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Analytical methods for human biomonitoring of pesticides. A review

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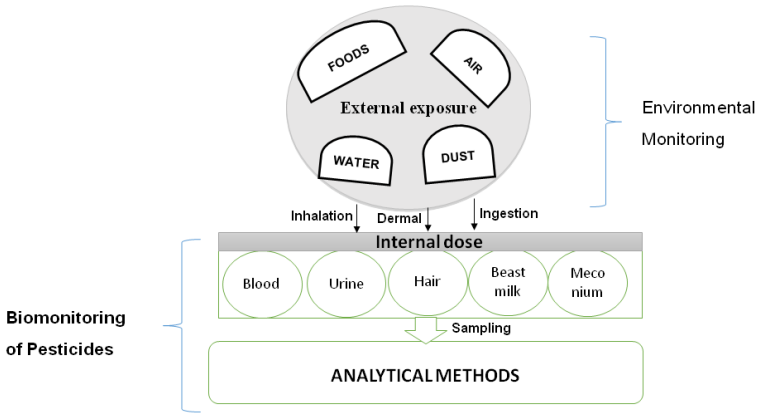
Graphical abstract



HIGLIGHTS

- We present an analytical review of methods for human biomonitoring of pesticides
- Urine and blood are the ~~main~~ primary matrices analysed for pesticide biomarkers
- Non-invasive matrices such as hair are emerging
- Future trends in this field are discussed

ABSTRACT

Biomonitoring of both currently-used and banned-persistent pesticides, is a very useful tool for assessing human exposure to these chemicals. In this review, we present current approaches and recent advances in the analytical methods for determining the biomarkers of exposure to pesticides in the most commonly used specimens, such as blood, urine and breast milk, and in emerging non-invasive matrices such as hair and meconium. We critically discuss the main applications for sample treatment, and the instrumental techniques currently used to determine the most relevant pesticide biomarkers. We finally look at the future trends in this field.

Keywords: Biomonitoring, Pesticides, blood, urine, hair.
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Contents

1. Introduction

There is a huge body of evidence supporting the hypothesis of pesticides being one of the main environmental stressors, and how this is related with diverse health outcomes. Many studies have established the epidemiological association between pesticide exposure and elevated rate of chronic diseases such as different types of cancers, diabetes, neurodegenerative disorders like Parkinson, Alzheimer and amyotrophic lateral sclerosis (ALS), mother and child health outcomes, birth defects and reproductive diseases[1, 2]. The population, including groups of higher risk such as children and mothers, are exposed to pesticides mainly through diet [3] and through household use of pesticides [4]. However, inhalation of polluted air could also be a relevant exposure pathway, mainly for those living near agricultural areas [5]. Even more so, farmers that handle or apply pesticides as well as other professional pesticide-handlers are potentially highly exposed through dermal and inhalation routes (Figure 1).

Considerable research has been dedicated to assessing external exposure to pesticides, particularly through surveying the occurrence of these chemicals in various environmental constituents (e.g., air, water, and food). Human risk assessment of exposure to pesticides is usually based on environmental monitoring, and follows the classical approach of estimating the external exposure and comparing it with health-based guidance values (HBGV) [6].

Apart from this conventional approach to assessing exposure, there is a growing interest in evaluating exposure to environmental chemicals using biomonitoring data (internal exposure approach). Therefore, at present, relevant efforts are being conducted to evaluate the presence of these compounds in biological specimens (urine, blood, breast milk)[7]. Human biomonitoring has become an increasingly relevant tool for (a) assessing the potential health risks associated with exposure to environmental chemicals; (b) identifying new chemical exposures; (c) evaluating trends and changes in exposure; (d) establishing distribution of exposure among the general population and identifying vulnerable groups and populations with higher exposures; (e) determining whether technological changes can affect human exposure; (f) conducting epidemiological studies; and, (g) evaluating the efficacy of regulatory actions [8]. When assessing human exposure to pesticides or others environmental chemicals using biomonitoring studies researchers need to select the most appropriate biomarkers and human specimens. A biomarker can be the chemical substance itself, its metabolite(s), or the products of interaction between the chemical and target biomolecules. Biomarkers of exposure, which link the biomarker measured to specific environmental exposures, are most frequently used for the biomonitoring of environmental pollutants [9, 10].

Analytical capabilities are at the core of monitoring for pesticides in the various matrices. Analytical methods currently used for the determination of pesticides in food and environmental matrices have been widely studied , and several outstanding critical reviews have recently been published [11, 12]. However, less attention has been paid to the analytical methods employed for biomarkers of exposure to pesticides. Margariti et al. [13] and Barr [14] published, respectively, in 2007 and 2008, two comprehensive reviews on the most important existing analytical methods for the biomonitoring of pesticides. The former paper mainly discussed methods developed for forensic and clinical purposes, whereas the latter focused its discussion on the analytical methods used for pesticide biomonitoring in the general population.

In view of the above considerations, the main objective of this review is to present the advances in the analytical methods used for biomonitoring of pesticides in environmental and occupational exposure, through the identification and discussion of the most relevant methods published in the literature in the past 7 years.

2. Compounds and matrices

Table 1 summarizes the pesticide biomarkers currently analysed in national biomonitoring programmes [15-18] or in specific studies [19-21]. Biomarkers of exposure are, broadly, generic or specific metabolites or the parent compounds present in human specimens in a free or conjugated form. Table 1 also shows the main human specimens used for determining exposure to each class of pesticide. The currently-used pesticides more frequently evaluated in the biomonitoring studies belong to the chemical classes of Organophosphates (OP), Pyrethroids (PYR), Carbamates (Ca), various classes of herbicides, Neonicotinoids (NEO) and Sulfonylurea Herbicides (SUH). However, many of the intact substances investigated are nowadays not approved for their use as plant protection product in agriculture (see Table 1).

Selection of the biological specimen for human biomonitoring of pesticides (as well as other chemicals) is driven by the chemical properties of the substance being monitored and its pharmacokinetics. To date, the most frequently used matrices for the biomonitoring of pesticides are blood and urine. Indeed, blood is an ideal matrix for most chemicals because blood plasma is in contact with every tissue in the body and is in steady state with all organs. In general, whole blood or its components (serum or plasma) are preferred when determining internal exposure to persistent pesticides such as organochlorine insecticides (the intact pesticide and/or its metabolite) which have long biological half-lives (several years) [14]. The main disadvantage of using blood in human biomonitoring is that it is an invasive matrix, and its collection needs the participation of qualified personal. In general, about 1 mL of blood is necessary, and an extensive clean-up is required.

By contrast, for assessing exposure to non-persistent pesticides such as pyrethroids, organophosphate insecticides, or different classes of herbicides (chlorophenoxy acids, atrazine,...), that have a short-life in the human body and consequently, are found in low concentrations in blood, urine is the matrix of choice. Likewise, the hydrophilic metabolites of non-persistent pesticides and, in some cases, the parent compound, are usually measured in urine, which is abundant and easy to collect. In general population studies the collection of spot samples are easier than 24-hours samples, so they are employed more often. To compensate for the varying volume and analyte concentrations in spot samples, an

adjustment of exposure level is performed, usually based on the level of creatinine [22]. A urine matrix is representative of recent exposure because non-persistent pesticides are rapidly metabolized and eliminated in a few days. Pesticide analysis in urine is performed using few mililitres (≤ 5 mL), and many approaches for sample preparation has been proposed.

Over the last few years, a growing interest has been observed in the use of alternative non-invasive matrices such as hair, meconium or breast milk [23]. While hair was initially used for forensic and clinical purposes [24], it is increasingly being used for biomonitoring of pesticides in the general population. Human hair presents numerous advantages, such as easy collection, transport and storage; stability, and information about short- and long-term exposures. However, there are some difficulties in differentiating between endogenous (internal dose through diffusion from blood capillaries) and exogenous deposition from the external environment and deposition from sweat or sebum secretions into the hair shaft. In spite of this, organoclorine insecticides, but also currently-used pesticides from various classes such as pyrethroids or organophosphates, have been investigated in hair, mainly through the analysis of their parent compounds [25]. The analysis of pesticides in hair is usually performed using between 50 and 200 mg, and some pre-treatments steps are normally applied.

Breast milk, in turn, is usually employed for monitoring mother and child exposure to lipophilic organochlorine pesticides and other persistent organic pollutants (POPs). After environmental exposure, some lipophilic chemicals are stored in the body in different tissues with high-fat content and can pass into the breast milk for excretion [26]. To compare pesticide levels, a lipid adjustment is necessary given lipid concentration in human milk is not constant [27]. The different analytical strategies for determination of pesticides in breast milk are using between 1 and 5 mL, and large sample preparation to remove proteins and fat is required.

Finally, meconium (the neonate`s first faeces) is an appropriate matrix for measuring prenatal exposure to pesticides. Chemicals accumulate in meconium from the third month of gestation onwards, until birth, so its analysis provides a long-term exposure of the foetus. It can be collected straightforwardly, and in large amount [28]. Additionally, meconium is a matrix that presents more sensitivity than cord blood and infant hair [28]. Various classes of

pesticides, including OPs, PYR, Ca, by measuring both parent compounds and some metabolites, have been determined in meconium [21]. Around 0.5 g (dry weight) is necessary to perform the analysis, and sometimes the substances are not easily extracted.

To our knowledge, other non-invasive matrices such as nails and saliva have still not been used for the biomonitoting of pesticides.

3. Analytical methods

Overall, the quantitative measurement of biomarkers of exposure to organic pollutants in human specimens includes a sample-pretreatment step, followed by an extraction and clean-up process, and finally by a separation and detection method. Tables 2 and 3 show a selection of the relevant analytical procedures proposed in recent literature.

Unlike newly developed methods for pesticide analysis in food and other environmental matrices[29], currently-used procedures in biomonitoring have a narrow scope, with the exception of a method developed by Cazorla-Reyes et al.[30] which, despite including more than 200 polar and non-polar parent pesticides, is of scarce application in biomonitoring programmes as it lacks the most relevant pesticide biomarkers. Consequently, sample preparation procedures have been developed, in general, to extract few analytes of the same class with similar physicochemical properties.

3.1 Sample pre-treatment

A pre-treatment of biological samples is often required to remove interference or to hydrolyze conjugated forms of the target biomarkers. Many of the specific metabolites of organophosphates, pyrethroids or neonicotinic pesticides are excreted in part as urinary glucuronide and sulfate conjugates. Deconjugation is usually done by an enzymatic hydrolysis treatment (or acid hydrolysis) with β-glucoronidase/sulfatase [31]. In other cases, the pre-treatment is a simple dilution of the urine with water or formic acid, to reduce the matrix effect and the matrix variability between samples that could otherwise affect analyte response.

Enzymatic and alkaline or acidic decomposition of hair have been proposed for increasing the extraction of chemicals [32]. However, this disintegration of hair is unsuitable for the analysis of organophosphate, carbamates and pyrethroid pesticides due to their lability[33]. In order to differentiate the external and internal deposition of pesticides in hair, the washing of hair with water and methanol is recommended [20]. After washing and drying, hair samples should be pulverized in a ball mill or cut into small pieces before the extraction of analytes [24].

Precipitation and denaturation of blood and breast milk proteins generally increase the efficacy of extraction of the lipophilic pesticides, and likewise facilitate (e.g., prevent clogging) solid-phase extraction (SPE) [34]. Formic acid and solvents such as n-propanol, iso-propanol, methanol or acetonitrile are the most commonly used reagents for this purpose [34].

3. 1 Extraction and clean-up strategies

Although direct analysis of samples is optimal, extraction and additional sample clean-up is usually necessary to reach reliable results and preserve instrument performance. While simple and generic sample treatment (e.g. "dilute and shot", QuEChERS [80]) are a present tendency in the analysis of pollutants [81], classical extraction and purification strategies, including liquid-liquid extraction (LLE), solid-liquid extraction (SLE), solid-phase extraction (SPE) and solid-phase microextraction (SPME), are still currently applied for pesticide biomarker analysis in human specimens. LLE has been used for many years as a routine technique, but it is time consuming and solvent demanding, and has been substituted by SPE in many of the recent applications.

SPE is one of the most important sample-preparation approaches for extracting and purifying pesticide biomarkers from liquid matrices (i.e. urine, milk), with both off-line and on-line configurations. Depending on the polarity of the extracted analytes, the appropriate SPE sorbent is selected, and the elution solvent, sample volume and pH conditions, optimized [82]. Several sorbents have been used for the extraction and purification of a wide range of pesticide metabolites from urine. Therefore, polymeric sorbents with a hydrophilic-hydrophobic balance, such as Oasis HLB, are especially proposed for specific metabolites of organophosphates, pyrethroids and herbicides [53] whereas Strata X-C (mixed-mode reversed-phase/strong cation-exchange) is suitable for atrazine metabolites [47]. A weak anion mixed mode sorbent (Strata X-AW) has been used for the extraction of acidic DAPs [45]; whereas for basic quaternary ammonium compounds, a weak cation mixed mode sorbent (Strata X-CW) has proved efficient [46]. A polymeric strong cation exchange sorbent (Bond Elute PCX) has been proposed for the extraction of neonicotinic metabolites in urine [36].

The demand of high-throughput analysis in biomonitoring programmes has led to the development of automated off-line and on-line SPE methods [44]. Tao et al. [54] developed a method for quantification of seven pyrethroid metabolites in urine using an automated SPE station with C18 cartridges, with recoveries ranging from 81-104 %. Similarly, Panuwet et al.[41] developed a rapid on-line solid-phase extraction–high-performance liquid chromatography method for the determination of seven atrazine metabolites in urine, useful for large-scale sample analysis. These on-line SPE methods tend to reduce about ten times the volume (0.1 mL) of sample required (see Table 1). In general, there are not substantial differences between the recoveries provided by the off-line and on-line SPE methods for pesticides in urine; usually both methods achieve recoveries higher than 80 %. The precision neither shows a significant difference between both approaches, and normally the RSDs are lower than 15%.

The simplification of sample treatment using generic extraction methods permits not only increasing the sample throughput, but also widening the scope of the methods. Although the QuEChERS approach is very common in the field of pesticide analysis in food [11], to our knowledge only a single recent study proposed the use of this generic and rapid sample treatment for the extraction of pesticide metabolites in urine. In this method, Roca et al. [59] use acetonitrile as solvent, without a dispersive phase clean-up step , for the extraction of 29 metabolites of several pesticide classes. A modified QuEChERS-based procedure was also used by Luzardo et al. [68] for the determination of 23 OC pesticides and other persistent compounds in human milk and colostrum using acetonitrile saturated with hexane for extraction and PSA in the clean-up step. The recoveries ranged from 76 to 105 %.

Solid liquid extraction is widely employed for the extraction of biomarkers from hair. The most frequently used solvents include methanol for the extraction of polar DAP metabolites [61], organophosphates [20]; acetonitrile (for different classes of pesticides of varied polarity)[64]; and hexane for the persistent ones [20]. SPE is also employed for the clean-up of hair extracts. Recently, Duca et al. [83] evaluated several solid-phase extraction cartridges for the purification of 67 metabolites and pesticides of different chemical classes extracted from hair. They concluded that a dual-layer cartridge of graphitized carbon black (GCB)/primary and secondary amines (PSA) was clearly the best suited to non-polar compounds such as organochlorines, pyrethroids and organophosphates,). For hydrophilic metabolites (e.g. dialkyl phosphates and other organophosphate metabolites, pyrethroid metabolites, phenols and carbamate metabolites), the best results were obtained with polystyrene divinylbenzene resin (PS-DVB). For hydrophilic parent pesticides (e.g.

neonicotinoids, carbamates, azoles) and metabolites without nucleophilic functions, the best recovery was obtained with a dual phase containing a strong anion exchange sorbent (SAX)/PSA, with recoveries ranging from 52.1% (3-hydroxycarbofuran) to 100.9% (3,4-dichloroaniline).

Solid-phase micro-extraction (SPME) has proven to be an efficient alternative to the more classical SLE and SPE approaches [66]. Schummer et al. [64] used this technique for the extraction of 50 pesticides from hair, including OC and currently used pesticides (CUPs) with recoveries ranging from 42 to 115 %.

Sample preparation of blood and blood products for persistent and lipophilic OC pesticides have also been developed to extract other classes of compounds simultaneously, including, PCBs PBDEs and dioxins. While LLE is a technique that has been extensively used in human biomonitoring studies, [84] today it is not as frequent, mainly because it is considered more labour intensive. Nevertheless, recently published methods still use LLE combined with a sulfuric acid clean-up [74] . However, over time, new techniques, that are less time consuming and require less sample volume (<1 mL), such as SPE, have arised. Many of the earlier SPE methods were based on alkyl bonded silica particles (C8, C18) and this sorbent still remains in several of the recently developed methods [72]. Blood is an invasive matrix with a very limited availability, mainly for studies in children and newborns. Consequently, the developments of methods that use very small amount of matrix are necessary. Currently, the methods that reduce the volume to less than 0.3 mL usually use on-line SPE and/or very sensitive instruments such as GC-HRMS (Table 2 and 3).

Wittsiepe et al. [69] developed a method for OC pesticides in small volumes of human blood (0.2 mL), using an automated on-line SPE with a silica-based octadecyl phase (HySphere C18 HD) in combination with a large volume injection into the GC/HRMS instrument. The method is recommended for situations requiring a high throughput and/or when only small amounts of sample material are available, including studies involving children. In addition, the hydrophilic-lipophilic-balanced reversed-phase sorbents are now widely used. Other techniques, such as gel permeation chromatography (GPC), coupled with GC and electron capture (ECD) or mass spectrometric detection have been proposed [78]. In fact, there is a great and recent interest in methods prioritizing blood volume reduction (50-300 µL) using extracts from dried blood spots (DBS) for newborn screening programmes and extensive

biomonitoring for assessing environmental exposures during the prenatal period [77]. Ma et al. [76] recently published a method for OC and PCBs that performs an L-L extraction and a GC-HRMS determination with LODs ranging from 0.003 to 0.017 ng mL^{-1}.

3.2 Instrumental techniques

The selection of the instrumental technique depends on the physicochemical properties of the target substances and their concentration. For the analysis of pesticide biomarkers, both GC-MS and HPLC-MS/MS are commonly used; the first technique is the best suited for the apolar and volatile compounds (e.g. OC, PYR, OPs) or the more polar compounds (e.g DAPs) with a previous derivatization step. However, the second technique, HPLC, is selected for more polar compounds with lower volatility or lower thermal stability, including the metabolites of OPs and PYR, triazine and phenoxyacid herbicide metabolites, or neonicotinic metabolites.

Recently published methods have a narrow scope, of usually between 1 and twenty five compounds. This is in clear contrast with the present tendency observed in the analysis of pesticides in water and food that presents a prevalence of multiresidue/multiclass methods for more than 150 substances on average [29].

Although for polar compounds such as DAPs, paraquat, pyrethroids and nicotinoids metabolites in urine, GC-MS have been used previous derivatization (Table 1), achieving low LODs (from 0.01 to 0.3 ng mL^{-1}), LC-S/MS has become more usual in determining polar compounds at sub µg L^{-1} level, providing high sensibility and good selectivity [12]. Current LC approaches mainly use silica-based, reversed-phase columns, being the octadecyl carbon chain (C_{18}) the most frequently used phase (Tables 2,3). However, other reversed-phases with phenyl groups are also proposed when more selectivity and retention capacity for polar compounds are required [41, 49]. A useful alternative for the highly-polar metabolites such as DAPs, or the quaternary ammonium compounds (paraquat, diquat), which have poor retention in the standard reversed-phase columns, is a hydrophilic interaction liquid chromatography (HILIC) [85] [45]. Generally, column sizes are in the range of 100 mm to 250 mm, with a particle size from 2.7 µm to 5 µm (Table 2). This is a typical LC set-up for the determination of a low number of compounds (e.g < 50). In this sense, only few methods [59] use the ultra-high performance liquid chromatography (UHPLC), which employs

columns with a lower particle size (< 2.1 µm), and provides relevant increases in resolution. Although UHPLC applications have increased significantly in recent years in the field of pesticide and other residue analysis [81], it is still barely used in pesticide biomonitoring.

Methanol and acetonitrile are the organic components of the mobile phases currently used in LC. Besides, solvent modifiers, mainly formic acid (proton donors) and ammonium acetate (proton acceptor), are added for enhancing ionization efficiencies or improving peak separation or peak shape of target compounds.

HPLC coupled to QqQ, operating in multiple reaction monitoring (MRM), with ESI ionization, is the most frequently proposed analytical system. Davis et al.[53] recently reported a multi-residue method for quantifying 12 specific metabolites of several classes of pesticides in urine, using LC-MS/MS, in the ESI positive/negative switching mode. The ESI interface is prone to ion suppression [86], so the authors use matrix-matched calibration and isotopically labeled internal standards to avoid matrix effect. The LODs ranged between 0.003 and 0.4 ng mL^{-1}. Likewise, LC-MS/MS was proposed by Jayatilaka et al.[50] for the determination of four OP pesticides and two metabolites of the bisdithiocarbamate fungicide family (ETU, PTU), employing an atmospheric pressure chemical ionization (APCI) source in positive ion mode. The authors reported LOQs between 0.004 ng mL^{-1} to 0.01 ng mL^{-1}.

For OPs metabolites in urine, the LODs reported using LC-MS/MS range from 0.044 to 2 µg mL^{-1}, lower than those achieved by GC-MS. However, for metabolites of pyrethroids, GC-MS provide lower LODs (around 0.05 µg mL^{-1}) than LC-MS/MS.

Conventional targeted analysis using LC-MS/MS is based on establishing a method to determine a list of known analytes, which requires the use of reference standards and purposeful chromatographic method development. . As an alternative, LC-HRMS offers the possibility of detecting hundreds of polar contaminants in a quantitative target approach due to its sensitivity and selectivity in full-scan analysis. Furthermore, it allows the detection of known compounds suspected of being present in environmental samples (suspect screening) without reference standards, even after measurement (post-target screening) and the screening of yet unknown non-target chemicals [87-88]. The use of HRMS, such as TOF or Orbitrap, for multiresidue analysis is largely driven by the advantages of using the full-scan acquisition mode with high sensitivity, combined with high-resolving power (> 50000 FWHM) and accurate mass measurement (1-5 ppm) [89].

Despite these advantages, to our knowledge only a single paper has been published using UHPLC-HRMS for biomonitoring of pesticides. In this study, Roca et al. [59] developed a comprehensive analytical strategy for biomonitoring of pesticides in urine that includes a target analysis of 29 metabolites of OPs, pyrethroids, herbicides and carbamates; and allows post-target screening of 60 others metabolites including pesticides, PAHs, phenols, and other environmental pollutants. The method uses an Orbitrap spectrometer, working at a resolution of 50.000 FWMH, and provides LOQs ranging from 0.8 to 3.2 μg L^{-1}.

For the analysis of biomarkers of pesticides in hair, blood and breast milk, published studies mainly used GC-MS and GC-HRMS. When polar metabolites such as DAPs were extracted from hair and analysed by GC-MS, such as in the study developed by Tsatsakis et al.[61], the required derivatization step was performed with pentafluorobenzylbromide (PFBBr) and the analyser was selected to work in ion-monitoring (SIM) mode.

The analysis of the parent pesticides in hair, (mainly OCs, PYR and OPs) is currently performed using GC-MS or GC-MS/MS. The use of tandem mass spectrometry provide lower LOQs, ranging from 0.01 to 5 pg mg^{-1}. Gas chromatography coupled to mass spectrometry in tandem (GC-MS/MS) was used for the analysis of 50 persistent and currently-used pesticides in hair by Schummer et al. [64]. The method utilizes both SPME and liquid injection, and provides LODs ranging from 0.01 pg mg^{-1} to 2 pg mg^{-1}. Others studies have also employed GC-MS/MS for determination of OC, OPs and pyretroids pesticides in hair, and in all cases two transitions were used, one for quantification and the second one for confirmation, presenting LODs from sub pg mg^{-1} to few pg mg^{-1} [60, 66].

The analytical methods for biomonitoring of persistent pesticides in blood and blood products (serum, plasma), breast milk and other rich-lipid matrices measure either the parent pesticide (e.g. DDT) or the biologically-persistent metabolites (e.g. DDE). In general, the persistent pesticides are analyzed together with other persistent organic pollutants (POPs) covered by the Stockholm Convention [90], including PCBs and dioxins. The effectiveness of this treaty has triggered the declining concentrations of the persistent pollutants in environmental and biological matrices (parts per trillion, ppt, levels). As a consequence, mores sensitive instrumental methods are required to quantify persistent pollutant amounts reliably. In breast milk, the analysis of persistent OCs using GC-MS/MS achieves LODs between 0.03 and 1.6 ng mL^{-1} (Table 3).

Gas chromatography coupled to high resolution mass spectrometry (GC-HRMS) is currently a routine method for quantification of persistent pesticides and other POPs in various environmental and biological matrices [91]. Salihovic et al.[75] recently published a rapid method for 23 POPs, including five OC, using GC-HRMS that requires a small amount of human plasma (0. 5ml). The analytes were chromatographically separated in a conventional capillary column (30 m x 0.25 mm i.d. x 0.25 µm DB-5), and detected in a mass spectrometer (MS) operating at > 10 000 resolving power. With the measurements performed in SIM mode and using the isotope dilution method using 13C-labeled standards for quantification, the authors reported LODs from 5.8-89 pg mL^{-1}.

Although today the analysis of persistent pesticides and other chlorinated and brominated POPs at ultra-trace levels in biological samples is mainly performed using established GC-(EI)-HRMS methods [34], other methods that use GC coupled to low resolution mass spectrometers (LRMS) working in SIM [63] or in tandem (MS/MS) mode [70] have recently been proposed .In this sense, Grimalt et al.[74] published an integrated method for the analysis of chlorinated pesticides and other halogenated organic pollutants in venous and umbilical cord blood sera, that uses ECD detector and LRMS after electron-capture negative ionization (ECNI) with ammonia as buffer gas. This method provides LODs from 1pg mL^{-1} to 10 pg mL^{-1} with a low sample volume (300 µL) and has been used in extensive biomonitoring programmes.

4. **Conclusions and future trends**

In the field of biomonitoring of pesticides, apart from the classical specimens (urine, blood), new non-invasive matrices are becoming increasingly relevant, including hair for studying exposure in the general population and meconium for studying fetal exposure. However, further studies are required to provide a better knowledge of the most suitable biomarkers of exposure for each pesticide in these matrices.

Even though conventional methods of sample preparation are still used in routine analysis, future trends could be marked by i) more generic extraction procedures (QuEChERS) that permit widening method scopes; ii) a reduction in sample volume (< 1 mL) to accommodate to sample limitation or to meet the increased demand for a reduction in size of the human

specimens collected; iii) automation (off-line, on-line) or simplification of sample treatment for high-throughput analysis forced by large scale biomonitoring programmes (Figure 2).

Although biomonitoring of persistent pesticides will remain, mainly linked to the international programmes for POP control, attention will be focused in evaluation of exposure to currently-used pesticides, which are more polar and consequently LC-amenable. Currently, this analysis is basically carried out by using conventional LC-columns (size> 2.5 µm). The introduction of UHPLC columns have increased resolution and require less time for multiresidue/multiclass analysis. A tendency to include this advantage in the field is now taking place; however, this approach has had so far little application.

The usual analytical system employed for polar biomarkers (metabolites or parent compounds) is the LC-MS/MS, but in a set-up for a reduced number of compounds. The incipient introduction of the recently developed high-resolution (> 50.000 FWHM) mass-accurate (< 3ppm) spectrometers, such as Q-TOF or Orbitrap, will continue, and this surely will lead to the routine implementation of combined quantitative target and post-run target analytical strategies for comprehensive determination of pesticide biomarkers, and other emerging contaminant biomarkers.

For analysis of chlorinated persistent pesticides in blood and mother milk, the currently-used GC-HRMS approach is well established, and provides enough sensibility for ultra-trace analysis. However there is a tendency to reduce the sample size and to increase the productivity in the context of massive biomonitoring programmes.

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Figure 1: Scheme of human biomonitoring and the analytical methods

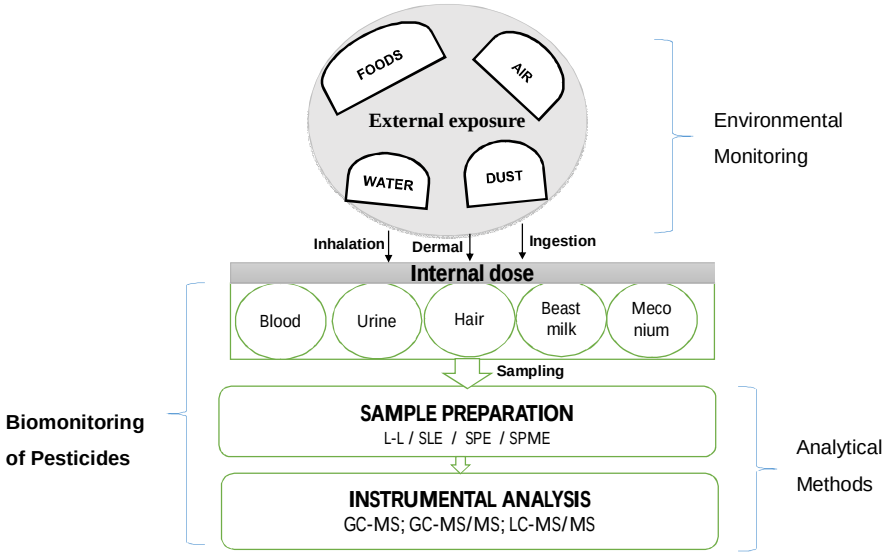


Figure 2: Trends in the analytical methods for biomonitoring of pesticides

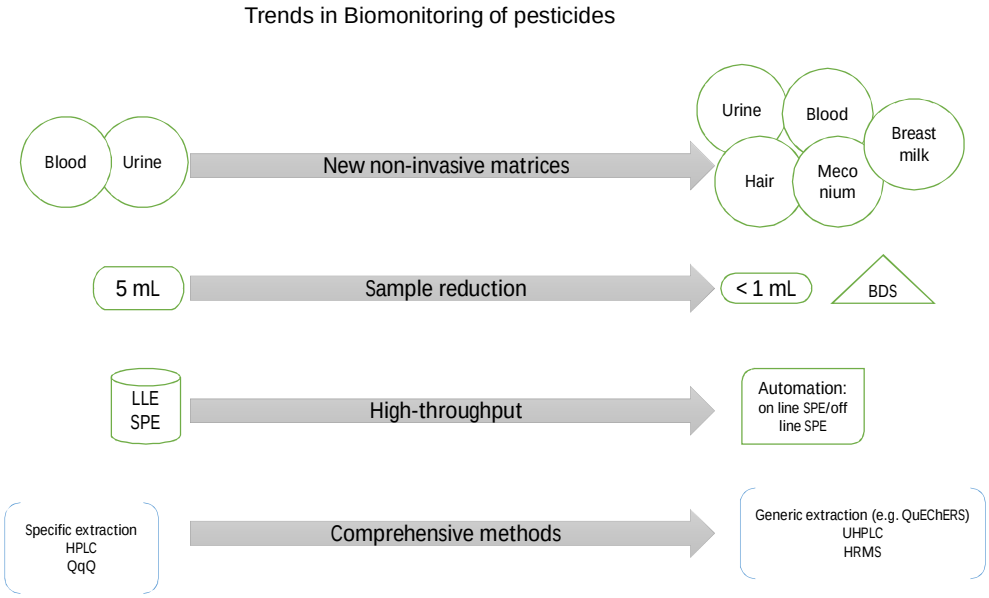
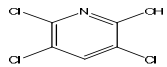
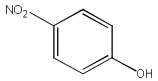
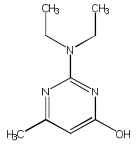
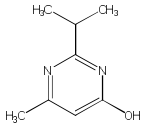
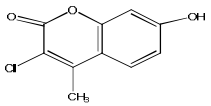
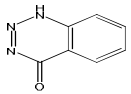
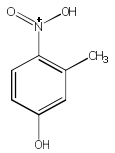
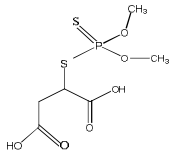
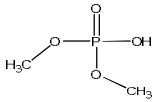
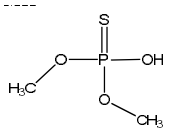
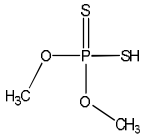
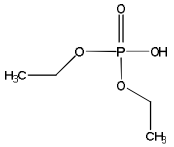
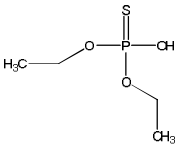
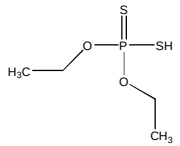
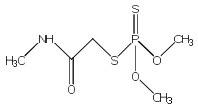
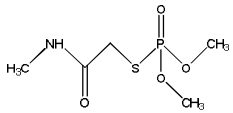
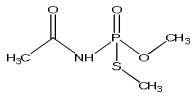
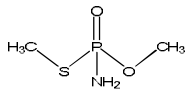
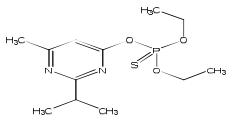
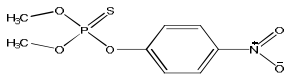
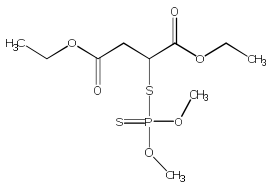
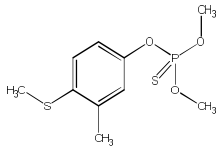
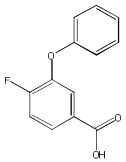
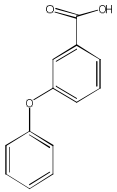
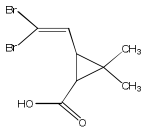


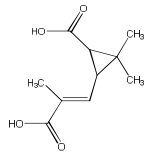
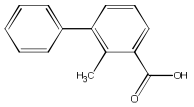
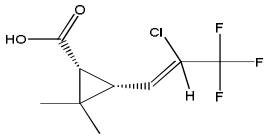
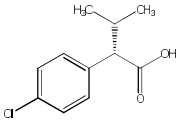
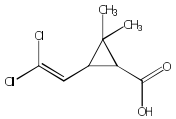
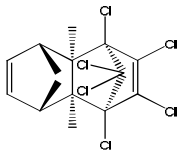
Table 1: Biomarkers of pesticides more frequently analysed in human specimens

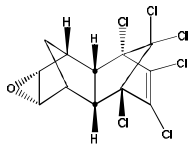
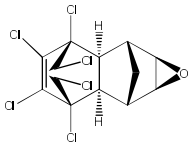
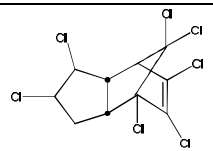
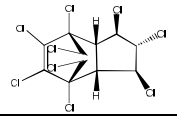
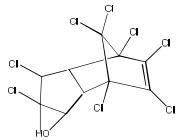
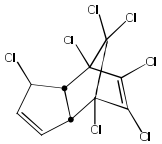
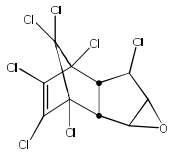
Biomarkers	Molecular structure	Parent (legal status)[1]	Matrix	Biomonitoring information
1. Organophosphate insecticides : specific metabolites			U	Elimination half-life range from hours to weeks. Urinary levels reflect recent exposures [16].
3,5,6-trichloro-2-pyridinol (TCPY)		Chlorpyrifos (in), chlorpyrifos-methyl (in)		
p-nitrophenol (PNP)		Parathion (out), methyl parathion (out)		
2-diethylamino-6-methyl-4-pyrimidinol (DEAMPY)		Pirimiphos-methyl (in)		
2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY)		Diazinon (out)		
3-Chloro-7-hydroxy-4-methylcoumarin (3-Chloro-4-methylumbelliferone) (CMHC)		Coumaphos (out)		
1,2,3-benzotriazin-4-one (BTA)		Azinphos-methyl (out)		
3-methyl-4-nitrophenol (MNP)		Fenitrothion (out)		

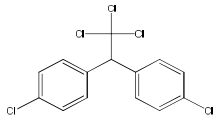
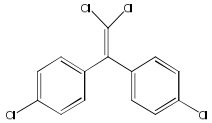
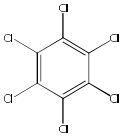
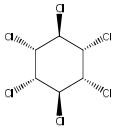
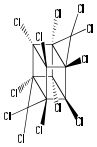
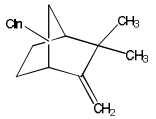
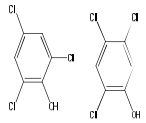
Malathion dicarboxilic acid (MDA)		Malathion (in)		
2. Organophosphate pesticides: dialkyl phosphate metabolites (DPAs)		Several OPs insecticides	U, H	Elimination half-life range from hours to weeks. Urinary levels reflect recent exposures [16].
Dimethylphosphate (DMP)				
Dimethylthiophosphate (DMTP)				
Dimethyldithiophosphate (DMDTP)				
Diethylphosphate (DEP)				
Diethylthiophosphate (DETP)				

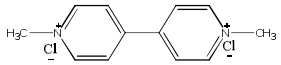
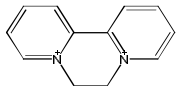
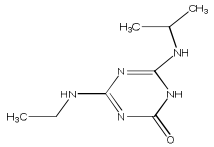
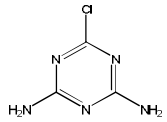
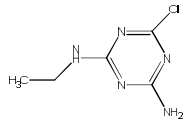
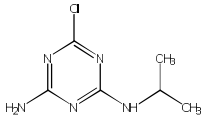
Diethyldithiophosphate (DEDTP)				
3. Organophosphate insecticides (intact)			U, H, B, M, Me	
Dimethoate (Dimet)		*(in)		
Omethoate (Omet)		*(out)		
Acephate (AP)		*(out)		
Methamidophos (MMP)		*(out)		
Diazinon		*(out)		
Methyl parathtion		*(out)		

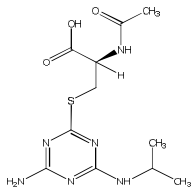
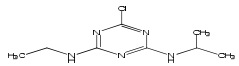
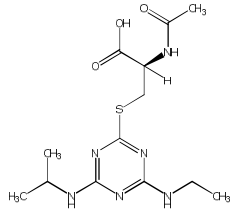
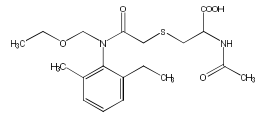
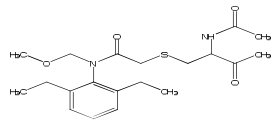
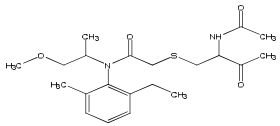
Malathion		*(in)		
Fenthion		*(out)		
Other parent OPs		*		
4. Pyrethroids			H, B, M, Me	Short biological half-lives, in the order of hours. After absorption these metabolites are eliminated over several days[15].
4-Fluoro-3-phenoxybenzoic acid (4-F-3-PBA)		Cyfluthrin (out), Beta-Cyfluthrin (in)	U	
3-Phenoxybenzoic acid (3-PBA)		Cyfluthrin (out), lambda Cyhalothrin (in), Cypermethrin (in), Deltamethrin (in),...	U	
cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (DBCA)		Deltamethrin	U	

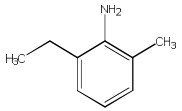
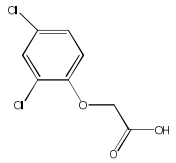
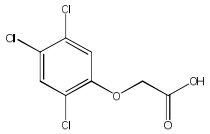
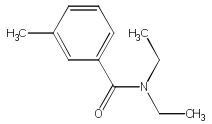
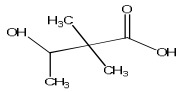
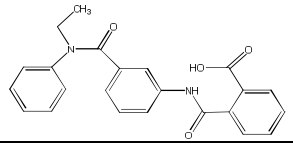
chrysanthemumdicarboxylic acid		Allethrin (out)	U	
2-methyl-3-phenylbenzoic acid (MPA)		Bifenthrin (in)	U	
3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid (HCBA)		Lambda-cyhalothrin (in)	U	
s-Fenvalerate acid (sFA)		Esfenvalerate (in)	U	
cis/trans-3-(2,2-dichlorovinyl)-2,2-imethylcyclopropane carboxylic acid (DCCA)		Cyfluthrin, Cypermethrin, Permethrin (out), ...	U	
Several parent pyrethroids		*	H, B, M, Me	
5. Organochlorine insecticides (OC)		out	B, **S**, P, H, M	OC bioaccumulate in fatty tissues. The elimination last from moths to several years. Most of them cross the placenta and are eliminated in breast milk [16].
Aldrin		*		Aldrin is metabolized to Dieldrin rapidly.

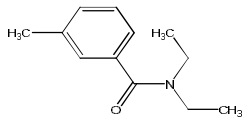
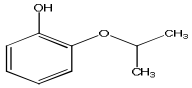
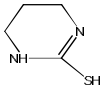
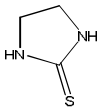
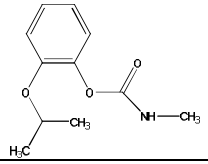
Dieldrin		Aldrin		
Endrin		*		Is metabolized rapidly to its metabolite. Usually not detected
Chlordane (α-Chlordane, γ-Chlordane, cis-Nanochlor,)		*		
trans-Nonachlor		Chlordane		
Oxychlordane		Chlordane		
Heptachlor		Chlordane		
Heptachlor epoxide		Heptchlor		

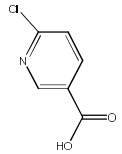
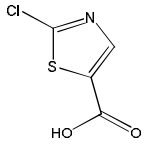
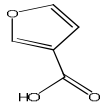
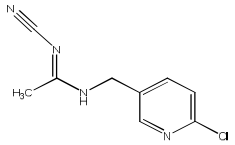
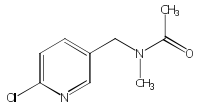
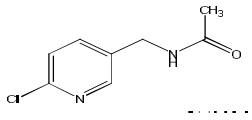
DDT		*		
DDE		DDT		DDE persist in the body longer than DDT. Serum levels is indicator of historic exposure
Hexachlorobenzene (HCB)		*		Accumulate in fatty tissues where persist for years. Serum concentrations reflect the body burden of HCH.
Hexachlorocyclohexane (HCH) (β-HCH, γ-HCH (lindane))		*		β-HCH half life: seven years
Mirex		*		Not metabolized in the body.
Toxaphene		*		
2,4,5 trichlorophenol (2,4,5-TCP); 2,4,6-TCP		Several OC: HCB, lindane,...		
6. Quaternary ammonium compounds (QAC)			U	Excreted unchanged by the kidney

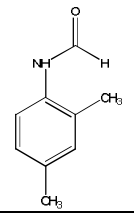
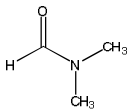
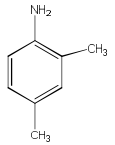
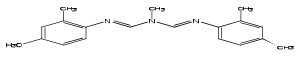
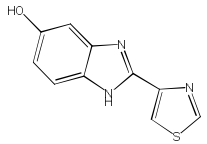
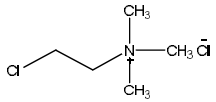
paraquat (PQ)		*		
diquat (DQ)		*		
7. Herbicides			U	
Hydroxiatrazine (HA)		Atrazine (out)		
Diaminochloratrazine (DACT)		Atrazine		
Deisopropylatrazine (DIA)		Atrazine		
Desethylatrazine (DEA)		Atrazine		

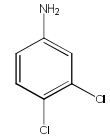
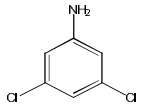
Desethylatrazine mercapturate (DEAM)		Atrazine		
Atrazine (ATZ)		Atrazine		Urinary levels reflect recent exposure
Atrazine mercapturate (ATZM)		*		Atrazines is metabolized and then eliminated in the urine in a few days
Acetochlor mercapturate		Acetochlor (out)		Urinary levels reflect recent exposure
Alachlor mercapturate		Alachlor (out)		Urinary levels reflect recent exposure
Metalochlor mercapturate		Metalochlor (out)		

2-methyl-6-ethylaniline		Methalochlor		
8. Phenoxyacid herbicides (PhA)		U, P, Me		Rapidly absorbed and excreted primarily unchanged in urine. Half- life of hours. Its presence in urine is reflective of exposure over the previous few days [15]
2,4-dichlorophenoxyacid (2,4D)		*(in)		
2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)		*(in)		
9. Insect repelent			U	
N, N-Diethyl-m-toluamide (DEET)		*		
N, Ndiethyl-3-hydroxymethylbenzamide (DHMB)		DEET		
3-(diethylcarbamoyl)benzoicacid (DCBA)		DEET		
10. Carbamates			U	

3-hydroxycarbofuran		Carbofuran out)		Quickly eliminated into urine. Reflects recent exposure
2-Isopropoxyphenol		Propoxur (out)		Quickly eliminated into urine. Reflects recent exposure
Propylenethiourea (PETU)		Propineb (in)		
Ethylenethiourea (ETU)		Ethylene bisdithiocarbamates: mancozeb in), maneb (in), metiram (in), nabam (out), zineb (out)		
Propoxur		*		
Other parent carbamates		*	U, H	
11. Sulfonylurea Herbicides (SUH) Chlorsulfuron, Foramsulfuron, Halosulfuron methyl, Mesosulfuron , ,Nicosulfuron, Oxasulfuron, Triasulfuron, …		*(in)	U	Rapid metabolism and excretion into both feces and urine. Urinary levels of the sulfonylurea herbicides reflect recent exposu
12. Neonicotinoid insecticides (NEOs)			U, S	It is considered reasonable to choose common NEO metabolites in urine as biomarkers [36]

6-chloronicotinic acid (6CN)		imidacloprid (in), nitenpyram(out) ,thiacloprid (in) and acetamiprid (in)		
2-chloro-1,3-thiazole-5-carboxylic acid (2CTCA),		thiamethoxam (in), clothianidin (in)		
3-furoic acid (3FA)		Dinotefuran (out)		
Several intact NEOs		*(in)	U,S	
N-desmethylacetamiprid		acetamiprid (in)		
5-(N-acetyl-N-methylaminomethyl)-2-chloropyridine (5-AMAM-2-CP)		acetamiprid		
5-(N-acetylaminomethyl)-2-chloropyridine (5-AAM-2-CP)		acetamiprid		
13. Others				

N-[2,4-(dimethylphenyl)-N' -methylformamidine (DMPF)		amitraz (out)	B	
2,4-dimethylformamidine (DMF)		amitraz	B	
2,4-dimethylaniline (DMA)		amitraz	B	
amitraz		*	B	
5-hydroxythiabendazole		thiabendazole (in)	U	
Chlormequat (chlorocholine chloride (CCC)		*	U	

3,4 Dichloroaniline (3,4-DCA)		Diuron (in), Linuron (in), Neburon (out), Prorpanil (out)		
3,5 Dichloroaniline (3,5-DCA)		Vinclozolin (out), Iprodione (in), Procymidone (out) (Chozolinate (out)	U	
	*Parent compound; [1]Legal status according with the Regulation (EC) 1107/2009 [37]. Data from the European Pesticide Database [38] In: approved; out: Not approved U: urine; S: serum, B: blood; H: Hair; M: breast milk; Me: meconium; P: plasma			

0

Table 2. Selected methods to determine pesticide biomarkers in urine

				Separation		Performance			
Compounds	**Sample (mL)**	**Extraction/Clean up**	**Analytical system**	**Mobile phase**	**Stationary phase**	**Recovery (%)**	**LOD (ng/mL)**	**RSD (%)**	**Ref.**
5 DAPs	1	SPE (SAX disk) Derivatization with CH_3I	GC-FPD		DB17 (30 m x 0.32 mm, 0.5 µm)	>80	5-11	< 7	[39]
6 : AP, Omet, MMP, Dmet, ETU, PTU	2	Lyophilization	LC-(APCI+)-MS/MS	MeOH (0.1%formic acid) Water (0.1%formic acid)	Zorbax SB-C3 (150 mm x 4.6 mm, 5 µm)	52-63	0.001-0.16	2.51-13.54	[40]
Atrazine and its 6 metabolites	0.2	Dilution 1:5 Online-SPE (Strata-X)	LC-(APCI+)-MS/MS	Methanol (0.1%formic acid) Water (0.1%formic acid)	Gemini C6-Phenyl (150 mm x 4.6mm, 3µm)	>80	0.1-2.8	4-20.5	[41]
6 DAPs	5	Lyophilization; Derivatization with PFBBr	GC-FPD	He	HP-5% phenyl methyl siloxane (30m x0.32 mm, 0.25 µm)	60-99	1-5		[42]
4 DAPs	2	SPE (BondElut PPL) Elution with ACN Derivatization with PFBBr	GC-(EI)-MS (SIM mode)	He	DB-5MS (30 m x 0.25 mm, 0.25 µm)	94-105	0.1-0.3	1-13	[43]
12 pesticides and metabolites	1.5	SPE online (ADS)	LC-UV	ACN Water (2.5mM ammonium formate)	Luna PFP (150 mm x 4.60 mm, 3 µm)	85-100	1-10	2.7-8.9	[44]
6 DAPs	0.6	Automated SPE (96-well Strata X-AW) Elution with TEA in ACN	LC-(ESI-)-MS/MS	93% of ACN 7% Water (100mM ammonium acetate)	Luna- HILIC (100 mm × 2.0 mm, 5µm)	40-100	0.04-1.54	18-21	[45]
2: PQ, 2Q	2	SPE (Strata-X-CW) Elution: 10% formic acid in ACN	LC-(ESI+-MS/MS	40% ACN 60% Water (250mM ammonium formate)	HILIC (150 mm x 2.1 mm, 5µm)	78-94	0.13-0.63	5-9%	[46]
Atrazine and its 6 metabolites	1	SPE (Strata X-C) Elution with 5% ammonium hydroxide in MeOH	LC-(APCI+)-MS/MS	MeOH Water (0,1 % formic acid)	Gemini C6-Phenyl (100mm × 4.6mm, 3 µm)	80-92	0.05-0.19	2,8-5	[47]
ETU	2	SPE (diatomaceous Chem Elut) Elution with DCM	LC-(APCI+)-MS	80 % Methanol 20% Water (0.1% formic acid)	Genesis C18 (250 mm x 4.6mm, 4µm)		2.5	11-14	[48]
18, DAPs and others alkyl phosphates	3	L-L (ACN) Dilution with water	LC-(ESI-)MS/MS		Luna Phenyl-Hexyl (2x 150 mmx 2 mm, 3 µm)	81-122	0.3-2.5		[49]

6: OPs,ETU, PTU	0.8	Lyophilization DCM/filtration in 96-well filtration plate. Reconstitution with MeOH	LC-(APCI+)-MS/MS	MeOH (0.1% formic acid) Water (0.1% formic acid)	Zorbax SB-C3 (150 mm x 4.6 mm, 5.0 µm)	70-90	0.004-0.01	9-15	[50]
chlormequat	0.1	automated SPE (HCX-Q) Elution with MeOH (1% formic acid)	LC/ESI+/MS/MS	ACN Water (0.05M acetic acid-ammonium acetate)	Atlantis HILIC (150 mm ×2.1 mm, 3µm)	92-105	0.1	5-9	[51]
11 Atrazine metabolites and hydrolysis products	0.5	Automated SPE (96 well Strata SCX) Elution with 2% NH_4OH in 80% MeOH	LC-(APCI+)-MS/MS	MeOH Water (0.5% formic acid)	Luna SCX (30 mm ×4.6 mm, 5µm) Gemini C6-Phenyl RP & Luna SCX (100 mm × 4.6 mm, 5 µm)		2.5–5	6-26	[52]
5 PYR Metabolites	5	L-L extraction (Hx in acidic conditions). Basic purification with sodium hydroxide	LC/ESI(-)/MS/MS	MeOH (5% water) Water (0.1% formic acid)	Atlantis T3 (150 mm x 2.1 mm, 5µm)	61-90	0.015	2-14.5	[31]
12 Pesticide metabolites	10	automated SPE (OASIS 96-well plate) Elution with Acetone	LC-(ESI-/+)MS/MS	ACN Water (5% MeOH & 1% acetic acid)	Betasil C18 (100 mm x 2.1 mm, 3µm)	50.5-92.9	0.03-0.4	5.6-25.1	[53]
7 PYR metabolites	2.5	automated SPE (SampliQC18) Elution with ACN	GC-(EI+)MS/SIM (2 ions, Q/q)	He	DB-5MS (30 m × 0.25mm, 0.25 µm)	81-100	0.02-0.08	2-6.6	[54]

DEET and two oxidative metabolites	0.1	on-line SPE (Chromolith Flash RP-18e monolithic column) mobile phase: 10% MeOH in 0.1% aqueous acetic acid.	LC-(APCI+)-MS/MS	ACN Water (0.1% acetic acid)	Phenyl-3 (PH-3) (100 mm × 4.6 mm, 5µm)	62-106	0.1-1	5,5-13	[55]
3 Nicotinoids metabolites	2	SPE (Bond Elute PCX) Elution with MeOH Derivatization with BSTFA-TMCS,	GC-(SIM-EI+)-MS	He	DB-5 MS (30 m × 0.25 mm, 0.25 µ m)	50-99	0.1	1,5-10	[36]
PQ	0.2	Derivatization with $NaBH_4$-$NiCl_2$ HE-SPME	GC-(SIM)-MS	He	Rtx-5 (10 m x 0.18 mm x 0.25 µm)	94-100	0.01	4-11	[56]
PQ	2	MSWCNTs)-d SPE elution: 5% TFA in ACN	LC-(ESI+)-MS/MS	60% ACN 40% Water (0.4% TFA)	Capcell Pak ST (150 mm x 2.0 mm, 2.6 µm)	92-108	0.94	0.-3.2	[57]
5-OH-TBZ	0.5	SPE (ISOLUTE-96ENV plate) Elution: ACN with 5% NH_3	LC-(ESI+)-MS/MS	gradient/ water-MeOH, with 0,1% formic ac.	Poroshell 120EC-C18 (233 mm x 4.6 mm, 2.7 µm)	100.3	0.05	5-9	[58]
200 polar and non-polar pesticides	5	SPE (C18) Elution with DCM	GC-IT-MS/MS LC-MS/MS	Methanol Water (0.01 % formic acid)	VF-5ms (30 m × 0.25mm, 0.25 µm; BEH C18 (100 mm × 2.1mm, 1.7 µm)	60-120	0.001 - 1.05	10-24	[30]
29 metabolites (Ops, PY, Her. Fun); post-target	5	QuEChERS (salts and ACN)	HPLC-(ESI+/-)HRMS (full scan); R= 50.000	MeOHl (0.1% acetic acid) Water (0.1% acetic acid)	Hypersyl Gold C18 (100 mm x 2.1 mm, 1.9 µm)	54-121	0.8-3.2	6-25	[59]

DAPs: Dialkylphosphates; ; AP: acephate. MMP: methamidophos. Omet: ometoqthe. Dimet: Dimethoate; ETU: Ethylenethiourea. PTU: propylenethiourea; PQ: Paraquat. DQ: Diquar; PFBBr : Pentqfluorobenzyl bromide; ADS: alkyldiol-silica; U: urine; M: breast milk; B: whole blood; S: serum; H: hair; At: adipose tissue; C: calostro; P: plasma; cB: cordon blood; DBS: dry blood spot; ACN: acetonitrile; TEA: Thriethylamine ; MeOH : methanol ; DCM : dichloromethane ;Hx: Hexane; L-L : liquid-liquid extraction; PYR: Pyrethoids; DEET: N,N-diethyl-m-toluamide; BSTFA-TMCS: N,O-bis(trimethylsilyl)trifluoroacetamide with 1%trimethylchlorosilane; HE-SPME: Head space- solid phase microextraction; MSWCNTs)-d SPE: magnetized single-walled carbon nanotubes-dispersive SPE; 5-OH-TBZ: 5-hydroxythiabendazole

Table 3: Selected methods to determine pesticide biomarkers in different biological specimens

					Separation		Performance			
Analytes	Matrix	Sample	Extraction/Clean up	Analytical system	Mobile phase	Stationary phase	Recovery (%)	LOD	RSD (%)	Ref
12 intact pesticides	H	50 mg	SLE with Hx	GC-(EI)-MS(SIM)	He	DB-5MS (30 m x 0.25mm x1.00 µm)	87-112	0.031-5.88 µg/g		[60]
6 OPs and OC	H	200 mg	SLE with MeOH LLE with EtAC and Hx	GC-MS(SIM)	He	HP-5MSI (30 m x 0.25 mm x 0.25 µm)	68-124	5 pg/mg		[20]
14 OC and OPs	H	200 mg	Incubation with HCl (3M) LLE with Hx-DCM (4:1) SPE (alumina+silica). Elution with Hx	GC-ECD/GC-MS(SIM)	He	HP-5 (30 m x 0.25 mm x 0.25 µm)	68-124	2.5-5 pg/mg		[20]
4 DAPs	H	100 mg	SLE with MeOH Derivatization with PFBBr	GC-(EI+)MS(SIM)	He	BPX5 (30m × 0.25mm × 0.25 µm)	84-116	3-6 pg/mg	10-13	[61]
16 intact pesticides	P	2 mL	SPE (ABS ELUT-Nexus) Elution with toluene	GC-(MID-EI+)-HRMS	He	DB-5MS (30m x 0.25 mm x 0.25 µm)	87-156	10 to 158 pg/mL	< 20	[62]
5 OPs	B		eDOSE (with Hx)	GC–(EI+)MS(SIM)	He	(Rtx-5MS (30 m x 0.25mm x 0.25 µm)	43-103			[63]
50 pesticides (OC and CUPs)	H	50 mg	SLE with ACN DI-SPME	GC-MS/MS	He	5 MS (30 m x 0.25x 0.25 µm)	42-112	0,01-2 pg/mg	1-37	[64]
chlorpyriphos, cypemethtin	c-B	0.3 mL	On-line SPE (Hypersil GOLD C8) Elution: MeOH & 20 mM ammonium acetate in Water	LC/ESI+/MS/MS	90% MeOH 10 % Water (20 mM ammonium acetate)	Hypersil GOLD C18 (50 mm ×2.1 mm x 1.9µm)	93-97	0.01- 0.05 ng/mL	8.8-12.3	[65]
22 OC and CUPs	H	50 mg	SLE with ACN DI-SPME	SPME-GC-MS/MS	He	HP-5MS (30 m x,0.25 mm x 0.25 µm)	72-84	0.05-10 pg/mg	>20	[66]
284 pollutants (57 pesticides)	At	5 g	SLE with ACN GPC (Bio-Beads S-X3, 360 mm ×25 mm i.d.); Mobil phase: Cyclohexane–EtAc (1 :1, v/v)	GC–(EI+)MS/MS	He	DB-1701 (30 m × 0.25 mm × 0.25 µm)	70-120	>0.01 mg/kg	< 20	[67]
57 organic pollutants (23 OC)	M	5 mL	QuEChERS (ACN saturated in n-Hx)	GC-(EI+)-MS/MS (QqQ)	He	BPX5 (30m x 0.25 mm x 0.25 µm)	76-105	0.05-0.5 µg/L	5-16	[68]
OC and PCBs	B,S	0,2 mL	On-line-SPE (HySphere C18 HD) Elution with n-Hx/dodecane (99:1, v/v)	GC-HRMS (SIR)		DB-5 MS 60 m x 0.1 µ m	30-114	0.007-0.017 µg/l	5-11	[69]
25 OC and CUPs	M	1ml	LLE with AcN & Hx SPE (GCB-PSA) Elution with ACN-toluene	GC-(EI+)-MS/MS	He	HP-5MS (30m x 0.25mm x 0.25 µm)	80-120	0.003-1.6 ng/ml	0.8-20	[70]
11 intact NEON and metabolites	U,S	1 mL	SPE (Extrelut®NT3) Elution with chloroform/2-propanol (3:1, v/v)	LC-(ESI+)-MS/MS	MeOH Water (ammonium acetate –0.1% formic acid)	Ascentis®C18 (150 mm x 2.1 mm × 0.3 µm)	81-106	0.1–1 ng/mL	8.3-13.2	[71]
2 Amitarz and its metabolite	B	1 mL	Automatic -SPE (Bon Elut C18) Elution with DCM-ACN-MeOH (2:1:1)	LC-(ESI+)-MS/MS	Me OH Water (0.1% formic acid)	Atlantis dC18 (150 mm × 3.9 mm x 5 µm)	90-104	0.5 µg/L	>15	[72]
9 OC, PYR	M	5 mL	SPD with Celite® LLE with n-Hx-Acetone (1:1, v/v) & n-HX-DCM (4:1, v/v)	GC-ECD	He	HP-5 30 m x 0. 250 mm x 0.25 µm)	3-20	0.002-0.057 µg/ml		[73]
45 OC and other halogenated pollutants	S, cB	0.3 mL	H_2SO_4+ Hx	GC-NICI-MS/GC-ECD	He	DB-5 (60 m x0.25 mm x 0.25µm)	90-120	1-10 pg/ml	0.1-14	[74]
23 POPs	P	0.5 mL	SPE (Oasis HLB) Elution with DCM/Hx H_2SO_4- Silica	HRGC-EI+-HRMS (SIM); R =10000	He	DB-5 (30m x 0.25 mm x 0.25 µm)	40-110	5.9-89 pg/ml		[75]
14 OC, PCBs	DBS	0.3 mL	Ultrasonic LLE with acetone-Hx-DCM	GC-HRMS (SIM), R>10000	He	ZB-5MSi (30 m x 0.25mm x 0.25 µm)	40-72	0.003-0.017 ng/mL	> 15	[76]

23 organic pollutants	DBS	0.05 mL	LLE (Hx-MTBE) SPE (H_2SO_4- Silica) Elution with Hx-DCM	GC-MS	He	DB-5 (30 m x 0.25mm x 0.25 µm)		183-300 ng/L		[77]
9 OC, PCBs	S	0.5	SPE (96-weell plate C18 disk) Elution with Hx-DCM GPC	GC-ECD/GC-MS(SIM)	He	DB-5 (50 m x 0.25 mm x 0.25 µm)	55-115	0.12-0.36 ng/mL	2-14.6	[78]
10 PYR	M	0.1 g dw	Sonication with Hx-DCM SPE (C18-basic alumina) Elution with ACN	GC–NCI-MS–MS (Ammonia)		DB-5MS (15 m × 0.25 mm x 0.1 µm)	76-104	32-1100 pg/g lipid	4-20	[79]
18 CUPs and metabolites	Me	0.5 g dw	SLE with MeOH-ACN SPE (Strata X-AW) Elution with MeOH (with formic acid)	LC-MS/MS	ACN Water (20 mM Amm. Act.	BEH Shield RP-18 (100mm x 2.1 mm x 1.7 µm)	20.2-94	0.2-200 ng/g	10-24	[21]

SLE: solid liquid extraction; LLE: liquid liquid extrqction; H: hair; P: plasma; M: breast milk; S: serum; cB: cordon blood; DBS: dry blood spot; Me: meconium; At: adipose tissue; EtAc: Ethyl Acetate. Hx: Hexane; DCM: dichloromethane; PFBBr: pentafluorobenzylbromide; eDOSE: Enzymatic Digestion-Organic Solvent Extraction; CUPs: currently used pesticides. ACN: acetonitrile; DI-SPME: direct immersion-solid phase microextraction; SIR: single ion recording; GCB-PSA: graphitized cabon black-primary secondary amine; NEON: neonicotinoids; SPD: solid-phase dispersion. R: resolution; DBS: dried blood spot; MTBE: methyl-t-butyl ether; PYR: pyrethroids; dw: dry weight