



Analytical strategies for chemical exposomics: exploring limits and feasibility

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Abstract

Tackling the challenges of chemical exposomics will require the implementation of diverse analytical strategies and technological advancements. Herein, high-resolution mass spectrometry-based methods applied in current chemical exposome studies have been surveyed and are shown to be limited. Notably, liquid chromatography separations almost exclusively employ reversed-phase C18 columns using water/methanol gradients with formic acid additive, while gas chromatography is underexploited in the field at this stage. A systematic evaluation of strategies applied in related disciplines (i.e. metabolomics, proteomics, multiresidue trace analysis) was undertaken to provide practical guidance for the development of chemical exposomics. The approaches were assessed on the basis of their costs (i.e. capital expenditure, overhead and maintenance fees, expertise required, consumables) and potential benefits (i.e. improvements to sensitivity, coverage, reproducibility, throughput, ease of use) to prioritize those with promise for chemical exposomics application. Alongside a need for technological investments (e.g. advanced hardware updates), numerous low-cost strategies showed high potential benefits (e.g. different column phases, enhanced sample fractionation) and are feasible for rapid adoption.

Keywords: trace analysis of environmental contaminants; suspect screening and non-target analysis; exposome and metabolome; SPE and passive sampling/SPME; LC-HRMS and GC-HRMS; chemicals of emerging concern.

INTRODUCTION

Coupling the comprehensive analysis of chemical exposures (i.e. chemical exposomics) with other omics (i.e. metabolomics, proteomics) is a promising strategy for linking chemical exposures to specific biological responses,¹⁻³ chemical prioritization^{2,4} and to lead exposure-based risk assessment.^{3,5-7} The profiling of small endogenous compounds and exogenous chemicals converge in terms of platforms used (e.g. high-resolution mass spectrometry [HRMS]⁸⁻¹²), m/z range covered^{3,13} and the analytical challenges faced, such as the need to increase coverage (i.e. physicochemical property range) and sensitivity. These challenges are amplified when profiling trace analytes and if working with low sample volumes/amount of complex matrix.¹⁴⁻¹⁶ Despite approaches to enhance the analytical sensitivity and coverage of endogenous compounds also having the potential to improve the detection of exogenous compounds,¹⁷ many remain underexploited for the profiling of chemical exposure agents, and further exchange between the fields of chemical exposomics and metabolomics should be encouraged.¹¹ Additionally, multiresidue methods to assay hundreds to thousands of target contaminants in complex matrices (e.g. food/plant) have recently been developed.¹⁸⁻²¹ Although these approaches are selective for known chemicals,

the breadth of chemicals assayed means sample preparation and chromatography is akin to profiling, and evidence that large-scale quantification of low abundant chemicals is feasible.

We have reviewed the gas chromatography (GC)-HRMS and liquid chromatography (LC)-HRMS approaches applied in chemical exposome studies and evaluated strategies used in related disciplines which may enhance analyte detection, coverage, sample throughput, and reproducibility. The strategies that warrant further investigation have been prioritized through an assessment of their relative costs and potential benefits of implementation in chemical exposomics. The review and assessment were restricted to GC- and LC-HRMS workflows, because their use is most widespread, but we guide readers to reviews of alternative platforms that provide appealing features for chemical exposomics, e.g. ion mobility-mass spectrometry²² and supercritical fluid chromatography.²³ In this article, we focus on the laboratory-based components and do not venture into the range of bioinformatic and computational approaches necessary for data analysis.

The work is articulated in 3 parts:

- 1) Analytical methodologies used for HRMS-based chemical exposomics were compiled from literature with the purpose

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to identify the most commonly applied approaches and pinpoint lacks and areas for improvement. From this survey, a clear trend emerged: rather uniform analytical conditions are applied for HRMS-based investigation of the chemical exposome.

- 2) Since the approaches applied in chemical exposomics are limited, we reviewed strategies used in related disciplines to identify those with potential for advancing chemical exposome research. Particular attention was paid to those applied in metabolomics because of the analytical and conceptual similarities with chemical exposomics,^{24,25} and applications from proteomics were considered. Targeted analysis of environmental contaminants was also considered if a broad range of compounds in complex matrix were assayed.
- 3) The costs and benefits of the reviewed strategies were assessed and evaluated with the aim to prioritize approaches with potential for application in chemical exposomics studies. Focus was upon strategies that could be adopted in the near future. The evaluation was based on literature findings and authors' experiences and a summarized perspective is presented.

Analytical approaches currently used for chemical exposomics are rather uniform

Literature was surveyed during December 2020 to identify analytical approaches applied in HRMS-based chemical exposomics. The keywords/phrases "non target analysis" (title), "non target analysis environmental chemicals" (topic), "suspect screening" (title), and "exposome" (topic) were used to search Web of Science.

Whereas targeted analysis methods selectively measure chemicals of already confirmed identity, non-target analysis, and suspect screening are typically performed with the MS scanning a wide m/z range to enable broad-scope chemical profiling.^{8,26,27} Non-target analysis aims to detect features without any a priori information while suspect screening relies on some a priori considerations, seeking to detect chemicals expected to be present in a sample.^{8,26,27} Unlike target analysis, non-target analysis and suspect screening provide a putative identification that requires subsequent efforts for confirmation.

The survey was a formal search of studies presented as chemical exposomics and suspect screening/non-target analyses of environmental chemicals in biological and/or environmental matrices.

It is acknowledged that the selected terms are not comprehensive but the survey is intended to provide an overview of the trends of recent studies in the field. The retrieved manuscripts were manually inspected by scientists with the appropriate expertise to identify studies that employed suspect screening/non-target workflows. In total, 144 publications reporting full-scan HRMS-based analysis were selected and compiled (see [Supplementary Data](#)). From this survey, it emerged that experimental conditions commonly used in chemical exposomics are rather uniform.

Solvent extraction and solid-phase extraction (SPE) are the most frequently used techniques for sample extraction of human matrices²⁸ and the same trend is evident when extending to other (non-human) biological specimens ([Tables S1.1](#) and [S2.1](#)). This was to be expected because minimally selective sample preparation/extraction methods are often advocated for chemical exposomics.²⁷⁻²⁹ Notably, no study applied pre-normalization procedures to correct for matrix effects or to reduce the dynamic

range of concentration (e.g. specific gravity, creatinine normalization).

Across all publications, LC is the most commonly used separation platform compared to GC: 172 LC-HRMS methods were reported for LC-HRMS (28 applied to biospecimens, 126 to other matrices, 18 to both; [Table S3.1](#)) while 29 methods were reported for GC-HRMS (9 applied to biospecimens, 18 to other matrices, 1 applied to both; [Table S4.1](#)).

The majority of LC studies used electrospray ionization (ESI), with acquisitions performed in both positive and negative polarity while atmospheric pressure chemical ionization (APCI) had limited application ([Table S3.1](#)). Reversed-phase (RP) chromatography was commonly performed by using C18-based columns and water/methanol gradients, with formic acid, at temperatures below 50°C ([Tables 1](#) and [S3.1](#)). Column lengths and particle diameters varied from 50 to 150 mm and 1.7-3.5 μm , respectively, while an internal diameter of 2.1 mm was common ([Tables 1](#) and [S3.1](#)).

Among the limited applications of GC separation, 5-type columns were generally used ([Table S4.1](#)) coupled with electron ionization (EI), operated in positive acquisition mode at 70 eV ([Table S4.1](#)).

The lack of versatility/diversity of current HRMS analytical approaches was recently highlighted as an obstacle limiting the comprehensive characterization of the internal chemical exposome.²⁴ It was evidenced that HRMS-based methodologies have limited capability to detect the wide range of environmental pollutants and consumer chemicals in human blood¹⁸; due to their abundance typically being orders of magnitude lower than many endogenous compounds and drugs/pharmaceuticals.^{24,30-32} Furthermore, the dynamic range of chemical concentrations in samples far exceeds that of MS detectors, meaning that multiple different extractions and enrichments will be essential for comprehensive detection. The diversification of methodologies must also be balanced with achieving highly reproducible workflows that provide a solid basis for harmonizing procedures across multiple laboratories to generate comparable data at large scale.

Analytical strategies to diversity chemical exposomics looking across related fields

Sample treatment

Pre-analytical procedures

Sample storage and stability

Sample storage, stability, and handling are crucial variables affecting analytical outcomes. The impact of pre-analytical procedures on coverage of endogenous metabolites has been comprehensively reviewed³³⁻³⁵ and guidelines for biological specimens elaborated.^{36,37} Chemical lability has been of minor concern for the measurement of exogenous compounds because the traditional chemicals of focus are persistent (e.g. persistent organic chemicals) or relatively stable (e.g. pesticides). These chemicals could potentially be measured many (tens of) years after sample collection with minimal provisions undertaken for storage. Yet, more comprehensive characterization of the chemical exposome extends the focus toward more polar and labile contaminants.³⁸ Cohort studies often involve long storage time (tens of years) and implementing greater protective measures for storage would enable the prospective integration with assays of less stable molecules (e.g. proteins). Hebel et al. reported that so long as consistent procedures have been implemented, biobank samples (stored at -80°C) would be suitable for meaningful multiomics analysis even after long storage periods (~15 years).³⁹

Table 1. LC Conditions commonly used in suspect screening and non-target studies

Ionization	Particle chemistry	Column ID	Mobile phase	Modifier	Oven temperature
ESI+	C18-based (84%) ^a (n = 137)	narrow bore (81%) (n = 135)	H ₂ O/MeOH or ACN (95%) ^b (n = 136)	FA or FA + AF/AA (75%) ^c (n = 132)	<50°C (97%) (n = 89)
ESI –	C18-based (86%) ^d (n = 116)	narrow bore (76%) (n = 114)	H ₂ O/MeOH or ACN (94%) ^e (n = 115)	FA or FA + AF/AA (48%) ^f (n = 108)	<50°C (90%) (n = 72)

FA: Formic acid. AF: Ammonium formate. AA: Ammonium acetate. MeOH: methanol. ACN: acetonitrile. ^a59% C18; 25% C18 modified for polar compound retention/hybrid phases. ^b55% H₂O/MeOH; 40% H₂O/ACN. ^c59% FA; 16% FA + AF/AA. ^d59% C18; 27% C18 modified for polar compound retention/hybrid phases. ^e57% H₂O/MeOH; 37% H₂O/ACN. ^f41% FA; 7% FA + AF/AA.

Studies comparing the long-term impact of different storage conditions are limited but some generic measures beneficial to preserve sample integrity include (1) quenching (e.g. dry ice, liquid nitrogen) immediately after sample collection and appropriate sample sealing to prevent losses of volatile compounds⁴⁰; (2) storage at –80°C or –196°C using liquid nitrogen, to prevent biotransformation reactions and prevent potential losses^{41,42}; (3) limiting storage time and frosting/defrosting cycles (e.g. by aliquoting subsamples in advance) and minimizing sample handling.^{40,42}

Further investigations to assess the long-term impacts of storage conditions on chemical components of biospecimen and environmental matrices are encouraged, and are especially critical for large-scale studies, where the analyzed samples have often been stored for different periods of time. The diversity of intracellular biochemical reactions that exogenously-derived chemicals are involved is typically less than for endogenous compounds⁴³ which facilitates comparatively more accurate prediction of biotransformation and modeling of chemical exposure than for metabolic networks. Approaches similar to the ones used to estimate chemical biodegradation could be attempted to model biotransformation reactions in biospecimens over storage. For example, Quantitative Structure-Biodegradability Relationships (QSBRs) are widely used to predict chemical biodegradability by using molecular descriptors.⁴⁴ Time and cost-efficient approaches to extrapolate half-lives reading across different environmental media have been recently proposed for small molecules, where activated sludges were used as accelerated reactors to calculate half-lives of various agrochemicals and regression models developed to extrapolate to soil half-lives.⁴⁵ Similarly, *in vitro* “reactors” with biological extracts enriched with degradative molecules and enzymes or genetically modified microorganisms (e.g. to over-express cytochrome P450 enzymes) could be used to provide biotransformation rates of chemicals in biological specimens to eventually extrapolate with temperature corrections (e.g. by Arrhenius equation)⁴⁶ or correlations with measurements at lower temperatures.

Sample normalization

To reduce variability across biological samples and obtain comparable data, sample normalization is fundamental, especially when approaching quantification.^{47,48} Otherwise, the correct interpretation of the results can be confounded by large inter-individual and intra-individual variation. For example, dilution factors for urine may vary up to a factor of 15,⁴⁹ leading to different detection limits and potential matrix interferences. For more details on normalization strategies for biological specimens (e.g. protein/lipid normalization, specific gravity/osmolality, weight/volume base methods), numerous reviews are available.^{34,47-49}

Pre-analytical normalization is more relevant for exocrine-related glands/tissues/fluids (e.g. urine, kidney, gastrointestinal tract) compared to endocrine (e.g. blood, thyroid, ovaries, etc.); a consequence of the role in maintaining homeodynamic

regulation. High variability limits comparative analysis of relative abundances due to varying limits of detection, and is further complicated, e.g. by signal ratio bias from non-linear ESI responses. This is particularly problematic for low abundant features because it reduces detection frequencies and sparsity cannot easily be corrected post-acquisition. On the other side, pre-analytical procedures to minimize signal variability typically involve dilution of samples, reducing the overall number of compounds detectable. Analyzing serial dilutions of representative samples to assess potential matrix effects by evaluating response linearity was recently recommended over procedures with extensive sample manipulation, with direct injection demonstrated to provide more reliable semi-quantification in many cases.^{50,51}

Post-analytical normalization strategies by using dedicated software and statistical approaches can be used to correct for sensitivity drifts and reduce variability across samples.⁴⁸ However, exogenously-derived compounds are present at lower abundance and with reduced frequency compared to constitutive metabolites and proteins, limiting applicability and validity of typical corrective procedures (e.g. noise thresholds and detection frequency filters, missing value imputation).⁵²

Longitudinal studies become even more complex because analytical batch variation is often larger than temporal changes, so randomizing measurements may not be enough to ensure data comparability. In addition, at the point of analysis, it is common that samples have been stored for varying lengths of time, adding further complexity. Consequently, the implementation of normalization procedures requires careful consideration for each chemical exposomics study and more long-term storage studies would also help to advance identification of suitable normalization parameters.

Sample extraction and purification

Finding the best compromise between selectivity and sensitivity can be challenging and careful consideration is needed with respect to the specific purposes of the study.²⁷ Sample homogenization is critical to obtain consistent extraction efficiency, especially for heterogenous matrices (e.g. tissues, fecal samples, cell cultures).⁵³ Sample clean-up can reduce recoveries for compounds of interest but greater removal of interferences and reduced matrix effects can enable increased concentration of extracts, providing better sensitivity to compensate losses.⁵⁴

Profiling sub-exposome components: partitioning/fractionation and selective recognition

Sample fractionation has been highlighted as a potential approach for the in-depth characterization of biospecimen because greater enrichment factors can be achieved through concentration of fractions and a combination of platforms can be used for more focused analysis of different fractions.²⁴ However, each stage of purification entails losses and the initial sample amount available is the main limiting factor.

Solvent partitions allow for the separation of analytes in different fractions based on their hydrophobicity that can be processed separately or purified and then pooled together. For example, Pourchet et al. proposed a non-targeted workflow for investigating halogenated compounds in human milk after protein and lipid removal by using solvent partition to separate analytes in two different fractions for GC-HRMS and LC-HRMS analysis.⁵⁵ Furthermore, hot solvent mixtures,¹⁴ microwave,⁵⁶ and ultrasound⁵⁷ can be used to enhance solvent extraction efficiency. SPE-based fractionations, via sequential elution^{58,59} or the use of sequential phases,⁶⁰ have been evidenced to increase metabolome coverage⁶¹ and can equally be applied for enhancing coverage of the chemical exposome, capitalizing on the wide range of SPE sorbents are commercially available. For example, step-wise elution from mixed-mode SPE was used to separate neutral and acidic polycyclic aromatic compounds in runoff water samples and fractions screened via GC- and LC-MS, uncovering numerous emerging biotransformation products,⁶² while a two-stage hydrophilic-lipophilic balance (HLB)-activated carbon SPE purification was applied to screen hundreds of chemicals in waters.⁶³ Plates and cartridges provide an alternative to the traditional precipitation techniques for removing proteins and lipids.^{64,65} For example, a phospholipid depletion followed by cation exchange SPE enabled greater concentration of plasma sample extracts prior to instrumental analysis compared to classical protein precipitation, showing increased sensitivity.⁵⁴

The use of molecularly imprinted polymers (MIPs) represents a promising approach to selectively purify specific structural classes of compounds, e.g. halogenated,⁶⁶ organophosphate,⁶⁷ and glucuronidated⁶⁸ chemicals, and shows greater cleanup efficiency to enable enhanced concentration of low abundant analytes.⁶⁹ Similarly, chemoselective probes that consist of a solid support containing a probe with specific sites to bind target groups and cleavage linkers to release the compound in certain conditions⁷⁰ have been successfully applied for enrichment in metabolite profiling studies,^{70,71} e.g. targeting amines,⁷² aldehydes, and ketones.⁷³ Protein receptors can be used as probes for selection of biologically-active chemicals⁷⁴ and multiple MIPs and/or probes can be used in tandem to enable highly resolved fractionation. However, applications are limited by the lack of commercially available chemistries.

Chemical and enzymatic treatments

Chemical reactions (e.g. deconjugation, derivatization) can promote analyte release from matrix components and/or enhance ionization efficiency. Deconjugation has been commonly used in target assays (e.g. for bisphenols) to measure the total concentration of the parent compounds⁷⁵ and recommended for suspect screening to confirm the identity of parent substances by comparing non-deconjugated and deconjugated samples.⁷⁶ Similar comparative profiling has also been conducted using glucuronidase and sulfatase with high activity and promiscuity for non-target analysis of glucuronide⁷⁷ and sulfate metabolites,⁷⁸ respectively, greatly increasing analyte detection. Adoption in chemical exposomics studies would advance toward more comprehensive screening of Phase 2 detoxification metabolism. However, obtaining such high activity requires multistep purification from commercial enzyme extracts, thus requiring a great level of expertise. Alternatively, typically more affordable chemical deconjugations could be used but their decreased specificity complicates annotation/identification.

Different reaction rates mean it is difficult to obtain complete deconjugation for a wide range of substances⁷⁹ and challenges to

standardize procedures and reaction conditions often leads to large intra- and inter-lab variability.²⁷ Furthermore, unexpected transformation products and degradation of parent compounds can occur⁸⁰ and information of biotransformation patterns can be lost, which is detrimental for fate and toxicity assessment across the chemical life cycle.

Besides deconjugation, enzymes can be used to assist extraction by degrading cell/membranes and matrix components.⁸¹ For example, Wawrzyniak et al. applied a mild proteinase K digestion to human plasma to misfold proteins and induce the release of associated metabolites.⁸² The method increased metabolome coverage compared to direct precipitation, more than doubling the number of annotated, reproducible features, though the formation of non-specific protein fragments was also noted. Application of a protease treatment step could also be beneficial for the enhanced detection of environmental chemicals since many are known to associate with proteins, such as per- and poly-fluorinated substances.⁸³ Similarly, the potential of other biological enzymes (e.g. lipases) could be explored as milder digestion alternatives to traditional strong acid/alkali treatments often employed.⁸⁴

Chemical derivatization can be used to make compounds more volatile for GC,⁸⁵ extend the hydrophobicity range covered by LC (e.g. for better separation of very polar compounds)⁸⁶ and increase detection sensitivity through the addition of moieties that improve ionization, e.g. halogens or alkyl groups. Various derivatization agents can be combined to target diverse groups of analytes⁸⁷ and improve confidence for annotation (e.g. comparing labeled and non labeled sample fractions/features), although libraries will need to be extended to include more derivatized compounds.⁸⁸ For example, Zhao et al.⁸⁷ proposed a 4-channel chemical isotope labeling method to quantify hydroxyls, amines/phenols, carboxylic acids, and ketones/aldehydes. However, optimization of reaction for each sub-class is complex. The slow reaction speeds can limit throughput for use of some derivatization agents⁸⁶ and the potential instability of derivatives makes it challenging to ensure reproducibility without automatization.^{89,90} Although usually performed in the latest stage of sample preparation,⁸⁶ derivatization before sample extraction can be used to increase the affinity of polar compounds toward polymeric phases (e.g. SPE cartridges)⁹¹ or solvents. The potential of this approach has been demonstrated for assessment of the urinary chemical exposome, with enhanced detection sensitivities from 2- to 1184-fold.⁹²

Microextraction techniques and passive sampling

The main microextraction techniques can be classified as solvent-based i.e. liquid-phase microextraction (LPME), or sorbent-based, i.e. solid-phase microextraction (SPME).⁹³ LPME consists liquid-liquid extractions using small solvent volumes, with the most common approaches being (1) dispersive liquid-liquid microextraction (DLLME), where droplets of a non-polar solvent are finely dispersed in an aqueous sample allowing for a rapid extraction; (2) hollow fiber-protected liquid-phase microextraction (HF-LPME), where analytes are extracted by a solvent placed in the pores of a hollow fiber and then transferred in an acceptor phase and (3) electromembrane microextraction (EME), which is similar to HF-LPME but supported by the application an electrical field. LPME present numerous advantages, such as reduced solvent consumption, low cost, broad selectivity, and rapidity. However, working with such small volume extracts can pose challenges for automation. More details on these techniques and applications can be found in recent reviews^{94,95} and their potential to improve

selectivity and efficiency in sample clean-up should be further investigated for chemical exposomics. The advantages of DLLME for broad extraction for multiple novel psychoactive compounds have been shown.⁹⁶

SPME has been more widely applied in metabolomics research,^{40,97} often showing performance comparable or even better than traditional approaches.⁹⁸⁻¹⁰¹ Non-exhaustive extraction methods, such as SPME, can provide better sensitivity for low abundant species since suppression from matrix components is reduced.^{98,99} Comparatively, a limited number of studies have been published on non-target analysis of environmental chemicals using passive sampling approaches,¹⁰²⁻¹⁰⁷ but applications in targeted studies show their potential for concentrating low abundant chemicals from complex matrixes (e.g. hydrophobic organic chemicals in waters or soil/sediment porewaters).^{108,109} Passive sampling (e.g. by SPME, silicone rods, etc.) provides many appealing features. First, sample extraction, clean-up, and concentration are conjugated in one step, enhancing reproducibility for sample preparation and throughput. Second, if operated in non-depletive mode, passive sampling allows insights of chemical activity because spontaneous processes such as partitioning will be driven by activity gradients until equilibrium is reached.^{110,111} Furthermore, the use of passive sampling devices provides a good balance between hydrophilic (mainly in freely dissolved form) and hydrophobic species (mainly associated with matrix components but usually with stronger affinity for polymeric phases),⁴⁰ and is a promising technique for *in situ*¹⁰⁴ and *in vivo*¹¹² applications. The potential of wearable passive samplers to measure a large array of chemicals across individuals was recently reviewed.¹¹³ Their low-cost, noninvasive nature make them practical for large-scale deployment and integration with ecological momentary assessments enables the spatiotemporal variability of an individual's chemical exposure to be captured. However, attention must be paid to potential artifacts with careful consideration to placement and sampling rate alteration, e.g. through obstruction such as coverage by clothing, personal care product use if in contact with skin, air velocity alterations for individual behaviors, etc.¹¹³

Sorbent choice is critical and most metabolomics applications have favored commercially available polydimethylsiloxane/carboxen/divinylbenzene (PDMS/Car/DVB) to provide broad coverage. Although currently limited to coatings made in lab, (HLB) particles have been shown to provide good performances in terms of sensitivity and extend coverage toward more polar analytes.^{100,114,115} For example, Vasiljevic et al. developed biocompatible SPME minitips coated with HLB particles and demonstrated their application for metabolomics and the targeted analysis of drugs of abuse,¹¹⁵ a combination polystyrene-divinylbenzene-weak anion exchange (PS-DVB-WAX)/HLB (50/50, w/w) fiber was demonstrated to cover a wide range of hydrophobicity ($-7 < \log P < 17$) and molecular weight (100-1000 Da)¹¹⁶ and Gionfriddo et al. proposed a polytetrafluoroethylene amorphous fluoroplastics (PTFE AF)/HLB combination suitable for both GC and LC applications.¹¹⁴ Xu et al. developed a biocompatible and stable polystyrene-polydopamine-glutaraldehyde SPME coating for *in vivo* analysis on pharmaceuticals in fish muscle and demonstrated sensitivities from 26 up to 42 times larger than traditional PDMS fibers,¹¹⁷ evidencing the potential for application of novel sorbents for the *in vivo* sampling of environmental chemicals.¹¹⁸

The main pitfalls of passive sampling for non-target applications are selectivity (similarly to SPE or extraction by solvents), extraction of unbound (freely dissolved) molecules, and lengthy sampling times; though these can be tackled via addition of matrix modifiers (e.g. salts, surfactants) and high temperatures can

speed up sampling rate,¹¹⁹⁻¹²² albeit with risk of increased degradation. When used for qualitative analysis or measurements of total concentrations, pushing passive sampling boundaries toward depletive mode by increasing the volume of the sampler (e.g. thickness of the SPME fiber) and or enhancing desorption from matrix (e.g. by using surfactants) to increase the amount of chemical extracted, can improve sensitivity.

Instrumental analysis

Advances in GC-HRMS analysis

Compared to LC-HRMS, GC-HRMS remains an underexploited tool in metabolomics and chemical exposomics, despite providing complementary compound coverage. GC separations can provide more reproducible retention than LC, with use of retention indices commonplace. Plus, the predominant mode of EI is largely standardized which enables highly reproducible fragmentation. The drawbacks are the limited range of compounds amenable to GC alongside the fact that EI is a "hard" (i.e. highly energetic) ionization mode and hinders annotation due to extensive in-source fragmentation. Furthermore, there is a substantial lack of open GC-HRMS spectral libraries¹²³ and spectra collected in low resolution may be not fully comparable to data acquired in HR in the abundances of certain ions.¹²⁴

Optimizing GC

Optimizing gas (and liquid) chromatography typically requires finding the best compromise between peak capacity/separation and sample throughput, selecting the best performing stationary phase and column characteristics (e.g. length, diameter, particle size), fitting the purposes of the work. An often overlooked approach to speedup the GC run and providing large sensitivity is the low pressure (so-called "vacuum-outlet") GC. This consists in connecting a restrictor in the inlet to a short (10-15 m) wide (typically i.d. ≥ 0.5 mm) column as vacuum outlet for the MS.^{18,125} High vacuum reduces the viscosity of the gas mobile phase allowing for a higher flow rate and mass transfer. Compared to other fast-GC approaches like using short capillary columns, fast thermal gradients or high flow rates, this method is much more robust because of the use of large columns. The numerous benefits of low-pressure GC led some authors to recommend it as the standard approach for GC analysis,¹⁸ with promising applications to HRMS suspect/non-target screening.¹²⁶ Possible drawbacks of this method regards increase of the column bleed, and potential narrowing of the peaks to durations incompatible with MS detector scan speed.¹²⁶

Comprehensive two-dimensional GC (GC \times GC) has been identified as a promising strategy for non-target analysis of environmental chemicals, providing greater peak capacity separations and often greater sensitivity.¹²⁷ The theory¹²⁸ and advances of this approach have been discussed for applications in, e.g. metabolomic,¹²⁹ biomedical,¹³⁰ and environmental¹³¹ research. Briefly, two GC columns are connected in series and multiple fractions/whole sample is sent from the first to the second column via a modulator. The increased commercial availability of GC \times GC modulators looks set to push GC \times GC towards routine use in the coming years.¹³² However, the reproducibility of GC \times GC remains to be demonstrated for large-scale profiling applications, particularly studies that would necessitate intervening maintenance (e.g. column replacements).

GC-MS ionization sources

The high fragmentation and absence/low intensity of a molecular ion when using hard ionization (e.g. EI) poses challenges for

processing and annotation workflows. Using lower ionization energies in EI has been proposed to reduce fragmentation.¹²⁷ However, conflicting results have been reported in literature with this approach shown to be highly dependent upon source design.¹³³ For example, significant improvements of molecular ion peak detection at 30 vs. 70 eV with a quadrupole-time of flight-mass spectrometer (Q-ToF-MS) were demonstrated,¹³⁴ while just slight differences between 12 and 70 eV were observed via GC-Orbitrap.¹³⁵ Another approach to enhance molecular ions is the use of Cold EI whereby a supersonic molecular beam interfaces the GC and MS and EI+ conducted on cold molecules.¹³⁶ This was later pushed further toward soft ionization by reducing electron energy (from 70 eV to 18 eV) and lowering helium pressure to limit collisions via increased nozzle-skimmer distance, which effectively limited in source fragmentation and was successful to obtain mainly molecular ions.¹³⁷ Cold EI has subsequently been linked to both LC and GC and shown favorable to ESI and conventional EI for identification,¹³⁸ yet only tested at low resolution.

The use of “soft” (i.e. low energy) ionization such as chemical ionization (CI), field ionization (FI), photoionization (PI), or APCI is relatively widespread. Recently the coverage domain of EI and CI (positive and negative mode) was investigated on a commercial set of metabolites and shown to be complementary.¹³⁹ Notably, of the 330 total compounds detected 81, 39, and 28 were unique for EI+, positive CI, and negative CI, respectively. However, sensitivity and coverage of soft ionization are on average reduced compared to EI+, and application usually additional rather than replacement for comprehensive profiling, decreasing throughput.¹²⁷ Notably, electron capture negative ionization/negative CI is widely applied for highly sensitive targeted analysis of chemicals containing groups with high electronegativity, particularly halogens. Although overlooked in chemical exposomics (Table S4.1), recent applications have shown promise for non-target analysis.¹⁴⁰⁻¹⁴²

Advances in LC-HRMS analysis

LC coupled to HRMS enables separation and detection of a wide array of compounds in a diverse spectrum of physicochemical properties, providing maybe the best compromise between broad analytical coverage and simple sample treatment.^{124,143} However, results obtained in terms of analytical detection, sensitivity, and separation, largely depend on specific conditions used since different columns phases, modifiers, and chromatographic run length and temperatures can remarkably affect analytical outcomes. Advances in LC have been fundamental for the development of many broad range suspect/non-target methods. Notably, the introduction of ultra-high-pressure LC (UHPLC) with sub 2-micron columns enabled higher separation efficiencies,¹⁴⁴ while core-shell/fused-core particles extended similar advantages to conventional high-pressure LC (HPLC).¹⁴⁵ To capture the large diversity of chemicals, novel developments should be addressed toward the combination/testing of different stationary and mobile phase chemistry, chromatographic conditions, and LC-MS interfaces.

Optimizing LC

Stationary phase

RP LC using C18 columns is typically favored because of the reliability and robustness, providing broad coverage, highly reproducibility retention times, and pH stability. Periat et al. tested 59 different columns for separation of 120 analytes which covered a wide range of hydrophobicity ($-4.1 < \log P < 14.76$), using RPLC and hydrophilic interaction chromatography (HILIC), including

mixed-mode columns and various pH conditions.¹⁴⁶ Pentabromobenzyl (PBrBz) was recommended as a versatile broad-spectrum stationary phase while bridged ethylene hybrid (BEH) C8 at pH 9 and charged surface hybrid non-encapped propylfluorophenyl columns were recommended for basic and acid compounds, respectively. Despite orthogonality, the additional benefits of complementing RP separations with HILIC were deemed to be low, offering just a 1% increase in unique chemicals showing acceptable peak shape beyond that possible for combining different RPs.¹⁴⁶ Conversely, Contrepolis et al. argued high complementarity by showing that fifty percent of the standard compound that showed unsatisfactory separation response via HILIC were acceptably detected through RPLC.¹⁴⁷ Similarly, Zhang et al. compared an RP, two HILIC, and two aqueous normal phase separations for 176 metabolite standards, and demonstrated that complementary use of the two HILIC methods attained the greatest coverage.¹⁴⁸

Further diversification of reversed-phase column choice also offers a rapid way to enhance coverage. For example, a combination of C8 and T3 (C18 modified for improved retention of polar groups) columns quadrupled the number of non-polar to semi-polar standards detectable compared to use of a single conventional C18 column.¹⁴⁹ Also, the application of C30 column showed better performance for separation for 49 lipid standards¹⁵⁰ and alternate selectivity for polar and non-polar analytes,¹⁵¹ yet are rarely used in comparison to traditional C18 columns. In addition, the use of porous graphitized carbon has been suggested as a RP alternative to HILIC separation, though considerations are needed to maintain retention stability¹⁵² and optimal choice between each dependent on analytes investigated and sample matrix.¹⁵³

The benefits and drawbacks of coupling of column with different phase chemistry and separation mechanism in series, in parallel or offline have recently been discussed.¹⁵⁴ Notably, many metabolomic studies have applied complementary RPLC and HILIC to simultaneously screen hydrophilic and hydrophobic chemicals,^{147,155-157} and the approach could be similarly applied in chemical exposomics.

Column dimensions (scale)

The miniaturization of LC-MS has recently been extensively reviewed¹⁵⁸⁻¹⁶⁰ and was highlighted by David et al. as a core development need to improve characterization of the human internal chemical exposome.²⁴ Miniaturization can increase sensitivity via enhanced ionization efficiency/reduced ion suppression,^{161,162} reduced sample dilution, and greater resolution i.e. sharper peaks.^{163,164} Furthermore, miniaturization reduces solvent consumption¹⁶⁵ and has been demonstrated to have reproducible performance with suitability for large-scale studies, including metabolomics applications.¹⁶⁶

Microscale columns (i.d.: 0.5-1 mm¹⁶²) provided good performances compared to narrow bore scale (2 mm¹⁶²) in metabolomic studies, by increasing sensitivity, signal to noise ratio (S/N), and number of features identified (Table 2), although some system updates (e.g. reduction of the tubing and ESI capillary diameters) can be necessary.¹⁶⁴ Moreover, Gray et al. reported excellent stability of microbore LC over time with no significant changes of peak shape, intensity, and backpressure after 1000 consecutive injections of human urine.¹⁶⁴ Similarly, experiment with capillary columns (i.d.: 0.1-0.5 mm)¹⁶² provided much better sensitivity than narrow bore scale.^{167,168} Nanoscale LC-ESI systems work with columns of 0.01-0.1 mm i.d.,¹⁶² flow rates of 10-1000 nL/min and ESI capillary with i.d. of 10-50 μm ¹⁶⁶ and offer a better

sensitivity than conventional narrow bore and microscale due to droplets 100-1000 fold smaller and reduced sample dilution.¹⁶⁹ For example, a 2- to 2000-fold improvement in detection limits has been demonstrated when reducing from a 1 mm to 0.1 mm column (Table 2).¹⁷⁰

However, compared to microscale and capillary separations major drawbacks of nanoscale LC are lowered robustness (e.g. column connections, tubing) increasing maintenance, slow conditioning and equilibration times,¹⁷¹ and the need for more careful sample preparation and purification required to avoid the clogging of the system.^{54,166} Each of these drawbacks reduces throughput and will need to be overcome for application to population-based chemical exposomics. To this end, applications of nano-UHPLC separations (i.e. using small diameter, small particle size columns) are increasing.¹⁷¹ Recently commercialized micropillar array columns with enhanced robustness and reproducibility are showing promise in proteomics¹⁷² and the coupling of SPME with nano-LC represents a promising approach to introduce a highly purified sample^{115,173} without the need of extensive clean-up steps.

Mobile phase modifiers

Most exposome studies used formic acid (typically 0.1%, with or without salt addition, e.g. ammonium acetate/formate) in water/methanol or water/acetonitrile gradients (Tables 1 and S3.1). However, promising results have been achieved with other modifiers. For example, Yanes et al. compared ammonium fluoride (NH₄F; 1 mM) against ammonium acetate (5 mM) and formic acid (FA; 0.1%) on a conventional C18 column in ESI⁻ and observed an average increase of 5.7-fold of the signal intensity for 36 standards.¹⁴ Ammonium fluoride has also been used for enhanced detection of emerging contaminants up to 5-fold¹⁷⁴ and xenoestrogens by RPLC, claimed favorable for both positive and negative ionization.¹⁵⁶ Similar results were obtained for HILIC, where NH₄F (2 mM) provided better ionization efficiency and

signal-to-noise ratios than ammonium acetate (10 mM) for 34 standards via ESI⁻.¹⁷⁶ However, attention must be paid when handling NH₄F solutions for potential hydrogen fluoride degassing.¹⁷⁵ Furthermore, studies on stability of this modifier over time should be performed since it may be microbiologically less stable than acidified solutions. Zhang et al. instead recommended acetic acid (1 mM) for improved detection of 26 metabolite standards when compared to both NH₄F and FA across a range of additive concentrations.¹⁷⁷ From a test of 17 different modifier conditions in ESI⁺ and 23 in ESI⁻, the observed compound-specific responses led to recommendation of using a combination of different modifiers for various ionization modes and analytes.¹⁷⁸ Furthermore, monitoring adduct mass and intensity shifts from use of isotopically enriched modifiers has recently been demonstrated to aid identification of potential metabolite features,¹⁷⁹ and could equally be applied to chemical exposomics.

LC temperatures

The use of high-temperature (HT) LC to increase separation efficiency and enable faster separations is a potential approach to increase throughput.¹⁸⁰ The decreased viscosity of the mobile phase can facilitate use of unconventional solvents (e.g. ethanol, isopropanol) to increase coverage, enable a reduction of the use of organic modifiers for elution and increase column lifetime by enhanced removal of, e.g. phospholipid matrix interferences. The main pitfalls are the limited availability of thermostable columns, potential loss of thermolabile compounds, and the need for dedicated instrumentation, e.g. pre-heating apparatus for the mobile phase, pressure regulation interface with MS.^{181,182} Though maximizing temperature is recommended for separation efficiency,¹⁸¹ the effect of mobile phase temperature on MS detection sensitivity was shown to be compound specific and gains in analyte signal intensity can be offset by increasing noise.¹⁸³ Despite this, applications utilizing HT-UHPLC-MS have demonstrated the benefits of increased ion detection and greater

Table 2. Performance comparisons of miniaturized LC and conventional narrow bore scale.

Internal diameter (mm)	Flow rate (μL/min)	Matrix	Results	Reference
1 vs. 2.1	136 vs. 500	Rat urine	Increase of S/N ratio (20% for a feature), reduction of the sample injected (from 10 to 2 μL)	Lenz et al. ¹⁶¹
1 vs. 2.1	140 vs. 600	Rat plasma	Increase in S/N (up to 3.6-fold across 5 pharmaceuticals), decrease of the solvent consumed (~ 4-fold)	Rainville et al. ¹⁶⁵
1 vs. 2.1	140 vs. 600	Human urine	Increase in peak area, peak height, and S/N of 2- to 3-fold for metabolites (7 analyzed in ESI ⁺ and 4 in ESI ⁻) and ~10% more features detected	Gray et al. ¹⁶⁴
0.32 vs. 2.1	10 vs. 600	Rat urine	Increase in sensitivity of ~100-fold and doubled ion detection	Granger et al. ¹⁶⁷
0.2 vs. 2.1	6 vs. 700	Human plasma	Increase in sensitivity (peak areas) from 6- to 49-fold among 11 drugs	Zhang et al. ¹⁶⁸
0.1 vs. 1	0.7 vs. 200	50/50 MeOH/water	Decrease of the instrumental limits of detection from a factor 2 to a factor of 2000, for 78 xenometabolites	Chetwynd et al. ¹⁷⁰

separation compared to conventional RPLC, for detection of both endogenous and exogenous compounds.^{184,185} This approach has been successful in reducing run time^{186,187} providing good peak capacity in rat and human urine metabolomic analysis, both by using conventional isotherm (90°C) with solvent gradient¹⁸⁴ and thermal gradient (up to 180°C).¹⁸⁸ These studies showed that reproducible HT-UPLC-MS is achievable for complex matrices (e.g. blood plasma), though the authors note HT LC still requires further development.

LC-MS ionization sources

Novel interfaces could extend LC-MS coverage and sensitivity. The use of multiple polarity and ionization modes has been evidenced to enhance coverage for metabolomics¹⁸⁹ and rapid polarity switching and dual/multimode sources common.¹⁹⁰ Rapid polarity switching can be less robust than separate acquisition (e.g. through disturbed spray) and increases cycle times limiting scans per peak; though dual emitters help tackle each problem.¹⁹¹ The main limitation is that the use of a single mobile phase can compromise coverage.¹⁹² Multimode ionization tends to show a reduction in sensitivity.¹⁹³ Alternatively, rapid switching between ESI and APCI sources was recently demonstrated without ion abundance loss and is a promising option for further development.¹⁹⁴ Novel ionization processes have been recently reviewed.¹⁹⁵ In particular, the electrospray ionization inlet (ESI^{II}) shows promise, offering increased ion abundance and lower background signal when compared to ESI, able to provide similar advantages as nano-ESI at microscale flowrates.¹⁹⁶

Finally, research advances coupling LC with EI ionization are rapidly progressing toward commercial viability.¹⁹⁷⁻¹⁹⁹ Combining the advantages of LC separations with EI ionization could significantly enhance coverage and provides more reproducible fragmentation though, at present, sensitivity requires improvement to be competitive with ESI. Optimizing source parameters (e.g. by using design of experiment, DoE) can improve analyte detection.^{15,200} For example, high in-source fragmentation was recently demonstrated to increase the proportion of annotated coverage.²⁰¹ Notably, in silico method development enables rapid testing and optimization of acquisition parameters^{202,203} while technological advances have seen the emergence of information-dependent acquisition modes to enable automated real-time parameter optimizations during analysis.²⁰⁴⁻²⁰⁶

An assessment of costs, benefits, and potential for chemical exposomics

Strategies reviewed in the previous section were assessed on the basis of their benefits and costs and a division proposed sorting out strategies with (i) low costs and high benefits; (2) low costs and low benefits; (3) high costs and high benefits; (4) high costs and low benefits (Table 3). Benefits considered were in terms of sensitivity (enhanced recovery/sample enrichment or instrumental response); physicochemical range coverage; reproducibility; sample throughput; practicality (strategies easy to apply, potential for automation/reducing manual operations, etc.). Costs accounted for were capital expenditure (dividing minor and major investment for capital equipment); installation (ready to use items vs. need for installations/specialist support); operational costs associated with maintenance (long-term financial costs to keep functionality); consumables (financial cost plus environmental burden), and commercial availability (niche/in-house applications were considered demanding high efforts). Access to

conventional LC and GC-HRMS instrumentation was considered a prerequisite.

Ideally, strategies with high benefit and limited costs/efforts should be readily tested and include alternative sample enrichment methods, more refined purification methods, chromatographic optimizations (Table 3). Promising results in enhancing coverage and detection of low abundant compounds have been obtained with sequential extractions and fractionations, micro-extraction techniques, and chemoselective probes which encourage their application for non-target analysis of environmental contaminants.

Some of these approaches have been widely applied in target studies while still underexploited for non-targeted analysis (e.g. SPME). However, commercially available materials (e.g. HLB SPME, novel imprinted polymers/probes) should be developed to simplify analysis and reduce manual operations. Fractionation methods, with different solvents or sorbent extraction, are included in recently developed workflows,^{59,67} and offer the benefit of differential enrichment of fractions and/or analysis via multiple platforms. Whenever possible, preparation methods should merge the steps needed for sample purification. For example, interferences that ionize in multiple modes and/or have detrimental effects on columns and signal suppression (e.g. lipids) should be removed prior to fractionation. Similarly, enzymatic/chemical treatments (e.g. protease/lipase treatment/derivatization) could be implemented prior to extraction, and may enable more selective clean-up.

Optimized chromatographic settings from other LC-HRMS-based omics (e.g. ammonium fluoride vs. formic acid; PBrBz vs. C18-based separation) should be more widely tested as they show potential to be largely beneficial with virtually no costs. Target studies support the potential of these strategies (e.g. utilization of ammonium fluoride as modifier).^{174,175}

Low-pressure GC has shown improved sensitivity and potential for routine implementation with minor hardware updates. This is a largely overlooked approach in chemical exposomics that should be prioritized for implementation, especially considering the possibility of loading large sample volumes that could be a valuable asset for low abundant chemicals. Remarkable improvements in instrumental sensitivity can be obtained just optimizing MS acquisition parameters with proper experimental design. Although this is commonly performed in-house, harmonized guidelines on how to do this when a broad range of chemicals is investigated are lacking.

The combination of GC and LC separation via workflows that separate polar and non-polar compounds prior to analysis would capitalize on the advantages of each platform. Compared to minimal sample preparation, this would allow to introduce cleaner extracts in the system, since potential matrix interferences would partition as well, and thus both coverage and sensitivity can be enhanced.

If limited resources are available, strategies with low costs and some benefits could be implemented (Table 3). These include mainly minor laboratory tips for sample handling/storage conditions and strategies to improve recovery (e.g. hot solvent and ultrasound extractions) and/or to obtain larger coverage (e.g. derivatization). Limited studies, for example, are available on chemical stability over storage and potential of deconjugation⁷⁶ and derivatization⁹² for suspect and untargeted workflows.

Reducing pitfalls of very promising strategies currently with elevated costs (Table 3) should be the goal of next research developments. These include advanced hardware developments with innovative interfaces that allow for better ionization and

instrumental sensitivity, though requiring development to become commercially available. For example, HT LC appears a promising strategy to enhance mass transfer and allow for high throughput analysis, yet is currently limited by high costs and maintenance. LC miniaturization needs to be coupled with advanced sample cleaning that can limit throughput. These strategies are largely unexplored in chemical exposomics.

Outlook and conclusions

A survey of the analytical conditions used in chemical exposomics underlined the need to diversify current methods and approaches to advance toward more comprehensive measures of chemical exposures. To enhance coverage and detection of low abundant environmental chemicals, a proficient use of knowledge and experience inherited from related fields was previously advocated. Therefore, the analytical approaches used in HRMS-based metabolomics and proteomics were reviewed, along with newly emerging wide-scope multiresidue target analysis methods, to identify potential strategies suitable for chemical exposomics. A pragmatic assessment of the costs and potential benefits of implementing the strategies in chemical exposomics was performed to prioritize those with most promise (Table 3). We hope this provides a practical aid for research groups developing chemicals exposomics and guides investments to address next research.

Beside analytical challenges, data processing constraints hinder the development of a comprehensive characterization of the chemical exposome and represent a bottleneck for untargeted workflows.^{24,27} Computational tools must cope with low intensities features at noise level, scarcity of the signals across samples, and large variation of the chemical response, due for example to matrix interferences, variations in dilution factors, and recovery issues; with consequent challenges for the statistical analysis of the data. The profiling of endogenous compounds has attained comparably high compound coverage in terms of detection and annotation than for the profiling of exogenous chemicals in bi-specimen. For example, measurement of ~100s-1000s of endogenous metabolites per human plasma sample is possible by LC-HRMS, compared to just 10s-100s commonly found exogenous chemicals via the same method/platform.²⁰⁷ The divide is largely a result of the average lower internal abundance of exogenous chemicals, coupled with greater inter- and intra-individual variability, and limitations in the detection and annotation/identification of low abundant features during data processing. It should be considered that the strategies proposed here should also be supported with modification and development of the data processing workflows, especially for the aggregation and/or integration of multiple diverse datasets acquired via complementary workflows.

Furthermore, harmonized standard procedures, quality assurance (QA)/ quality control (QC) protocols, and minimum reporting standards should be implemented for chemical exposomics, similar to efforts in, e.g. metabolomics^{42,208,209} to assess overall quality of the data produced and comparability across laboratories. Steps toward this direction have been recently proposed.^{210,211} Ideally, the recommended guidelines should be as streamlined and simple as possible,^{53,212} to avoid encountering a lack of uptake scenario, commonly exemplified in metabolomics.^{213,214}

To conclude, we wish that this manuscript encourages further exchanges and discussions across experts from different fields. In our vision, sharing knowledge, tools, and analytical hints across fields is fundamental to push forward the limits of the

current approaches and integrate findings. Integrating experiences from related fields is a critical step to avoid reinventing the wheel in the emerging field of chemical exposomics. A plethora of approaches and novel strategies must be developed, tested, and integrated to push toward more comprehensive measures of chemical exposures and there is no time to waste.

Supplementary material

Supplementary material is available at *Exposome* online.

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Conflict of interest statement

G.W.M. receives royalties for his books *The Exposome: A Primer and The Exposome: A New Paradigm for the Environment and Health*. He has no other conflicts of interest. The other authors declare they have no actual or potential competing financial interests.

Authors' contributions

All authors contributed to the work presented in this manuscript, provided final approval and are accountable.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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