

Pesticide residue monitoring in the European Union Agricultural Sector via modern analytical techniques.

A review on Organophosphates

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Abstract — Organophosphate exposure, via food products circulated within the EU member states, is monitored by various researchers and the results are provided to their corresponding national authorities or to official European monitoring bodies. Different analytical methods for the detection of pesticide residues in food products are applied, although the most preferable method used lately in private or educational laboratories is the QuEChERS method (a solid phase extraction technique) whereas the ultra-high pressure liquid chromatography (UHPLC) system is gradually gaining ground in regard to pesticide residue trace analysis. This review focused on analyzing, from collected published data, the preferred methods for the detection of organophosphate pesticide residues in food products from the European Union Agricultural Sector. Even though a European legislation exists and applies strict guidelines, regulations and even bans, in order to reduce the use of persistent pesticides and to encourage the development of target specific pesticides, this review shows the current state for monitoring and detecting pesticide residues in general, as well as their possible drawbacks and possible active solutions for pesticide monitoring in today's agriculture sector. In addition, results in the existing literature

are sufficient to demonstrate the difference in efficiency for monitoring and detecting organophosphate pesticide residues, however, more studies are needed to evaluate the available analytical techniques so as to strengthen the existing literature and to confirm the existing data.

Keywords — pesticides, organophosphates, residual analysis, agricultural sector, European Union

I. INTRODUCTION

Agricultural products are produced throughout the world, providing the basis of human nutrition with vitamins, minerals and fibers [1], while many national economies depend on agricultural practices. Yesterday's agriculture was based on conventional cultivation methods, and focused only on mass production, without quality control schemes and insufficient control measures. These cultivation methods act like a chain, starting with producers experiencing continuous fluctuations and instability in their production and hence, their income, next the consumers are not able to distinguish if a product falls within the acceptable safety region, its quality or its origin, and finally the increased risk to human health and the

environment as shown from the following years of such practices [2, 3, 4]. Methods of agriculture, however, have changed considerably over the last decades. The industrial takeover produced monocultured agricultural production in developed countries, with local economies evolving into global economies and focused more on qualitative production than quantitative. The introduction of greenhouse cultivation, mainly in the Mediterranean region, offered a “controlled environment” in order to produce specific products otherwise not possible. The introduction of a wide variety of pesticides for all types of treatment, fertilizers for all types of deficiencies, agricultural tools and instruments and new irrigation systems, gave a new impulse to the agriculture sector, increasing gradually the agricultural income, but at the same time, introducing new or recurring problems (contaminated soil and ground water, polluted air, food-borne illness, toxic chemicals in foods, animal feed and fiber) both in the rural exploitation and in the wider environment, and consequently in the social structure [5]. One of the main reasons this occurs is due to the thoughtless and reckless use of pesticides from non-trained farm producers.

II. METHODOLOGY OF THE REVIEW

In this review, a significant number of European (EU) studies published on the detection of pesticide residues in food produce were collected. Studies that did not measure specifically organophosphate levels via an analytical technique were excluded. Food description, sample preparation, detection apparatus and method validation characteristics are parameters taken into account for studies considered. The importance of this review is to present the ongoing status of the detection of pesticide residues, focusing primarily on organophosphates, based on ongoing practices and their retrospective EU legislations.

III. EU REGULATIONS AND DIRECTIVES

In Europe, certain legislations, regulations and directives have been established by the European Parliament focusing on: placing of plant protection products on the market [Regulation (EC) No 1107/2009], maximum residue levels of pesticides in or on food, and feed of plant and animal origin [Regulation (EC) No 396/2005]; laying down rules for making available on the EU market fertilizer products [Regulation (EU) 2019/1009] and biocidal products [Regulation (EU) No 528/2012]; and establishing a framework for Community action to achieve the sustainable use of pesticides (Directive 2009/128/EC) [6, 7, 8, 9]. Pesticide monitoring reports are published for all European countries every year [10]. Additionally, European Community’s Rapid Alert System for Food and Feed [11], publishes weekly overviews of alerts and information notifications on its website, with chemical hazards being one of the reported hazard categories. Additionally, the European Commission publishes EU statistics on pesticide sales in regard to geographical location, year, unit of measure and pesticide groups, and an updated EU pesticide database that categorizes products/active substances as “approved” or “not approved”.

IV. PESTICIDE USAGE AND HEALTH EFFECTS

The basic agricultural pesticide categories, based on plant protection activity, are insecticides (insects), fungicides (fungi) and herbicides (weeds). The most common non-organic insecticides applied are

organochlorines, organophosphates, carbamates, neonicotinoids and synthetic pyrethroids. Although specific categories have been banned in EU countries (e.g. organochlorines, and recently specific organophosphates).

Even though pesticides are designed to target specific organisms, this is not guaranteed. Chemical structures differ in both within and between these categories and their induced modes of action cause collateral damage to unwanted species. This can lead to an abrupt change in the ecosystem, destabilizing the natural processes of the environment and increasing the health risks to humans, as the induced toxicity varies widely within each type of group [12, 13, 14, 15, 16, 17, 18].

Pesticide residue exposure plays a pivotal health role for producers and their families that are exposed directly, as well as the general population that is exposed indirectly via marketing and application of pesticides, domestic vegetable cultivation, close proximity to rural areas of agricultural fields, and by the consumption of exposed food and water [19, 20, 21, 22, 23, 24, 25, 26]. This has been noted especially for intensely cultivated European countries (i.e. greenhouse in Spain, Portugal, Italy, and Greece), where pesticides have been used at least for three decades. The lack of proper information (what pesticide for what crop, adverse health effects, safety measures etc.), false estimations (pesticide dosage per crop) and training (correct clothing and storage, application tools, how and when to apply the pesticide mixture, safe harvesting post period, disposal of the empty packages etc.) result in a silent chronic exposure detrimental to human health, and in some cases, of acute exposure, are fatal to human health.

V. ORGANOPHOSPHATES

Organophosphate pesticides were one of the most important preferred categories due to their increased activity against a wide spectrum of insects at relatively low application doses. Organophosphates and carbamates had replaced organochlorines worldwide as they were regarded safer for their toxic impact, although today most organophosphates have started to be banned as they are now being replaced by other insecticidal products (especially synthetic pyrethroids) that have an even less toxic impact. Sales of insecticides based on organophosphates, in regard to the 27 geopolitical entities of the EU, for the year 2018, show the immense level of this preferred insecticide group as shown in Fig 1.

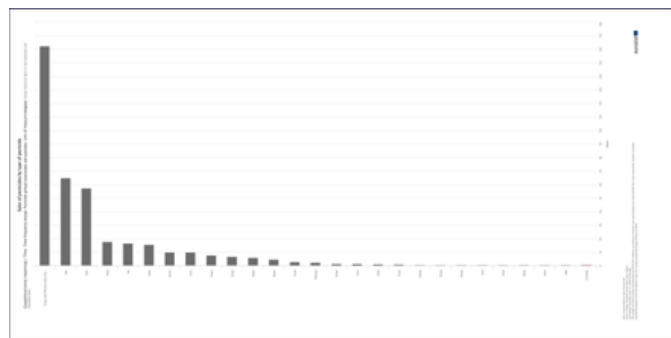


Fig1. Volume of pesticides sold by type of pesticide (based on organophosphates) within the current 27 EU member states for the year 2018. [Eurostat (2020). Sales of pesticides by type of pesticide. Retrieved from:

<https://ec.europa.eu/eurostat/databrowser/view/tai02/default/bar?lang=en>]

A few of the most used organophosphates, that are not approved based on the [EU pesticide database](#), are Parathion, Phoxim, Chlorpyrifos, Azinphos-Methyl, Fenitrothion, Dichlorvos, Diazinon amongst others, although some are approved, but as for all active substances, there are specific restrictions of use (Phosmet, Malathion i.a). They were the most widely sold pesticides in the USA and European countries, while some are even registered for public health applications [27, 28, 29, 30]. Organophosphorus pesticides are esters of phosphoric acid and their derivatives have a main mode of action via inhibition processes of acetylcholine esterase (AChE). AChE is a known key enzyme of the cholinergic system responsible for the breakdown of acetylcholine that functions as a neurotransmitter. Inhibition of this enzyme results in accumulation of the neurotransmitter acetylcholine (ACh) at the synapses and overstimulation of nerves and muscles. Organophosphorus pesticides exhibit a high activity/low persistence time, are relatively unstable in the environment and degrade quickly to toxic biproducts. Nearly 75% of organophosphate pesticides are metabolized to dialkyl phosphate metabolites (DMP, DMTP, DMDTP, DEP, DETP, DEDTP), which do not inhibit acetylcholinesterase enzymes. These metabolites are not considered toxic, but rather provide an exposure marker of the parent pesticide, suggesting a possible recent exposure. Since dialkyl phosphate metabolites may also exist in the environment as an organophosphate degradation product, the detection of these metabolites in urine may also indicate the person's exposure to the metabolite itself. Additionally, since each of these six urinary metabolites can be produced from more than one parent pesticide, the metabolite detection alone is not enough exposure evidence to a specific pesticide and further investigation is needed. In contrast to dialkyl phosphate metabolites, other specific metabolites can also be produced by organophosphate pesticides, from one or only a few parent pesticides (e.g. malathion dicarboxylic acid can be produced from malathion). The detection of the specific metabolites suggests both exposure to parent pesticide and presence of metabolite in person's food or environment, [31]. Health concerns linked to organophosphate exposure are either acute or chronic. All levels of exposures have a particular toxic impact that is amplified or reduced based on age, underlying diseases, demographics etc.

VI. ORGANOPHOSPHATE RESIDUAL ANALYSIS

Pesticide residual analysis is a methodology, used for the investigation and quantitation of banned or registered pesticides that may occur in an agricultural product. A residue is a trace (mg/kg, µg/kg, ng/kg) of a substance, present in a matrix. The European Union has established a pesticide maximum residue level (MRL) for each legal pesticide, in order to avoid high limits of residual quantities (default lowest limit in EU law is 0.01 mg/kg) and to ensure that the maximum level of a pesticide residue is legally acceptable when pesticides are applied correctly [Good Agricultural Practice (GAP)]. The EU sets new MRL's and amends or removes any existing one after EFSA's (European Food Safety Authority) opinion. The Commission then adopts Regulations for the purpose. The analytical techniques for the identification and quantification of pesticide residues are being developed and improved continuously. Gas chromatography (GC) is good for detecting volatile and thermally stable compounds, high-performance liquid chromatography (HPLC) is for non-volatile and high molecular weight samples, and capillary electrophoresis (CE) is best for less consumptions of organic solvents with faster compound separation processes [75, 76]. Although official analytical methods exist (e.g. AOAC International: Association of Analytical Communities,

SANCO documents from European Commission Directorate General Health and Consumer Protection, Ministry of Public Health in Netherlands etc.), multi-residue in-house methods are developed and followed by the vast majority of laboratories throughout Europe, mainly due to the great variety in sample preparation and detection apparatus that can be used. Considering organophosphate analysis in vegetables, a number of sample preparation steps are followed before the analysis can take place. Subsampling, homogenization, extraction and clean-up procedures are considered major important steps since they can introduce a significant uncertainty into the final result. Vegetable samples reach laboratories in certain quantities (usually 1 kg or more), consisted from a number of individual products (e.g. 1-2 cucumbers, 3-5 tomatoes, 5-10 peppers). In order to acquire a representative result, all individual items are chopped, homogenized in a blender and further divided into smaller quantities (e.g. 50 or 10 g). These subsamples can be stored at -20°C or immediately utilized. After sample preparation the extraction step is followed in order to move (extract) the organic contaminants, such as the organophosphate pesticides, from the solid phase of the vegetable to a liquid one. Analytes are isolated from the primary matrix and their concentration is increased above the detection limit, for the specific analytical technique to be followed [32]. During the extraction of vegetable samples, a number of interfering compounds are co-extracted with the analytes (e.g. fats, carbohydrates, water, chlorophyll) [32], hence the need of an additional analytical step in order to purify the extract (clean-up step). Solid phase extraction (SPE), gel permeation chromatography (GPC), sulphuric acid treatment or saponification are some of the techniques used for purification purposes. Liquid-liquid extraction (LLE) is a widely used method used for the isolation of pesticides from fruit and vegetable matrices, initially based on two basic approaches: acetone followed by partitioning with dichloromethane and petroleum ether and extraction with ethyl acetate at the presence of sodium sulfate. These two approaches have been modified by many laboratories in order to take into consideration health and environmental aspects [33]. Even though acetone is completely mixed with water there is a need of a nonpolar solvent in order to enhance a distinct separation from the water phase, a fact that may lead to lower recoveries. Ethyl acetate is not completely miscible with water, hence water can be removed by the excess of sodium sulfate [34]. A typical example of liquid-liquid extraction is the addition of ethyl acetate, anhydrous Na₂SO₄ and NaCO₃ to the weighted sample, homogenization with Ultra-Turrax, filtration and concentration prior to analysis [35]. In recent years acetonitrile extraction has an increased use, particularly after the development of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method, where anhydrous NaCl with MgSO₄ are used, leading to increased recoveries of polar compounds [36].

LLE can be used without further purification steps, or it can be combined by various clean-up steps, in order to minimize matrix effect, improve ruggedness, decrease LOQ and prohibit contamination in detection apparatus [35, 37]. Supercritical Fluid Extraction (SFE), Solid-Phase Microextraction (SPME), Stir Bar Sorption Extraction (SBSE) and Matrix Solid-Phase Dispersion (MSPD) are additional purification steps after the first extraction of the vegetable matrix. SFE can lead to a pure extract but with high instrumentation cost and a large number of parameters needed to be optimized [38, 39, 40], while SPME can provide a fast and cheap method more oriented to qualitative analysis [41]. SBSE has been reported as an efficient method for less polar pesticides [42] and MSPD can utilize reversed phase material (C₁₈ and C₈) and Florisil sorbent for more polar pesticides [43, 44, 45].

SPE initially used Florisil and Silica sorbents, due to strong adsorption of organophosphates, while later graphitized carbon black (GCB) sorbents were used, in order to increase pigment removal from fruit and vegetable extracts [46, 47, 48, 49]. Since carbon sorbents did not eliminate matrix problems, the use of reverse phase C_{18} was introduced in order to remove non-polar co-extractives from vegetable extracts [50, 51, 52]. Chemically bonded stationary phases (aminopropyl, $-NH_2$), primary-secondary amine (PSA) and strong anion exchanger ($-SAX$) were also used in multi-residue pesticide methods. The bonded normal phase SPE columns (with PSA and $-NH_2$) has been reported as an effective step in order to reduce fatty acids, while C_{18} and $-SAX$ remove less matrix co-extractives from the vegetable extract. Researchers [50, 51] have also reported the combination of two or three SPE columns (GCB, C_{18} , $-SAX$, PSA, $-NH_2$) as an effective clean-up step of sample extracts. SPE sorbents were also used by Anastassiades et al. [36] during the development of the QuEChERS method with improved removing of matrix co-extractants and reduced laboratory handling. Letohay and others [53, 54] further modified the QuEChERS method, in order to improve recoveries for a wide range of pesticides from various non-fatty matrices, with pH from 2 to 7. Dispersive solid-phase extraction (DSPE) is a method where, after liquid-liquid extraction, the SPE material is mixed with a portion of the vegetable crude extract. DSPE with a combination of PSA and GCB can be used for vegetable samples high in carotinoides (e.g. red pepper) or chlorophyll (e.g. lettuce), while QuEChERS method with a combination of C_{18} and PSA has been used for a large number of pesticides from various food matrices, [33]. GPC is usually used for fatty matrices with high molecular weight co-extractants, but with an increase in solvents amounts and laboratory time.

Organophosphate pesticide detection is usually performed with gas chromatography (GC) coupled with nitrogen-phosphorous detectors (NPD), following specific temperature programs. The use of auto-samplers facilitates analysis and eliminates errors. The use of programmed temperature vaporizer (PTV) can facilitate large volume sample injections. Confirmation of positive samples can be performed with mass spectrometers-detectors (MS or MS/MS), usually either with electron impact ionization (EI) in full scan mode (as a means to evaluate clean-up efficiency) or selective ion monitoring (SIM) (evaluation of recovery efficiency) or atmospheric pressure chemical ionization mode (APCI). LC can also be used coupled with mass selective detectors with atmospheric pressure chemical ionization (Pizzutti, 2007). Ultra-high pressure liquid chromatography (UHPLC) systems, working with higher operating pressures than in normal LC are gradually gaining ground in pesticide analysis [56].

VII. REVIEW OF RESULTS

This review found a limited number of scientific publications of pesticide residue analysis within the European Union Agricultural Sector and selected representative information presented in Table 1, initially categorized by country, vegetable commodity, number of samples monitored and number of various organophosphates analyzed. The majority of these countries were mainly from the Mediterranean region and from central European countries (Spain, Greece, Netherlands, Portugal and Italy). This is because these countries are the main source of vegetable production throughout Europe, due to excellent climatic conditions. Nevertheless, other countries are entering the vegetable market too, such as Bulgaria and the Czech Republic.

In regard to sample preparation, extraction and purification

techniques of organophosphate residue analysis of the aforementioned countries, it was shown that laboratories sampled a significant amount of vegetable (1 or 2 kg), consisting of various vegetable items, washing was avoided, vegetables were chopped and then homogenized in mixers. Subsampling is usually used, in order to end with a representative quantity of the initial sample and not jeopardize the trace analysis. Extraction is followed by all researchers, with liquid extraction being the most common method, using solvents such as acetone (C_3H_6O), ethyl acetate ($C_4H_8O_2$) or acetonitrile (C_2H_3N). There are cases where supercritical fluid extraction (SFE), single drop microextraction (SDME), accelerated solvent extraction (ASE), matrix solid phase dispersion (MSD) or stir bar sorptive extraction (SBSE) are used. Some researchers did not use any further purification steps, while those who use clean-up procedures (clean-up of matrices is the first and most critical step for both broad-spectrum screening and accurate determination of pesticides) prefer solid phase extraction with GCB (graphitized carbon black) or SAX (strong anion exchange) and PSA (primary-secondary amine sorbent).

The modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method with acetonitrile as a solvent, seems to be the most recent preferred method used in private or educational laboratories, since it improves the extraction efficiency [53, 57] and provides acceptable recovery rates, resulting in a simple, cheap and reliable sample preparation technique (extraction and purification) in order to meet the low levels required by European MRLs standards [58]. The range of QuEChERS covers many applications and can be compared with alternative methods for various applications. Such methods, can include but are not limited to, Solid Liquid Extraction (SLE), Solid Phase Extraction (SPE), Accelerated Solvent Extraction (ASE), Microwave Assisted Extraction (MAE), Ultrasound Assisted Extraction (UAE), Gel permeation chromatography (GPC), Vortex-Assisted Liquid-Liquid Microextraction (ME-IL-VALLME), Dispersive liquid-liquid microextraction (DLLME) and Ion Mobility Spectrometry (IMS). Besides the availability of other analytical techniques, QuEChERS remains a preferred choice that has the main advantage to provide in general more results and less drawbacks especially in crop product analysis. Nanotechnology nowadays is also participating as a new uprising detection method for pesticide residue [e.g. the polystyrene-coated magnetic nanoparticle (Pst/MNPs) technique].

Therefore, pros and cons of sample preparation techniques, used for extraction and purification of vegetable samples before the sample analysis, exhibit a number of variations in initial matrix amount required, solvent usage, toxic or flammable chemicals disposal, time and laboratory work required.

LLE techniques are reported as time consuming with large amount of solvent usage. The use of the Soxhlet apparatus reduces laboratory cost but it still requires a large amount of solvents, while microwave assisted Soxhlet extraction reduces sample preparation time. Ultrasonification extraction has been reported to facilitate the solvent leaching process. In order to further reduce extraction time and solvent usage, MAE, ASE and SFE techniques can be used, providing a better analyte desorption and diffusion from the solid matrix. These techniques have advantages such as lower temperature requirements, higher extraction rates, less solvent usage, while in certain cases (e.g. SFE) can produce extracts with no further clean-up step required [32]. Additional to liquid extraction, solid adsorbent materials can be used for extracting analytes from the vegetable matrix. SFE, SPME, MSPD and SBSE are reported to provide more advantages compared to LLE techniques (simultaneous concentration of analytes and removal of interfering compounds, less solvent usage,

more samples analyzed in shorter times), [32]. Solid phase extraction techniques involve purification of analyte extracts while gel permeation is mainly used to purify extracts with an increased fat content.

Organophosphate detection is carried out with GC-NPD, while there are cases where GC-ECD methods have been developed. Confirmation is performed by the majority of researchers, usually using GC-MS, GC-MS/MS or LC-MS/MS. Currently, the use of LC-MS or triple quadrupole MS for pesticide trace analysis is increasing, despite the high purchase cost for private or education laboratories. Additionally, all laboratories have developed some sort of validation procedure, evaluating recovery (Re %), relative standard deviation (RSD %), limit of detection (LOD in mg/kg or µg/kg) and limit of quantitation (LOQ mg/kg or µg/kg), (Table 1), along with other parameters not shown in Table 1 (e.g. precision, linearity, uncertainty etc.). These values were found mostly within SANCO requirements [59]. There is not enough information about the accreditation status of these laboratories (ISO 17025) or the participation in official proficiency tests.

VIII. CONCLUSIONS

Organophosphates seem not to be so intriguing to monitor and research, despite being still some of the most frequently applied pesticides in the world for cultivation purposes. Although, within the EU, their ban is in force since early 2020. Traceability and food labeling techniques provide evidence for certain production methods (e.g. organic food), food product origin, and MRLs violation cases. Certification paradigms used for vegetable cultivation in European countries (such as GLOBALGAP, formerly known as EUREPGAP) should become a prerequisite, not only for produce intended to be sold abroad, but also for produce that is sold inside each country, eliminating in that way possible adverse effects from organophosphates exposure, especially in local markets that do not carry a “free from pesticide residue” certification.

Thus, assuming all agricultural products exported to other European countries are certified and the requirements of each certification scheme are strictly followed, we could conclude that public health is not questioned, but limitations can arise as it cannot be 100% guaranteed of what happens inside each European country, in regard to correct produce and selling activities, given certifications of sold agricultural products, implemented regulations and directives followed, and having only national authorities responsible for inspection and monitoring. Future steps should focus on more cost-effective ways of monitoring pesticide residues, via improved, more efficient analytical techniques and additional dedicated analytical laboratories (private and governmental laboratories that collaborate) per region, so as to cover the entire agricultural sector for all product batches that need to be analyzed prior to being available on the consumer market.

The non-target extraction, analysis, qualification and quantification of more chemical compounds are regarded to be one of the ongoing priorities of the EU.

In conclusion, all different analytical methods used for the detection of pesticide residues in food products, have the same purpose, that is to ensure a correct, safe and sustainable solution for cultivation processes, specific product origins, and marketing strategies based on consumer demands.

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Conflicts of interest

The authors declare no conflicts of interest.

Abbreviations

Sno: number of samples analyzed,
OPPs no: number of organophosphate analytes analyzed
Re (%): recovery %
RSD (%): relative standard deviation %
LOD (mg/kg): limit of detection
LOQ (mg/kg): limit of quantitation

PTV – GC-ITMS: programmable temperature vaporization injector – gas chromatography with ion trap mass spectrometry
GC-NPD: gas chromatography – nitrogen phosphorous detector
GC – MS (SIM): gas chromatography – mass spectrometry with selected ion monitoring
GC/PFPD: gas chromatography – pulse flame photometric detection
RPLC – GC: reversed phase liquid chromatography – gas chromatography
LVI-GC-NPD: large volume injection – gas chromatography – nitrogen phosphorous detector
LC-MS: liquid chromatography – mass spectrometry
GC-ECD: gas chromatography – electron capture detector
GC-NCI-MS: gas chromatography – negative chemical ionization mass spectrometry
GC-EI-MS: gas chromatography – electron impact ionization – mass spectrometry
UHPLC-TOF MS: ultra-high performance liquid chromatography – time of flight mass spectrometry
MD-GC/MS: multidimensional gas chromatography – mass spectrometry
LP-GC-MS: low pressure gas chromatography – mass spectrometry
SLE: Solid Liquid Extraction
SPE: Solid Phase Extraction
ASE: Accelerated Solvent Extraction
MAE: Microwave Assisted Extraction
UAE: Ultrasound Assisted Extraction
ME-IL-VALLME: Vortex-Assisted Liquid-Liquid Microextraction
IMS: Ion Mobility Spectrometry
GPC: Gel permeation chromatography
DLLME: Dispersive liquid-liquid microextraction
Pst/MNPs: polystyrene-coated magnetic nanoparticle
LLE: Liquid-liquid extraction
SFE: Supercritical Fluid Extraction
SPME: Solip-Phase Microextraction
SBSE: Stir Bar Sorption Extraction
MSPD: matrix solid-phase dispersion
PTV: programmed temperature vaporizer
MS or MS/MS: mass spectrometers-detectors
EI: electron impact ionization
SIM: selective ion monitoring
APCI: chemical ionization mode
UHPLC: Ultra high pressure liquid chromatography

Table 1. Selected published data on pesticide monitoring with focus on organophosphates

Food description			Sample preparation (Extraction, and/or Clean-up steps)	Detection Apparatus (Characteristics)	Method Validation Characteristics (range)				References
Matrix Country	Sno	OPPs			Re (%)	RSD (%)	LOD (µg/kg)	LOQ (µg/kg)	
Spain Vegetables	23	2	Liquid extraction (acetonitrile) Solid phase extraction (multi – layer Superclean Envi CarbII / PSA SPE cartridge, acetonitrile : toluene)	PTV – GC- ITMS	80 - 96	1 – 7	1 - 2	1- 4	[60]
Greece Vegetables	13	8	Single – drop microextraction (SDME)	GC - NPD	29.9 – 51	4.9 - 8.7	0.6-10.0	-	[61]
Spain Vegetables	-	5	Liquid extraction (acetonitrile, acetone, ethyl- acetate)	GC – NPD GC – MS (SIM)	71.3-115	2.1 - 7	0.1-3.8	0.5-12.6	[62]
Austria Vegetables	-	22	Modified QuEChErs method (acetonitrile)	GC - MS	70-132	2.9-17.3	0.4-48.2	1.2-161	[63]
Spain Vegetables	-	1	Liquid extraction (dichloromethane)	GC / PFPD	89.2 – 92	8.2-14.9	3	10	[64]
Spain Vegetables	-	5	Supercritical fluid extraction (SFE)	RPLC – GC LVI-GC-NPD	-	-	1.49-7.66	-	[65]
Spain Fruits	429	11	Liquid extraction (ethyl - acetate)	GC-NPD	60-92	5-18		0.01-100	[66]
Spain Fruits	116	1	Liquid extraction (ethyl - acetate)	LC-MS	72	19	-	20	[45]
Bulgaria Fruits Vegetables	-	5	Liquid extraction (acetone) Solid-phase extraction (GCB, SAX-PSA)	GC-ECD GC-MS	74-114	2-16	1- 5	5 - 10	[67]

Slovak Republic Fruits	-	7	QuEChERS method (acetonitrile)	GC-NCI-MS GC-EI-MS	-	1.7-8.1 6-7.5	0.0068- 0.113 0.14-0.5	0.0234- 0.160 0.36-1.66	[68]
Czech Republic	-	37	QuEChERS method (acetonitrile) (clean up- PSA)	UHPLC-TOF MS	-	2.25-13.63	-	<10	[69]
Portugal Fruits	28	11	Dispersive liquid-liquid microextraction (DLLME)	MD – GC/MS	66-106	3-20	0.2-4.1	0.06-1.24	[70]
Portugal Fruits	-	7	QuEChERS method (acetonitrile)	LP-GC-MS	74-90	4-12	1.2-4	-	[71]
Czech Republic Fruits	-	2	Liquid extraction (acetonitrile)	LC-MS/MS GC-MS	93-94	7-8	-	9-11	[72]
Slovak Republic Fruits Vegetables	-	4	QuEChERS method (acetonitrile)	GC-EI-MS GC-NCI-MS	-	3.4-5.3 7.5-12	0.033- 0.055 0.08-0.34	0.11-0.18 0.27-1.13	[73]
Spain Vegetables	-	2	QuEChERS method (acetonitrile)	GC-MS (SIM) GC-MS/MS	84.9-98.2 -	2.0-4.6 6.0-7.5	0.2-0.3 0.1-0.3	-	[74]