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ANALYTICAL OVERVIEW AND SYSTEM ANALYSIS OF ORGANOPHOSPHATES FILTRATION IN THE AGRICULTURAL SECTOR OF THE EUROPEAN UNION

Abstract. *This research focused on reviewing organophosphate monitoring in European countries that was carried out by various researchers independently to National authorities or official European monitoring bodies, during the last ten years. A modified QuEChERS method (a solid phase extraction technique for detection of pesticide residues in food) seems to be the most preferable method used lately in private or educational laboratories, while ultra high pressure liquid chromatography (UHPLC) systems, are gradually gaining ground in trace analysis. Considering the collected published data, organophosphates seem not to be so intriguing to monitor and research, besides being still one of the most frequently applied pesticides used for cultivation purposes today. The question is, will public health be exposed to adverse effects of produce sold, especially in local markets, without the necessary "free from pesticide residue" certification? Traceability and food labeling techniques can provide evidence for certain production methods (eg organic food) or specific food product origin, while facilitate traceability in case of MRLs violation.*

Key words: *pesticides, organophosphates, residual analysis, agricultural sector.*

АНАЛІТИЧНИЙ ОГЛЯД ТА СИСТЕМНИЙ АНАЛІЗ ФІЛЬТРАЦІЇ ФОСФОРОРГАНІЧНИХ КИСЛОТ В АГРАРНОМУ СЕКТОРІ ЄВРОПЕЙСЬКОГО СОЮЗУ

Анотація. *Це дослідження було зосереджено на перегляді моніторингу фосфорорганічних кислот у європейських країнах, який проводився різними дослідниками незалежно від національних органів влади або офіційних європейських моніторингових органів протягом останніх десяти років. Модифікований метод QuEChERS (метод твердофазної екстракції для виявлення залишків пестицидів у харчових продуктах), здається, є найбільш переважним методом, який останнім часом використовується у приватних чи освітніх лабораторіях, тоді як системи рідкої хроматографії надвисокого тиску (UHPLC) поступово завойовують свою популярність. аналіз. Беручи до уваги зібрані опубліковані дані, органофосфати, здається, не настільки інтригуючі для моніторингу та досліджень, крім того, що вони досі є одним із найчастіше застосовуваних пестицидів, які використовуються для вирощування на сьогоднішній день. Питання полягає в тому, чи буде громадське здоров'я піддане негативному впливу реалізованої продукції, особливо на місцевих ринках, без необхідної сертифікації «без залишків пестицидів»? Методи відстеження та маркування харчових продуктів можуть надати докази для певних методів виробництва (наприклад, органічних харчових продуктів) або специфічного походження харчових продуктів, одночасно полегшуючи простежуваність у разі порушення МДГОВ.*

Ключові слова: *пестициди, органофосфати, залишковий аналіз, аграрний сектор.*

Graphical Abstract

Overview. *Agriculture.*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 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].

Ways of agriculture however, have changed considerably during the last decades. The industrial takeover produced mono-cultured 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 in 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) so much in the rural exploitation, as well as 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.

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 market of EU fertilizing 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], while 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 the reported hazard categories. Additionally, the European Commission publishes EU statistics on pesticide sales in regards 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".

Pesticide Usage. 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 (eg organoclorines).

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 abruption in the ecosystem, un-stabilizing the natural processes of the environment and increasing the health risk to humans as the induced toxicity varies widely within each type of group [12; 13; 14; 15; 16; 17; 18].

The importance of pesticide residue exposure plays a pivotal health roll 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 agriculture fields and by the consumption of exposed food and water [19; 20; 21; 22; 23; 24; 25; 26]. This has been noted especially for intense 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, fatal to human health.

Organophosphates. Organophosphate pesticides was one of the most important preferred categories due to its increased activity against a wide spectrum of insects at relatively low application doses. Organophosphates and carbamates had replaced organochlorines world-wide 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.

Organophosphate insecticides have been widely used for more than three decades in the U.S. accounting for almost 30% of the global insecticide market sectors [EPA, 2017].

Sales of insecticides based on organophosphates, in regards to the 27 geopolitical entities of the EU, of the year 2018, show the immense level of this preferred insecticide group as shown in Fig. 1.

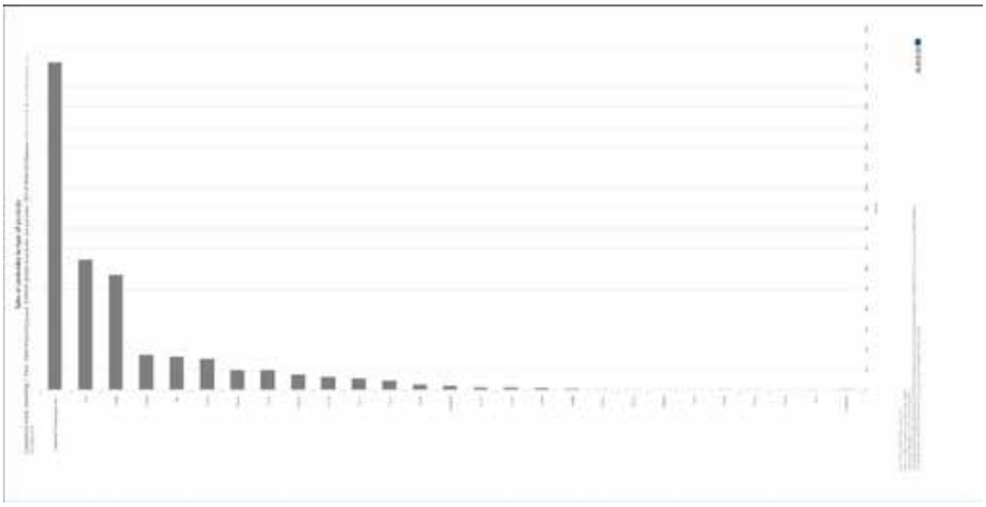

Fig. 1. Volume of pesticides sold by type of pesticide (based on organophosphates) within the current 27 EU member states for the year 2018. [Eurostat (2020). Sales of pesticides by type of pesticide. Retrieved from: https://ec.europa.eu/eurostat/databrowser/view/tai02/default/bar?lang=en]

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 pesticides sold in USA and European countries, while some of them 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 function 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 degrad quickly to toxic bi-products. 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 they 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 contrary to dialkyl phosphate metabolites, other specific metabolites can also be produced by organophosphate pesticides, from one or only a few parent pesticides (eg 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.

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 levels (MRLs) for each legal pesticide, in order to avoid high limits of residual quantities.

The analytical techniques for the identification and quantification of pesticide residues are being developed and improved continuously. Gas chromatography is good for detecting volatile and thermally stable compounds, high-performance liquid chromatography 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 (eg 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 takes place. Sub-sampling, homogenization, extraction and clean-up are considered of major importance steps since they can introduce a significant uncertainty into the final result.

Vegetable samples are reaching laboratory in certain quantities (usually 1 Kg or more), consisted from a number of individual products (eg 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 in smaller quantities (eg 50 or 10g). These sub-samples 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]. Many extraction techniques have been reported in the literature, with the most frequent to be : liquid-liquid extraction (LLE), solid-phase extraction (SPE), accelerated solvent extraction (ASE), gel permeation chromatography (GPC), microwave-assisted extraction (MAE), matrix solid-phase dispersion (MSPD), supercritical fluid extraction (SFE). During the extraction of vegetable samples a number of interfering compounds are co-extracted with the analytes (eg 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, gel permeation, sulphuric acid treatment or saponification are some of the techniques used for purification purposes.

Liquid-liquid extraction 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 non-polar 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 Na_2SO_4 and $NaCO_3$ 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 method, where anhydrous NaCl with $MgSO_4$ are used, leading to increased recoveries of polar compounds [36].

Liquid-liquid extraction can be used with out 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), solip-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 (C_{18} and C_8) and Florisil sorbent for more polar pesticides [43; 44; 45].

Solid-phase extraction (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-extractans 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) was called the method, where after liquid-liquid extraction the SPE material was 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 (eg red pepper) or chlorophyll (eg 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]. Gel permeation chromatography (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). Liquid chromatography (LC) can also be used coupled with mass selective detectors (MS, MS/MS) 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].

Review of Results – Discussion. This paper focused on reviewing organophosphate pesticide monitoring in European countries that was carried out by various researchers, independently to National authorities or official European monitoring bodies, during the last five years. Results showed a limited amount of information available for organophosphate pesticides. Even though hundreds of papers are published about pesticide monitoring in agricultural products and the vast majority of work deals with the development of new multi-residue in house methods, new sampling preparation methods, modern analysis apparatus, validation of new methods or evaluation of various pesticide analysis parameters, organophosphates are not preferred by private or educational laboratories in their analysis schedule. Organophosphates seem to be forgotten by researchers these days. Nevertheless, the review found a limited number of scientific publications and selected representative information of these data is presented in Table 1, so as to highlight the various sample preparation techniques, the analysis apparatus used, as well as any validation that is performed in order to prove method suitability, as far as organophosphate analysis is considered.

Data are presented in Table 1, initially categorized by country, vegetable commodity, number of samples monitored and number of various organophosphates analyzed. The majority of countries found during this review to deal with organophosphates are: Spain, Greece, Netherlands, Portugal and Italy. This is well explained since these countries are the main source of vegetable production through out Europe, due to excellent climate conditions (most of them being Mediterranean countries). Nevertheless other countries are entering vegetable market such as Bulgaria or Czech Republic.

Considering sample preparation for organophosphate analysis, all laboratories sample a significant amount of vegetable (1 or 2 Kg), consisted of various vegetable individual items, washing is avoided, vegetables are chopped and homogenized in mixers. Sub-sampling is usually used, in order to end with a representative quantity of the initial sample and not jeopardize the trace analysis. Efforts are taken, in order sample preparation and the following extraction or purification steps to be identical and with small variations in the final procedure to end with different extracts for different pesticide categories. Extraction is followed by all researchers, with liquid extraction being the most common method, using acetone, ethyl acetate or acetonitrile. 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 prefer solid phase extraction with GCB or SAX and PSA. Modified QuEChERS method with acetonitrile seems to be the most preferable method used lately in private or educational laboratories, since it improves extraction [53; 57] and provides acceptable recovery rates, resulting to 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 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. Liquid – liquid extraction techniques are reported as time consuming with large amount of solvents usage. The use of Soxhlet apparatus reduces laboratory cost but it still requires large amount of solvents, while microwave assisted Soxhlet extraction reduces sample preparation time. Ultasonification extraction has been reported to facilitate the solvent leaching process. In order to further reduce extraction time and solvent usage microwave assisted extraction (MAE), accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE) can be used, providing a better analyte desorption and diffusion from the solid matrix. These techniques can lead to lower temperature requirements, higher extraction rates, less solvent usage, while in certain cases (eg SFE) can produce extracts with no further clean up step required [32]. Additionally to liquid extraction, solid adsorbent materials can be used for extracting analytes from the vegetable matrix. Solid-phase extraction (SFE), solid-phase microextraction (SPME), matrix solid-phase dispersion (MSPD) and stir-bar sorptive extraction (SBSE) are reported to provide more advantages comparing to liquid – liquid extraction 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 (eg 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.

Table 1

Selected published data on pesticide monitoring with focus to 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 QuEChеRs 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]

Conclusions – Recommendations. Even though European legislation exists and applies strict guidelines and regulations in order to reduce the use of persistent pesticides and encourage the development of target specific pesticides, this review shows a tendency in pesticide research towards more new and modern chemical molecules that are used in today's agriculture. Organophosphates seemed to be old fashioned among various laboratories in European countries, even thought they are still used for cultivation purposes. Certainly, we should take into consideration the existence of National laboratories in each European country, which are monitoring pesticides and informing European Union in order to produce specific reports. Considering private laboratories in each country, we should acknowledge that routine analysis is taking place and certainly organophosphates are monitored among the other pesticide categories. In this case, we should underline that private laboratories operate with agricultural samples originating from independent farmers, farmer groups, super-markets or factories that export vegetables abroad, to other European countries and the majority of samples are analyzed as a prerequisite for certification purposes. So, 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, what happens inside each European country, where certification is not necessary for selling agricultural products (as a marketing requirement) and products reach consumers, sold in local markets or supermarkets, with only National authorities been responsible for inspection and monitoring? Analysis cost, insufficient laboratory methods (eg pesticide metabolites that are not covered by in-house methods), new pesticides or foreign imported pesticides, are some of the parameters that may limit the successful pesticide monitoring from National authorities. Not only organophosphates but all pesticides are in question and there is not substantial evidence (published data) to prove that farmers have been using legal-registered pesticides or they have followed the necessary pre-harvest days after pesticide usage (strictly defined for each pesticide).

Finally, we should underline the importance of accreditation to all major pesticide categories from National laboratories and the usefulness of specific agricultural sampling protocols, which would take into consideration cultivation process, product origins, marketing strategies, as well as weather fluctuation and consumer demands. Certification schemes 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. Traceability and food labeling techniques can provide evidence for certain production methods (eg organic food) or specific food product origin, while facilitate traceability in case of MRLs violation.

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

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