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Review article

Characterization of the internal working-life exposome using minimally and non-invasive sampling methods - a narrative review

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ABSTRACT

During recent years, we are moving away from the 'one exposure, one disease'-approach in occupational settings and towards a more comprehensive approach, taking into account the totality of exposures during a life course by using an exposome approach. Taking an exposome approach however is accompanied by many challenges, one of which, for example, relates to the collection of biological samples. Methods used for sample collection in occupational exposome studies should ideally be minimally invasive, while at the same time sensitive, and enable meaningful repeated sampling in a large population and over a longer time period. This might be hampered in specific situations e.g., people working in remote areas, during pandemics or with flexible work hours. In these situations, using self-sampling techniques might offer a solution. Therefore, our aim was to identify existing self-sampling techniques and to evaluate the applicability of these techniques in an occupational exposome context by conducting a literature review. We here present an overview of current self-sampling methodologies used to characterize the internal exposome. In addition, the use of different biological matrices was evaluated and subdivided based on their level of invasiveness and applicability in an occupational exposome context. In conclusion, this review and the overview of self-sampling techniques presented herein can serve as a guide in the design of future (occupational) exposome studies while circumventing sample collection challenges associated with exposome studies.

1. Introduction

The impact from chemicals and other substances within the environment on human health, in particular at workplaces, has been a concern for a long time. Additionally, not all job-related exposures are chemicals (working hours, shift work, noise etc.), and can still affect the health. Observational studies in the distant past already pointed towards an association between occupational exposure and health, e.g., associations between soot exposure and testicular cancer by Percival Pott

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Nomenclature

Abbreviations

2D-PAGE	Two-Dimensional Polyacrylamide Gel Electrophoresis
BTEX	Benzene, Toluene, Ethylbenzene, Xylene
COVID-19	Coronavirus Disease 2019
CRP	C-Reactive Protein
DBS	Dried Blood Spot
EBC	Exhaled Breath Condensate
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
FASP	Filter Aided Sample Preparation
FID	Flame Ionization Detector
GC-MS	Gas Chromatography (GC) Coupled With Mass Spectrometry (MS)
GC-MS/MS	Gas Chromatography-Tandem Mass Spectrometry
HIV	Human Immunodeficiency Virus
HPLC	High-Performance Liquid Chromatography
HPV	Human Papillomavirus
ICTP	Pyridinoline Cross-Linked Carboxyterminal Telopeptide Of Type I Collagen
LC-MS/MS	Liquid Chromatography Coupled To Tandem Mass Spectrometry
MN	Micronucleus
mtDNA	Mitochondrial DNA
mtDNAcn	Mitochondrial DNA Copy Number
NGS	Next-Generation Sequencing
PAF	Platelet-Activating Factor
PAH	Polycyclic Aromatic Hydrocarbon
PCR	Polymerase Chain Reaction
qFISH	Quantitative Fluorescent In Situ Hybridization
RIA	Radioimmunoassay
RNA-seq	RNA-Sequencing
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RT-qPCR	Reverse Transcription Quantitative Real-Time PCR
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SCE	Sister Chromatid Exchange
S-PMA	S-Phenylmercapturic Acid
SPME	Solid Phase Microextraction
TL	Telomere Length
TRF	Terminal Restriction Fragment
UHPLC	Ultrahigh Performance Liquid Chromatography
UV	Ultraviolet
VOC	Volatile Organic Compound

et al., 1755. More recent examples include air pollution e.g., from diesel engines and respiratory health, benzene and leukemia, asbestos and mesothelioma, and shift work and cancer.

Early studies involved clinical cases or observational studies in which a direct association was inferred between external exposure and health effects. However, as the latency between a particular exposure and health effect may be lengthy, the establishment of causal associations is often complicated and prone to confounding factors. Markers for exposure (biomonitoring), to verify the actual internal exposure, as well as predictive markers of biological effect (biological effect monitoring), preferably detectable prior to the onset of disease development, are therefore useful and warranted. With the advent of medicine and physiology, clinical chemistry, analytical chemistry, immunology, toxicology, clinical chemistry, cellular biology and molecular epidemiology, the inclusion of these markers in occupational health research has going on since the 1980s. Examples are 1-OH pyrene as a biomarker of polycyclic aromatic hydrocarbon (PAH) exposure (Jongeneelen et al., 1986), cytogenetic damage in relation to benzene exposure (including attempts to establish quantitative exposure response relationship from these (Scholten et al., 2020).

Although these developments have certainly fostered the understanding of the relationship between exposure and disease for single exposures, it became apparent that multifactorial exposures may even further complicate the link between exposures and health effects. The exposome has been coined as a promising concept for exploring the complex relationships between multiple exposures from the environment and disease outcomes, which conceptually encompasses all non-genetic risk factors experienced during a person's life (external exposome) and its relation to biological responses inside the human body (internal exposome) (Wild, 2005). Exposome research includes three domains: the internal, general external and specific external domain. The internal domain comprises metabolism, endogenous hormones, physical activity, oxidative stress etc. The general external domain includes education, financial status, psychological and mental stress among others. The specific external domain contains e.g. chemical contaminants and environmental pollutants, diet and lifestyle (Wild, 2012). This explanation of the exposome by Wild has further evolved and Miller and Jones (2014), further defined the exposome as "*The cumulative measure of environmental influences and associated biological responses throughout the lifespan, including exposures from the environment, diet, behavior, and endogenous processes*". To this definition three concepts were added: (1) the concept of cumulative biological responses, this refers to the response of the body to external factors (e.g. chemicals); (2) behavior (interactions with our surroundings) and (3) endogenous processes (Miller and Jones, 2014). The EU project EPHOR (Exposome Project for Health and Occupational Research) is embracing the exposome concept to enable health research in occupational settings and defines "the working-life exposome as all occupational and related non-occupational factors (general and socio-economic environment, lifestyle, behavior)" (Pronk et al., 2022). This also includes the internal exposome, the totality of internal exposures, in particular in relation to early health effects.

Knowledge on the internal exposome related to working life will inform, ideally at individual level, on the need for exposure reduction in relation to disease risk, and/or confirm the efficacy of personal protective equipment to reduce exposure. Unravelling the exposome is a challenge by itself, yet, shaping working life exposome research via inclusion of biomarkers of exposure and/or health effects is an additional challenge. The reason being that collection of the biomaterial samples should ideally: (I) not be invasive to the worker; (II) not impede the progress of the work; (III) yield cost-effective and actionable biomarker read outs to characterize the internal exposome. As a potential strategy to accomplish this, the collection of biomaterials via non-, or only minimally, invasive self-sampling can be considered. Examples of suitable matrices are urine, saliva, breath, sweat, oral buccal cells, and nasal swabs. In more general settings, the use of self-sampling for minimally invasive testing is already quite common. Examples to document biological effects are pregnancy tests (e.p.t. In-Home Early Pregnancy Test, since 1978) and home glucose monitoring for diabetes mellitus patients (Dextrostix, the clinical application already described in the mid-1960s (e.g., Marks and Dawson, 1965) and in use in home settings since the 1970s). Likewise, examples for detecting exposures include the personal breath test for ethanol, the possession of which was mandatory (until 2020) in France while driving. An even more recent example is the self-detection of COVID-19 antigen in nasal swabs, the latter being developed very soon after the discovery of the Corona virus and swiftly implemented in public health strategies worldwide to combat the pandemic. Therefore, it can be assumed that the principle of biological self-sampling is by now well known to the general population and therefore application of self-sampling strategies in occupational settings

seams generally feasible at least from a practical perspective. As part of the EPHOR project, the development and application of non-/minimally invasive self-sampling methodologies is piloted, as the use of self-sampling can increase the number of subjects enrolled into occupational studies. To prepare for this, we performed an extensive literature review to determine the feasibility of self-sampling to characterize the internal occupational exposome. In particular, we extracted which biological matrices are compatible with self-sampling, which relevant occupational exposures can be possibly documented, which early markers of biological effects can be included and whether the various combination of these can help to resolve exposures and/or early health effects at the individual or population level.

2. Methods

2.1. Aim

The first aim of this review was to synthesize existing information on self-sampling techniques for biological samples that can be used for evaluation of a range of biomarkers of exposure and effect, which are already used or could be used in an (occupational) exposome context. Self-sampling is defined here as the collection of one's own biological material by the individual him-/herself. Second, we aimed to evaluate and estimate the feasibility of using these self-sampling techniques in the occupational exposome context.

2.2. Search strategy and selection criteria

In order to extract a strategy for self-sampling of biological materials, a literature study was performed to identify relevant existing studies employing self-sampling and to extract relevant information in the context of biomonitoring, exposure monitoring and biological effect monitoring. The concepts and specific keywords used in building the search string were chosen based on the outcomes of interest for the EPHOR project and were further expanded to include BTEX (benzene, toluene, ethylbenzene, xylene) and cytogenetics (Pronk et al., 2022). First the concepts were defined as 'self-sampling' AND 'biomarkers of exposure'/'biomarkers of effect'/'biomarkers of exposure and effect' AND 'inclusion/exclusion criteria' (Table 1).

Controlled terms (MeSH and Emtree terms) were combined with free-text words to first build a general search string and secondly build a specific search string for each biomarker. The general search strings consist of two parts. The first part includes keywords on self-sampling, the second part comprises the inclusion and exclusion criteria. Specific search strings consist of three parts, the two fixed parts from the general search string and a variable part for each biomarker, resulting in 15 separate search strings.

Each search string consists of three parts: two fixed parts on self-sampling and inclusion/exclusion criteria, (shown in a darker shade in the table), one variable part for each biomarker (shown in white). The table only shows the number of papers resulting from the search string, for some subsections additional papers have been added.

The complete search strategy can be found in the Supplementary Table S1. PubMed contains millions of records focused on biomedical and life sciences and EMBASE even further expands on this, therefore, the search strings were run in both EMBASE (https://www.embase.com) and PubMed (https://pubmed.ncbi.nlm.nih.gov/) databases. All results were imported into EndNote between January and March 2021. After removing the duplicates, papers were imported into Excel for title and abstract screening for inclusion or exclusion. Only papers on research in humans and using self-sampling techniques, or on research in humans and using techniques that could be used for self-sampling were included. The following exclusion criteria were used: reviews, papers published between 2010 and 2021, papers not written in English, animal studies, *in vitro* studies. The search strings for metals, clinical chemistry, hormones, melatonin and cortisol resulted in an unmanageable number of papers, for these sections a narrative approach was used based on reviews and key papers selected by the authors. Since the exposome is a rapidly evolving field, we do not aim for completeness, but want to give an overview of the most relevant and recent information, therefore papers were only retrieved if they were published after 2010. Discrepancies were resolved by an independent author, not involved in the writing of a specific subsection.

2.3. Data extraction

After title and abstract screening, relevant papers were further assessed. A predesigned data extraction sheet was used to extract information from the selected papers. The following information was extracted from the papers: authors, year of publication, title, abstract, chemical, domain of research, study type, matrix collected, time of collection, if sampling amount was equal to or below 300 μL, methods used for collection, methods used for pre-processing, storage conditions, if the analyzed volume was equal to or below 300 μL, analytical method,

Table 1
Overview of search string design and retrieved papers.

Self-sampling	Biomarker		Inclusion/ exclusion criteria	Number of total hits before screening	Number of papers retained for data extraction
-Keywords on self-sampling	Biomarker of exposure	Metals	Included:	Narrative	Narrative
-Keywords on non-/minimally invasive matrixes and sampling methods	to xenobiotics	Polycyclic aromatic hydrocarbons	-Research in humans	99	25
		Cotinine	Excluded:	17	8
		BTEX	-Reviews	58	19
	Biomarkers of effect	Differential cell count, immune factors	-Papers published before 2010	732	33
		Clinical chemistry, hormones, melatonin, cortisol	-Papers not written in English	Narrative	Narrative
		Genomics	-Animal studies	218	24
		Epigenomics	-*In vitro* studies	203	41
		Telomere length		22	1
		mtDNA copy number variation		42	2
		Transcriptomics		204	13
		Oxidative damage, fluorescent oxidation products		213	49
		Proteomics		282	71
		Cytogenetics		34	18
	Biomarkers of exposure and effect	Volatile organic compounds		49	13

biomarkers measured, study limitations. Reviewers indicated if the study was a technical validation study. In case the study was not a validation study, the reference of the validated method was extracted.

2.4. Narrative synthesis

A narrative synthesis was performed for the results of the search, structured around self-sampling techniques for biological samples that can be used for quantification of occupational biomarkers of exposure and effect, for biomarkers used in the exposome context and for biomarkers to determine specifically the occupational exposome.

3. Results & discussion

In this review, we provide an overview of different self-sampling methodologies, structured according to the type of biomarker (biomarkers of exposure, biomarkers of effect and biomarkers of exposure and effect) (Fig. 1). Cotinine, metals, polycyclic aromatic hydrocarbons, benzene, toluene, ethylbenzene and xylene (BTEX) are discussed in this review as biomarkers of exposure. Differential cell count, immune factors, clinical chemistry, hormones, melatonin, cortisol, genomics, epigenomics, proteomics, transcriptomics, telomere length, mtDNA copy number variation, oxidative damage, fluorescent oxidation products and cytogenetics were included as biomarkers of effect, as these are relevant to address the occupational exposome. Volatile organic compounds (VOCs) are considered both as biomarkers of exposure and of effect. They can originate from external sources such as diet, medication and the environment or from metabolic processes within the body. All biomarkers of exposure, except VOCs, are interpretable at the individual level while some biomarkers of effect (e.g. omics) are interpretable only at the population level due to high inter-individual variability. For biomarkers of effect a subdivision is made in the results section where biomarkers interpretable at the individual levels are discussed first, followed by the ones only interpretable at the population level. The applicability of the presented self-sampling methods in an occupational exposome context will be discussed in the discussion section.

3.1. Biomarkers of exposure (interpretable at the individual level)

3.1.1. Metals

Metallic and metalloid elements can be toxic to any organ in the body. Some of them have well known toxic properties, for example neurotoxicity by lead and methylmercury, and chronic kidney damage by inorganic mercury and cadmium. Some can act as mutagenic and carcinogenic agents, endocrine disrupters, immune-regulatory agents, etc. Metals are present both in the general and the work environment and they are therefore important to biomonitor. For metal analysis a lot of well-established methods reporting the use of non-invasive matrices are in use, and therefore a tailored approach was used for this section where high quality reviews served as the basic for this section.

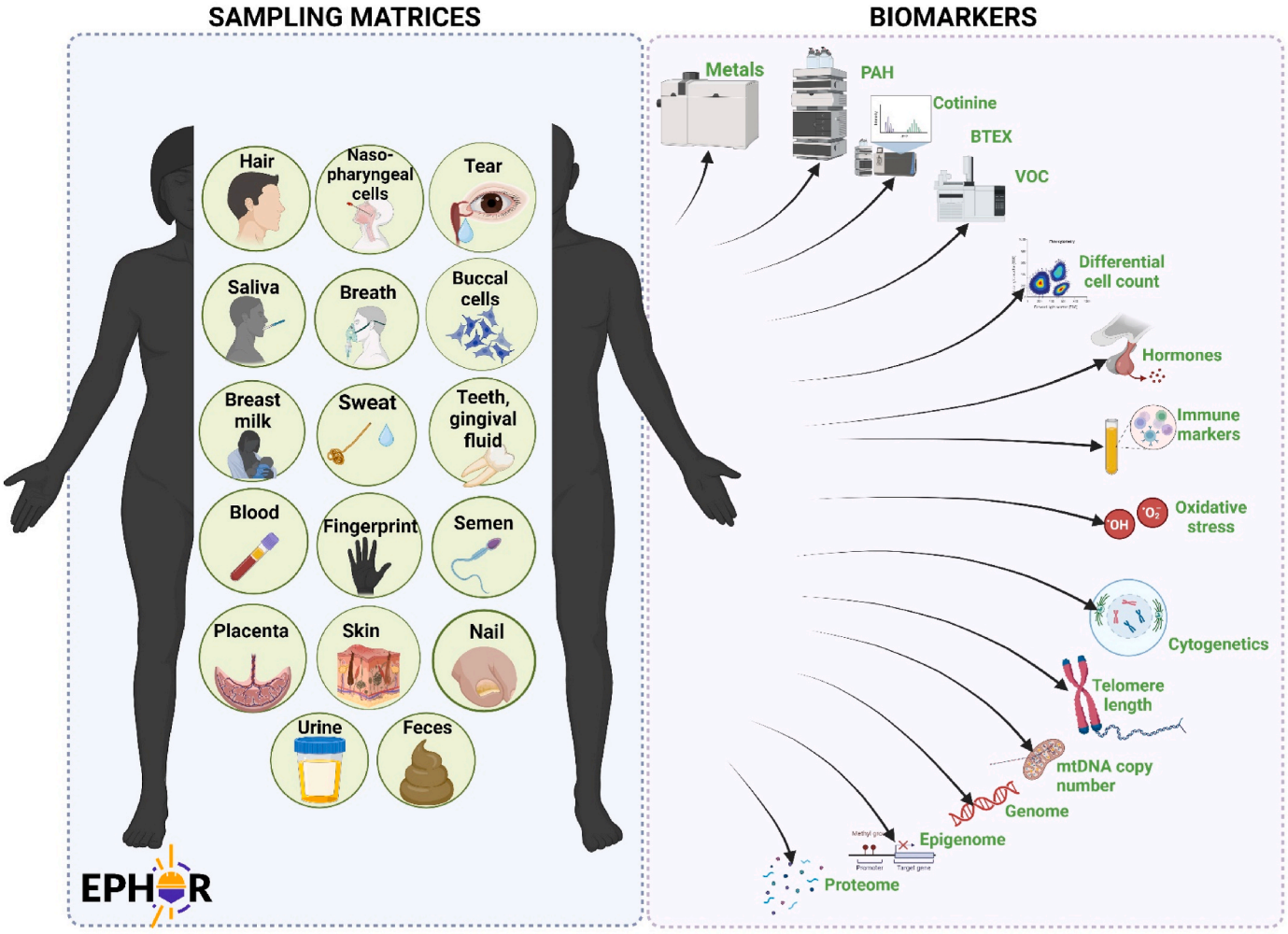

Fig. 1. Overview of different (minimally invasive) sampling matrices for characterization of internal work life exposome (biomarkers of exposure and effect) reviewed in the manuscript; "Created with BioRender.com."
PAH: polycyclic aromatic hydrocarbons; BTEX: benzene, toluene, ethylbenzene, xylene; VOC: volatile organic compound; mtDNA: mitochondrial DNA.

Among the matrices, urine is a well-established, non-invasive and preferred matrix for exposure assessment of several metals, particularly arsenic, cadmium and inorganic mercury (Nordberg, 2022). Furthermore, hair has for decades been used for measuring exposure to methylmercury and arsenic (Cernichiari et al., 1995; Skröder et al., 2017). Both urine and hair are well suited for self-sampling. Less used biological matrices such as placenta, nails, teeth, breast milk, meconium and dried blood spots (DBS) were further explored here.

Placental tissue is increasingly used as a non-invasive matrix for prenatal exposure of the child but also for evaluating how metal exposure influences the placental growth and function. The placenta is complex as it consists both of maternal and fetal tissues and different cell types, which makes it a challenge to harmonize sampling for comparability between studies (Esteban-Vasallo et al., 2012). Lead, arsenic and methylmercury easily cross the placental barrier to the child whereas cadmium accumulates in the placenta (Nordberg, 2022; Kippler et al., 2010). The most used method for measurement of metals in placenta is inductively coupled plasma-mass spectrometry after mixing of the samples in acid followed by digestion in a microwave digestion system.

A recent review of 88 original articles concluded that although toenails have been extensively used as non-invasive biomarker, the lack of standardization in sample collection, quality control, analytical techniques, along with the conflicting results among studies, render toenails not a valid biomarker of exposure to toxic metals in general (Salcedo--Bellido et al., 2021). However, toenail mercury seems to be promising as a biomarker of exposure to methylmercury, as it consistently showed positive associations with fish intake. Toenails are considered a valid matrix for measuring exposure to arsenic, with the strongest relationship being drinking water–arsenic exposure (Adair et al., 2006). Relatively less is known about the relationship between arsenic in food and in nails.

Metals can successfully be measured in teeth and the most used method is laser ablation-inductively coupled plasma-mass spectrometry (Hare et al., 2011). This method has the advantage that when measuring in deciduous teeth, a non-invasive matrix, one can assess metal concentrations during different developmental stages. The neonatal line (a histological feature formed in enamel and dentine at birth) provides a landmark that distinguishes the prenatal and postnatal compartments of teeth (Austin et al., 2013). Lead and manganese are by far the most studied metals in teeth.

Breast milk has been used to assess metal exposure for mothers and offspring, including lead, mercury, cadmium, and arsenic (Rebelo and Caldas, 2016; Solomon and Weiss, 2002). Even though metals do not bind to fat and therefore not usually accumulate to higher concentrations in breast milk, metals in breast milk are important as an additional pathway of exposure and as an indicator of likely prenatal exposures. Breast milk has been used to assess metal exposure around the world (Grandjean et al., 1994; Somogyi and Beck, 1993). In most methods, the milk is submitted to microwave acid digestion under controlled temperature and pressure and analyzed with atomic absorption spectrometry or mass spectrometry detection.

Saliva emerges as a promising non-invasive medium, also for metals. It is easy to store, requires less manipulation compared to e.g., blood sampling, and the procedure is cheaper (Augusto-Oliveira et al., 2021). Saliva has been used to assess metal exposure to e.g., mercury, cadmium, lead, silver (Davis et al., 2006; Ilea et al., 2019; Koh and Koh, 2007). Microanalytical based sensor methods are being developed, also to be used for saliva, with good agreement between urine, blood, and saliva (Yantasee et al., 2007).

A recent review evaluated fetal exposures to metals through meconium (Michelsen-Correa et al., 2021). They evaluated 32 studies, and identified results for numerous metals including arsenic, chromium, copper, iron, lithium, lead, mercury, nickel, and zinc. Because meconium represents accumulation of material over approximately six months, analysis of meconium for metals can be used to assess longer-term (second and third trimester) fetal exposures. Several analytical methods have been used to determine metal concentrations in meconium including atomic absorption and fluorescence spectrometry, mass spectrometry, and various colorimetric methods. Sample preparation methods included acid digestions, or dry-ashing (Michelsen-Correa et al., 2021).

Dried blood spots (DBS) are advantageous to use for measurement of metals compared to blood tests in venous blood. DBS require less invasive sampling and storage and transport are cheaper and easier, which makes DBS advantageous for field-based research. However, there are also some limitations. Because the blood volume in a blood spot is small (up to 75 μL), it can be difficult to obtain sufficient sensitivity for some metals. Further, the filter papers contain metals, and there is a great risk of contamination during self-sampling and preparation of samples. Nevertheless, there are several studies that developed sampling of metals via DBS and used this method in field studies (Chaudhuri et al., 2009; Funk et al., 2013; Nyanza et al., 2019).

In general, most studies using non-invasive sampling were considering environmental exposure, but quite a few studies focusing on occupational exposures are available. In a recent review on toxic metals in toenails a range of metals (e.g., nickel, cadmium, lead, welding fumes) were assessed in an occupational setting (Salcedo-Bellido et al., 2021). Placental tissue has been used to measure trace elements (e.g., manganese, zinc, lead) in order to evaluate the impact of paternal mining (Van Brusselen et al., 2020). Furthermore, placental content of cadmium was assessed in an intensive e-waste pollution site (Li et al., 2011). We could not identify studies where occupational exposures specifically were assessed in breast milk samples despite an interest in the topic 40 years ago (Wolff, 1983), but multiple studies on environmental metal exposure are available, for example (Dórea, 2021), where lead, mercury and cadmium were measured. Saliva has been used to assess heavy metals (e.g., chromium, cadmium, nickel, lead, zinc) among workers producing surgical instruments (Junaid et al., 2016), and lead levels in more industries (aluminum, plastic, detergent and pain) (Shawahna et al., 2021). Finally, DBS have been used to assess mercury in e-waste workers (Santa Rios et al., 2021a,b) and small-scale mining (Santa-Rios et al., 2021b), as well as leads levels in e-waste workers (Rodríguez-Saldaña et al., 2021).

3.1.2. Polycyclic aromatic hydrocarbons

Exposure to polycyclic aromatic hydrocarbons (PAHs) is often monitored as exposure can lead to organ damage and even cancer. The title and abstract of 99 papers were screened. After screening, 34 articles were included for full text screening. Based on the full text, 9 papers were excluded, and 25 papers were included.

Most of the studies were conducted in the domain of public health and included mainly cross-sectional studies. In 23 out of 26 papers urine was collected for polycyclic aromatic hydrocarbon analysis (Cheong et al., 2011; Díaz de León-Martínez et al., 2021; Duan et al., 2018; Gill et al., 2020; González-Martín et al., 2020; June et al., 2011; Kim et al., 2021; Klink and Schmitz, 2016; Li et al., 2021; Li et al., 2019b; Liljedahl et al., 2021; Luo et al., 2021; Murawski et al., 2020; Naccarato et al., 2018; Park et al., 2018; Puttaswamy et al., 2020; Saad et al., 2019; Tuakuila et al., 2013; Urbancova et al., 2017; Wang et al., 2017; Weiss et al., 2020; Yu et al., 2021b; Zhang et al., 2015). In the two remaining studies PAHs were measured in hair (Leung et al., 2020) or breast milk (DiScenza et al., 2018).

Urine samples were often collected in the morning, before potential exposure, and/or at predetermined timepoints after exposure to PAHs. Murawski et al. (2020), Díaz de León-Martínez et al. (2021), Yu et al. (2021b), Weiss et al. (2020), Cheong et al. (2011), and Tuakuila et al. (2013) collected spot urine in a container (polyethylene, polypropylene or polystyrene), whereas Liljedahl et al. (2021) collected urine over 48 h (Liljedahl et al., 2021). Li et al. (2021) collected spot urine in pre-treated frozen storage tubes (Li et al., 2021). Urbancova et al. (2017) collected urine from newborns, complicating standard urine collection (Urbancova et al., 2017). Therefore, they used a plastic adhesive urine bag. Samples were immediately analyzed without any pre-processing steps or

storage by Puttaswamy et al. (2020), Park et al. (2018), Duan et al. (2018), Klink and Schmitz (2016) and González-Martín et al. (2020). Others did store samples at −80 °C (June et al., 2011; Zhang et al., 2015), −70 °C (Wang et al., 2017) or −20 °C (Cheong et al., 2011; Kim et al., 2021; Li et al., 2021; Naccarato et al., 2018; Saad et al., 2019; Tuakuila et al., 2013; Urbancova et al., 2017; Yu et al., 2021b) without pre-processing. In the remaining studies, urine was aliquoted to avoid degradation of compounds after repeated freeze/thaw cycles before storage at −80 °C or −20 °C. In some studies urine needed to be transported from the collection site to the site of analysis. In that case, urine was first stored at −22 °C (Weiss et al., 2020) or at room temperature (Liljedahl et al., 2021) before being transported to the lab. After transport, Liljedahl et al. (2021) stored samples at 5 °C in the lab until aliquots were prepared and stored at −20 °C (Liljedahl et al., 2021). While urine volume is often not a limiting factor, three papers used methods requiring a low sample volume equal to or below 300 μL (Luo et al., 2021; Saad et al., 2019; Wang et al., 2017).

Methods for sample preparation include urine filtration, enzymatic hydrolysis, centrifugation, liquid-liquid extraction or (micro)solid phase (micro)extraction. In almost all studies, the samples were submitted to enzymatic hydrolysis prior to extraction and analysis. Since most PAHs are excreted as conjugates in urine, enzymatic hydrolysis is a standard step in the sample preparation process to enable quantification of total PAH concentration. Park et al. (2018), Weiss et al. (2020), Luo et al. (2021) and Díaz de León-Martínez et al. (2021) implemented an additional derivatization step to increase the volatility of hydroxy-PAHs (Díaz de León-Martínez et al., 2021; Luo et al., 2021; Park et al., 2018; Weiss et al., 2020). All but two research groups used gas or (ultra (high)/high performance) liquid chromatography to separate PAHs depending on the volatility of the compound. Klink and Schmitz (2016) used supercritical-fluid chromatography which has an advantage that it allows faster analysis and analysis of thermolabile compounds (Klink and Schmitz, 2016). However, the limited polarity of the mobile phase is a disadvantage. After separation, compounds were analyzed using a time-of-flight mass spectrometer. Gill et al. (2020) used multi-segment injection-capillary electrophoresis-tandem mass spectrometry to avoid using LC coupled to MS/MS or fluorescent detection and GC-MS, which is often accompanied by low sample throughput and complex sample preparation (Gill et al., 2020).

Leung et al. (2020) used hair as a matrix to measure PAH exposure (Leung et al., 2020). Hair samples were cut from the occipital area of the head, close to the scalp. Hair samples were first decontaminated and pulverized. After hydrolysis, PAHs were extracted, and samples were analyzed using GC-MS/MS or LC-MS/MS. While urine analysis only reflects recent exposure to PAHs, hair samples can give an insight into long-term exposure. PAHs were measured in breast milk by DiScenza et al. (2018). After collection, samples were sterilized and frozen. Breast milk samples were analyzed by use of GC-MS; however, no PAHs were detected.

Most of these methods enable quantification of a wide range of PAHs. Some research groups however only measured 1-hydroxypyrene as this is considered a general indicator of PAH exposure (Klink and Schmitz, 2016; Liljedahl et al., 2021; Tuakuila et al., 2013).

Three out of 25 papers were occupational exposure studies (Díaz de León-Martínez et al., 2021; Li et al., 2019b; Weiss et al., 2020) and none of the papers studied PAHs in an exposome context. In the future, non-invasive urine collection for PAH analysis can be easily implemented at the workplace for occupational exposure studies in an exposome context.

3.1.3. Benzene, toluene, ethylbenzene and xylene

Benzene, toluene, ethylbenzene and xylene (BTEX) exposure is often monitored because exposure can result in neurological impairment, aplastic anemia and cancer. The designed search string resulted in a total of 58 studies. Based on title and abstract screening 20 papers were included for full text screening. After screening the full text, one paper was excluded, and 19 studies were selected for data extraction.

Benzene, toluene, ethylbenzene and xylene exposure is generally measured through quantification of their metabolites in urine. Sixteen studies included measurements in urine, while only one study measured BTEX in breast milk and another in exhaled breath (Behbahani et al., 2018; Cao et al., 2021; Cattaneo et al., 2021; De Maria et al., 2020; Erb et al., 2019; Frigerio et al., 2019; Ghamari et al., 2016; Mergen et al., 2010; Orru et al., 2020; Schwedler et al., 2021; Shan et al., 2020; Singaraju et al., 2012; Tevis et al., 2021; Tranfo et al., 2018, Tranfo et al., 2010; Vidya et al., 2019; Zhao et al., 2021). Urine samples from occupationally exposed workers were often collected as spot samples after and sometimes also before their work shift. Urine spot samples from environmentally exposed humans were either collected before and after personal air sampling (Cattaneo et al., 2021) or as a convenience spot sample (Cao et al., 2021). Urine samples were generally collected in polyurethane, polystyrene or polypropylene containers, after which they were sometimes aliquoted, before storing them at −20 °C or −80 °C until further analysis. Breast milk can also be collected through self-sampling and was collected at least 30 days post-partum by manual expression or a breast-pump into a Vacutainer® to a target volume of 7.5 mL sealed with a butyl rubber stopper. The samples were stored at 4 °C until further analysis within 2 weeks of sample collection (Blount et al., 2010). The study in which the measurement in exhaled breath was described concerned the development of a skin-attachable biosensor that can be implemented as a human-interactive wearable device to measure volatile organic compounds (including toluene) that are found in exhaled breath of lung cancer patients (Jin et al., 2018).

Methods for sample preparation of the urine samples included filtration (Frigerio et al., 2019), centrifugation and/or acid treatment generally followed by (head space) solid phase (micro)extraction (Behbahani et al., 2018; Cao et al., 2021; Cattaneo et al., 2021; De Maria et al., 2020; Erb et al., 2019; Orru et al., 2020; Tranfo et al., 2018). Ghamari et al. (2016) used hollow-fiber liquid-phase microextraction. Breast milk samples were analyzed using solid-phase microextraction headspace sampling in conjunction with gas chromatography and selective ion monitoring mass spectrometry (Blount et al., 2010), while exhaled breath was analyzed using an intrinsically stretchable polyurethane electrolyte volatile organic compound sensing channel (Jin et al., 2018).

In most studies BTEX metabolites in urine were measured using high-performance liquid chromatography (HPLC) or gas chromatography (GC) coupled with mass spectrometry (MS), ultraviolet (UV) or flame ionization detector (FID) detection. In one study HPLC/MS/MS measurements of S-phenyl mercapturic acid (S-PMA) were compared to measurements of a commercial anti-S-PMA monoclonal antibodies with chemiluminescence detection assay (Tranfo et al., 2010). The HPLC/MS/MS measurements showed good correlations with airborne benzene concentrations. However, the authors concluded that the immunoassay cannot be recommended to monitor low exposure to benzene and further investigations in its use to determine urinary S-phenylmercapturic acid (S-PMA) are needed, particularly with regard to cross reactivity with the urine matrix and the correlation with airborne benzene exposure (Tranfo et al., 2010). Other studies used colorimetric determination of phenol, a metabolite of benzene (Singaraju et al., 2012), UV–Visible Spectrophotometry to determine urinary methyl hippuric acid, a metabolite of xylene (Vidya et al., 2019), or a luminescent sensor to measure hippuric acid, a metabolite of toluene (Zhao et al., 2021).

Most of the studies were experimental studies to optimize analytical methods, but in six studies urine samples from occupationally exposed workers were analyzed. None of the studies used an exposome approach in which exposure from multiple sources and/or exposures throughout different life-time phases were included. However, non-invasive, self-sampling of urine to analyze BTEX metabolites can be easily implemented within occupational exposure studies in an exposome context.

3.1.4. Cotinine

Cotinine is used as a biomarker to map the smoking behavior of individuals and exposure to tobacco smoke. The designed search string resulted in a total of 17 studies. Based on title and abstract screening nine papers were included for full text screening. After screening the full text, one poster abstract was excluded and eight studies were selected for data extraction.

Cotinine is conventionally measured in urine; however, saliva offers a good alternative for urine as it is a less complex matrix and enables non-invasive, easy collection. Urine can be collected by urinating in a container, while saliva is collected by use of a cotton swab or by spitting in a tube. While most research groups stored urine samples at −40 °C or −20 °C without any pre-processing (Hautekiet et al., 2020; Hou et al., 2012; Piller et al., 2014; Tranfo et al., 2018), some immediately analyzed the samples (Cheng et al., 2013; June et al., 2011). Whereas in these papers the density of urine was not measured, it was measured by Malafatti et al. (2010) before storage at −20 °C to measure hydration and standardize cotinine levels. Additionally, aliquots were prepared by Lee et al. (2020) before storage at −80 °C (Lee et al., 2020a; Malafatti et al., 2010). Aliquoting samples is common practice to prevent degradation of compounds due to repeated freeze/thaw cycles. While sample volume is comparatively less of a challenge for urine samples as compared to other matrices such as blood or saliva, three studies used urine volumes equal to or below 300 μL for analysis (Hautekiet et al., 2020; Hou et al., 2012; Piller et al., 2014).

Methods for sample preparation include centrifugation, sample dilution, filtration or solid-phase extraction to purify the sample for subsequent analysis with (ultra/high performance) liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), or gas chromatography coupled to a nitrogen phosphorus detector (Hautekiet et al., 2020; Hou et al., 2012; Malafatti et al., 2010; Piller et al., 2014; Tranfo et al., 2018). Others used immunoassays (Cheng et al., 2013; June et al., 2011; Lee et al., 2020a). Lee et al. (2020) developed a platform with integrated cotton swab collector, directly delivering the sample to the electrochemical immunosensor to avoid extensive sample preparation often required for liquid/gas-chromatography and to enable sensitive analysis (Lee et al., 2020a). Both LC-MS/MS and enzyme-linked immunosorbent assay (ELISA) methods are commonly used for cotinine quantification. LC-MS/MS is however the preferred method due to its higher sensitivity and specificity compared to ELISA.

Most of the studies were method development studies and none of the studies were occupational exposure studies. This can be explained by the nature of exposure, tobacco smoke itself may not be a primary occupational exposure, however, it could be included in occupational exposure studies as confounding factor. Urine and saliva can be easily collected on site and could thus also be used in an occupational context. Nevertheless, due to the non-invasive nature of sample collection, low volumes for analysis and because reliable methods for cotinine quantification are already in place, cotinine could also be studied in an exposome context.

3.2. Biomarkers of effect

3.2.1. Markers interpretable at the individual level

3.2.2.1. Immune response. Immune factors are a broad category of molecules, primarily polypeptides active in the immune system in different ways. Overall, immune factors regulate the immune recognition and immune responses in the innate and the adaptive immune system.

The designed search string resulted in 732 articles. After title/abstract screening, 49 full papers were screened, and 33 were included in the final evaluation. From the systematic search we included papers on breast milk, feces, gingival fluid, hair, saliva, skin, sweat, tears and urine. Papers describing conventional blood sampling were not included. Moreover, our search did not pick up DBS methodology, and therefore we in addition included results from two recent review papers on immune factors and DBS (Amini et al., 2021; Fischer et al., 2019).

Nine papers described the use of breast milk for assessing immune factors (Demers-Mathieu et al., 2021; Díaz-Gómez et al., 2014; O'Rourke et al., 2018; Rabe et al., 2020; Ruiz et al., 2017; Saso et al., 2019; Takahashi et al., 2019; Yu et al., 2021a; Zheng et al., 2020b). Breast milk was collected either with (electric) breast pumps or with manual expression. The volume sampled varied between 4 and 5 mL and up to at least 20 mL. In the majority of studies, it was stored at −70/-80 °C. The volume analyzed was unknown for most studies but amounts between 100 μL and 1 mL was stated for some of the papers. A variety of immune factors were analyzed including antibodies (e.g., total IgA, total IgM, IgG) and a wide variety of cytokines (e.g., IL-6, TGF-α, IFN-γ, IL-1b, IL-2, IL-4, IL-5, IL-10, IFNg, TGF-b1, TNF) using ELISA. Furthermore, leucocyte count and subtypes of macrophages using flow cytometry were described.

Twelve studies used saliva (Balmasova et al., 2019; Bartolini et al., 2018; Belstrøm et al., 2017; Ghazali et al., 2019; Khozeymeh et al., 2016; Nauwelaerts et al., 2020; Polz-Dacewicz et al., 2016; Riis et al., 2020; Tamaki et al., 2015; Wang, 2014; Ween et al., 2021; Zaura et al., 2020). Saliva was mostly collected as unstimulated (drooling) or stimulated (e.g., chewing gum) saliva fluid. One study used swabs (Bartolini et al., 2018). The stated volume sampled varied between 1 and 5 mL. In most studies, the processed material was stored at −80 °C. The volume analyzed was not stated in most studies but varied between 3 μL and 2 mL in studies with the needed information. A variety of immune factors was analyzed including chemokines (e.g. IL-8, MCP-1, 1β, MIP-1β), growth factors (e.g. G-CSF, GM-CSF, IL-7), pro-inflammatory cytokines (e.g. 1β, IL-1β, IL-6, TNFα, IL-17A), cytokines related to humoral immunity (e.g. IL-4, IL-5, IL-13), cytokines related to cell-mediated immunity (e.g. IL-2, IL-12p70, IFNγ), and immunosuppressive cytokines (e.g. IL-10). Also, antibodies were identified (IgG).

Two papers assessed immune factors in feces (Asaro et al., 2021; Zhang et al., 2020a). They assessed pro-inflammatory cytokines (IL-8, TNF-a, and MCP-1) using ELISA.

Raj et al. (2018) assessed immune factors in gingival fluid, collected using volumetric microcapillary pipettes (Raj et al., 2018). Very low amounts of material were required (3 μL). IL-35 was assessed using ELISA.

One paper assessed immune factors in hair, collected using depilation forceps (Yoshikawa et al., 2013). Four μg of RNA for microarray and 1 μg for PCR was used to assess the expression of IL-32, IL-1B, IL-8, and CXCL1.

Another paper assessed immune factors in skin, collected using ELIPatch, a thumbnail-size patch with immunospot array for multiplexed protein detection from human skin surface (Oh et al., 2018). Using 6-plex immunoassays, Oh et al. (2018) assessed TNF-α, IL-1α, and IL-17A (Oh et al., 2018). Jagannath et al. (2020) and Upasham et al. (2021) used sweat, collected with patches and stored at −80 °C (Jagannath et al., 2020; Upasham et al., 2021). Three μL and 2 mL were analyzed, respectively. CRP and IL-1beta were assessed using ELISA, while cortisol and TNF-α were assessed using Electrochemical immunoassay.

Engelbrecht et al. (2020) and Geoffrion et al. (2021) measured immune factors in tear samples. Tear samples were collected using microhematocrit capillary tubes or micropipettes and were stored at −80 °C or −150 °C. The volume analyzed was 25 μL. A wide variety of cytokines was assessed using a Milliplex® Map kit with 41-Plex Premixed Beads or a 27-plex Luminex magnetic bead-based multiplex immunoassay. Among others they assessed GF, FGF-2, Eotaxin, TGF-α, G-CSF, Flt-3L, GM-CSF, Fractalkine, IFN-α2, IFN-γ, GRO, IL-10, MCP-3, IL-12p40, MDC, IL-12p70, PDGF-AA, IL-13, PDGF-AB/BB, IL-15, sCD40L, IL-17a, IL-1ra, IL-1α, IL-9, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, RANTES, TNFα, TNFβ and VEGF.

In addition, urine have also been used (Fellström et al., 2021; Malhotra et al., 2020; Nauwelaerts et al., 2020; Nayak et al., 2021). The urine was kept at −70/-80 °C and analyzed for TIMP-2, NGF-β, TGF-β2, IL-18, KIM-1, NGAL, MCP-1, and YKL-40, among others.

DBS have been used extensively to assess specific antibodies towards a range of infections, among others HIV, Hepatitis, measles, rubella, HPV, and SARS-CoV-2 as reviewed by Amini et al. (2021) . The majority of studies used ELISA for analysis of antibodies, and few used other methods such as agglutination assay, or chemiluminescence immunoassay. The DBS cards were stored between room temperature and −80 °C. Overall, there was a good agreement between antibody concentrations in DBS and serum/plasma samples, but limited data on anti-bacterial antibodies. Protocols for analysis of other immune factors are also available, e.g., for CRP and IL-6 (Fischer et al., 2019).

Based on this review it is evident that biomarkers of immune factors can be measured in several (non-invasive) ways suitable for self-sampling. Of note, in the included papers, immune factors were not assessed as a response to occupational exposures, but as a response to e.g., probiotics, respiratory disease, chronic periodontitis, infections, and other chronic disease. For one study immune factors were assessed in saliva after e-cigarette exposure (Ween et al., 2021). However, the methods reviewed can be used to assess immune factors also as a response to occupational exposures. Until now, immune factors in occupational studies have traditionally been measured using methods not well suited for self-sampling, for example blood sampling (e.g., specific IgE in relation to occupational farming exposures ("Elholm et al., 2018); CRP in relation to occupational exposure among greenhouse workers (Madsen et al., 2021), nasal lavage (e.g., acute phase response) in relation to welding (Ali et al., 2018), and interleukin response among biofuel workers (Zheng et al., 2014).

3.2.2.2. Clinical (bio)chemistry. Clinical chemistry tests are discussed in this review as biomarkers of effect in an occupational context. It is common to measure blood glucose levels, lipids other metabolic substances, electrolytes, enzymes and proteins in a variety of epidemiologic studies. These tests allow physicians and health researchers to determine if an individual has diabetes, hyperlipidemia, as well as several other health outcomes. Due to the vast amount of literature found using the determined search string, the discussion of clinical chemistry tests is in the form of a narrative review.

Clinical chemistry tests are most commonly performed using whole blood, plasma or serum ("Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Laboratory Protocol. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and P," n.d.). Specific analyses, such as blood sugar levels may be performed in urine (Sacks et al., 2011). However, some tests can be performed on DBS, an easier blood-based method than requiring participants to be sampled in a laboratory setting. DBS techniques would benefit from further study and advancement in defining standard methodologies. However, these methods offer a much less invasive and resource intensive assessment of standard clinical chemistry analyses and have been widely applied (Affan et al., 2014).

DBS samples are collected by using a lancet to prick the participants and collecting drops of blood on filter paper. Samples are then dried and placed in sealed bags for transportation and storage (Affan et al., 2014; Gruner et al., 2015). Ideally DBS cards are stored at a temperature of −20 °C or lower as soon as possible, but storage at a temperature of −4 °C or even ambient temperature is possible for up to 14 days (Matos et al., 2020). To process, a blood spot is punched out of the filter card, transferred to a plate well and then the spot is mixed with solvent (commonly an alcohol/methanol mixture) and placed in a shaker to elute the DBS. The elution process generally takes 2 or more hours (Lakshmy et al., 2012; Matos et al., 2020), and following elution, the solution is transferred to microcentrifuge tubes and centrifuged to free the supernatant. This can then be used for the analysis of many endpoints using an enzyme-linked immunosorbent assay (ELISA) microplate reader or commercially available kits suited to the analytes of interest (Gruner et al., 2015; Lakshmy et al., 2012).

3.2.2.3. Hormones: cortisol and melatonin. Hormone tests, including melatonin and cortisol measurement are discussed in this review as biomarkers of effect in an occupational context. Hormone levels are of interest, especially in studies of circadian disruption among populations exposed to night shift work. Because of the vast amount of literature found using the determined search string for hormone tests, this section is in the form of a narrative review. Because of the diurnal variation in hormones, it is most appropriate to use a sampling technique that allows for the repeated collection of samples. As such, venous blood sampling in a laboratory setting is more burdensome to the patient and less applicable.

Commonly melatonin and cortisol are measured from urine or saliva, both non-invasive sampling methods. The use of saliva for the quantification of melatonin and cortisol is very common (Gomez-Gomez et al., 2020; Vining et al., 1983; Voultsios et al., 1997), with slight variations in methods. This method is extremely easy for the use in field studies because participants can collect their own samples and the samples are stable for several days in the refrigerator or even at room temperature prior to analysis or long-term storage. Urine is another common matrix (Benloucif et al., 2008; Levine et al., 2007). Urine samples should be stored in a refrigerator after collection and for long-term storage, should be aliquoted and stored at −80 °C. A common technique for processing of urine involves combining 5 mL urine, buffer and internal standard solutions. Several steps including hydrolysis, centrifugation, drying and evaporation are performed until finally the dry residue is reconstituted and ready for analysis (Agrawal et al., 2021; Geyer et al., 1997), which is commonly completed using radioimmunoassay (RIA) or ELISA with commercial kits available for both methods (Mirick and Davis, 2008).

In addition to cortisol measurement using saliva and urine samples, cortisol can be measured in the hair (Raul et al., 2004), providing information on the chronic status of cortisol (not subject to daily circadian rhythm variation). Cortisol can be measured reliably in hair up to 4–5 cm in length, representing 4–5 months of hormone activity, after this time there may be a leaching effect and a decline in hormone levels (Wright et al., 2015).

Furthermore, DBS-based methods for hormone analyses have emerged (Wong et al., 2004), and finally, depending on the type of study and which participants are enrolled, another matrix that may be used to analyze cortisol or melatonin levels is breast milk (Aparici-Gonzalo et al., 2020).

3.2.2. Markers interpretable at the population level

3.2.2.1. Oxidative damage. Oxidative damage as a result of oxidative stress results in disruption of cellular signaling and several diseases (e.g., Alzheimer's disease, Parkinson's disease, cancer). The designed search string resulted in a total of 213 papers after removing duplicates. Seventy-one papers were selected after abstract screening. Following full text screening, 49 papers were selected for data extraction.

The studies were conducted in different domains such as oncology, chronic diseases and metabolism, neurosciences and oral health sciences with the most usual study type being experimental studies (biomonitoring method development), followed by case-control studies, cross-sectional studies and randomized controlled trials. The matrix used by most studies was urine but there were a few studies that used saliva (Gornitsky et al., 2016; Hong et al., 2020; Tanaka et al., 2020), breast milk (Tsopmo et al., 2011), feces (Brubaker et al., 2021), gingival crevicular fluid (GCF), and exhaled breath condensate (EBC) (Hopf et al., 2019; Singh et al., 2019).

Urine was used in 44 studies, with the most common biomarkers of

oxidative damage measured being 8-hydroxy-2' -deoxyguanosine (8-OHdG) or 8-Oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) (Bonnema et al., 2015; Borrego et al., 2013; Chen et al., 2020; Fan et al., 2016; Govindasamy et al., 2019; Hopf et al., 2019; Jelinek et al., 2021; Kim et al., 2017; Matulakul et al., 2020; Misawa et al., 2020; Osredkar et al., 2019; Pelclova et al., 2019; Rocha et al., 2017; Sancilio et al., 2021; Shih et al., 2020; Singh et al., 2019; Tao et al., 2018; Utari and BudiawanAuerkari, 2020; Yagi et al., 2016; Ye et al., 2020; Yu et al., 2021b; Zhang et al., 2019), 8-OHG (Bonnema et al., 2015; Chen et al., 2020; Pelclova et al., 2020; Pelclova et al., 2019; Shih et al., 2020), 8-isoprostane (Hopf et al., 2019; Osredkar et al., 2019; Pelclova et al., 2020; Pelclova et al., 2019; Singh et al., 2019), malondialdehyde (MDA) (Hopf et al., 2019; Kim et al., 2017; Pelclova et al., 2020; Pelclova et al., 2019), alcohols (C6–C12) (Pelclova et al., 2020; Pelclova et al., 2019), xanthine (Ferrag et al., 2020; Wang et al., 2011), homocysteine (Beitollahi et al., 2020; Xiao et al., 2011), leukotrienes (LTs) (Pelclova et al., 2019; Singh et al., 2019) while other biomarkers were only measured by a single study (Adem et al., 2020; Aubron et al., 2012; Diaz-diestra et al., 2017; Du et al., 2011; Duan et al., 2020; Fan et al., 2014; Ferrag et al., 2020; Govindhan et al., 2015; Hildebrand et al., 2020; Hobo et al., 2010; Jelinek et al., 2021; Lin et al., 2013; Mergola et al., 2013; Osredkar et al., 2019; Pelclova et al., 2019; Reischl et al., 2011; Sancilio et al., 2021; Singh et al., 2019; Wong et al., 2018; Xiao et al., 2011; Yamkamon et al., 2018; You and Tseng, 2019; Zeng et al., 2017; Zhang et al., 2010). For additional information see supplementary table S2.10. Regarding the urine sample collection time, spot samples were common (Bonnema et al., 2015; Pelclova et al., 2020, Pelclova et al., 2019; Rocha et al., 2017; Yamkamon et al., 2018; Yu et al., 2021b) as well as first morning samples (Borrego et al., 2013; Chen et al., 2020; Kim et al., 2017; Sancilio et al., 2021; Shih et al., 2020; Tao et al., 2018). Storage conditions varied with samples usually being stored at −80 °C (Bonnema et al., 2015; Borrego et al., 2013; Chen et al., 2020; Osredkar et al., 2019; Pelclova et al., 2020; Rocha et al., 2017; Sancilio et al., 2021; Zhang et al., 2010) and −20 °C (Aubron et al., 2012; Ferrag et al., 2020; Jelinek et al., 2021; Kim et al., 2017; Matulakul et al., 2020; Shih et al., 2020; Singh et al., 2019; Utari and BudiawanAuerkari, 2020; Yamkamon et al., 2018; Yu et al., 2021b). Methods for pre-processing urine samples included centrifugation (Aubron et al., 2012; Bonnema et al., 2015; Chen et al., 2020; Duan et al., 2020; Govindhan et al., 2015; Kim et al., 2017; Lin et al., 2013; Matulakul et al., 2020; Osredkar et al., 2019; Tao et al., 2018; Xiao et al., 2011; Yamkamon et al., 2018; Ye et al., 2020; Zeng et al., 2017; Zhang et al., 2019), filtering (Aubron et al., 2012; Govindhan et al., 2015; Lin et al., 2013; Tao et al., 2018; Xiao et al., 2011; Ye et al., 2020) and aliquoting (Borrego et al., 2013; Duan et al., 2020; Kim et al., 2017; Osredkar et al., 2019; Sancilio et al., 2021; Singh et al., 2019). The biomarkers were measured with LC-MS/MS (Bonnema et al., 2015; Borrego et al., 2013; Chen et al., 2020; Hildebrand et al., 2020; Hopf et al., 2019; Kim et al., 2017; Pelclova et al., 2020; Pelclova et al., 2019; Reischl et al., 2011; Rocha et al., 2017; Shih et al., 2020; Singh et al., 2019; Utari and BudiawanAuerkari, 2020; Yu et al., 2021b; Zhang et al., 2010), sensors/probes (Adem et al., 2020; Beitollahi et al., 2020; Diaz-diestra et al., 2017; Du et al., 2011; Duan et al., 2020; Fan et al., 2016, (Fan et al., 2014); Ferrag et al., 2020; Govindhan et al., 2015; Hobo et al., 2010; Lin et al., 2013; Matulakul et al., 2020; Mergola et al., 2013; Tao et al., 2018; Wong et al., 2018; Ye et al., 2020; You and Tseng, 2019; Zeng et al., 2017; Zhang et al., 2019), commercial enzyme-linked immunosorbent assay (ELISA) kits (Aubron et al., 2012; Jelinek et al., 2021; Osredkar et al., 2019; Sancilio et al., 2021; Singh et al., 2019), colorimetric methods (Misawa et al., 2020; Xiao et al., 2011; Yagi et al., 2016; Yamkamon et al., 2018) and inductively coupled plasma mass spectrometry (ICP-MS) (Hildebrand et al., 2020).

Four papers used saliva. The biomarkers of oxidative damage measured in saliva were 8-OHdG (Gornitsky et al., 2016), cystatin C, myeloperoxidase (MPO), matrix metallopeptidase (MMP) −8 and −9, lactoferrin (Hong et al., 2020), IL-1β, IL-6, prostaglandin E2, resolvin D1 and resolvin D2 (Singh et al., 2019) and oxidation-reduction potential (ORP) and dissolved hydrogen concentration (DH) (Tanaka et al., 2020). The oxidative damage biomarkers were measured using ELISA kits (Gornitsky et al., 2016; Hong et al., 2020; Singh et al., 2019) and DH and ORP meters (Tanaka et al., 2020).

Two papers assessed oxidative damage biomarkers in EBC (MDA, 8-isoprostane, 8-OHdG, formate, NO2–/NO3–, fractional exhaled nitric oxide (Hopf et al., 2019) and triglycerides (Singh et al., 2019) and the methods used for their determination were HPLC-MS/MS and standardized commercial devices (Hopf et al., 2019) and a fluorometric kit (Singh et al., 2019).

In the one study that breast milk was used, it was collected using a mechanical breast pump and stored at −20 °C and oxygen radical absorbance capacity (ORAC) of peptides was measured using fluorescence (Tsopmo et al., 2011). Another study assessed a biomarker of oxidative damage (myeloperoxidase) in feces using ELISA (Brubaker et al., 2021). GCF was used in one study to assess PAF, cathepsin B and ICTP levels which were determined using ELISA kits; the methods for sample collection and pre-processing were well described (Hong et al., 2020).

Only in one study, biomarkers of oxidative damage were measured in workers (Pelclova et al., 2020); however, the sampling methods included in the rest studies can be easily used in occupational settings.

3.2.2.2. Cytogenetics. Cytogenetic alterations have been implicated as a risk factor in the development of cancer. Assays to evaluate cytogenetic biomarkers involve the determination of chromosomal aberrations (CAs), micronuclei (MN) and sister chromatid exchanges (SCE). As the micronuclei assays can be performed in almost any cell type, including easy to collect buccal mucosa cells, this assay has become more widely used to estimate the genetic risk of exposure to toxic chemicals.

The designed search string resulted in a total of 34 studies, whereof only 18 papers were included for full-text screening based on title and abstract screening. Full-text screening resulted in 18 studies to be included for data extraction.

In all studies micronucleus assays were performed using oral buccal mucosa cells, except for one study which used cervical exfoliated cells (Yildirim et al., 2019). Oral buccal mucosa cells can be easily collected by rubbing or scraping the inside of the cheeks with a brush or spatula, generally yielding samples with a volume <300 μL. Although, self-sampling was not applied in any of the studies, the self-sampling of oral buccal mucosa cells is feasible. In a clinical trial on the optimization of collection conditions of buccal cells for RNA yield, it was shown that self-collection of buccal cells for RNA was feasible with 36 of 50 (72%) samples passing quality control (Geiger et al., 2021). The timing of the sample collection was not reported in most of the studies, except for some studies investigating occupationally exposed workers where the samples were mostly collected at the end of the work shift. In most studies the collected buccal mucosa cells were not stored, but immediately smeared on slides, fixed, air dried and stained using various stains, including PAP, Giemsa solution, acridine orange, Feulgen reaction. All studies evaluated the number of micronuclei, but some also reported the number of binucleated cells or other nuclear alterations such as karyorrhexis, pyknosis, karyolysis, nuclear buds, broken eggs, or condensed chromatin. Using a combination of different biomarkers within the micronucleus assay makes it possible to not only evaluate cytogenic but also cytotoxic endpoints, illustrating that it is possible to evaluate multiple endpoints within the same assay on the same samples.

Ten studies investigated occupational exposure to different chemicals or mixtures, including pesticides (de Adad et al., 2015), electronic waste (Berame et al., 2020), calcite dust (Diler and Ergene, 2010), toxic agents among rubber industry workers (Gemitha and Sudha, 2013), silica (Halder and De, 2012), metal working fluids (Hopf et al., 2019), PAHs (Khan and Sudha, 2012), benzene (Singaraju et al., 2012), boron (Yildirim et al., 2019) or xylene (Vidya et al., 2019). Other studies investigated air pollution (González-Santiago et al., 2021), human

papilloma virus (HPV) (Pal et al., 2018; Yildirim et al., 2019), smoking and smokeless tobacco (Das et al., 2022; Pradeep et al., 2014; Zamani et al., 2011), gutkha (a betel quid substitute) (Siddique et al., 2012) and radiography for dental treatment (Agarwal et al., 2015). In most of the studies, differences in the number of micronuclei and/or other biomarkers measured in the MN assay were observed between exposed and control samples. None of the studies used an elaborated exposome approach in which exposure from multiple sources and/or exposures throughout different life-time phases were included. However, since non-invasive, self-sampling of cytogenetic assays, such as the MN assay in oral buccal cells or urothelial cells (de Oliveira Costa Júnior et al., 2018; Espinoza et al., 2019) can measure different chemicals and mixtures, this method can be applied in an exposome context.

3.2.2.3. Telomere length. Alterations in telomere length (TL) have been implicated as risk factors in several diseases, including cardiovascular disease, diabetes mellitus, obesity, and cancer (Ennour-Idrissi et al., 2017). However, few studies have investigated changes in TL using self-sampling methods. The designed search string resulted in a total of 22 studies, whereof only four papers were included for full-text screening based on title and abstract screening. Full-text screening resulted in one study to be included for data extraction. This study by Rej et al. (2021), aimed to investigate the feasibility of DBS from capillary finger prick sampling for TL analysis, and in addition performed a meta-analysis to evaluate minimally invasive sampling techniques (DBS, saliva and buccal cells) to measure TL (Rej et al., 2021). Here, the DBS cards were air-dried at RT overnight and stored at −20 °C until DNA extraction, using a commercial DNA extraction kit and TL analyzed by qPCR (Rej et al., 2021). Although other matrices were not identified using the designed search string in the present review, Rej et al. (2021) suggests that in addition to DBS, saliva is a promising proxy for venous blood (Rej et al., 2021). Furthermore, DNA from saliva has been previously used to study alterations in TL in relation to breast cancer risk in night shift workers (Samulin Erdem et al., 2017). Several methods for analysis of TL are available including qPCR, TRF (Terminal Restriction Fragment) analysis, and qFISH (quantitative Fluorescence In Situ Hybridization) methods. While DBS, saliva and also buccal samples can be suitable self-sampling matrices for TL analyses in occupational studies, restrictions on matrices and amount of DNA extracted due to self-sampling further limit the suitability of various TL analysis methods e.g., TRF analysis requires large amounts of starting DNA and qFISH methods utilize cell or tissue samples. Furthermore, the relation between TL in non-invasive samples and venous blood, which is the most utilized matrix for TL measurement, is not fully established and needs further research.

3.2.2.4. Mitochondrial DNA copy number. Mitochondria, the main energy-producing organelle in eukaryotic cells, have multiple copies of extra-nuclear circular DNA. The mitochondrial DNA copy number (mtDNAcn) increases in response to oxidative stress (Lee et al., 2000), which can be generated naturally during cellular respiration by the mitochondria itself or due to external toxic insults. Alterations in mtDNA have been implicated as risk factors in several diseases, including cardiovascular disease, kidney disease, and cancer (Castellani et al., 2020). mtDNAcn is in most studies measured in peripheral blood or in diseased tissue samples by using quantitative polymerase chain reaction (PCR) (Xing et al., 2008). However, few studies have investigated changes in mtDNAcn using self-sampling methods.

The designed search string resulted in a total of 42 studies, whereof seven papers were selected for full-text screening based on title and abstract. Full-text screening resulted in two studies (Anderson et al., 2021; Brandhagen et al., 2018) to be included for data extraction.

The study by Anderson et al. (2021) utilized venous blood dripped on filter paper that was stored at room temperature for subsequent mtDNAcn analysis by qPCR (Anderson et al., 2021). The filter paper-derived mtDNAcn was compared with mtDNA measured in venous blood stored in the EDTA tubes. The mtDNAcn was analyzed with predesigned TaqMan Copy Number Assays for human mitochondrially encoded 12S rRNA and nuclear encoded Ribonuclease P RNA component H1 sequences. The authors found that mtDNAcn values were higher, on average, when measured in the DBS than in the whole blood samples, and that measurements from the two sample types were moderately correlated. This suggest that body fluids sampled on filter paper can be used for mtDNAcn analysis.

In the study by Brandhagen et al. (2018), shaft hair samples stored for up to 50 years at 4 °C or at room temperature were used for comparison of quantity and quality of mtDNAcn and nuclear DNA by using shotgun sequencing (Brandhagen et al., 2018). The study showed that mtDNA quantity and quality decrease along the length of the hair shaft, and accordingly, any sampling of hair should be made consistent. Nuclear DNA was present in shed hair and abundant relative to mtDNA, even in the most distal fragments, showing that shaft hair can also be used for nuclear DNA analysis.

Saliva samples could also be an alternative self-sampling matrix for mtDNAcn analyses; however, the search string did not identify any studies fulfilling the criteria for the present review.

3.2.2.5. Multi-omics

3.2.2.5.1. Genomics. Genomics is studied to help unravelling diseases and find an association between genetic variants and a specific phenotype. The designed search string resulted in a total of 218 studies. Based on title and abstract screening 37 papers were selected for full text screening. After screening the full text, 24 studies were selected for data extraction.

Researchers have traditionally used blood as the source of DNA for genotyping in clinical studies, however there are several studies using alternative matrices, such as saliva, milk, urine and buccal cells. These matrices open up possibilities for self-collection and saliva and urine have the advantage that the DNA remains stable in the long-term at ambient temperature. Buccal cells have been collected by buccal swabs (Boxer and Garneau, 2015; Cuevas-Sierra et al., 2020; Goecker et al., 2020; Katsarou et al., 2018; Li et al., 2019a; Loomis et al., 2010; Loureiro Tonini et al., 2020; Northrop et al., 2010). Commercial nucleic acid isolation kits are used to extract the DNA from the swabs, such as Maxwell® 16 Buccal Swab LEV DNA Purification Kit (Cuevas-Sierra et al., 2020). Oral mucosal cells can also be extracted by brushing and stored in saline solution; this technique was used in a case-control study of patients with periodontitis using real-time PCR to detect gene variants (Cimões et al., 2014).

Two birth cohort studies collected human milk for DNA extraction and genotyping analysis by PCR (Bai et al., 2018; Boxer and Garneau, 2015). In the study by Bai et al. (2018) subjects self-collected their milk and subsequently froze the sample (−20 °C) in their kitchen freezer, before transporting it within 1 week to the laboratory on dry ice.

Several studies used Oragene® DNA kits for the collection, stabilization and transportation of DNA from saliva (Drogou et al., 2020; Goodman et al., 2020; Kidd et al., 2014; Sebastian et al., 2014). DNA from saliva collected in Oragene containers should be stable for at least 5 years at ambient temperature. The DNA extracted from all matrices was analyzed by a range of array-based, PCR and NGS methods.

Other sources of DNA for genotyping that are convenient and suitable for self-sampling are hair, nails and urine. One study described the extraction of DNA from alkaline lysates of hair root for allele specific PCR of alcoholism-related genes (Shirasu and Kuroki, 2014). Another study used self-collected nail clippings, packed in a small envelope, and sent by participants via regular postal mail to the laboratory (Truong et al., 2015). They showed that nail clippings are a feasible alternative source of DNA but there are limitations; nail DNA is unsuitable if long PCR amplicons are desired, and the genotyping success rate is lower.

An exposure study of Slovenian children to bisphenols used urine,

collected in the morning, for DNA extraction and genotyping when blood was not available (Tkalec et al., 2021). Most of the other studies were cohort studies in human genetics, and either cohort or case-control studies for different diseases and conditions. However, the sample collection methods and downstream analysis could be applied to study the occupational exposome.

3.2.2.5.2. Epigenomics. Epigenomics is used to get an insight in gene regulation and helps to find associations between epigenetic variants and a phenotype. From a total of 203 identified studies, 64 studies were selected for full-text screening based on title and abstract, from which a total of 41 papers were picked out after the full text-screening. Two papers that were not identified using the search string were added manually. Most of the studies included investigated DNA methylation changes using both targeted and untargeted approaches. Several studies also investigated the role of non-coding RNAs as an epigenetic regulator. However, in the identified studies, histone modifications were not examined.

Similar to genomics analysis, epigenetic analysis and more specifically DNA methylation studies have been traditionally performed on DNA extracted from blood (peripheral whole blood/peripheral blood mononuclear cells). Less frequently, self-sampling methods such as saliva, urine, buccal cells etc. have been used as matrices for the extraction of DNA or RNA and evaluation of epigenetic alterations. In this review, we have only included the studies that used the self-sampling approaches.

Majority of the studies used saliva as a matrix of choice while many studies used urine, buccal cells and stool samples. However, some studies used semen, vaginal swab, hair, menstrual blood, blood spot and breast milk (Hao et al., 2021; Priskorn et al., 2021; Soubry et al., 2017; Wang et al., 2022). In most studies, the storage temperature for collected samples was either −20 °C or −80 °C. For extracting genomic DNA from saliva samples, four studies (Bakulski et al., 2021; Erdem et al., 2020 Erdem et al., 2017; Goodman et al., 2020) used a commercial self-collection kit from Oragene, whereas other studies stored saliva in a collection tube and extracted DNA by standard centrifugation procedures (Lim et al., 2016; Puglia et al., 2020). Saliva samples were also used to extract RNA. Similar to DNA extraction procedures, researchers used either a commercial self-collection kit such as an Oracollect RNA swab (Hicks et al., 2018) or collection tubes (Cressatti et al., 2020; Pietro et al., 2018; Yang et al., 2020). For collecting buccal epithelial cells, commercial swab kits were used such as blackPREP Swab DNA Kit (Mundorf et al., 2021), CytoSoft cytology brush (Lovinsky-Desir et al., 2014), and OmniSwab cotton stick (Stonawski et al., 2020). After sample collection, DNA was extracted from the buccal epithelial cells, by following the standard procedures. However, DNA, extracted from buccal epithelial cells was only used for genomic DNA-based analysis, but not for RNA-based analysis. In most cases first voided urine was used for both DNA-based assays (Alsaleh et al., 2019; Engström et al., 2015; Rozalski et al., 2016; Soubry et al., 2017; Zhang et al., 2014) and RNA-based assays (Ferrero et al., 2017; Ku et al., 2021; Magayr et al., 2020; Rashid et al., 2021; Snowdon et al., 2012). Stool samples were used in five studies. Of them, three studies (Klerk et al., 2021; Mo et al., 2021; Wang et al., 2022) used genomic DNA for epigenetic analysis while two used RNA-based epigenetic analysis (Ferrero et al., 2017; Rashid et al., 2021).

The extracted RNA samples were used primarily for the analysis of miRNA expression changes (Cressatti et al., 2020; Hicks et al., 2018; Lee et al., 2020b; Magayr et al., 2020; Pietro et al., 2018; Rashid et al., 2021; Snowdon et al., 2012; Wang et al., 2022; Yang et al., 2020), using both targeted and untargeted approaches. With the targeted approaches, miR-4484, miR-3663-3p, miR-153, miR-223, miR-125b, miR-126, miR-143, miR- 200a, miR-122, miR-21 and miR-451a were found to be epigenetic regulators where the qPCR-based arrays were mainly used for the assessment. For untargeted miRNA profiling, the commercial kits used in the studies were TaqMan OpenArray, Affymetrix® GeneChip® miRNA 4.0 Arrays, nCounter Human v3 miRNA Expression Assay Kits, Illumina TruSeq Small RNA, and miScript miRNA PCR Array.

Prior to the DNA methylation analysis, extracted DNA was treated with sodium bisulfite, which is a gold standard procedure for detecting methylation changes (Li and Tollefsbol, 2011). Bisulfite-converted DNA was used for both targeted and untargeted-based assays. To study whole genome DNA methylation, Illumina Human Methylation 450K Bead Array was used by a number of studies (Bakulski et al., 2021; Goodman et al., 2020; Mundorf et al., 2021; Nissen et al., 2016; Richmond et al., 2018; Stonawski et al., 2020) except Mundorf et al. (2021) used Illumina Infinium Methylation EPIC array (Mundorf et al., 2021), covering a nearly double number of CpG sites. Bisulfite-converted DNA was also used for pyrosequencing and methylation-specific PCR assay to study DNA methylation in target regions or genes (Erdem et al., 2020, Erdem et al., 2017; Gao et al., 2018; Hao et al., 2021; Klerk et al., 2021; Lim et al., 2016; Lovinsky-Desir et al., 2014; Mo et al., 2021; Portales-Casamar et al., 2016; Puglia et al., 2020; Soubry et al., 2017; Wang et al., 2021).

Among 43 studies that used self-sampling methods for epigenomic analysis, only two studies were on occupational exposure (Erdem et al., 2020; Erdem et al., 2017). In these two occupational exposure studies, DNA, used for detecting methylation changes, was extracted from saliva. Saliva was even found to be the most popular self-sampling matrix in the included epigenomic research due to its easy access and cost-effectiveness. Therefore, saliva, which is used for both DNA- and RNA-based epigenetic assays, can be a suitable replacement for invasive sampling approaches in occupational exposure studies to detect DNA methylation changes.

3.2.2.5.3. Transcriptomics. Transcriptome profiling on human subjects can be used to better understand the cellular and molecular effects of the exposome. The designed search string resulted in a total of 204 studies. Based on title and abstract screening 37 papers were included for full text screening. After screening the full text, 13 studies were selected for data extraction. One additional relevant paper was identified and included for data extraction.

Transcriptomic analyses of biomarkers of effect are traditionally performed on peripheral blood or tissue samples, however, other matrices such as saliva, finger stick blood, urine or feces could be promising self-sampling alternatives. Saliva collected by spitting or passive drooling can either be stabilized in an RNA stabilizer (Barlow et al., 2017; Yasukochi et al., 2020) or immediately frozen on dry ice (Gandhi et al., 2020) to halt gene expression changes, inhibit destructive ribonucleases (RNases), and limit microbial overgrowth. In the study by Barlow et al. (2017), the stabilized saliva samples were kept at −20 °C for long-time storage prior to RNA extraction (Barlow et al., 2017). Devices that enable the collection of RNA swabs are an alternative for saliva collection. In the study by Hicks et al. (2018), saliva collected by an Oracollect RNA swab (DNA GenoTek) at the base of the tongue and bilaterally between the gums and buccal mucosa was used for RNA extraction (Hicks et al., 2018).

Few publications are available on finger prick blood for transcriptomic analysis purposes; however, recent studies show that the RNA extracted has sufficient quantity and quality for transcriptomic analysis e.g., RT-PCR and RNA sequencing. Sampling of finger prick blood can be performed using a capillary straw retrieving capillary blood which must be immediately mixed with RNA stabilizer (Rinchai et al., 2017; Toma et al., 2020). According to Toma et al. (2020) the ratio of human blood vs. stabilizer volume should be kept at 1:2 as loss in RNA quality and quantity will be observed if this ratio is altered. After collecting, the blood sample should be kept cold at 4 °C no longer than 48 h and kept at −20 °C or −80 °C for long-term storage.

Fecal samples are commonly used in assessment of the intestinal microbial composition; however, one recent publication used fecal samples to assess the relationship between the intestinal exfoliated cell transcriptome and the microbiome (He et al., 2020). Here, the fecal samples were immediately placed in a denaturing regent to stabilize the RNA and stored at −80 °C until RNA extraction was performed (He et al.,

2020). In addition to blood and fecal samples, nasopharyngeal swab samples have been used for transcriptomic analysis. In a recent study by Nicolas De Lamballerie et al. (2021), RNA from nasopharyngeal swabs stabilized in RNA later was used for transcriptomic analysis (Nicolas De Lamballerie et al., 2021). Although the study did not have an occupational focus, the method is a non-invasive alternative when investigating biomarkers of effect following pulmonary exposure. An additional study was identified in which total RNA has been extracted from hair follicles for whole transcriptome profiling using bead arrays (Herrera-Rivero et al., 2020).

It is also possible to perform transcriptomic analysis using RNA extracted from urine and milk. However, there is a need for additional immediate pre-processing steps such as centrifugation and filtration prior to RNA extraction (de Andrés et al., 2018; De Palma et al., 2016; Dooley et al., 2020; Mussack et al., 2019; Nommsen-Rivers et al., 2012).

For all the matrices RNA extraction was performed either by commercial kits or by a standard Trizol method, and transcriptomic data was assessed either on selected candidate genes by RT-qPCR, microarray or by NGS approaches. Although none of the included studies had an occupational exposure focus, saliva, finger prick blood, and nasopharyngeal samples can easily be collected at site and could thus also be used in an occupational context. However, urine and milk samples require immediate pre-processing in laboratory settings limiting their suitability as self-sampling matrices.

3.2.2.5.4. Proteomics. Proteomics studies proteins and their expression patterns. The titles and abstracts of 282 records were screened. One hundred eighty-two studies were excluded (including reviews, not original research studies, studies done *in vitro* or in silico, animal studies, studies in non-adult populations). In total 71 full texts were reviewed reporting sample collection of urine, saliva, breast milk, feces, skin, tears, buccal cells and fingerprints.

Urine was the most frequently used matrix that allows for non-invasive sampling and used in proteomics studies based on the review results. Feces samples were also used in the analysis of proteomics related to the gut microbiome. Breast milk samples also allow for non-invasive self-sampling and have been used by different studies especially in the study of the proteome of the sensitive post-pregnancy time window. Fingerprint analysis, skin, buccal cells and tears were also matrices that have been used in proteomics, but less frequently. Several of the retrieved studies referred to clinical settings or to description of methods or to large proteomics datasets. Different studies reported the use of hair for proteomics analysis. In hair sampling, self-sampling was not explicitly reported. Due to the difficulties of collecting one's own hair it was assumed that hair sampling was not a self-sampling process in the retrieved proteomics studies (Adeola et al., 2020; Chu et al., 2020; Goecker et al., 2020; Laatsch et al., 2014; Mohamed Nasir et al., 2020; Plott et al., 2020), although it could be argued that in different scenarios, such hair-based self-sampling might be feasible.

A total of 25 articles using urine as a non-invasive biospecimen for proteomics analyses were identified that were either collected alone (Anyanwu et al., 2018; Bakun et al., 2012; Banach et al., 2019; Brown et al., 2020; Chen et al., 2011; da Silveira et al., 2020; Dhondt et al., 2020; Husi et al., 2018; Husi et al., 2013; Klein et al., 2014; Liu et al., 2012; Sigdel et al., 2018; Wörn et al., 2021; Wu et al., 2021; Zheng et al., 2020a), or together with other matrices (e.g., blood) (Gaipov et al., 2020; Guo et al., 2019; Magagnotti et al., 2010; Navarrete et al., 2013; Oros et al., 2020; Sakai et al., 2020; Santucci et al., 2014; Yu et al., 2021c; Zhang et al., 2020b; Zhao et al., 2013). The urine collection time was not uniform among studies with some relying on spot, 24-h, first-, or second-morning urine void collection schemes. Both targeted and untargeted proteomics platforms were deployed for the majority of the selected studies. The main bioanalytical platform for the estimation of the proteomic profile was almost exclusively based on mass spectrometry that was only occasionally coupled or substituted by immunoassays, such as ELISA or fluorometric protein assays (Anyanwu et al., 2018; Dhondt et al., 2020).

Eighteen articles used saliva collected in the morning (Belstrøm et al., 2016; Cecchettini et al., 2019; Chee et al., 2016; Contini et al., 2020; Gonzalez-Begne et al., 2011; Heo et al., 2013; Jehmlich et al., 2016; Khurshid et al., 2017; Krapfenbauer et al., 2014; Meleti et al., 2020; Rabe et al., 2019, Rabe et al., 2018; Saitou et al., 2020; Serrao et al., 2020; Sivadasan et al., 2015; Srinivasan et al., 2018; Sun et al., 2014; Zhang et al., 2020a). The approach to collect salivary fluid varied with some studies focusing on unstimulated free flowing saliva (Cecchettini et al., 2019; Chee et al., 2016; Contini et al., 2020; Khurshid et al., 2017; Meleti et al., 2020; Serrao et al., 2020; Sivadasan et al., 2015; Srinivasan et al., 2018; Sun et al., 2014; Zhang et al., 2020a), while others focused on stimulated saliva collection methods (Belstrøm et al., 2016; Gonzalez-Begne et al., 2011; Heo et al., 2013; Jehmlich et al., 2016; Krapfenbauer et al., 2014; Rabe et al., 2019, Rabe et al., 2018; Saitou et al., 2020); a limited number relied on a marketed approach (Salivette) (Rabe et al., 2018), pre-coated tubes (Krapfenbauer et al., 2014) or chemicals (like citric acid) (Gonzalez-Begne et al., 2011; Heo et al., 2013). In all studies, minimal volumes were needed to perform (<300 μL) the analytical measurements of proteomics. Both targeted and untargeted proteomics platforms were deployed for the majority of the selected studies. The main bioanalytical platform for the estimation of the proteomic profile was almost exclusively based on mass spectrometry that was only occasionally coupled or substituted by nuclear magnetic resonance platform (Meleti et al., 2020), or the use of immunoassays (e.g., ELISA) (Srinivasan et al., 2018) or gel electrophoresis (Khurshid et al., 2017; Krapfenbauer et al., 2014).

In six out of eleven studies, the human milk proteome was the outcome of interest (Aslebagh et al., 2018; Hahn et al., 2020; Liao et al., 2011; Zhang et al., 2016a, Zhang et al., 2016b, Zhang et al., 2013) while more specific proteomics analysis was performed in the rest studies; N-glycoproteins (Kim and Dallas, 2021), O-glycosylated and N-glycosylated proteins (Kwan et al., 2021), peptides (Picariello et al., 2019), inflammatory proteins and cytokines (Rabe et al., 2020) and N-glycopeptides (Zhu et al., 2020). The methods of proteomics analysis included different types of LC-MS (Hahn et al., 2020; Kim and Dallas, 2021; Kwan et al., 2021; Liao et al., 2011; Picariello et al., 2019; Zhang et al., 2013) i.e. LC-MS, LC/MS-QTOF, LC-MS/MS, HPLC-Orbitrap MS/MS, LC-ESI/MS/MS, a combination of methods (Aslebagh et al., 2018; Rabe et al., 2020; Zhang et al., 2016a, Zhang et al., 2016b; Zhu et al., 2020) like Bradford assay, Coomassie and silver-stained gels, 2D-PAGE and nano LC-MS/MS, Proximity Extension Assay (PEA) and Enzyme-Linked Immunosorbent Assay (ELISA) for Cytokine Quantification, filter aided sample preparation (FASP) and dimethyl labelling combined with LC-MS/MS or with nano LC LTQ-Orbitrap XL MS, while in one article an automated platform using hydrophilic-interaction chromatography (HILIC)-based cartridges was used. The volume of milk sample collected was higher than 300 μL in six studies (Kwan et al., 2021; Liao et al., 2011; Picariello et al., 2019; Rabe et al., 2020; Zhang et al., 2016a, Zhang et al., 2016b; Zhu et al., 2020), while the rest did not report this type of information. In most studies, milk samples were provided by donors while in only two studies, the samples were from archived milk banks.

Untargeted proteomics were also conducted in fecal samples (Cantarel et al., 2011; Chin et al., 2018; Gathercole et al., 2020; Gonzalez et al., 2020; Hickl et al., 2019; Rechenberger et al., 2019; Tanca et al., 2015; Verma et al., 2019). In a clinical setting, Rechenberger et al. (2019) used fecal samples to describe the proteome of acute leukemia patients and colorectal cancer patients, respectively (Chin et al., 2018; Rechenberger et al., 2019). Tanca et al. (2015) described a proteomics dataset collected from a stool sample of a single volunteer (Tanca et al., 2015). Feces samples from humans have been used for proteomics analysis of infectious agents such as *Vibrio cholerae*, as performed in a study that also combined genomics analysis (Verma et al., 2019). LC/MS-MS was most frequently used in the untargeted proteomics analyses of fecal samples (Cantarel et al., 2011; Chin et al., 2018; Gathercole et al., 2020; Gonzalez et al., 2020; Hickl et al., 2019;

Rechenberger et al., 2019; Tanca et al., 2015; Verma et al., 2019).

Kaleja et al. (2020) provide a description of extraction methods from skin proteome in samples collected using tape strips in volunteers (Kaleja et al., 2020). Liu et al. (2020) as well as Ma et al. (2020) reported on the analysis of skin using LC-MS/MS and UHPLC methods, respectively (Liu et al., 2020; Ma et al., 2020). The study of Oro et al. (2014) reported on the use of a patch for collection of biomarkers from the surface of the skin (Orro et al., 2014). The described process is non-invasive and theoretically could be used by study participants in a self-sampling protocol.

Three studies used tears. Guntermann et al. (2019) described a proteome dataset derived from tear samples collected using the Schrimer test strips (Guntermann et al., 2019). Reflex tear collection was used in the collection of samples in the data description of the tear proteome in patients with *Aspergillus flavus* infection (Kandhavelu et al., 2016). The tear proteome was also assessed by Nättinen et al. (2019). Although not explicitly mentioned, tear collection can be considered non-invasive and can be conducted in occupational-based self-sampling protocols (Guntermann et al., 2019; Kandhavelu et al., 2016; Nättinen et al., 2019).

One study reported analysis of buccal cells which were collected after rinsing and cheek brushing (Grek et al., 2014). The study was done in a clinical environment and buccal cell collection was performed using a non-invasive approach.

Schulte et al. (2021) used touch samples for proteomic analysis, i.e., fingerprints (Schulte et al., 2021). Fingerprints were collected on glass slides and later analyzed using electrophoresis and mass spectroscopy.

None of the studies were conducted in an occupational setting, however all of the reported sampling methods in the selected studies could be applied to an occupational exposome context.

3.3. Biomarkers of exposure and/or effect

3.3.1. Volatile organic compounds

Exposure to VOCs can cause several health effects (e.g., irritation of eyes, nose, throat, lungs, nausea, organ damage, cancer, etc.), and in such cases VOCs are measured as biomarker of exposure (e.g. aromatic and aliphatic hydrocarbons as biomarker for exposure to volatile compounds in jet fuel (Tu et al., 2004)). On the other hand, VOCs can also serve as biomarker of effect as they can be formed within the body as a consequence of oxidative stress. In total, 49 papers were retained after removing duplicates. Fourteen papers were selected for full text screening after title and abstract screening. Thirteen articles on measuring volatile organic compounds (VOCs) were included in this review.

The studies were conducted in different domains such as oncology, public health and chronic diseases among others and they mainly are experimental studies. VOCs can be measured in wide range of biological matrixes. Six papers measured VOCs in urine (Aggarwal et al., 2020; Bannaga et al., 2020; Cheong et al., 2011; Erb et al., 2019; Mochalski and Unterkofler, 2016; Pinto et al., 2020), two in feces (Ahmed et al., 2020; Deianova et al., 2020), one in both urine and feces (Conte et al., 2020). Our search results suggest that breath, transcutaneous blood gas, saliva and tears are less conventional matrixes for measuring VOCs with only one paper for each of these biological matrixes (Iitani et al., 2020; Saasa et al., 2019; Vogel et al., 2020; Yao et al., 2020). First morning urine or pre- and/or post-shift samples were collected in a container, bottle, tube or glass headspace vial. In some studies, urine was first aliquoted to avoid repeated freezing and thawing samples and subsequent compound degradation (Aggarwal et al., 2020; Erb et al., 2019), however most of the research groups immediately stored the urine samples at −20 °C or −80 °C (Bannaga et al., 2020; Cheong et al., 2011; Conte et al., 2020) or directly analyzed the samples (Mochalski and Unterkofler, 2016; Pinto et al., 2020). None of the studies used small volumes below 500 μL for analysis, but Conte et al. (2020) used a small amount of 200 μg of urine (Conte et al., 2020). All but one research group used solid phase microextraction (SPME) (Bannaga et al., 2020; Conte et al., 2020) or headspace-SPME (HS-SPME) (Aggarwal et al., 2020; Erb et al., 2019; Mochalski and Unterkofler, 2016; Pinto et al., 2020). Mochalski et al. (2016) first diluted urine and incubated the headspace vials containing urine at 37 °C for 20 min while stirred at 750 rpm (Mochalski and Unterkofler, 2016). Only Cheong et al. (2011) extracted VOCs using solid-phase extraction or salting-out liquid-liquid extraction (Cheong et al., 2011). The main strategy for VOC analysis in urine is gas chromatography with mass spectrometry (GC-MS) coupled to (HS-)SPME. Other research groups used gas chromatography with a time-of-flight mass spectrometer coupled to HS-SPME (Mochalski and Unterkofler, 2016) or coupled to metal oxide sensors (Bannaga et al., 2020). Cheong et al. (2011) used high-performance liquid chromatography (HPLC) coupled to a UV detector after solid-phase extraction or salting-out liquid-liquid extraction (Cheong et al., 2011).

In addition to urine, VOCs can also be measured in feces. Deianova et al. (2020) collected the first stool production of the day from newborns' diapers and transferred it to a container (Deianova et al., 2020). In the study of Conte et al. (2020) feces were collected in the morning and transferred in a sterile tube (Conte et al., 2020). All research groups stored the samples at −20 °C or −80 °C until analysis. Only 200 μg of feces was necessary for analysis using SPME coupled to GC-MS according to the method of Conte et al. (2020), while Ahmed et al. (2020) used samples ranging from 50 to 100 mg (Ahmed et al., 2020; Conte et al., 2020). Deianova et al. (2020) used an electronic nose device (Cyranose 320®) to obtain a VOC profile (Deianova et al., 2020). Furthermore, VOCs have also been measured in saliva. In the study of Vogel et al. (2020) unstimulated whole saliva samples were collected over 24 h and stored at 4 °C until analysis (Vogel et al., 2020). VOCs were extracted by liquid-liquid extraction and analyzed by GC-MS. Iitani et al. (2020) used an alcohol dehydrogenase-immobilized mesh and 2D Mako with skin-gas cam to measure transcutaneous blood ethanol and acetaldehyde (Iitani et al., 2020). In the study of Saasa et al. (2019) breath was collected in the morning by exhaling in a Tedlar bag (Saasa et al., 2019). Breath samples were immediately analyzed with HS-SPME/GC-MS. Finally, Yao et al. (2020) collected tears with Schirmer paper (Yao et al., 2020). As little as 10 μL was sufficient for direct analysis with paper spray MS. Most of these methods enable analysis of a wide selection of VOCs and their metabolites. Conte et al. (2020) for example was able to measure up to 110 VOCs in feces by use of a modified method based on Garner et al. (2007) (Conte et al., 2020; Garner et al., 2007). The methods used by Bannaga et al. (2020) and Deianova et al. (2020) did however not allow identification of the exact compounds (Bannaga et al., 2020; Deianova et al., 2020). There are thus already quite some methods available that enable measurement of VOCs in a non-invasive way.

Only two out of thirteen papers are occupational exposure studies in which urine is collected (Cheong et al., 2011; Erb et al., 2019). Feces, saliva, tears, and breath samples could however also be easily and quickly collected at the workplace and should therefore be further explored in the future. A major advantage of the method of Yao et al. (2020) is that no sample preparation is required which makes it low-cost and less time consuming (Yao et al., 2020). Advantages of breath sampling are that breath is inexhaustible, allows whole body monitoring and often does not require training. The search did not result in any papers on the exposome. The reported collection methods could nonetheless be applied in an exposome context.

4. General remarks and synthesis

Our primary aim was to present an overview of current self-sampling methodologies to characterize the internal exposome by conducting a literature review. Our secondary aim was to evaluate the feasibility of applying this broad range of sampling methodologies in the specific context of the occupational exposome. We were able to compose such an overview of currently used self-sampling techniques (Table 2). Initially,

Table 2
Overview of self-sampled matrices most commonly used for biomarker analysis.

			Easy implementation in occupational exposome setting							Potentially applicable in occupational exposome setting						Not applicable in occupational exposome context				
			Non-invasive							Minimally invasive			Can be perceived as invasive			Non-invasive		Can be perceived as invasive		
			Urine	**Feces**	**Saliva**	**Breath**	**Sweat**	**Fingerprint**	**Skin**	**Blood**	**Nasopharyngeal cells**	**Oral buccal/ mucosal cells**	**Hair**	**Nails**	**Tears**	**Deciduous teeth**	**Placenta**	**Gingival fluid**	**Breast milk**	**Semen**
Biomarkers of exposure	**Interpretable at individual level**	**Metals**	○	○	○					○			○	○		○	○		○	
		Cotinine	●		●															
		PAHs	●										●						●	
		BTEX	●			●													●	
Biomarkers of effect	**Interpretable at individual level**	**Immune factors**	●	●	●		●		●	○			●		●			●	●	
		Clinical chemistry	○							○										
		Cortisol	○		○					○			○						○	
		Melatonin	○		○					○									○	
	Interpretable at population level	**Genomics**	●		●					○		●	○	●					●	
		Epigenomics	●	●	●					●		●	●						●	●
		Proteomics	●	●	●			●	●	○		●	○		●				●	
		Transcriptomics	●	●	●					●	●								●	
		Telomere length			●					●		●								
		mtDNAcn			○					●			●							
		Oxidative damage	●	●	●	●												●	●	
		Cytogenetics										●								
Biomarkers of exposure and/or effect	**Interpretable at population level**	**VOCs**	●	●	●	●			●						●					

●: based on search string, ○: based on narrative sections/expert opinion; mtDNAcn: mitochondrial DNA copy number

our search strings were limited to the occupational setting, however this resulted in a very limited number of studies. Therefore, the search strings were adapted and as a consequence our results include occupational as well as non-occupational settings. Only the minority of the papers included in this review were thus occupational exposure studies, suggesting that the application of these methods is relatively rare in occupational settings, and they have been mainly applied in non-occupational settings. The impact and utility of this review can thus go beyond the occupational health sciences. However, when focusing on an occupational exposome context, the methods used should preferably be minimally invasive, enabling repeated sampling in a large population and over a long time period. Therefore, we categorized each matrix according to level of invasiveness, applicability in an occupational exposome context and level of interpretation of the outcome (individual or population level). Based on the level of invasiveness and the applicability in an occupational exposome setting, we will discuss three different scenarios: (I) easy to implement in an occupational exposome setting, (II) potentially applicable in an occupational exposome setting and (III) not applicable in an occupational exposome setting. While interpreting these findings we need to be careful about the limitations of this review.

A first remark is that for some well-studied biomarkers (i.e., metals, clinical chemistry and hormones) no systematic screening of the literature was performed, but a more narrative approach was used. Second, this review only gives a summary of most commonly used techniques for specific analyses. Cotinine could for example also be measured in sweat, however this is rarely done since measurement in urine is widely accepted (Koster et al., 2014). Our aim was to identify commonly used matrixes suitable for characterization of different exposome markers and methods used for collection of those matrixes, the review does not focus on the quality of the methods used for sample analysis. Another remark here is that our search was focused on self-sampling matrices estimating biomarkers of exposure/effect in relation to (occupational) exposure. As a consequence, we did not include papers reporting on e.g. markers of diagnosis. Finally, we do acknowledge the importance of time-dependency and sensitivity of different biomarkers, toxicokinetics, to choose the best moment of sampling, how quality of samples is influenced by self-sampling and the influence of stability, storage, intra-individual variability and matrix effect. However, including an evaluation of these parameters would go beyond the scope of this review and would make this review too extensive. Nonetheless many of these parameters will be taken into account in the pilot study of the EU EPHOR project (see 4.5 Application in occupational exposome studies – an example).

4.1. Easy to implement in an occupational exposome setting

Collection of urine, feces, saliva, breath, sweat, fingerprint and skin is non-invasive. Urine enables analysis of most biomarkers (metals, cotinine, PAHs, BTEX, immune factors, clinical chemistry, cortisol, melatonin, genomics, epigenomics, proteomics, transcriptomics, oxidative damage, and VOCs) and allows easy collection, however for transcriptomics, immediate pre-processing in laboratory settings is needed which limits its suitability for this biomarker in an occupational exposome setting. As is the case for urine, feces is also easy to collect and can be used for analysis of immune factors, epigenomics, proteomics, transcriptomics, oxidative damage and VOCs. Meconium in specific was used to measure metals, but the use of this matrix is limited due to its narrow time window for collection. Furthermore, at the time of meconium collection, the subject is likely not working at that time. Saliva also covers most biomarkers and has the advantage of being easy to collect at the workplace and being cost-effective compared to traditional blood collection. Saliva can provide information on metal exposure, cotinine, immune factors, cortisol, melatonin, genomics, epigenomics, proteomics, transcriptomics, telomere length, mtDNAcn, oxidative damage and VOCs. Breath, sweat, fingerprints and skin have not been studied very often yet and our search only resulted in a limited number of papers using these matrixes. Breath was mentioned in only four studies; however, it has many advantages. Breath is inexhaustible, allows for whole body monitoring and often does not require training ("7 Reasons to Collect Breath Samples," n.d.). Breath can be used to measure BTEX exposure, oxidative damage and VOCs. Two papers mentioned the use of sweat for immune factor analysis, allowing monitoring over longer time periods with one single skin patch. Disadvantages of using sweat are the high variability between subjects and in volume of sweat produced. In addition, the possibility of environmental contamination needs to be considered. Only one paper used fingerprints for proteomics analysis. Fingerprints used for proteomics analysis showed to have robust quantity and quality regardless of the age of the sample and across subjects (Schulte et al., 2021). Skin is another matrix that can be used for analysis of immune factors, proteomics and VOCs. Skin samples can easily be collected by adhesive tape but could potentially cause skin irritation. All these matrixes and methods can thus easily be collected and implemented at the workplace by self-sampling and are non-invasive.

4.2. Potentially applicable in an occupational exposome setting

Collection of blood by use of a fingerstick, collection of nasopharyngeal cells and oral buccal cells by use of a swab include inserting an instrument through the skin or into a body opening and are thus invasive sampling methods. Nonetheless, the blood can be easily collected by one single fingerstick. In addition, people have been getting used to using nasopharyngeal and oral buccal swabs in the context of COVID-19 (antibody self-) testing. Self-sampling methods for collection of blood, nasopharyngeal and oral buccal cells can thus be described as minimally invasive sampling techniques. The strength of using fingerstick blood is that the blood can be stored for years at room temperature on blood spot cards. DBSs can be used for analysis of metals, immune factors, clinical chemistry, cortisol and melatonin, providing information on the individual level and epigenomics, transcriptomics, telomere length and mtDNAcn, providing information on the population level; the use of nasopharyngeal cells has been described for transcriptomics analysis; oral buccal cells allow studying of genomics, epigenomics, proteomics, telomere length and cytogenetics. As per definition collection of hair, nails and tears is not invasive, however this might be perceived as invasive depending on the individual, which makes it more challenging to implement these methods in a (occupational exposome) study. The main advantage of using hair and nails is that they can offer a view on an individual's long-term exposure. The use of tears on the other hand is very limited and is only relevant in case of ocular diseases. Hair is used to provide information on metal and PAH exposure, immune factors, cortisol, genomics, epigenomics, proteomics and mtDNAcn, nails can be used for metals and genomics and tears can provide information on immune factors, proteomics and VOCs.

4.3. Not applicable in an occupational exposome setting

Collection of samples from deciduous teeth and placenta (after delivery) is non-invasive and can provide information about metal exposure. These methods are not applicable in an occupational exposome context because sampling of these matrixes can only be done during narrow time windows, and they do not reflect occupational exposure. Methods for collection of gingival fluid and breast milk can be perceived as invasive and provides information on immune factors and oxidative damage (gingival fluid/breast milk), metal, PAH and BTEX exposure, cortisol, melatonin, genomics, epigenomics, proteomics and transcriptomics (breast milk). In addition, semen can be used for epigenomic analysis, however this can also be perceived as invasive and is only applicable in case of male subjects.

4.4. Application in occupational exposome studies – an example

Even though this review shows there is a lot of potential for using self-sampling methods, most of these methods have not been applied in an occupational exposome context. This review can be used as a guidance in the design of future occupational exposome studies and help to overcome common challenges associated with exposome studies (e.g., large cohorts, multiple timepoints, shift work, people living/working in remote areas, pandemics). The EU EPHOR project can serve here as an example in which self-sampling methods play an important role. In the EPHOR project, the working-life exposome and its relation to non-communicable diseases is studied (Pronk et al., 2022). This includes the assessment of internal exposures and effects through biomonitoring and omics. In EPHOR we also face previously mentioned challenges accompanying sample collection in large cohorts. Making use of the self-sampling methods listed in this review offers a solution here. To evaluate the applicability of self-sampling methods in this setting, we are conducting a pilot study. The pilot was designed to resemble the case studies that are part of EPHOR to enable optimization of the workflow and of methods. We asked participants to fill in questionnaires on e.g., socio-demographics, lifestyle and medical history and we collected several biological samples (exhaled breath condensate, followed by exhaled breath, saliva, urine, finger prick blood and the traditional blood samples) at two timepoints. These samples are used to develop, optimize, validate and compare alternative self-sampling methods with conventional methods. In addition, the general workflow was optimized during the pilot study, resulting in different standard operating procedures (SOPs) for sample collection, pre-processing and storage of biological samples, made available in the EPHOR toolbox ("Biological sampling — We Expose," n.d.). These protocols are eventually being implemented in the two EPHOR case studies in which the relation between the working life exposome and (I) respiratory health or (II) shift work is studied. In conclusion, this review served as a guide for modifying our sampling strategy and for selecting self-sampling methods to overcome challenges caused by mainly the COVID-19 pandemic.

5. Conclusion

Our aim was to create an overview of existing self-sampling methods in the literature that could be used to assess the internal exposome. We succeeded in offering an overview of self-sampling techniques for collection of biological samples in occupational as well as non-occupational settings and evaluated for each matrix the applicability in an occupational exposome context. We found that urine, saliva and oral buccal cells allow for analysis of all biomarkers included in this review and offer simple, rapid and non-/minimally invasive sampling methods. Furthermore, this review can serve as a guidance in different situations to overcome challenges of lower participation rates. Due to the COVID-19 pandemic, people are now more accustomed to self-sampling. Finally, we demonstrated the use of this review as a guide for designing occupational exposome studies by means of the EU EPHOR project. In the future, this review will thus serve as a tool in the design of occupational exposome studies using self-sampling strategies and will help to increase the participation rate.

Credit author statement

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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

Data supporting the findings formulated in this paper are available within the article or the supplementary material.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.117001.

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