

## Editorial

# The Use of High-Resolution Metabolomics in Occupational Exposure and Health Research

In this issue of the *Annals of Work Exposures and Health* [Baker et al. \(2017\)](#) report on the use of high-resolution metabolomics (HRM) to identify biological signatures of manganese (Mn) exposure. Metabolomics concerns the study of metabolomes, the complement of naturally occurring (endogenous) and exogenous (e.g. environmental, occupational pollutants), low-molecular-weight metabolites present within biological systems ([Viant et al., 2017](#)). The authors were able to identify and validate several mass spectrometry (MS) features that differentiated between exposed and unexposed workers of which several displayed an exposure-response relation with measured air concentrations of Mn. Unfortunately, the authors were not able to annotate (name or empirical structure) these mass spectrometry features limiting biological interpretability and hampering the assessment if these features are truly exposure related or driven by some unmeasured confounders. As such this study may not sound as a big step forward in occupational exposure and health research but this notion would be incorrect. As [Baker et al. \(2017\)](#) note for many occupational exposures we currently lack good biomarkers of exposure. Moreover, knowledge about how occupational exposures exert their health effects is for most occupational exposures still very limited. In this regard, Mn is a perfect example where good biomarkers of exposure and effect are lacking hampering both exposure and risk assessment. The study by [Baker et al. \(2017\)](#) demonstrates how HRM allows for the agnostic screening of such markers by measuring thousands of metabolic features.

While metabolomics has been used successfully to identify biomarkers of physiological alterations related to disease status ([Jones, 2016](#)) the application of this technique to identify exposure related biomarkers and/or their biological effects has been much more limited. One of the first applications in this regard were in the

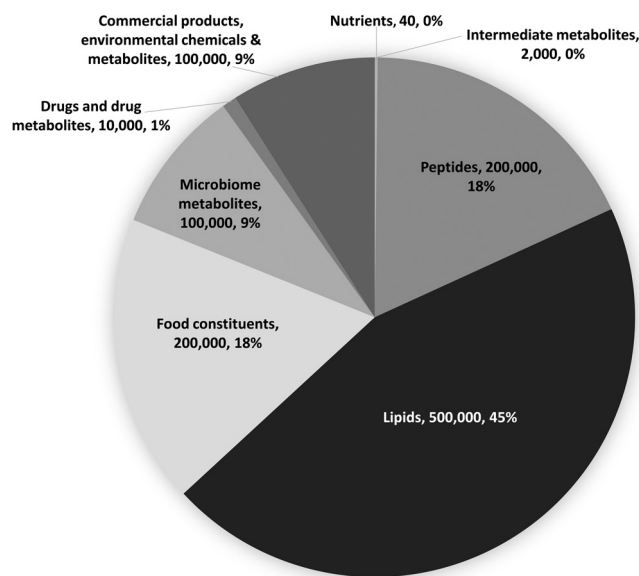
nutritional field. For example in the INTERMAP study, urinary metabolic measurements, using  $^1\text{H}$  NMR spectroscopy, showed clear differences in metabolic phenotypes between 17 populations across China, Japan, UK, and USA ([Holmes et al. 2008](#)). Among the discriminatory metabolites, urinary formate, alanine and hippurate excretion were associated with blood pressure supporting the association between dietary patterns and cardiovascular health. More recently, [Cheung et al. \(2017\)](#) reported on a metabolic study of urinary biomarkers of meat and fish intake, using HRM. A total of 249 mass spectrometry features related to meat and/or fish intake were identified. Using 18 of these features highly predictive classifiers for meat and fish intake could be developed. Among these 18 features, 8 were successfully annotated resulting in specific biomarkers for chicken (anserine), fish (trimethylamine-N-oxide), and meat (carnosine) intake. The identification of these biomarkers is potentially a game changer in nutritional research as it allows now their application in epidemiological studies on meat and fish intake and health which previously depended on self-reported information. The applications in environmental and occupational research are more limited. In 2013, [Saber Hosnijeh et al. \(2013\)](#) published on serum metabolomic perturbations among workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) using HRM. More recently, more studies on occupational exposures and circumstances have appeared. For example on the metabolic patterns among shiftworkers ([Campagna et al., 2016](#)) and military personnel ([Liu et al., 2016](#)), DDT and HCB exposed individuals ([Salihovic et al., 2016](#)), and workers exposed to trichloroethylene (TCE) ([Walker et al., 2016b](#)) and benzo(a)pyrene ([Walker et al., 2016a](#)). Similar as to the study of [Baker et al. \(2017\)](#), these studies identified several metabolic features related to the occupational circumstance or exposure of interest but

only very limited number of metabolic features could be positively annotated.

So what is governing the increasing use of HRM in occupational exposure and health research and what are the current obstacles? One of the major advances in recent years has been the coverage that mass spectrometry is providing. For example, in the late 1950s, 30 features could be approximately detected while currently over 50 000 features can be detected using HRM (Jones, 2016). This in combination with improvements in the quality of the measurements have made it possible to obtain stable metabolic measurements covering both exogenous (environmental, occupational, and lifestyle) and endogenous molecules. This allows metabolomics currently both to be used to directly measure internal dose of exogenous and endogenous compounds as well as the biological perturbations these exposures have on the biological system. The study by Walker *et al.* (2016b) is an example of this where among TCE exposed workers known and unknown metabolites of TCE were identified. These TCE metabolites were linked to metabolites and biological measures indicative of immune- and nephrotoxicity, two known adverse effects of TCE. Although, current platforms provide an extensive coverage of the metabolome they are still far off from the estimated more than 1 million chemicals constituting the human metabolome (Figure 1; Jones, 2016). However, recent innovations in mass spectrometry will likely bring the technology a step closer to global coverage of the

metabolome including two-dimensional chromatography mass spectrometry, and capillary electrophoresis (Kamphorst and Lewis, 2017). Although this increased coverage may not be directly necessary for identifying perturbations in major biological pathways (it has been argued that a coverage of 50 000 chemicals should be sufficient for precision medicine; Jones 2016) it will be necessary to delve deeper in the environmental metabolome especially concerning exogenous environmental and occupational exposures which occur at much lower concentrations than endogenous compounds (e.g. lipids, peptides), food constituents, and drugs and drug metabolites (Rappaport *et al.*, 2014).

The second advancement, but at the same time still the largest obstacle, that has been made in recent years is the improved annotation of metabolic features. Annotation is crucial as in occupational exposure and health research we are not so much interested in building prediction classifiers but are predominantly interested in identifying specific metabolites of the exposures of interest and/or in understanding the biological perturbations these compounds cause. Meaningful biological inferences can only be drawn from metabolomic datasets when MS-features can be structurally identified. The advancement in annotation has mostly been achieved in annotation of endogenous compounds (e.g. human metabolomics database [HMDB]; METLIN, and mzCloud) but is much less developed for exogenous compounds. Currently, several efforts are ongoing to



**Figure 1.** The human metabolome contains over one million chemicals. The environmental metabolome constituting out of food constituents, microbiome metabolites, drugs and drug metabolites, commercial products, environmental chemicals and metabolites is likely to include over 400 000 chemicals (categories given with estimated number of chemicals and percentage of total).

improve the annotation of these exogenous compounds by compiling metabolic databases dedicated to biomarkers of exposure to environmental risk factors (e.g. <http://exposome-explorer.iarc.fr/>). This improved annotation of exogenous compounds will be crucial for the success of HRM to identify new exposure and effect biomarkers for environmental and occupational exposures. Currently, approximately 80% of the metabolic features measured with HRM are still uncharacterized and it is likely that most of the informative markers are still lurking in the dark matter of the metabolome (Liu *et al.*, 2016).

The third advancement that has been made recently is in computation and bio-informatics which is crucial given that the technical innovations in HRM are resulting in a continual increase in the size and complexity of metabolomics datasets (Kamphorst and Lewis, 2017). For example, the xMSanalyzer package offers utilities for data extraction, detection of overlapping and unique metabolites in multiple datasets, and batch annotation of metabolites (Uppal *et al.*, 2016). Another advancement has been the use of approaches to predict biological activity directly from mass spectrometry data using pathway analysis programs such as Mummichog (Li *et al.*, 2013), MetaboAnalyst (Xia and Wishart, 2016) or MetaCore ([thomsonreuters.com/metacore/](http://thomsonreuters.com/metacore/)). Although pathway analysis are challenging and our knowledge of metabolic pathways is still evolving such approaches allow for the direct linkage of occupational exposures to biological effects, providing a more integrated interpretation of the data than would be possible based on single metabolites. As our knowledge of the metabolome increases bio-informatic tools will help to further elucidate adverse-outcome pathways related to occupational exposures aiding the hazard assessment process and potentially can function as agnostic screening tools for new exposures.

Although, it is early days in the use of HRM in occupational exposure and health research these first set of studies have given some promising indications that the use of agnostic HRM approaches may lead to the identification of novel biological signatures of occupational exposures and circumstances.

Roel Vermeulen, Institute for Risk Assessment Sciences, Utrecht University, the Netherlands, E-mail: [r.c.b.vermeulen@uu.nl](mailto:r.c.b.vermeulen@uu.nl)

## References

- Baker M, Simpson C, Lin Y *et al.* (2017) The use of metabolomics to identify biological signatures of manganese exposure. *Ann Work Exposures Health*;1.
- Campagna M, Locci E, Piras R *et al.* (2016) Metabolomic patterns associated to QTc interval in shiftworkers: an explorative analysis. *Biomarkers*; 21:607–13.
- Cheung W, Keski-Rahkonen P, Assi N *et al.* (2017) A metabolomic study of biomarkers of meat and fish intake. *Am J Clin Nutr*;25:146639.
- Holmes E, Loo RL, Stamler J *et al.* (2008) Human metabolic phenotype diversity and its association with diet and blood pressure. *Nature*;453:396–400.
- Jones DP. (2016) Sequencing the exposome: a call to action. *Toxicol Rep*;3:29–45.
- Kamphorst JJ, Lewis IA. (2017) Editorial overview: recent innovations in the metabolomics revolution. *Curr Opin Biotechnol*;23:30011–3.
- Li S, Park Y, Duraisingham S *et al.* (2013) Predicting network activity from high throughput metabolomics. *PLoS Comput Biol*;9:e1003123.
- Liu KH, Walker DI, Uppal K *et al.* (2016) High-resolution metabolomics assessment of military personnel: evaluating analytical strategies for chemical detection. *J Occup Environ Med*;58:S53–61.
- Rappaport SM, Barupal DK, Wishart D *et al.* (2014) The blood exposome and its role in discovering causes of disease. *Environ Health Perspect*;122:769–74.
- Saberri Hosnijeh F, Pechlivanis A, Keun H *et al.* (2013) Serum metabolomic perturbations among workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Environ Mol Mutagen*;54:558–565.
- Salihovic S, Ganna A, Fall T *et al.* (2016) The metabolic fingerprint of p,p'-DDE and HCB exposure in humans. *Environ Int*;88:60–6.
- Uppal K, Walker DI, Jones DP. (2016) xMSannotator: an R package for network-based annotation of high-resolution metabolomics data. *Anal Chem*;15:15.
- Viant MR, Kurland IJ, Jones MR *et al.* (2017) How close are we to complete annotation of metabolomes? *Curr Opin Chem Biol*;36:64–9.
- Walker DI, Pennell KD, Uppal K *et al.* (2016a) Pilot metabolome-wide association study of benzo(a)pyrene in serum from military personnel. *J Occup Environ Med*;58: S44–52.
- Walker DI, Uppal K, Zhang L *et al.* (2016b) High-resolution metabolomics of occupational exposure to trichloroethylene. *Int J Epidemiol*;45:1517–27.
- Xia J, Wishart DS. (2016). Using MetaboAnalyst 3.0 for comprehensive metabolomics data analysis. *Curr Protoc Bioinformatics*;55:14.10.1–14.10.91.