Policy Brief: Science based support to policy positioning of innovative chemical profiling approaches

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Executive summary

This policy brief summarises the positioning of innovative chemical profiling approaches such as suspect screening (SS), non-targeted screening (NTS) and effect-directed analysis (EDA) as a support to policy in the field of chemical risk assessment. These new approaches may contribute by several ways to the assessment of the human and environmental chemical exposome, notably in the frame of the zero-pollution ambition and the chemical industry package revision:

- In the short term, these methods can help revealing qualitative but extended real-life chemical mixtures present in environment, food, and human samples, useful to complement conventional methods typically focused on a limited number of chemicals.
- In the medium term, these methods can generate semi-quantitative data useful for prioritization based on real exposure data, trends analyses of chemical exposures, and contribution to early exposure warning system.
- In the long term, these methods can generate new data useful for novel marker discovery, new research hypotheses, as well as retrospective analyses.

Significant technical and scientific capacities already exist at this point in Europe for developing and implementing SS/NTS/EDA approaches in support of chemical risk assessment and related policies. However, they still require maintained efforts to reach the expected higher level of harmonization, consolidation and network structuration currently on-going thanks to several EU initiatives.

Background

Given the large and growing number of chemicals used in industrial processes, agriculture and consumer products, our environment has become a vector of exposure to thousands of chemicals, some of which may be harmful for ecosystems and humans. Spatial and temporal variations in chemical emissions along with different physical, chemical and biological processes make the real-life chemical exposure mixtures even more complex. This complexity is recognized in the exposome concept, describing exposures from a variety of sources and over the full lifetime. However, it also presents a major challenge to the scientific community as well as public and environmental health agencies since current approaches for exposure, hazard, and risk assessment as well as risk management still largely rely on a compound per compound basis, often addressing environmental, food and human compartments separately. This single substance approach is also facing limitations with regards to the understanding of the links between exposure to chemical mixtures and health outcomes. Analytical chemical methods for quantitative exposure assessment typically capture only a small fraction of chemicals at the same time. On the other hand, developing quantitative targeted methods for all chemical candidates of the exposome would be unrealistically time-consuming and costly. Favoured by the growing development of the exposome concept and the technological progress and innovation, new methodological approaches have emerged that enable us to expand our knowledge on environmental and human chemical exposure.

Setting the scene definitions

Suspect and non-targeted screening (SS/NTS) refer to comprehensive chemical profiling approaches that aim at identifying suspected or unknown compounds in a sample. Typically based on advanced high-resolution mass spectrometry (HRMS) instrumentation, SS/NTS use non-selective analytical protocols and a combination of analytical chemistry and bioinformatics resources and competences for compound identification. While NTS aims at detecting compounds present in given sample(s) neither *a priori* criteria nor hypothesis, SS focusses on compounds for which specific characteristics and identity are known and compiled in a suspect list that is used to evaluate the acquired data. Suspect lists are either accessible in online repositories or created in-house. In EDA, an orthogonal approach is embedded by the complementary use of effect-based methods, e.g. *in vitro* bioassays that identify a biological effect without a priori knowledge of the chemicals causing this effect. Combining SS/NTS with EDA can pinpoint the identification of chemicals to an observed effect in a bioassay that is linked to an adverse outcome *in vivo*. This approach focuses more on those chemicals that pose a hazard to the environment and humans. As illustrated in figure 1, SS/NTS presents a promising strategy to advance our knowledge on the chemical exposome, to identify new or overlooked chemicals, to create early warnings of potential new environmental and health issues, and to provide directions for more systematic monitoring efforts or new risk assessment initiatives. SS presents a useful strategy for studies on pre-defined groups of compounds, using existing or project-tailored suspect lists. While still time-consuming, SS studies can be a cost-effective compromise between NTS and targeted analyses.

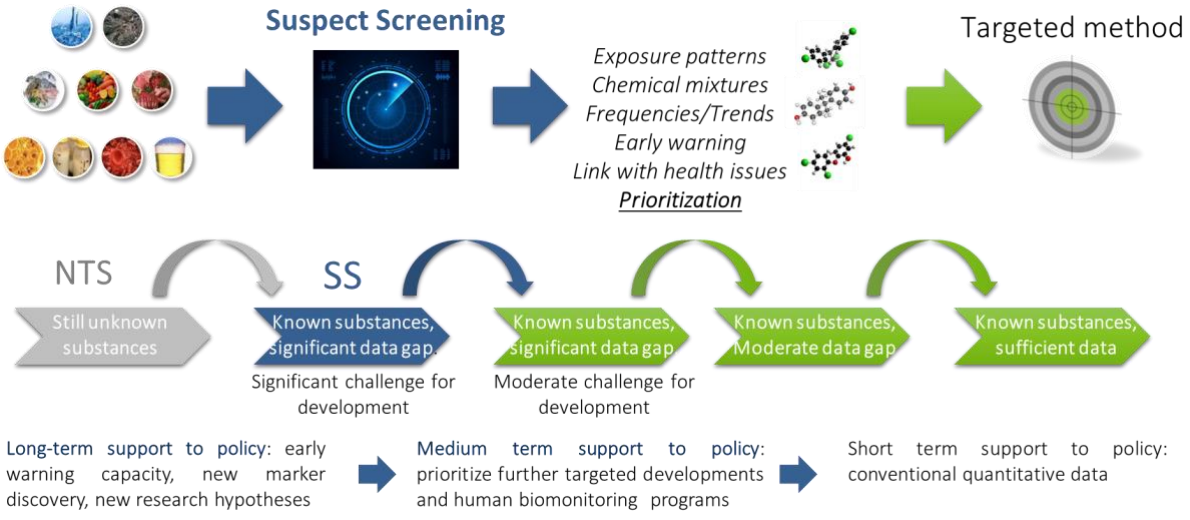

Figure 1: Global positioning of suspect and non-targeted screening methods (SS/NTS) within exposure assessment and support to policy context.

Typical output

The **first level of results** obtained from SS/NTS approaches typically consists of qualitative list(s) of chemicals identified in the samples, possibly combined with aggregated indicators such as detection frequency. These lists of identified chemicals are useful to characterise real-life exposure mixtures and chemical drivers present in the considered samples. For each of the identified features, an associated confidence level should be provided, reflecting the certainty of the correct identification. A number of tools exist for a transparent and standardised documentation of the confidence of identification of a compound, for instance the well-recognised Schymanski[1] scale, as well as the Identification Point score system[2] which is used in the food safety area. However, a better harmonized detailed confidence level scoring scale is needed to align and compare reports from different laboratories. Also needed is a harmonized simplified scale that can translate the highly technical aspect of the original scoring into a decision making tool useful for supporting policy, considering the given level of uncertainty (*e.g.* three levels covering green-orange-red).

A **second level of result** consists of semi-quantitative results relying on the abundance of the identified chemicals in the samples. This semi-quantitative use of SS/NTS typically proceeds through two possible strategies.

Strategy 1: A first option is to report the absolute HRMS signal intensity measured for each feature in each analysed sample, as this signal is known to be directly related to the quantity of the chemical. This approach, however, presents several limitations. Firstly, instrumental response factors differ for different chemicals, so no conclusions can be drawn on the quantities of different compounds. Secondly, the signals depend on instrumental parameters and performance and can vary between analytical runs, impairing the interlaboratory comparability.

Strategy 2: A second option is to report a normalized signal intensity, *e.g.* where the signal abundancy of each identified chemical is normalized to the sum of abundancies observed for the totality of the identified chemicals. In that way the uncertainty related to the semi-quantification results can be reduced to some extent and inter-sample comparisons are improved. This generates real-life exposure patterns suitable for trend analyses and inter-sample comparisons. However, this approach does still not permit to compare quantities of different chemicals due to uncharacterised instrumental response factors.

A **third** and last **level** of result consists in reporting concentration estimates, based on calibrations available from pure reference standards or structurally similar chemicals that are assumed to have comparable response factors. This approach is however limited to those compounds for which this availability of standard or analogue is effective, and usually results in higher uncertainties compared to concentration output generated from targeted methods. However, this is a field of ongoing research where more developments can be expected in the short to medium term future.

[1] https://pubs.acs.org/doi/10.1021/es5002105
[2] https://eur-lex.europa.eu/eli/reg_impl/2021/808/oj
[3] https://www.norman-network.net/
[4] https://www.hbm4eu.eu/

Current state of development and implementation

SS/NTS/EDA approaches have been applied for several years in the environmental field, with most experience from studies on water compartments. The NORMAN Network[3] has played an important role in this field through the organisation of interlaboratory studies and the establishment of database infrastructures. The same approaches are also of increasing relevance in the food safety area, including actions from the European Food Safety Authority (EFSA), although not yet routinely implemented. The Human biomonitoring for Europe (HBM4EU[4]) initiative has similarly led to a significant structuration and methodological harmonization basis with regard to SS/NTS applied to human samples. Finally, laboratories involved in the metabolomics research community also increasingly using SS/NTS chemical profiling approaches, with a focus on markers of effects rather than markers of exposure and approaching the question of unknown or suspected chemicals from a specific research question rather than a from a risk assessment and management angle.

Current scientific challenges

NTS is a highly challenging data driven approach which requires expertise not only in analytical chemistry but also in bioinformatics that is not yet widely implemented in routine laboratories. While SS approaches may be more time and cost effective than NTS analyses, they usually show lower performances compared to conventional targeted methods at this point of their development and implementation, i.e. lower sensitivity and higher uncertainties regarding both the identification of the detected chemicals and their semi-quantification. Both NTS and SS approaches have in common that the sampling and sample preparation directly affect the chemical space accessible for analysis. This is the case also for conventional approaches, but in case of SS/NTS the technical choices operated in term of sample preparation are even more challenging considering the objective of wide chemical coverage. Furthermore, generic instrumental methods might not be equally suitable for all compounds, even if various techniques are combined. SS outputs also directly depend on the comprehensiveness of reference databases used for compound identification, that differ among laboratories. Although complementary capacities positively expand the knowledge on the real-life chemical exposome and contribute to general method development, they also result in difficulties in the comparison of SS results between laboratories. Although standardisation should not be a main objective in this field (where complementarity of approaches needs to be preserved), this one still lacks established reference procedures and harmonised criteria for documenting method performances and ensuring reliability and comparability of results produced by different laboratories. Clearly defining and communicating the scope of individual methods, establishing and implementing a minimal common set of QA/QC provisions, and creating an harmonized approach for reporting results appear to be the three key challenges that need to be prioritized in the areas of environment, food, and human health. Importantly, these innovative global chemical profiling approaches also come with major challenges in terms of ethical issues and public communication, in particular with regards to their application in the environmental health and human biomonitoring fields. The implementation of SS/NTS approaches in human biomonitoring programs (and related research projects) are facing the ethical difficulty of *a priori* not knowing the chemicals that might be detected, while these studies are supposed to inform the participants before study begin about the compounds they are monitored on. From the communication perspective, once such global chemical profiling is performed and a list of detected chemicals is generated in a given human tissue, their reporting back to the individuum may appear a challenge in case of unexpected observations that may give rise to a problematic risk assessment.

Policy recommendations

- Harmonization of the main conceptual and technical aspects related to SS/NTS across the environmental, food and human biomonitoring communities in the scope of an aligned support to policy.
- Harmonization of the procedures and criteria used to assess the performance of individual SS/NTS methods as a basis for transparency and assessment of method comparability.
- Harmonization of SS/NTS results reporting, including reporting of confidence levels, through unified templates as a basis for data sharing.
- Improvement of the detectability capacity of SS/NTS (reduction of false negatives), reduced by inevitable selectivity in sampling, sample preparation, and instrumental analysis.
- Improvement of the semi-quantitative capacity of SS/NTS through methodological guidelines and systematic assessment/reporting of uncertainty.
- Improvement of the interpretability of results generated by SS/NTS approaches to support policy, with respect to current regulation, but also in a prospective manner.
- Improvement of the strength of the network of laboratories working on SS/NTS.
- Improvement of the interdisciplinary connexion between chemical profiling and bioassay communities for better integrated exposure and hazard characterization.

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Document references