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PESTICIDE CHEMISTRY AND RISK ASSESSMENT

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1.1 INTRODUCTION

> *And he gave it for his opinion that whoever could make two ears of corn or two blades of grass to grow upon a spot of ground where only one grew before, would deserve better of mankind, and do more essential service to his country, than the whole race of politicians put together.*
>
> Jonathan Swift, 1667–1745

Plant protection, worldwide, has a very important role in the food production. One of the most important ways of protecting plants and plant products against harmful organisms, including weeds, and of improving agricultural production is the use of plant protection products (pesticides). Pesticides have brought to the world the most abundant, safe, and cheap food in its history. Pesticides, like pharmaceuticals, are the most thoroughly tested chemicals in the world, and only those that pass strict government testing are authorized for use. Active substances (pesticides) should only be included in plant protection products where it has been demonstrated that they present a clear benefit for plant production and they are not expected to have any harmful effect on human or animal health or any unacceptable effects on the environment, especially if placed on the market without having been officially tested and authorized or if incorrectly used.

Human exposure to pesticides and their metabolites through the food chain could be direct, through the consumption of treated foods, or indirect, through the transfer of residues into products of animal origin from treated feed items. Regulatory agencies, internationally, have provided pesticide regulations increasingly stringent in terms of establishment of the maximum residue limits (MRLs) for pesticides in food of plant and animal origin. Monitoring studies are organized annually by national authorities to enforce compliance with MRLs and to ensure food safety for consumers.

The unlimited number of pesticides and their metabolites, in conjunction with their low concentration levels in various food commodities and environmental matrices, makes the analysis of pesticide residues one of the most challenging and complex areas of analytical chemistry. Pesticide residue methods have been developed worldwide using hyphenated confirmatory techniques, such as gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS) for the determination of trace concentration levels.

Mass spectrometry (MS) platforms are widely applied in pesticide residues for (i) the determination of pesticide residues and their metabolites in food to ensure safety of the food supply, (ii) the investigation of the contamination of water resources from pesticides and their relevant metabolites, and (iii) the structure elucidation of *unknown* metabolites or degradation/transformation products (TPs) that sometimes can be more toxic than the parent pesticides.

This chapter provides information regarding the chemistry and toxicity of pesticides, their metabolites, and TPs. Risk assessment topics are discussed. Definitions and explanations in various topics of pesticides are also included.

Mass Spectrometry for the Analysis of Pesticide Residues and Their Metabolites, First Edition. Edited by Despina Tsipi, Helen Botitsi and Anastasios Economou.

1.2 PESTICIDE CHEMISTRY

1.2.1 Historical Perspective

The International Union of Pure and Applied Chemistry (IUPAC) defines a pesticide as any substance or mixture of substances intended for preventing, destroying, or controlling any pest (Holland, 1996). Looking back over the years, the modern pesticide history begins in 1939 with the synthesis of dichlorodiphenyltrichloroethane (DDT) from Paul Muller in Geigy (Switzerland). In 1948, after the successful widespread use of DDT as insecticide to protect human health from diseases (like malaria) and also in agriculture practice, Paul Muller was awarded the Nobel Prize (The History of Pesticides, 2008).

After the synthesis of DDT, a plethora of organic chemical compounds with insecticide, herbicide, and fungicide action started to be synthesized. Later in the 1960s, laboratory studies in the United States proved that some chemical compounds belonging to the class of organochlorine insecticides such as dieldrin, endrin, and aldrin are not degraded in the environment and bioaccumulate in living organisms. In the same time period, DDT residues have been detected in river waters in the United States, while in 1963, the phenomenon of dead fish in Mississippi was attributed to the presence of aldrin in river water (Delaplane, 2000). In 1972, mainly due to their high environmental persistence and bioaccumulation, organochlorine insecticides were banned first in the United States and later in Europe.

Nowadays, more than 1600 pesticides belonging to more than 100 chemical classes are in use worldwide for food production. Information on synthetic and commercially available pesticides is readily found at "The Pesticide Manual" (*The Pesticide Manual*, 2012). Furthermore, the electronic Compendium of Pesticide Common Names (http://alanwood.net/pesticides/) contains data sheets for more than 1700 different active ingredients and for more than 350 ester and salt derivatives used in pesticide formulations.

The challenge of providing new molecules to control pests is a straightforward task with high rates of scientific success and considerable commercial reward. In no other field of chemistry has been such a diversity of structures arising from the application of the principles of chemistry to the mechanisms of action in pests to develop selectivity and sensitivity in agents toward certain species while reducing toxicity to other forms of life. The dramatic advances and the rapid changes in pesticide chemistry are presented, over the past 50 years, in the conferences in pesticide chemistry of the IUPAC taking place at 4-year intervals.

1.2.2 Identity and Physicochemical Properties of Pesticides

The systematic names of chemicals are derived from the IUPAC and the Chemical Abstracts Service (CAS). In addition to a systematic name, CAS assigns a registry number to each chemical. Since systematic names of pesticides are not convenient for general use, the widely accepted common names have been assigned by standard bodies. The Technical Committee 81 of the International Organization for Standardization (ISO) has devised a system for naming pesticides, with the aim of ensuring that common names indicate similarities between related compounds, do not conflict with any other names, and are suitable for use in many languages. New common names of chemicals for pest control are provisionally approved each year by the committee and are then used in the literature and on product labels. The ISO standards related to the selection of common names for pesticides are ISO 257:2004 (Pesticides and other agrochemicals—Principles for the selection of common names), ISO 765:1976 (Pesticides considered not to require common names), and ISO 1750:1981 (Pesticides and other agrochemicals—Common name) and its amendments.

Evaluation of pesticides begins with clear identification of their physical and chemical properties. Knowledge of the physical and chemical properties of a substance is a necessary prerequisite to understanding its general behavior in metabolism, analytical methods, formulations, and the environment.

Residues of pesticides on/in food commodities are also a function of many factors, which are mainly linked to the physicochemical properties of active ingredients. In the study performed by Thorbek and Hyder (2006), the relationship between physicochemical properties of the active ingredients and residue limits in foodstuffs was explored for fungicides, herbicides, and insecticides, using artificial neural networks. The authors concluded that the physicochemical properties of the active ingredients and crop type explained up to 50% of the variation in residue limits.

Pesticides currently used worldwide belonging to different chemical classes have different physicochemical properties. Physicochemical parameters of pesticides are usually measured according to well-established protocols recognized by national and international agencies (US Environmental Protection Agency (EPA) guidelines, Organization for Economic Co-Operation and Development (OECD), European Union (EU) protocols, etc.). Most of the physicochemical data are measured in the laboratory under well-defined experimental conditions. The main physicochemical data—water solubility, vapor pressure, volatility, stability in water, photodegradation, water–octanol partition coefficient, and acid–base properties—are characteristic of the single pesticide molecule. Short definitions of physicochemical properties are presented here with a commentary aspect on their relevance to various domains like the pesticide–environment interactions, its mode of application, and its analytical determination.

1.2.2.1 Water Solubility The water solubility of a pesticide is defined as its maximum concentration dissolved in water when that water is both in contact and at equilibrium

with the pure chemical. Data on pesticides' water solubility reported are usually measured in mg/1 at 20°C (PPDB IUPAC, 2014, Stephenson et al., 2006). Pesticides with high water solubility will be transported away from the application site by runoff or irrigation water to reach the surface water (PAN PD). Data on water solubility of a compound is needed for interpreting the routes of mammalian excretion, understanding its environmental behavior and its behavior in analytical methods.

The experimental procedures determining the solubility of pesticides in water are time-consuming and expensive. A highly effective tool depending on a quantitative structure–property relationship (QSPR) has been recently developed to predict pesticides' solubility in water; QSPR models were developed using multiple linear regression, partial least squares, and neural network analyses (Deeb and Goodarzi, 2010).

1.2.2.2 Vapor Pressure Vapor pressure (Vp) is defined as the partial pressure of a chemical, in the gas phase, in equilibrium with pure solid or liquid chemical (PAN PD). Vapor pressures are temperature dependent, measured at the temperature of 25°C, and expressed in Pa (mPa) or in mmHg (PPDB IUPAC, 2014). This parameter governs the distribution between liquid and gas phase or between solid and gas phase. The vapor pressure of a pesticide can serve as a potential indicator of its volatility, allowing a prediction of pesticides prone to evaporate from leaf and soil surfaces after application. Knowledge of pesticide volatility is also important to check the appropriateness of a gas chromatographic determination method and/or the implementation of evaporation steps in the extraction procedure.

1.2.2.3 Henry's Law Constant ($\mathbf{H}$ or $\mathbf{K}_H$) Henry's law constant (H or K_H) is a partition coefficient defined as the ratio of a chemical's concentration in air to its concentration in water at equilibrium. The tendency of pesticides to volatilize from water solution into air is largely determined by their H values: a high value favoring volatilization while H values $< 10^{-5}$ Pa $m^3 mo1^{-1}$ show little tendency to volatilize. The H values of compounds are more appropriate indicators of their volatilization than the single value of the Vp because they represent partitioning coefficients. Samples containing pesticides with high H values must be handled carefully in order to avoid loss; evaporation steps should not be included in the sample preparation process, while headspace analysis and/or solid-phase microextraction (SPME) techniques may be alternatively applied. The value of H can be expressed in either a dimensionless form or with units (PPDB IUPAC, 2014). In the dimensionless form, the same units of concentration are used in both the air and the water phases. The dimensionless form can be converted into the dimensional form by multiplying by RT (R is the ideal, or universal, gas constant, equal to the product of the Boltzmann constant and the Avogadro constant, and T is the absolute temperature of the gas), thus converting the air concentration to units of pressure, with the use of ideal gas law. By use of Henry's law, H can be conveniently calculated as the ratio of the liquid or solid vapor pressure and solubility. Therefore, H is often reported in Pa $m^3 mo1^{-1}$ with the vapor pressure in Pa and the solubility of the chemical in water expressed as a molar fraction in mol m^{-3}. The H values can be also estimated from experimentally determined solubilities and vapor pressures.

1.2.2.4 Acid–Base Ionization Constants ($p\mathbf{K}_a$) The acid ionization constant, K_a, is related to the equilibrium concentration of the nonionic and ionized forms by

$$\text{Acid} \quad HA \rightleftharpoons H^+ + A^-, K_a = ([A^-][H^+])/([HA])$$

$$\text{Base} \quad BH^+ \rightleftharpoons H^+ + B, K_a = [B][H^+]/[HB^+]$$

The ionization constant is usually expressed as pK_a ($=-\log K_a$). The higher the pK_a value, the weaker is the acid and its tendency to be ionized. Phenoxyalkanoic acids, sulfonylureas, and other herbicides such as bromoxynil, dicamba, ioxynil, and fluroxypyr have pK_a values around 3–4 (PPDB IUPAC, 2014). Since ionic pesticides behave differently from nonionic pesticides, it is important to know which pesticides are capable of ionization within the normal soil/water environmental pH range of 5–8 to predict leaching or retaining of pesticides. Knowledge of pK_a is also important for performing trace analysis of pesticides and especially for extractions from water, because it is much easier to extract a nonionic compound than an ionic one performing a simple pH adjustment of the sample (Barceló and Hennion, 1997).

1.2.2.5 Octanol–Water Partition Coefficient ($\mathbf{K}_{ow}$, log $\mathbf{P}_{ow}$) The octanol–water partition coefficient, K_{ow}, is defined as the ratio of the equilibrium concentrations of the two-phase system consisting of water and *n*-octanol. More specifically, K_{ow} is the ratio of the concentration of pesticide in the *n*-octanol layer to the concentration of the pesticide dissolved in the water layer (Stephenson et al., 2006). This parameter is usually reported as a logarithm usually as log K_{ow} or log P_{ow}. This partition coefficient is characteristic of the lipophilicity of the molecule and gives an indication of the pesticide's tendency to accumulate in biological membranes, living organisms, and foods. It is generally considered that substances with a log K_{ow} value higher than 3 can show accumulation; persistent organochlorines withdrawn from the market had log $K_{ow} > 4$ (PPDB IUPAC, 2014). The polarity of a molecule is also strongly correlated with K_{ow}. Nonpolar pesticides are characterized by log K_{ow} values above 4–5, whereas polar analytes have log K_{ow} values below 1 or 1.5. Between these two values, pesticides are classified as moderately polar. Knowledge of K_{ow} is useful when

choosing liquid chromatography conditions for pesticide analysis and reversed-phase sorbents for pesticide extraction where hydrophobic interactions are involved in the retention mechanism. K_{ow} has also proved valuable for the prediction of mobility and persistence in soils and of soil sorption since hydrophobic interactions also occur in the sorption of pesticides to soils containing large amounts of organic matter (PAN PD, Barceló and Hennion, 1997).

1.2.2.6 Soil Partition Coefficient ($\mathbf{K}_d$) The soil partition coefficient (K_d) is defined as the experimental ratio of a pesticide's concentration in the soil to that in the aqueous (dissolved) phase at equilibrium. The K_d is a distribution coefficient reflecting the relative affinity of a pesticide for adsorption by soil solids and its potential for leaching through soil (Stephenson et al., 2006).

1.2.2.7 Normalized Soil Sorption Coefficient ($\mathbf{K}_{oc}$) Various studies have demonstrated that for soil partition coefficient (K_d) values measured in a range of soils, good correlations were obtained between K_d and the organic matter content of the soil, probably due to interactions between the pesticide and the organic matter of the soil. Therefore, the adsorption coefficient has been normalized to take into account the different soil organic matter or organic carbon content; K_d values are expressed per unit of organic matter as K_{om} or per unit of organic carbon as K_{oc} (Stephenson et al., 2006):

$$K_{om} = \frac{100K_d}{\%\ \text{organic matter}}$$

$$K_{oc} = \frac{100K_d}{(\%\ \text{organic carbon})}$$

The K_{oc} values are more commonly reported in the literature than K_{om} values; they are expressed in $cm^3\ g^{-1}$. The environmental relevance of this parameter is important for leaching properties in groundwater. Pesticides with K_{oc} values below 50 are considered to be highly mobile compounds.

1.2.2.8 Half-Life ($\mathbf{T}_{0.5}$) Half-life ($T_{0.5}$) is defined in the case of a reactant in a given reaction, as the time required for its concentration to reach a value that is the arithmetic mean of its initial and final (equilibrium) values. For a single reactant that is entirely consumed (e.g., pesticide degradation), it is the time taken for the reactant concentration to fall to one-half its initial value (Stephenson et al., 2006).

The degradation of pesticides is often described using a modified first-order equation:

$$C_t = C_0\ \exp[-k(t - t_0)]$$

where C_t and C_0 are the concentrations at times t and 0 (units typically in days) and k is a time constant expressed in the same reciprocal units (Barceló and Hennion, 1997).

The *half-life*, $T_{0.5}$, is defined as the time required for the pesticide to undergo degradation to half of its initial concentration. If the above equation is appropriate, the half-life is independent of the initial time and concentration. However, the *half-life* measurements for pesticides depend strongly on the environmental conditions, and consequently, the exponential decay function can only be an approximation.

1.2.3 Pesticide Classification

Pesticides are classified by their chemical classes (e.g., organochlorines, organophosphates (OP)) or based on their target action (e.g., acaricides, herbicides, insecticides) or by their biochemical mode of action (MoA). The classification of the commercially used pesticides in categories based on their action on target organisms is presented in Table 1.1 (the Compendium of Pesticides Common Names).

Definitions and/or explanations of relative terms along with data on the individual substances of each group of pesticides or on the chemical classes included in each group (e.g., nematicides, plant growth regulators, and insecticides) are discussed in this chapter. Examples of representative compounds are given with their common (ISO) names and their chemical classes. Chemical groups of the major pesticides classes (i.e., acaricides, insecticides, herbicides, fungicides) are also presented. The chemical groups involved are not equivalent in terms of the number of compounds, for example, the organophosphorus may contain about 90 different compounds, currently used (Casida and Durkin, 2013a), while the neonicotinoids contain very few compounds (Tomizawa and Casida, 2005); both groups are classified as insecticides. Individual compounds or even chemical groups of active ingredients can occur in more than one class of pesticides, for example, organophosphorus, organochlorines, and pyrethroid groups are used as insecticides, acaricides, and/or nematicides (PAN PD, the *Compendium of Pesticides Common* Names):

TABLE 1.1 Classification of pesticides based on their action on target organisms

Acaricides	Algicides	Antifeedants	Avicides
Bactericides	Bird repellents	Fungicides	Herbicides
Herbicide safeners	Insecticides	Insect attractants	Insect repellents
Mammal repellents	Mating disrupters	Nematicides	Plant activators
Plant growth regulators	Rodenticides		

- *Acaricides*: A pesticide that is used to kill mites and ticks or to disrupt their growth or development. Compounds of different chemical groups are used as acaricides. Many of them are also classified as insecticides. The main chemical groups used are presented in Table 1.2. The numbers in parentheses indicate the number of chemical subclasses in each chemical group.
- *Algicides*: A pesticide that is used to kill or inhibit algae. Compounds of different chemical groups like phenylureas (diuron, isoproturon), diphenyl ethers (oxyfluorfen), triazines (cybutryne, simazine, terbutryn), and amides (quinonamid) are included in this class.
- *Antifeedants*: A pesticide that is used to prevent an insect or other pests from feeding. The commonly used compounds in this category are pymetrozine, fentin (organotin group), quazatine (guanidines), and chlordimeform (formamidines).
- *Avicides*: A pesticide that is used to kill birds. Different chemical compounds like fenthion (organothiophosphates), endrin (cyclodiene organochlorines), strychnine (botanical), and 4-aminopyridine are applied.
- *Bactericides*: A pesticide that is used to kill or inhibit bacteria in plants or soil. Chemical compounds like the bridged diphenyls dichlorophen and hexachlorophene, the pyridines dipyrithione and nitrapyrin, and antibiotics like chloramphenicol, kasugamycin, streptomycin, and oxytetracycline are used as bactericides.
- *Bird repellents*: A pesticide that is used to deter birds from approaching or feeding on crops or stored products. Anthraquinone, the OP diazinon, the methyl carbamates methiocarb and trimethacarb, and the dithiocarbamates thiram and ziram are usually applied as bird repellents.
- *Fungicides*: A pesticide that is used to kill fungi in plants, stored products, or soil or to inhibit their development. Chemical groups of fungicides are presented in Table 1.2. The numbers in parentheses indicate the number of different chemical subclasses in each chemical group, that is, the dicarboximides include the dichlorophenyl dicarboximides (e.g., iprodione, procymidone, vinclozolin) and the phthalimide dicarboximide (e.g., captafol, captan, folpet) subclasses.
- *Herbicides*: A pesticide that is used to kill plants or to inhibit their growth or development. The chemical groups of compounds of this class are presented in Table 1.2. The numbers in parentheses indicate the presence of chemical subclasses in each chemical group. A sound example is the case of triazine herbicide group that includes the chlorotriazines (e.g., atrazine, cyanazine, simazine, terbuthylazine), the fluoroalkyltriazines, the methoxytriazines (e.g., prometon, secbumeton), and the methylthiotriazines (e.g., ametryn, prometryn, simetryn, terbutryn).
- *Insecticides*: A pesticide that is used to kill insects or to disrupt their growth or development. The chemical groups of compounds with insecticidal activity are shown in Table 1.2. The numbers in parentheses indicate the number of chemical subclasses in each chemical group such as in the group of carbamates that includes the benzofuranyl methylcarbamate (MC) (e.g., carbofuran, benfuracarb), the dimethylcarbamate (e.g., pirimicarb), the oxime carbamate (e.g., aldicarb, methomyl), and the phenyl MC (e.g., methiocarb, propoxur) subclasses.
- *Nematicides*: A pesticide that is used to kill nematodes in plants or soil. Avermectin compounds (abamectin), carbamates (e.g., carbofuran, aldicarb), and organophosphorus compounds (e.g., fenamiphos, cadusafos, chlorpyrifos) may also act as nematicides.
- *Plant growth regulators*: A substance that alters the expected growth, flowering, or reproduction rate of plants. Antiauxins (e.g., clofibric acid), auxins (e.g., 2,4-D, 2,4-DB, dichlorprop, 2,4,5-T), cytokines, defoliants (e.g., ethephon, endothall, tribufos), gametocides (maleic hydrazide), gibberellins, growth inhibitors, growth retardants, and growth stimulators are included in this class of pesticides.
- *Rodenticides*: A pesticide that is used to kill rats, mice, and other rodents. Lindane, pyrinuron, and the coumarins—coumachlor, flocoumafen, and bromadiolone—are compounds included in the list of rodenticides.

1.2.4 Modes of Action (MoA)

The toxic effects of pesticides are compound specific and include several known mechanisms of action. Pesticides are bioactive compounds, intended to disrupt a primary target in the pest. Enzymes, receptors, or channel sites at which specific binding initiates the physiological change can act as target sites of pesticides. For a bioactive molecule, used as pesticide, a defined MoA describes the specific biochemical interaction to which its bioactivity is mainly attributed. Nearly a hundred of different biochemical targets (MoA) in pest insects, weeds, and fungi have been investigated for the major groups of insecticides, herbicides, and fungicides (Casida, 2009). Most insecticides disrupt neurotransmission to alter insect behavior or survival in a short period of time, whereas herbicides generally target the weed's specific functions necessary for their survival (Insecticide Resistance Action Committee (IRAC), Herbicide Resistance Action Committee (HRAC)). Fungicides act on many cellular functions essential for the survival of microorganisms (Fungicide Resistance Action Committee (FRAC)).

TABLE 1.2 Chemical classes of acaricides (A), fungicides (F), herbicides (H), and insecticides (I)

Chemical classes	A	F	H	I	Chemical classes	A	F	H	I
Amides		*v*(7)	*v*(3)		Phenoxy acids			*v*(3)	
Aromatics		*v*	*v*(4)		Phthalimides	*v*			*v*
Benzimidazoles		*v*			Pyrazoles	*v*(1)		*v*(2)	*v*(1)
Benzothiazoles		*v*	*v*		Pyrethroids	*v*(2)			*v*(3)
Benzoylcyclohexanediones			*v*		Pyridazines			*v*	
Benzofuranyl alkylsulfonates			*v*		Pyridazinones			*v*	
Bridged diphenyls	*v*	*v*			Pyridines		*v*(1)	*v*	
Carbamates	*v*(1)	*v*(2)	*v*(1)	*v*(4)	Pyrimidinamines	*v*			*v*
Carbonates			*v*		Pyrimidines		*v*		
Carbazates	*v*				Pyrroles	*v*			*v*
Cyanoacrylates		*v*			Quaternary ammoniums	*v*	*v*	*v*	*v*
Diamides				*v*	Quinolines		*v*		
Dicarboximides		*v*(2)	*v*		Quinones		*v*		
Dinitroanilines			*v*		Quinoxalines	*v*	*v*		
Dinitrophenols	*v*	*v*	*v*	*v*	Strobilurins	*v*	*v*(4)		
Dithiocarbamates		*v*(2)	*v*		Sulfoximines				*v*
Dithiolanes		*v*			Tetrazines	*v*			
Formamidines	*v*			*v*	Tetronic acids	*v*			*v*
Hydrazides	*v*				Thiazoles		*v*		
Imidazoles		*v*			Thiazolidines	*v*	*v*		*v*
Imidazolinones			*v*		Thiocarbamates	*v*	*v*	*v*	
Macrocyclic lactones	*v*(2)			*v*(3)	Thiocarbonates			*v*	
Morpholines		*v*			Thiophenes		*v*		
Neonicotinoids				*v*(3)	Thioureas	*v*		*v*	*v*
Nitriles			*v*		Triazines		*v*(4)	*v*	
Organochlorines	*v*			*v*(1)	Triazinones			*v*	
Organophosphorus	*v*(5)		*v*	*v*(7)	Triazoles		*v*	*v*	
Organotins	*v*	*v*			Triazolones			*v*	
Oxadiazines				*v*	Triazolopyrimidines		*v*	*v*	
Oxadiazolones			*v*	*v*	Ureas		*v*(3)	*v*	*v*
Oxazoles		*v*	*v*		Uracils			*v*	

The numbers in parentheses indicate the number of chemical subclasses in each chemical group.
v: symbol used for indication of chemical classes belonging to acaricides (A), fungicides (F), herbicides (H), and insecticides (I).

1.2.4.1 Insecticides and Acaricides Insecticides are used to kill insects or to disrupt their growth or development. The majority of the commercially available insecticides target the functionality of the nervous system of insects at the synapse or the axon (Casida, 2009); at least eleven biochemical targets—MoA—have been identified in the insect nervous system for lipophilic insecticides (Casida and Durkin, 2013b). The cholinergic system is the major insecticide nerve target with OP and MC compounds inhibiting acetylcholinesterase (AChE) responsible for the hydrolysis of acetylcholine (ACh) at synaptic regions. AChE inhibition by OP and MC insecticides involves phosphorylation and carbamylation, respectively, of serine in the enzyme esteratic site, provoking ACh accumulation and prolonged stimulation of cholinergic receptors. The nicotinic acetylcholine receptor (nAChR) is the target site of neonicotinoids—the newest class of potent insecticides. Neonicotinoids are similar to nicotine in their structure and action as agonists of the nAChR, but they are more toxic to insects than mammals due to differences in their binding site interactions at the corresponding nAChRs (Tomizawa and Casida, 2005, 2009). The γ-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter of insects and mammals and acts as agonist for opening the pentameric transmembrane Cl^- channel; synaptic neurotransmission at that channel is the target for polychlorocycloalkanes (PCCAs) and phenylpyrazoles. Cross-resistance between some of the PCCAs was the first sign of a common target and defined MoA, that is, compounds acting at the same binding site. The insect Na^+ channel proteins consist of four homologous domains, each one with six transmembrane segments. Pyrethroids and DDT analogues act both on axonal neurotransmission at insect voltage-gated Na^+ channel recognition sites to block Na^+ transport, enhance channel inactivation, prolong the course of the Na^+ current during depolarization, and induce a residual slow-acting current. Four other sites in insect Na^+ channels are targets for the synthetic insecticides, oxadiazines and semicarbazones, without cross-resistance to pyrethroids and DDT (Casida and Durkin, 2013a, b). Insecticidal activity is also achieved at the mitochondrial respiratory electron transport chain, for example, the insecticide chlorfenapyr is one of the pesticidal uncouplers of oxidative phosphorylation (Casida, 2009). Furthermore, insecticides may interfere at the hormone-guided processes of growth and development acting as insect growth regulators (IGRs) through different pathways. MoA of insecticides correlated with the main chemical groups and their representative compounds are presented comprehensively in Table 1.3 (IRAC, 2014).

TABLE 1.3 Modes of Action (MoA) of representative chemical classes of insecticides

Insecticides			
Chemical classes	Target system	Biochemical modes of Action (MoA)	
Organophosphates	**Nervous and muscle system**	**Cholinergic**	Acetylcholinesterase (AChE)
Methyl carbamates			
Neonicotinoids			nAChR[1] competitive agonist
Spinosyns			nAChR[1] allosteric agonist
Pyrethroids		**Sodium channel**	Modulator
DDT analogues			
Oxadiazines			
Semicarbazones			Voltage-gated sodium channel blocker
Cyclodiene organochlorines		**Chloride channel**	GABA[2]-gated chloride channel antagonist
Phenylpyrazoles			
Avermectins, milbemycins			Glutamate-gated chloride channel activator
Flavonoids	**Respiration**	**Inhibition of mitochondrial electron transport**	Complex I NADH[3] oxidoreductase
Quinazolines			
Pyrazoles			Complex II
Naphthalenes			Complex III
Arylpyrroles		**Oxidative phosphorylation**	Uncouplers via disruption of proton gradient
Thioureas, cyclohexanes			Inhibition of mitochondrial ATPase[4]
Haloaliphatic acid			Inhibition of aconitase
Juvenile hormone analogues, phenoxyphenoxy ethers	**Growth regulation**	**Insect growth regulators**	Juvenile hormone mimic
Diacylhydrazines		**Molting disruptor**	Ecdysone receptor agonist
Triazines			Dipteran
Benzoylureas			Inhibition of chitin biosynthesis

nAChR[1], nicotinic acetylcholine receptor; GABA[2], γ-aminobutyric acid; NADH[3], nicotinamide adenine dinucleotide; ATPase[4], adenosine triphosphatase.

1.2.4.2 Herbicides Herbicides disrupt the plants' unique process of converting light energy to the chemical energy of adenosine triphosphate (ATP), necessary for their survival and development, by inhibiting photosynthesis and pigment synthesis. About 50 commercial herbicides of the chemical groups of triazines, triazinones, uracils, ureas, amides, nitriles, and others target the photosystem II (PSII), whereas compounds of chemical types like thiadiazoles, oxadiazoles, and diphenyl ethers act on the protoporphyrinogen IX oxidase (HRAC). Inhibition on pigment synthesis due to herbicides acting on phytoene desaturase, lycopene cyclase, and 4-hydroxyphenylpyruvate dehydrogenase leads to bleaching and weed death (Casida, 2009). Phytotoxic compounds like glyphosate, sulfonylureas, and glufosinate interfere in the aromatic or branched chain amino acid biosynthesis of plants, while compounds of different chemical types like trifluralin and propyzamide alter the microtubule assembly process. Moreover, a variety of compounds exert their inhibitory action on the fatty acid synthesis processes in plants, while some herbicides act on targets related with the respiration and the growth processes.

1.2.4.3 Fungicides Fungicides exert inhibitory action on several vital biochemical systems of microorganisms essential for their development and survival; more than 40 targeted biochemical systems—MoA—have been defined until now by the FRAC. Many fungicides, like triazoles and imidazoles, block ergosterol (the fugal sterol) biosynthesis by inhibiting the C14α-demethylase (CYP51), while morpholines act on the Δ^{14} reductase and the $\Delta^{8} \rightarrow \Delta^{7}$ isomerase. Diverse chemotypes exert inhibitory action on the other two sterol synthesis targets. Fungicide targets involved in nucleic acid biosynthesis are selected by compounds like acylalanines, isoxazoles, and others, while antibiotic fungicides inhibit protein synthesis. Phospholipid and glucan biosynthesis is blocked by phosphorothiolates, dithiolanes, carboxylic acid amide groups, and antibiotics with fungicidal activity, whereas methionine biosynthesis is inhibited by aniline pyrimidine compounds. The antibiotic fungicides streptomycin, kasugamycin, oxytetracycline, and blasticidin-S block protein synthesis on fungi, while antitubulin fungicides like benomyl and thiophanate-methyl affect β-tubulin assembly in mitosis. Respiration targets such as the ubiquinol oxidase at Q_o site and the ubiquinone reductase at Q_i site of complex III and oxidative phosphorylation targets are affected by strobilurins, sulfonamides, and dinitrophenols, whereas other compounds like thiazoles and thiadiazoles may act as fungal disease development regulators or host plant defense inducers (Casida, 2009).

A major limiting factor in the continuing use of pesticides is the emergence of resistance developed by pests (Casida, 2009, Casida and Durkin, 2013a, b). A sound example is the resistance of houseflies to DDT soon after its application due to the selection of less sensitive strains with cross-resistance to some pyrethroids. All the PCCA insecticides lost their initial effectiveness with cross-resistance due to a low sensitivity target site in the GABA-gated chloride channel (Tomizawa and Casida, 2009). Pesticide management is a major aspect of pest control in order to slow the resistance development and fight the emergence of resistant pest strains. The importance of pesticide management led to the establishment of the resistance action committees, that is, the *HRAC, the FRAC, and the (IRAC)*, to define resistance groups. Listings of pesticides' primary target sites in the pests revealed near a hundred of MoA for insecticides, herbicides, and fungicides. Metabolomic studies have greatly contributed to the discovery of the MoA of herbicides, insecticides, acaricides, fungicides, and antibiotics (Aliferis and Jabaji, 2011). Metabolomics is defined as the comprehensive qualitative and quantitative profiling of a large number of metabolites of a biological system (Fiehn et al., 2000). Metabolomics enables the simultaneous and comprehensive monitoring of global metabolite networks of biological systems and their alterations triggered by biotic and/or abiotic factors. Within this framework, metabolomics have been applied in pesticide research and development to investigate the MoA of these bioactive compounds, the assessment of their toxicological and ecotoxicological risk, and the discovery of new bioactive compounds (Aliferis and Jabaji, 2011, Aliferis and Tokousbalides, 2011). Nuclear magnetic resonance (NMR) spectroscopy and MS analyzers are the main analytical platforms employed in metabolomic studies. GC–MS was the MS platform initially used for MS metabolomics (Fiehn et al., 2000, Liu et al., 2010), whereas LC–MS with triple quadrupole (QqQ), time-of-flight (TOF), and hybrid quadrupole time-of-flight (QTOF) analyzers have shown a great potential in metabolomic studies (Allen et al., 2004, Taylor et al, 2010). Two powerful MS detectors—Fourier transform ion cyclotron resonance/MS (FT-ICR/MS) and Orbitrap-MS—have been successfully introduced in high-throughput metabolomic studies (Oikawa et al, 2006, Xiao et al., 2012). Technological advancements in MS applied in studies involved in the pesticide research have contributed to the development of novel and more efficient pesticides safer for the consumer and the environment (Aliferis and Tokousbalides, 2011).

1.3 PESTICIDE METABOLITES AND TRANSFORMATION PRODUCTS

Pesticides can be transformed in plants, animals, and the environment through biological, chemical, and physical processes into a large number of degradation products, commonly defined as Transformation Products, TPs; other terms such as metabolites or pesticide *derivatives* are also used. Pesticide metabolites and TPs may have different physicochemical properties from the parent compound and can be more toxic and persistent than parent compounds. *Relevant* metabolites and TPs should be

included in monitoring studies of food products as being incorporated in the residue definition of the parent compound in the MRLs established for food products of plant and animal origin. Furthermore, pesticides and their TPs derived from a variety of biotic and abiotic degradation pathways in the environment suspected of entering the environment and causing adverse effects on health should be included in environmental studies.

1.3.1 Biotransformation

The terms biotransformation and metabolism are often used synonymously, particularly when applied to xenobiotics. The term metabolism is often used to describe the total fate of a xenobiotic, which include absorption, distribution, biotransformation, and elimination. However, metabolism is commonly used to mean biotransformation as the products of xenobiotic biotransformation are known as metabolites (Casarett and Doulls, 2001).

Metabolism studies are necessary to understand the fate of pesticides, identify the metabolites, and provide data for human dietary risk assessment. The qualitative and quantitative nature of pesticide residues in plants and livestock is dependent on the following processes:

- Absorption: The movement of the pesticide across membranes. Pesticides can be transferred into and out of cells of a biological system by passive diffusion, osmosis, or active transport mechanisms. Physicochemical properties of pesticides, such as lipophilicity (log P) and acidity (pK_a), influence the absorption process following their application on the plant, along with the cell membrane types and the electrochemical potential in the cells (Skidmore and Ambrus, 2004).
- Distribution: Transport within the biological system. In the case of livestock, pesticides entering the systemic circulation are distributed in tissues by the same mechanistic processes as above; the distribution will be dependent on the blood–tissue dynamics and the tendency of pesticides to bind with plasma proteins. The distribution of pesticides in plant is dependent on their entry into the transport system of the plant that uses a network of vascular conduits, xylem and phloem, to transfer nutrients and water. The passage and retention of pesticides in the phloem are also influenced by their physicochemical characteristics, mainly their log P and pK_a values.
- Metabolism: Biological or chemical transformation of pesticides resulting from natural processes in the biological systems.
- Elimination: The pesticide and its metabolites are eliminated through active cell processes.

A great number of complex biotransformation pathways may occur within biological systems during the metabolism of pesticides. Pesticide metabolites resulting from these processes can be characterized into one of four categories as follows (Dorough, 1980):

- Phase I: Free metabolites derived from reactions introducing functional groups into the pesticide molecule
- Phase II: Conjugated metabolites
- Phase III: Bound residues
- Phase IV: Naturally incorporated

1.3.1.1 Phase I and Phase II Biotransformation

Phase I metabolism involves oxidation, reduction, and hydrolytic reactions (Skidmore and Ambrus, 2004). Typical oxidative reactions occurring in plants and livestock include aliphatic hydroxylation, alicyclic hydroxylation, aromatic hydroxylation, benzylic oxidation, O-,N-dealkylation, N-,S-oxidation, etc. Oxidative reactions may be mediated by a range of enzymes such as microsomal cytochrome P450 (CYP450) isozymes and peroxidases. Hydrolysis reactions can be both chemical and enzyme mediated; ester hydrolysis is important in the case of the arylphenoxypropionic acid herbicides whose alkyl esters are readily hydrolyzed to the active moiety. Hydrolysis reactions resulting in the opening of heterocyclic ring systems have been also reported: the hydrolytic cleavage of the oxazolidone ring of the dicarboximide fungicides vinclozolin and procymidone and the cleavage of the triazine or pyrimidine heterocyclic ring of sulfonylurea herbicides. Reduction reactions may include the reduction of nitro groups, aldehydes, ketones, and alkenes, common in both livestock and plants (Roberts and Hutson, 1999, Skidmore and Ambrus, 2004).

Phase II metabolism includes the conjugation reactions where the pesticide (exocon) is chemically bonded to an endogenous substrate (endocon). Conjugation reactions occur mainly with glutathione (GSH), sugars, and amino acids; lipophilic and sulfate conjugation reactions (sulfation) have been also reported.

Most phase II biotransformation reactions result in a large increase in xenobiotic hydrophilicity; hence, they greatly promote the excretion of chemicals.

GSH is a common tripeptide in plants and animals composed by glutamine, cysteine, and glycine; the conjugation reaction results from the nucleophilic attack of the thiolate anion on an electrophilic center and is catalyzed by the enzyme glutathione-*S*-transferase. Multiple isoforms of this enzyme have been isolated from various species, while GSH conjugates have been reported for chloroacetanilides, triazines, sulfonylureas, thiocarbamates, and organophosphorus pesticides. The initially formed GSH conjugate is catabolized to the cysteine conjugate, which is further catabolized to a complex mixture of metabolites.

Sugar conjugates of pesticides with endogenous sugar molecules in plants and animals are usually in the form of

glycosides in plants and glucuronides in animals. A number of O-, S-, and N-glycoside conjugates in plants and their respective glucuronide conjugates in animals for several classes of pesticides, parent compounds, and phase I metabolites—for example, pyrethrins, triazoles, dithiocarbamates, and strobilurins—have been reported. In plants, sugar conjugates may be subjected to further conjugation with extra sugar molecules or with malonic acid, while in animals, the glucuronic acid conjugates can be further conjugated by sulfation (Roberts and Hutson, 1999, Skidmore and Ambrus, 2004).

Amino acid conjugation has been observed with various amino acids like glycine, glutamic acid, aspartic acid, alanine, serine, aspartate, and glutamate for various pesticides and their metabolites (Bounds and Hutson, 2000). A significant plant metabolite of triazole fungicides is an amino acid conjugate, the triazolylalanine, derived by the reaction of 1,2,4-triazole with serine; triazolylalanine may be further catabolized to the triazolylacetic acid.

Lipophilic conjugation of pesticides increases the lipophilicity of the parent molecule leading to its stronger retention within the biological system. Conjugation of pyrethroids with cholesterol, fatty acids, and glycerol has been evidenced, while conjugation of haloxyfop and tebufenozide with triglycerol has been also reported.

Sulfate conjugates are commonly observed in animals, and in many cases, they are competitive to glucuronide conjugates; their formation has been attributed to an enzyme-catalyzed transfer of sulfate from 3-phosphoadenosine-5-phosphosulfate to the pesticide. Sulfate conjugates of kresoxim-methyl, thiabendazole, and deltamethrin in livestock have been identified (Skidmore and Ambrus, 2004).

1.3.1.2 Metabolic Pathways in Plants and Animals Metabolic studies of pesticides are usually performed with radiolabeled pesticides, allowing for a rapid and sensitive detection by using analytical techniques such as liquid scintillation counting and phosphorimaging following chromatographic separation. Technological advancements in MS analyzers have provided new sensitive and selective tools for the detection and identification of pesticide metabolites that can act complementary to the established radiolabeled techniques.

A typical example is the case of imidacloprid, a neonicotinoid systemic insecticide. Initially, the metabolic studies of imidacloprid in a number of plant applications were conducted using its radiolabeled available analogue, [^{14}C]imidacloprid (Roberts and Hutson, 1999). Later, the evaluation of primary and secondary toxicity mechanisms of neonicotinoids was performed using liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Casida, 2011, Dick et al., 2005, Ford and Casida, 2006). Casida (2011) concluded that phase I metabolism of neonicotinoids is dependent mainly on microsomal CYP450 isozymes with selectivity in hydroxylation, desaturation, dealkylation, sulfoxidation, and nitro reduction, while phase II metabolism involves methylation, acetylation, and conjugation with sugars, amino acid, sulfate, and GSH. The metabolic pathway of imidacloprid based on radiolabeled and LC–MS/MS techniques is depicted in Figure 1.1 (Casida, 2011, Roberts and Hutson, 1999).

Recently, ultrahigh-performance liquid chromatography (UHPLC) combined with a high-resolution and high-mass-accuracy quadrupole time-of-flight mass analyzer (QTOF-MS) was applied in a metabolism study of imidacloprid in onions (Thurman et al., 2013). Since primary standards of plant metabolites were not available, accurate mass analysis was used as a tool for structure elucidation of metabolites. A combination of five techniques—that is, database mining using the accurate masses from known chemical structures, chlorine filters using accurate mass formula generation with chlorine, fragmentation studies of the parent pesticide and its diagnostic ions, Mass Profiler software, and MS/MS studies and metabolite analogy—enabled the identification of imidacloprid new TPs. The putative structures of these newly discovered plant metabolites as proposed by the authors (Thurman et al., 2013) are shown in Figure 1.2.

Overall, high-resolution mass spectrometry (HRMS) is an attractive methodology for investigating the pesticide metabolites in food samples using comparative studies of blank and treated samples from field trials. Each chromatographic peak found in the treated sample, but not in the control sample, is subjected to further investigation to assign a molecular formula from the accurate masses and the isotopic and fragmentation pattern observed in the MS and MS/MS spectra (Hernández et al., 2008).

The presence of pesticide metabolites in pesticide-positive food samples has been studied using ultrahigh-pressure liquid chromatography coupled with hybrid quadrupole time-of-flight mass spectrometry (UHPLC–QqTOF-MS/MS) (Hernández et al., 2009). Accurate mass measurements of both parent (MS) and product ions (MS/MS) allowed the determination of elemental compositions of metabolites. The common MS fragmentation pathway between the parent pesticide and its metabolites has been considered to search for metabolites in two positive market samples (imazalil in lemon, chlorpyrifos in grape). This approach allowed the discovery of two metabolites of imazalil, 1-[2-(2,4-dichlorophenyl)-2-oxoethyl]-1*H*-imidazole (IMZ-M1) and 1-[2-(2,4-dichlorophenyl)-2-hydroxyethyl]-1*H*-imidazole (Fig. 1.3).

Liquid chromatography–high-resolution mass spectrometry (LC–HRMS) has been shown to be particularly useful for the identification of pesticide glycoside conjugates, an important group of pesticide metabolites in plants. For example, the hydroxyl derivatives of tebuconazole and tebuconazole glucoside in a sample of cherries containing tebuconazole residues were detected using ultrahigh-performance

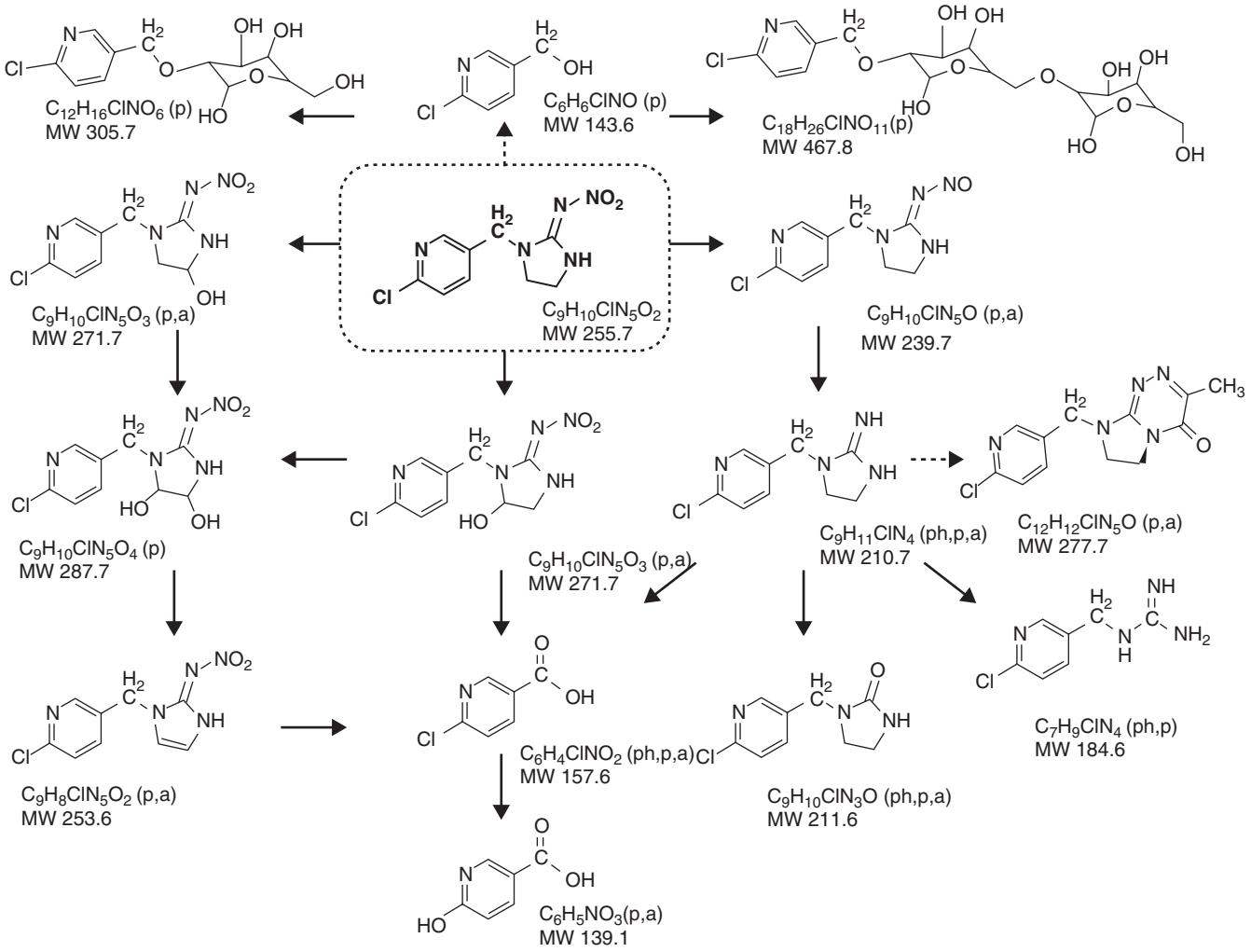


FIGURE 1.1 Metabolic pathways of imidacloprid in plants (p) and animals (a) based on radiolabeled and LC–MS/MS techniques. Metabolites mentioned with (ph) have been also found as photolytical products.

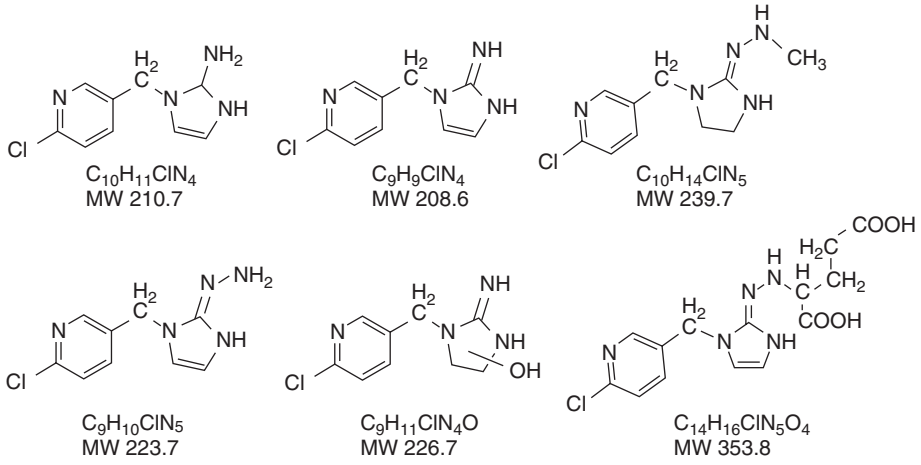


FIGURE 1.2 Imidacloprid metabolites in plants identified using LC–QqTOF-MS.

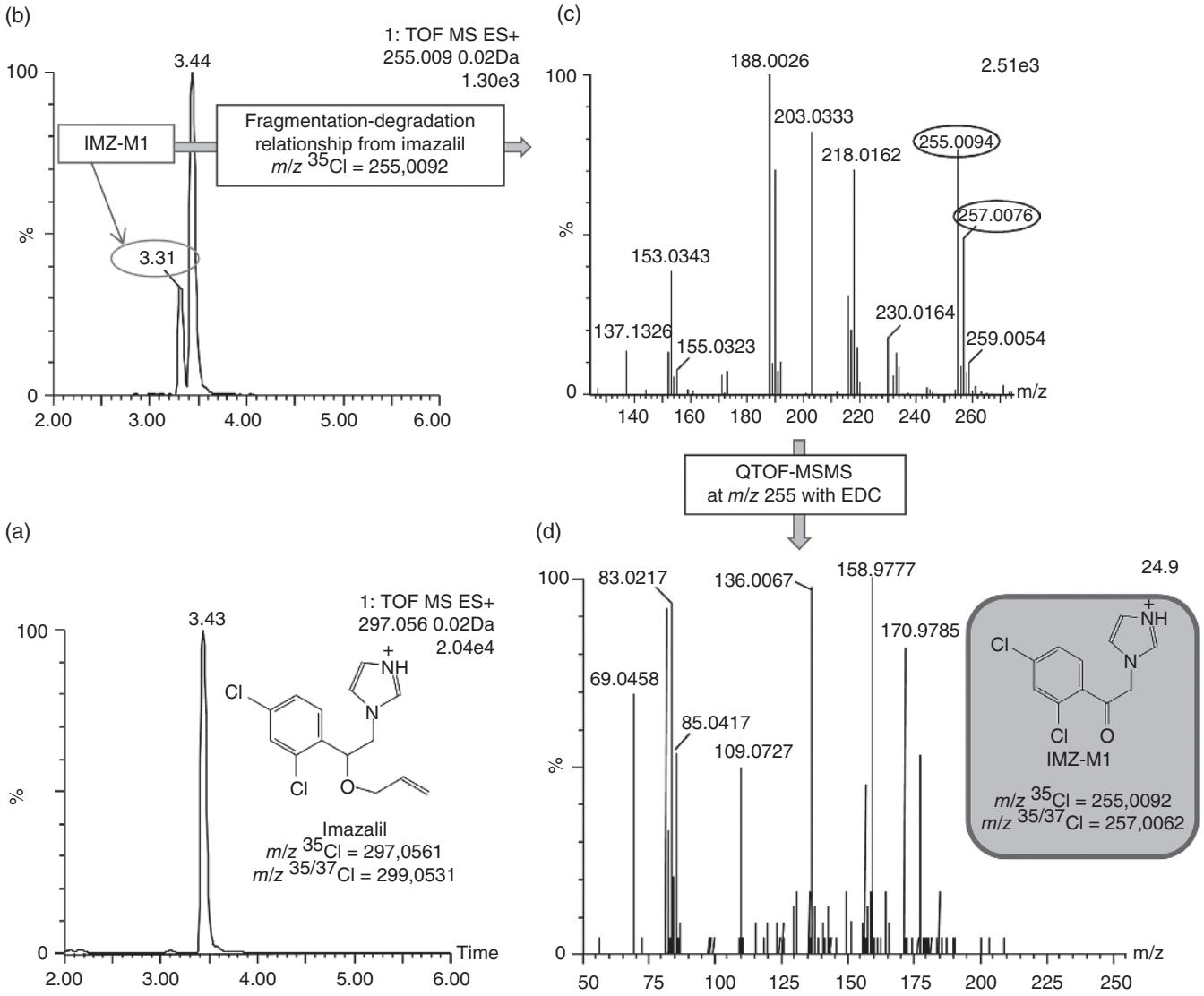


FIGURE 1.3 UHPLC/ESI(+)-TOF nw-XIC chromatograms of an imazalil-positive lemon sample at *m/z* (a) 297.0561 and (b) 255.0092, corresponding to the ion $[M+H]^+$ of imazalil and one of its fragments, respectively. (c) Combined spectrum of potential metabolite IMZ-M1. (d) Product ion QTOF-MS/MS spectrum of metabolite IMZ-M1 (precursor ion *m/z* 255) with EDC centered at *m/z* 130 and collision energy of 20 eV. Chemical structure proposed for metabolite IMZ-M1. (Reproduced with permission from Hernández et al., 2009.)

liquid chromatography–time-of-flight mass spectrometry (UHPLC–TOF-MS) (Lacina et al., 2010).

A screening methodology was also reported for the detection of pesticide metabolites including glycosides in fruits and vegetables using liquid chromatography–time-of-flight mass spectrometry (LC–TOF-MS) (Polgar et.al, 2012). This approach was based on (i) search for parent pesticide molecules; (ii) search for their metabolites in the positive samples, assuming common fragmentation pathways between the metabolites and parent pesticide molecules; and (iii) search for pesticide conjugates using the data from parent species and their diagnostic fragmentation. An accurate mass database was constructed consisting of 1396 compounds (850 parent compounds, 447 fragment ions, and 99 metabolites). The screening process was performed by the software in an automated fashion. The proposed screening methodology was evaluated with incurred samples. In some cases, the pesticide glycoside derivatives were found in a relatively high ratio, drawing the attention to these kinds of metabolites and showing that they should not be neglected in multiresidue methods and monitoring studies.

Several pesticide metabolites have been identified in food matrices using mass spectrometric techniques with low- and high-resolution mass analyzers. In many cases, new *unknown* TPs have been identified; several reviews are focused on the inherent advantages of mass analyzers, TOF-MS, Orbitrap

and their hybrid platforms, QqTOF-MS, Q-Orbitrap, and LTQ-Orbitrap for pesticide metabolite identification (Farré et al., 2014, Fernández-Alba and García-Reyes, 2008, García-Reyes et al., 2007, Gómez-Ramos et al., 2013, Hernández et al., 2011, Kaufmann, 2012, Martínez Vidal et al., 2009, Soler and Picó, 2007).

1.3.2 Environmental Fate

The fate of pesticides in the environment, mainly in water and soil, depends on their physicochemical properties, on their vulnerability to various transformation and transport processes, and on environmental conditions, biota, water composition, and soil and sediment characteristics. Transformation pathways of pesticides can be biotic—aerobic and anaerobic metabolism—or abiotic occurring through reactions such as hydrolysis, photolysis, oxidation, and/or reduction. The extent of degradation may vary from minor modification of the pesticide molecule to complete mineralization with end products, such as carbon dioxide, ammonia, water, and inorganic salts; moreover, the degradation rate varies widely with half-lives of pesticides from minutes to years (Holland and Sinclair, 2004).

In the environment, photodegradation and hydrolysis are mainly involved in the decomposition of pesticides, especially within aquatic systems; photodegradation may occur through direct photolysis or more commonly through indirect photolysis induced by other molecules (photosensitizer) (Burrows et al., 2002, Katagi, 2004, Pehkonen and Zhang, 2002). During direct photolysis, a photon is absorbed by the target compound resulting in bond cleavages; this pathway is selected by compounds that absorb light within the solar spectrum ($\lambda > 290$ nm). During indirect photodegradation, the photon is absorbed by a photosensitizer to produce reactive species capable to degrade the pesticide. Dissolved organic matter (DOM) is acting as a photosensitizer in indirect photodegradation of pesticides within aquatic systems, producing oxidants such as hydrated electrons and singlet oxygen ($^1O_2$), superoxide radical anion ($\bullet O_2^-$), hydroxyl radicals ($\bullet$OH), hydrogen peroxide (H_2O_2), carbonate radical ($\bullet CO_3^-$), and alkyl peroxy radicals (ROO$\bullet$) (Katagi, 2004, Remucal, 2014). Likewise, naturally occurring nitrate ions (NO^{3-}), nitrite, H_2O_2, and iron in surface water may serve as a primary source for sunlight-induced hydroxyl radical production involved in the phototransformation of pesticides (Vaughan and Blough, 1998). The photodegradation rate of a pesticide is the sum of direct and indirect photolytical processes. Different chemical groups of pesticides follow different photolytical degradation mechanisms including losses of alkyl, halogen, or hydroxyl groups; cleavage of alkyloxy, amide, amino-alkyl, and ester bonds; photoisomerization; and hydroxylation (Burrows et al., 2002, Katagi, 2004); decarboxylation reactions have been observed in pesticides containing acidic groups in their molecules (Pinna and Pusino, 2012), while photonitration reactions occurred in pesticides containing aromatic groups in the presence of nitrate and nitrite (Chiron et al., 2009). The role of photodegradation in the environmental fate of pesticides has been addressed in a number of reviews focused on different pesticide classes like phenylurea (Amine-Khodja et al., 2004) and sulfonylurea herbicides (Headley et al., 2010), triazines, and organophosphates, OP (Stangroom et al., 2000).

Hydrolysis is significantly involved in the degradation pathways of many pesticides occurring abiotically or biotically. Hydrolysis reactions are strongly pH dependent and related to the presence of H_2O molecules, H_3O^+ and OH^- ions, and the pK_a of the compound (Holland and Sinclair, 2004). Hydrolysis products are usually more polar than the parent compounds and more water soluble. Climate conditions and temperature may also influence the hydrolytic decomposition rate of pesticides in environmental matrices (Agüera López et al., 2014).

Biological processes, mainly microbial transformations, are very effective in the degradation of pesticides, especially in soils and groundwater. Under appropriate conditions, microorganisms may use certain synthetic organic compounds as nutrients enabling mineralization. Nonetheless, many pesticides have chemical structures or attached groups not biodegradable as, for example, the organochlorine pesticides and their metabolites that they can still be found in environmental samples. Microbial transformation is the most important way of triazine dissipation in soils even though the S-triazine ring is quite resistant to the microbial attack; a great variety of triazine degradates have been identified in environmental samples (Agüera López et al., 2014).

The transformation and degradation processes are dependent on the type of pesticide and matrix, and consequently, different TPs of the same parent compound may occur in the environmental samples. The degradation process of some pesticides can produce TPs more toxic than the parent compound; many TPs can have different physicochemical properties from the parent compound; for instance, due to their different mobility in the soil/water environment, TPs may occur in environmental areas such as groundwater deposits where parent compounds can hardly reach. The detection and characterization of pesticide TPs are important tasks in order to evaluate their formation, kinetics, stability, and toxicity. Pesticide TPs are included in the group of the *emerging contaminants*, which encompasses compounds not commonly monitored in the environment but suspected of entering in it and causing adverse effects on health (Agüera López et al., 2014). A thorough risk assessment should consider the parent compound and all the TPs possibly formed and occurred in the environment; consequently, metabolism and monitoring studies are necessitated to detect and identify *known* and *unknown* TPs in the environment and to assess their environmental impact (US EPA, 1999, 2002, 2004, Regulation (EC) No 1107/2009, Commission Regulation (EU) No 283/2013, Commission Regulation (EU) No 284/2013).

MS techniques hyphenated with gas and liquid chromatography, GC–MS and LC–MS, have greatly contributed to the detection and identification of pesticides and their TPs in environmental matrices (Díaz et al., 2012, Farré et al., 2014, Masiá et al., 2014a, b). Reemtsma and co-authors have developed a multiresidue method for the determination of 150 pesticide TPs with LC–MS/MS in groundwaters and surface waters (Reemtsma et al., 2013). Moschet and colleagues reported the optimization of a *suspect* screening strategy with LC–HRMS using only the exact mass as a priori information for the detection of pesticides, parent compounds, and TPs in surface water samples; a number of TPs not previously reported were detected in surface water samples applying this approach (Moschet et al., 2013). This strategy of target screening approach combined with the *suspect screening* can offer a more efficient and comprehensive pesticide screening in the assessment of surface water quality (Moschet et al., 2014).

1.4 RISK ASSESSMENT

> *What is there that is not poison? All things are poison and nothing is without poison. Solely the dose determines that a thing is not a poison.* (Paracelsus 1493–1541)

Pesticides because of their intended use may involve risks and hazards for humans and the environment. Risk assessment is therefore necessary to estimate a level of human and environmental exposure that will not result in adverse human health effects. Risk assessment is a process based on scientific considerations defining the risk associated with a specified use pattern for a pesticide.

Risk assessment process of any compound involves four integrated steps (Holland, 1996):

1. Hazard identification
2. Dose–response assessment
3. Exposure assessment
4. Risk characterization

The first stage of the risk assessment process is *hazard identification*. This is the identification of the adverse effects that a substance has an inherent capacity to cause. For pesticides, toxicity data and physicochemical information are accumulated in order to identify hazards. The second stage, *dose (concentration)–response (effect) assessment*, is the heart of toxicology. It is the estimation of the relationship between the level of exposure and the incidence of an adverse effect. The third stage, *exposure assessment*, is defined as an estimation of the concentration level and time to which the human population is exposed. The final stage, *risk characterization*, combines the information received from the three previous stages into the estimation of the incidence and severity of adverse effects due to actual or predicted exposure to a substance.

The issues relating to hazard and risk assessment and risk management for chemicals are discussed in detail in the World Health Organization (WHO) Environmental Health Criteria monograph on *Principles for the Assessment of Risks to Human Health from Exposure to Chemicals* (WHO, 1999).

In the global level, the *Joint Meeting on Pesticide Residues* (JMPR) is an expert ad hoc body administered jointly by the Food and Agriculture Organization (FAO) and WHO with the purpose of harmonizing the requirement and the risk assessment on pesticide residues. The JMPR comprises the WHO Core Assessment Group and the FAO Panel of Experts on Pesticide Residues in Food and the Environment. The WHO Core Assessment Group is responsible for reviewing pesticide toxicological data and estimating acceptable daily intakes (ADIs) and acute reference doses (ARfDs). The FAO Panel is responsible for reviewing pesticide data residues and for estimating MRLs, supervised trials median residue (STMR) values, and highest residues (HRs) in food and feed.

In the EU, the food safety *integrated approach* started with European Commission (EC) Regulation 178/2002 (Regulation (EC) 178/2002), fixing the food safety principles and establishing an independent body for risk assessment, the European Food Safety Authority (EFSA, 2014). Since 2002, the EU has decided to compartmentalize the process of risk analysis for food safety into two steps: the first is the risk assessment carried out by the EFSA, and the second is the risk management by the European legislative bodies.

Since 2003, the EFSA has provided scientific advice on food safety, including such issues as having a direct or indirect impact on the safety of food and feed supply chains (e.g., animal health and welfare, plant health, and nutrition) to the EC, member states, and the European Parliament. The scientific Panel of Plant Protection Products and their Residues of EFSA (PPR Panel) provides advice on risk factors related to pesticides. Since 2002, through different legislative tools (regulations, directives, or decisions), the EU has been developing procedures for the evaluation of risks in the area of pesticide residues in the food chain.

The US EPA (2014) has the responsibility to conduct human health and environmental fate and effect risk assessments for pesticides. To evaluate the risks posed by pesticides in the diet, the EPA follows standard risk assessment guidelines. The agency uses different procedures for cancer risks and noncancer risks (US EPA, 1995, 2005).

1.4.1 Safety Factors

1.4.1.1 No-Observed-Effect Levels Toxicology studies allow the determination of the daily dose of a pesticide or chemical that can be given over a certain period of time by a particular dose route at which no effects are observed. This is known as the no-observed-effect level (NOEL). This level has been defined (WHO, 1990) as the highest dose of a substance

that causes no changes distinguishable from those observed in normal (control) animals. The no-observed-adverse-effect level (NOAEL) is the highest dose of a chemical at which no toxic (i.e., adverse) effects are observed (WHO, 1990). Whether a NOEL or NOAEL is used will depend on technical policy considerations in different regulatory agencies. Sometimes, there will also be differences of scientific opinion about whether a particular finding in a toxicology study is necessarily *adverse*. In reviewing the toxicity of a particular chemical, it is customary to set a NOEL or NOAEL for each repeat-dose toxicology study conducted on the chemical.

1.4.1.2 Acceptable Daily Intake The WHO, in order to assess health risks from chemicals, has established ADIs for pesticides (WHO, 1962). For each pesticide, an exposure level representing an acceptable/minimal risk for the human population has been set by the JMPR, namely, ADI, which is "the daily intake of a chemical that, if ingested over a lifetime, appears to be without appreciable risk." ADI values, expressed in mg/kg of body weight per day, are calculated from NOEL values divided by safety factors (SF) according to the following equation:

$$\text{ADI} = \text{NOEL or NOAEL/SF}$$

Safety factors are not rigidly defined and can vary from 100 to 2000, depending on the supporting toxicological database. When NOELs are based on studies in animals, the usual safety factor used to derive an ADI is 100, made up of a factor of 10 for interspecies extrapolation and an extra factor of 10 to allow for variations between individuals in human populations. Lists of internationally accepted ADIs are published by the International Programme on Chemical Safety (IPCS) of the WHO.

Although it is commonly taken to be synonymous with the *ADI*, the US reference dose (RfD) is distinctly defined (Dourson and De Rosa, 1991, Dourson et al., 1985). Developed by the US EPA for the assessment of risks associated with systemic toxicity, the RfD is an estimate of a daily exposure to the human population, including sensitive subgroups, that is likely to be without appreciable risk of deleterious effects during a lifetime (Hamilton and Crossley, 2004). However, it does not assume that all doses below the RfD are *acceptable* (or risk-free), nor that all doses that exceed the RfD are necessarily *unacceptable* (i.e., result in adverse effects). The equation for its derivation is as follows:

$$\text{RfD (mg/kg bw/d)} = \text{NOAEL/UF} \times \text{MF},$$

where UF is an uncertainty factor and MF is a modifying factor.

The ADI and RfD are quite similar, as it is referred in the relevant US EPA Background Document (Hamilton and Crossley, 2004).

1.4.1.3 Acute Reference Dose The need to consider the acute effects of pesticide residue intake has been acknowledged for many years, and the concept of the ARfD was developed by the JMPR in 1994. Since then, there has been a progressive increase in the establishment of ARfDs for particular pesticides to address potential exposure to residues in food and drinking water at relatively higher doses for short-term periods, due to accidental or incidental events. The JMPR has continuously updated its procedure on the setting of ARfDs.

The ARfD is defined as "an estimate of the amount of a substance in food or drinking water, normally expressed on a body weight basis that can be ingested in a period of 24 h or less without appreciable health risks to the consumer on the basis of all known facts at the time of the evaluation."

The JMPR has recently developed a guidance on the setting of ARfD for pesticides, which was published as a scientific publication in a peer-reviewed journal (Solecki et al., 2005). The new WHO guidance on the setting of ARfDs builds upon existing guidance developed by the JMPR and by national regulatory authorities and provides a comprehensive guide on the process of selecting and evaluating appropriate toxicological endpoints for acute guidance values. The guidance presents a stepwise approach on the derivation of ARfDs and provides specific guidance on relevant toxicological endpoints, namely, hematotoxicity, immunotoxicity, neurotoxicity, liver and kidney toxicity, endocrine effects, and developmental effects.

1.4.2 Ecological Risk Assessment for Pesticides

In the ecological risk assessment, the harmful ecological effects may be caused after exposure to one or more pesticides is evaluated. The effects can be direct (e.g., fish die from a pesticide entering waterways) or indirect (a hawk becomes sick from eating a mouse dying from pesticide poisoning) (US EPA, 2004). An ecological risk assessment employs the most current scientific methods to determine if a pesticide meets the requirements for registration and will not significantly harm wildlife.

The studies used by the US EPA for ecological risk assessments define the chemical properties of the pesticide, how the pesticide behaves in the environment, and its impact on plants and animals not targeted by the pesticide (US EPA, 2004). The Ecological Data Requirements of the US EPA for Pesticide Registration are described as follows:

- Wildlife and aquatic organisms: How the pesticide affects various animal species
- Plant protection: How the pesticide affects various plant species
- Nontarget insect: How the pesticide affects insects other than the ones the pesticide is intended to kill

- Environmental fate: What happens to the pesticide in soil, water, and air after being released into the environment
- Residue chemistry: How much pesticide remains after application over time
- Spray drift: How much the pesticide drifts off-site when sprayed from the air (exposure of no-target organisms)

Within the EU, the current pesticide risk assessment is performed on one hand under Regulation (EC) 1107/2009, dealing with issuing plant protection products to the market (Regulation (EC) No 1107/2009), together with Directive 2009/128/EC, for the *sustainable use* (Directive 2009/128/EC) and on the other hand the Water Framework Directive (WFD) (Directive 2000/60/EC). Conceptually very different, these texts might lead to contradictions. The WFD addresses both short-term risks, expressed as maximum acceptable concentrations environmental quality standards (MACs-EQSs), and long-term risks, expressed as annual average environmental quality standards (AA-EQSs), and does not follow a tiered approach. Pesticide Regulation (EC) 1107/2009 also distinguishes short- and long-term risks but follows a tiered approach (Brock et al., 2011).

In the EU, the environmental risk assessment of a chemical substance is performed by comparing the predicted environmental concentration (PEC) with the predicted no-effect concentration (PNEC) (Technical Guidance Document, 2003).

The PEC is an indication of the expected concentration of a substance in the environment, taking into account the amount initially discharged to the environment, its distribution, and the probable methods and rates of environmental degradation and removal, either forced or natural. The PNEC is the concentration below which exposure to a substance is not expected to cause adverse effects to species in the environment.

If the PEC>PNEC, this indicates that adverse effects may be caused by the substance in the environment. The PEC/PNEC risk ratios above one would trigger the inclusion of substance into monitoring program and the derivation of legally binding thresholds, its EQSs.

Considering the vast number of existing pesticides produced mainly at industrial scale, surface water pollution, caused through emissions of manufacture facilities, presents a threat to the aquatic environment with effects such as acute and chronic toxicity to aquatic organisms, accumulation in the ecosystem, and loss of habitats and biodiversity, as well as a threat to human health (von der Ohe et al., 2011).

1.4.2.1 Endocrine Disruptors Several decades ago, researchers began expressing their concern for not desirable estrogenic effects of environmental xenobiotic chemicals (Kavlock et al., 1996, US EPA, 1997). In addition to potential human health effects, reports have accumulated that many chemicals released into the environment can disrupt normal endocrine function in a variety of aquatic life and wildlife. Some of these effects observed in animals have been attributed to some persistent organic chemicals such as polychlorinated biphenyls (PCBs), DDT, and dioxin; pharmaceuticals, personal care products; and some pesticides, for example, vinclozolin (Martinovic-Weigelt et al., 2011). Adverse effects include abnormal thyroid function and development in fish and birds; decreased fertility in shellfish, fish, birds, and mammals; decreased hatching success in fish, birds, and reptiles; demasculinization and feminization of fish, birds, reptiles, and mammals; defeminization and masculinization of gastropods, fish, and birds; decreased offspring survival; and alteration of immune and behavioral function in birds and mammals. It has been proposed that the above adverse effects may be due to an endocrine-disrupting mechanism.

The mechanism of endocrine disruption includes various pathways. Some chemicals mimic a natural hormone, fooling the body into overresponding to the stimulus (e.g., a growth hormone that results in increased muscle mass) or responding at inappropriate times (e.g., producing insulin when it is not needed). Other endocrine-disrupting chemicals (EDCs) block the effects of a hormone from certain receptors (e.g., growth hormones required for normal development). Some other chemicals directly stimulate or inhibit the endocrine system and cause overproduction or underproduction of hormones (e.g., an over- or underactive thyroid). Recent publications (EEA, 2012, Kortenkamp et al., 2011, WHO/UNEP, 2013) have analyzed in detail the evidence for endocrine disruption in humans, wildlife, and animal models.

According to WHO/IPCS 2002 (WHO 2002) definition:

> An endocrine disrupter (ED) is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations.

Recently, the International Endocrine Society published a statement of principles on endocrine disruptors (ED) and public health protection in which another definition of an ED has been proposed (Zoeller et al., 2012):

> An ED is an exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action.

National and international authorities are in the process of establishing testing programs and strategies to assess the safety of currently used chemicals with regard to their potential to interact with the endocrine system of human and wildlife, resulting in potential impacts on reproduction, growth, and/or development (WHO, 2002).

One of the leading international programs is the Endocrine Disruptor Screening Program (EDSP) of the US EPA, which employs a battery of *in vitro* and *in vivo* screening assays to

assess the ED potential of a chemical (www.epa.gov/endo). The focus of this two-tiered program has been first to develop *in vitro* and *in vivo* assays to identify and classify substances relative to their potential interaction with the endocrine system (Tier 1) and then to develop concentration–response relationships in animal models (Tier 2). The EDSP Tier 1 battery consists of 11 diverse yet complementary *in vitro* and *in vivo* screening assays as recommended by the FIFRA SAP (SAP, 2008).

In 2009, the EPA announced the list of the first group of chemicals for screening in the agency's EDSP based on the approach focused on human exposure-related factors rather than a combination of exposure- and effect-related factors (US EPA, 2009). This *First List of Pesticide Chemicals for Initial Screening* in the EDSP included 67 pesticide active ingredients and high-production-volume (HPV) chemicals used as pesticide inert ingredients. Because this list of chemicals was selected on the basis of exposure potential only, it should neither be construed as a list of known or likely endocrine disruptors nor characterized as such. On November 17, 2010, the *Second List of Chemicals* for Tier 1 screening has been published. This list includes 134 chemicals and substances that have been listed as priorities within EPA's drinking water and pesticides programs under the Safe Drinking Water Act (SDWA). This EDSP is currently proceeding on developing and validating Tier 2 tests and selecting chemicals for screening and testing.

In 1999, the EU developed short-, medium-, and long-term strategies to address this issue. The short-term strategy of the EU was to compile a priority list of candidate substances for further evaluation of their ED properties (Commission of the European Communities, SEC, 2007). The first list included 575 substances, and their ED effects were investigated. The 320 substances showed evidence or potential evidence for ED effects. An assessment of the legal status of the substances with evidence or potential evidence of endocrine-disrupting effects showed that the majority of them are already subject to a ban or restriction or are addressed under existing community legislation, although for reasons not necessarily related to endocrine disruption.

The medium-term strategy of the EU was to fund the research projects linked to ED including studies in the area of human and wildlife health and development of test methods, strategies, and risks assessment tools (http://ec.europa.eu/research/endocrine). Furthermore, the EC has worked closely with its member states to coordinate the EU input into the OECD to develop internationally harmonized test methods and testing strategies for EDCs. Many of these efforts are still ongoing.

Long-term strategies of the EU are on the development and adaptation of legislative framework and actions that enable hazard identification, risk assessment, and risk management of EDCs. According to the EU's Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) legislation, no authorization shall be granted for pesticides with similar properties as the so-called substances of very high concern (SVHCs) in accordance with the new Regulation (EC) No 1907/2006 (Regulation (EC) No 1907/2006). Regarding wildlife, the new *endocrine cutoff criterion* states: "An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines, it is not considered to have endocrine disrupting properties that may cause adverse effects on non-target organisms unless the exposure of non-target organisms to that active substance in a plant protection product under realistic proposed conditions of use is negligible" (Regulation (EC) No 1107/2009). Thus, a paradigm shift in regulatory decision making was introduced for pesticides with endocrine properties: instead of the established risk-based approach, a hazard-based approach is now required (i.e., only the proven presence or assumed absence of endocrine-disrupting properties shall be decisive for a [non]authorization). However, specific scientific criteria to support regulatory decision making on substances with endocrine-disrupting properties both under REACH and the new pesticide regulation are lacking today and thus have to be developed and agreed upon (at least in the EU) within the near future.

Extensive efforts are currently underway at the EU and international levels to improve the quality, reliability, use, and integration of *in silico* tools. An example of the potential application of computational toxicology approach is discussed in a recent EFSA opinion on ED (EFSA, 2013a).

1.5 DIETARY EXPOSURE TO PESTICIDES

The risk assessment of dietary exposure to pesticides involves the following considerations: (i) which pesticides/in what foods/at what levels (monitoring programs), (ii) what amount of each food is consumed by the population (diet), and (iii) how the evaluation of the human exposure to chemical substances can be correlated with the toxicological studies in order to assess the risk.

Dietary exposure assessment combines food consumption data with data on the concentration of chemicals in food. The resulting dietary exposure estimate may then be compared with the relevant health-based guidance value for the food chemical of concern, if available, as part of the risk characterization. Assessments may be undertaken for acute or chronic exposures, where acute exposure covers a period of up to 24 h and long-term exposure covers average daily exposure over the entire lifetime.

The general equation for both acute and chronic dietary exposure would be expressed as follows:

$$\text{Dietary exposure} = \sum(\text{food chemical concentration} * \text{food consumption})/\text{body weight}$$

1.5.1 Acute Exposure or Short-Term Intake

Acute exposure or short-term intake for a pesticide is evaluated for each commodity by comparing the high intake and MRL with ARfD. International estimated short-term intakes (IESTIs) are assessed for a pesticide by the JMPR when it has established (or intends to establish) an ARfD. Exposure is calculated by multiplying the HR values by the highest 97.5th percentile of food consumed on a single day.

1.5.2 Chronic Exposure or Long-Term Intake

Chronic exposure or long-term dietary intake risk assessment for a pesticide is performed by comparing the average daily exposure with ADI. International estimated daily intakes (IEDIs) are assessed for a pesticide by the JMPR. Exposure is calculated by multiplying the median concentrations of residues by the average daily per capita consumption estimated for each commodity on the basis of the Global Environment Monitoring System (GEMS)/Food Consumption cluster diets. IEDIs are expressed as a percentage of the ADI for a 55 kg or 60 kg or 70 kg (EFSA, 2012) person, depending on the cluster diet.

1.5.3 Cumulative Exposure to Multiple Substances

The presence of more than one pesticide in the same product, although within acceptable limits, is of concern because the defining of the limits is based on the individual toxic effect of each pesticide, while possible interactions or additive effects are not considered. Despite the tremendous recent developments in toxicology, there are still gaps as far as the simultaneous effect of mixtures of toxic compounds on health is concerned. Due to the gaps and uncertainties, the whole issue of mixture toxicity for several decades was not covered by a general legislation, and so it was tackled on the basis of preventive approach: "food contamination should be limited to the lower possible level by the least possible number of chemicals."

Historically, the EPA has generally evaluated the safety of pesticides on the basis of single-chemical and single-exposure pathway scenarios. In 1993, a report by the National Research Council (NRC) made several recommendations on how to improve the assessment of health risks posed by pesticides in the diets of infants and children (NRC, 1993). One recommendation included consideration of all sources of dietary and nondietary exposures to pesticides and assessment of risks from exposure to multiple pesticides that cause a common toxic effect (an example was provided for five organophosphorus pesticides).

The Food Quality Protection Act (FQPA) of 1996 provides that when determining the safety of a pesticide chemical, the EPA shall base its assessment of the risk posed by the pesticide chemical on aggregate (i.e., total food, drinking water, residential, and other nonoccupational) exposure to the pesticide. The EPA is also required to consider available information concerning the combined toxic effects to human health that may result from dietary, residential, or other nonoccupational exposure to chemicals that have a common mechanism of toxicity.

A cumulative risk assessment begins with the identification of a group of chemicals, a common mechanism group (CMG), that induce a common toxic effect by a common mechanism of toxicity. The Office of Pesticide Programs (OPP) of the US EPA has developed a general framework for identifying the chemicals that belong in that group (US EPA, 1999). Once a CMG has been established, the next step is to evaluate registered and proposed uses for each CMG member in order to identify potential exposure pathways (i.e., food, drinking water, residential) and routes (i.e., oral, inhalation, dermal). During the hazard characterization phase, the various endpoints associated with the common mechanism of toxicity are identified, as well as the test species/sex that might serve as a uniform basis for determining relative potencies among the chemicals of interest. The common effect is also evaluated to determine if it is expressed across all exposure routes and durations of interest for each CMG member. The temporal aspects (e.g., time to peak effects, time to recovery) of the common mechanism of toxicity are characterized to determine the critical window of its expression.

For the first time, the EFSA performed an indicative cumulative risk assessment taking into account the results of the 2010 monitoring program with the purpose of exploring possible deficiencies in the monitoring data (e.g., if the level of detail of the data reported was sufficient) and other limitations, which may impede the practical implementation of the cumulative risk assessment methodologies currently under development (EFSA, 2013c).

Cumulative risk is assessed by combining the exposures of different compounds expressed as functions of their toxicities. A basic consideration in cumulative risk assessment is the identification of the cumulative assessment group (CAG), defined by the EFSA (2008) as a group of chemicals that could plausibly act by a common MoA, not all of which will necessarily do so. The EFSA (2008) described the methods by which toxicity from exposures to different substances in the same CAG can be combined in a cumulative assessment. In order of increasing complexity, this can be by using a hazard index (HI) or adjusted hazard index (aHI), a reference points index (RfPI), relative potency factors (RPF), or physiologically based toxicokinetic and toxicodynamic modeling approaches. The HI and aHI are sums of the ratios of the individual compound exposures to their respective toxicological reference values. The results of this cumulative risk assessment are presented in the annual report of EFSA (2013c).

1.6 PESTICIDE RESIDUES IN FOOD

1.6.1 Maximum Residue Limits

To ensure food safety for consumers and to facilitate international trade, international bodies have established *MRLs* for pesticide residues in food commodities, that is, "the maximum amount of pesticide residue and its toxic metabolites that might be expected on a commodity if good agricultural practice was adhered to, during the use of the pesticide."

Residue means one or more substances present in or on plants or plant products, edible animal products, drinking water, or elsewhere in the environment and resulting from the use of a pesticide, including their metabolites, breakdown or reaction products considered being of toxicological significance. *Metabolite* means any metabolite or a degradation product of a pesticide, formed either in organisms or in the environment. A metabolite is deemed relevant if there is a reason to assume that it has intrinsic properties comparable to the parent substance in terms of its biological target activity or that it poses a higher or comparable risk to organisms than the parent substance or that it has certain toxicological properties that are considered unacceptable (Directive 2009/128/EC, Regulation (EC) No 1107/2009).

According to the definition of the IUPAC, the MRL is the maximum concentration of a residue that is legally permitted or recognized as acceptable in, or on, a food, agricultural commodity, or animal feedstuff as set by the Codex or EU or a national regulatory authority. The term *tolerance* used in the United States is, in most instances, synonymous with MRL, normally expressed as mg/kg fresh weight (Holland, 1996).

It is important to note that the MRLs are not maximum toxicological limits. They are based on good agricultural practice, and foods derived from commodities that comply with the respective MRLs are intended to be toxicologically acceptable.

In principle, MRLs are set on the bases of the following (FAO, 2009):

a. Supervised agricultural residue trials.
b. Using appropriate consumer intake models.
c. Data from toxicological tests on the pesticide allow for the fixing of an *ADI*.
d. If the estimated daily consumer intake for all commodities calculated under (b) is lower than the ADI calculated under (c), then the residue level under (a) is set as the MRL.

For pesticides that are not still in use or have been banned, an *extraneous maximum residue limit (EMRL)* has been established (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor). *EMRL* is the maximum concentration of a pesticide residue arising from environmental sources including pesticides in which their uses have been nationally banned but, because of their persistent properties, the residues still exist in agricultural commodities.

The pesticide MRLs for foodstuff and feed were first established in 1961 by the Codex Alimentarius Commission (CAC) of FAO and WHO, which is responsible for setting MRLs (Codex MRLs (CXLs)) and EMRLs at a global level (Codex Alimentarius) (http://www.codexalimentarius.net). In the EU level, the commission is fixing MRLs for pesticides currently or formerly used in or outside the EU based on recommendations by the EFSA (EU MRL database; http://ec.europa.eu/sanco_pesticides/public/index.cfm). The US EPA establishes tolerances for pesticides that may be found on foods and can also revoke tolerances determined to be unsafe to public health and the environment. The list of tolerances and exemptions is compiled in the electronic Code of Federal Regulations (e-CFR, 2014).

The US National Pesticide Information Center (NPIC) has created an electronic tolerance search tool to help find tolerances for food published in the Code of Federal Regulations (CFR) available at http://npic.orst.edu/reg/tolerance.html.

In 2005, the OPP of the US EPA and Health Canada's Pest Management Regulatory Agency (PMRA) jointly released a spreadsheet to calculate statistically based MRLs or tolerances (EPA). The North American Free Trade Agreement (NAFTA) MRL Harmonization Working Group developed this spreadsheet to better coordinate the pesticide regulatory framework among NAFTA partners with the aim of minimizing trade barriers of pesticide-treated commodities between the United States and Canada. The last version of the NAFTA MRL Calculator was published in 2008.

With the aim of harmonizing the calculation of MRLs across the member countries of the OECD, in 2008, an expert group to propose a new MRL calculation procedure was commissioned. The members of this OECD MRL Working Group include some of the same individuals from the NAFTA MRL Harmonization Work Group, but also representatives from other international agencies responsible for regulating pesticides and other stakeholder OECD members. In November 2010, the Working Group on Pesticides (WGP) officially approved the OECD MRL Calculator, and in March 2010, it became available at http://www.oecd.org/env/ehs/pesticides-biocides/oecdmaximumresiduelimitcalculator.htm (OECD, 2011).

Both Health Canada's PMRA and EPA's OPP have agreed that the OECD MRL Calculator provides statistically robust and scientifically defensible MRLs. The OECD MRL Calculator has replaced and superseded the NAFTA MRL Calculator.

1.6.1.1 MRLs for Processed Food Changes to the levels and nature of residues in food can be also affected by

processing practices for food production. For example, fat-soluble pesticides tend to partition into the oil when oilseeds are processed. Water-soluble residues in grape have a higher possibility to reach wine than the water-insoluble compounds. The laboratory experiments on food processing studies can simulate the real commercial processing practices and evaluate the processing factors (PF) of pesticides. The Codex evaluates processing studies to derive PF used to estimate residue concentrations in processed foods and feeds for dietary risk assessments and, if necessary, recommends MRLs for processed foods and feeds (FAO, 2009).

The Codex Committee for Pesticide Residues (CCPR):

a. Establishes MRLs for important processed foods and feeds moving in international trade (e.g., Codex MRL of fenthion in olive oil = 1 mg kg^{-1})
b. Establishes MRLs for processed foods and feeds only if the resulting value is higher than the MRL established for the corresponding raw agriculture commodity (RAC), processing factor > 1.3 (PF > 1.3)
c. Continues the practice of establishing MRLs for processed foods and feeds where, due to the nature of the residues during some specific process, significant amounts of relevant metabolites appear or increase
d. Supports the current JMPR practice of evaluating all processing studies provided and including in each evaluation or review a summary table of all validated PF

Recently, the EU has started the procedure of evaluation of PF used to estimate residue concentrations in processed foods. The *Annex VI* of the EU Regulation (EC) No 396/2005 will include the list of conversion factors of MRLs for processed commodities. This annex has not been published yet.

One of the first established EU PF is the PF for virgin olive oil that was set at 5, taking into account an olive oil production standard yield of 20% of the olive harvest (Commission Regulation (EU) 1274/2011).

1.6.2 Residue Definition

The *MRL definition* (or *residue definition*) for a number of pesticides includes not only the parent compound but also its isomers, its metabolites, and/or its TPs considered *relevant* (*total MRL* or *MRL sum*). In the EU MRL Pesticides database (EU-MRL Pesticides database), 19% of the established MRLs include more than one compound in their definition. The three different cases of the total MRLs or MRL sum are the following:

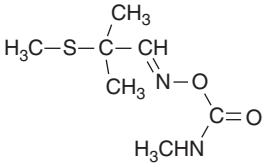
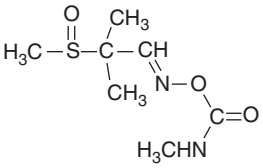
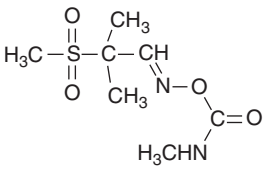
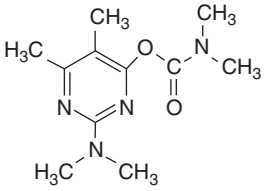
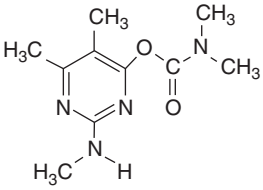
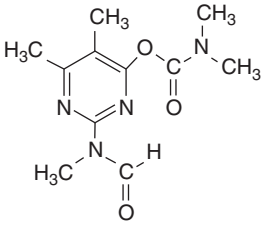
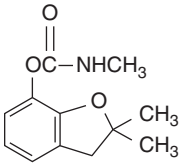
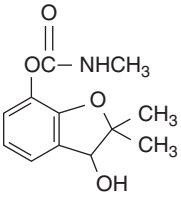
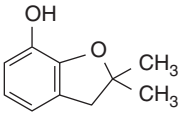
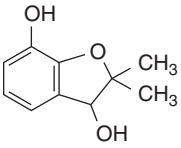
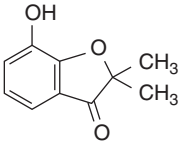
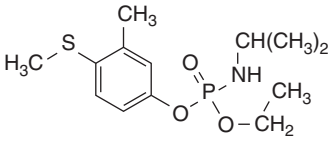
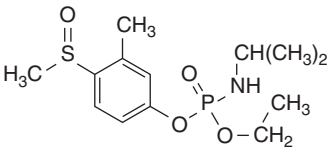
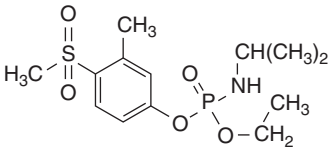
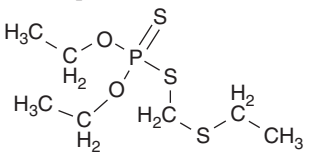
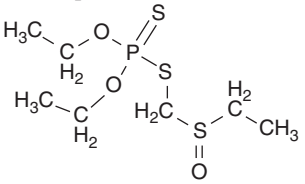
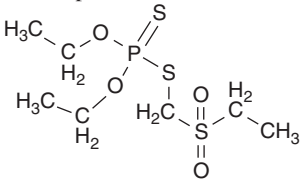
a. The total MRL definition including the parent pesticide and its salts, its esters, and its conjugates constitutes about 22% of the sum MRL cases. Pesticides belonging mainly to the aryloxyalkanoic acids group (2,4-D, 2,4-DB, MCPA, etc.), the aryl-phenoxypropionic herbicides (diclofop, haloxyfop, fluazifop, etc.), and the nitrile herbicides (bromoxynil, ioxynil) have a such MRL definition. Similar residue definitions can be found for these compounds in their Codex Alimentarius Residue Limits (CXLs) (Codex Alimentarius DB).
b. The total MRL definition consisting of the sum of the parent compound and its isomers covers about 27% of the *sum MRL* cases. These pesticides belong to the chemical groups of pyrethrins (cypermethrin, fenvalerate and esfenvalerate, permethrin), organochlorine insecticides (DDT, dicofol), anilide fungicides (benalaxyl, metalaxyl), and others. Similar residue definitions have been established for these groups of pesticides in their Codex Alimentarius Residue Limits (CXLs).
c. The total MRL definition involving the parent compound and its *relevant* metabolites covers about 51% of the sum MRL cases. Pesticides belonging to the chemical groups of organophosphates, carbamates, organochlorines, etc. have this type of MRL definition.

The metabolites of a certain pesticide included in the MRL definition were identified during the metabolic studies in controlled laboratory experiments, and this data is required for pesticide authorization. The mechanism of biotransformation and degradation of the parent compound in plants and animals is extensively studied before a new pesticide is placed on the market. When different pesticide metabolites have been identified in plants and animals, the MRL definition might include different metabolites dependent on the foodstuff origin.

The metabolic pathways and resulting metabolites of a pesticide depend strongly on its chemical nature and may be differentiated for compounds of the same chemical group; different metabolic pathways may dominate in each case. Consequently, the kind of metabolites included in the *residue* definition may be different for pesticides of the same chemical group. Examples of pesticides, parent compounds and metabolites included in their MRL definitions, are shown in Table 1.4.

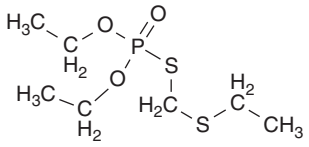
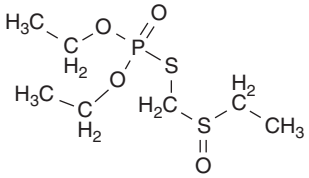
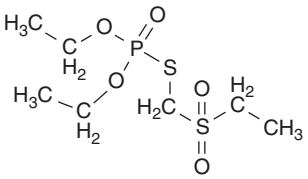
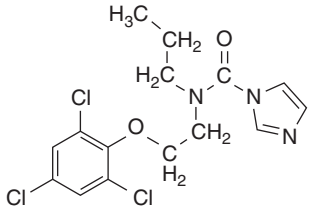
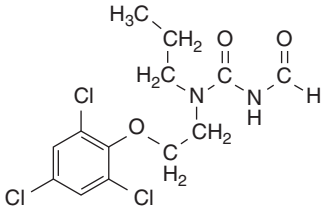
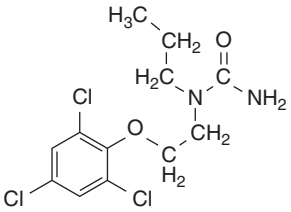
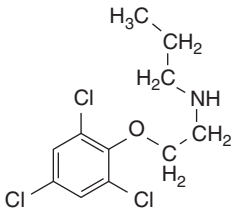
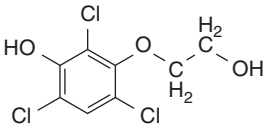
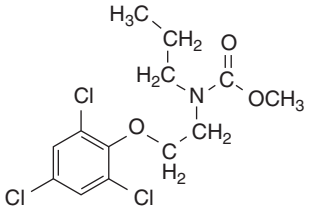
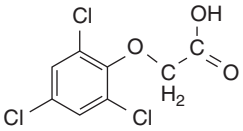
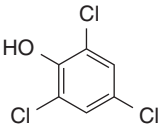
For example, EU MRL and Codex MRL definitions of the carbamate insecticide aldicarb include both the sum of the parent compound and its sulfoxide and sulfone derivatives, as shown in Table 1.4 (Regulation (EU) No 396/2005, Codex Alimentarius DB). The US EPA residue definition of aldicarb also includes its two metabolites, aldicarb sulfoxide and aldicarb sulfone. Aldicarb sulfoxide is considered

TABLE 1.4 Examples of pesticides and metabolites included in their residue definitions

Pesticides (chemical family)	Parent compound	Metabolites included in the residue definition	
Carbamates			
Oxime carbamates	Aldicarb	Aldicarb sulfoxide	Aldicarb sulfone
			
Dimethyl carbamates	Pirimicarb	Desmethyl-pirimicarb	Desmethylformamido-pirimicarb
			
Benzofuranyl methyl carbamates	Carbofuran	Carbofuran-3-OH	Carbofuran-phenol
			
		3-OH-carbofuran-phenol	3-Keto-carbofuran-phenol
Organophosphates			
Phosphoramidates	Fenamiphos	Fenamiphos sulfoxide	Fenamiphos sulfone
			
Organothiophosphates	Phorate	Phorate sulfoxide	Phorate sulfone

(*continued*)

TABLE 1.4 (*Continued*)

Pesticides (chemical family)	Parent compound	Metabolites included in the residue definition		
				
	Phorate-oxon	Phorate-oxon sulfoxide	Phorate-oxon sulfone	
Conazoles				
	Prochloraz	BTS 44596	BTS 44595	
				
		BTS 40348	BTS 54906	
				
		M590F040	BTS 9608	BTS 45186 or TCP

to have similar potency to the parent compound in terms of toxicity, while aldicarb sulfone is less potent (US EPA). The CXL definition of pirimicarb, a carbamate aphicide, integrates the parent compound, the desmethyl, and desmethylformamido metabolites (Table 1.4) for plant commodities, but the EU MRL includes the parent compound and its desmethyl metabolite. The EU MRL definition of carbofuran, another carbamate insecticide, in products of plant origin is the sum of carbofuran and 3-OH-carbofuran, expressed as carbofuran (Table 1.4). The CXL definition of carbofuran also includes the sum of carbofuran and 3-OH-carbofuran, expressed as carbofuran (Codex Alimentarius DB). The US EPA tolerances are established for the combined residues of carbofuran and its metabolites, 3-OH-carbofuran, carbofuran-phenol, 3-OH-carbofuran-phenol, and 3-keto-carbofuran-phenol, in or on the various raw agricultural commodities (National Pesticide Information Center, NPIC).

The MRL definition of OP compounds commonly consists of the parent compound and the sulfoxide and

sulfone metabolites as in the case of fenamiphos (Table 1.4), while the MRL definitions of fenthion and phorate include also their oxon analogues derived through oxidative desulfurization (Table 1.4). The EU and Codex MRLs share common residue definitions for these pesticides.

The EU MRL definition for the pesticides amitraz, flufenacet, and prochloraz does not include certain metabolites but the sum of metabolites containing a common chemical moiety in their molecules. The residue definition of amitraz includes the metabolites containing the 2,4-dimethylaniline moiety, while for flufenacet, all the compounds containing the *N*-fluorophenyl-*N*-isopropyl moiety are included in its *residue* definition. The MRL definition of prochloraz is defined both by the EU and Codex as "the sum of prochloraz and its metabolites containing the 2,4,6-trichlorophenol moiety" for plant and animal commodities (Regulation (EU) 396/2005). The chemical structures of prochloraz and relevant metabolites are shown in Table 1.4. For risk assessment, it was agreed by the EFSA experts to define the residue as the "sum of prochloraz and its metabolites containing the 2,4,6-TCP moiety, expressed as prochloraz." For monitoring, the EFSA experts proposed to define the residue as the "sum of prochloraz and its metabolites BTS 44595 and BTS 44596, expressed as prochloraz" since BTS 44595 and BTS 44596 were the only available analytical standards at that time (EFSA, 2011). The noninclusion of BTS 40348 in the residue definition proposed by the EFSA leads to a strong deviation from the currently existing EU residue definition of prochloraz (the sum of prochloraz and its metabolites containing the 2,4,6-trichlorophenol (TCP) moiety), according to which a hydrolysis step is required to transform prochloraz and its metabolites to TCP prior to their determination, as TCP, by using GC–MS. Recently, an alternative analytical approach has been proposed by the European Reference Laboratory for Single Residue Methods (EURL-SRM) based on the LC–MS/MS analysis of the parent compound and its metabolites BTS 44595, BTS 44596, BTS 40348, BTS 9608, and TCP. The prochloraz metabolite, BTS 40348 (*N*-propyl-*N*-2-(2,4,6-trichlorophenoxy) ethyl amine), has been identified in citrus extracts using LC–TOF-MS and ion trap tandem mass spectrometry (Thurman et al., 2005). The Codex residue definition of imidacloprid, a widely applied neonicotinoid insecticide, is defined as the "sum of imidacloprid and its metabolites containing the 6-chloropyridinyl moiety, expressed as imidacloprid" for plant and animal commodities, both for compliance with the CXL and for estimation of dietary intake, while the EU MRL definition includes only the parent compound. A great number of compounds containing the 6-chloropyridinyl moiety have been identified as imidacloprid metabolites (Casida, 2011, Thurman et al., 2013).

In the EU legislation (Regulation (EU) 396/2005), MRL definitions for 40 compounds, recognized with the symbol (R), are differentiated between foods of plant and animal origin.

The different MRL definitions are grouped in the following categories:

- The EU MRL definition of pesticides, listed in Table 1.5, in food commodities of animal origin includes more metabolites than in foods of plant origin. The MRL of propyzamide in foods of plant origin includes only the parent compound, while the residue definition in foods of animal origin includes the parent compound and all the metabolites shown in Table 1.5. Similarly, the MRL of acetamiprid in foods of animal origin includes the parent compound and the metabolite *N*-desmethyl-acetamiprid (IM-2-1) (Table 1.5), whereas only acetamiprid is included in the EU MRLs for foods of plant origin. Similar CXL definitions have been set by the Codex for acetamiprid for foods of plant and animal origin.
- The MRL definition for food commodities of animal origin includes less and/or completely different metabolites than for foods of plant origin. For example, the MRL of flonicamid, a neonicotinoid insecticide, for foods of animal origin includes the parent compound and the metabolite TFNA-AM, whereas the parent compound and the metabolites TNFG and TNFA are included in the MRL definition for foods of plant origin. In the MRL definition of spirotetramat, the ketohydroxy, monohydroxy, and enol metabolites and the enol-glucoside conjugate are included along with the parent compound, based on the metabolic pathway observed in all plant groups tested (EFSA, 2013b, Regulation (EU) 396/2005). In that case, the *residue* definition encompasses not only metabolites of the parent compound but also the glucoside conjugate of the enol metabolite of the parent compound. Conversely, the MRL definition of spirotetramat for foods of animal origin includes the parent compound and the enol metabolite. The US EPA tolerances are similarly established for residues of the insecticide spirotetramat, including the same metabolites and degradates in or on various food commodities, calculated as the stoichiometric equivalent of spirotetramat (NPIC).
- Only metabolites are included in the MRL definition in foods of animal origin, as for the pesticides listed in Table 1.6, while the parent compound is not included in the MRL definition. For example, in the case of spiroxamine, its MRL includes spiroxamine carboxylic acid expressed as spiroxamine, whereas the MRL of kresoxim-methyl includes two metabolites (Table 1.6).

TABLE 1.5 Examples of pesticides with different EU MRL definitions in foods of plant (p) and animal (a) origin

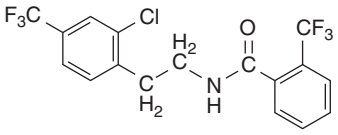
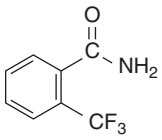
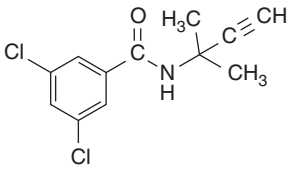
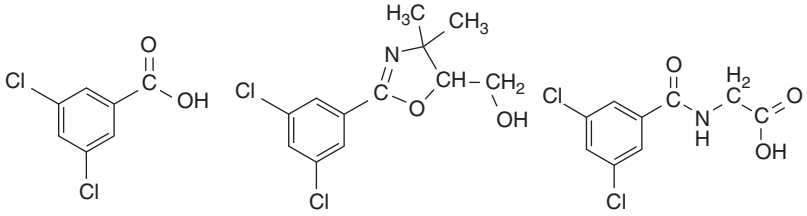
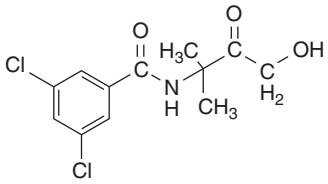
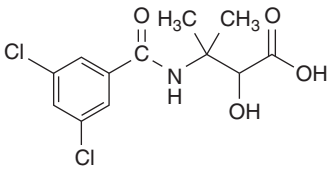
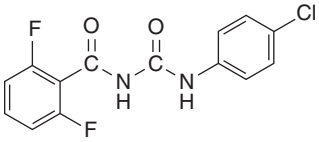
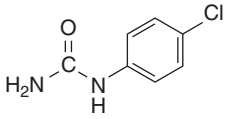
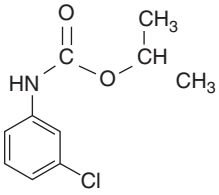
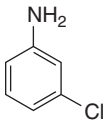
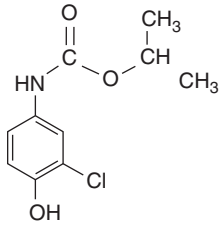
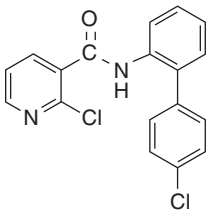
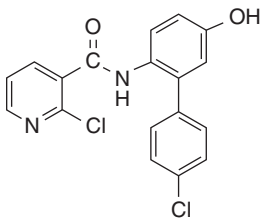
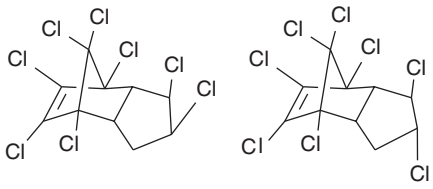
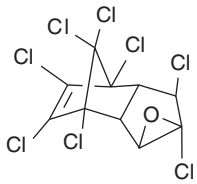
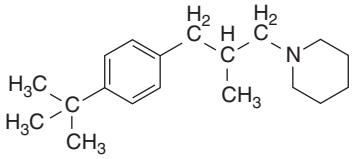
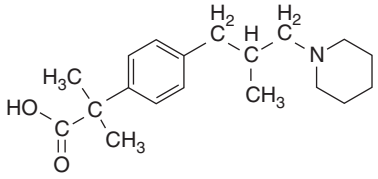
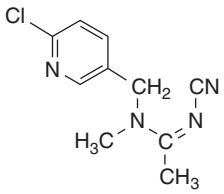
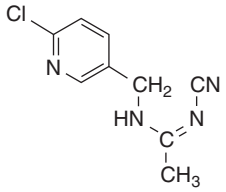
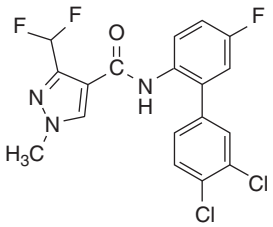
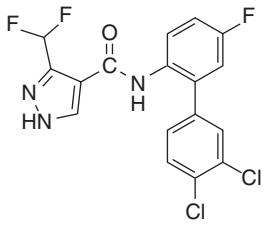
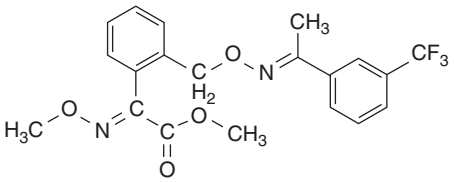
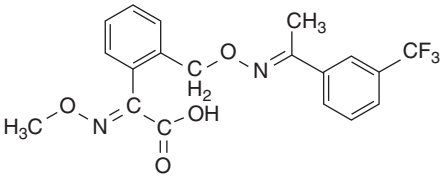
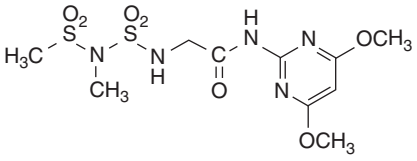
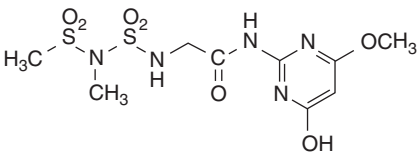
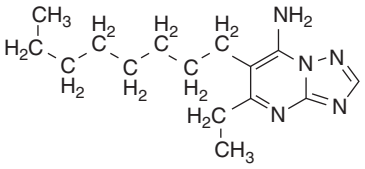
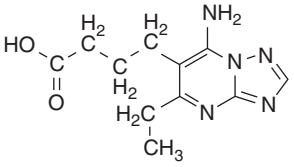
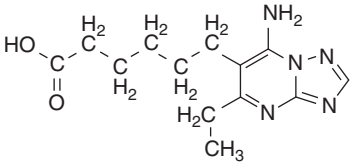
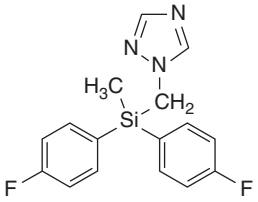
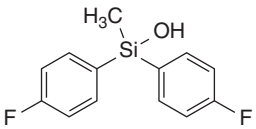
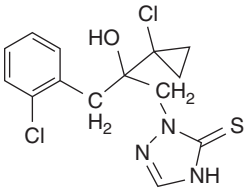
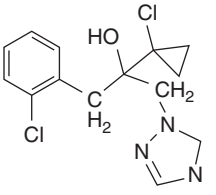
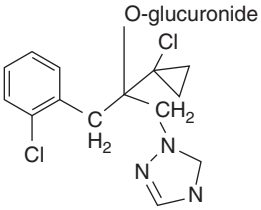
Pesticides (chemical classes)	Parent compound	Metabolites included in the residue definition of foods of animal origin	
Benzamides			
	Fluopyram (p, a)	2-(Trifluoromethyl)benzamide (a)	
			
	Propyzamide (p, a)	(I) 3,5-Dichlorobenzoic acid (DCBA) (a) (II) (2-(3,5-Dichlorophenyl)-4,4-dimethyl-4,5-dihydrooxazole-5-yl) methanol (a) (III) 2-(3,5-Dichlorobenzamido) acetic acid (a)	
			
		3,5-Dichloro-N-(4-hydroxy-2-methyl-3-oxobutan-2-yl)benzamide (a)	3-(3,5-Dichlorobenzamido)-2-hydroxy-3-methylbutanoic acid (a)
Benzoylureas			
	Diflubenzuron (p, a)	4-Chlorophenylurea (CPU) (a)	
Carbamates			
	Chlorpropham (p, a)	3-Chloro-aniline (p)	4′-Hydroxychlorpropham-O-sulfonic acid (4-HSA) (a)

TABLE 1.5 (*Continued*)

Pesticides (chemical classes)	Parent compound	Metabolites included in the residue definition of foods of animal origin
Carboxamides		
	Boscalid (p,a)	2-chloro-N-(4′-chloro-5-hydroxy-[1,1′-biphenyl]-2-yl) nicotinamide (M5110F01), free and conjugated (a)
Organochlorines		
	Chlordane (*cis*- and *trans*-isomers) (p, a)	Oxychlordane (a)
Morpholine Piperidines		
	Fenpropidin (p, a)	2-methyl-2-(4-(2-methyl-3-(piperidin-1-yl)propyl)phenyl)propanoic acid (CGA 289267) (a)
Neonicotinoids		
	Acetamiprid (p, a)	*N*-desmethyl-acetamiprid (a)
Pyrazoles		
	Bixafen (p, a)	Bixafen-desmethyl (a)

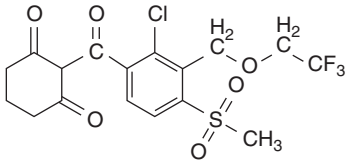
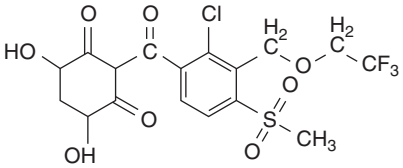
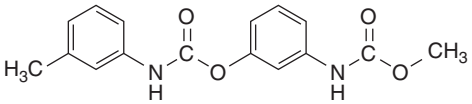
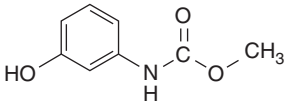
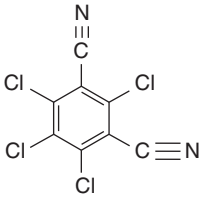
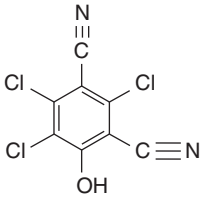
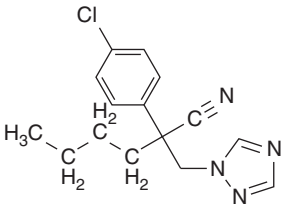
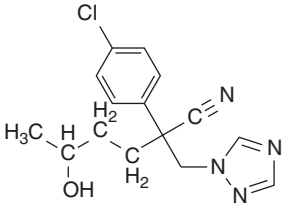
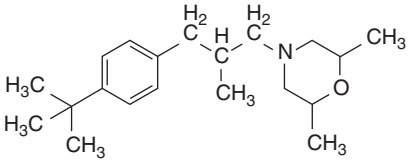
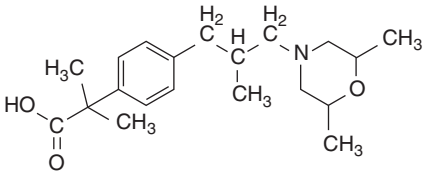
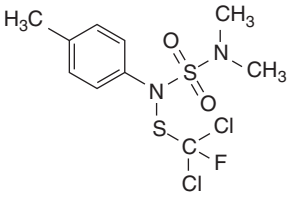
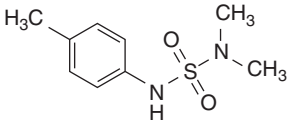
(*continued*)

TABLE 1.5 (*Continued*)

Pesticides (chemical classes)	Parent compound	Metabolites included in the residue definition of foods of animal origin	
Strobilurins			
	Trifloxystrobin (p, a)	(E)-2-(methoxyimino)-2-(2-((((E)-(1-(3-(trifluoromethyl)phenyl) ethylidene) amino) oxy) methyl) phenyl) acetic acid (a)	
Sulfonylureas			
	Amidosulfuron (p, a)	N-(4-hydroxy-6-methoxy pyrimidin-2-yl)-2-((N-methyl-N-(methylsulfonyl) sulfamoyl) amino) acetamide (AEF 101630) (a)	
Triazole pyrimidines			
	Ametoctradin (p, a)	4-(7-amino-5-ethyl-[1,2,4] triazolo[1,5-a]pyrimidin-6-yl)butanoic acid (M650F01) (a)	6-(7-amino-5-ethyl-[1,2,4] triazolo[1,5-a]pyrimidin-6-yl) hexanoic acid (M650F06) (a)
Triazoles			
	Flusilazole (p, a)	Bis(4-fluorophenyl) (methyl)silanol (a)	
			
	Prothioconazole	Prothioconazole-desthio (p, a)	Prothioconazole-desthio-glucuronide (a)

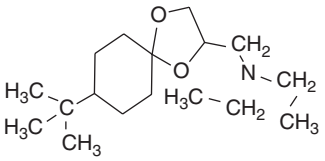
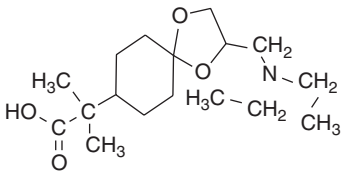
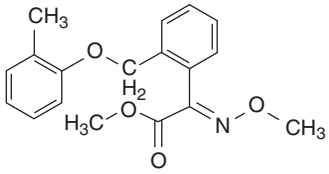
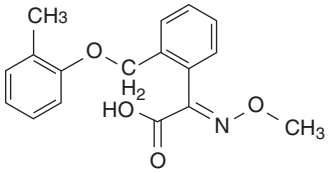
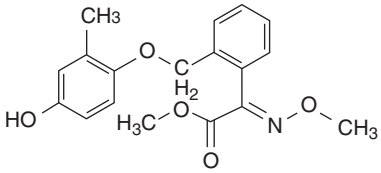
(p), plant; (a), animal.

TABLE 1.6 Pesticides with EU MRL definitions in foods of animal origin including only metabolites

Pesticides (chemical classes)	Parent compound	Metabolites included in the residue definition in foods of animal origin
Benzoyl cyclohexanediones		
	Tembotrione (p)	Tembotrione-dihydroxy (a)
Carbanilates		
	Phenmedipham (p)	methyl (3-hydroxyphenyl)carbamate (a)
Chloronitriles		
	Chlorothalonil (p)	2,4,5-trichloro-6-hydroxyisophthalonitrile (a)
Conazoles		
	Myclobutanil (p)	2-((1H-1,2,4-triazol-1-yl)methyl)-2-(4-chlorophenyl)-5-hydroxyhexane nitrile (RH 9090) (a)
Morpholines		
	Fenpropimorph (p)	Fenpropimorph carboxylic acid (a)
Phenylsulfamides		
	Tolylfluanid (p)	DMST (a)

(*continued*)

TABLE 1.6 (*Continued*)

Pesticides (chemical classes)	Parent compound	Metabolites included in the residue definition in foods of animal origin	
Spiroketalamines			
	Spiroxamine (p)	Spiroxamine carboxylic acid (a)	
Strobilurins			
	Kresoxim-methyl (p)	(E)-2-(methoxyimino)-2-(2-((*o*-tolyloxy)methyl)phenyl)acetic acid (a)	(E)-methyl 2-(2-((4-hydroxy-2-methylphenoxy)methyl) phenyl)-2-(methoxyimino) acetate (a)

(p), plant; (a), animal.

- The MRL definition for bentazone, fluroxypyr, and phosmet in foods of animal origin includes only the parent compound contrary to their MRLs for foods of plant origin where the parent compound is not included.
- The MRL of carbendazim includes carbendazim and benomyl in foods of plant origin where the respective MRL for foods of animal origin includes carbendazim and thiophanate-methyl.

1.6.3 Reporting of Results

Whenever reporting a concentration result value for a pesticide, its residue definition should be taken into account. In the case of multicomponent pesticide residue definitions, the total concentration is obtained by applying conversion factors, for each one of the components in the definition, by different ways, depending on the residue definition.

The EU Multiannual Control Program for pesticide residues in food of plant and animal origin, for the years 2012, 2013, and 2014, includes some pesticides with multicomponent residue definitions amenable by multiresidue methods (Commission Regulation (EU) 788/2012). The European Union Reference Laboratories (EURLs) in order to help the participant laboratories to apply properly conversion factors for calculation of the concentration of the multicomponent pesticide residue definitions in food have created an e-learning tool, which is available at www.eupt.es/e-learning.

When the residue definition is "the sum of the parent compound and its transformation products expressed as parent compound," the concentrations of the TPs should be adjusted according to their molecular weight (conversion factor Cf = MWparent/MWmetabolite) being added to the total residue concentration (SANCO/12571/2013).

For example, the MRL definition for the insecticide fenthion includes its sulfoxide and sulfone metabolites and their oxygen analogues (oxons), and all these compounds (Fig. 1.4) should be included in the residue analysis.

The total concentration of fenthion ($C_{\text{FenthionSum}}$) is calculated by the following equation:

$$C_{\text{FenthionSum}} = 1.00 \times C_{\text{Fenthion}} + 0.946 \times C_{\text{FenthionSO}} + 0.897 \times C_{\text{FenthionSO}_2} + 1.06 \times C_{\text{Fenthionxon}} + 1.00 \times C_{\text{FenthionoxonSO}} + 0.946 \times C_{\text{FenthionoxonSO}_2}$$

1.6.4 Residue Analysis

The diversity of pesticides, their metabolites, and TPs produced in the different food matrices is an issue of major concern for laboratories participating in the monitoring programs aiming to ensure food safety. The large number of pesticides to be monitored, parent compounds, and metabolites requires

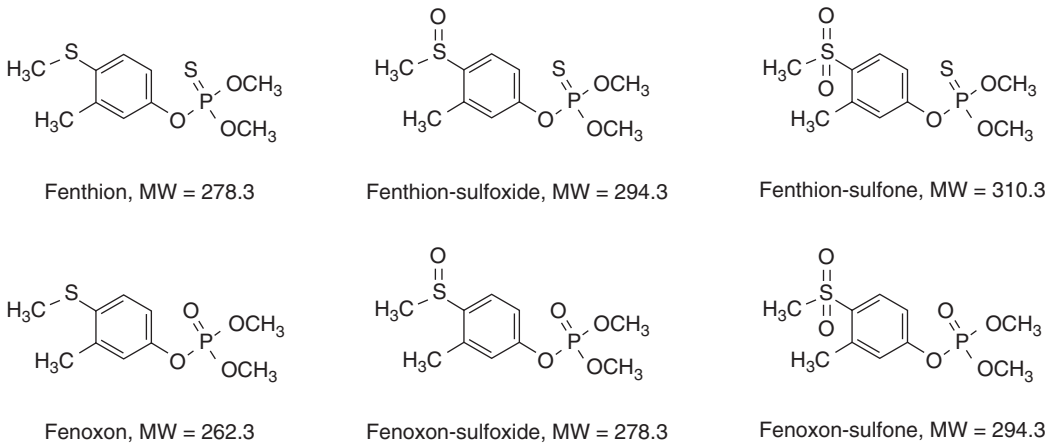


FIGURE 1.4 MRL definition for fenthion, parent compound, and metabolites.

sensitive and selective multiresidue methods for their identification and quantification. International and national authorities in order to assist official laboratories in addressing an effective pesticide residues analysis have published detailed analytical methods to be used for food and environmental control (US EPA, FDA Manual, FSSAI, 2012). The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has published a database of detailed analytical methods for the analysis of food contaminants and pesticides (Food Contaminant and Residue Information System (FCRIS) Pesticide Methods Database) that included several commonly used multiresidue methods and a Pesticide Attributes Database (FCRIS-PAD) for food safety/environmental laboratories. Recent regulations and guidelines on food and environmental analysis especially in the EU and the United States (European Commission DG-SANCO, No 12571/2013, FDA, 2003) require the screening for pesticides using confirmatory techniques, such as GC–MS and LC–MS.

The determination of pesticides and their metabolites included in their residue definition is usually performed by target analysis using gas chromatography–tandem mass spectrometry (GC–MS/MS) or liquid chromatography-tandem mass spectrometry LC–MS/MS with triple quadrupole (QqQ) analyzers due to their high sensitivity, selectivity, and specificity (Fernández-Alba and García-Reyes, 2008, García-Reyes et al., 2007, Hernández et al., 2008). A number of methodologies presented over the last years, based on LC–QqQ-MS, are dealing with the simultaneous trace analysis of hundreds of pesticides and their TPs in food products (Botitsi et al., 2011). Recently, a fully automated system was developed using two-dimensional LC–MS/MS for the determination of more than 300 pesticides (parent compounds and TPs) in and on various food commodities (Kittlaus et al., 2013).

HRMS instruments offer several advantages for the analysis of pesticides and their metabolites such as high resolution, high mass accuracy, and full-scan spectrum acquisition. TOF-MS and Orbitrap platforms enable the screening of the sample for a great number of pesticides and metabolites; the data file of each sample can be examined for a theoretically unlimited number of compounds and/or even reprocessed a posteriori for additional compounds (Gómez-Ramos et al., 2013, Hernández et al., 2008, Lacina et al., 2010, Mol et al., 2012, Polgár et al., 2012).

Advances in MS instrumentation in the forthcoming years are expected to provide a fast, reliable, and effective screening of food samples for a large number of pesticides and their metabolites to ensure food safety.

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