Chapter 18. Application in pesticide analysis: Liquid chromatography - A review of the state of science for biomarker discovery and identification

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18.1 A brief introduction to exposure biomarkers for environmental contaminants

18.1.1 Environmental Contaminants

Throughout history economic growth has led to an increased utilization of natural resources. This increased usage has manifested in the deposition of industrial wastewater, medical waste, and other waste residuals into the environment and the generation of polluted air. The widespread use of biohazardous pesticides, artificial food additives, pharmaceuticals, neutraceuticals, nanomaterials, and mineral-based beauty and skin care products also contribute to this environmental burden. The delicate balance between the growth of living organisms, resource utilization, and environmental stressors is being challenged. The persistence of environmental contaminants in many environmental compartments presents a potential risk to humans and ecosystems. Human exposures leading to adverse health outcomes can increase as the variety and quantity of contamination sources increase.

Environmental contaminants can enter into the body through different routes of exposure (e.g., dermal, inhalation, ingestion) leading to various outcomes. The harmful effect caused by a pollutant depends on the route of exposure as well as its innate toxicity, the effective exposure dose, and host factors such as age, gender, nutritional state, and developmental stage [1].

18.1.2 Biomonitoring

The conventional approaches used for environmental monitoring make it difficult to determine causal connections between contaminants and biological responses. Depending on the chemical properties of the xenobiotic, exposures can be determined by measuring parent compound, metabolites, conjugates or other biomarkers induced by the exposure. Biomonitoring is based on measuring the concentration of these biomarkers in biological fluids such as blood, urine, saliva, sputum, sweat, and cerebrospinal fluid. The Centers for Disease Control and Prevention has stated that "biomonitoring measurements are the most health-relevant assessments of exposure because they measure the amount of the chemical that actually gets into people from all environmental sources, such as the air, soil, water, dust, or food combined" [2]. Biomonitoring is an effective approach for assessing human exposure to chemicals and to reduce uncertainties along the source-to-outcome continuum [3]. Environmental monitoring coupled with biomonitoring has become an increasingly important research area to study the interactions of contaminants with humans and ecosystems. Tracking the levels of environmental contaminants enables an assessment of exposure and the effects of environmental pollutants on an organism as well as the environment. Biomonitoring measurements aid in identifying contaminants of potential ecological or public health concern and provide data for risk assessments and effective risk management.

Biomonitoring has been used to monitor chemical exposures for decades; however, advances in analytical methodologies such as with high performance liquid chromatography (HPLC) in tandem with mass spectrometry (MS) enable the discovery and identification of new

biomarkers in complex matrices, as well as the measurement of more chemicals, in smaller concentrations, using smaller sample sizes [2]. Improvements in HPLC such as the use of smaller particles (< 2 μm i.d.) and ultra high pressures up to 15,000 pounds per square inch (psi) enables the analysis of trace concentrations at low parts per trillion [4-6]. Through these advancements, biomonitoring has become more widely used for a variety of applications, including public health research, evaluation of environmental regulations and risk assessment and management.

18.2 Biomarkers

Biomarkers have emerged as very powerful indicators of xenobiotic exposure and augment the traditional methods of environmental monitoring used to determine the presence and potential harmful effects of environmental contaminants. An increasing number of research papers from various fields, including exposure science, environmental epidemiology, toxicology, occupational and environmental medicine, analytical chemistry, public health and pharmacology report on the utility of biomarkers [7].

The biomarker approach for environmental toxicology and ecological risk assessment and monitoring has been adopted from medical toxicology and pharmacology [8]. The term biomarker is used in a broad sense dependant on the research application and biological level of analysis [e.g., 9-13]. For environmental applications a generally accepted definition of a biomarker is that it is a substance in a biological fluid or tissue that is measurable at the biochemical, cellular, physiological, or behavioral level that reflects exposure to an environmental agent resulting in cytotoxic or other biologic effects [9,10,12-14]. For example, a biomarker could be defined as an alteration in conformation and/or function induced by a genetic mutation caused by exposure to an environmental contaminant [15].

Exposure to a diversity of environmental contaminants may result in various cellular toxic response mechanisms that are mediated by different signal transduction pathways. Several studies have demonstrated that many pollutants can exert toxic effects on living organisms first by interacting with cellular macromolecules, such as proteins and nucleic acids, leading to DNA damage, apoptosis (programmed cell death), and heritable epigenetic mutations [16-24]. The toxic effects of pollutants can be further reflected at the tissue (histopathological), organ (malfunction), and organismal (deformity, carcinogenisis) evels, as well as at the different levels of organization in an ecosystem (Table 18.2) [25-29].

18.2.1 Biomarker Characteristics

Biomarkers can be used to measure the sensitive alterations in the state of biological systems before cellular damage advances to become a serious adverse condition. A list of criteria for an ideal biomarker is [30]:

1) Sensitivity
2) Specificity
3) Reliability (able to measure accurately)
4) Extensively field-validated
5) Easy to obtain

18.2.2 Biomarker Classification

Biomarkers can be classified on the basis of different parameters for particular applications. Three main categories of exposure, effect (sometimes referred to as response) and susceptibility are typically used to classify biomarkers [9]. This classification is not exact and may require clarification e.g., DNA adducts could be considered as a biomarker of both exposure and effect [31].

18.2.2.1 Biomarkers of exposure

External exposure to environmental contaminants may result in absorption leading to an internal dose, followed by distribution, metabolism and excretion. The U.S. National Academy of Sciences, Institute of Medicine defines a biomarker of exposure as: "a constituent or metabolite that is measured in a biological fluid or tissue that has the potential to interact with a biological macromolecule". Biomarkers of exposure can be divided into three sub-groups: potential dose or external dose, internal or absorbed dose, and biologically effective dose [32]. This type of biomarker is an effective tool to assess exposure to environmental contaminants. Compared to conventional methods that measure trace amounts of xenobiotics in biological media, biomarkers are able to detect and quantify the metabolic products of xenobiotic metabolism as well as the interaction between xenobiotics and their internal targets, such as macromolecules or cells [33].

18.2.2.2 Biomarkers of effect

Ideally, a biomarker of effect is a quantitative measurable alteration that depending on its magnitude can be directly linked with a pathological change [14]. These biomarkers are biological indicators of the body's response to external exposures and may indicate the presence of early sub-clinical problems or the impairment of normal physiological function which may lead to an adverse health outcome.

Biomarkers of effect can track early biological effects including mild biochemical modifications in target tissues, that may develop into non-reversible, severe functional and/or structural damage [32,34], such as chromosome aberrations [35], and targeted gene mutations [36]. For example, micronuclei formation, detected in cultured peripheral lymphocytes has been used as a biomarker to assess the genotoxic potential of exposure to high concentrations of organic solvents such as toluene [37].

18.2.2.3 Biomarkers of susceptibility

Biomarkers of susceptibility are indicators of an inherent or acquired ability of an organism to respond to the challenge of being exposed to a foreign substance [14]. Biomarkers of susceptibility can be inherited or induced, and may indicate differences between individuals or populations affecting their response sensitivity [9,38,39]. Genetic polymorphism, pre-existing conditions (e.g., obesity, diabetes, diminished organ function), genotypic characteristics, differences in metabolic rate, variations in serum immunoglobulin concentrations, and cellular regeneration rate from environmental insults are examples of biomarkers of susceptibility [38,40-44].

Genetic biomarkers provide an estimate of how genetic variations influence an individual's susceptibility to an environmental agent. Following a genomics approach, inherited genetic susceptibility has proven to be an important factor in the toxicological response

manifested, leading to a new focus on identifying biomarkers of genetic variations in xenobiotic metabolism genes [39,45-47].

The metabolism of xenobiotic agents is typically a process to convert toxic molecules into more readily excretable hydrophilic compounds via the renal and biliary systems [48,49]. Polymorphisms in genes that encode xenobiotic-metabolizing enzymes have been extensively used as biomarkers for the evaluation of increased susceptibility to environmental toxins [40,50].

18.2.3 "Omic" approaches for biomarker discovery

Several "Omic' approaches are being employed to determine biomarkers for both clinical and environmental exposure applications. HPLC-MS based proteomics is a rapidly developing technique suitable for both qualitative and quantitative assessment of proteins particularly for monitoring protein profiles expressed by cell cultures that have been exposed to xenobiotics when stable isotope labeling is used. Figure 18.1 provides the overall approach for determining biomarkers using cell cultures and HPLC-MS/MS. Stable isotope labeling with amino acids in cell culture (SILAC) is a simple and accurate approach that depends on the incorporation of amino acids containing substituted stable isotope nuclei (e.g., ^{2}H, ^{13}C, ^{15}N) into proteins in living cells [51]. In addition to genomic and proteomic platforms, other "omic" techniques such as metabolomics, lipidomics, glycomics, and secretomics are also being used and many require HPLC-MS capability (Table 18.1).

18.3 A description of liquid chromatography techniques used for biomarker studies

In the mid 1970s, Csaba Horváth first introduced the acronym HPLC standing for high pressure liquid chromatography in his Pittcon presentation. The continued development of chromatographic performance has allowed the use of very small particles, small column diameters, and very high fluid pressures to achieve enhanced separations in shorter periods of time. Thus, the acronym HPLC has become known as high performance liquid chromatography, rather than "high pressure".

Table 18.4 lists several commercially available detectors for use in HPLC for environmental and biological monitoring. Each detector has its own merits and distinctions for application to different target chemicals. A data acquisition system collects the data information from the HPLC and presents it as chromatogram, providing qualitative data (retention time) and quantitative data (area under curve).

Traditional HPLC detectors include ultraviolet-visible (UV-Vis), fluorescence, refractive index, electrochemical and mass spectrometry, as shown in Table 18.4. MS detection provides the most sensitivity and specificity. MS data provides valuable information about the molecular weight, structure, identity and quantity of the target analyte.

18.3.1. Components of mass spectrometer

A mass spectrometer consists of four main components: inlet system, ion source, mass analyzer, and detector. An ion source is an electro-magnetic device to generate charged ions. These ions are then transferred by electromagnetic fields to a mass analyzer [97]. Improvements in ion source techniques such as atmospheric pressure ionization (API) have greatly expanded the number of compounds that can be successfully analyzed by HPLC-MS [98]. Sample molecules are ionized under atmospheric pressure and the ions are then mechanically and electrostatically

separated from neutral molecules. A mass analyzer sorts and identifies ions by their mass/charge (m/z) ratios [90]. Four basic types of mass analyzers are most often used in HPLC-MS: quadrupole, ion trap, time of flight (TOF), and Fourier transform-ion cyclotron resonance (FTICR) [98]. An MS detector can generate both two-dimensional abundance data and three-dimensional mass spectra data to determine the molecular weight and structure [98]. A full description of all types of ion sources, detectors and their underlying mechanisms is outside the scope of this chapter. We present a brief overview of MS techniques used in tandem with HPLC for developing biomonitoring methods.

18.3.2 Basic principles of mass spectrometric detection

Mass spectrometers weigh molecules electronically by ionizing molecules and then sorting and identifying the ions according to their *m/z* ratios [99]. The sample is introduced into the mass spectrometer and vaporized forming ions. The ions of charge z (where z is the elementary charges carried by the ions) are produced by an ion source within the instrument. All of the ions are then accelerated by an electric field so that they have similar kinetic energy. The ions with different m/z ratios are separated in various ways such as by a quadruple mass filter. The ions at each m/z value are detected in proportion to their abundance producing a mass spectrum (a plot of ion abundance versus m/z ratios) [100]. The ions generated from the sample provide information concerning the nature and the structure of their precursor molecule [101]. The resulting mass spectrum provides information to determine the molecular weight and structure of the sample compounds, and the relative abundance of a specific compound in the sample matrix [102].

Biomarker analyses are frequently performed by HPLC in tandem with two mass analyzers (HPLC-MS/MS). The first mass analyzer is a non-destructive mass analyzer, such as a quadrupole or an ion trap, that initially separates the sample components into parent ions and sequentially releases parent ions of known m/z ratios. In a collision cell, the parent ions interact and collide with molecules of an inert gas (helium, neon, argon, or nitrogen) to break into pieces of daughter ions [103]. These daughter ions then move into the second mass detector such as a time of flight analyzer, providing a full spectrum of mass data for the daughter ions to compliment the parent ion mass spectrum data. MS/MS can detect trace amounts of the target in complex samples with exceptional sensitivity.

HPLC-MS/MS has been used as a very powerful analytical technique for separating and quantifying trace quantities of biomarkers from complex mixtures. For the quantification of very complex samples, MS/MS has the advantage over the conventional MS to select and direct particular ions of interest to the second mass detector for further investigation. Since the matrix-induced background signal is greatly reduced, sample cleanup prior to chromatographic separation becomes much less critical.

18.3.3 Isotope dilution mass spectrometry coupled with HPLC

Stable isotope dilution mass spectrometry (SIDMS) is a quantitative technique providing high accuracy and precision. It is able to determine ultra-trace concentrations (< pg/g) of compounds in biological and environmental matrices [104]. The term SID refers to the use of a known concentration of a stable isotope-labeled internal standard spiked into the sample. The stable isotope-labeled analog is identical to the endogenous target analyte with the exception of having

a heavier mass [105]. The isotope-labeled internal standard has identical physicochemical properties as the target analyte and can act as a surrogate during the extraction and analysis steps preventing loss of the target analyte [105,106]. Thus, the internal standard can verify the presence of the compound and normalize experimental variables, such as matrix effects and system instabilities.

SIDMS in combination with HPLC (LC-SIDMS) can provide a very high degree of specificity for quantification. LC-MS/MS is applicable to the analysis of a wide range of biomarkers and is now widely used in the discovery and validation of exposure biomarkers [105].

Lindh et al. have studied the plant growth regulator chlormequat in human urine as a biomarker of exposure by HPLC-MS using an Atlantis HILIC column (3μm particle size, 150 mm x 2.1 mm) and 0.05 M acetic acid/ammonium acetate buffer (pH 3.75) in water and acetonitrile as the mobile phase. The experiment was carried out using the selected reaction monitoring in the positive ion mode. [$^{2}H_4$] labeled chlormequat was used as the internal standard for quantification. The limit of detection was determined to be 0.1 ng/ml with a reproducibility of 3-6% [75].

Norrgran et al. have proposed a method for quantification of six herbicide metabolites (atrazine mercapturate, acetochlor mercapturate, metolachlor mercapturate, 2,4,5-trichlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid) by HPLC-MS using a Betasil Hexylphenyl column (3 μm particle size, 4.6 mm x 100 mm) and automated liquid delivery of internal standards and acetate buffer. Isotope dilution calibration was used for quantification of all analytes. The mobile phase was acetic acid in water or acetonitrile. The limit of detection ranged from 0.036 to 0.075 ng/ml [107].

Lindh et al. developed a method to quantify 3,5-dichloroaniline (3,5-DCA) as a biomarker of the fungicides vinclozolin and iprodione in human urine by LC-MS/MS using a Rapid Resolution Zorbax Eclipse XDB C_{18} column (1.8 μm particle size, 2.1 mm x 50 mm). The mobile phase consisted of water and methanol, with 0.5% acetic acid. The urine samples were treated by basic hydrolysis to degrade the fungicides, their metabolites and conjugates to 3,5-DCA. The 3,5-DCA was then extracted using toluene and derivatized using pentafluoropropionic anhydride (PFPA). Analysis of the derivative was carried out using selected reaction monitoring in the negative ion mode. Quantification of the derivative was performed using [$^{13}C_6$]-labeled 3,4-DCA as an internal standard with good precision and linearity in the range of 0.1-200 ng/ml urine. The limit of detection was determined to be 0.1 ng/ml [83].

18.3.4 Liquid chromatography/electrospray ionization-tandem mass spectrometry

Electrospray ionization (ESI) is a common atmospheric pressure ionization technique [98]. ESI uses electrical energy to transfer sample compounds into gaseous phase ions for analysis. Column effluent is sprayed into a chamber at atmospheric pressure in the presence of a strong electrostatic field and a heated drying gas. The electrostatic field further breaks down the compounds to form droplets. The heated drying gas causes the solvent in the droplets to

evaporate. As the droplets shrink, the charge concentration in the droplet increases and ions are ejected into the gas phase and exported into the mass analyzer [98].

HPLC coupled with electrospray ionization-tandem mass spectrometry (HPLC/ESI-MS/MS) is a very powerful technique capable of analysing both small and large molecules of various polarities in a complex sample. With the additional separation capacities of MS/MS, sample purification prior to HPLC may not be necessary [108].

Sams et al. developed a method to quantify two major metabolites [2-(dimethylamino)-5,6-dimethylpyrimidin-4-ol (DDHP) and 5,6-dimethyl-2-(methylamino)pyrimidin-4-ol (MDHP)] of the carbamate insecticide pirimicarb in human urine by HPLC-MS. HPLC-MS was carried out on an Agilent 1100 chromatograph interfaced to an ion trap mass spectrometer. A Genesis C18 column (3 μm particle size, 250 mm x 2.1 mm) was used for chromatographic separation of the analytes. The MS was operated using positive ESI. Nitrogen was used as the nebuliser gas at 15 psi and as the drying gas at 61/min, 350 °C. Metabolites were detected at the following m/z ratios: MDHP (154); DDHP (168); and internal standard (168) [109].

Li et al. developed a method to detect pesticide adducts in tryptic digests of butyrylcholinesterase (BChE) as biomarkers of pesticide exposure in human plasma from patients poisoned by pesticides. BChE was purified from 2 ml serum by ion exchange chromatography at pH4, followed by procainamide affinity chromatography at pH7. A 5 ml aliquot of HPLC-purified, tryptic BChE peptides were injected onto a Vydac C_{18} polymeric reverse-phase nanocolumn for HPLC separation. Peptides were separated with a 90 min linear gradient of acetonitrile (0-60%) and formic acid (0.1%) and then electrosprayed through a fused silica emitter directly into a hybrid quadrupole linear ion trap mass spectrometer (QTRAP 4000) [110].

18.3.5 Ultra-high-pressure liquid chromatography with mass spectrometry

The evolution of chromatographic methods has been in part due to the reduction in the particle size of the column packing materials. However, a decrease in the particle diameter leads to an increase in the column backpressure. A column backpressure exceeding 10000 psi (~ 700 bar) is referred to as ultrahigh-pressure liquid chromatography (UHPLC) [111]. UHPLC has a higher separation and throughput for the rapid discovery and monitoring of biomarkers via the utilization of sub-2 micron column particle sizes at high linear velocities of 3.5-6 mm/s [112]. Columns packed with sub-2 micron particles are generally divided into two categories: 1) short columns (less than 5 cm); and 2) capillary columns (inner diameter less than 100 μm). Recent studies have shown that the efficiency of 1.0 mm id columns (15 cm long) packed with 1.5 μm packing materials is approximately twice as high as 150 mm analytical columns packed with 3 μm materials [111].

Most UHPLC biomarker analyses are performed using reversed phase (RP) columns such as the Acquity High Strength Silica T3 and the Acquity C_{18} with a bridged ethylsiloxane-silica hybrid adsorbent [113-117]. Hydrophilic interaction chromatography (HILIC) is sometimes used, but it is applicable only for the separation of polar metabolites [113]. Mass spectrometry is normally the detector for UHPLC separation using positive or negative electrospray [118]. Heat assisted electrospray ionization (HESI) is also used when it is necessary to eliminate potential

interferences and improve sensitivity [119]. Triple quadrupole, quadrupole-time of flight (Q-TOF), and Orbitrap instrumentation have all been used as mass analyzers [113, 120-123].

Chen et al. described a method to determine nine environmental phenols (bisphenol A, 2,3,4-trichlorophenol; 2,4,5-trichlorophenol; pentachlorophenol; triclosan; 4-tert-octylphenol; 4-n-octylphenol; 4-n-nolyphenol; and benzophenone-3) in human urine by UHPLC-ESI-MS/MS. A [$^{13}C_6$]-labeled internal standard was added to the samples before the analytes were extracted and preconcentrated with solid-phase extraction. The chromatographic separation was carried out on an Acquity UPLC BEH, C_{18} column (1.7 μm particle size, 2.1 mm x 100 mm) maintained at 35 °C equipped with a filter (Frit, 0.2 μm, 2.1 mm) and a Van Guard BEH, C_{18} pre-column (1.7 μm). The mobile phases were methanol and water. The ions were detected by a Waters Quattro Premier mass spectrometer using an ESI probe in the negative ion mode and with a multiple reaction monitoring mode. The flow rate was 0.25 mL/min (11 min run time). The LOD for all nine compounds ranged from 0.02 to 0.90 ng/mL [124].

Alwis et al. have developed a sensitive and high throughput method to simultaneously measure 28 metabolites as biomarkers of exposure to volatile organic compounds in human urine using reverse-phase UHPLC coupled with ESI/MS/MS. The chromatographic separation was performed using an Acquity UPLC HSS T3 column (1.8 μm particle size, 2.1mm x 150 mm). The mobile phase was 15 mM ammonium acetate pH 6.8 and acetonitrile. The eluent from the column was ionized using an electrospray interface (-4000v) and the mass spectrometer was operated in the multiple reaction monitoring mode for negative ions. The ion source temperature was 650 °C. The LOD for all 28 metabolites ranged from 0.5 to 20 ng/mL [125].

Hsiao et al. have described a sensitive and high throughput method to detect the conjugate 2,5-dichlorophenol glucuronide (2,5-DCPG) as a biomarker of exposure to1,4-dichlorobenzene in human urine using solid-phase extraction for sample preparation and UHPLC-MS/MS with negative ESI for detection. The mobile phase was ammonium acetate buffer and methanol [126].

18.3.6 Nano-HPLC

Nano-liquid chromatography was first introduced by Karlsson and Novotny in 1988 as a complementary separation method to conventional HPLC [127]. Nano-HPLC has several advantages such as higher efficiency, ability to work with minute sample sizes, lower consumption of mobile phases (reducing the use of organic solvents), and better compatibility with MS [127,128]. Nano-HPLC is highly compatible with MS due to the relatively low flow rate (40-600 nL/min) that allows the transfer of the entire effluent from the column [128,129].

The chromatographic separation is performed in capillary columns of ids in the range between 10 and 100 μm. Capillary columns are typically fused silica or polyetheretherketone (PEEK™). Several HPLC stationary phases are applicable to both silica and PEEK™. The development of smaller particle sizes (3-5 μm d_p) with uniform pore sizes improves efficiency, resolution, and selectivity, all with a shorter analysis time. A drawback to this technique is the

increase in backpressure. More recently, particles of 1.5-1.8 μm d_p have been successfully employed in ultra performance LC [128].

MS can be easily coupled with nano-LC instrumentation through the different nanospray interfaces available. Normally, the capillary column used for the chromatographic separation is connected to the emitter tip through a zero dead volume union attached to a power supply (voltages between 1000 and 2500 V) [128-131].

Nano-LC has been applied to the separation of a wide number of compounds in different areas such as proteomic and pharmaceutical research [128]. Nano-LC has not been widely used for the analysis of compounds of environmental interest up to now, although HPLC is one of the major techniques for the analysis of pollutants and their metabolites. Very few studies report the application of nano-LC in environmental analysis [128]. One study reported by Cappiello et al. described the use of a new nano-LC gradient generator coupled to a modified direct electron ionization LC-MS interface for the analysis of pesticides, nitropolynuclear aromatic hydrocarbons (PAHs), and hormones [132]. Rosales-Conrado et al. proposed the enantiomeric separation of phenoxy acid herbicides mecoprop, dichlorprop, and fenoprop in their acid form by nano-LC using a 75 μm i.d. capillary column packed with vancomycin-modified silica particles of 5 μm. This separation capability is important as the (R) isomers of the phenoxy acid herbicides show much higher herbicide activity and a different metabolism than the (S) isomers [133]. Aryal et al. described an approach for the detection and quantification of phosphorylated BChE activity as an exposure biomarker of organophosphates and nerve agents by coupling magnetic bead-based immunoaffinity purification with LC-MS/MS. They purified BChE protein by biotinylated anti-BChE polyclonal antibodies conjugated to streptavidin magnetic beads. The peptide samples were analyzed using an automated nano-flow, metal-free nano-LC system with an i.d. capillary column (40 cm x 50 μm) packed with 3 μm Jupiter C_{18} silica. The heated capillary was maintained at 200 °C and the ESI voltage was held at 2.2 kV [134].

Summary

Exposure to environmental contaminants may result in human health effects and may also negatively impact the balance within an ecosystem. Environmental monitoring is needed to provide environmental fate and transport information to determine the deposition of contaminants and identify potential exposure sources and routes. Biomonitoring is critical to detect exposures and early biological changes caused by environmental contaminants that may lead to an adverse outcome. Both monitoring approaches require reliable analytical methods to provide data of known quality.

Biomarkers are effective tools for biomonitoring studies to assess exposure to environmental contaminants. Biomarkers are vital to understanding the relationships between exposure and adverse outcomes in humans and the environment. Liquid chromatography particularly in combination with mass spectrometry has been extensively utilized for the discovery and identification of new biomarkers to determine metabolites, biotransformation enzymes, biotransformation products, stress proteins, and other markers. The continued advancements in LC methods will undoubtedly keep pace with the increasing variety and chemical composition of environmental contaminants and enable the discovery and identification of new biomarkers to safeguard human health and the environment.

Sensitive and high throughput analytical methods are essential for the detection of various biomarkers of exposure to support biomonitoring studies such as the National Biomonitoring Program conducted by the Centers for Disease Control and Prevention [135], and U.S. EPA environmental monitoring studies. The combination of data from both types of monitoring studies provides insight to the complete exposure scenario from source-to-outcome.

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