. Generally, there are six or more replicates for each sample at different fortification levels, sometimes in relation with either quantification limits or maximum residue limits (MRLs) [3,10,12,27,35,46].

Methods developed for the determination of pesticides from the same class mostly reach the guideline rates of recovery for all the studied pesticides. However, in multi-residue methods it is very difficult to obtain satisfactory results for all of the analysed compounds because of the diverse physical and chemical properties of the different classes of agricultural chemicals [10,73]. Then, recommended recovery rates are not always reached for all the tested pesticides (Table 3). For instance, triazines and anilines are not usually recovered from fatty food samples by the same methods as OCPs and OPPs due to the difficulty in resolving the chemically diverse range of pesticides from dietary fat [96]. Similarly, in a

multi-residue study on 117 pesticides from various classes, Hopper [82] reported recovery rates of 82 pesticides ranging from 73 to 115% while 4 compounds were recovered poorly and 31 were not recovered at all through the clean-up procedure. In a method proposed for the analysis of 24 typical OPPs in whole milk, recovery rates of 22 residues ranged from 72 to 109% but two of them, omethoate and dimethoate, were not recovered. The authors [97] claimed that the analytical conditions did not favour the elution of these polar compounds from the SPE used in this study.

Recovery rates vary depending on various parameters such as the matrix, the sample processing procedure, analyte properties, and analyte concentration. Poor recovery rates are presumably connected to different causes such as losses during the extraction, partitioning, and evaporation steps, analyte adsorption on clean-up material, and degradation into GC.

Residue amounts have been reported to influence recovery values [38]. Indeed, Argauer et al. [15] observed different recovery rates at different spiked levels. However in most cases, no apparent differences in recovery rates between the spiking levels studied have been reported [3,10,56,60]. Garrido-Frenich et al. [38] assessed the recovery factor with the analyte concentration in meat samples for the determination of OCPs and OPPs by GC–MS/MS. The obtained values indicated that the recovery factor did not depend on the analyte concentration, except when close to the quantification limit.

The extraction step has also been described as a critical point. For instance, Argauer et al. [33] reported low recovery rates for four PYRs (tefluthrin, bifenthrin, deltamethrin, and permethrin) out of the 10 included in their study, probably because of the solvent partitioning step. The non-polar/polar extraction solvent ratio has been claimed to have a strong influence on the recovery in the case of PYRs [15]. Similarly, Przybylski and Segard [78] noted that the acetonitrile/hexane ratio during the washing step was a crucial point. Severe losses of apolar residues can occur when hexane volume is increased above a critical value. Likewise, Park et al. [28] obtained good recovery rates for 19 pesticides but poor ones for three DDT metabolites, probably related to their persistence in the n-hexane layer during solvent partitioning.

Losses during the evaporation steps as well as adsorption of pesticides on clean-up or analytical columns [78] may also be responsible for poor recovery rates. For instance, the highly volatile HCB [3] and the low-boiling dichlorvos [43,96] can be lost during evaporation of extraction solvents, inducing low recovery rates and high coefficients of variation. Acidic treatment can cause low recovery of certain pesticides such as HEPO, an epoxide compound which can be converted into the corresponding diol or sulphate, which is much more hydrophilic and is then not extracted by organic solvents [53].

Losses can also occur during the chromatographic run [78]. For instance, Rosenbaum et al. [96] reported degradation of DDT into DDD in the GC inlet, leading to apparently high DDD recovery rates. In multi-residue methods using gas chromatography coupled with ion-trap mass spectrometry (GC–ITMS), the intrinsic low mass spectrometry response can also be a source of poor recovery rates for some compounds [78].

7.2. Matrix chromatographic interference

The presence of co-extractives has a major influence on accuracy as well as on the sensitivity of analytical methods. Matrix effects can lead to abnormally poor or high recovery rates and high limits of detection/quantification. Food matrix and fat levels have been related to poor recovery rates of certain residues in some cases [15,78]. For instance, it was reported that the lipid content of ground beef meat samples lowered the PYR recovery rates [33].

In GC, such problems caused by matrix components may occur both at the injector/column and the detector sites [18]. Using detection methods such as ECD, FPD, and NPD, co-elution of matrix components with pesticide residues may result in different problems such as masking of analyte peak (false negative result), identifying impurities as analytes (false positive result), increasing the detector signal (overestimated result), and quenching of the detector signal (underestimated result) [17,18]. Mass spectrometry (MS) has overcome some problems due to higher specificity based on the analyte's molecular structure, but some drawbacks still remain, *e.g.* matrix components can induce the diminution of the signal corresponding to an analyte by decreasing the ionisation potential [78]. MS/MS offers additional specificity with secondary fragmentations, and thus may circumvent co-elution problems. Increasing the separation power of the chromatographic system may be an alternative solution. Using two columns with stationary phases of different polarities was reported to reduce the risk of obtaining false positive data due to co-elution [43,84,86,90]. Recently, a comprehensive two-dimensional technique (GC × GC) coupled with time-of-flight (TOF) mass spectrometry was claimed to make an important contribution to the residue analysis field [105]. This new methodology has not yet been applied to the analysis of pesticide residues in foods of animal origin, although Hajšlovšá and Zrostlíková [17] have argued that GC × GC/TOF-MS will undoubtedly become a technique of choice in the future whenever unbiased determination of multiple trace analytes in very complex matrices is needed.

However, a thorough clean-up procedure may help in substantially reducing matrix interference since recovery values obtained have demonstrated the absence of a significant matrix effect, even from high-fat foods [80,99]. For instance, Argauer et al. [15] reduced co-extractives by using acetonitrile pre-saturated with iso-octane in their extraction solvent system. Another study showed no difference in recovery rates while analysing OCP and OPP residues in composite food samples with low, medium, and high fat contents [35]. Studying the influence of the matrix's nature, Garrido-Frenich et al. [37] also obtained similar recovery values in chicken, pork, and lamb meats for 46 pesticides from different classes (each at two different fortification levels).

7.3. Matrix enhancement effects

Besides detection difficulties due to co-extractives, the matrix components can also block active sites in the GC inlet and column, thus protecting the pesticide compounds from adsorption and/or decomposition. As a consequence, transfer of those analytes in the matrix-containing solution from injector/column to detector is more complete than when analyte standards are dissolved in matrix-free solvent. This occurrence, called matrix-induced response enhancement, results in recovery rates in excess of 100% for residue-free extracts spiked with pesticides when standards prepared in residue-free solvents are used for calibration [106]. This phenomenon can also occur in the ion volume when using MSD [78]. Different recovery values were obtained both for polar and thermally labile pesticides when comparing pesticides in composite food matrices *vs.* pesticides in solvent [96]. Similar results were observed for several residues from all pesticide classes when recovery rates of pesticides extracted from meat-based baby food were compared to values obtained for the same pesticides in solvent [78].

Matrix effects in pesticide multiresidue analysis by LC–MS in fruits and vegetables were recently reviewed [107]. The co-extraction of matrix constituents may reduce the lifetime of the LC column and affect the ionisation process in LC–MS analysis, causing the suppression or enhancement of the analyte response [76]. The ion suppression/enhancement effect can drastically affect both identification and quantification of pesticide residues since

the response of the analytes in pure standard solution or in a real sample extract differs significantly as a consequence of the absence/presence of matrix components during analyte ionisation [17,19,20]. This effect has also been observed when analysing foods of animal origin. Indeed, Mol et al. [76] observed extensive matrix effects which decreased for the matrixes in the order meat > milk > egg.

Matrix enhancement prevention techniques are well documented in the analysis of pesticides in fruits and vegetables, but less so in the analysis of residues in fatty foods of animal origin. Nevertheless, most of the proposed solutions can probably be adapted to pesticide analysis in meat products. Two alternative approaches can be considered to avoid overestimation due to matrix-induced response enhancement: (1) elimination of causes, and/or (2) effective compensation [17].

7.3.1. Elimination of causes

In practice, obtaining totally deactivated GC inlets and/or columns is unrealistic. Additionally, the wide range of physicochemical properties of pesticides complicates the efficient removal of co-extracted matrix components from crude extracts. Schenck and Lehotay [102] observed that even the use of three SPE cartridges did not eliminate the matrix enhancement effect from fruit and vegetable extracts for the most affected pesticides. Obtaining matrix-free extracts from fatty foods of animal origin therefore appears unlikely. Nevertheless, efficient clean-up procedures may substantially reduce matrix enhancement effects and contribute to the overall performance of a given analytical method. On the other hand, as most of the problems are encountered in the hot vaporising injectors, significant elimination of analyte adsorption can be partially achieved using an on-column injector [106], programmed temperature vaporisation (PTV) injector [78], pulsed splitless injector [105], and direct sample introduction (DSI) [108]. Bennett et al. [10] adopted an on-column injector in track-oven mode, but without any mention of the matrix effect. Some recovery rates were over 120% for several pesticide residues in this study. A few other authors [14,15,37,38] used split/splitless programmed-temperature injectors with good recovery rates, but without commenting on any relationship between injection mode and matrix enhancement effects. Finally, Rosenbaum et al. [96] investigated the use of a temperature-programmable pre-separation column in the GC inlet as a final step in the separation of pesticides from dietary fats. These authors reported the ability of such a temperature-programmable pre-column separator to separate most of the co-extracted fats from pesticide analytes.

Improving sample preparation and optimising chromatographic conditions can help to reduce matrix effects in LC methods. Modification of the mobile phase composition is another alternative. Thus, Mol et al. [76] reduced matrix effects using acetonitrile as an extraction solvent instead of methanol or acetone, for all the tested animal matrixes. Bogialli et al. [57] increased ion signal intensities for some poorly recovered carbamates from milk extracts by decreasing the strength of the LC mobile phase.

7.3.2. Effective compensation

Calibration with matrix-matched standards and/or addition of analyte protectants to sample extracts have been proposed for masking active sites and limiting matrix effects [77]. Matrix-matched calibration standards are prepared in a solution that corresponds as closely as possible to the composition of the sample solution. Such matrix calibration standards are prepared by the addition of pure pesticides to blank extracts, i.e. ones that have undergone the clean-up procedure, as varied as a medium fat composite food [96], meat-based baby food [78], and chicken, pork and lamb meats [37,38]. Several drawbacks associated with matrix-match calibration have been reported. These included (a) problems obtaining totally residue-free matrices for some food products [50], (b) difficulties getting a blank matrix for every commodity or alternatively a representative blank extract for a defined range of food-stuffs [19], (c) gradual accumulation of non-volatile matrix components in the GC system, thus reducing column lifetime and ruggedness of the method [109], (d) additional workload, thus increasing the time and cost of analyses [17]. The calibration approaches, calibration using external matrix-matched standards and calibration using internal standards, can also compensate for the matrix effect in pesticide residue analysis by LC–MS [17,19]. This approach respects the previously discussed limitation of obtaining totally residue-free matrices for every sample type. Another approach in GC is the use of analyte protectants. Using compound additives to minimise the matrix-induced response enhancement was first attempted in the early 1990s by Erney and Poole [110]. Later, various additives were evaluated as analyte protectants for overcoming the matrix effect in pesticide analysis in fruits and vegetables [108,109,111]. To our knowledge, the use of analyte protectants has not been mentioned in the published scientific literature on pesticide analysis in animal tissue. However, the use of analyte protectants is officially considered as a practical alternative approach to minimising matrix effects in GC analyses [77]. Another option, reported as the most effective way to negate matrix enhancement or suppression effects, is calibrations using isotope-labelled analytes as internal standards [77]. However, some restrictions should be pointed out, (1) availability of such compounds, (2) cost, (3) not well suited to multi-residue methods [19].

8. Conclusion

The determination of pesticide residues in the environment and in foods is necessary for ensuring that human exposure to contaminants, especially by dietary intake, does not exceed acceptable levels for health. Consequently, robust analytical methods have to be validated for carrying out both research and monitoring programmes, and thus for defining limitations and supporting enforcement of regulations. In this field, reproducible analytical methods are required to allow the effective separation, selective identification, and accurate quantification of pesticide analytes at low levels in food-stuffs including foods of animal origin.

One analytical challenge in the food safety is to present reliable results with respect to official guidelines, as fast as possible without impairing method properties such as recovery, accuracy, sensitivity, selectivity, and specificity. Classical extraction procedures including homogenisation of food matrices, solvent extraction of analyte compounds, liquid–liquid partitioning, and clean-up steps are tedious and time-consuming, require a lot of handling, and use large volumes of solvents. These conventional methods are still widely used because they have been proved to be reliable. However, advances in sample preparation aim to minimise laboratory solvent use and hazardous waste production, save employee labour and time, and reduce the cost per sample, while improving the efficiency of the analyte isolation. Newer developments in extraction and clean-up steps involve alternative techniques such as accelerated solvent extraction (ASE), solid-phase extraction (SPE), and matrix solid-phase dispersion (MSPD). As described in this review, a few drawbacks mean that additional improvements are required in order to adapt these methods for use with the various food matrices of animal origin. However, these techniques appear promising.

In the 1990s, mass spectrometry (MS) was mostly used to confirm identification of analytes after quantification by means of specific detection methods such as ECD, FPD, and NDP. During the last decade, MS has tended to be used largely for direct identification and quantification of the pesticide compounds in the

extracts. However single quadrupole MS is restricted to screening purposes since these instruments do not meet the more recent criteria set by the EU, especially those regarding the requested number of identification points. In the 2000s, tandem-MS was also used for identification and quantification of pesticide residues in food. The enhanced selectivity afforded by tandem-MS detection may also contribute to the simplification of the extraction procedure, if attention is paid to ion suppression phenomena. At this point, the use of triple quadrupole and ion trap analysers has been widely reported in the scientific literature in this field of research. Recently, LC–TOF-MS was used for screening pesticide residues, confirming the identification of contaminant compounds, and elucidating the structure of their metabolites in honey and plant extracts. In the near future, therefore, it would be interesting to assay this high-resolution mass spectrometry detection technique for research purposes on pesticide residues and their possible metabolites in foods of animal origin. Indeed, in the food safety research field, it could be valuable for identifying pesticide degradation products neo-formed during various food processes.

On the other hand, two-dimensional gas chromatography (GC × GC) coupled with TOF-MS has been adapted to the determination of multiple pesticide residues in fruit [105]. In the same way, future developments could explore the adaptation of this technique to meat, fish, milk and related matrices. This technique appears effective at eliminating the matrix effect on the detector site.

However, the study of new and effective analyte protectants for GC analysis would be an appreciable improvement in this topic. In the meantime, the use of matrix-matched standards and standard addition calibration is a good solution for reducing the matrix enhancement effect.

Finally, the role of LC–MS in analysing pesticide residues, their metabolites and degradation products in fruits and vegetables has recently been expanding. No doubt, this will soon have repercussions on the analysis of foods of animal origin.

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