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Liquid and gas-chromatography-mass spectrometry methods for exposome analysis

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ABSTRACT

Mass spectrometry-based methods have become fundamental to exposome research, providing the capability to explore a broad spectrum of chemical exposures. Liquid and gas chromatography coupled with low/high-resolution mass spectrometry (MS) are among the most frequently employed platforms due to their sensitivity and accuracy. However, these approaches present challenges, such as the inherent complexity of MS data and the expertise of biologists, chemists, clinicians, and data analysts to integrate and interpret MS data with other datasets effectively. The "omics" era advances rapidly, driven by developments of AI-based algorithms and an increase in accessible data; nevertheless, further efforts are necessary to ensure that exposomics outputs are comparable and reproducible, thus enhancing research findings. This review outlines the principles of MS-based methods for the exposome analytical pipeline, from sample collection to data analysis. We summarize and review both standard and cutting-edge strategies in exposome research, covering sample preparation, focusing on MS-based platforms, data acquisition strategies, and data annotation. The ultimate goal of this review is to highlight applications that enable the simultaneous analysis of endogenous metabolites and xenobiotics, which can help enhance our understanding of the impact of human exposure on health and disease and support personalized healthcare.

1. Introduction

There is mounting evidence that environmental factors play a significant role in human health. Exposome research aims to explore the effects of these environmental factors by capturing chemical, biological, and physical stressors and their association with human (patho)physiological responses [1,2]. By determining external exposures, biological responses to exposures, and/or host susceptibility at a systems level, it is possible to establish links between the exposome and health outcomes [3]. Nevertheless, characterizing an individual's exposome is challenging because exposome profiles are highly dynamic, individual-based, and associated with both (a) nonchemical parameters, including lifestyle, socioeconomic, and demographic factors, and (b) chemical profiles of inorganic and organic substances that can be explored through analytical approaches. In this review, we will focus on analytical approaches applied to a comprehensive chemical profiling of organic substances, including both endogenous metabolites as well as xenobiotics, with a focus on environmental pollutants.

The chemical space in the human exposome includes the endogenous metabolome (human and gut microbiota-derived metabolites) and xenobiotics coming from various external sources, such as diet, medication, as well as non-intentional exposure to environmental pollutants and their chemically and biologically transformed products [2]. These substances are of utmost relevance due to their known (and unknown) environmental impact and effects on human health [2]. It is estimated that over 350,000 man-made chemicals and their mixtures have been registered for large production and use worldwide [4]. Only in the European Union (EU) market, by the latest survey, over 26,000 new exogenous chemicals were registered under the European Chemical Agency (ECHA) regulations; nonetheless, the current chemicals on the market at volumes below one ton (which includes several compounds as polymers, pharmaceuticals, biocides, pesticides) are not counted in the list (https://chem.echa.europa.eu/). This list includes persistent organic pollutants (POPs) and pseudo-persistent chemicals with shorter

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half-lives, including polycyclic aromatic hydrocarbons (PAHs), surfactants, polychlorinated biphenyls, pesticides, dioxins, polyfluorinated alkyl substances (PFAS), flame retardants (FR), pharmaceuticals and personal care products. Notably, not only has the number of newly manufactured chemicals dramatically increased during the past decades, but their release and dispersal have accelerated markedly in the last half-century, as confirmed by analysis of core sediments and retrospective analysis of human samples [5].

Alertly, the impact of exogenous chemicals on human health, and particularly the impact of their combined and interactive effects, needs to be better characterized. Most of the research exploring the effects of exogenous chemicals on human health has been focused on the impact of single groups of chemicals, even though studies revealed chemical mixtures can cause different types of combination effects, including additive and synergistic effects, and, less often, antagonistic effects [6–9] (Fig. 1). Therefore, rather than purely targeting selected pollutants, screening a broad coverage of known and unknown compounds is of the utmost importance. It is also fundamental to explore longitudinal and retrospective studies to investigate the intricate associations between exposure, metabolism, and health outcomes. Fortunately, gas chromatography (GC) and liquid chromatography (LC) coupled with mass spectrometry (MS) can be designed to meet this challenge.

Due to the complexity of the chemical space in exposome research, analytical approaches applied in their characterization are often based on a combination of multiple methods, including target, non-target, and suspect screening methods, and combinations of these [10–17]. Unlike classic target workflows that aim for the accurate and quantitative determination of selected compounds, the goal of GC and LC-MS-based approaches in exposome research is to comprehensively cover endogenous and exogenous substances with some tradeoffs in the accuracy of all compounds detected. At present, since there is not a "fit for all purpose" analytical approach able to cover the entire chemical space, the goal is to cover the majority of the exposome with as few pipelines as possible while maintaining good coverage, precision, and accuracy for the compound classes that are suitable to be analyzed by the chosen analytical platforms. While current methods applying non-target or suspect screening approaches are mostly semiquantitative, it is possible to combine these with quantitative analysis of preselected compounds whose internal standards and/or native standards are available, thereby improving output information [18].

Undoubtedly, MS-based approaches provide better coverage than other techniques applied in exposome research, such as nuclear magnetic resonance [19], but defining pre-analytical steps, separation technique(s), and post-analysis methods will vary according to the fraction of the exposome being explored. From an analytical perspective, MS-based analysis is the approach of choice in exposome research due to its ability to detect and identify a broader range of environmental substances at trace concentrations. Still, MS-based protocols face several challenges, from sampling and extraction to analysis, data processing, compound annotation, and data interpretation. Here, we provide an overview of the main steps of MS-based analytical approaches in exposome research, from sample extraction to GC- and LC-MS analysis and data processing (Fig. 2).

2. Mass spectrometry in exposome analysis

2.1. Sample collection and extraction

The analysis of the human (i.e., internal) exposome is conducted in a range of biological matrices, mainly using urine and blood but also feces, saliva, breath biopsy, breast milk, as well as other organs and tissues [10]. Additionally, studies may integrate other organisms (*e.g.*, cells, rodents) to elucidate potential underlying mechanisms on complex biological systems and non-biological matrices (e.g., water, soil) to provide complementary environmental information. While many studies focused on developing methods to characterize the chemical exposome comprehensively, more effort is needed to define reporting guidelines for sampling in exposome research, including procedures for fasting (or non-fasting) conditions in human biomonitoring, sampling devices, and conditions for sample pre-processing [20]. As in any other "omics" workflow involving biological samples, sample analysis in exposomics must reflect the nature level of the chemical exposome. Thus, potential contamination during sampling should be accounted for, ensuring that the identification and quantification of exogenous compounds represent the intrinsic levels of biological matrices. This is particularly important in exposomics, as many exogenous substances

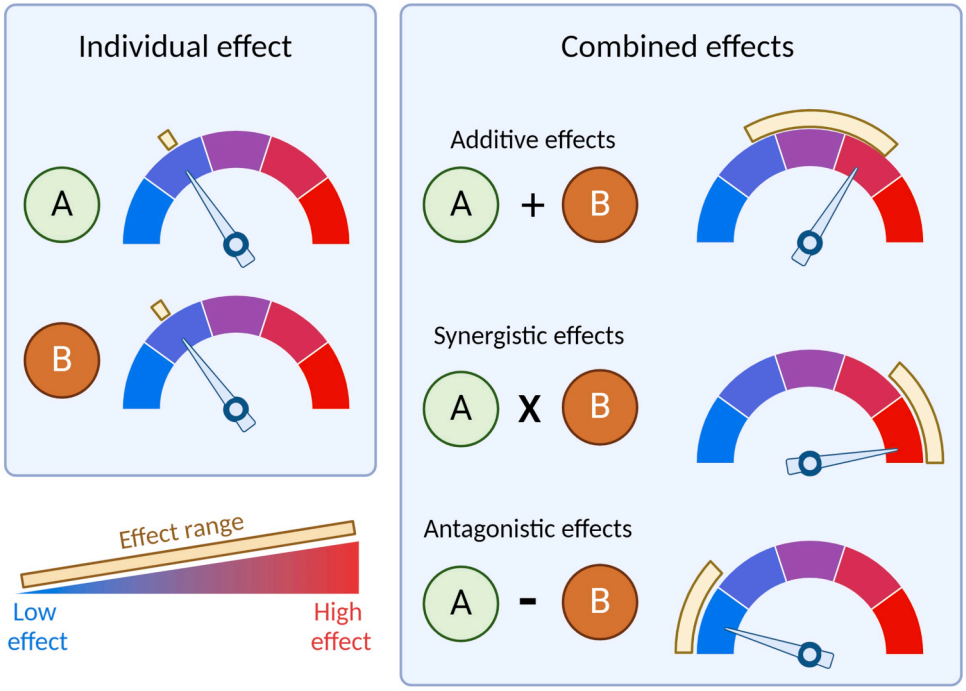

Fig. 1. Combined effects of environmental pollutants (e.g., compounds A and B) are usually additive, synergistic and, less often, antagonistic.

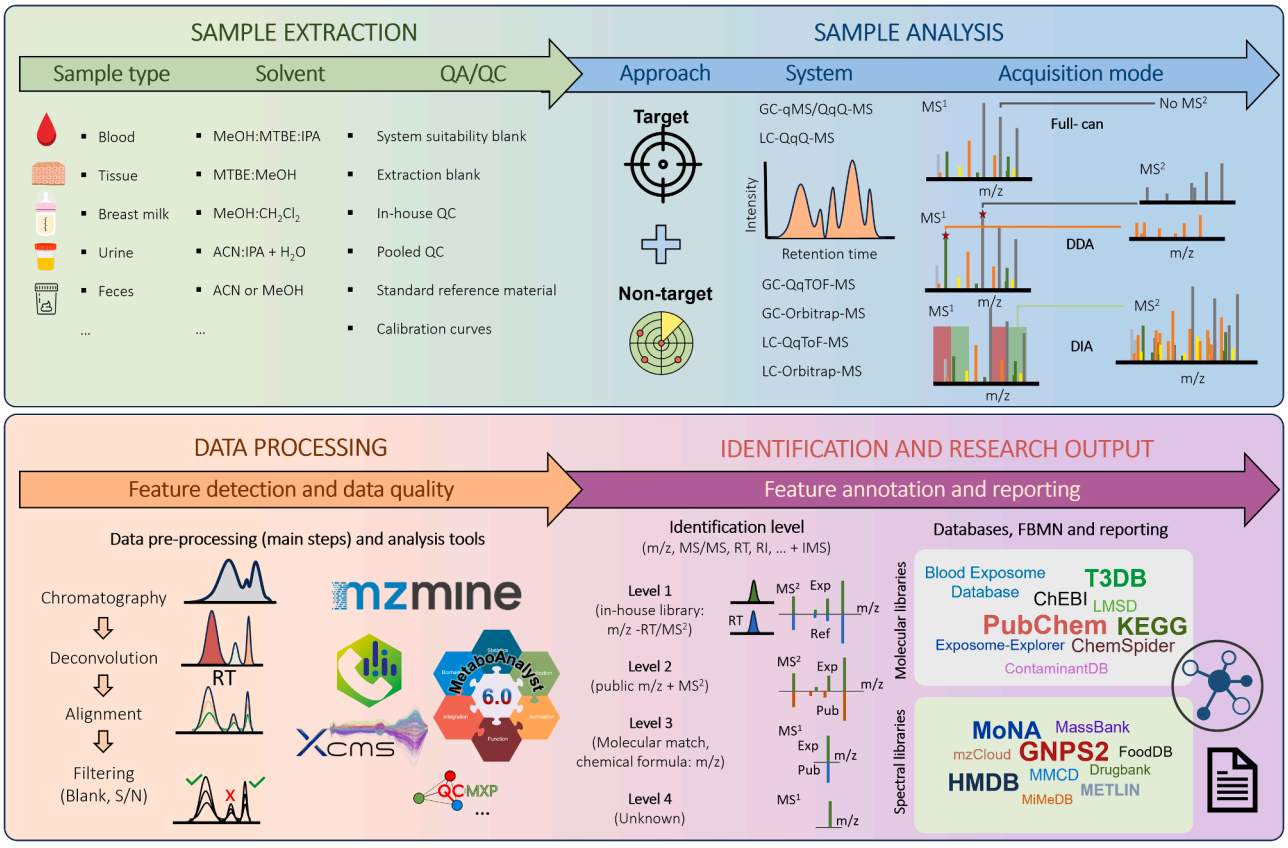

Fig. 2. Overview of sample preparation and GC- and LC-(HR)MS analysis workflows in exposomics research.

posing toxicity are also found in sampling materials [21].

In addition to potential contamination sources, quenching procedures and sampling time are necessary to allow analysis of extracts reflecting endogenous levels, thereby ensuring adequate biological interpretation in highly metabolically active systems such as cells and tissues. One way to improve sampling routines for exposome is to employ pre-analytical procedures universally applicable for non-target metabolomics whenever possible [22–26].

Sample preparation methods in exposomics have been explored more thoroughly than sampling strategies and were also similar to those described for non-target metabolomics analysis. As recent and comprehensive reviews on exposomics sample preparation are available in the literature [27,28], we will focus on overarching concepts rather than defining specific methods. In metabolomics, smaller volumes are typically sufficient because most endogenous analytes are present at relatively high concentrations. However, this is not the case for some exogenous substances, which might be detected near the detection limit of MS technologies. Consequently, different sample preparation methods, such as liquid extraction and solid-phase extraction, have been developed to explore the exposome considering different matrices and chemical space covered.

For comprehensive exposome analysis, the sample preparation methods should be ideally nonselective. It is important to note that matrix effects can hinder the detection of low-abundance compounds and compromise analytical reproducibility. Therefore, a primary objective in selecting a sample preparation method is to minimize these effects while maintaining good coverage and preserving the integrity of the analytes. As discussed further, the choice of nonselective approaches leads to complex extracts that often reduce chromatographic and ionization performance In addition, the sample preparation techniques should be selected based on the analytical method, such as LC or GC. Due to the wide range of polarities of both exogeneous and endogenous compounds, it is difficult to have a single extraction method with sufficient analytical coverage, as illustrated in Fig. 3. Recently, single-phase liquid-liquid extraction (LLE) and tandem solid-phase extraction (SPE) methods have been developed for metabolomics and environmental monitoring, offering relatively high recoveries for both polar and non-polar compounds [29,30]. Recently, the use of chemical derivatization in LC-MS-based methods have shown great potential as it enhances the detectability and separation of compounds that are often challenging to analyze with standard LC-MS but are better suited for GC–MS [31]. Similar approaches would also be promising for inclusion in exposomics pipelines. Examples regarding the combination and usage of these methods are discussed in the following section.

2.2. *Mass spectrometric-based analysis*

GC or LC combined with high-resolution mass spectrometry (HRMS) are the most widely employed methods in exposome analysis. Despite the appreciated sensitivity and peak resolution capabilities of GC, LC can cover a broader chemical space and, therefore, has been applied more frequently in exposome studies. A recent systematic review revealed that more than 60 % of exposome studies employ LC—HRMS while remaining studies mostly rely on GC-based methods [32]. Other authors indicate that the fraction of LC—HRMS-based studies in exposomics is even higher, up to 80 % [33]. Targeted quantification using low-resolution (triple quadrupole) mass spectrometry (QqQ-MS) remains in use to some extent due to its sensitivity, but primarily as a complementary technique. Other MS-based techniques, including capillary electrophoresis-MS (CE-MS) [34], selected ion flow tube MS (SIFT-MS) [35], and MS imaging (MSI) [36], also have been employed for selected applications.

In addition to the need to cover a broad chemical space, concentration levels also pose a challenge in exposome research (Fig. 3). Due to

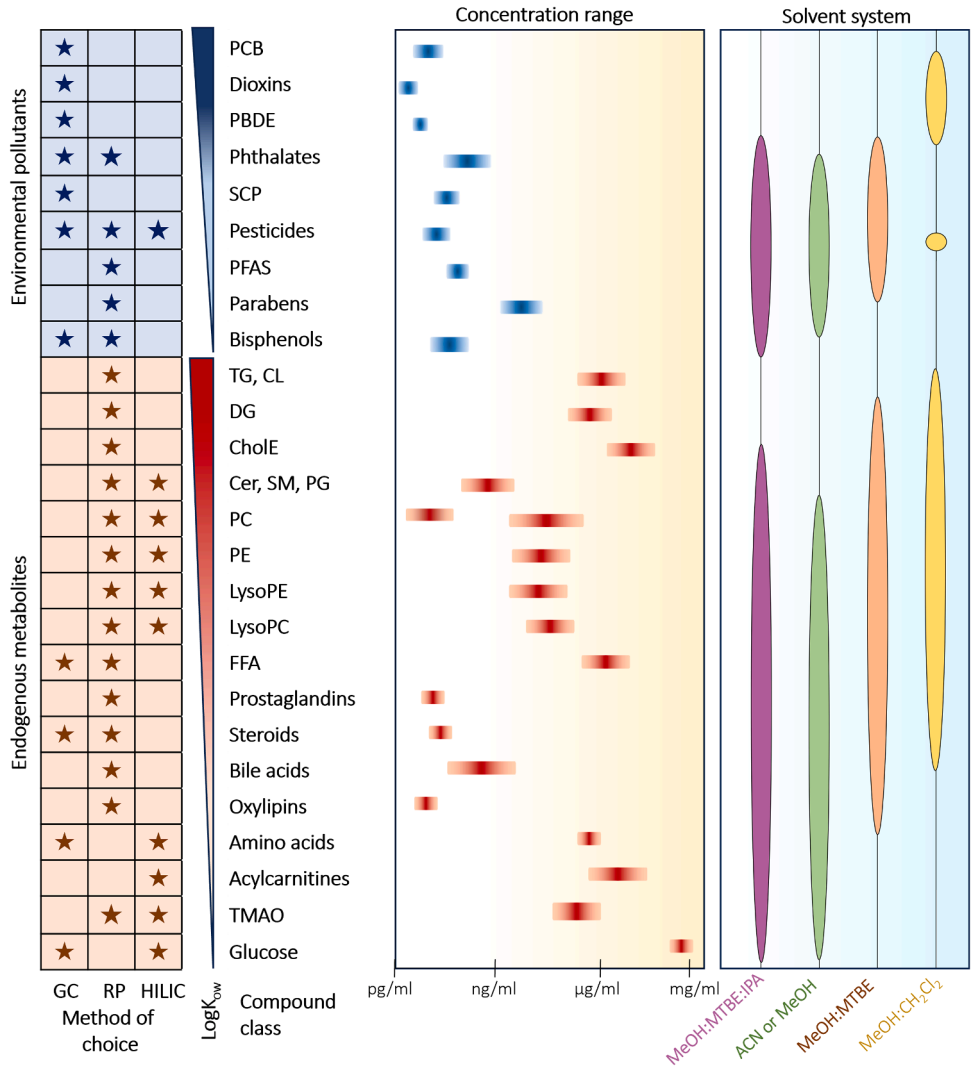

Fig. 3. Blood concentration ranges of persistent organic pollutants (POPs) and endogenous metabolites, their partition coefficient, methods of choice, and solvent systems often used for extraction. Abbreviations: Cer, ceramides; Chol, cholesterol; CholE, cholesteryl esters; CL, cardiolipins; DG, diacylglycerols; FFA: free fatty acids; LPC, lysophosphatidylcholines; LPE, lysophosphatidylethanolamines; PC, phosphatidylcholines; PE, phosphatidylethanolamines; PG, phosphatidylglycerols; SM, sphingomyelins; TG, triacylglycerols; TMAO, trimethylamine N-oxide. Figure adapted from Orešič et al. [2] and Cajka and Fiehn [115].

the extensive chemical diversity and concentration range of the so-called chemical exposome, it is often not feasible to capture a comprehensive chemical profile with a single method. Current studies either focus on a narrow chemical space using a single analysis platform or try to combine different platforms to achieve broader coverage. A non-exhaustive list of MS-based techniques employed for exposome analysis and practical examples are highlighted in Table 1.

Exposome applications are constantly following new technological advances in MS instrumentation (and data analysis tools). In the current scenario, hybrid quadrupole time-of-flight (QTOF) and Orbitrap MS appear to be the most widely used HRMS detectors. Other HRMS analyzers, including Fourier Transform-Ion Cyclotron Resonance (FT-ICR), as well as hyphenation between HRMS and ion mobility spectrometry (IMS), offer advantages including increased resolution and improved separation/annotation, respectively, but exposome studies employing these technologies are still less common compared to more conventional (and affordable) HRMS. The proportion of studies employing LRMS is also decreasing because new HRMS instrumentation can provide similar sensitivity with superior selectivity. Nevertheless, there is still a need for technological improvement in HRMS resolving power and hyphenation with other techniques to achieve unambiguous and high-throughput annotation pipelines. Here, we will focus our discussion on LC- and GC—HRMS, which represent the core analytical techniques employed in exposome research.

2.2.1. *LC-(HR)MS-based methods*

LC can cover compounds with an extensive range of molecular weights up to 100,000 Da without limitations in volatility or thermal stability, as observed in GC analysis [37]. Indeed, LC typically requires relatively simple sample treatment and provides good analytical coverage, ranging from ionic species of interest to macromolecules, including labile natural products and contaminants. Thus, it is unsurprising that most of the focus in exposome has been given to LC-based separation techniques [33]. However, as endogenous and exogenous substances of interest vary in their polarity, from highly polar compounds, such as trimethylamine N-oxide (TMAO), polar pesticides, and sugars, to relatively large and nonpolar neutral lipids, such as triacylglycerols, no single LC method can provide good separation of the entire chemical space in exposome. Thus, different LC-based separation techniques are often applied, from more conventional reversed-phase chromatography (RP) to hydrophilic interaction (HILIC) and ion exchange (IEX) chromatography. Sometimes derivatization, which is a procedure commonly used to increase GC analysis coverage, is also applied in LC analysis, for example, in the analysis of amino acids [38,

Table 1
GC- and LC-MS-based target and non-target techniques employed for exposome research and practical examples.

Methodology	Platforms	Chemical coverage: Examples	References
LC target	LC-QqQ-MS	Bile acids, carnitines, SCFAs, amino acids, oxylipins, environmental phenols (e.g., Bisphenol A), polycyclic aromatic hydrocarbons (e.g., 1-Hydroxynaphthalene), Pesticides (e.g., 4-Nitrophenol), volatile organic compounds (VOCs), tobacco alkaloids and its secondary metabolites, drugs of abuse.	[122–124]
LC non-target	LC-QqTOF-MS, LC—Orbitrap-MS	Lipidomics, polar and semipolar metabolites, drugs of abuse, surfactants, biocides, industrial chemicals, personal care products, additives, corrosion inhibitors, pesticides, and perfluorinated compounds.	[125,126]
GC target	GC-qMS, GC-QqQ-MS	Volatile and semi-volatile organic metabolites, chlorinated pesticides, non-polar EDCs, mono- and dehydroxylated PHAs, brominated flame retardants, dioxins, PCBs, OCPs, polybrominated diphenyl ethers.	[60,61,63, 66,90,127]
GC non-target	GC-QqTOFMS, GC—Orbitrap-MS	Volatile and semi-volatile organic metabolites and environmental pollutants, such as Phthalates, PAHs, FR, Dioxins, and PCBs.	[128–135]
Others (target and non-target)	MS imaging	Nucleotides, nucleosides, amino acids, carboxylic acids, phosphorylated sugars, enzyme co-factors, lipids, pharmaceuticals, dietary compounds, and toxic ionic liquids.	[136–140]
	CE-MS	Amino acids, peptides, mono-, di-, and triphosphate nucleosides, organic acids, sugar phosphates, polar metabolites, pesticides, drugs, PAHs, and EDCs.	[141–144]
	SFC-QqQ-MS	Neutral lipids, phospholipids, sphingolipids, and lipid-like pharmaceutical drugs	[145]

39] and short-chain fatty acids (SCFA) [40–42]. In the case of SCFA, derivatization improves their chromatographic retention in RP and their ionization efficiency, thus enhancing MS sensitivity [41] .

In addition to unidimensional separation techniques, orthogonal two-dimensional liquid chromatography (2D-LC) has also been employed for exposome analysis [43]. In 2D-LC, the second-dimension separation can resolve closely eluting peaks that could not be resolved using one-dimensional chromatography, increasing peak capacity, resolution, and sensitivity. 2D-LC can be configured with different separation modes, making it versatile and adaptable to various separation challenges. However, despite its versatility, it also requires specialized instrumentation and method development expertise, which is also more challenging for data processing [44]. The separation performance (as well as detection and sensitivity) in LC largely depends on specific chromatographic conditions since not only separation methods but also mobile phase composition, the presence of modifiers, the chromatographic run gradient, and, to a lesser extent, temperature can remarkably affect the analysis outcome.

Regarding ionization techniques, most LC-based exposomics studies have been using electrospray ionization (ESI) for both positive and negative ionization modes due to its high sensitivity and ability to ionize a broad range of analytes. In contrast, atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) have been employed in less than 5 % of studies, mainly as an alternative to improve the sensitivity of certain chemicals compared to ESI [33]. From an analytical perspective, the choice of ESI over APCI/APPI is somewhat expected as, in general, ESI gives better performance for a broader range of polar, semi-polar, and non-volatile molecules, which is needed for good coverage of the chemical space. However, despite the relatively small coverage of APCI/APPI compared to ESI, the ideal would be to use these ionization techniques complementary since APCI/APPI has the advantage of both ionizing weakly polar compounds and being less susceptible to interferences in sample matrix for small molecules [45]. Also, for specific environmental contaminants of biological relevance, such as (nitro-)polycyclic aromatic hydrocarbons (PAH), APCI/APPI are techniques of choice for ionization [46].

Even though ESI has better overall performance than APCI/APPI, the former is more susceptible to ion suppression during the analysis of complex matrices. This occurs mainly due to changes in the spray droplet solution properties caused by compounds with lower volatility, decreasing the evaporation efficiency of co-eluting compounds and, thus, the number of charged ions in the gas phase [47]. Low-abundant compounds can co-elute with compounds with relatively high abundance, leading to the non-detection of the former [48]. That said, even though advances in HRMS data pre-processing tools have allowed efficient deconvolution and integration of co-eluting features in complex matrices used in exposome research, chromatographic separation is still needed to some extent to avoid missing information due to ionic suppression [47]. Concurrently with the improvement of separation in conventional LC techniques, 2D-LC can be used to improve chromatographic separation but with trade-offs in costs and analysis throughput due to both analysis time and more complex HRMS data handling compared to LC [44].

2.2.1.1. LC-(HR)MS data acquisition. HRMS systems are not a single entity and different technologies will pose different aspects of data acquisition, which may result in different outputs. In general, LC—HRMS data can be obtained using three approaches: full-scan, data-dependent acquisition (DDA), and data-independent acquisition (DIA).

Conventionally, LC—HRMS non-targeted approaches are carried out by first performing LC—HRMS in full scan acquisition mode to obtain accurate m/z and relative abundance for all detected features. The generated information is further pre-processed and filtered using quality control (QC/QA) approaches to obtain a high-confidence list of features (for more information about QC/QA aspects in exposomics, please refer to the recent review by Lennon et al. [49]). Then, statistical methods are applied to identify features of interest according to the research question. From there, LC-tandem MS approaches, often MS^2 (MS/MS) but also MS^n, can be applied to the analysis of a pooled sample to gather information on the fragmentation pattern of relevant features to either confirm or improve annotation. This conventional approach has been well established and is often employed in exposome studies. However, it separates the quantification process from structure annotation, reducing analysis throughput [50] and does not allow for further application of feature-based molecular networks and other strategies to explore the relationship between structural patterns of known and unknown features. Thus, other acquisition methods, such as data-dependent acquisition (DDA) and data-independent acquisition (DIA), can be applied to circumvent this limitation.

In DDA, a pre-selection of precursor ions for MS^2 is defined before analysis. Shortly, a preselected narrow m/z-retention time (RT) window produces MS^2 spectra with minimal interference. In this setting, both MS full-scan and MS^2 spectra can be acquired in the most common HRMS systems used in exposome research, i.e., QTOF and Orbitrap. However,

the current HRMS commercially available can only perform MS^2 while maintaining sensitivity in a limited number of the most intense MS precursor ions *per* scan, typically the top 3 to 20 most intense precursor ions [51]. Thus, less abundant MS^1 signals can be missed. Moreover, due to the simultaneous collection of MS^1 and MS^2, a large portion of the acquisition time is used for MS^2 spectra generation, thus reducing the signal intensity for MS^1 features. No extra information about fragmentation patterns is provided for the non-selected compounds, which might be an issue, especially in sample retrospective analysis. Fortunately, new DDA strategies are being rapidly developed and prototyped *in silico*, such as in Virtual Metabolomics Mass Spectrometer (VIMMS) [52], and further instrumentally validated [53]. Additionally, including time-staggered precursor ion lists as inclusion lists, automated exclusion lists of previously DDA scanned features during repeated injections [51], and machine learning predicting spectra [54] combined with tools for RT prediction [55] can improve the output of DDA-based workflows.

In DIA, there is no previous selection of precursor ions, i.e., all ions are sent into the collision cell for fragmentation, and data processing algorithms are used to connect the fragment ions to the parent compounds [50]. For this approach, an exclusion list can be prepared to avoid acquiring unwanted MS^1 signals (e.g., previously known interferences arising from methodological contamination and reference ions). The exclusion list reduces data load and allows fragmentation of less abundant, co-eluting molecules. However, like in DDA, sensitivity is an issue in DIA for the co-eluting features with lower abundance. In addition, since a large number of MS^2 spectra is obtained at the same scan, it is challenging for algorithms to establish the correct precursor-fragment link. In this context, DIA approaches including scanning sequential window acquisition of all theoretical spectra (SWATH) [56] and SONAR [57], as well as orthogonal use of IMS [58] can improve MS^1 selectivity, helping to reconstitute MS^1-MS^2 parent-daughter associations. The main LC-MS^2 data acquisition approaches are summarized in Table 2.

When comparing DDA and DIA approaches, DDA is the preferred approach for MS^2 library generation and database searches due to its ability to easily generate MS^2 for defined *m/z* at defined RT windows. At the same time, DIA is better for retrospective analysis and feature-based molecular networking. As mentioned, both approaches cover only a subset of the features detected in full-scan mode. Furthermore, DDA and DIA are designed mainly for qualitative analysis instead of performing quantitative or semiquantitative analysis with fragment ions, as done with targeted approaches using QqQ-MS systems.

A recent study performed a broad comparison between full-scan, DDA, and DIA modes in LC—HRMS-based untargeted approaches to explore a standard mixture and urine samples [50]. As expected, full scan mode detected two to three times more features than DIA and DDA. The number of features in DIA was slightly higher compared to DDA. However, these two approaches showed qualitative- and quantitative differences regarding MS^2 data. While DIA provided a considerably higher number of MS^2 spectra than DDA, up to two times, DDA resulted in better MS^2 spectral quality. These findings highlight the complementarity of different acquisition approaches when employing LC—HRMS for exposome analysis (Fig. 4).

2.2.2. GC-(HR)MS-based methods

GC–MS-based approaches offer intrinsic advantages over LC-MS methods. These include using inert carrier gas as the mobile phase and fewer separation techniques, leading to standardized and widely accepted retention time indexes. GC–MS also benefits from electron ionization (EI) spectral libraries that are independent of the chromatographic setup, unlike LC-MS. Despite these benefits, the use of GC–MS in exposomics research remains relatively underexploited [33]. In fact, it is estimated that only 32 % of exposomics studies are performed using GC-(HR)MS as a standalone technique, and around 16 % are employed in combination with LC-(HR)MS approaches [59]. The underuse of GC–MS compared to LC-MS can be partially linked to the nature of compounds analyzed in exposome studies, in which the LC is preferable for higher coverage of more polar and less volatile compounds, making

Table 2
Conventional LC-MS^2 acquisition approaches and their characteristics.

Technique	DDA	DIA		
		Conventional DIA	SWATH	Scanning SWATH
Conventional instruments	QqQ, QqTOF, Orbitrap	QqTOF, Orbitrap	QqTOF, Orbitrap	QqTOF
Precursor selection mode	Time-based MS^1 survey scan	None	Pre-defined	Pre-defined
Precursor selection rules	Top N (most intense)	N/A	N/A	N/A
Precursor selection width	1 Da	Full mass range	Wide (e.g., 10–50 Da)	Wide (50–100 Da)
Precursor selection range	Defined by survey scan	Full mass range	Selected	Scanned
Product ion mass range	Full mass range	Full mass range	Full mass range	Full mass range
Product ion generation	CID of selected precursor	CID of entire range; alternates with low energy (precursor) scan	All precursors in the selected window	All precursors in the window
Precursor-product determination	Known from selected precursor	Match LC profiles	Match LC profiles	Match LC profiles
Compound identification	Library search	Library search	Library search	Library search
Qualitative performance	Very good	Fair	Good	Good
Quantitation modes	Precursor ion chromatograms Mass peak ratio to labeled standard	Fragment chromatograms Precursor chromatograms	Fragment chromatograms Precursor chromatograms	Fragment chromatograms Precursor chromatograms
Quantitation performance	Fair	Good	Very good	Very good
Strengths	Efficient use of time, good selectivity	Fast	Reproducible, consistent data acquisition	Reproducible, consistent data acquisition
Acquisition method development	Minimal	No	Minimal	No
Weaknesses	Non-systematic selection of precursor and quantitation only from MS1 or ion ratios	No MS precursor selectivity. Difficult to assign MS^1-MS^2 associations	Data is large and complex and requires special processing tools	Data is large and complex and requires special processing tools

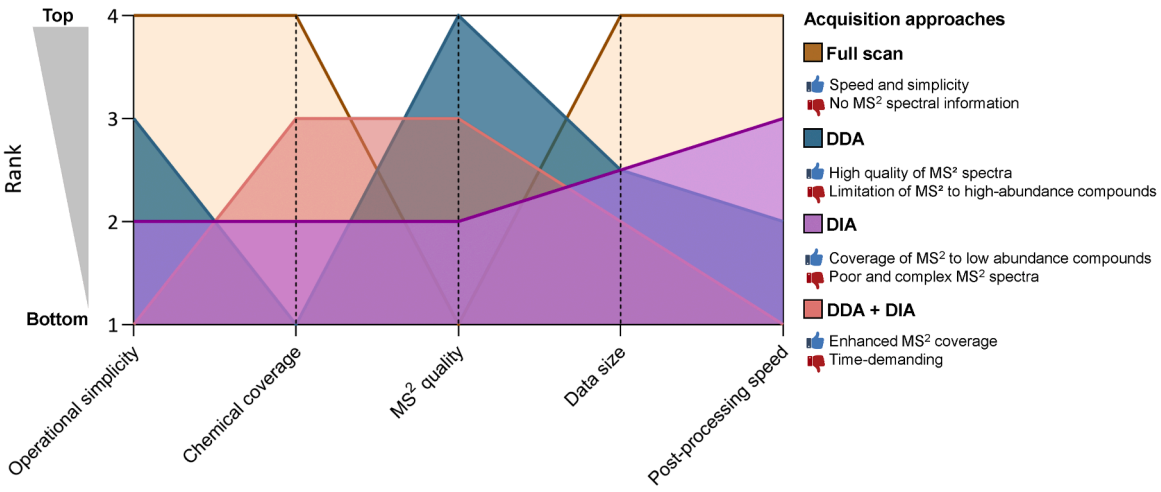

Fig. 4. Qualitative comparison between different aspects of acquisition method. Full scan, data-dependent acquisition (DDA), data-independent acquisition (DIA) and combined DDA and DIA were ranked (4 = top; 1 = bottom) based on easiness to set up, chemical coverage, MS^2 spectral quality, data file size, and post processing speed.

up a more significant portion of the exposome; however, the use of GC–MS-based methods can enhance the exposome coverage, as it can be employed as a complementary technique for high sensitivity and selectivity detection of non-polar volatile and semi-volatile pollutants compounds such as specific endocrine disruptor chemicals (EDCs), organochlorine pesticides (OCPs), PCBs, and PAHs, which are not easily accessible using LC-MS [60–63]. Other POPs analyzed in human samples mainly by GC–MS methods include flame retardants (FR) and dioxins [64–66], which, despite their long-standing restrictions or bans, they continue to persist in the environment and human biomatrices due to their long half-lives. Moreover, after derivatization, GC–MS offers good analytical coverage for a large number of metabolites, including amino acids, TCA cycle metabolites, free fatty acids, (simple) sugars, and phenolic compounds, which are often challenging to analyze with a single LC method [67–69].

A bottleneck of GC–MS application in exposomics (and metabolomics) studies is directly associated with not all organic compounds being analyzed by GC without prior chemical derivatization to increase the volatility and improve separation [19]. Still, the current trends in developing automated, high-throughput derivatization techniques of polar xenobiotics have made the GC–MS-based approaches increase in popularity in exposome research [33,70,71].

The chromatographic separation used in GC–MS-based exposomics methods is mainly based on low-polarity GC columns [33,64], such as 5 %-phenyl-95 %-methyl-polysiloxane or equivalent, which are the most employed due to their ability to provide reliable separations of a wide range of semi-volatile compounds (such as PCBs, PAHs, FR, and other POPs). A current and promising trend in the use of GC-based methods in exposomics is the employment of not only the conventional (one-dimensional) GC but also the use of comprehensive two-dimensional (2D) GC approaches [72]. Applying the 2D-GC methods in human exposome analysis of complex mixtures can increase chromatographic separation and resolution power compared to conventional GC, which can facilitate metabolite annotation and biomarker discovery [73,74]. Like 2D-LC, the second dimension of separation in GC provides increased resolving power; however, this comes at the tradeoff of introducing more constraints and new approaches concerning data handling [75]. Open-source and commercial software tools for data processing and high-throughput screening followed by pattern analysis [76] have been employed successfully to identify key chemical/biological signatures of interest in complex and processing-demanding GCxGC data [77].

As for LC, even though data pre-processing algorithms have dramatically improved the throughput of (2D-)GC—HRMS analysis, improved separation before MS analysis is still needed to reduce spectra complexity and improve annotation and quantification. In this scenario, 2D-GC holds promise for developing novel ways of detecting the dark matter of exposome in often complex biological samples. Indeed, both traditional (1D) and 2D- GC present some advantages over LC, especially in sensitivity and batch-wise annotation performance due to extended libraries with information on both mass spectral fragmentation and retention indexes. Nonetheless, there is still relatively limited and standardized information available on GC—HRMS data [33], so fragmentation patterns obtained through GC—HRMS approaches are often analyzed as LRMS (i.e., nominal) data.

2.2.2.1. GC–MS ionization methods. EI is the most used ionization technique due to its commercial availability and available spectral library. In EI, molecules are ionized by high-energy electrons from an electron beam, generating fragment ions that are characteristic of the molecular structure of the analytes. EI is defined as a "hard" ionization technique, meaning that it generates high-energy ions that can cause significant fragmentation and may lead to poor sensitivity for specific compounds due to the absence of a molecular ion [33]. To overcome this drawback, it has been proposed to use lower ionization energies in EI to reduce the fragmentation of more labile compounds [78]. However, conflicting results are being reported as different HRMS systems, such as QTOF and Orbitrap (or even the same systems from different vendors), appear to provide different fragmentation patterns for the same molecules when using the same ionization energies below 70 eV threshold [79,80]. Complementary to the positive ionization mode, applying different ionization energies in the negative ionization mode has enhanced the annotation of previously unknown exogenous compounds in environmental samples [81–83]. This is particularly important for analyzing halogenated compounds, whose sensitivity is usually better in negative ion mode. Nevertheless, the often-extensive fragmentation in EI is a double-edged sword: while EI fails to detect molecular ions of important features, information about fragmentation patterns in EI mode at 70 eV is widespread across libraries, providing harmonized information for feature annotation. Information about fragmentation patterns has also helped to establish links between features with similar fragmentation patterns through molecular networking, which can provide, at least partly, biological interpretation of data on unknown features [84].

Similarly to using multiple ionization modes in LC—HRMS approaches, "hard" and "soft" ionization approaches are ideally used as complementary techniques in GC–MS-based exposome analysis. For example, in addition to the compound annotation using EI, the

determination of molecular ions to enhance the accuracy and confirm the compound annotation can be done using "soft" ionization techniques such as CI, field ionization (FI), photoionization (PI), and atmospheric pressure ionization (API). Overall, the most widespread "soft" ionization technique employed in GC approaches is CI, in which a reagent gas with different proton affinity (*e.g.*, methane, ammonia, isobutane) is ionized and reacts with the analyte molecules to form ions [85]. Another crescent wave in "soft" ionization techniques for GC–MS methods in exposomics research is the application of API mode [86], due to its ability to be easily adapted for operation in combination with ion mobility spectrometry (IMS) [87], although its widespread use is still hampered due to the lack of spectral libraries [88]. GC-API has shown promising results for specific classes of xenobiotics, such as OCPs and pyrethrins [89,90]. Using an API source in combination with further fragmentation provides benefits, as fragmentation at the ionization source is not necessarily the same as in the collision cell, allowing the acquisition of both molecular ions and specific fragmentation patterns. This approach can significantly increase sensitivity compared to the EI mode while maintaining selectivity because of the latter fragmentation step; however, like DIA in LC-based approaches, it is still challenging to unambiguously reconstitute MS^1-MS^2 parent-daughter associations.

2.2.3. *Hyphenation of MS and ion mobility spectrometry (IMS)*

Recently, there has been a significant development in the hyphenation of conventional HRMS (e.g., QTOF) and ion mobility spectrometry (IMS), allowing, in some cases, to resolve isomers or isobars, thus improving the resolution and coverage of exposome analysis [58]. From a biological perspective, implementing IMS as an orthogonal technique to HRMS is of great value since compounds with the same molecular formula and similar molecular structures can play different roles in biological systems and, therefore, should be ideally annotated as separate entities [91]. With the addition of collisional cross-section values (CCS) provided by some IMS instruments, there has also been an improvement in database quality. Additionally, the additional separation dimension can provide more clean mass spectra for detected features, thus making the identification more reliable (Fig. 5**A**).

There are several commercial IMS-HRMS systems, including drift tube IMS (DTIMS), traveling wave IMS (TWIMS), trapped IMS, and field

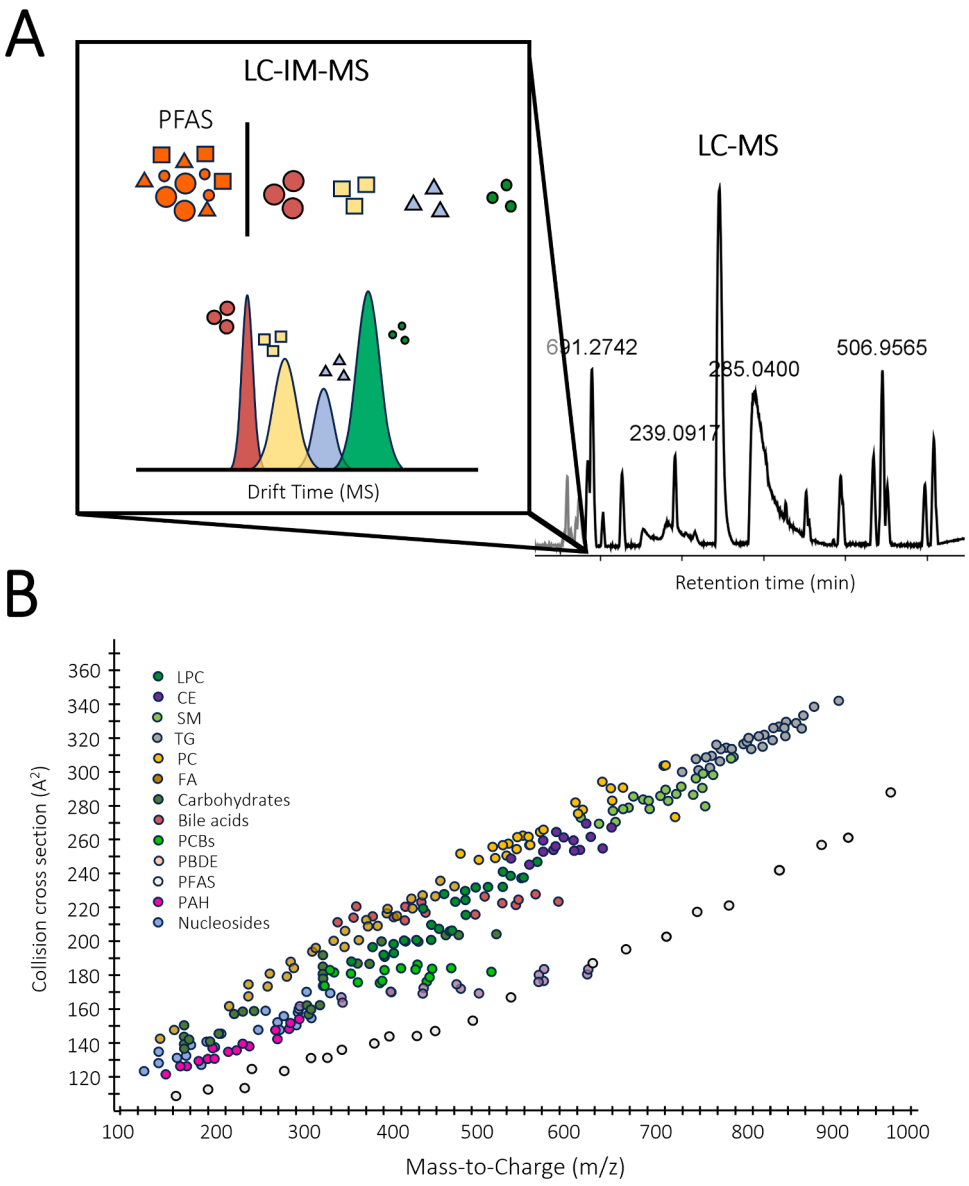

Fig. 5. A) Enhanced chromatographic resolution and spectral quality using LC-IMS-HRMS-based approaches in exposomics. **B)** Endogenous molecules and halogenated compounds show distinct *m/z* x CCS patterns due to the increased *m/z* of halogens compared to hydrogens, despite slight size difference. Adapted with permission from reference [97]. Copyright (2022) American Chemical Society.

asymmetric ion mobility (FAIMS), among others [92]. DTIMS appears to be the most promising alternative for exposome analyses, *i.e.*, analysis of small molecules, as it does not require external calibration approaches, unlike most IMS-based platforms [19]. However, similarly to 2D-LC, there are still challenges when processing and interpreting IMS-HRMS data due to its orthogonal nature and high-demanding tools for data processing, particularly the need for tools to seamlessly align HRMS and IMS information. In this respect, there has been rapid development in open-source data pre-processing tools, such as MZmine [93] and DEIMoS [94]. Another challenge is that the nature of IMS separation also demands an ion gating or trap-and-release mechanism involving a loss of duty cycle and (in the latter case) the possibility of ion losses, or interactions between different ions in the trapping environment, which also can cause additional fragmentation for labile, (de)protonated, substances [19]. In addition, IMS-HRMS requires delicate engineering and parameter optimization for in-field use, and matrix effects may influence IMS response similarly to HRMS.

Despite the abovementioned challenges, IMS-HRMS is a powerful and promising tool that can increase the much-needed coverage in exposome research. In addition to providing isobar separation and increasing the possibility of unambiguous annotation, recent reviews summarized that IMS alone can be employed to differentiate endogenous metabolites and specific xenobiotics [95,96]. Endogenous biomolecules have higher slopes in the CCS versus mass-to-charge ratio (m/z) plots than molecules with more halogen atoms, such as per- and polyfluoroalkyl substances (PFAS) and polybrominated diphenyl ethers (PBDE). This occurs due to increased m/z of halogens versus hydrogen but a slight size difference, as demonstrated in Fig. 5**B** [97]. These findings offer great potential for developing novel post-analysis techniques that use CCS-m/z pairs to classify exogenous compounds in biospecimens.

2.3. Data processing, annotation and quality control

2.3.1. Data processing for exposomics

There has been a continuous improvement in user-community algorithms and other tools to untangle the complexity of LC- and GC—HRMS data. Fortunately, exposomics can take advantage of previous advances in data pre-processing tools for metabolomics, which is an essential step prior to compound annotation and quantification in HRMS studies. Open-source data processing tools (see examples in Table 3) can offer a cost-effective and flexible way to process and analyze large datasets.

In LC—HRMS-based exposome approaches, data pre-processing pipelines involve multiple stages, such as filtering, feature detection, peak alignment, and gap-filling. The pipeline also includes componentization/deisotoping, or annotation of adducts, and ideally reporting potential in-source fragments based on feature-based molecular networking or correlation analysis [98]. For GC—HRMS, isotopic patterns are often maintained to support annotation, and additional spectral deconvolution of multiple fragments generated during EI is required to generate a "pure" EI spectrum for each feature. The open-source software MS-DIAL performs deconvolution based on the grouping of the EIC peaks of the fragments; at the same time, the ADAP-GC algorithm in MZmine uses mainly multivariate curve resolution. This latter strategy omits selecting model peaks, improving the separation of coeluting components, and detecting less low-intensity peaks. However, this leads to a significant increase in processing time and produces model peaks that might generate different peak shapes compared to the actual shape in real EIC peaks [99]. Similarly to metabolomics, there are post-processing filtering steps in both GC- and LC-based analysis workflows to remove artifacts and unwanted features from the final peak list. These strategies include, but are not limited to, using extraction/process blank, checking variation of single features in pooled samples injected throughout the analysis batch, a dilution series prepared from a pooled sample, and frequency occurrence in all samples or within chemical

Table 3
Examples of open-source data pre-processing tools used in LC- and GC–MS-based exposomics research.

Software	Availability	Language	Comments	References
MZmine	GNU (GPL-3.0)	Java	MZmine provides an easy-to-use workflow for MS data deconvolution, peak detection, alignment, and annotation. It's widely used for visualization and exploratory analysis of LC-MS(MS) and GC–MS(MS) data.	[93]
MS-DIAL	CC-BY 4.0	C#	MS-DIAL is a user-friendly tool for MS data. It has multiple compound deconvolution algorithms for alignment, peak picking, and MS^2 processing.	[101]
OpenMS	BSD 3 license	C++	OpenMS is designed for ease of use and extensibility in proteomics and exposomics/ metabolomics workflows. It is usually applied for peak picking, quantification, and peptide identification.	[146]
MetaboAnalyst	GNU (GPL-2.0)	R	MetaboAnalyst is a web-based platform designed for comprehensive metabolomics data analysis. It supports both targeted and untargeted metabolomics, offering tools for statistical and functional analysis and streamlined analysis for quantitative data.	[102]
Asari	BSD 3-Clause License	Python	Asari is designed to process efficient and scalable data in LC-MS-based metabolomics. It is particularly noted for its ability to handle LC-MS data with transparency and scalability.	[147]
MAVEN	GPL-3.0	Java	MAVEN is a user-friendly tool that enables intuitive data interaction and offers key steps for LC-MS data analysis, such as peak alignment and visualization. It allows fast metabolite quantitation from multiple reaction monitoring data or	[148]

(*continued on next page*)

Table 3 (*continued*)

Software	Availability	Language	Comments	References
			high-resolution full-scan MS data.	
OpenChrom	EPL-1.0	Java	OpenChrom is an open-source software for processing and analyzing chromatographic data (GC and LC). It offers components for metabolomics/exposomics MS data processing workflows such as data visualization, peak detection, and integration.	[149]
XCMS	GNU (GPL>2)	R	XCMS is an open-source R package that analyzes MS-based metabolomics/exposomics data. It provides tools for peak detection, alignment, and annotation, enabling the identification and quantification of metabolites in complex datasets.	[103]
MetFrag	GPL-3.0	Java	MetFrag is a web-based tool for metabolite identification from tandem MS data. It uses *in silico* fragmentation for computer-assisted identification of unknown compounds.	[150]
ORIGAMI	GPL-3.0	Python	ORIGAMI is suitable for analyzing MS and ion mobility MS datasets. It enables easy data extraction, in-depth handling of large datasets, and statistical analysis.	[151]

classes.

Despite the availability of several data processing tools, the increased need for using simultaneous MS^2 data acquisition and hybrid detectors such as IM-HRMS in exposome studies requires more efficient algorithms for data (pre-)processing. Optimally, these tools should be able to process MS^2 signals automatically, align signals with corresponding precursor ions from the MS^1 data, link with MS^2 library search, and optionally export MS^2 spectra (and IMS information) in a compatible format for other software programs or applications. In this context, MZmine [100] and MS-DIAL [101] have been shown to be the most promising tools.

MZmine and MS-DIAL have been used primarily for metabolomics research and offer a range of features for data processing, analysis, and visualization; they are currently able to handle multiple file formats from different MS vendors in addition to popular open-source formats such as mzML and mzXML. This allows more seamless integration and comparative analysis from multiple instruments and technologies. MZmine also provides a range of normalization and scaling methods to account for variations in data quality between samples, while information on RI for GC-based analysis is better integrated within MS-DIAL pipelines. Both MZmine and MS-DIAL also have advanced data filtering and annotation features, including spectral and retention time filtering, adduct and isotope annotation, and improved automatic peak annotation using public databases. MZmine can be integrated with other open-source software tools such as MetaboAnalyst [102] and XCMS [103]. Additionally, it can be integrated with online metabolomics tools such as the Global Natural Products Social Molecular Networking (GNPS) and lipidomics platforms [104]. Not least, the range of data visualization and analysis tools on both MZmine and MS-DIAL helps identify endogenous and exogenous compounds that are differentially expressed and explore associated pathways and biological processes. Of course, using these tools requires a steep learning process, but the number of tutorials and resources available has increased along with their popularity.

2.3.2. *Annotation of unknowns: databases and in silico tools*

Advanced LC and GC techniques coupled with HRMS are accessible for chemical profiling and comprehensive characterization of biological specimens. Yet, only a small fraction of chemicals can be unambiguously identified and quantified. Classical identification through the confirmation of IS retention and MS fragmentation pattern is not available in most cases, and remaining signals, which often constitute more than 80 % of detected features in exposome studies, are generally termed "known-unknows" or "unknown-unknowns." The "known-unknows" are compounds with known structure and suspect presence but lacking information for unambiguous identification and routine quantitative measurement, while "unknowns-unknowns" are detected features without previous information that could associate them with a specific chemical entity [105].

By definition, (HR)MS-based exposome approaches can be classified as targeted, suspect, or non-targeted. However, as mentioned earlier, recent studies combine these approaches by including the quantification of compounds of high relevance whose standards are available using an external calibration curve and semi-quantitative information on suspect/putative compounds and unknown features.

Suspect screening lists have been combined with untargeted analysis in MS^1 and MS^2 experiments to a similar extent that molecular networking has been employed for GC-ESI and LC-MS^2 analysis. In suspect screening, the first step is creating a suspect list of compounds in samples to be analyzed, including endogenous metabolites and xenobiotics. The spectral libraries should contain m/z values and RT (or predicted RT), isotopic patterns, as well as MS^2 data, and CCS values, whenever available**.** For RT prediction in LC-(HR)MS, multiple tools have been developed, including PredRet [106], the RTI platform [107], Retip [55], and simple linear regression using log Kow or log P [108]. However, it should be noted that due to the large selection of different column chemistries, solvents, buffers, and broad chemical space, it is difficult to model all variables, thus making prediction models challenging to transfer between methods.

Exposome research has greatly benefited from strategies that annotate specific endogenous metabolites and xenobiotics based on their MS^2 spectra behavior. Studies also have achieved increased sensitivity by combining MS patterns with the orthogonal use of IMS. Nevertheless, feature annotation remains a challenge, particularly for small molecules. Multiple databases are available for metabolomics, exposomics, and environmental pollutants (Table 4). For metabolomics, the Human Metabolome Database [https://hmdb.ca/]) has currently over 200 K metabolites [109]. For environmental contaminants, publicly available exposome-related databases include blood exposome [https://bloodexposome.org/) [110], the toxic exposome database (T3DB) [http://www.t3db.ca/] [111], Exposome-Explorer [http://exposome-explorer.iarc.fr/] [112].

2.3.3. *Quality control and standard references*

Quality control (QC) and quality assurance (QA) samples have

Table 4
Molecular and spectral databases used in exposome research.

Database	Methods	Chemical Space	Comments	Ref
The Blood Exposome Database	Merging literature data from blood-related publications from PubMed, PubChem, PMC, and HMDB	65,957 unique isomer structures and their salts	Endogenous and exogenous chemicals that are expected and detected in human blood specimens	[110]
ChEBI	Small molecules	59,511 compounds	Incorporating an ontological classification and providing a graph view of the entry within the ChEBI Ontology	[152]
ChemSpider	Small molecules	114,000,000 chemical structures and 273 data sources	The list of contributing Data Sources, details summary, and count of compounds deposited.	[153]
ContaminantDB	Xenobiotic molecules	54,249 compounds	Includes IARC Carcinogens Group 1, 2A, 2B, 3 and 4, Drugbank drugs and metabolites.	contaminantdb.ca
Drugbank	Small molecules drugs: MS/MS spectra, CCS, RT, RI	11, 891 small molecules drugs	Drug, drug-target, and related pharmaceutical information	[154]
Exposome-Explorer	Data collected from peer-reviewed publications and organized to make it easily accessible.	Over 1000 linked-exposure compounds	Comprehensive data on all known biomarkers of exposure to pollutants, contaminants, and dietary factors measured in population studies.	[155]
Fiehn GC–MS Database	Volatile and semi-volatile metabolites: MS, RT, and RI	1200 spectra and retention time index	Over 1000 primary metabolites below 550 Da cover a wide range of small metabolites detectable by GC–MS.	[156]
FoodDB	MS spectral information of food-derived metabolites	Over 28,000 compounds	Most comprehensive resource on food constituents, chemistry, and biology.	[157]
GNPS2	MS, MS/MS, MSn	over 1 M MS/MS spectra	Molecular networking, Data Repository	[104]
Golm Metabolome Database	MS, RT, RI	Several hundreds of entries of MS and RI for metabolites	Metabolomics database designed to support the analysis of plant and microbial volatile and semivolatile metabolites	[158]
Microbial Volatile Organic Compound Database (mVOC)	MS information	Over 3500 unique compounds	Database containing information on microbial volatile compounds produced by bacteria and fungi, with applications in linking agricultural and ecological information to human health	[159]
HMDB	MS, MS/MS, and NMR	64,830 experimental LC-MS/MS spectra, 1325,670 predicted LC-MS/MS spectra and 220,945 metabolites	Detail description of biological function and clinical information of metabolites	[109]
KEGG	Metabolites	18,000 metabolite entries	Pathway mapping from molecular-level information	[160]
LMSD	Lipids	48,691 lipid structures	Masses are generated by computational methods or are present in the LIPID MAPS® Structure Database (LMSD)	[161]
MassBank	MS, MS/MS, MSn	20,148 MS spectra and 65,824 MS/MS spectra	All are experimental spectra	[162]
METLIN	MS, MS/MS, and FTMS	Over 1 M high-resolution MS data of metabolites	All are experimental spectra. An updated and commercial version, METLIN GEN2, has 50 times more data on molecular standards than METLIN	[163]
MiMeDB	MS spectra	Over 25,000 metabolites or exposure chemicals and 648 861 MS spectra	Comprehensive, multi-omic, microbiome resource tool for connecting the human microbiome and its chemicals	[164]
MMCD	MS and NMR data	Over 20,000 compounds	A database focusing on small molecules and spectral data to support NMR and MS-based analytical techniques	[165]
MoNA	MS, MS/MS, MSn	693,174 spectra	Other free spectral databases are pickable for combined search	mona.fiehnlab.ucdavis.edu
mzCloud	MS, MS/MS, MSn	19,541 compounds and 8550,418 spectra	High-resolution tandem mass spectra, spectral trees, filtered and recalibrated spectra	mzcloud.org
PubChem	Small molecules	110,667,566 compounds and 275,430,544 substances	Detail description of chemical properties of compounds	[166]
Toxic Exposome Database (T3DB)	Toxins and toxins-associated compounds	3678 toxic compounds	A database holding 3678 toxins, including pollutants, pesticides, drugs, and food toxins, which are linked to toxin target records	[111]

become essential in modern mass spectrometry-based exposomics to achieve reliable results. These practices ensure high-quality data and consistent instrument performance monitoring throughout the analytical workflow. Recent reviews have comprehensively outlined standard operating procedures and recommendations for QC and QA in analytical protocols [113,114].

For QA, routine injections of "blank samples" containing pure solvent (*e.g.*, MeOH, ACN) are recommended every 5 to 10 sample injections to minimize the carryover effect and clean the column, thus allowing assessment of robustness throughout the analysis batch. Additionally, extraction blank samples processed identically to experimental samples should be included in the analysis batch to help identify possible contaminants and artifacts during the extraction step.

Several types of QC samples can be added to ensure the quality of acquired data and track instrument performance, particularly in clinical studies, where batch effects may pose a challenge when comparing samples analyzed in different batches or platforms. Several types of QC samples, including *in-house* QC, pooled QC, and inter-lab QC, can be used based on study objectives and injection sequence length [22]. In-house QC samples serve as intra-lab QC, allowing monitoring of instrument performance across analysis batches over time. This provides insightful information regarding the instrument performance and helps to compare results between projects or projects analyzed on different batches. The pooled QC sample is a composite of all mixed samples and thus represents the collective sample matrix and components. Pooled QCs are typically injected periodically throughout the running sequence, equally spreading after a certain number of injections from the beginning to the end of the run. The pooled QC is crucial to exploring

intra-batch performance, often by calculating the relative standard deviation (RSD) of features in pooled QC injections to filter out the non-stable detected features. In addition, especially in clinical exposomics, pooled QC can be used for batch effect correction, including correction of intensity drift or retention time shifts [115]. Finally, the certified standard references can be considered the inter-lab QC; they are certified samples with known quantities of a broad spectrum of chemicals, thus facilitating data comparison and harmonization across laboratories. Depending on the type of samples, the reference standards can be chosen. The National Institute of Standards and Technologies (NIST) 1950 and 1957 are often the regular plasma and serum choices in exposome analysis. There have also been efforts to develop additional reference standards for other matrices, such as fecal samples [116].

2.4. Selected applications on combined methods for the analysis of human metabolism and xenobiotics

The current trend in exposome analysis is to include both endogenous compounds, mainly metabolites, and xenobiotics compounds, such as environmental pollutants and diet-derived compounds, in a single analysis and to combine targeted and non-targeted approaches. However, there are still a limited number of studies using this approach. Unfortunately, the majority of the studies have been focused on the analysis of environmental contaminants separately from human metabolism. Although studies focusing solely on environmental contaminants can be useful for biomonitoring and when associated with health outcomes, they do not provide tools to understand the mechanistic effects at the metabolic level. Here, we highlight some studies that have used the combined, overarching approach.

A study by González-Domínguez et al. [117] utilized UHPLC-QqQ-MS for the semiquantitative analysis of various metabolites, drugs, chemicals from personal care products, plastic additives, and environmental pollutants in urine and plasma samples. Several sample preparation methods were tested, and the best results were obtained when urine samples were simply diluted and filtered, while for the plasma samples, protein precipitation (PP) with acetonitrile was employed. A single internal standard was used for the analysis, and calibration curves showed linear responses over 3–5 orders of magnitude within the concentration range of 0.1–10 000 μg/L, highlighting the complexity of studying the human exposome. The matrix effects were negligible for most compounds, except those eluting at the void, supporting the possibility of using calibration curves prepared in solvent instead of matrix-matched calibrations to simplify the analytical workflow. The sample volume was 20 μL of urine or 100 uL of plasma. The majority of the compounds identified were endogenous metabolites, identified based on authentic standards, but some personal care products (e.g., parabens, phthalates, benzophenones) were also detected.

We also have been applying a combined targeted and nontarget approach for metabolomics and exogeneous compounds in multiple human studies [118–120]. The sample preparation includes either PP with acetonitrile or LLE using a methyl-tert-butyl ether/-methanol/Isopropanol mixture, followed by RP-UPLC-QqTOFMS analysis. If PP is used, lipid analysis is done separately using a modified Folch extraction, as PP does not quantitatively recover neutral, nonpolar lipids, such as triacylglycerols and cholesterol esters. We incorporate many internal standards, both for metabolites as well as for exogenous compounds, during the sample preparation step calibration curves for the quantitation of target compounds such as amino acids, bile acids, free fatty acids, and perfluoroalkyl substances (PFAS). Identification is based on a target list currently consisting of >1500 compounds (metabolites, gut microbial metabolites, diet-derived compounds, pollutants, drugs), as well as suspect screening lists for additional exogenous compounds compiled from online databases, e.g., the Norman network database (https://www.norman-network.com/nds/SLE/). The analytical coverage, using the PP, is from polar to semipolar chemicals, and the sensitivity is sufficient for detecting several environmental chemicals such as PFAS, parabens, some mycotoxins, and plasticizers in about 40 μL of human plasma and serum.

Flasch et al. [18] proposed a novel approach for simultaneously measuring the endogenous human metabolome and the chemical exposome by combining a dual column (RP and HILIC) method and fast polarity switching in a single analytical run in UHPLC—Orbitrap-HRMS. In this approach, pooled urine and plasma samples were spiked with the internal standard mix of xenobiotic compounds, followed by the extraction using an acetonitrile/methanol mixture. This approach showed good limit of detection (LODs) for endogenous metabolites (0.02 to 1068 ng/mL) and xenobiotics (0.01–5.7 ng/mL), indicating the feasibility of assessing the endogenous metabolome and the chemical exposome both simultaneously and quantitatively.

Moreover, different evaluations of LLE methods have been tested to achieve a broader metabolome coverage by UPLC-QqTOF-MS. Liu et al. [121] tested four extraction protocols, i.e., methanol PP, SPE, and different two-step extraction protocols using chloroform/methanol followed by methanol/water or using dichloromethane/methanol followed by methanol/water, and explored results concerning number of features, reproducibility, and recovery in serum samples. Under the tested conditions, the protocol employing methanol PP proved less efficient. In contrast, the two-step extraction using dichloromethane/methanol followed by methanol/water showed superior performance, yielding more extracted features, improved reproducibility, and better recovery. Based on the identified compounds, authors also suggest that the selected two-extraction step protocol effectively captures a wide range of both endogenous metabolites and xenobiotics in human serum samples.

3. Conclusions

The advancement of mass spectrometry (MS) techniques in recent years has greatly enhanced our ability to investigate the health impacts of chemical exposures, even at trace levels. However, for a comprehensive overview of the complex chemical space of the human exposome, GC- and LC-based approaches need to be applied to improve detection capacity. This review highlights essential steps for conducting GC- and LC-MS-based exposomics, from sample collection to data analysis, emphasizing the need for broad coverage and robust data acquisition. Comprehensive exposomics workflows require nonselective extraction methods and robust GC- and LC-MS techniques, which can be hyphenated with new approaches, such as IMS. All of this must be followed by proper quality control and consistent reporting standards to ensure robustness and reproducibility. Importantly, exposomics is not just about environmental monitoring; it encompasses a broader chemical space aiming at exploring the relationship between xenobiotics and human metabolism, providing a comprehensive understanding of how exposure affects human health. Ultimately, when properly approached and integrated with other data including environmental monitoring, dietary and physical status, and health outcomes, exposomics holds great potential to uncover complex human-environment interactions and advance personalized healthcare.

CRediT authorship contribution statement

Victor Castro-Alves: Writing – review & editing, Writing – original draft, Visualization. **Anh Hoang Nguyen:** Writing – review & editing, Writing – original draft, Visualization. **João Marcos G. Barbosa:** Writing – review & editing, Writing – original draft, Visualization. **Matej Orešič:** Writing – review & editing, Conceptualization. **Tuulia Hyötyläinen:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

No data was used for the research described in the article.

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