HBM4EU science and policy for a healthy future

3rd HBM-PT

Workshop on Human BioMonitoring in Portugal

18 November 2020
Online edition

Book of abstracts

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HBM4EU science and policy for a healthy future

Activities of the Portuguese National Hub - Past, present and future

Rita Cavaleiro | The National Hub Contact Point for Portugal (FCT)

For the 3rd consecutive year, the Portuguese National Hub on Human Biomonitoring* is pleased to welcome you to the Workshop on Human Biomonitoring in Portugal (HBM-PT), this year as a fully online event, given the COVID-19 pandemic circumstances.

The Portuguese National Hub on Human Biomonitoring was created in 2017 as a result of the Portuguese participation in the European Human Biomonitoring Initiative (HBM4EU), a European Joint Programme that aims to provide better evidence of the actual exposure of European citizens to chemicals and to strengthen the link with policy makers, so that measures can be designed to reduce exposure. The work carried out by the National Hub in each of the 30 participating countries is fundamental to harmonize methodologies, so that comparable data on human exposure to chemicals are generated.

Thus, from the beginning, the Portuguese National Hub responded to ~70 requests from the HBM4EU Work Package Leaders, facilitating the collection of relevant national data on human exposure to chemicals and contributing to the results from the HBM4EU project itself, including the participation in collaborative aligned national studies and occupational studies on the exposure to several priority substances.

In addition, the National Hub has been making efforts at the level of dissemination, not only through the collaboration in the dissemination activities of the HBM4EU project, but also through the organization of national events, including the three editions of the Workshop HBM-PT and one focus group with citizens.

Strengthening the links between science, regulatory and policy actions to improve human health is of major relevance. The political commitment of Portugal with regard to national participation in the future Partnership for the Assessment of Risk from Chemicals (PARC), under Horizon Europe, is of paramount importance to pave the way for the creation and development of a sustainable national platform on Human Biomonitoring, where the influence of relevant national research institutions, industry, citizens, regulators and policy makers can be ensured.

*The Portuguese National Hub for Human Biomonitoring is currently composed of the national institutions participating in the HBM4EU project: Fundação para a Ciência e Tecnologia, I.P. (FCT), Instituto Nacional de Saúde Dr. Ricardo Jorge, I.P. (INSA), Direção-Geral da Saúde (DGS) and Agência Portuguesa do Ambiente, I.P. (APA), Faculdade de Medicina, Universidade de Lisboa (FMUL) and Escola Superior de Tecnologia da Saúde de Lisboa (ESTeSL), Instituto Politécnico de Lisboa.

Rita Cavaleiro is a science officer at the Foundation for Science and Technology (FCT) since 2012, currently working at the Department for International Relations, where she represents FCT in several transnational cooperation scientific networks. Within the HBM4EU project, she is the National Hub Contact Point for Portugal. Prior to that, she was a biomedical researcher in the area of immunology of HIV infection at the Institute of Molecular Medicine and she was awarded with the 2008 Pfizer Award in Clinical Research, attributed by the Society for Medical Sciences of Lisbon. She was a Secondary level teacher and University professor, a product support specialist and scientific advisor at the company Citomed and an R&D and Innovation Junior Manager at Eurotrials Scientific Consultants. Rita holds a PhD in Biomedical Sciences and a MSc in Human Molecular Biology from the University of Lisbon and a Licenciatura degree in Applied Chemistry from the NOVA University of Lisbon.

Opening session

09:00 - 09:05 **Welcome**
João Lavinha, Ambassador of the Portuguese National Hub for Human Biomonitoring

09:05 - 09:20 **Activities of the Portuguese National Hub - Past, Present and Future**
Rita Cavaleiro, National Hub Contact Point (Fundação para a Ciência e a Tecnologia)

Session 1 | How HBM supports risk assessment and policy action
Chair: Susana Viegas (ENSP-NOVA), Ricardo Assunção (INSA), Carina Ladeira (ESTeSL)

09:20 - 09:50 **Communicating science for impact**
Catherine Ganzleben (European Environment Agency)

09:50 - 10:20 **Anchoring HBM4EU data to chemicals risk assessment**
Maria João Silva (Instituto Nacional de Saúde Doutor Ricardo Jorge)

10:20 - 10:30 *Coffee break*

10:30 - 11:00 **How human biomonitoring has facilitated chemical restrictions - the risk assessment perspective**
Mark Blainey (European Chemicals Agency)

11:00 - 11:30 **Human biomonitoring in Food Safety and Nutrition: (im)possibilities and challenges**
Hans Verhagen (Ulster University | Food Safety & Nutrition Consultancy)

11:30 - 11:40 *Coffee break*

Session 2 | Free communications
Chair: Sónia Namorado (INSA), Paula Alvito (INSA), Isabel Moura (APA)

11:40 - 11:55 **Risk assessment of combined occupational exposure to hexavalent chromium, nickel, and PAHs: a literature-based approach**
A. M. Tavares, S. Viegas, H. Louro, and M. J. Silva

11:55 - 12:10 **How Human Biomonitoring data increases knowledge base to support Circular Economy – a case study in the E-Waste industry aiming to assess occupational exposure to chemicals**
S. Viegas, C. Ladeira, E. Ribeiro, H. Louro, and M. J. Silva

12:10 - 12:25 **Biomonitoring of occupational exposure to a known carcinogen: formaldehyde**
S. Costa, C. Costa, J. Madureira, V. Valdiglesias, A. Teixeira-Gomes, B. Laffon, and J. P. Teixeira

12:25 - 12:40 **Urinary neonicotinoids profiles in adults from Aveiro District, NW Portugal**
A. C. Sousa, Y. Ikenaka, T. Ichise, S. M. M. Nakayama, S. Souto-Miranda, A. Marques,

12:40 - 12:55 **Biomonitoring of Portuguese nursing mothers: levels of biomarkers of exposure to polycyclic aromatic hydrocarbons and infant´s health risks**
M. Oliveira, S. Duarte, C. Delerue-Matos, A. Pena, and S. Morais

12:55 - 14:00 *Lunch break*

Session 3 | Can HBM provide relevant information for tackling actual and new challenges in Public Health?
Chair: Teresa Borges (DGS), Henriqueta Louro (INSA), Ana Tavares (INSA)

14:00 - 14:30 **Human biomonitoring (HBM) - a tool to support the health argument in addressing major environmental challenges**

Dorota Jarosińska (WHO European Centre for Environment and Health)

14:30 - 15:00 **"It's those things that the plastics we use in our daily life have...": Portuguese citizens' attitudes and beliefs on chemical exposure and human biomonitoring**

Ricardo R. Santos (Instituto de Saúde Ambiental, Faculdade de Medicina da Universidade de Lisboa)

15:00 - 15:30 **Perfluoralkyl substances (PFAS): an example for priority substances under HBM4EU**

Maria Uhl (Environment Agency Austria)

15:30 - 15:40 *Coffee break*

Session 4 | Free communications
Chair: Carlos Dias (INSA), Edna Ribeiro (ESTeSL), Joana Costa (FMUL)

15:40 - 15:55 **The genotoxicity of an organic solvent mixture: a human biomonitoring study and translation of a real-scenario exposure to *in vitro***

C. Ladeira, G. Gajski, M. Meneses, M. Gerić, and S. Viegas

15:55 - 16:10 **Biomonitoring of metals and minerals in urine in a population of Portuguese school-aged children: deriving reference values**

I. Rebelo, V. C. Fernandes, E. Keating, J. C. Leite, D. Teixeira, M. L. Maia, S. Norberto, E. Pinto, A. Moreira-Rosário, D. Sintra, B. Moreira, A. Costa, S. Silva, V. Costa, I. Martins, F. C. Mendes, P. Queirós, A. Guerra, M. Fontoura, I. P. Carvalho, R. M. Lima, C. Martins, C. Delerue-Matos, A. Almeida, L. Azevedo, C. Calhau, and **D. Pestana**

16:10 - 16:25 **Biomarker-validated maternal smoking and environmental tobacco smoke exposure status and its associations with perinatal outcomes**

A. I. Silva, A. Camelo, J. Madureira, A. T. Reis, F. Barbosa Jr, J. P. Teixeira, and C. Costa

16:25 - 16:40 **Human trials addressing environmental toxicant exposures: scoping review of immunological and endocrine markers and protective strategies**

D. Marques-da-Silva, P. Videira, and R. Lagoa

16:40 - 16:55 **Feasibility of a silicone membrane for mimicking skin absorption of environmental toxicants: experimental studies with polycyclic aromatic hydrocarbons**

J. Silva, D. Marques-da-Silva, and **R. Lagoa**

16:55 - 17:10 **Concluding remarks and closure**

João Lavinha, Ambassador of the Portuguese National Hub for Human Biomonitoring
Maria João Silva, Instituto Nacional de Saúde Doutor Ricardo Jorge

Table of contents

Invited communications

HBM4EU science and policy for a healthy future

Communicating science for impact

Catherine Ganzleben | European Environment Agency (EEA)

The effective communication, dissemination and exploitation of human biomonitoring results are fundamental to ensuring societal impact. Impact can be achieved by feeding evidence into risk assessment and policy making, as well as through efforts to increase public awareness and promote responsible lifestyle changes amongst citizens. As such, communication strategy should start by identifying key audiences in the risk assessment and the policy making community, the scientific and stakeholder communities, and the general public. Understanding the knowledge needs of end users is critical to ensuring that knowledge outputs match user needs through ongoing dialogue and engagement with users. To achieve impacts, results must actively disseminated through engagement with relevant networks, including with EU and national policy makers as well as stakeholders, who can amplify messages through their own networks. Targeting users with tailored communication products that specifically address their concerns at an appropriate level of technical detail will enable their exploitation of results.

The HBM4EU project, the European Human Biomonitoring Initiative, is bridging science and policy, exploring current questions to deliver answers that help policy makers protect human health. Our evidence will support policy makers at different stages of the policy cycle, including inception and design, implementation (including risk assessment) and evaluation. For HBM4EU results to be used by policy makers, they must answer specific policy-relevant questions, clearly communicate science, and be delivered at the right moment in the regulatory cycle. To meet these criteria, HBM4EU partners are actively identifying openings in regulatory processes on chemicals where the project might feed in evidence, in collaboration with the EU institutions. Under the European Green Deal, there is currently a focus on both chemicals legislation and policies that aim to reduce releases of chemicals from consumer products and industrial processes, as well as from the agriculture, energy and transport sectors. This implies that there are opportunities for the human biomonitoring community to demonstrate how people's bodies are contaminated with chemicals, how this impacts on health and how efforts to reduce upstream emissions can benefit health in Europe.

As Head of Group on air pollution, environment and health at the European Environment Agency (EEA), Catherine Ganzleben is responsible for strands of work on a range of environmental risks to health, including air pollution, noise and chemicals, as well as how ecosystems support human well-being. She has been working at EEA on environmental health since 2014. Prior to that, Catherine was a Senior Policy Advisor at Milieu Ltd. for seven years, delivering support to public sector institutions on environment and health issues. From 2005-6, Catherine worked at the United Nations Institute for Training and Research, delivering technical assistance in support of multilateral environmental agreements on chemicals. Catherine has a post doctorate from the United Nations Institute of Advanced Studies in Tokyo and a doctorate from the University of Oxford.

Anchoring HBM4EU data to chemicals risk assessment

Maria João Silva | Department of Human Genetics, Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), and Centre for Toxicogenomics and Human Health (ToxOmics), NOVA Medical School, Universidade NOVA de Lisboa, Portugal.

A major goal of the European Human Biomonitoring Initiative, HBM4EU, is to coordinate and advance human biomonitoring across Europe and reinforce its application in different regulatory frameworks for chemicals.

To illustrate the development of state-of-the-art approaches and the potential of human biomonitoring (HBM) data to refine exposure assessment and, thereby, risk assessment, this presentation will be focused on the work carried out on hexavalent chromium, [Cr(VI)], an important occupational lung carcinogen, which is currently authorized in Europe for several industrial activities. Cr(VI) was considered a priority substance under the HBM4EU Project, indicating the need for generating and analyzing data on human exposure, despite the recently agreed binding limit value for occupational exposure established in the European Union.

The anchoring of HBM to risk assessment and management practices will be evidenced through the information generated from three different studies, namely: i) a critical review on effect biomarkers to link Cr (VI) exposure to health outcomes, ii) a multinational, collaborative study to support management of occupational exposure to Cr(VI), and iii) the development of a case study on co-exposure to Cr (VI), nickel and polycyclic aromatic hydrocarbons, to advance the identification of mixture health effects and to progress towards a more refined risk assessment.

These research efforts to integrate HBM into new risk assessment approaches need to be supported by mechanistic knowledge obtained from *in vitro*/*in vivo* studies, toxicokinetic data and the development of adverse outcome pathways, as well.

Funding: work co-funded by the HBM4EU project (Grant Agreement No: 733032) and by national funds through FCT/MCTES (UIDB/00009/2020 and UIDP/00009/2020).

Maria João Silva is a senior researcher at the Human Genetics Department of the Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), Lisbon, where she leads the Genetic Toxicology Research Group since 1997 and a member of the Center of Toxicogenomics and Human Health (ToxOmics), NOVA Medical School, Universidade Nova de Lisboa. Her main scientific area is human and environmental genotoxicity and her current research interests include human biomonitoring, toxicity assessment of chemicals (and mixtures) and associated mechanisms, and nanotoxicology. She coordinates the European Initiative HBM4EU scientific activities at INSA.

How human biomonitoring has facilitated chemical restrictions - the risk assessment perspective

Mark Blaney | European Chemicals Agency (ECHA)

In recent years, there has been the growing use of human biomonitoring (HBM) data for risk assessment of chemicals and to support regulatory actions by authorities. The Registration, Evaluation, Authorisation and Restriction of CHemicals (REACH) regulation by the European Union entered into force in 2007, and it has played a key role in protecting human health and the environment from hazardous chemicals. REACH applies to all chemical substances and identifies industries as the main responsible party for the safe manufacture and use of chemicals. Chemicals of concern are identified by combining hazard assessment, exposure assessment (through modelled or exposure data, where HBM data can also be included) and risk assessment. Two restrictions case-studies (phthalates and Bisphenol-A) will be discussed to illustrate the link between HBM data and risk assessment. Finally, the need for easily accessible, comprehensive and harmonised HBM data to support regulatory actions will be stressed.

Mark Blainey is leading one of the risk management units in the European Chemicals Agency (ECHA responsible for the restrictions process and also is involved in applications for authorisations. A toxicologist by training, Mark has worked for the Health and Safety Executive, for the European Commission and for his own company all on chemical-related issues.

Human biomonitoring in Food Safety and Nutrition: (im)possibilities and challenges

Hans Verhagen | Ulster University, and Food Safety & Nutrition Consultancy

Chemicals in food can be nutrients, non-nutrients, contaminants, food additives, pesticides, etc. EU Regulation 178/2002 lays down that food needs to be safe. The *risk analysis* paradigm, distinguishes between risk assessment, risk management and risk communication. The *risk assessment* paradigm identifies and characterizes hazards, and assesses the exposure, which together lead into risk characterisation.

As concerns exposure assessment, dietary exposure is the most frequently estimated by combining food composition information with food consumption data. This results in levels in food that are safe for humans upon consumption. Such levels can be regulated in laws and can be enforced.

A more precise quantification of exposure can be achieved by using biomarkers. Biomarkers can be used to estimate internal levels of exposure and markers of effect. The outcome of these are values in e.g. blood or urine that are essentially safe for humans. However, internal values cannot be regulated in laws and cannot be enforced.

Priorities and challenges for external and internal monitoring are similar. Priorities: Harmonization, Standardization, Accessibility, Transparency, and Quality. Challenges: Improving data quality and representativeness, Foster data re-use and innovation, Promote direct access to data and the development of collaborative platforms, Collaboration with other exposure domains through multi-disciplinary projects or initiatives, Awareness of (im)possibilities, and Communication on (im)possibilities.

EFSA has used human biomonitoring only for some contaminants and pesticides. Also, EFSA has used human biomonitoring in the derivation of some dietary reference values. The use of biomarkers is a prerequisite in the scientific substantiation of article 14 health claims.

Hans Verhagen held management positions at the National Institute for Public Health and the Environment and TNO - innovation for life, in The Netherlands, and in the European Food Safety Agency. Hans worked in Unilever Research and at the Universities of Maastricht, Nijmegen, and Ulster. He has published widely on subjects related to toxicology and nutrition. He is a board-certified toxicologist and a board-certified nutritionist. He is a visiting professor at the University of Ulster, Northern Ireland, since 2009. From November 2020, he is the owner and founder of the Food Safety & Nutrition Consultancy in The Netherlands.

Human biomonitoring (HBM) - a tool to support the health argument in addressing major environmental challenges

Dorota Jarosinska, Irina Zastenskaya, Francesca Racioppi | WHO Regional Office for Europe, European Centre for Environment and Health

From a public health perspective, HBM is one of essential tools to assess environmental exposure to different chemicals; in some instances, it allows assessing the related health risks. HBM is very useful for awareness raising, and can drive public policies as well as exposure reduction actions. Critical for producing reliable assessments, planning, monitoring, and meaningful comparisons is the proper design, selection of biological matrices and biomarkers, quality of analytical methods, and analysis of the data. WHO has been working with experts and stakeholders on HBM, including the development and implementation of large scale projects, development of standard operating procedures, as well as capacity building in Member States. Experiences from the HBM activities coordinated by WHO, focusing on priority hazardous chemicals, such as mercury and lead, or categories of chemicals, like persistent organic pollutants, will be presented. In the context of global environmental and climate change, challenges facing the use of HBM data include addressing temporal and spatial dimensions, or tracking the links with specific environmental change.

Dorota Jarosinska MD, PhD, has been working on the health and environment issues at national and international levels for nearly 30 years. At the WHO European Centre for Environment and Health, she manages the programme working on the health aspects of chemical safety, air quality and climate change mitigation, environmental noise, environmentally sustainable health systems, and workers' health. Before joining the WHO in 2014, she worked at the European Environment Agency on environment and health issues, and contributed to the major assessments, such as the joint EEA/JRC report on Environment and Health, and synthesis of the State of the Environment Reports. Prior to this, she was leading the first outpatient clinic of environmental medicine in Poland, and coordinated human biomonitoring work on lead and mercury. She was a Fulbright scholar at the National Institute of Environmental Health Sciences, and is a Fellow of Collegium Ramazzini.

«It's those things that the plastics we use in our daily life have...»: Portuguese citizens' attitudes and beliefs on chemical exposure and human biomonitoring

Ricardo R. Santos, Joana Costa, Osvaldo Santos, Ana Virgolino | Environmental Health Behaviour Lab, Institute of Environmental Health, Faculty of Medicine, University of Lisbon, Portugal

HBM4EU is a programme that intends to generate evidence of the potential health impact of exposure to chemicals by European citizens to support policy makers and ultimately to improve public health. In fact, citizens are daily exposed to many chemicals through different routes and exposed to many sources of information. The efficacy of policies aimed at reducing risk is significantly associated to human behaviour, which is closely connected to risk perception. Knowing perceptions, attitudes, and beliefs of citizens towards chemical exposure and human biomonitoring is crucial to facilitate risk communication, to improve the acceptance of policies of human biomonitoring, and to engage communities in local governance, including risk management. For this purpose, it was developed a cross-sectional observational qualitative study. Data was collected through a semi-structured focus group composed of 10 Portuguese citizens recruited following a purposive sampling. The content was analysed independently by two researchers based on the grounded theory principles. In terms of results, participants were able to recognise the existence of a biomonitoring communication process system composed by three main domains: science, policy makers, and citizens. Communication and reliance were the two topics that participants addressed across the three domains and considered to be critical for people to recognise the effective importance of the HBM4EU programme and the human biomonitoring for their life and the life of future generations at both individual and community levels.

Ricardo R. Santos is a Board-certified biologist with advanced education in Philosophy, Bioethics, and Science Communication. He is currently a researcher at the Environmental Health Behaviour Lab, Institute of Environmental Health, Faculty of Medicine, University of Lisbon.

Perfluoralkyl substances (PFAS): an example for priority substances under HBM4EU

Maria Uhl | Environment Agency Austria

Per and polyfluoroalkyl substances are among the priority substance groups in HBM4EU. Therefore, a scoping document was published and policy questions were defined to be answered in the course of the project. Activities in all three pillars of the five-year HBM programme were designed in order to answer these questions related to exposure, health risks, and impact and effectiveness of policy. Existing European studies were collected, their results evaluated. Further aligned studies in European teenagers of all four regions – north, east, south and west - were performed. In the so called research pillar questions around toxicokinetics, modes of actions and adverse outcome pathways were addressed. HBM4EU performed a policy mapping on PFAS and supports policy actions, which will feed into the new chemicals strategy for sustainability, which addresses PFAS specifically due to the growing concerns related to human health and the environment.

Maria Uhl is a toxicologist with more than 20 years of experience in the field of environmental health. She holds a Master's degree in biology from the University of Vienna and a PhD and Master's degree in toxicology from the Medical University of Vienna. Maria Uhl is Austria's national coordinator within the European Human Biomonitoring Initiative HBM4EU and she also coordinates the Austrian platform for human biomonitoring. She is head of the team Studies and Consulting of the Umweltbundesamt Laboratories dealing specifically with Human and Environmental Monitoring. As senior scientist, Maria Uhl contributed to projects for the European Commission and was project leader of several national and European projects in the context of environmental health. Maria Uhl has been involved in risk assessment and evaluation processes according to Chemicals legislation and International treaties (e.g. REACH, UNEP: Stockholm Convention on Persistent Organic Pollutants, Minamata Convention). Maria Uhl is Chemical Group Lead of perfluoroalkylated substances (PFAS) within HBM4EU and member of the PFAS working group at ECHA.

Free communications

HBM4EU science and policy for a healthy future

Risk assessment of combined occupational exposure to hexavalent chromium, nickel, and PAHs: a literature-based approach

Ana Maria Tavares [1*], Susana Viegas [2], Henriqueta Louro [1,3], Maria João Silva [1,3]

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Occupational exposure is usually characterized by a complex mixture of chemicals, originating from different raw materials and transformation processes. Co-exposure to hexavalent chromium (Cr(VI)), nickel (Ni) and polycyclic aromatic hydrocarbons (PAHs) can occur in some workplaces. These substances display well-known genotoxic and carcinogenic effects, especially in the respiratory tract, sharing similar modes of action. However, reference values for occupational exposure only account for individual components exposure and not for a potential mixture effect.

In the scope of HBM4EU Initiative, we performed a mixtures risk assessment (MRA) based on literature from occupational studies conducted in the European Union that contain human biomonitoring (HBM) data on Cr(VI), Ni and/or PAHs. After HBM data extraction, Hazard Quotient (HQ) and Hazard Index (HI) were calculated for binary and tertiary mixtures. Exposure was considered of concern if HI>1.

Twenty-four articles were selected, most (n=18, 75%) presenting Cr(VI) and Ni exposure biomarkers. Among these, HI>1 was obtained for all studies on welding activities, in which chromium was the main driver of toxicity with HQ>1 in most measurements. Only two studies in waste incineration setting reported exposure to the three substances, and again all HI>1. Noteworthy, for some of the analysed studies, although exposure levels were below the reference values, still the mixture was considered of concern (HI>1).

Our findings show the limitations of applying occupational exposure reference values defined on a single substance basis to workplaces, highlighting the relevance of MRA as a more realistic approach to provide more suitable risk management measures in occupational settings.

KEYWORDS | mixtures risk assessment; hexavalent chromium; nickel; polycyclic aromatic hydrocarbons; occupational exposure

How Human Biomonitoring data increases knowledge base to support Circular Economy – a case study in the E-Waste industry aiming to assess occupational exposure to chemicals

Susana Viegas [1,2,3*], Carina Ladeira [3], Edna Ribeiro [3], Henriqueta Louro [4,5], Maria João Silva [4,5]

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Circular economy is a strategy to promote sustainability with the aim to reuse and recycle as much as possible. However, potential health impacts of the transition to a circular economy may appear in the context of recycling, such as chemical exposure of workers, especially in the expanding e-waste management sector. E-waste is defined as 'various forms of electric and electronic equipment that have ceased to be of value to their users or no longer satisfy their original purpose" and it is expected to grow. The e-waste stream contains a broad range of chemicals, including metals, flame retardants, phthalates and many others that were legal at the time they were manufactured but are now either restricted or banned, thus recycling of these materials can result in workers exposure to chemical mixtures during the e-waste processing.

In the scope of HBM4EU, a new study focused on chemicals occupational exposure in the e-waste setting is planned. This study aims to identify the most relevant compounds that workers are exposed to. In this study, besides Portugal, more eight European countries will participate in the biomonitoring campaign planned.

The results of this study will contribute to increase awareness for the potential presence of these chemicals, tackling also the exposure to mixtures and promoting good work practices. Additionally, the study will provide important information to support new regulatory actions to guarantee that dangerous chemicals are removed from the waste loop and, with that, assure that circular economy reaches the main goal: contribute for the sustainable development.

KEYWORDS | occupational health; exposure assessment; risk assessment; sustainable development; science to policy framework

Biomonitoring of occupational exposure to a known carcinogen: formaldehyde

Solange Costa [1,2*], Carla Costa [1,2], Joana Madureira [1,2], Vanessa Valdiglesias [3], Armanda Teixeira-Gomes [1,2], Blanca Laffon [3], João Paulo Teixeira [1,2]

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Formaldehyde (FA) is a high-volume production chemical with a large range of industrial and medical uses for which exposure is associated with an increased risk of nasopharyngeal cancer and myeloid leukaemia. Based on sufficient evidence from epidemiological studies and experimental data, FA status was revised and reclassified as a human carcinogen. Many people are exposed to FA both environmentally and occupationally, however the highest level of human exposure to FA occurs in occupational settings, namely in anatomy pathology laboratories. The aim of the present study was to evaluate the occupational exposure to FA (n = 85) relating the exposure with different biomarkers and individual susceptibility, a control group (n = 87) was also evaluated. Genotoxicity was assessed by means of cytogenetic alterations and DNA damage, by comet assay (%TDNA). Percentages of different lymphocyte subpopulations were selected as immunotoxicity biomarkers. The effect of polymorphic genes of xenobiotic metabolising enzymes and DNA repair enzymes was also assessed. The mean level of FA-exposure was 0.38 ± 0.03 ppm. All cytogenetic endpoints and DNA damage were significantly increased in FA-exposed workers compared to controls. The exposed group also presented significant alterations in the percentage of cytotoxic T lymphocytes, NK cells and B lymphocytes. In addition, results suggest that polymorphic genes involved in the metabolism of FA (GSTP1 and GSTM1) and repair (FANCA) are associated with increased genetic damage in FA-exposed subjects. Data obtained emphasise the need of more effective measures to protect workers from potentially hazard health effects due to occupational exposure to FA.

Urinary neonicotinoids profiles in adults from Aveiro District, NW Portugal

Ana Catarina Sousa [1,2*], Yoshinori Ikenaka [2], Takahiro Ichise [2], Shouta M. M. Nakayama [2], Sara Souto-Miranda [3,4], Alda Marques [3,4], Carla Valente [5], Luís Taborda-Barata [6,7], M. Ramiro Pastorinho [8,9], Mayumi Ishizuka [2]

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Neonicotinoid insecticides (Neonics - NNs) are systemic insecticides widely used in agriculture to control insects. Due to their broad-spectrum insecticide activity, they are also used in the domestic environment and on animals, including household pets. Owing to their toxicity towards non-target organisms, particularly honeybees, the agricultural outdoor use of some neonics was already banned. Nevertheless, they can still be used in indoor activities. Neonics' residues have been detected in food, water and indoor dust and, consequently, humans are exposed to these insecticides. However, human biomonitoring data is limited to a few studies worldwide, with no data for Portugal. In this study, levels of neonicotinoids namely acetamiprid (and its metabolite dm-acetamiprid), clothianidin, dinotefuran, imidacloprid, nitenpyran, thiacloprid and thiamethoxan, were quantified in spot urine samples provided by 46 volunteers from Aveiro district. The volunteers were recruited from RESPIRA project, an ongoing study that aims to evaluate the role of environmental contaminants in the progression of respiratory diseases. Overall, the obtained results disclose that 81.4% of the individuals were exposed to at least one neonicotinoid. Dinotefuran and dm-acetamiprid showed the highest detection frequencies (46.5%), followed by imidacloprid (41.9%), whereas nitenpyran and thiacloprid were never detected (bellow detection limit). The neonics with the highest concentrations were dm-acetamiprid (max: 1443 ug/g creatinine, average: 39.1 ug/g creatinine) and thiamethoxan (max: 152 ug/g creatinine, average: 6.9 ug/g creatinine). These results are in general accordance with previous reports that disclosed dm-acetamiprid as one of the most frequently detected NN in human urine samples.

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KEYWORDS | exposure assessment; pesticides; urine; acetamiprid; dinotefuran

Biomonitoring of Portuguese nursing mothers: levels of biomarkers of exposure to polycyclic aromatic hydrocarbons and infant´s health risk

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Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants known for their toxic, mutagenic, and carcinogenic properties. Human milk represents the main source of exposure to persistent organic pollutants, including PAHs, during the first postnatal period. Concentrations of PAHs biomarkers of exposure, 18 un-metabolized PAHs and 6 major metabolites, were assessed in the breast milk of 65 nursing mothers (21-40 years).

Concentrations of un-metabolized and metabolized PAHs varied between 55.2-1119 ng/g fat and from 6.66 -455 ng/g fat, respectively. Naphthalene, dibenz(a,h)anthracene, benzo(g,h,i)perylene, and phenanthrene were the predominant un-metabolized compounds while 1-hydroxynaphthalene and 1-hydroxyacenaphthene were the most abundant metabolites. The carcinogenic benzo(a)pyrene and its major metabolite, 3-hydroxybenzo(a)pyrene, were never detected in breast milk. PAHs levels were increased in older nursing mothers (>30 years) and in those whose child was born with less than 3.0 kg. Breast-fed infant presented a PAHs daily intake of 1.41 μg/kg body weight and an exposure to total four PAHs (benz(a)anthracene, benzo(b)fluoranthene, benzo(j)fluoranthene, and chrysene) established by the European Food Safety Authority of 0.024 mg/kg body weight. Despite being a secure food for newborns, biomarkers of exposure to PAHs should be included in biomonitoring surveillance studies to prevent potential health risks for infants.

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This work was approved by the Ethics Committee of University School Vasco da Gama (Coimbra, Portugal). Authors are thankful to all participants.

KEYWORDS | human biomonitoring; breast milk; biomarkers of exposure; polycyclic aromatic hydrocarbons (PAHs); infant exposure assessment

The genotoxicity of an organic solvent mixture: a human biomonitoring study and translation of a real-scenario exposure to *in vitro*

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Humans are environmentally exposed to a large and diverse number of substances and it has been recognized that simultaneous exposure to more than one chemical or physical agent that is potentially hazardous to health is common. The aim of this study was to evaluate the occupational exposure to a styrene and xylene mixture through environmental monitoring and identify the potential genotoxic effects resulting from their exposure through biological monitoring. With the obtained environmental concentrations of both xylene and styrene either alone or in mixture, exposed *in vitro* human peripheral blood cells to understand their mechanism of action. The results obtained by air monitoring were below the occupational exposure limits for both substances. The urinary metabolites for all workers exhibited values below the detection limit of the method (mandelic and phenylglyoxylic acids = 0.16 g/L; methylhippuric acid = 0.55 g/L). All biomarkers of effect, except nucleoplasmic bridges, had higher mean values in workers (N=17) compared to the corresponding control group (N=17). There were statistically significant associations between exposed individuals and the presence of nuclear buds and oxidative damage. As for *in vitro* results, there was no significant influence on primary DNA damage in blood cells as evaluated by the comet assay. On the contrary, we did observe a significant increase of micronuclei and nuclear buds, but not nucleoplasmic bridges upon *in vitro* exposure. In summary, both styrene and xylene have the potential to induce genomic instability either alone or in combination, showing higher effects when combined.

KEYWORDS | biomonitoring; chemical mixtures; risk assessment; *in vitro*

Biomonitoring of metals and minerals in urine in a population of Portuguese school-aged children: deriving reference values

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Human biomonitoring (HBM) encompass the measurement of internal levels of chemicals/metabolites in easily accessible biological fluids or tissues, aiming to understand environmental health threats and assist policy measures, namely in susceptible populations such as children. Experience with HBM differs among European countries, and despite increasing efforts there is still a lack of data in Portugal.

This study was conducted to assess children´s levels of metals and minerals in urine from a cross-sectional study of a random representative sample (n= 2018) conducted in three regions of northern Portugal. The levels of major toxic metals (**As**, **Cd**, **Pb**, **Ni**) and other metals and micronutrients (**Al**, **Sb**, **Co**, **Cu**, **Sn**, **Mn**, **Mo**, **Se**, **Tl**) were measured using the first-morning urine by ICP-MS. The median levels (µg/L) were 6.54 for Al, 0,068 for Sb, 30.04 for As, 0.616 for Pb, 0.714 for Co, 18.66 for Cu, 0.265 for Cd, 0.60 for Sn, 1.214 for Mn, 56.222 for Mo, 4.583 for Ni, 37.614 for Se, 0.275 for Tl. Regarding reference values (RV), the P95 (µg/L) were 25,96 for Al, 0.18 for Sb, 146.28 for As, 2.03 for Pb, 2.41 for Co, 45.85 for Cu, 0.68 for Cd, 4.54 for Sn, 3.77 for Mn, 143.55 for Mo, 17.81 for Ni, 79.29 for Se, 0.95 for Tl.

These preliminary results emphasise the need to further evaluate the health impacts of both metals and minerals levels in school-aged children. In this context, we will further assess the determining factors of exposure and the potential impacts on health.

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KEYWORDS | biomonitoring; children; metals; minerals; reference values

Biomarker-validated maternal smoking and environmental tobacco smoke exposure status and its associations with perinatal outcomes

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Tobacco consumption and environmental tobacco smoke (ETS) are associated with an increased risk of multiple adverse perinatal outcomes. Within the framework of the NEOGENE project, the aim of this study was to validate smoking and ETS exposure status among pregnant women, to more precisely ascertain its associations with perinatal outcomes, using urinary cotinine (UC), a biomarker of tobacco smoke exposure.

This cross-sectional study enrolled 582 pregnant women who sought prenatal care on Centro Hospitalar de São João, in Porto (Portugal), from May 2017 to August 2018. Data on smoking habits and ETS exposure, sociodemographic characteristics and anthropometrics was collected through a structured questionnaire, completed for each participant after delivery, during the hospital stay. Perinatal outcomes, such as birth weight, length and head circumference were retrieved from medical records. Cotinine concentrations were determined by solid-phase competitive ELISA, in maternal urine samples, collected on the day of delivery. Maternal smoking and ETS exposure status in the third trimester of pregnancy were categorized based on the optimal UC cut-off values, identified specifically for the study population.

The results obtained in this study reinforce the need for increased public health policies and awareness campaigns not only to encourage smoking cessation among pregnant women, but also to limit their exposure to ETS, with the overall goal of improving perinatal outcomes in Portugal.

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KEYWORDS | urinary cotinine; perinatal outcomes; tobacco smoke

Human trials addressing environmental toxicant exposures: scoping review of immunological and endocrine markers and protective strategies

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Introduction: Clinical trials enable the research of the human health effects of environmental exposures and protective strategies. The aim of this work was to determine the immunological and endocrine aspects used to biomonitor the effects of environmental toxicants' exposure and to identify strategies being explored to counteract these effects.

Methods: Clinical trials addressing environmental and occupational toxicants' effects were searched at the ClinicalTrials.gov database and screened for immunological or endocrine outcomes described. The potential protective interventions were also accessed.

Results: A total of 156 observational and interventional (non-randomized and randomized) completed trials were identified, including for priority substances as bisphenol A, chemical mixtures and polycyclic aromatic hydrocarbons. The immunologic outcomes analyzed are mainly the levels of inflammatory cytokines, as IL-6, IL-8, TNF-a and IL-1ß, while more specific cells profiling or immune-mediated toxicity markers are not monitored. Regarding endocrine outcomes, we found studies targeting cortisol, estradiol and thyroid hormones, among others, but the hormonal dynamics is clearly less characterized. The protective strategies tested against a condition of environmental toxicant exposure were: 1) intake of beet or orange juice; 2) antioxidant/anti-inflammatory dietary supplements; 3) L-arginine; 4) omega-3 fatty acids; and 5) polyphenols.

Conclusions: The number of studies found addressing environmental toxicant exposure reveals the concerns about the risks to human health, and the immunological and endocrine consequences are starting to be considered under the scope of clinical trials. Moreover, protective strategies are being drawn to minimize the harmful effects of environmental toxicants' exposure, giving insights to future research.

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KEYWORDS | environmental toxicants; immune system; endocrine system; dietary interventions

Feasibility of a silicone membrane for mimicking skin absorption of environmental toxicants: experimental studies with polycyclic aromatic hydrocarbons

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Polycyclic aromatic hydrocarbons (PAHs) are toxic organic compounds ubiquitous in the environment. In addition to inhalation and ingestion, recent data highlight the relevance of skin absorption in human exposure to PAHs outdoors and in workplaces.

In general, skin models point toward a permeation ability in increasing order: benzo[b]fluoranthene (BbF) < benzo[a]pyrene (BaP)≈ benz[a]anthracene (BaA)< anthracene (Ant)< naphthalene (Nap). However, conflicting results can be found and non-animal models to investigate toxicant absorption are lacking.

In this work, permeation assays were carried with a silicone membrane (Lintec Co.) and pig skin mounted on Franz cells. Penetration of BbF, BaP, BaA, Ant and Nap into the receptor compartment was analyzed by HPLC. Kinetic assays were also carried for Ant applied over the membrane with organic and aqueous vehicles. Our results were compared with data of PAH permeability in human skin from the literature.

The results obtained for the 5 compounds, both with pig skin and silicone membrane, gave further support to the above-mentioned relative permeability of PAHs and reproduced the observations by different authors using human skin. At 5 hours, Nap and Ant reached concentrations above 50 pmol/mL inside the Franz cells' receptor, while BbF did not exceed low pmol/mL levels. Remarkably, the amounts of PAHs (except Nap) penetrated through the synthetic membrane and pig skin were significantly correlated (R^2=0.996). Kinetic assays with Ant afforded fluxes >20 ng/cm^2/h, similar to the reported for human skin.

These results suggest silicone membrane as a skin-equivalent non-animal model for PAHs dermal absorption in research and biomonitoring applications.

KEYWORDS | environmental toxicants; polycyclic aromatic compounds; dermal exposure; non-animal models

e-Posters

HBM4EU science and policy for a healthy future

Review of the analytical methodologies for the human biomonitoring of short-term exposure biomarkers to acrylamide

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Acrylamide is a processing contaminant found in carbohydrate-rich foods that have been heated at high temperatures, as a result of a reaction between asparagine and reducing sugars named Maillard reaction. It is present in a wide range of frequently consumed foods and in tobacco smoke, making human exposure unavoidable. The concern about the possible carcinogenic effects of acrylamide (IARC classification 2A: probably carcinogenic to humans) justifies the need to carry out studies that allow to know the body burden of this chemical and the level of exposure to it. In this sense, the implementation of human biomonitoring is an useful tool to evaluate the exposure through the study of the levels of its biomarkers in biological matrixes, the comparison with reference values and the interpretation of values measured in a context of health risk analysis. Acrylamide is extensively metabolised to the epoxide glycidamide that might be measured in serum, but they have a short half-life. On the other hand, mercapturic acids of acrylamide and glycidamide (AAMMA and GAMA, respectively), excreted in urine, and hemoglobin adducts of acrylamide and glycidamide (AAVal and GAVal) are stable compounds. A bibliographic review of the analytical methodology for short-term exposure to acrylamide evaluation has been carried out. Urine, a non-invasive matrix, is the preferred matrix for the measurement of the biomarkers N-acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA), N-acetyl-S-(2-carbamoyl-2- hydroxyethyl)-L-cysteine (GAMA), iso-GAMA and AMMA sulfoxide. The determination is generally performed by Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS) and includes previous clean-up steps usually by solid phase extraction (SPE).

KEYWORDS | acrylamide; urinary metabolites; human biomonitoring

In vitro combined toxicity of chromium, nickel and PAHs in human lung cells as support for mixtures risk assessment

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Risk assessment of chemicals mainly relies on exposure to single chemicals and their hazardous effects, although the real scenario in occupational settings is more commonly characterized by exposure to chemical mixtures. Chromium (Cr), Nickel (Ni), and Polycyclic Aromatic Hydrocarbons (PAHs) are often present in occupational settings, such as aeronautic industries, where workers are exposed primarily through inhalation. Considering that those substances are recognized lung carcinogens acting mainly by genotoxic mechanisms, it is likely that interactive effects occur, indicating that the risk from occupational exposure to these chemicals should be assessed as a mixture. Such issue is being addressed in the HBM4EU Initiative, where a real scenario of occupational exposure has been studied.

In order to provide support to the hazard assessment of the referred mixture, the present work aimed to evaluate the combined toxicity of Cr(VI), Ni, and benzo(a)pyrene (BaP), using a human lung cell line (A549 cells). MTT assay was performed for each individual chemical and a dose-response curve was established, enabling the determination of the IC50. The combined toxicity of the Cr and Ni mixture as well as that of Cr, Ni and BaP were determined comparatively to the single chemicals' toxicity to ascertain whether additive effects or deviations from additivity towards synergism or antagonism was obtained. The results will be presented and discussed and are expected to contribute to the overall mixture's risk assessment.

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KEYWORDS | mixtures; risk assessment; occupational health; hexavalent chromium, nickel and PAHs

earlyMYCO: assessing the risk associated to early-life exposure to mycotoxins

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A number of health disorders has been associated to exposure to hazardous chemicals during the first 1000 days of life. Therefore, a proper risk assessment built on accurate data assumes particular importance to evaluate the potential impact that early-life exposure could represent in adulthood. Mycotoxins are secondary fungal metabolites that might cause harmful effects in humans and animals. Recent studies showed that Portuguese young children are exposed to multiple mycotoxins through food consumption which could constitute a health concern. However, earlier exposure to these compounds remains unexplored.

earlyMYCO – Early-life exposure to MYCOtoxins and its impact on health, a national funded project, intends to contribute to clarify this issue evaluating the health effects of early-life exposure of Portuguese mother-and-child pairs to mycotoxins and assessing the associated risk. The Estimated Daily Intake, using human biomonitoring data, will be compared with reference dose values. For those mycotoxins representing a health concern, an estimate of the associated probable health-impact will be performed by calculating the associated burden in terms of disability-adjusted life years (DALY).

Preliminary results of exposure to mycotoxins through food consumption (cereal-based foods) in young children (≤ 3 years old) revealed a potential adverse health effect for percentiles of intake of aflatoxins above or equal to P50 (corresponding to 0.041 ng/kg body weight/day or higher).

Our results will contribute to reach an accurate risk assessment framework and to establish and prioritize preventive measures to reduce exposure to chemicals, especially for vulnerable population groups as pregnant women and infants.

This work was funded by FCT/MCTES, PTDC/MED-TOX/28762/2017 and co-funded by CESAM (UIDP/50017/2020+UIDB/50017/2020), through national funds.

KEYWORDS | early-life exposure; mycotoxins; risk assessment; human biomonitoring; health

Firefighters' biomonitoring: a review on urinary biomarkers of exposure

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Firefighters are involved in the front-line response to fires, being at a high risk of suffering potential health impacts due to their chronic exposure to numerous health-hazardous pollutants during firefighting activities. Human biomonitoring is an essential tool to evaluate firefighters' occupational exposure through the use of urinary biomarkers of exposure. This work presents a critical review of the existing reports of the last twenty-five years on the concentrations of the main urinary biomarkers of exposure, i.e. polycyclic aromatic hydrocarbons and their metabolites, metals and metalloids, pesticides, environmental phenols, phthalates, benzene, toluene, methoxyphenols, and levoglucosan in occupationally exposed firefighters. Overall, firefighting activities contribute to increased concentrations of several persistent and non-persistent organic pollutants and/or their respective metabolites in the urine of exposed firefighters comparatively with non-exposed firefighters. Also, urinary levels of different biomarkers of exposure were predominantly higher in (exposed/non-exposed) firefighters than in the general population. More studies are needed to better evaluate firefighters' occupational safety and health and to support the implementation of preventive measures and mitigation strategies to promote the protection of firefighters.

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KEYWORDS | fire emissions; hazardous pollutants; firefighters' occupational exposure; biomarkers of exposure.

Dietary acrylamide intake and risk of cancer: a systematic review

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Objectives: To explore the association between dietary acrylamide exposure and the risk of different cancers in prospective studies.

Methods: PubMed, Web of Science and Scopus databases were searched for studies published in English, until 18 June 2020. Eligible studies included adults, assessment of dietary acrylamide and cancer incidence, reported as hazard ratio (HR) for acrylamide intake as a continuous variable (per 10µg/day increase). Breast, endometrial, ovarian, and renal cell cancers were excluded. Quality of papers was assessed using the NIH's Quality Assessment Tool for Observational Cohort and Cross-sectional Studies.

Results: Out of the 855 papers identified, 10 were included, providing a dataset of 526,151 participants, of which 13,629 developed cancer. The mean follow-up period was 14.5 years. All studies were found to have fair quality. Multivariate-adjusted pooled HR showed no clear association for almost all cancer diagnosis included per 10µg/day increase of exposure. As exceptions, in men, pooled HR showed an increased association between a consumption increment of 10µg/day and the following cancers: cutaneous malignant melanoma [HR=1.13 (95%CI:1.10-1.26)], follicular lymphoma [HR=1.28 (95%CI:1.03-1.61)], and multiple myeloma [HR=1.14 (95%CI:1.01-1.27)]. In women a decreased risk of lung cancer was noted [HR=0.82 (95%CI:0.69-0.96)].

Conclusions: This systematic review suggests that dietary acrylamide exposure may increase the risk of cutaneous malignant melanoma, follicular lymphoma and multiple myeloma in men only. More studies are needed to confirm the effect of dietary acrylamide in the development of each cancer type, to improve current knowledge regarding the risks associated to the current exposure of the population to this substance.

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KEYWORDS | acrylamide; dietary exposure; neoplasms; epidemiological studies; systematic review

Non-invasive human stress biomarkers detection-Project Stressense

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The study of human stress and emotions is a recent field with high importance to all modern society. Imbalanced parameters to external stimuli induce stress both physical as psychological that has been related to several diseases and discordant social behaviour. In the STRESSENSE project, funded by Fundação para a Ciência e Tecnologia, civilian, military and business' association researchers (from FCT-NOVA, CINAMIL/Academia Militar and CITEVE) work together to study and early detect stress biomarkers, through non-invasive methodologies where sweat is the target biological fluid. The ultimate goal is to build a sensor able to operate in field with real-time monitoring and integrated in wearables. The multidisciplinary team has complementary expertise in electrochemistry, analytics, biochemistry, materials and data-processing and machine learning, together with knowledge associated to individuals' emotional and physical behaviour. The project volunteers are from Portuguese Army (Academia Militar) and take part of a case-of-study allowing attaining the proof-of-concept of the sensing methodology. After validation, the novel sensors are predicted to be integrated in fabrics to attain a wearable device. The novel sensing devices in development present high interest in different fields from medicine, sports to health care services.

KEYWORDS | Ssress; sweat; non-invasive; biomarkers

Nutritional and body composition assessment in patients with bronchiectasis

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Introduction: Bronchiectasis is a respiratory disease characterized by bronchial dilation. Increased susceptibility to respiratory infections, chronic inflammatory condition, and catabolic status may result in changes in nutritional status and body composition.

Objective: Study the relationships of clinical characteristics, nutritional status, and body composition with disease severity (DS) among patients with bronchiectasis.

Methods: Anthropometric and body composition assessment were performed by bioimpedance, with subsequent calculation of phase angle (PA), among 56 outpatients (60.7% women) attending a follow-up bronchiectasis appointment. DS was assessed using Bronchiectasis Severity Index (BSI).

Results: According to a classification suitable for chronic respiratory diseases, 35.3% of women and 22.7% of men were thin, 44.1% of women and 27.3% of men had normal weight and 20.6% of women and 50.0% of men were obese. Median PA was significantly higher among males (5.57 ° *vs.* 4.81 °; $p = 0.002$). The biological and diagnostic age were positively associated with DS, both among females ($r = 0.648$, $p = 0.001$; $r = 0.473$, $p = 0.005$) and males ($r = 0.479$, $p = 0.024$; $r = 0.468$, $p = 0.028$). Among males a positive association was observed between the percentage of fat mass and DS, while among females lower PA was associated with higher DS.

Conclusions: Due to the pathophysiology of bronchiectasis, which may differ regarding effects on body cell mass, hydration, and maintenance of cellular integrity, it would be important to study the prognostic value of PA in these patients. In addition to assessing nutritional status, PA seems to be a likely indicator of DS.

KEYWORDS | bronchiectasis (BSI); nutritional status; body composition; phase angle (PA)

Biomonitoring of Ochratoxin A in breastmilk: a global health study

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Currently, human milk is considered essential for the normal growth and health of the baby, preferably as the only source of food during the first six months of life. However, through breast milk, different food and environmental contaminants can be transferred which can lead to a toxicologically relevant exposure of breastfed babies, considered a particularly vulnerable population.

Ochratoxin A (OTA) is a mycotoxin with recognized nephrotoxic effects, besides acting as a liver toxin, an immunological suppressor, a teratogenic and carcinogenic agent (IARC group 2B). Previous biomonitoring studies in relation to the Portuguese adult population in general confirmed that exposure to OTA exists and is widespread.

Given the above, we analyzed 81 (convenience) samples of breast milk of nursing mothers in Portugal (n = 42) and Angola (n = 39), along with a written informed consent, a sociodemographic questionnaire and a dietary questionnaire (semi-quantitative).

The results showed a generalized contamination, in Portugal (97.6%) and Angola (100%), with maximum values of 560 and 2527 ng/L, respectively. Mean contamination values were higher in Angolan milk samples (Portugal: 305±114 ng/L; Angola: 690±472 ng/L. The occurrence and OTA levels determined were higher than previously reported, particularly in Angola.

The results highlight the need to strengthen surveillance and biomonitoring programs in both countries in order to decrease the presence of OTA in human milk and the resulting exposure of lactating newborns.

Exposure and cumulative risk assessment to non-persistent pesticides in Spanish children using biomonitoring

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The main objective of the present research is to evaluate the exposure to pesticides in children (n= 568) from the Valencian Region (Spain). Six non-specific and 20 specific metabolites of organophosphate pesticides (OPs), herbicides (Herb), and pyrethroids (Pyr) were analyzed in urine samples. The biomarkers with the highest detection frequencies (N70%) were diethyl phosphate, p-nitrophenol, 3-phenoxybenzoic acid, and 3,5,6-trichloro-2-pyridinol, whose geometric mean concentrations (ng·ml-n1) were 1.53, 1.03, 1.51 and 1.19, respectively. Robust regression models showed that the province of residence and the recent consumption of vegetables, legumes and cereals were some of the most important predictors of pesticide exposure. Pesticide risk assessment is estimated using two different strategies: one based on the pesticides' mode of action (MoA); and the other based on cumulative assessment groups (CAGs), proposed by EFSA. The estimated daily intakes (EDIs) ranged from 0.08 (chlorpyrifos) to 1.62 µg·kgbw^{-1} (λ-cyhalothrin). The MoA approach resulted in hazard quotients ranging from 0.01 (chlorpyrifos) to 0.65 (λ-cyhalothrin), and a hazard index for OPs lower than 1. Similarly, the risk assessment based on CAGs led to total margins of exposure (MOETs) far from 100. In conclusion, both risk assessment strategies does not reveal any evidence of a potential health risk due to pesticide exposure in Spanish children.

KEYWORDS | pesticides; urine; biomonitoring; children; risk assessment

Pyrethroid pesticide metabolite in urine of Portuguese pregnant women: a preliminary study of the IoMum cohort

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Exposure to pesticides in the population is widespread, especially via dermal, ingestion and inhalation routes. Like many other insecticide compounds, pyrethroids pesticides are known to be neurotoxic. Pyrethroids are metabolized and excreted in the urine. Pyrethroid metabolite, 3-phenoxybenzoic acid (3-PBA) has been associated with stillbirths, low birth weight, prematurity, congenital malformation, and neurobehavioral function disorder. The aim of this work was to quantify levels of 3-PBA in a sample of Portuguese pregnant women.

Methods: Pregnant women attending routine 1st trimester ultrasound scan (T1) from April 2018 to April 2019 at S. João Hospital Center were invited to participate. Those in the 10-13 gestational weeks, with confirmed fetal vitality and who signed the informed consent were included in the study. A simple solid-phase extraction (SPE) followed with derivatization prior to GC/MS was performed for 3-PBA analysis.

Results: Median age (in years) was 33 (IQR: 29.0-36.0). 44% of cohort had low educational level (≤12 years) and 74% had BMI within normal weight. Preliminary results of 69 spot urine samples showed a detection rate for urinary 3-PBA of 52% and a median urinary 3-PBA of 0.370 µg/L (IQR: 0.180-0.390). Regarding the positive samples, the values ranged between 0.120 µg/L (Min)– 0.550 µg/L (Max).

Conclusion: This study shows that Portuguese pregnant women of the IoMum are exposed to pyrethroid pesticides potentially affecting fetal growth. Future work will focus on analyzing 3-PBA levels seasonality and 3-PBA exposure connections with pregnancy (gestational age at birth) or birth outcomes (weight, length and head circumference of the newborn).

KEYWORDS | 3-PBA; pyrethroid pesticides; newborn; anthropometry

Biomonitoring occupational exposure to a mixture of organic solvents: genotoxic effects according to the workplace

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Styrene and xylene are used in large quantities as organic solvents in a variety of industries. The aim of this study was to evaluate occupational exposure to a styrene and xylene mixture through environmental monitoring and identify the potential genotoxic effects using cytokinesis blocked micronucleus assay and comet assay in workers. The workers may be allocated in the following workplaces: (1) Shift Chief; (2) Production and Analysis Technician; (3) Reactor Operator; (4) Cargo Preparer; (5) Filling Operator. The results obtained by air monitoring were below the occupational exposure limits for both substances and the urinary metabolites for all workers exhibited values below the detection limit of the method (mandelic and phenylglyoxylic acids = 0.16 g/L; methylhippuric acid = 0.55 g/L). In a general analysis, all biomarkers of effect, except nucleoplasmic bridges, had higher mean values in workers (n=17) compared to the corresponding control group (n=12). Specifically regarding the workplaces occupied by the exposed individuals, the Shift Chief is the one that has a higher average of micronuclei (11.67 ± 3.93). On the other hand, the Cargo Preparer has the higher average for nuclear protusions (3.00 ± 2.04) and DNA damage (32.32 ± 10.08). A higher mean of nucleoplasmic bridges was found in the Fill Operator (1.00 ± 1.00) and the highest mean of oxidative damage was observed in the Reactor Operator (39.14 ± 17.27). Results obtained demonstrate that effect biomarkers can support the identification of higher risk groups of workers and to shape/guide the definition of health surveillance programs.

KEYWORDS | biomarkers; genotoxicity; monitoring; occupational; workstation

Use of whole blood and UV-light in the Challenge-Comet assay for DNA repair capacity determination

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Several methods have been attempted to evaluate individual's functional DNA repair capacity, but most of them are neither precise enough nor easily adopted for population studies when many samples need to be processed simultaneously and quickly. Among available assays, the Challenge-Comet assay has been used in population studies to determine capacity in DNA damage response and repair (DDRR) function. Although the Challenge-Comet assay has gained acceptance, its use has been traditionally based on the application of X-rays as the challenge agent and on isolated peripheral blood mononuclear cells (PBMC) as cell specimen. To enhance the usefulness of the assay, our objective was to investigate the suitability of using whole blood samples for this assay, and to use ultraviolet (UV) light as a challenge agent to detect nucleotide excision repair (NER) capacity. Human fresh and frozen blood samples were challenged with UV light, and DNA damage was evaluated by the Comet assay at four time points (0-180min); results were compared with those from PBMC. Maximum expression of DNA damage was obtained at 30min. All sample types showed higher initial DNA damage as compared to controls, and progressive repair was evident in all of them. In addition, PBMC and fresh blood cells reached the initial basal DNA damage levels at the end of the repair period. No significant differences were observed in the basal DNA damage levels. In conclusion, whole blood is a proper biomatrix for the Challenge-Comet assay, and UV light can be used as challenge agent for detecting NER alterations.

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KEYWORDS | challenge-comet assay; whole blood samples; ultraviolet light

Glyphosate and AMPA: urine biomonitoring of a Portuguese rural population

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Glyphosate (N-(phosphonomethyl)glycine) is an organophosphorus broad-spectrum systemic herbicide, and the most widely used. Its main metabolite, aminomethylphosphonic acid (AMPA) has a comparable toxicity profile. Human exposure results from the inadequate protection during its production/ application, or from its ingestion through contaminated food/water. The human health impact is currently controversial. It has been recently thoroughly assessed in the EU, and is currently approved until December 2022, however, the IARC has classified it as probably carcinogenic to humans (Group 2A). Urine biomonitoring is thus essential and a non-invasive valuable tool to evaluate individual exposure.

Our goal was to evaluate glyphosate and AMPA, by GC-MS-MS, in first-morning urine (winter/summer sampling) of a Portuguese rural population. Volunteers (18 males; 19 females) aged between 19 and 74. Most of the subjects practice subsistence farming, using herbicides, and consume more than 75% of self produced/local products. The validated methodology allowed LOQs of 0.24 µg/L (glyphosate) and 0.60 µg/L (AMPA).

Out of the 74 analyzed samples, 45 (60.81%) contained glyphosate up to 8.74 µg/L, with an average of 0.62 µg/L. For AMPA, 46 samples (62.16%) were positive, with an average of 0.40 µg/L. No statistical differences were found between winter and summer sampling.

Only 14.86% and 1.35% of glyphosate and AMPA concentrations were ≥LOQs (the majority regarding male samples). This might be explained by glyphosate/AMPA reduced oral absorption and fast elimination via urine. Moreover, since it is presumed the little metabolization of glyphosate by humans, one can conclude that this exposure was mainly from ingestion and not occupational.

KEYWORDS | glyphosate; AMPA; biomonitoring; rural population

Air pollution in urban environments: implications for citizens´ health

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Currently, 54% of worldwide population lives in urban areas. While growing urbanization causes environmental pollution and energy demand, it has also adverse implications on health, living conditions and lifestyle habits of the respective citizens. To protect human health, numerous organizations have implemented guidelines to limit environmental pollution, or even proposed recommendation on human activity, but how do these translate to the exposure and risks of the respective population? This work aimed to explore how urban development, using time series data from 2015 to 2018, might influence air quality and potential health risks of citizens living in these zones.

$PM_{2.5}$ and PM_{10} levels were retrieved from urban and suburban air monitoring stations of Portuguese Environmental Agency (North-Porto) during 2015–2018. Portuguese age-specific anthropometric data were obtained.

In spite of growing urbanization, the results demonstrated that health based protective guidelines were fulfilled. $PM_{2.5}$ and PM_{10} obliged annual limits during all 3 years, possibly also due to improved urban planning and use of clean energy. Lifestyle habits strongly impacted estimation of individual dose (range 0.28–1.36 μg kg^{-1}; 0.08–0.34 μg kg^{-1}) and potential effects on health.

While the ambient data monitoring is relevant component of national and international public health programs, personal exposure measurements should be integrated in order to fully protect human health of citizens.

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KEYWORDS | ambient monitoring; particulate matter (PM); urbanization; risks

Salivary leucocytes as suitable biomatrix for the hOGG1-modified comet assay

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The human 8-oxoguanine DNA glycosylase (OGG1)-modified comet assay has been widely used in human biomonitoring studies to detect oxidative DNA damage, specifically oxidized purines (mainly 8-oxoguanines). Peripheral blood leucocytes, isolated from whole blood, are the most common sample type used for this assay, but their obtaining involves an invasive procedure. Thus, the objectives of this study were to test the validity of leucocytes isolated from saliva samples as a proper biomatrix for the OGG1-modified comet assay, and to determine whether frozen salivary leucocytes are still suitable for detecting oxidative DNA damage. To that aim, isolated salivary leucocytes, both from fresh and frozen samples, were exposed to three concentrations of potassium bromate, which induces oxidative DNA lesions, but does not generate DNA strand breaks *per se*. Oxidative DNA damage was evaluated by means of the OGG1-modified comet assay, and results were compared with those obtained for peripheral blood leucocytes (PBL). Exposure caused significant increases in the net OGG1 sensitive sites for the two highest concentrations tested in the three sample types, with a slight non-significant increase for the lowest concentration, and concentration-response relationships were significant in all cases. No differences were observed in the basal oxidative genetic damage in the three sample types assayed. In conclusion, the results obtained showed that salivary leucocytes, both fresh and frozen, can be suitably used as biomatrix for the OGG1-modified comet assay. Frozen salivary leucocytes are a very convenient sample for evaluating oxidative DNA damage in large human biomonitoring studies.

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KEYWORDS | biomonitoring, frozen samples, OGG1-modified comet assay, oxidative DNA damage, salivary leucocytes

Concluding remarks and closure: towards a toxic-free environment

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In these uncertain times, in which we are going through a health, social and economic crisis that we have never experienced, the desire to continue organizing the HBM-PT prevailed and proved to be an excellent opportunity to update and share knowledge in human biomonitoring and related sciences.

During this 3rd HBM-PT workshop, we have witnessed the quality and diversity of the work done by a large and enthusiastic bunch of people working in vibrant places, namely R&D centres, national regulatory agencies or international organisations. The latter included the European Centre for Environment and Health – WHO, the European Environment Agency (EEA), the European Chemicals Agency (ECHA) and the European Food Safety Authority (EFSA). Altogether, their members attending the workshop nicely put the Portuguese situation into a broader context and perspective.

As an example, Dorota Jarosinska (WHO) emphasized the role of HBM in facilitating intersectoral and multi-stakeholder involvement and action. In fact, beyond the health and environment sectors, HBM brings to participation other societal interests from agriculture to fisheries, from work conditions to mobility and transportation, from education to science & technology.

Regarding the public awareness of the environmental health hazards and risks, it was underlined the need to overcome the prevailing positivistic view of the scientific endeavour, perceived by the public as essentially uncertainty-free, which is far from being a reality.

It was questioned whether the business-as-usual (unlimited growth) model of chemicals usage is sustainable in human health and environment terms? To this respect, a special consideration should be given to the air, water and soil environmental domains. It was also questioned, having in mind the finitude of the Earth's natural resources, how we produce food and other consumer goods, how we use energy and the way we travel from A to B.

A diversity of themes was further approached and exciting results from national and international projects, either on occupational health or on environmental determinants of disease, were communicated. The former studies included, among others, workers' exposure to organic or inorganic substances and their mixtures highlighting the value of human biomonitoring data in occupational surveillance programs and their relevance to trigger preventive or mitigation measures for health protection. Other studies addressed human exposure to environmental factors, e.g., heavy metals, and related health effects, as assessed by effect biomarkers. Noteworthy was the number of biomonitoring studies that were focused on vulnerable population groups, like children and pregnant women, who may be at a higher risk of developing environment-related outcomes impacting on their (or their descendants) health or quality of life.

A possible clue to hope may stem from the so-called European Green Deal, proposed by the European Comission, with its different lines of action: chemicals strategy for sustainability, from farm to fork, biodiversity promotion, circular economy, just transition (to a greener economy) and the zero-pollution goal.

Wishing that we truly move towards a toxic-free environment and a healthier future, we hope to meet you in good health and bringing more exciting themes to the next HBM-PT in 2021.

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