1 Prioritised substance group: Emerging Chemicals

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1.1 Background Information

Emerging Chemicals should be understood as chemicals of emerging concern (CECs), which can reach human tissues via direct usage of consumer products or uptake via the environment and food. Most of them are manufactured or manmade and their toxicity or persistence are likely to significantly alter the metabolism of a living being (Sauvé and Desrosiers, 2014). Those substances are not yet included in existing HBM programs, partially due to the absence of analytical method available to determine the considered chemical or its metabolites in human specimen. In any case there is a lack of knowledge about the burden of the general population with these so-called emerging substances.

Chemicals can be considered as emerging substances when: (i) they are really '**new substances'** (e.g. recently developed substitutes for substances currently under regulation or which have been banned) or (ii) substances possibly already present for a while in the environment-food-human continuum, but '**causing a new concern'**. Such new concerns can arise due to sensitivity improvements of analytical methods, allowing the detection at low concentrations of formerly not detected substances in the environment or human. In addition, new application fields developed by the chemical industry for a known chemical can open up a new route of exposure. Alongside, recent toxicological facts including increasing presence in the environment and effects on environmental species can be an alert and can also change the perspective for human risk assessment on a given chemical. At a regulatory and policy level, the main challenge associated to CECs is to develop early warning capability to rapidly handle these chemicals through biomonitoring program and further risk assessment process. At a scientific level, the main challenge associated to the detection of CECs and relevant chemical mixtures is to develop new methodological strategies to rapidly document the reality of exposure and the related health impact for these chemicals, then to detect and prioritise these chemicals on the basis of relevant and well integrated exposure and toxicological data.

In interaction with the prioritisation process established within WP4, a complementary list of emerging chemicals candidates is being generated by WP16. This inventory is based on existing lists of emerging chemicals elaborated at international level (including ECHA, REACH, NORMAN, US EPA…) but also on bottom-up suggestions originated from WP16 partners daily involved in the characterisation of the Human chemical exposome in various contexts. Importantly, this inventory will also include metabolites of the concerned CECs mainly known at a first stage as parent compounds, with the support to biocomputing modelling. This inventory will be shared and crossed with the WP4 related activity, and further prioritisation will occur by considering available exposure, toxicological, and metabolism data as well as analytical considerations.

Besides this *a priori* inventory based approach, the development and application of suspect and non-targeted approaches will be operated within WP16 in the scope of revealing, then identifying, new (i.e. not yet known) markers of exposure related to chemicals of concern for HBM (parent compound or metabolite).

Suspect screening approaches

”Suspects” are known compounds in terms of chemical name and structure which are expected (“suspected”) to be present in a sample. The typical approach applied in this case is large-scale suspect screening aiming to generate semi-quantitative data and contribute to better prioritisation for further targeted developments. The same approaches are also helpful to elucidate the composition of complex mixtures by simultaneously generating exposure data for a wide range of markers from each individual sample. In most cases, analytical standards are not readily available and therefore, relevant analytical methods are not validated and compound identities not definitive. To some extent, suspect screening can be considered an extension of multi-class/multi-residue analysis, whereby some markers may be unambiguously identified and possibly quantified as per a targeted method, while others are mostly qualitatively measured. This qualitative annotation step refers to the assignment of a given compound identity to a signal detected by suspect or non-targeted approaches and relies on the elaboration and implementation of reference libraries to match the generated experimental data with structural descriptors (e.g., m/z, experimental or predicted Rt, MS/MS spectra) indexed from a list of a priori defined chemical compounds. Different levels of confidence for the annotation depending on the availabiliy of chemical descriptors need to be provided.

Non-targeted screening approaches

Non-targeted screening aims to detect “unknown unknowns” compounds without any a priori criteria, to identify potential new markers of exposure and toxicological concern. Generally, sample preparation and data acquisition are similar for suspect and non-targeted screening whereas data analysis/mining are different. Although highly challenging, this approach represents the most promising strategy to advance our knowledge of the human chemical exposome. In addition, it will enable better anticipation of future health threats and related risk assessment and regulatory dispositions. The development and implementation of NTS requires advanced capabilities and good integration of new front-of-science data management aspects (advanced data acquisition and processing facilities, bioinformatics and modelling tools). A solid basic knowledge of chemistry (MS, NMR, chemical synthesis) and biochemistry is essential to allow the unambiguous structural elucidation and relevant interpretation and contextualisation of compounds besides the revealed signals. NTS is then coming with new paradigm modifying the conventional hypothesis-driven research approach to a data generating hypothesis-driven approach, as a really open way to characterise biological samples.

Globally, work on emerging chemicals within the HBM4EU project aims at providing anticipation and early warning, and generating exploratory human data for guiding next orientations of HBM in terms of relevant targets. Concretely the outputs of this dedicated chemical group and associated WP16 are expected to contribute to further in terms of quantitative method development and inclusion of some exposure markers in future HBM programs. This is also referring to a reactivity process and ambition to minimise the delay before warning and real measurement at HBM scale. It is globally based on a principle of reality-driven approach, and a bottom-up characterisation of current human exposome as observed to help prioritisation of further investments and methodological effort targeted toward certain biomarkers of exposure rather than others.

Now all this proposed work in relation with emerging substances still remains a front-of-science associated to a significant level of necessary innovation and methodological research besides these clearly finalised objectives.

1.2 Categorisation of Substances

Emerging chemicals may fall in two categories.

The first one is related to a priori already identified substances. The second one is related to not yet known/identified substances. For the first category, the prioritisation process and related criteria established within WP4 will be used as a basis for dispatching the different compound candidates between Cat. C and Cat. D. In particular, main criteria considered for this categorisation will rely on (i) the investment needed in term of method development and (ii) the knowledge gap in term of exposure data. Indeed, the total number of substances finally classified into Cat. C after application of the systematic process developed within WP4 is expected to be very high. One part of these substances will be handled in WP9 with regard to the development and/or adaptation of appropriate quantitative methods. But realistically this will not be the case for the whole set of compound candidates. For some of these substances (constituting the Cat. D group), the development and application of a semi-quantitative suspect screening approach is then proposed in WP16, with the objective to generate a first level of data enabling to document the reality of human exposure and better justify further investment in a full quantitative and validated method development.

For the second category (constituting the Cat. E group), non-targeted screening approaches coupled to identification of unknowns capabilities and competences will be developed and applied in order to reveal, and further identify, new (i.e. not yet known) markers of exposure related to chemicals of concern for HBM (parent compound or metabolite). From a methodological point of view, this main component of the WP16 work plan will be based on the last generation of mass spectrometric technologies, that offer a unique and never achieved perspective for such global and untargeted sample characterisation. High resolution mass spectrometry, already in place in several labs in EU, will be the main support of these investigations, coupled to hyphenated competences in terms of data processing and analysis for extracting the relevant information from the generated global chemical profiles.

Table 1-1: Substances included in the substance group, listed according to availability of toxicology and human biomarker data

Cat.	Abbrev./ Acronym	Systematic name	Regulation
A	-	-	-
B	-	-	-
C	-	-	-
D	*a priori* already identified compounds but not yet measured in humans to be measured by suspect target screening	To be defined as a result of the first year prioritisation process	-
E	substances measured by non-target screening and (1) described in chemical databases or (2) not yet described (unknowns)	-	-

1.3 Objectives / Policy-related questions

1. Providing early warning of presence of unknown and emerging concern chemicals in EU population

2. Inform REACH process to identify substances of very high concern
3. Inform development of strategy for a non-toxic environment (7th Environment Action Programme)

1.4 Research activities to be undertaken

Table 12-2: Listing of research activities to be carried out to answer the policy questions

Substance	Policy question	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
D	Early warning of presence in EU population	Different inventories of emerging chemicals exist internationally in the field of environment, food safety, registration of chemicals for the REACH process, occupational exposures	Inventarise existing lists or databases related to emerging chemicals at international level to get a good overview (WP16 Y1) Check whether it is analytically feasible to monitor substances on these lists in human samples. ➢ Develop prioritisation tool for analysis of these chemicals based on kinetics and toxicological properties, production volume and policy/societal concerns (WP4). ➢ Improve suspect and non-targeted screening methods to allow detection of emerging chemicals (WP16) including effect directed screening assays (WP14), improve and apply these methods for different human matrices (urine, blood, placenta, maternal milk, adipose tissue, meconium…) including sample preparation, information extraction, data processing and provide guidelines for method validationperformance assessment and QA/QC consolidation. ➢ Select biobanked samples for screening. ➢ Screen human matrices for the presence of emerging chemicals. Collate existing data on mammalian metabolism/distribution/excretion of the selected Cat. D emerging chemicals. If not available: predict potential metabolites using computer models/software and existing data as input for the screening above (WP12).
	Inform REACH process to identify substances of potential concern	REACH uses IT screening tools to get information of potential concern	Provide information on biological half-life in human matrices and if possible also linkage to effect and health outcomes (WP12).

Substance	Policy question	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
	Development of strategy for a non-toxic environment -> first step		➢ Develop an indicator to monitor in humans the bioaccumulation of the above identified chemicals of potential concern. ➢ Develop an indicator to monitor in humans the decrease of total chemical load of environmental chemicals (WP5).
			WP16
E			➢ Improve non-targeted screening methods to detect not yet identified emerging chemicals in human matrices including sample preparation, information extraction, data processing and provide guidelines for method performance assessment and QA/QC consolidation.. ➢ Select biobanked samples for first screening steps. ➢ Screen human matrices (urine, blood, placenta, maternal milk, adipose tissue, meconium…) for the presence of unknowns. ➢ Generate databases for identification of the unknowns in human samples, based on mass spectral information

1.5 References

1. Sauvé, S and Desrosiers, M (2014): A review of what is an emerging contaminant. Chemistry Central Journal 8:15.
2. Norman network: http://www.norman-network.net/?q=node/81