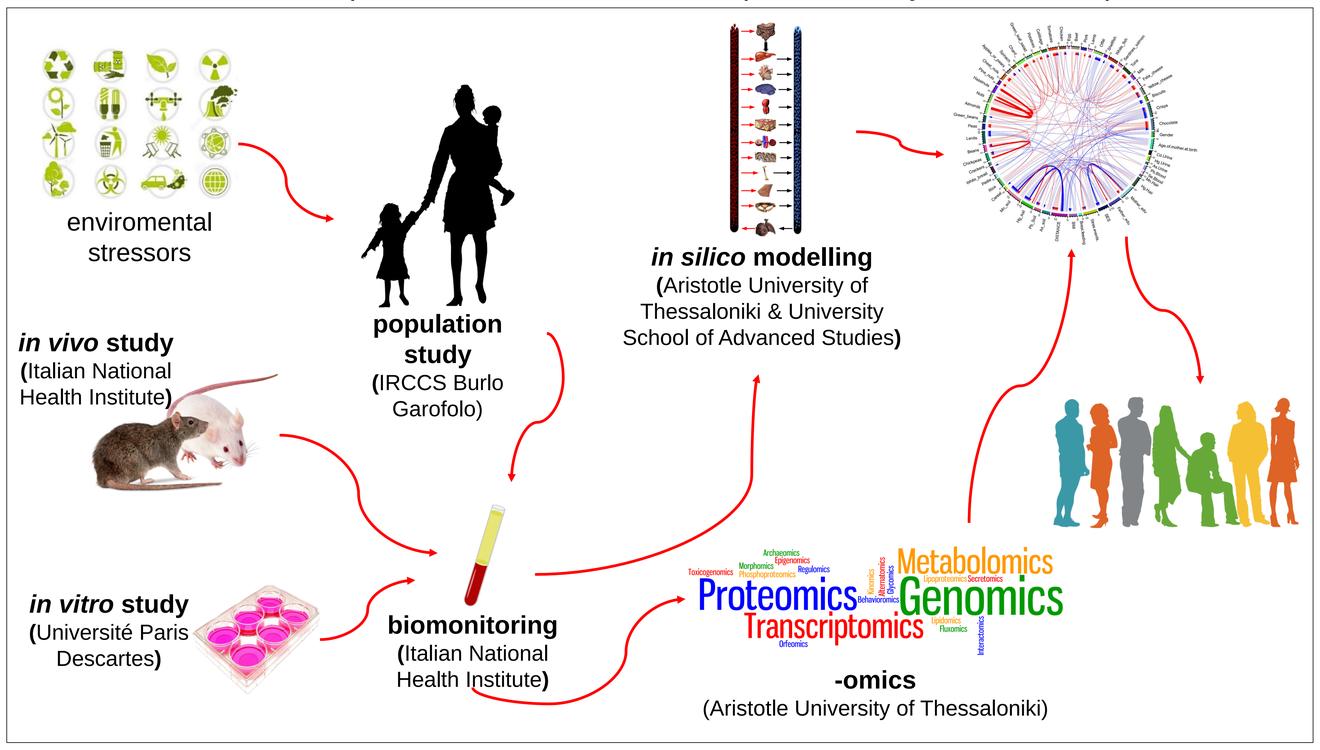
NEUROSOME: A Multidisciplinary Training Network To Explore The Neurodevelopmental And Neurological Exposome

D.A. Sarigiannis, A. Stratidakis, V. Kokaraki, I. Petridis, V. Dzhedzheia, R. Rezaee, (Aristotle University of Thessaloniki & University School of Advanced Studies, Greece/Italy); G. Calamandrei, A. Alimonti, L. Ricceri, F. Ruggieri, O. Dinckol, B.F. Fuentes Juarez, (National Health Institute, Italy); J. O. Grimalt, M. Capodiferro, (Spanish Council for Scientific Research, Spain); M. Horvat, B. Kontić, A. Runkel, T. Bizjak, (Jožef Stefan Institute, Slovenija); X. Coumoul, C. Chauvet, L. Lopez Suarez (Université Paris Descartes, France); M. Schuhmacher, Deepika, (Universitat Rovira i Virgili, Spain); A. Tsatsakis, O. Anesti, (ToxPlus SA., Greece); L. Ronfani, P.d'Adamo, I. Fragkiadoulaki, (IRCCS Burlo Garofolo, Italy)

NEUROSOME is an Innovative Training Networks funded in 2017 within H2020 Marie Sklodowska-Curie Actions. The main objective of the NEUROSOME project is the development of an integrated model based on real human biomonitoring data (HBM) to identify causal associations between early environmental exposures, the human genome, and the risk of neurodevelopmental disorders and neurodegenerative diseases. In particular, the project is based on the evaluation and re-analysis of biological samples collected in existing birth cohorts (PHIME, INMA, PROBE) and in the context of a cross-Mediterranean cohort study set up specifically within NEUROSOME. The project is focused on exposure to mixtures of heavy metals and organic compounds (phthalates, plasticizers, pyrethroids, organophosphate pesticides and brominated and organophosphate flame retardants), but will also consider the role of modulation and the synergistic or additive effects of other intrinsic (such as genetic susceptibility) and extrinsic (such as diet and socio-economic status) environmental stressors. This requires the synthesis among different scientific disciplines, including environmental and exposure modelling, recent advances in toxicology (including *in vitro*, *in vivo* and *in silico* aspects) with a special focus on omics technologies and bioinformatics, as well as environmental epidemiology, taking stock of gene- and exposome-wide associations. We present here the work so far performed by some of the partners, based on the real exposure scenario of children to heavy metals.

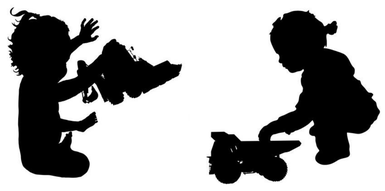


POPULATION STUDY

Common gene variants may induce susceptibility to environmental factors by increasing or decreasing physiological responses to adverse effects from environmental toxins, through the mother's internal or external environment. Single nucleotide polymorphisms (SNPs) may help predict an individual's susceptibility to environmental pollutants, and risk of developing particular diseases.

The experimental part is based on the analysis of existing bio-samples (cord blood/cord tissue) of the PHIME Mediterranean cohort, recruiting in Italy, Slovenia, Greece and Croatia. The PHIME database provides information regarding the maternal and infant levels of exposure to metals (Hg, Mn, Cu, Zn, As, Se, Cd, Pb), neuropsychological scores (through Bayley III test) of children at 18 months of age and socio-economic information. Regarding the Italian part of the cohort, total number of samples corresponds to 601 and DNA extraction has been performed in all of them with the use of "EZI Advanced XL" instrument by QIAGEN. Number of samples in which genome-wide genotyping has already been performed using Infinium HD arrays by Illumina, is 411.

Future plans include genome profiling, bioinformatic analysis (PLINK: Whole genome data analysis toolset) and wide-association analysis. After completion of the genome-profiling of the individuals of the PHIME cohorts, SNPs identification will be performed in specific genes related either to toxicology or to metabolism (Phase I & II) of xenobiotics.



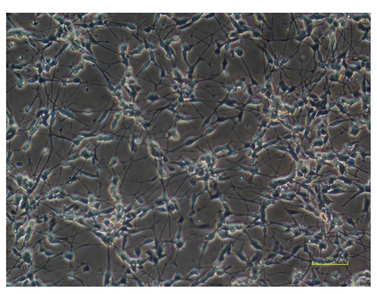
IN VITRO MODELS

Rationale:

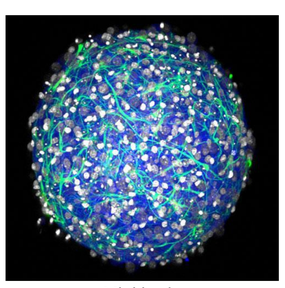
- Clarify the effects of the environmental toxicants in the nervous system identifying the response of relevant nervous system cells to them.
- Test the hypothesis that lipophilic chemicals could facilitate the absorption of hydrophilic compounds across the BBB.

Methodology:

- Several cell lines of neurons (including SH-SY5Y human neuronal cells), glial cells and representative BBB epithelial cells will be treated with environmentally representative contaminants mixtures including metals.
- We will also assess the effect of these chemicals in a novel induced Pluripotent Stem Cells (iPSC)-derived human 3D brain microphysiological system (BMPS), developed at JHU.



SH-SY5Y Cells



Mini brain
https://news.brown.edu/articles/2015/10/minibrain

- **Cell lysates** and **supernatants** will be harvested in order to perform transcriptional, proteomics and metabolomics analysis.

Supported by Horizon 2020 MSCA-ITN Grant No. 766251
http://www.neurosome.eu

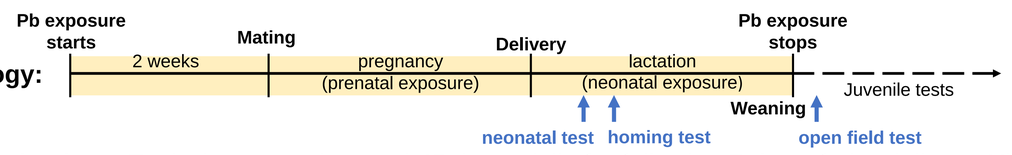
IN VIVO MODELS

Rationale: Identifying early behavioural changes (with translational value for child neurodevelopment) in newborn rodents exposed to low dose of heavy metals selected from biomonitoring,.

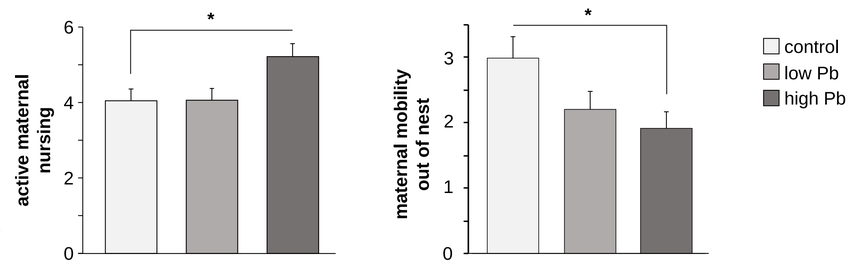
First study on Lead (Pb);

Treatment: **control group**: 0 mgPb/L **low Pb group**: 25 mgPb/L **high Pb group:** 100 mgPb/L

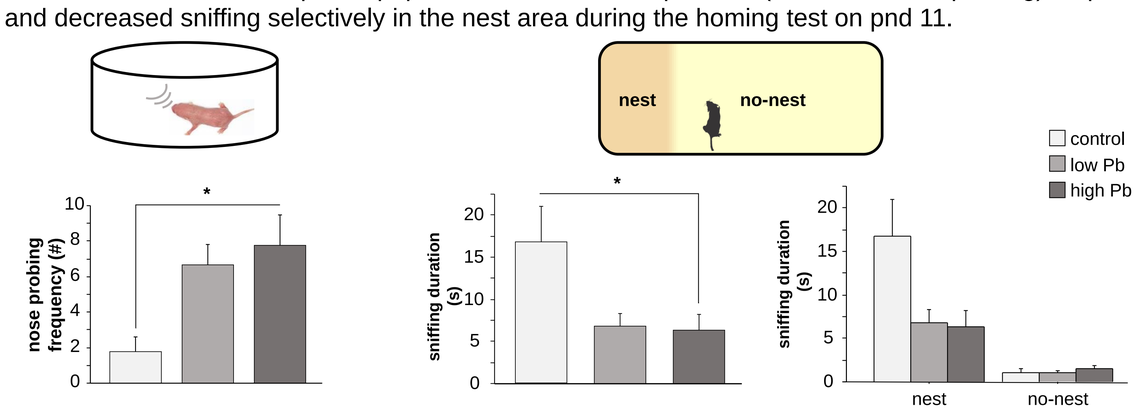
Methodology:



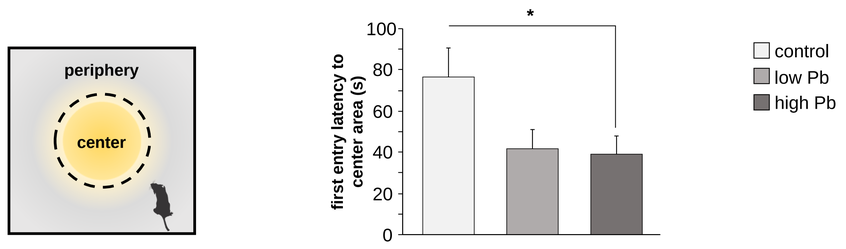
Maternal behaviour: Each dam was observed 45 times daily for following 7 daysof the delivery. High Pb exposed dams show higher levels of active maternal nursing and less activity out of the nest.



Neonatal behaviour: Pb exposed pups show altered motor patterns (increased nose probing) on pnd 8 and decreased sniffing selectively in the nest area during the homing test on pnd 11.



Juvenile behaviour: Pb exposed mice show an altered anxiety profile on pnd 23 (decreased latency to enter the central area of the arena).



Future plans: Next steps of the *in vivo* study would be examining effects of low dose exposure to heavy metals' mixture (Pb, Hg, Mn) via pregnancy and lactation on maternal behaviour and developmental behaviour to model human population studies.

IN SILICO MODELS

Exposure reconstructions/simulations for identifying exposure levels starting from HBM data were carried out on a modelling platform that provides realistic exposure scenarios coupled with a generic physiologic based bio-kinetic (PBBK) model and numerical "reversal" techniques for exposure reconstruction. The process starts from ancillary exposure-related data that are fed into the exposure model taking into account multiple exposure routes. Results are evaluated against the biomonitoring data distributions, aiming at the reduction of uncertainty in back-calculating doses, by minimizing the error between the predicted and the actual HBM data. This was applied for the reconstruction of cadmium intake levels, starting from EU wide child and mother HBM urinary data.