



science and policy  
for a healthy future

**2nd HBM-PT**

# Workshop on Human BioMonitoring in Portugal

HBM: assessing exposure for a healthier future in Portugal

**October 25, 2019**

Auditorium of the Portuguese Environment Agency  
Lisbon, Portugal



## Book of abstracts



## 2nd HBM-PT

# Workshop on Human BioMonitoring in Portugal

HBM: assessing exposure for a healthier future in Portugal

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# welcome message

Dear participants in the 2nd Workshop on Human BioMonitoring in Portugal (HBM-PT),

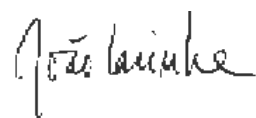
It is my great pleasure to welcome you to Lisboa and APA, the Portuguese Environment Agency, to participate in 2019 main event of our human biomonitoring community, a multifaceted network of researchers, experts in environmental or occupational health, regulators, chemical industry professionals and other stakeholders.

This year's HBM-PT programme showcases some of the remarkable recent advancements in organisation, data gathering and field work on the environment and health interface in Portugal and elsewhere. This represents a substantial progress as compared to the 2018 workshop, materialised in the number, quality and thematic diversity of the communications.

Scientific meetings like the 2nd HBM-PT are indeed an excellent opportunity to strengthen the links between science, policy, regulatory actions and their implementation to prevent, mitigate or adapt to the effects of the life-long human exposure to chemicals in different environmental domains and societal settings.

When the extension (for the 2022-2027 period) of the current European Union Human Biomonitoring Initiative is under discussion, it is paramount to set up sound knowledge basis and capabilities to support the political commitment of Portugal and the other EU member states towards human biomonitoring coordination at the national and international levels.

Enjoy the meeting!



**João Lavinha**

INSA

Ambassador of the Portuguese  
National Hub







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# PROGRAMME

09:30 - 10:00

## Opening session

Nuno Lacasta, President of the Agência Portuguesa do Ambiente  
Beatriz Fernandes, Vice-President of the Escola Superior de Tecnologia da Saúde de Lisboa  
Fausto J. Pinto, Director of the Faculdade de Medicina da Universidade de Lisboa  
Diogo Cruz, Subdirector-General of the Direção-Geral da Saúde  
Cristina Abreu dos Santos, Board of directors of the Instituto Nacional de Saúde Doutor Ricardo Jorge  
José Paulo Esperança, Vice-President of the Fundação para a Ciência e a Tecnologia  
Rita Cavaleiro, National Hub Contact Point (Fundação para a Ciência e a Tecnologia)

## Session 1 | HBM from the science perspective

Chair: Teresa Borges (DGS)

10:00 - 10:20

### The Human Biomonitoring Initiative for Europe (HBM4EU): An overview

Maria João Silva (INSA)

10:20 - 10:40

### Why bother with infertility when the world is overpopulated?

Paulo Navarro-Costa (ISAMB, FMUL & IGC)

10:40 - 11:00

### Integrating environmental and human monitoring: a case study of parental and pre-natal exposure to harmful substances in Aveiro region

Susana Loureiro, Ana C. Alves, Marta Fraga, Madga Henriques, Teresa Herdeiro, Margarida Fardilha, Carlos Gravato, Amadeu M.V.M. Soares, Marta S. Monteiro

11:00 - 11:30

## Coffee break and poster session

## Session 2 | Free communications

Chair: Paula Alvito (INSA)

11:30 - 11:45

### Exposure assessment of Portuguese women of childbearing age to methylmercury: first results of a human biomonitoring study

S. Santiago, S. Namorado, C.M. Dias, C. Martins, C. Carvalho, and R. Assunção

11:45 - 12:00

### The impact of secondhand smoking in intrauterine growth

T. Varandas, C. Ribeiro, A. R. Marinho, A. Pinto, and E. Ramos

12:00 - 12:15

### Selenium and Selenoproteins in Blood and Plasma of Pregnant Women from the Island of Madeira: Considerations About Biomarkers and Confounding Factors

V. Branco, L. Carvalho, C. Barboza, M. E. Mendes, A. Cavaco, and C. Carvalho

12:15 - 12:30

### Urinary levels of Manganese, Copper, Tin and Antimony in Portuguese Children

L. Correia-Sá, V. Fernandes, M. da Luz Maia, S. Norberto, C. Delerue-Matos, C. Calhau, and V. F. Domingues

12:30 - 12:45

### Plasma concentration of brominated flame retardants and breast cancer risk: A nested case-control study in the French E3N cohort

F. R. Mancini, G. Cano-Sancho, P. Marchand, M.-C. Boutron-Ruault, G. Severi, J.-P. Antignac, and M. Kvaskoff

12:45 - 14:00

## Lunch and poster session



# PROGRAMME

## Session 3 | The role of HBM on human health and environment policies

Chair: Teresa Nuncio (APA)

- 14:00 - 14:20 **HBM in support of human health and environmental policies**  
Teresa Borges (DGS)
- 14:20 - 14:40 **HBM relevance in occupational exposure and risk assessment – a case-study on chromium VI**  
Susana Viegas (ESTeSL-IPL & ENSP-UNL)
- 14:40 - 15:00 **Risk assessment of methylmercury in susceptible populations**  
Cristina Carvalho (iMed, FFUL)
- 15:00 - 15:30 **Discussion Forum | HBM as a useful tool for risk assessment**  
Moderator: Cesaltina Ramos (DGS)
- Bárbara Oliveira (DGAV - Direção de Serviços de Meios de Defesa Sanitária)  
Inês Almeida (DGAV - Divisão de Gestão e Autorização de Medicamentos Veterinários e Biocidas para Uso Veterinário)  
Narcisa Bandarra (IPMA - Divisão de Aquacultura, Valorização e Bioprospeção)
- 15:30 - 16:00 **Coffee break and poster session**

## Session 4 | Free communications

Chair: Joana Costa (ISAMB/FMUL)

- 16:00 - 16:15 **Population Exposure to Particulate-Matter and Estimated Excess Mortality Due to the Portuguese Wildfires in October 2017 Driven by Storm Ophelia**  
S. Augusto, N. Ratola, P. Tarín-Carrasco, P. Jiménez-Guerrero, M. Turco, M. Schuhmacher, S. Costa, J. P. Teixeira, and C. Costa
- 16:15 - 16:30 **Biomonitoring of Wildland Firefighters: Levels of Urinary Biomarkers of Exposure to Polycyclic Aromatic Hydrocarbons and Genotoxic/oxidative-effect Biomarkers**  
M. Oliveira, S. Costa, J. Vaz, A. Fernandes, K. Slezakova, C. Delerue-Matos, J. P. Teixeira, M.C. Pereira, and S. Morais
- 16:30 - 16:45 **Co-exposure of a Portuguese mining population to lead, manganese and arsenic**  
V. Lopes de Andrade, D.C. Serrazina, M. Cota, M.L. Mateus, and A. P. Marreilha dos Santos
- 16:45 - 17:00 **The Usefulness of Early Effect Biomarkers in Monitoring Occupational Exposure to Hexavalent Chromium**  
C. Afonso, B.C. Gomes, H. Louro, A. Nogueira, H. Pinhal, S. Reis, C. Ventura, C. Ladeira, E. Ribeiro, T. Santonen, S. Viegas, and M. J. Silva
- 17:00 - 17:15 **Human Biomonitoring of Multiple Mycotoxins in the Portuguese Population: Strengths and Limitations under Risk Assessment**  
C. Martins, M. De Boevre, S. De Saeger, C. Nunes, D. Torres, A. Goios, C. Lopes, R. Assunção, P. Alvito, and A. Vidal
- 17:15 - 17:45 **Concluding remarks and closure**  
João Lavinha (INSA, Ambassador of the Portuguese National Hub)





# invited speakers





# The Human Biomonitoring Initiative for Europe (HBM4EU): An overview

**Maria João Silva** | Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), Lisboa and ToxOmics, NOVA Medical School, Universidade Nova de Lisboa, Portugal

The Human Biomonitoring Initiative (HBM4EU, <https://www.hbm4eu.eu/the-project/>) is a Horizon 2020 Framework Project aimed at understanding the European citizens' exposure to chemical substances from environmental sources, food intake, consumer products or occupational activities. The HBM4EU is organized in three pillars, namely, science to policy, Human Biomonitoring (HBM) platform, and exposure and health, all working to generate harmonised HBM data across the 28 participating countries. To coordinate activities, a National Hub (NH) was established in every country, resulting in the creation of a European HBM network.

Following consultation to stakeholders and EU organizations, several substances were prioritized for study, e.g., phthalates, bisphenols, PAHs, cadmium, chromium, mercury, pesticides, and mycotoxins. A chemical group leader (CGL) was assigned to each substance/substance group, to formulate relevant policy questions and propose activities to be undertaken; a Portuguese team is the CGL for mycotoxins. In addition to the analysis of existent data sets for each substance, new and harmonized data on internal exposure to some of them and to mixtures is being produced, which will contribute to fill gaps in knowledge and policy needs.

The Portuguese participation in the HBM4EU has allowed to lay the foundations of what could become a wider national HBM network, e.g., by raising awareness of scientists, policy makers and stakeholders about the role of HBM in chemicals risk assessment or contributing to capacity building in some biomarkers analyses. Furthermore, new and comparable data on occupational exposure to hexavalent chromium and on Portuguese population exposure to several chemicals is being generated, which will feed into a pan-European publicly available database. The data will be used to evaluate current chemicals policies and to support the development of new ones, in order to reduce or prevent exposure to hazardous substances and the associated health outcomes, for a healthier future in Portugal and in Europe.

Work co-funded by the HBM4EU project, Grant Agreement No: 733032.

**Bio.** With a background in Pharmaceutical Sciences and specialization in Human Genetics and Toxicology, Maria João Silva is the PI of the Research Group in Genetic Toxicology at the Human Genetics Department, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisbon. She also co-leads the thematic line on Environmental and Genetic Determinants of Human Disease, Center of Toxicogenomics and Human Health, NMS-FCM, Universidade Nova de Lisboa. Her main research interests comprise environmental genotoxicity, chemicals and mixtures hazard assessment, nanotoxicology and human biomonitoring. She has participated as PI or researcher in national and international funded projects and currently coordinates the HBM4EU Project activities at INSA.



# Why bother with infertility when the world is overpopulated?

**Paulo Navarro-Costa** | Instituto de Saúde Ambiental, Faculdade de Medicina da Universidade de Lisboa and Instituto Gulbenkian de Ciência

Although one out of every ten couples of reproductive age are infertile, the world population is growing at an unprecedented rate. In the age of in vitro fertilization and of other even more advanced assisted reproduction techniques, should we still consider infertility a public health issue?

In this talk I will address myths and facts about infertility, and identify some of the areas where our collective knowledge about this disease is still surprisingly incomplete. I will present evidence suggesting that climate change may pose unexpected constraints to reproduction, and highlight the importance of combining basic and clinical research to tackle several outstanding challenges in human reproductive health. Given the sociodemographic, environmental, and behavioral correlates of infertility, the need for robust population-level data collection systems emerges as a priority in the field.

**Bio.** Paulo Navarro-Costa is an expert on oocyte and sperm cell development. He is principal investigator of a multidisciplinary project on the genetics of male infertility. Paulo develops his research at the Gulbenkian Institute (IGC) and at the Environmental Health Institute (ISAMB) in Portugal. He is a visiting professor at Lisbon's Medical School, assessor for the European Molecular Genetics Quality Network and member of the Editorial Board of the official journal of the American Society for Reproductive Medicine. Paulo has authored 10 scientific papers and his work has been cited more than 200 times. He has received 10 awards and distinctions.

# Integrating environmental and human monitoring: a case study of parental and pre-natal exposure to harmful substances in Aveiro region

**Susana Loureiro**<sup>1</sup>, Ana C. Alves<sup>1</sup>, Marta Fraga<sup>1</sup>, Madga Henriques<sup>2</sup>, Teresa Herdeiro<sup>2</sup>, Margarida Fardilha<sup>2</sup>, Carlos Gravato<sup>3</sup>, Amadeu MVM Soares<sup>1</sup>, and Marta S. Monteiro<sup>1</sup>

<sup>1</sup>Departamento de Biologia & CESAM, Universidade de Aveiro; <sup>2</sup>Instituto de Biomedicina-iBiMED, Universidade de Aveiro; <sup>3</sup>Faculdade de Ciências da Universidade de Lisboa & CESAM, Universidade de Lisboa

Urban and industrial areas with different potential sources and emissions of contaminants, such as potentially harmful elements (PHEs) and polycyclic aromatic hydrocarbons (PAHs) are prone to induce environmental and health problems. Increasing evidences indicate that the human reproductive function is affected by environmental contaminants, as well as the prenatal period, a particular sensitive windows of development.

Mercury is one of the metal contaminants that evidences concerns in terms of fertility and adverse reproductive outcomes. But, few studies have evaluated the molecular effect of Hg in reproductive health and the results are controversial. Thus, it is important to unravel the molecular mechanisms underlying the associated decline in human fertility. In addition, in the Aveiro district, parturient–newborn pairs were monitored in order to understand how life traits/habits influence long term contaminants' accumulation. Maternal hair presented total Hg levels with a mean value of 900 ng/g, which is lower than the USEPA and WHO acceptable threshold. However, 32% of all parturient hair samples analyzed were above 1000ng/g (USEPA) and 6% were higher than Hg levels considered acceptable by WHO (2000 ng/g). Furthermore, higher Hg content in placental tissues were found in comparison to previous reports from other European countries. Higher levels of other PHEs in placenta, such as aluminum, chromium, cadmium and nickel were associated with parturient resident in rural areas of Aveiro district. The element aluminum presented significantly higher levels in parturient from Estarreja county. Relatively to PAHs exposure, increased levels of naphthalene and phenanthrene equivalents in placenta were associated with parturient exposure to vehicle exhaust in their area of residence, while high levels of benzo[a]pyrene equivalents were associated with exposure to tobacco smoke at work.

Further research should be conducted in order to understand how environmental contaminants impair human reproduction along with other adverse health effects.

**Bio.** Susana Loureiro is an Assistant Professor with Habilitation at the University of Aveiro, department of Biology. She is a member of CESAM- Centre for Environmental and Marine Studies where she coordinates the Thematic Line Ecology and Functional Biodiversity. Susana Loureiro has been the PI of several funded national and international projects regarding environmental contamination, exposure, fate and effects to aquatic and soil biota. She has particular interest in emergent contaminants like nanomaterials and on mixture toxicity, in environmental and human health studies. In the recent years she has been involved in research projects that aim to biomonitor human populations regarding environmental contaminants' exposure.

# HBM in support of human health and environmental policies

**Teresa Borges** | Direção-Geral da Saúde

A major obstacle for a better assessment and management of chemical risks in Europe is that information about citizens exposure to chemical substances is insufficient, non harmonized therefore non comparable.

HBM supports that human exposure (general public and workers) can be assessed based on the measurement of chemical substances and/or their metabolites in human fluids and tissues exposure biomarkers), collected through controlled experimental studies.

Better information on the internal level of human exposure to hazardous chemicals from different sources can alert to early signs of disease and contribute to setting risk preventive measures.

In this sense, validated HBM data can be useful in regulatory chemical risk assessment and is the cornerstone to be achieved.

Better safety, better environment, better health!

**Bio.** Member of the Scientific Committee for Health, Environmental and Emerging Risks (SCHEER) of DG SANTE. Giving scientific advice to the European Commission on risks related to Consumer Health. Expertise in human health effects assessment, exposure assessment and risk assessment of chemicals under the Biocidal Product Regulation (BPR), REACH and CLP. National Coordinator for the human health Toxicological section of the Biocidal Competent Authority Reports (CAR) for the evaluation of active substances. Member of the Committee of Biocidal Products (BPC) of ECHA. Member of Nanomaterial WG (ECHA) for the development of information requirements for the registration of nanoforms under REACH. Expertise in hazard assessment of chemicals with regard to the application of CLP criteria (Reg. EU 1272/2008). Responsible for the CLH Reports for human health hazards of the biocidal active substances. National Program Owner in the European Human Biomonitoring Project – HBM4EU. LTP Partner in the Work Package 5, Task 5.3: Role of the HBM in the optimization of Chemical Risk Assessment and Policy Decision-Making.

# HBM relevance in occupational exposure and risk assessment – a case-study on chromium VI

**Susana Viegas** | Escola Superior de Tecnologias da Saúde de Lisboa, Instituto Politécnico de Lisboa & Escola Nacional de Saúde Pública, Universidade Nova de Lisboa

Human Biomonitoring (HBM) allows to assess the magnitude of internal exposure to environmental substances and how exposure is changing over time. In occupational health interventions, the main role of HBM is to assess exposure by all routes and to complement information obtained by workplace environmental monitoring (e.g. air monitoring). From the risk management angle, HBM can help to answer several questions related with the efficacy of the risk management measures in place, what are the highly exposed groups and much more. However, HBM is seen frequently as only a health surveillance data and not as an exposure assessment tool. A case study developed in the scope of HBM4EU project will be presented and discussed. The relevance of this study for the European regulatory framework in place and for more effective occupational health interventions will be demonstrated. It will be also shown the contribution of HBM4EU project for the harmonization of HBM protocols and the utility of HBM for assessing workers exposure and risk management measures efficacy.

**Bio.** Susana Viegas is graduated in Environmental Health from Lisbon School of Health Technology and have two Master degrees in Safety and Ergonomics from Universidade de Lisboa and Applied Toxicology from University of Surrey. She has a PhD in Public Health from National School of Public Health/Universidade NOVA de Lisboa. Actually, she is professor in National School of Public Health in the Department of Occupational and Environmental Health. The principal research topics are occupational and environmental toxicology, exposure and risk assessment, exposure to mixtures and food safety. She was a co-opted member in the Risk Assessment Committee in European Chemicals Agency between 2015 and 2018. Since 2019, she is working as an expert adviser for Portuguese Environment Agency in issues related with authorizations in the scope of REACH. Previously, Dr. Viegas has worked in different industrial companies performing exposure and risk assessment/management.

# HBM relevance in occupational exposure and risk assessment – a case-study on chromium VI

**Cristina Carvalho** | Research Institute for Medicines and Pharmaceutical Sciences (iMed.Ulisboa), Faculty of Pharmacy, University of Lisbon, Portugal.

Methylmercury (MeHg) is a fish contaminant with a severe impact in public health mainly due to its neurotoxicity. Portugal is one of the largest consumers in the world and the first fish consumer of Europe, therefore it is mandatory to assess the risk of the populations especially those more susceptible to the contaminant. It is known that MeHg exposure during pregnancy can have irreversible effects on the neurobehavioral development of children due to multiple deficits in neurons and glia including motor disturbances, dysesthesia, decreased IQ, impaired visual perception and speech, leading to neurodevelopmental negative effects on fetuses.

The populations' chosen to assess the exposure to MeHg have been pregnant women consuming seafood, their newborns as well children of different regions of Portugal.

To assess mercury exposure we have been using diaries and food frequency questionnaire (FFQ) with self-reported information on number of times and species of fish ingestion per week. Often estimated exposure was correlated with the levels of mercury in blood or hair, as biomarker of exposure. Data analysis has been used to calculate levels of risk. Uncertainties will be discussed for the different estimates.

JECFA established a tolerable daily intake (PTWI) of 1.6 µg/kg body weight per week for children, pregnant women and women at their fertile age. Among the studied populations 20-30% ingested a higher dose of mercury, indicating the existence of risk due to fish consumption and consequent MeHg exposure.

The levels of risk estimated justify the adoption of a strategy involving all stakeholders to adequately communicate risk to the populations. Fish consumption should be readjusted to include species containing lower Hg levels. Toddlers, babies, breast-feeding women, pregnant women, and women on fertile age should be particularly protected and aware of the potential danger of mercury, especially in the form of methylmercury.

**Bio.** Cristina Carvalho licentiate in Pharmaceutical Sciences in 1991 by Faculty of Pharmacy, University of Lisbon and took a PhD in Biotechnology in Instituto Superior Técnico in 1999. Presently she is an Associated Professor of Toxicology at Faculty of Pharmacy, University of Lisbon and a member of Research Institute for Medicines (iMed.Ulisboa). Her research interests include the study of the molecular mechanisms of mercury toxicity and the environmental and human risk assessment to mercury. Her group has been working in 1) the importance of the thioredoxin system in human health and disease (e.g. cancer); 2) selenium and selenoproteins as antagonists of mercury toxic effects; 3) exposure assessment of populations at risk of mercury; 4) identification of targets and mercury biomarkers.



# oral communications







# Exposure assessment of Portuguese women of childbearing age to methylmercury: first results of a human biomonitoring study

S. Santiago<sup>1,\*</sup>, S. Namorado<sup>1,2</sup>, C. M. Dias<sup>1,2</sup>, C. Martins<sup>1,3</sup>, C. Carvalho<sup>4</sup>, and R. Assunção<sup>1,3</sup>

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Methylmercury (MeHg) is a toxic compound that undergoes bioaccumulation in the aquatic food chain. Fish, especially predatory species such as tuna or swordfish, are an important human exposure source. Portugal presents the highest consumption of fishery and aquaculture products in the European Union (EU), above the EU and world average. The central nervous system is the main target of MeHg and the prenatal period represents a period of greatest vulnerability regarding neurodevelopmental effects on the fetus. As recommended by EFSA, the risk of exceeding the MeHg tolerable dose through consumption of fish and seafood should be properly assessed by each member state. Total mercury concentration in blood is usually considered a suitable biomarker for estimating short-term internal exposure to methylmercury, in individuals with regular fish consumption.

The present study aimed at evaluating the exposure of Portuguese women of childbearing age to MeHg through human biomonitoring. For this study, 300 Portuguese women of childbearing age (25 to 44 years) were randomly selected among the participants in a cross-sectional epidemiological study carried out in Portugal (INSEF, <http://www.insef.pt/>). Total mercury content was determined in whole blood samples collected in 2015 by thermal decomposition and amalgamation atomic absorption spectrophotometry (TDA/AAS).

Two samples had total mercury levels below LOQ (0.5 µg/L), and in the remaining (n=298), mercury levels ranged from 0.6 to 35 µg/L. Almost 52% of the samples had values below 5 µg/L, a HBM value below which no adverse health effects are expected. However, 48% of samples revealed levels above 5 µg/L, and therefore presented an increased risk of adverse health outcomes.

This study reinforces the need to develop and implement in Portugal strategies regarding risk communication focused on the selection of fish species with lower MeHg concentration in order to prevent human exposure to this compound, especially in particularly susceptible populations.

**keywords** Methylmercury, Human biomonitoring, Fish, Women of childbearing age

# The impact of secondhand smoking in intrauterine growth

T. Varandas<sup>1,2,\*</sup>, C. Ribeiro<sup>1,2</sup>, A. R. Marinho<sup>1,2</sup>, A. Pinto<sup>1,2</sup>, and E. Ramos<sup>1,2</sup>

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**Background/Objective:** Environmental exposure to tobacco during pregnancy causes adverse effects, such as restriction of fetal growth, abnormal development of the nervous system, preterm birth, placental abruption and perinatal mortality. Nowadays it is well known the effect of maternal smoking, however, less is known about the effect of secondhand exposure. The objective was to evaluate the effects of environmental exposure to tobacco - maternal and secondhand exposure (father or others) - during the gestational period on birth weight and length.

**Participants and Methods:** As part of the BiTwin cohort, we evaluated 477 singletons. The anthropometric parameters were obtained through medical records and questionnaires were used to evaluate environmental exposure to tobacco during pregnancy (in the 3 months before, in the 1st trimester and after the 3th trimester). Exposure was classified as “not smoked and not exposed” if the mother never smoked or was not exposed to secondhand smoke, “not smoked, but exposed to secondhand” if she never smoked, but was exposed to secondhand smoke, and “smoked either exposed or not” if she smoked at some or all pregnancy and was exposed to secondhand smoke. ANOVA test was used to compare means.

**Results:** In this sample, 64% mothers were “not smoked and not exposed”, 13% mothers “not smoked, but exposed” and 23% mothers “smoked either exposed or not” to external smoke. The mean (standard deviation) of birth weight (grams) was 3262.83(447.92) among “not smoked and not exposed”, 3210.41(390.70) among “not smoked, but exposed” and 3134.68(436.87) among “smoked either exposed or not”, ( $p=0.031$ ). Similar trend was found for length [49.41(2.15), 49.15(1.74) and 48.83(2.40),  $p=0.055$ ].

**Conclusion:** Although the effect was lower than maternal smoking, exposure to secondhand smoking during pregnancy induces growth reduction.

Health and Environment-wide Associations based on Large population Surveys (FP7-ENV-2013-603946)

**keywords** Secondhand smoke, Intrauterine development, Birth weight, Length

# Selenium and selenoproteins in blood and plasma of pregnant women from the Island of Madeira: Considerations about biomarkers and confounding factors

V. Branco<sup>1,\*</sup>, L. Carvalho<sup>1</sup>, C. Barboza<sup>1</sup>, M. E. Mendes<sup>1</sup>, A. Cavaco<sup>1</sup>, and C. Carvalho<sup>1</sup>

<sup>1</sup>Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Professor Gama Pinto, 1649-003 Lisbon - Portugal

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**Introduction:** Selenium (Se) is a micronutrient that can be found in several food sources, namely fish, meat, eggs and nuts. A proper Se intake has been linked to beneficial effects over the cardiovascular and immune systems and related with improved fertile function and reduced mortality. Also, Se has been pointed as being a key factor in the detoxification of mercury which is especially relevant in pregnant women due to foetal susceptibility to this neurotoxic compound.

**Methods:** We evaluated the Se status of 513 pregnant women from the Madeira Archipelago by quantifying Se in blood/plasma and the activity of the selenoenzymes glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) and of the enzyme thioredoxin (Trx). Moreover, the confounding effect of exposure to methylmercury on Se levels and enzyme activities was addressed since these enzymes (TrxR in particular) are important targets for mercury.

**Results:** The Se levels in whole blood ( $65 \pm 13.1$  µg/L) and low plasma GPx activity ( $69.7 \pm 28.4$  U/L) suggest a sub-optimal Se status for this population. On the other hand, TrxR being a high priority selenoenzyme, was kept a physiological relevant levels ( $12.3 \pm 5.6$  ng/mL). Surprisingly, TrxR was not affected by blood mercury levels which suggests the available Se is enough to maintain its activity. Interestingly, the activity of Trx, was negatively affected by increasing Hg levels.

**Conclusions:** Overall, these results emphasize the necessity to: (1) enhance consumption of Se rich foods or supplement intake by pregnant women of Madeira; (2) properly establish the levels of Se needed to optimize activity of GPx and TrxR in plasma considering co-exposure to potentially confounding factors such as Hg; (3) evaluate how Se levels and enzymatic activities in the foetus reflect the observations in maternal blood/plasma namely concerning Hg exposure to properly estimate risk.

**keywords** Selenium, Selenoproteins, Thioredoxin, Mercury

# Urinary levels of Manganese, Cooper, Tin and Antimony in Portuguese children

L. Correia-Sá<sup>1,2,\*</sup>, V. C. Fernandes<sup>1,2</sup>, M. L. Maia<sup>1,2</sup>, S. Norberto<sup>2</sup>, C. Delerue-Matos<sup>1</sup>, C. Calhau<sup>2,3</sup>, and V. F. Domingues<sup>1</sup>

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Many studies revealed the relationship between metals and obesity, but the results were not consistent. Thereat, there is a growing interest in evaluating the health effects of exposure to metals in trace levels.

The aim of the study was to evaluate the relationship between obesity and urinary levels of Manganese, Cooper, Tin and Antimony in Portuguese children.

Children were recruited from the pediatric appointment at Hospital de S. João, and several local schools, in 2014 and 2015, and were from Oporto and Aveiro, two Portuguese districts, in the North and Central region of the country.

The children were divided in two groups: the group “normal weight” included healthy children which were normal-/underweight; the group “obese/overweight” included children diagnosed for obesity/overweight without other known associated diseases. First morning urine samples were collected from each participating child. The study was approved by the ethics committee of the Centro Hospitalar S. João/FMUP and all the parents provided written consent. Urine analysis was performed by inductively coupled plasma-mass spectrometry (ICP-MS).

Urinary concentrations of Manganese, Cooper, Tin and Antimony were investigated in 110 urine samples, from 4 to 18 years old Portuguese children. The study group was composed of 55 % girls and 55 % boys, with a median age of 10 years old. The majority of the children was overweight/obese (61 %; n=67). Statistical differences (in µg/L) were observed ( $p<0.05$ ), between the two studied groups, for Manganese, Cooper, Tin and Antimony.

Obese/overweight children presented higher median values for these compounds compared to the normal-weight children except for Tin. Risk assessment also showed that the maximum values determined for Antimony and Cooper were some orders of magnitude above reference values thus, alerting for further need of research on this topic/field (future monitoring interventions in the country).

# Plasma concentration of brominated flame retardants and breast cancer risk: A nested case-control study in the French E3N cohort

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**Introduction:** Brominated flame retardants (BFRs) are lipophilic substances characterized by endocrine-disrupting properties. Although there is increasing interest in understanding the contribution of BFRs to breast cancer (BC) incidence, evidence of an association between environmental exposure to these chemicals and BC is still inadequate to draw firm conclusions. This study aimed to investigate the associations between plasma concentrations of two main groups of BFRs, namely PBDEs (penta-bromodiphenyl ethers) and PBBs (polybrominated biphenyls), and the risk of breast cancer in women in a case-control study nested in the E3N cohort.

**Methods:** 197 incident BC cases and 197 controls with a blood sample collected between 1993 and 1995 were included in the study. Plasma levels of PBDE congeners (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154) and PBB-153 were measured by gas chromatography coupled to high-resolution mass spectrometry. Conditional logistic regression models, adjusted for potential confounders, were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) of BC risk in relation to plasma concentration levels of PBDEs and PBB-153.

**Results:** The results highlighted overall a lack of association between plasma levels of PBDEs and PBB-153 and BC risk. However, a non-linear inverse association was observed between levels of BDE-100 and BDE-153 and BC risk with the exposure expressed as ng/L plasma, while no association was found when using the exposure expressed as ng/gr of lipids in the plasma. Similar results were obtained after stratification by tumor hormone receptors expression and body mass index categories (<25 and ≥25).

**Conclusion:** Our results suggest no clear association between internal levels of PBDEs and PBB-153 and risk of BC, though they need to be carefully interpreted, taking into account study limitations due to the study design and availability of data. More studies are warranted to further investigate the relationships between PBDEs and PBBs exposure and BC risk.

**keywords** Brominated flame retardants, Pentabromodiphenyl ethers, Polybrominated biphenyls, Breast cancer

# Population exposure to Particulate-Matter and estimated excess mortality due to the Portuguese wildfires in October 2017 driven by Storm Ophelia

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**Introduction:** In October 2017, hundreds of wildfires ravaged forests in the north and centre of Portugal. The fires were fanned by strong winds as tropical storm Ophelia brushed the Iberian coast. The storm has dragged up smoke from the Portuguese wildfires, together with Saharan dust from NW Africa, into western Europe, including the United Kingdom.

**Methods:** Here we estimate the excess mortality in those populations exposed to particulate matter (PM) due to the Portuguese wildfires in October 2017, one of the worst fire sequences over the last decade. We analysed space- and ground-level observations to track the smoke plume and dust trajectory over Portugal and Europe, and to access PM concentrations during the wildfires.

**Results:** The population in Portugal was exposed in average to additional PM<sub>10</sub> levels that varied from 16.2 to 120.6 µg/m<sup>3</sup> in smoky days with dust and from 6.1 to 20.9 µg/m<sup>3</sup> in dust-free smoky days. Considered together, and assuming a 0.5%-2% increase in all-cause mortality per 10 µg/m<sup>3</sup> derived from epidemiological studies, these exposure levels may have been associated with 56-224 cases of additional mortality (112 cases, central estimate), which represents 0.11% of the country mortality in 2017. In the United Kingdom (England, Wales, Scotland and North Ireland), the population was exposed in average to an additional PM<sub>10</sub> level of 11.7 µg/m<sup>3</sup> during seven smoky days (three were with dust), which may have been associated with 0.022% of the country mortality in 2017.

**Conclusions:** With increasing temperatures and frequency of storms due to climate change, PM from Iberian wildfires together with NW African dust will be dragged up more often into Northern European countries. In urban areas, where PM levels are already high, this increment in PM might become an emerging threat to human health.

**keywords** Wildfires, Air pollution, Climate change, Human health

# Biomonitoring of Wildland Firefighters: Levels of urinary biomarkers of exposure to Polycyclic Aromatic Hydrocarbons and Genotoxic/oxidative-effect biomarkers

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Firefighting represents one of the riskiest occupations, yet due to the logistic reasons, limited information exists. This work assesses firefighters' occupational exposure by six biomarkers of exposure (OHPAHs: 1-hydroxynaphthalene, 1-hydroxyacenaphthene, 2-hydroxyfluorene, 1-hydroxyphenanthrene, 1-hydroxypyrene, and 3-hydroxybenzo(a)pyrene) and two biomarkers of effect (basal and oxidative DNA damage).

A total of 171 firefighters filled a questionnaire, signed an informed consent and were divided into three different groups: A) non-smoking and non-exposed subjects to fire combat (Control group), B) non-smoking and exposed individuals (who participated in firefighting), and C) smoking and exposed firefighters. Firefighters' arterial oxygen saturation, blood pressure and cardiac frequency were registered; sampling of spot urine and venous blood samples was also performed. Urinary OHPAHs and creatinine levels were determined by previously validated methodology [1] and genotoxic/oxidative-effect biomarkers were assessed by the comet assay [2].

Concentrations of total OHPAHs (up to 340%;  $p \leq 0.05$ ), 1-hydroxynaphthalene+1-hydroxyacenaphthene (82-88% of total OHPAHs) and 2-hydroxyfluorene (5-15%) were significantly higher in exposed firefighters than in Control group. Levels of biomarker for oxidative stress were increased in non-smoking exposed workers than in control group (316%;  $p \leq 0.001$ ); data for basal DNA damage was inconclusive. Positive correlations were found between cardiac frequency, urinary OHPAHs and the oxidative DNA damage of non-smoking (non-exposed and exposed) firefighters; however, no causal relationship was found. More studies are needed to validate these findings.

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[1]Oliveira et al. J. Hazard. Mater. 323 (2017) 184-194.

[2]Abreu et al. J. Toxicol. Environ. Health A 80 (13-15) (2017) 596-604.

**keywords** Firefighting, Tobacco smoking, Urinary monohydroxyl metabolites, DNA damage



# Co-exposure of a Portuguese mining population to lead, manganese and arsenic

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Miners are repeatedly exposed to chemical mixtures, with lead (Pb), manganese (Mn) and arsenic (As) among the major toxicants present in their working environment, causing these populations a chronic exposure to this toxic mixture. Pb, Mn and As induce common adverse outcomes, such as anemia and changes in the nervous system and reproduction, possibly justifying the appearance of some adverse effects at concentrations below environmental quality guideline levels for individual mixture components. There is little information regarding the levels of metal mixture exposure of miners in Portugal being this knowledge fundamental so that subclinical effects may be identified at an early stage [1].

The objective of this work was to evaluate the exposure of a mining population to the three referred metals and compare the results with a control population, as well as with limit values proposed by international regulatory agencies.

Blood and urine samples of workers were obtained from a Portuguese mining industry (N=60) and compared with a control population (N=80). The levels of Pb, Mn and As were determined in blood and urine by atomic absorption spectrophotometry.

The miners' concentrations of the three metals in blood and urine were higher and significantly different ( $p < 0.05$ ) from controls. The average concentration of Pb found in the miner's blood was higher than the limit values proposed by ACGIH [2] and SCOEL [3] agencies, while Mn values were superior to the German MAK Commission limit [4]. Concerning As, the values found in the miners' blood suggest an occupational exposure after comparing with non-occupationally exposed subjects [5]. Overall, this work revealed that the working population is co-exposed to Pb, As and Mn near limit values. We conclude that biological monitoring of this working population regarding metals body burden is advisable, since prevention may contribute for a better health and quality of life.

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**keywords** lead, manganese and arsenic co-exposure; occupational exposure; mining population; biological monitoring

# The usefulness of early effect biomarkers in monitoring occupational exposure to Hexavalent Chromium

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**Introduction:** In the frame of the Project HBM4EU (<https://www.hbm4eu.eu/>) a biomonitoring study is being conducted across Europe to characterize occupational exposure to hexavalent chromium [Cr(-VI)], a recognized human lung carcinogen. Herein we present the results of biomarkers of exposure and effect characterized in a group of workers from a Portuguese aircraft maintenance company, where Cr(VI) replacement is not expected in the near future.

**Methods:** All participants signed an informed consent and responded to a questionnaire to collect contextual information. Cr(VI) exposure was assessed by the analysis of personal air samples; total Cr was measured in urine and blood samples from 50 workers and 26 controls. The effects biomarkers included the alkaline comet assay and the cytokinesis-block micronucleus assay in blood in leukocytes.

**Results:** The concentrations of Cr in the air samples were variable, depending on the activities performed. Noteworthy, workers engaged in painting exterior surfaces with Cr(VI)-reach paints were exposed to the highest Cr(VI) levels. Results from biomonitoring confirmed the occurrence of internal exposure. Furthermore, significantly increased micronuclei frequencies ( $p < 0.001$ , Fisher's exact test) and DNA damage levels were identified in leukocytes from workers comparatively to controls. Interestingly, among the painters showed up a group of 6 workers who were exposed to a mixture of Cr, organic solvents, silica, and other chemicals and who displayed an elevated level of micronucleated cells comparatively to matched controls.

**Conclusions:** An association between Cr(VI) exposure and genotoxicity biomarkers was found, raising concern about a potentially increased cancer risk. Furthermore, these results suggest that the effect biomarkers selected are useful to strengthen the exposure data, in that more exposed or vulnerable groups can be more reliably detected. This is relevant to allow the prioritization of occupational hygiene and risk management measures implementation in the workplace.

Work co-funded by the H2020 HBM4EU project (GA 733032).

**keywords** Biomarkers, Occupational exposure, Hexavalent chromium

# Human Biomonitoring of Multiple Mycotoxins in the Portuguese Population: Strengths and Limitations under Risk Assessment

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Mycotoxins constitute a relevant group of food contaminants with associated health outcomes such as estrogenic, immunotoxic, nephrotoxic and teratogenic effects. Although scarce data are available in Portugal, human biomonitoring (HBM) studies have been globally developed to assess the exposure to mycotoxins at individual level.

This study aimed to present data for mycotoxins' urinary biomarkers within a human biomonitoring study developed to assess the exposure of the Portuguese population, and to characterize the risk associated to the exposure.

In the scope of the National Food, Nutrition, and Physical Activity Survey of the Portuguese General Population (2015-2016), 24h-urine samples from 94 participants were analyzed by liquid chromatography–mass spectrometry (LC-MS/MS) for the simultaneous determination of 37 mycotoxins' urinary biomarkers. Data obtained were used to estimate the probable daily intake as well as the risk characterization applying multiple imputation, reverse dosimetry and hazard quotient approaches.

Results revealed the exposure of Portuguese population to zearalenone, deoxynivalenol, ochratoxin A, alternariol, citrinin and fumonisin B<sub>1</sub>. Risk characterization data revealed a potential concern to some reported mycotoxins since the reference intake values were exceeded by some of the considered participants. The use of data at individual level, the collection of 24h urine samples, the performance of analytical method and the use of multiple imputation approach were identified as the main strengths of this study. The limitations identified were related with the use of excretion data obtained within animal studies and the absence of health based guidance values for urinary biomarkers that would allow a direct comparison.

The present study generated, for the first time and within a HBM study, reliable data on internal exposure to multiple mycotoxins at individual level for the Portuguese population. These data contributes for supporting risk managers in the establishment of preventive policy measures to ensure public health protection.

**keywords** Human biomonitoring, Risk assessment, Biomarkers of exposure, Mycotoxins



# discussion forum





- Do you consider important to use HBM to assess human exposure to chemicals?
- Which constraints do you anticipate when using this tool for chemical risk assessment?



**Cesaltina Ramos (DGS, Moderator)**

Public Health Doctor.

Deals with EU discussions and national implementation of regulations REACH, CLP and Biocides, since 2002.



**Inês Martins de Almeida (DGAV)**

Degree in Veterinary Medicine.

Head of the Veterinary Medicines Unit.

Deals with EU discussions and national implementation of regulations VMP and Biocides of Veterinary use since 2008.



**Ana Bárbara Oliveira (DGAV)**

Degree in Agronomic Engineering.

Agronomist.

Head of Services Directorate competent for the Authorisation of Plant Protection Products & Biocides (Wood Preservatives).



**Narcisa Bandarra (IPMA)**

Degree in Applied Chemistry.

Head of Aquaculture, Upgrading and Bioprospecting Division and Researcher

Deals with the evaluation of risk benefit of seafood products.







# posters





# Health studies: Opportunities for the development of HBM in Europe

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**Introduction:** Human Biomonitoring (HBM) and health studies are very similar in terms of the infrastructure and procedures necessary for their implementation, as in either type of studies data is collected through fieldwork, which constitutes one of the largest expenditures for such studies. Thus, combined studies could result in more cost-effective ways to conduct health and environmental monitoring. As such, within the HBM4EU project an inventory of the health studies available which could include an HBM component was performed.

**Methods:** An online questionnaire was developed to collect information on recently conducted, ongoing and planned health studies, which could be linked to an HBM study. The link to the questionnaire was distributed with the help of the National Hub Contact Points of the HBM4EU project.

**Results:** From the 58 different studies included in this inventory, half were longitudinal and presented the possibility of introducing an HBM component in the future. Most of the studies for which data was reported had public funding, either from the government or from public grants (national or European). The vast majority of the studies included the collection of biological samples and the most frequently stored samples were blood, plasma, serum or DNA. More than 50% of the studies reported that the measurement of chemicals was already performed or was planned to be performed. The most frequently measured chemicals were phthalates, bisphenols and cadmium.

**Conclusions:** In vast majority of the studies included in the inventory biological samples are collected and stored, posing the opportunity to use them in HBM studies for the analyses of chemicals of interest. About 50% of these studies already had ethical approval to measure chemicals from collected samples.

**Funding:** HBM4EU has received funding from the European Union's Horizon 2020 research and innovation programme (grant agreement 733032).

**keywords** Human biomonitoring, Health examination survey, Biological samples, HBM4EU

# Environmental occurrence of contaminants as indicators for Human bioMonitoring? A based-exercise for Portugal

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**Introduction:** The “One Health Triad” requires the implementation of strategies aimed to an integrated collaboration on different scientific fields. Based on this principle and taking into account the results of an occurrence exercise in surface water in Portugal for pesticides (Directives 2000/60/EC; 2013/39/EU) we identified several hot points exposure-scenarios, which may be used as indicators for possible human bio monitoring. The main goal of our work comprises a prioritization approach for the pesticides detected as well as their relevant inherent properties, metabolites, residues and possible effects in humans.

**Methods:** The sampling was done during 3 years in 17 stations located in rivers from north to south of Portugal, in a time period concerning the field treatment. The pesticides were detected qualitatively using 2 POCIS (polar organic integrative sampler) disks/each sampling station. The samples were analyzed by system liquid chromatography (LC) coupled with high-resolution mass spectrometry (HRMS) with quadrupole-time-of-flight (Q-TOF). To identify the “hot-point exposure scenarios”, the pesticides were prioritized by their detection frequency by region and/or station, environmental fate and human kinetic / effect.

**Results:** 87 different pesticides were detected, some of them not authorized in Portugal. The “hot-point exposure scenarios” were located essentially in Algarve (Ria Formosa), Rio Tejo and Rio Ave. Specific contaminants, as simazine, terbutylazine, tebufenozide or rabenzazol reached high percentage of frequency of detection.

**Conclusion:** The present work has identified several acceptable exposure scenarios as possible indicator for human bio-monitoring. The pesticide groups ranged from fungicides to insecticides and herbicides. For instance, rabenzazol is a fungicide mainly used in cereals and fruit/vegetables and its residues are often found in food (1) In our study, a detection frequency of 65% has been observed.

(1) Yanwei Fu et al. A comprehensive analysis of 201 pesticides for different herbal species-ready application using gas chromatography–tandem mass spectrometry coupled with QuEChERS, 2019, Journal of Chromatography B, Vol. 1125, 121730

**keywords** Pesticides, Exposure-scenarios, POCIS, Rabenzazol

# INSEF-ExpoQuim: Assessing the exposure of the Portuguese population to chemicals

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**Introduction:** Exposure to hazardous chemicals may endanger human health and pollute the environment. To assess and minimize the risks associated with the use of chemicals it is essential to know whether and to what extent these substances are present in the human body. We report preliminary results of the recruitment phase in the study of Exposure of the Portuguese Population to Selected Environmental Chemicals: INSEF-ExpoQuim.

**Methods:** INSEF-ExpoQuim is an epidemiologic study nested in INSEF 2015 targeting 300 noninstitutionalized individuals aged 28-39 years, living in Portugal for more than 12 months, able to follow an interview in Portuguese. Fieldwork started in June 2019 and is ongoing. Procedures are according to the guidelines of the HBM4EU project. Selected individuals receive an invitation letter and are later contacted by phone to schedule sample collection and the telephone interview. Urine samples for determination of heavy metals, bisphenols and Polycyclic Aromatic Hydrocarbons are collected, as well as data on sociodemographic characteristics, living conditions and residential history, habits/lifestyle, nutrition, health, occupation and substance specific information covering nearly all exposure pathways.

**Results:** Up to date contacts 384 of the 848 eligible individuals were successfully contacted (45,3%). of which 172 accepted to participate in INSEF-ExpoQuim corresponding to a participation rate of 20%.

**Conclusions:** Results from INSEF-ExpoQuim will contribute to reduce the health impact that could result from the exposure of the population residing in Portugal to environmental chemicals, by producing high quality data on the actual exposure of the Portuguese population to hazardous chemicals, in order to support the development and implementation of policy measures aimed at minimizing exposure to those chemicals.

**keywords** Human biomonitoring, Heavy metals, Bisphenols, PAHs.

# Portuguese citizens' perceptions on Human Biomonitoring: preliminary results of a qualitative approach

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**Introduction:** People of all ages are daily exposed to a wide range of chemical substances present in the air, food, water and materials, namely at workplace. The diversity of exposure routes and pathways are wide, and a great part is virtually inevitable due to the variety of human activities. The HBM4EU is a joint initiative that aims to generate evidence of the actual exposure of citizens to chemicals and the possible health effects to support policy making. Considering that both parts of the science-politics dyad are driven by people's needs, it is crucial to know perceptions on Human Biomonitoring (HBM) to efficiently communicate about the initiative's leitmotiv and relevance to citizens.

**Methods:** One focus group (FG) was conducted with citizens selected through purposive sampling. Written informed consent was obtained from participants. Audiotaped record was fully transcribed, and content analysis of the full anonymized corpus was made.

**Results and discussion:** The FG included 10 Portuguese citizens (five males) with an average age of  $46,6 \pm 14,6$  years and heterogeneity regarding educational level. Preliminary results show concerns regarding pollutant exposure, particularly the link between sources and pathways of pollution. Environmental determinants of human and animal health were identified, with concerns on long-term impact of exposure to low dose of chemicals. The majority of participants have not achieved a definition of HBM but the idea that we all do a sort of individual-level HBM emerged. Several participants reported to have information deficits, and one mentioned that an excess of information is also harmful (potentially leading to alarmist responses). Despite a generalized distrust on politics, national authorities and industry sponsoring science, participants expressed hope that HBM4EU results would be effectively translated into political measures for the common good. Adequate information policy and communication strategies were considered necessary for making people recognize the importance of HBM.

**keywords** Human biomonitoring, perception, citizens, focus group

# Pesticides, mycotoxins and children: biomonitoring and risk assessment

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**Introduction:** Cancer is a leading cause of death for children, with 300,000 new cases diagnosed each year among children. For many chemicals, such as pesticides and mycotoxins, the health impacts associated with exposure remain unknown. It is also of concern the exposure to mixtures of chemicals in this vulnerable population. Urine and breast milk biomonitoring is non-invasive valuable tool to evaluate individual exposure. Our main goals were the determination of pesticides (glyphosate; IARC 2A) and mycotoxins (ochratoxin A-OTA; IARC 2B and aflatoxin M1-AFM1; IARC 1) in urine of Portuguese children and breast milk to better characterize the health risk of this population.

**Methods:** The analytical methodologies were based on IAC-HPLC-FD and competitive ELISA. Urine samples were collected from healthy children from the Central zone of Portugal, aging between 2 and 13 years old. Breast milk samples were collected up to 27 months post-partum. Volunteers completed a questionnaire concerning anthropometric data and diet habits.

**Results:** Children's' urine biomonitoring showed high OTA incidence (92.9%) with levels up to 0.052 ng/mL. Depending on the OTA excretion rate considered for the PDI calculation, the risk assessed for the average OTA contamination varied from 10% to 194%, indicating a potential concern. Regarding glyphosate, a high incidence was also observed (97.6%) with a maximum level of 4.58 ng/mL. Concerning breast milk, OTA contaminated 97.6% of the samples up to 560 ng/L, whereas AFM1 appeared in 32.8% of the samples with maximum of 1.9 ng/L. Estimated risk was higher for young babies for OTA (<7kg; 720,7%) and for AFM1 (<7kg; 530%).

**Conclusions:** These are the first reported studies regarding OTA, AFM1 and glyphosate exposure in Portuguese children, showing a clear exposure and highlighting the need for further surveillance research work. Both biological samples revealed to be suitable for biomonitoring approach to assess exposure in this vulnerable population.

**keywords** Pesticides, Mycotoxins, Biomonitoring, Children

# Mycotoxins under the Human Biomonitoring Initiative (HBM4EU): contribution for an update on human exposure across Europe

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The Human Biomonitoring Initiative HBM4EU (<https://www.hbm4eu.eu/the-project/>) is a joint effort of 28 countries and the European Environment Agency under the EC Horizon 2020 Programme. Since 2017, and during 5 years, HBM4EU is generating evidence on the current exposure of European citizens to chemicals and the possible health effects. Portugal, as member of HBM4EU, created a National Hub to answer project questions as well as contribute to gather national expertise on human biomonitoring. Following a systematic prioritization exercise, which brought together national and EU level policy needs for knowledge on chemical exposure and health outcomes, mycotoxins (Deoxynivalenol and Fumonisin B1) were, in the 2nd prioritisation round, considered as priority substances being Portugal selected as Chemical Group Leader.

Mycotoxins are secondary fungal metabolites often found as natural contaminants in agricultural commodities all over the world and their occurrence poses a risk for human and animal health. Currently, the main human and animal health burdens of mycotoxin exposure are related to chronic toxicity, such as carcinogenic, teratogenic, immunotoxic, nephrotoxic, and endocrine disrupting effects. Moreover, the expected climate change scenario will affect mycotoxin incidences and levels on the agricultural crops that might result in higher exposure for European citizens. Within the three pillars of the HBM4EU, namely Science to Policy, European HBM Platform and Exposure and Health, the most relevant policy questions for mycotoxins were identified. These included the identification of knowledge gap on the current levels of human exposure, time and geographical trends in Europe, and highly exposed subgroups (including workers), the availability of toxicokinetics data, methods for exposure biomarkers analysis in human samples, the usefulness of effect biomarkers, the use of biomonitoring data under a risk assessment framework, derivation of HBM guidance values, exposure to mixtures of mycotoxins and potential interactive effects.

Work co-funded by the HBM4EU project, Grant Agreement No: 733032.

**keywords** Mycotoxins, Human biomonitoring, HBM4EU



# The association between phthalate metabolites and body mass index: a meta-analysis in children and adults

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Exposure to environmental chemicals has become one of the major concerns in the past decades. Phthalates are a family of synthetic organic chemicals used in the manufacture of plastics, solvents, and personal care products. These compounds are considered as endocrine-disrupting compounds (EDCs) since they may interfere with the endocrine system and disrupt its physiologic function. The purpose of this work is to synthesize results from published literature on the association between the exposure to phthalates and adiposity in adults and children.

We searched PubMed from inception up to 01 August 2019, to retrieve original papers reporting the association between EDCs and adiposity. The study variables, characteristics and association measures were extracted from the papers. Studies examined several adiposity measures, but body mass index (BMI) was the most commonly reported. Hence, we used beta coefficients regarding BMI and odds ratios when BMI outcome was categorical (obesity or overweight) to perform the meta-analysis. Data from the studies were combined using fixed effects meta-analyses to compute summary regression coefficients or odds ratios and corresponding 95% confidence intervals. Heterogeneity between studies was assessed by the I<sup>2</sup> statistic.

The systematic review included 29 publications addressing the association between phthalate metabolites and adiposity. In children, the meta-analysis showed negative associations for MiBP, MCPP and MbzP metabolites and a null association for MBP, although none of them presented statistical significance. For adults, meta-analyses were possible to perform only for MEP and MEHP. A positive association was found for MEP and, conversely, a negative association was found for MEHP, but none reached statistical significance. These compounds presented similar results in children.

The inconsistencies found between studies did not allow to reach a definitive conclusion. However, most of the results in the systematic review presented a positive association between phthalates and adiposity measures.

**keywords** Endocrine Disruptors; Phthalic Acid; Adiposity, PubMed

# Contribution to Hazard Characterization of the Next-Generation Plasticizer DINCH®: Cytotoxicity and Genotoxicity Assessment in Human Cells

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The chemical diisononyl cyclohexane-1,2-dicarboxylate (Hexamoll® DINCH®), a cyclohexanoate plasticizer, has been employed as a safer alternative to restricted phthalates, to increase flexibility and elasticity of many consumer products made of PVC or polystyrene, namely food packaging, children's toys, and medical devices. Concomitantly, the detection of DINCH in human surroundings and in biological matrices has increased during the last decade. This prompts the establishment of biomonitoring guidance values for DINCH metabolites in urine, as a measure of precaution; however, the studies about potential adverse effects of DINCH in humans are still scarce. DINCH is not classified as reprotoxic nor genotoxic and mutagenic however, there are limited data available regarding safety assessment, especially regarding cytotoxic and genotoxic effects.

Since liver and kidney are DINCH target organs in animal models, the aim of this study was to assess DINCH cytotoxic and genotoxic effects in a human liver (HepG2) and kidney cell lines (HK-2). The methodology included the MTT cell viability, micronucleus, conventional and FPG-modified comet assays to detect cytotoxicity and genotoxicity.

The results showed that DINCH was moderately cytotoxic for kidney cells exposed for 48h, but not for liver cells. No chromosomal damage was induced after short-term or longer exposures of both cell lines. However, DINCH was able to induce oxidative DNA damage in liver cells exposed for 3h, which decreased after a more prolonged exposure. The occurrence of oxidative lesions, even transiently, implies that mutation fixation may occur leading to adverse effects in liver. Overall, the present work provides new insights into the potential toxicity of this next-generation plasticizer in kidney and liver cells, in spite of public reports in which DINCH is classified as non-genotoxic agent. On the other hand, human biomonitoring studies are fundamental to confirm the current levels of human internal exposure to DINCH, as well as to detect early biologic effects.

**keywords** DINCH; Cytotoxicity; Genotoxicity; Hazard characterization

# Heavy metals and arsenic exposure in Portuguese pregnant women and the anthropometric profile of the offspring at birth: results from the IoMum cohort study

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**Introduction:** Portuguese evidence of prenatal exposure to environmental pollutants such as heavy metals and metalloids is lacking. The aim of our study was to assess exposure to lead (Pb), cadmium (Cd) and arsenic (As) and its correlation with the anthropometric profile of the offspring at birth, in a cohort of Portuguese pregnant women.

**Methods:** Pregnant women attending routine 1st trimester ultrasound scan (T1) from April 2018 to April 2019 at São João Hospital Center were invited to participate. Those in the gestational week between 10 and 13, with confirmed fetal vitality and who signed the informed consent were included in the study. After exclusion criteria application (levothyroxine intake, twin pregnancy, delivery after the 6th of June 2019), 199 mother:newborn pairs were obtained. Pb, Cd and As were measured by inductively coupled plasma mass spectrometry (ICP-MS) in T1 spot urine samples. Multiple linear regression models were used to analyze the association between exposure to Pb, Cd and As and birth weight, length and head circumference, considering the co-variables smoking habits, maternal age, weight gain until T1, pre-gravid body mass index, gestational age at delivery and newborn sex.

**Results:** The median levels of heavy metals fell within the corresponding reference ranges. Negligible exposure to Cd was found (median of 0.00 µg/L), with only 1% of the population presenting Cd levels above the upper limit of the reference interval. However, 19 and 32% of women had levels of Pb and As above the corresponding reference ranges. Additionally, it was found that Pb exposure was positively associated with birth weight and length (adjusted mean difference in birth weight and length between the group of mothers with Pb levels above reference and those within the reference range (beta) of 180.6 grams [ $p=0.006$ ] and 0.676 cm [ $p=0.022$ ], respectively).

**Conclusion:** This study evidences that Portuguese pregnant women are exposed to heavy metals that may affect fetal growth.

**keywords** Heavy metals, Pregnancy, Birth outcomes

# Selenium urinary levels in Portuguese pregnant women and the anthropometric profile of the offspring at birth: results from the IoMum cohort study

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**Introduction:** Selenium (Se) is a component of the antioxidant system and its deficiency has been associated with poor pregnancy outcomes. The aim of our study was to determine the Se urinary levels in a cohort of Portuguese pregnant women and test for an eventual correlation with the anthropometric profile of the offspring at birth.

**Methods:** Pregnant women attending routine 1st trimester ultrasound scan (T1) from April 2018 to April 2019 at São João Hospital Center were invited to participate. Those in the gestational week between 10 and 13, with confirmed fetal vitality and who signed the informed consent were included in the study. After exclusion criteria application (levothyroxine intake, twin pregnancy, delivery after the 6th of June 2019), 199 mother:newborn pairs were obtained. Se was measured by inductively coupled plasma mass spectrometry (ICP-MS) in T1 spot urine samples. A multiple linear regression model was used to analyze the association between Se exposure and birth weight, length or head circumference, considering the following co-variables: smoking habits, maternal age, weight gain until T1, pre-gravid body mass index (BMI), gestational age at delivery and newborn sex.

**Results:** Median urinary Se (24.9 µg/L) fell well within the reference range. Nevertheless, 29 and 17% of women presented Se urinary levels below and above the reference interval, respectively. Se levels tended to vary with the municipality of residence: 2 times higher in Maia (median 30.8 µg/L) when compared to Gondomar (median 17.5 µg/L). Additionally, it was found that Se levels were positively associated with birth weight and head circumference (adjusted mean difference in birth weight and head circumference between the group of mothers with Se levels above reference and those within the reference range (beta) of 175.4 grams [ $p=0.010$ ] and 0.592 cm [ $p=0.021$ ], respectively).

**Conclusion:** This study evidences the importance of Se during pregnancy, namely its impact on fetal growth.

**keywords** Selenium, Pregnancy, Birth weight, Head circumference

# Risk-benefit assessment associated to fish consumption by the Portuguese population: A representative overview

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Fish is a key component of Portuguese diet. Moreover, fish consumption has been recommended due to its assumed health benefits. Indeed, fish are rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which prevent health problems, such as cardiovascular disease. Fish are also a source of essential elements, such as selenium (Se), which, among other benefits, reduce the negative effects of toxic metals (e.g., methylmercury, MeHg). In particular, fish species are the main dietary source of MeHg, exposure to which can affect the neurocognitive development of children. Thus, it is important to weigh benefits versus risks in fish consumption. In order to fulfill this objective, a state-of-the-art mathematical-statistical modeling was used for integrating the available data regarding MeHg, Se, and EPA + DHA contents in fish. For a more realistic assessment of the benefit-risk binomial, culinary treatments and bioaccessibility/bioavailability aspects were considered. It was also evaluated the Se health benefit value (Se-HBV) to predict the benefit of Se against MeHg toxicity. Accordingly, new insights into the assessment of recommended levels of consumption of small pelagic species, such as sardine, and large carnivorous fish on the top of the food web, such as tuna, were achieved. Concerning EPA+DHA, species such as sardine provide maximum benefit with a daily meal and a negligible risk regarding MeHg. For tuna, although Se-HBV value was positive, consumption of tuna subjected to specific culinary treatments, such as grilling, should not be frequent. Moreover, risk-benefit analysis showed that blue shark, regardless of the way it is cooked, displayed high probabilities of surpassing the MeHg tolerable weekly intake (TWI), >22 %. For this shark, Se did not neutralise MeHg toxicity. Hence, this species should be rarely consumed. On the whole, it was shown that fish consumption benefits outweigh risks for almost all species and culinary treatments.

**keywords** Risk-benefit assessment, Portuguese population, Fish consumption, EPA+DHA/Se/MeHg

# Mercury levels in high school students from Lisbon

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Human health is intimately linked to environmental quality and this intricate link has been recognized not only by scientists all over the world but also by policy makers and citizens. Citizens, in particular, are more than ever interested in environmental and health problems associated with exposure to noxious chemicals. In this context, concerns over the levels of well know contaminants such as mercury, has increased over the past few years. This interest is emerging not only in adults, but also in younger generations, particularly teenagers. In this work, a group of teenagers from a school in Lisbon together with their professors and researchers from the academia conducted a monitoring survey on mercury levels in the students from a private school in Lisbon.

The recruitment of the volunteers was performed by the students enrolled in this project. The study protocol was approved by the Pedagogical Board of Colégio Valsassina and authorized by the CNPD. A total of 95 students were recruited, with ages ranging from 12 to 18 years. All volunteers answered a questionnaire with specific questions on possible mercury exposure sources (e.g. seafood consumption, dental amalgams). Hair samples were analyzed by atomic absorption spectrometry with thermal decomposition and gold amalgamation alongside with human hair certified reference material.

The concentrations of total mercury in hair varied between 12.6 and 3314.74 ng g<sup>-1</sup>, with an average of 1085±661 ng g<sup>-1</sup>. Significantly higher levels of mercury were registered in students that consumed fish more than once a week; however, given the small sample size, such results should be interpreted with caution. Overall, our results disclose that 12% of the studied population, exhibit levels higher than the reference dose set by the WHO (2000 ng g<sup>-1</sup>). Given that mercury is a serious threat to human health, measures to minimize exposure are proposed.

**keywords** Mercury, Students, Public engagement, Citizen science

# Occupational secondhand smoke effect on nasal epithelial proteoma

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**Introduction:** The tobacco is one of the biggest public health threats, smoking kills more than 7 million people/year worldwide and more than 890,000 are deaths resulting from exposure to Second Hand Smoke (SHS). In adults, SHS is associated to cardiovascular and respiratory diseases, including coronary heart disease and lung cancer, through pathological and molecular mechanisms not yet understood. In this study, we aimed to investigate the SHS effects on nasal epithelial proteome in exposed workers.

**Methods:** Nasal epithelium was collected from hospitality workers (non-smokers=40; smokers=12) long-term exposed and non-exposed to SHS at the workplace. Samples were analyzed by shotgun proteomics using an ESI-LQT Orbitrap XL mass spectrometer. The generated MS raw data was submitted to bioinformatics search engines for peptide identification and relative quantification by label-free. Golden rules were apply to obtain reliable data such, the inferred proteins must have at least one unique peptide and be detect in at least 80% of each group from the population cohort.

**Results:** Five proteins were found to be differentially expressed in the no-smokers exposed to SHS compared with control: BPI fold-containing family A member 1 (BP1FA1), Heat shock Protein Beta-1 (HSPB1), Tubulin beta-4B chain (TUBB4B), Glyceraldehyde-3-phosphate dehydrogenase (GAPDHS) and Alcohol dehydrogenase 1C (ADH1C). BP1FA1 plays a role in the airway inflammatory response after exposure to irritants substances, HSPB1 is a regulator of actin filament dynamics, and TUBB4B is the major constituent of microtubules; its modulation can foresee a possible role in the dynamics of lung epithelium differentiation in presence of SHS. GAPDHS is been related with human lung cancers.

**Conclusions:** Our findings support the indication that in non-smokers the prolonged exposure to SHS can lead to nasal epithelial proteome modulation. When validated, the uncovered proteins can be promising candidates to “susceptibility/risk” and/or “response” biomarkers for SHS exposure.

**keywords** Occupational exposure, Second Hand Smoke, Nasal epithelial proteome modulation, Biomarkers



# Synthetic musk in adipose tissue of bariatric patients from the Portuguese Region of Porto

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Synthetic Musks (SM) are present in personal care products (PCPs) such as perfumes, body lotions, shampoos, deodorants and antiperspirants. SM were described as bioaccumulative and persistent xenobiotics, that could lead to distinct types of dermatitis, carcinogenesis and endocrine disorder. In Europe, the Regulation (EC no.1223/2009) prohibited the use of muskamberette, musk-tibetene and musk-moskene and limited the concentrations in cosmetics for musk-ketone, musk-xylene, phantolide and tonalide. The general population is potentially chronically exposed through PCPs use, but the actual exposure is poorly documented. Dermal absorption is thought to be the major route of human exposure to SM. Being these contaminants lipophilic, the adipose tissue (AT) is the preferred matrix for SM assessment. Not included in routine monitoring, SM data on health impact are short. Nonetheless, literature supports the conclusion that SM bioaccumulate in human body and can even be passed on through breast milk or perinatal exposures.[1]

Four SM were extracted with n-hexane and the extract was clean with dispersive SPE: 50mg PSA, 150mg MgSO<sub>4</sub> and 100mg C18EC.[2] The quantification of SM was performed by gas chromatography mass spectrometry (GC-MS).

The presence of galaxolide, tonalide, musk-ketone and musk-xylene were evaluated in human AT of three patients undergoing bariatric surgery at Hospital de São João collected in Porto, Portugal (protocol approved by the Ethics Committee of Hospital São João).

Our work shows the suitability of this easy and rapid method for efficient isolation of SM in AT, prior GC-MS analysis for human biomonitoring. More studies are needed to fill the lacking information regarding SM bioaccumulation properties and the consequently impact on human health.

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[1] H. Dong, et al., *Talanta*.120(2014) 248-254.

[2] V.C. Fernandes, et al., *Biomed Chromatogr.* 26(2012) 1494-1501.

**keywords** Synthetic musks, Adipose tissue, Human biomonitoring, Bioaccumulation



# Micro and Nanoplastics: From Ocean to Humans

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The plastic tsunami that is flooding our lives with new plastic polymers is not only an environmental issue by itself but a health hazard for everybody.

Plastic litter is a major pollution issue and Microplastics and Nanoplastics have a too vast distribution in the oceans causing a silent Pandemia in the sense that they're commonly not perceived as a major pollutant, although they persist for centuries, accumulate and transfer toxins in the food chain.

The possible contamination for humans depending on distribution of the particles, and because of unique properties like biopersistence, bioaccumulation and biomagnification Micro and Nanoplastics can build up on all marine species and may serve as an important source of human exposure.

Other key factor contributing to their bioavailability and entrance at lower level of food chain, are their heterogeneous physico-chemical properties.

We use a race boat "Turn the Tide on Plastic", during the Volvo Ocean Race that carried a special instrument equipped with filters that captured and measured Microplastic debris from samples of ocean water around the world.

The results from ocean observation indicate that sub-surface waters are polluted by MPs to a much higher content than expected.

Also the data shows contamination of the world's most remote stretches of the high seas.

Mirpuri Foundation work involves increasing awareness of the problems faced by our planet and society.

However Microplastics as emerging contaminants are a Pandora's box of a kind or at least an infinite source of research questions.

**keywords** Microplastics, Ocean pollution, Human contamination

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