Issue No. 3,
March 2020

NEUROSOME

# Neurosome Newsletter

Many chemicals in everyday life and industrial usage has the potential to cross blood-brain barrier and develop the neurotoxicity in human beings.

Integration of different studies human biomonitoring, in-vivo studies, in-vitro and in-silico is one of the reliable way to find relationship between chemicals and neurodevelopmental disorders.

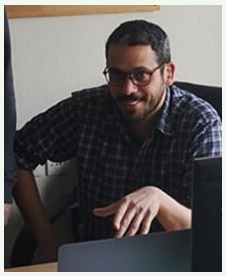
NEUROSOME is a Europe integrated training network funded in the frame of Marie Sklodwska-Curie action. This project brings together beyond-the-state-of-art advances in human biomonitoring and systems biology, exposure monitoring and toxicological testing technology and advanced tools for computational analyses of the exposure-to-health effect continuum according to the exposome paradigm.

It will improve the scientific knowledge between chemicals and neurodevelopmental disorders taking into account exposure and health effect modification due to intrinsic and extrinsic factors.

**In this Issue:**

- Update about each ESR work for continuation of project
- It includes the experiments, results, training and courses attended by the ESRs.
- Dissemination activities for the Project
- Interested Forth coming events for ESRs & Neurosome participants.

***Welcome to the third issue of Neurosome Newsletter!***



## ESR1: Functional integration of different omics results using bio-informatics tools towards development of AOPs for neurodevelopmental Disorders

Antonios Stratidakis, D.Sarigiannis, IUSS

My research project focuses on developing an integrative biology approach along with the use of bioinformatics tools for pathway analysis for developing AOPs for neurodevelopmental disorders. For the use of bioinformatics tools, I obtain omics results from other ESRs. The compounds of interest of my research project include **3 heavy metals (lead, mercury and cadmium)** and **3 phthalates (DEHP, DINP and BB2P)**. All compounds of interest are well known to pose AOs to human health.

The most crucial step regarding the understanding of metabolites' role, proteins role and genes role included their identification, as biological meaning can only be found if compounds are identified. For this reason we have been done a very thorough review on metabolite identifiers.

After a very thorough review of all available identifiers (Figure 1), **Human Metabolome Data-Base (HMDB)** and **Metlin databases** were used, and KEGG ID annotations for the identification of metabolites. The sets of data, generated by analyzing samples using untargeted metabolomics techniques, were characterized by high complexity and required high performance bioinformatics tools. In this study, pathway analysis has been performed with the combination of two modules of GeneSpring called **Mass Profiler Professional (MPP)** and **Pathway Architect**. Hypergeometric test was used to calculate the probability of the metabolites of the given list to be enriched with a particular pathway.

The urea cycle has been co-mapped in case of data integration using **transcriptomics** and **proteomics**, due to the statistically significant difference in the expression of arginase-1, arginase-2 (mitochondrial), arginosuccinate synthase, carbonyl-phosphate synthase (mitochondrial), ornithine carbamoyltransferase (mitochondrial) and argino-succinate lyase (Fig1). The identification of the urea pathway is of particular interest since has been also identified in human samples in PHIME cohort using untargeted metabolomics analysis on plasma samples. Co-mapping of proteomics and metabolomics data revealed that common drivers are responsible for the allostasis of metabolic pathways related to choline, phosphatidylcholine, phospholipases and triacylglycerol metabolism, due to alterations in the expression levels of phosphatidylcholine, and 1,2-diacyl-sn-glycerol-3-phosphate.

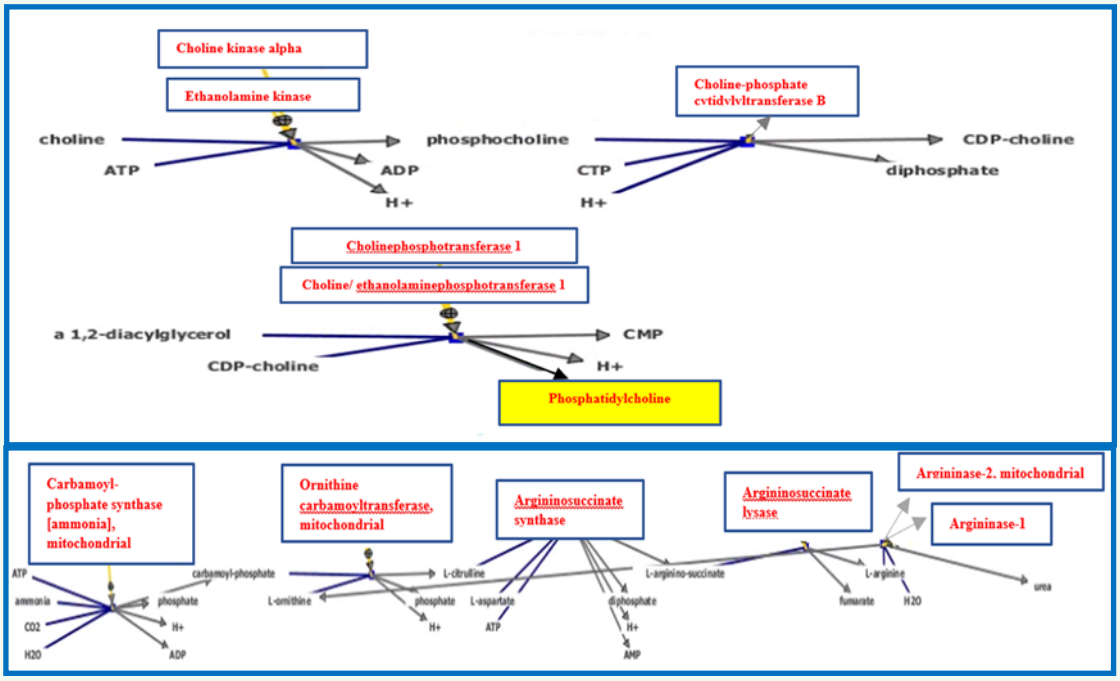



**Fig 1:** Mapping of urea cycle

Future work includes the development of AOPs based on the **omics data**, aiming on the identification of the common nodes of toxicity, in this case to identify where the various **AOPs related to exposure to heavy metals and plasticisers** towards adversities related to neurodevelopment.

## ESR 2: Development of an integrated exposure model coupled to PBBK model for mixtures of neurotoxicants

Kokaraki Venetia, D.Sarigiannis, IUSS

My research focuses on developing a mathematical model that simulates the concentration of chemical over time in tissues and blood, by taking into account the rate of the chemical's absorption into the body, distribution in tissues, metabolism and excretion (Fig 2). PBPK models have the unique capability to take into account the **interaction between chemicals** at the level of hepatic metabolism where the effect of the interaction is evaluated according to the potential mechanism of action (competitive, non-competitive, and uncompetitive).

**Competitive inhibition** occurs when chemicals compete for the same active site of the enzyme resulting in decreased metabolism of the parent compound, which leads to increased toxicity. **Non-competitive inhibition** occurs when a chemical binds to the enzyme at a site that is away from the active site (Fig 3).

This binding changes the **conformation** of the enzyme, resulting in a decreased catalytic activity and the less frequently encountered uncompetitive inhibition occurs when a chemical binds to the **enzyme-substrate complex**.

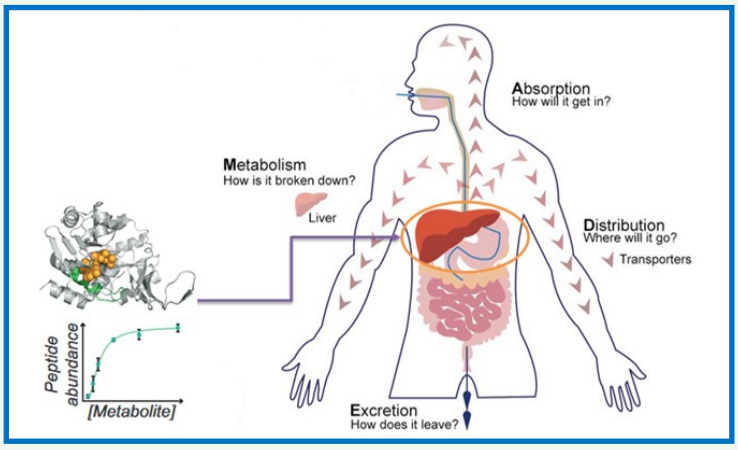



**Fig 2:** Diagrammatic representation of movement of chemical inside human body.

In order to qualify and quantify the interactions among chemicals, I collected some data related to the **metabolism of bisphenols, polycyclic aromatic hydrocarbons (PAHs) and heavy metals** related to enzymes that take place in the metabolism of each chemical and specific metabolic parameters. The knowledge of the **maximal velocity (Vmax )** and **apparent affinity (Km)** is essential to describe saturable metabolism of each mixture component and the metabolic inhibition constant, Ki, is required additionally for describing the interaction among chemicals.

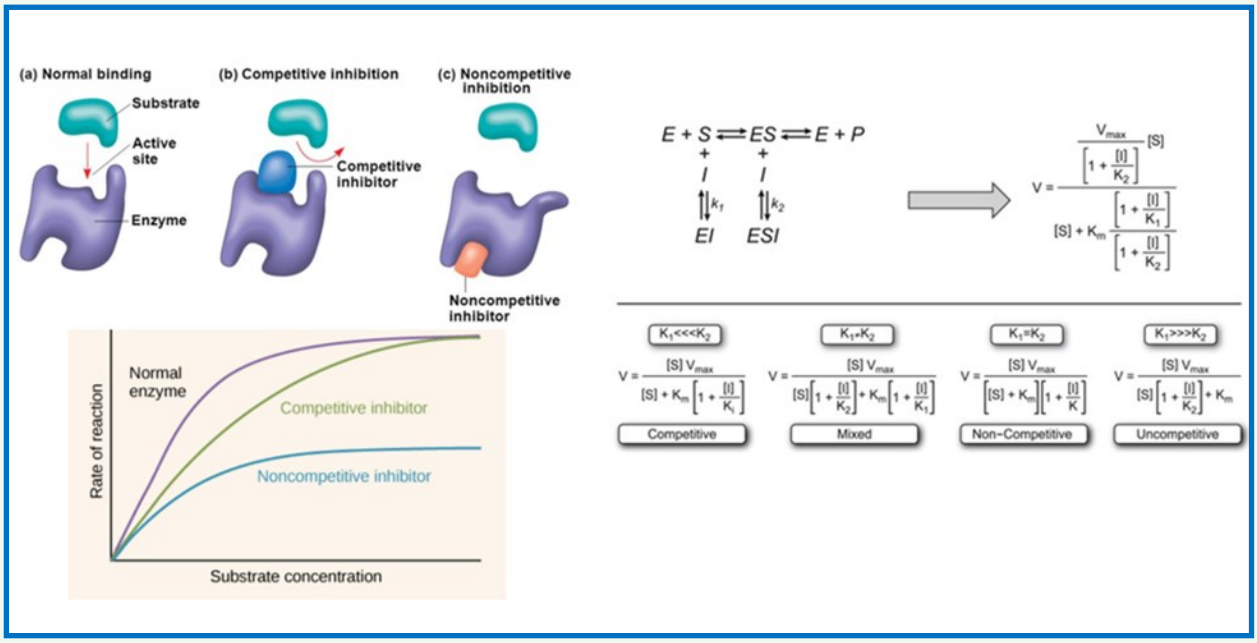



**Fig 3:** Mechanism of inhibition taking into account mixture interaction

## ESR 3: Human biomonitoring of heavy metals associated to neurodevelopmental Disorders

Byron Fuentes, Dr. Anna Pino, ISS

Following the analysis from biomarkers on mice exposed to lead (Pb) concentration control, low and high groups, a new set of samples from brain and bones where analyzed.

The results show a weak relationship between the group of exposure and the concentration found in the biomarker, nevertheless, for bones biomarkers a strong relation was found among the concentration and group (shown in figure 4)

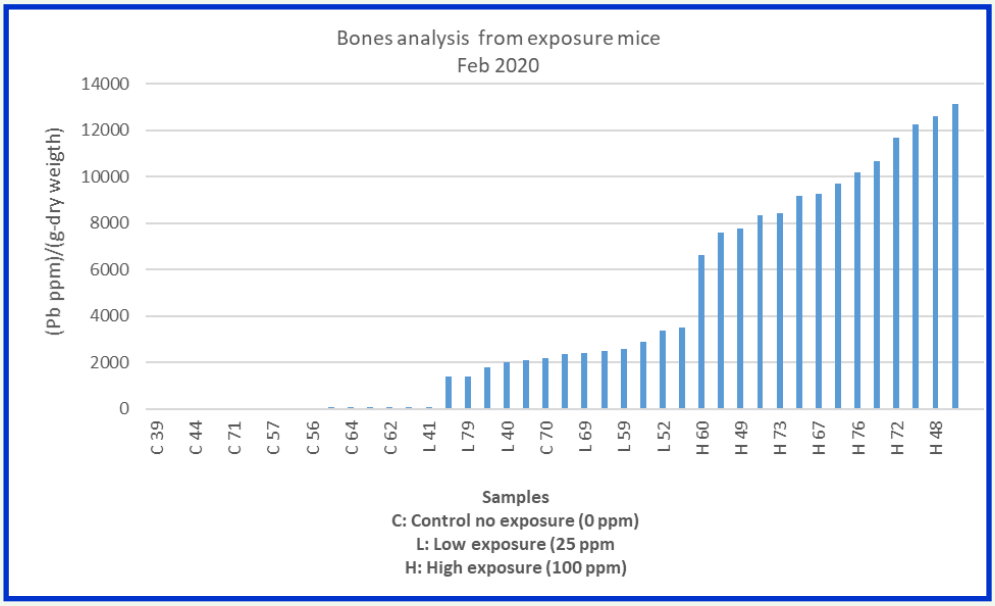



**Fig 4 :** Bone analysis representing lead concentration in different groups of mice

Additionally first trails from brains region that included hypothalamus, prefrontal cortex and cortex, to setup the methodology of analysis.

Using Thermo Scientific™ Neptune™ MC-ICP-MS, **isotopic signatures** from the source of lead acetate (Pb $(CH_3COO)_2$) used as source of Pb for exposure was taken. The measurement was done by mixing different proportions of the **lead acetate** and **isotopic standard solution (European Reference Material ERM AE142)** (Table 1).

**Table 1:** It represents variation between European Reference Material and lead (Pb).

| Variation between the % of ERM and AcPb | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Sample** | **Mean value** | **206Pb/204 Pb (1)** | **207Pb/204 Pb (2)** | **208Pb/204 Pb (3)** | **206Pb/207 Pb (4)** | **206Pb/208 Pb (5)** | **207Pb/208P b (6)** |
| 100% ERM - 0% AcPb | Mean | 21,10937 | 15,94009 | 39,81334 | 1,32436 | 0,53021 | 0,40035 |
| 90% ERM - 10% AcPb | Mean | 20,84687 | 15,90384 | 39,65034 | 1,31084 | 0,52578 | 0,40110 |
| 80% ERM - 20% AcPb | Mean | 20,60615 | 15,88102 | 39,52165 | 1,29745 | 0,52138 | 0,40185 |
| 70% ERM - 30% AcPb | Mean | 20,33590 | 15,83313 | 39,32691 | 1,28444 | 0,51710 | 0,40259 |
| 60% ERM - 40% AcPb | Mean | 20,10288 | 15,80574 | 39,19113 | 1,27188 | 0,51295 | 0,40330 |
| 50% ERM - 50% AcPb | Mean | 19,88020 | 15,78589 | 39,07425 | 1,25940 | 0,50880 | 0,40400 |
| 40% ERM - 60% AcPb | Mean | 19,65027 | 15,75695 | 38,93608 | 1,24703 | 0,50468 | 0,40470 |
| 30% ERM - 70% AcPb | Mean | 18,87045 | 15,66724 | 38,48161 | 1,20446 | 0,49038 | 0,40714 |
| 20% ERM - 80% AcPb | Mean | 18,87045 | 15,66724 | 38,48161 | 1,20446 | 0,49038 | 0,40714 |
| 10% ERM - 90% AcPb | Mean | 19,00013 | 15,67564 | 38,54392 | 1,21204 | 0,49293 | 0,40669 |
| 00% ERM - 100% AcPb | Mean | 18,80260 | 15,65511 | 38,43290 | 1,20104 | 0,48925 | 0,40735 |

The future aim is compare with the **biomarkers analyzed** and find **contamination source** which explains the result obtained.

From this work, a paper is planned to be published.

## ESR 4: Targeted *in vivo* testing of neurodevelopment focusing on exposure to heavy metals

Oyku Dinckol, G. Calamandrei, ISS

Our project aims to mimic human neurotoxic metals exposure during **pregnancy and lactation** and to evaluate the **neurodevelopmental outcomes** in an *in vivo* mouse model. To accomplish this purpose, our first study included two doses of a single metal contaminant, lead (Pb).

We assessed the **neurodevelopmental effects of prenatal and neonatal Pb exposure**. Female mice were exposed to two different, human-related low-level concentrations of Pb (2.5 and 10 mg/kg bw/day) via drinking water for 2 months including pre-conceptional period, gestation and lactation. Firstly, to assess the effects of Pb on maternal behaviour, dams were observed daily for the first 7 days after parturition. Secondly, to assess neurodevelopmental effects, offspring was examined by different behavioural tests during different development stages of life. **Behavioural tests** were conducted to test social, locomotor, olfactory and cognitive skills and anxiety levels. Finally, evaluation of Pb levels in different tissues **(blood, brain, and bone)** was carried out during young adulthood and adulthood in collaboration with ESR3 at ISS. Below figure show different behavioural patterns related to exploration and sociality of Pb exposed mice. On postnatal day (PND) 8, spontaneous movement test was performed and 100-ppm pups showed increased nose probing (a spontaneous response associated with nipple searching). On PND 11, during the homing test (an olfactory-based spatial task), 100-ppm pups showed a reduced sniffing response to the nest-odour, although time to reach the nest area was not different from controls. On PND 23, during the open field test assessing spontaneous exploration and activity (Fig 5), 100-ppm mice showed less hesitation on entering the central area (usually considered as a more hazardous zone by mice).

At young adulthood, offspring were tested in **three -chamber social interaction test** (Fig 6) and 100-ppm male mice (Blood lead level-BLL: 2.546 µg/dL) showed a decreased preference for the social stimulus; no alterations of social responsiveness in the 25-ppm group (BLL: 1.021 µg/dL) were evident. "At adulthood, Pb-dependent alterations on olfactory skills and learning/memory were not found; 6 months after the end of the exposure, BLL of 100-ppm group (0,197 µg/dL) was significantly higher than the control group whereas 25-ppm BLL (0,141 µg/dL) did not differ from controls. "

Preliminary data were presented in a poster at the 17th biannual meeting of the international Neurotoxicological Association. Final results will be presented in FENS forum 2020. As the next step, a widened new project will start with **co-exposure of neurotoxic metal mixture.**

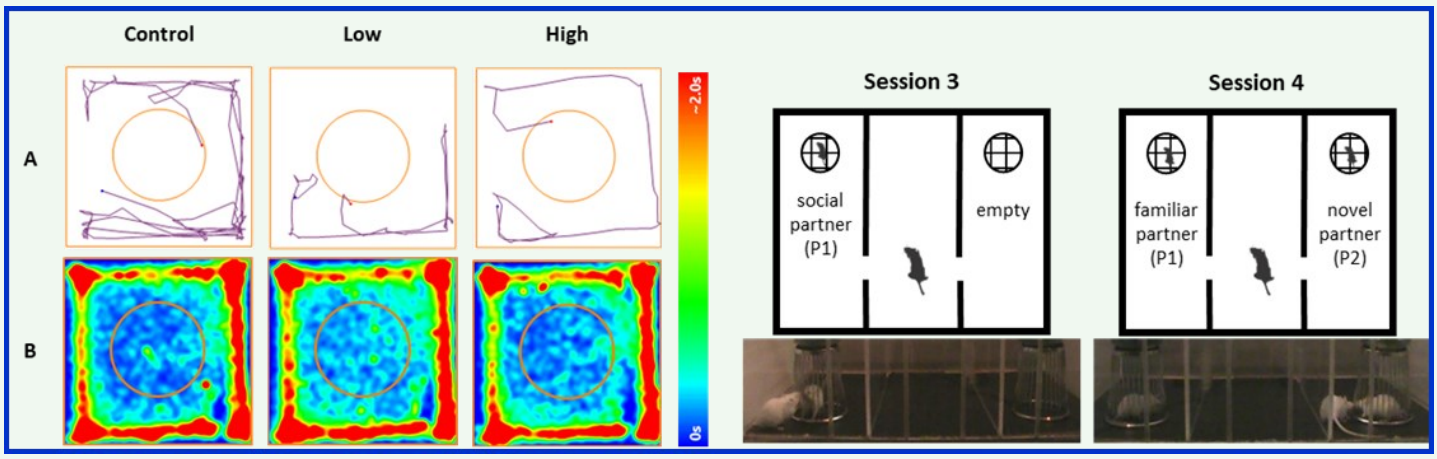



**Fig.5:** Open field (OPF) test. The square represents the OPF arena and the circle represents the central area. **(A)** Representative track plots show wended path until the first entrance to central area. **(B)** Mean heat map of 15-minutes OPF test.




**Fig.6:** Three chamber social interaction test set-up scheme and images from the experiment. Test animals first encounter with a social partner (P1) and an empty cage in session 3 then in session 4, a novel partner (P2) is introduced instead empty cage.

## ESR5: Development of an analytical framework of environmental samples from different media towards exposome assessment

Marco Capodiferro, J.Grimalt, CSIC

Primarily, a study was performed on the levels of different chemicals on Greek soils after a huge fire. The levels of different **persistent organic pollutants (POPs)** were analyzed, in particular **polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides and polychlorinated biphenyls (PCBs), polybromodiphenyl ethers and dioxins.** In addition, metals and sugars were also determined.

A study on **mercury levels** in fish samples from the western Mediterranean has just been completed. Eight sampling campaigns were carried out in different sites scattered in the western part of Europe and more than a thousand samples were collected, classified and analyzed.

Now, further analyses will be performed in order to identify what are the sources contributing with the inputs of this metal. For this purpose, a three-month secondment has been planned in the near future at the Jožef Stefan Institute.

**Organochlorine compounds (OCs)** and **mercury** were found in a cohort of four-years old children from Menorca. Data relating food habits and pollutant concentrations found in samples of these children were provided to other ESR 9. The contribution from each food group to the total dietary intake of specific group of chemicals from this cohort was also examined (Figure 7).

Further, a paper in collaboration with ESR 9 is under development for calculation of dietary exposure through modelling and experimental results.

Currently, we are developing a new analytical methodology to analyze POPs in **human serum** that includes compounds that are eliminated by sulphuric acid digestion, e.g. vinclozolin, endosulfans and the endrin group. Some of these compounds are **strong hormone disruptors**.

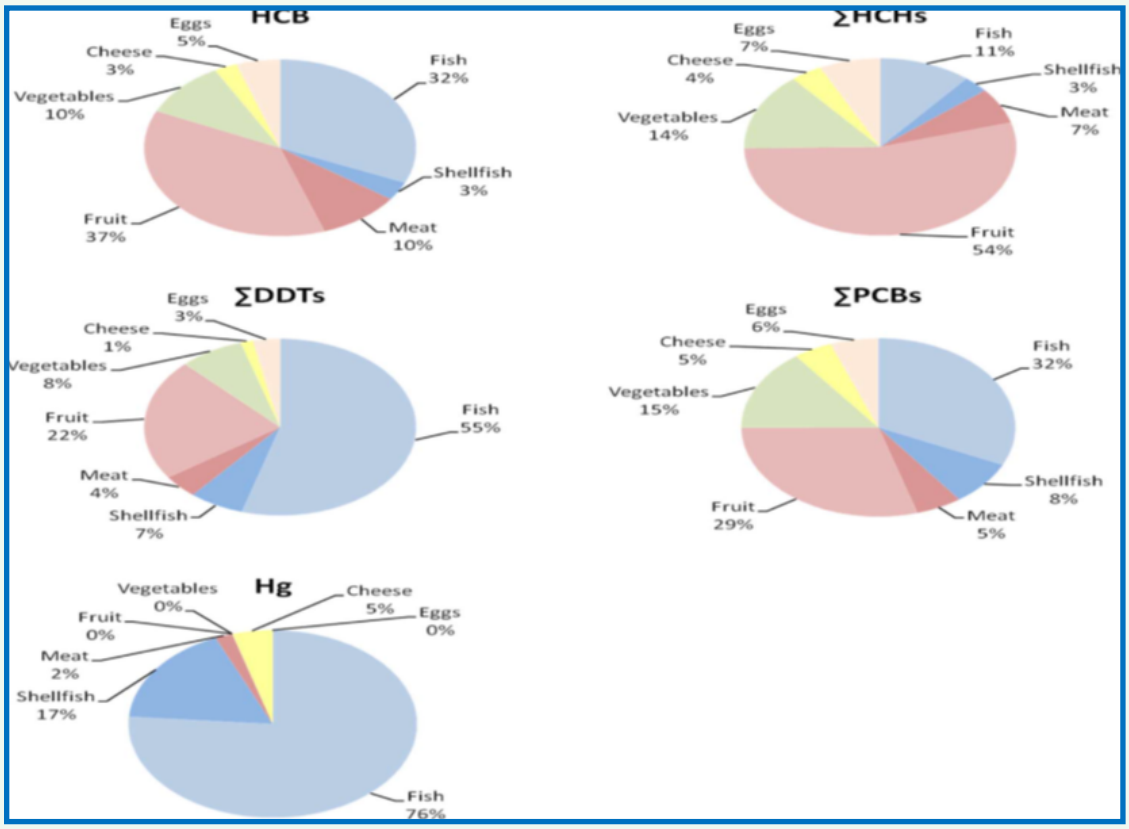



**Fig 7:** Contribution from each food group to the dietary intake of HCB, ΣHCHs, ΣDDTs, ΣPCBs and Hg in the Menorca cohort.

## ESR 6: Analysis of human biosamples for biomarker Quantification (ESR6)

Agneta Runkel, M.Horvat, JSI

The main task of my project is the **analysis of organic contaminants in human samples** that are related to neurotoxic outcomes. In the light of this, an article on phthalate exposure of the Slovenian population is currently under review.

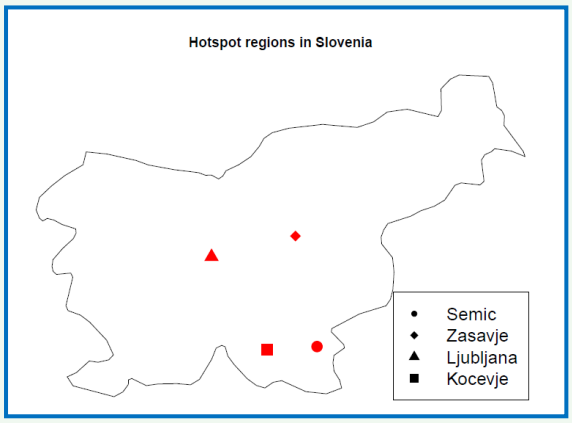



**Fig 8:** Locations in Slovenia with the highest levels of POPs in human samples.

An article on exposure to **persistent organic contaminants (POPs)** is currently in preparation. Samples from Slovenian men and first-time mothers were taken between 2008 and 2014 in 12 regions throughout Slovenia and analyzed for Aldrin, Chlordane, Dieldrin, dichlorodiphenyltrichloroethane (DDT), Endrin, Heptachlor, hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDE) and polychlorinated dibenzodioxins and furans (PCDD/Fs) (Fig 8). Results from this study show regional differences in exposure to POPs throughout country.

POPs were measured in individual samples of **serum (men)** and **maternal milk** as well as in **pooled samples of plasma (men)** and **maternal milk**. In individual samples, most compounds were below the limit of detection, except for p.p. DDE, PCB 153, PCB 138, and PCB 180 (in maternal milk) and p.p. DDE and the sum

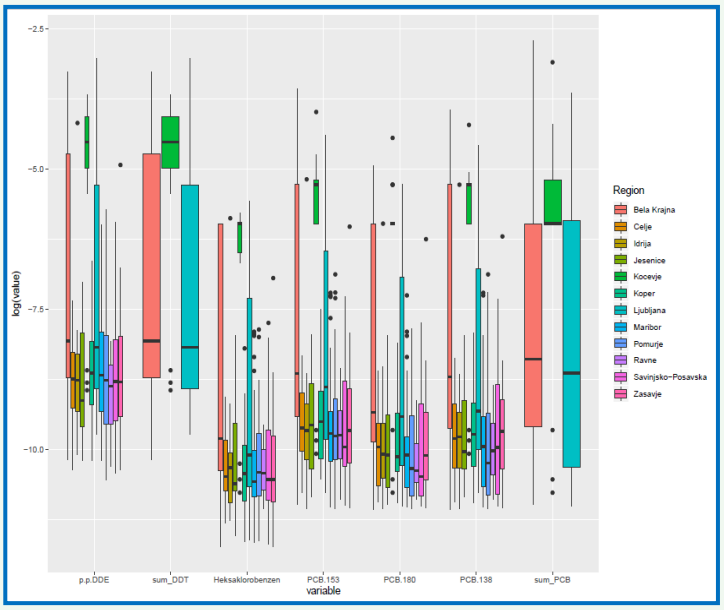



**Fig 9:** Regional differences in POPs exposure in individual samples of maternal milk

of measured PCBs in serum (Fig 9).

In pooled milk samples, all measured tetra to octa PCDD/F were detected in 12% - 100% of samples. PCBs were detected in 100% of samples except for PCB 81, where 28% were below LOD. All analyzed **PBDEs were detected** in all samples, except BDE 183, where 12% were below LOD. The highest median concentrations of dioxins/furans, PCB, and PBDE were obtained for: 1,2,3,4,6,7,8,9 OCDD (24.5 pg/g), PCB 118 (2700 pg/g), and BDE 47 (505 pg/g). In pooled plasma samples, detection rates of PCDD/F lay between 3 and 27%, of PCBs between 9 and 100%, and of PBDEs between 18 and 100%. The highest concentrations observed for each of the three groups where 0.1 pg/g (1,2,3,4,6,7,8,9 OCDD), 9.8 pg/g (PCB 118), and 1.4 pg/g (BDE 99). The regions Ljubljana, Zasavje, Kocevje, and Semic are standing out for exposure to PCBs (Ljubljana, Kocevje, Semic) and dioxins (Zasavje). These levels of higher exposure could be linked to industrial activities (Zasavje), urban environment (Ljubljana), and a history of pollution (PCB pollution river Krupa).

Within the same study, 515 samples of **Slovenian men and women** were analyzed for bisphenol A (BPA), bisphenol S (BPS), bisphenol F (BPF), triclosan (TCS), methyl paraben (MeP), ethyl paraben (EtP), *iso*-propyl paraben (iPrP), propyl paraben (PrP), *iso*-butyl paraben (iBuP), butyl paraben (BuP), and benzyl paraben (BzP). Among parabens and bisphenols, the highest concentrations were observed for methyl paraben (geometric mean 15.5 ng/mL) and bisphenol A (geometric mean 2.3 ng/mL).

## ESR 7: Integration of HIA, sustainability appraisal and environmental impact evaluations with development planning emphasizing the role of HBM in ex-ante HIA

Tine Bizjak, B.Kontic, JSI


In the last few months, my research focused on the survey that aims to identify differences in understanding of the **health risk assessment (HRA)** and **health impact assessment (HIA)** and their usefulness in the context of informing decisions. I have distributed the survey among NEUROSOME partners, selected professionals in Slovenia and among participants of the educational activity I attended course at **Harvard T.H. Chan School of Public Health in Boston, USA**. I am still gathering answers.

In addition, I focused on a publication that will build on the commentary "**Auditing in addition to compliance monitoring: a way to improve Public health"** that was published last year in the International Journal of Public Health. In December, 2019 I attended the NEUROSOME meeting in Athens, Greece. In January, I attended **HERA consortium meeting in Barcelona**, Spain. The main topic of the meeting was the interim research agenda for the environmental, climate and health area. The meeting was an opportunity to exchange opinions about the understanding of my research area; especially, since the assessment of health impacts is identified as one of the important future research areas in the proposed interim research agenda.

I have also attended the **SciShops final meeting and symposium in Brescia**, Italy that focused on the importance, benefits and ways of facilitating community based participatory research. During the symposium I helped to present the Centre for Participatory Research at the Jožef Stefan Institute and its work. I have also participated in an educational activity at the Harvard T.H. Chan of Public Health "Environmental Health Risk: Analysis and Applications", where I met many different HRA professionals and consolidated my understanding of the HRA, risk perception, risk communication etc.


## ESR 9: Development of multimedia environmental contamination and human exposure model

Deepika Deepika, Marta Schuhmacher, URV

The main objective of the project is to develop a comprehensive **multi-media environmental contamination** and **human external and internal exposure model** for estimating contamination levels of contact media (air, water, soil, food wed, settled dust), indoor exposure and food items and validate it under different scenarios taking into account uncertainty.

Multimedia models provide a comprehensive picture of environmental behavior of chemicals with spatial information by utilizing different parameters like physico-chemical properties, emission rates and environmental parameters. Currently, we have compared different multimedia models for exposure assessment.

Based on literature review, we have selected **INTEGRA** and **Merlin** for exposure assessment. We have also acquired the data from ESR 5 which contains the concentration of different **contaminants (metals, pesticides)** analyzed from food and other samples across different areas of Spain. It will be used to calculate the dietary exposure.

Also, paper will be published in collaboration with ESR 5 for calculation of dietary exposure for neurotoxic chemicals with modelling and experimental results.

For human internal exposure, we have established **brain sub-compartment model** which will be incorporated in lifetime PBPK model (Fig 10). It consists of 6 compartments: hippocampus, cortex, cerebrospinal fluid, intracranial blood, blood brain barrier and rest of brain. For developing the **BBB compartment**, P-gp substrate, plasma protein binding, metabolic modification by barrier enzymes and permeation property of molecule are considered. In case of no literature data for certain areas in brain, volume is scaled from *in vitro* and *in vivo* to human. Physiological parameter for the model are given in table 2.

**Table 2:** Physiological parameters for the brain

| Volume of brain parts | | Flow rate to brain parts | |
|---|---|---|---|
| Spinal CSF | 30 ml | CSF formation | 0.3-0.4 ml/min |
| Cranial CSF | 130 ml | CSF absorption | 38% of total CSF production |
| Hippo-campus | 5.68 ml | CSF (spinal to cranial) | 7 ml/h |
| Cortex | 283 ml | Hippocam-pus to CSF | 0.00114 ml/min |
| CSF | 160 ml | Cortex to CSF | 0.0566 ml/min |

For performing the validation of model, group of chemicals (metals, pesticides) will be considered to assess the neurotoxicity in different brain areas. Workflow of model building is shown in below figure. The model is made in R studio.

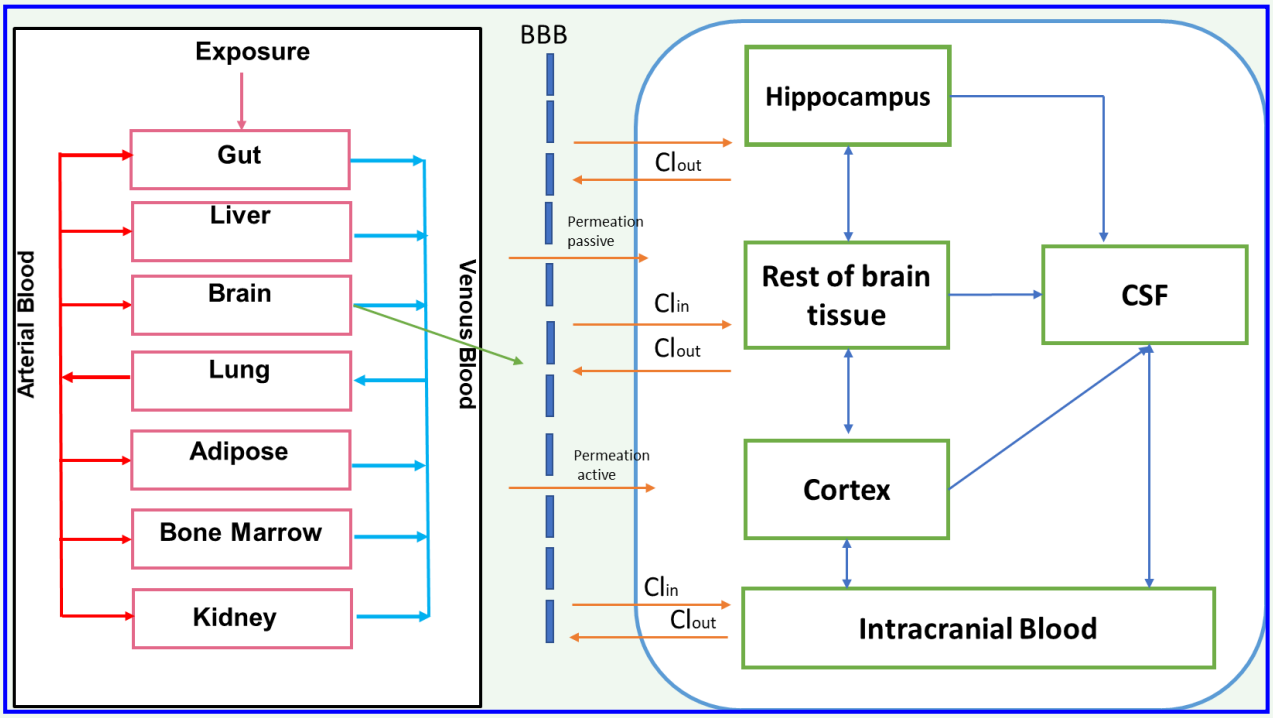
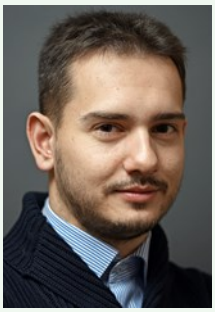



**Fig 10:** Brain Sub-compartment PBPK model for Neurotoxicity (Cl: Clearance, BBB: Blood brain barrier, CSF: Cerebrospinal Fluid)

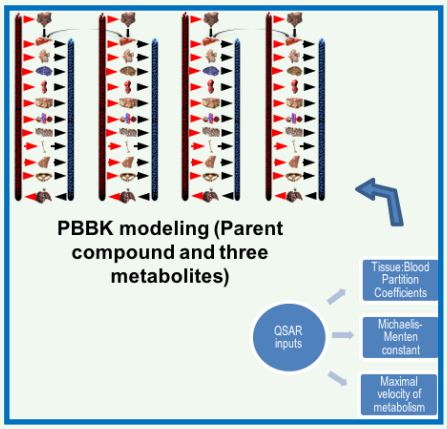
## ESR 10: Development of Quantitative Structure Activity Relationships for use in biokinetic models

Ioannis Petridis, Prof. D. Sarigiannis.AUTH



Our research focuses on the use of physiologically based biokinetic models for assessing the **life cycle of chemicals** with **neurodegenerative effects** inside the human body.

Data gathering and analysis is being performed to create and optimize these models. In the meantime, we use existing **PBBK models** and **INTEGRA** platform to reconstruct exposure levels based on human biomonitoring data (HBM), aiming to perform risk assessment calculations for selected chemicals (Bisphenols - BPA, Phthalates - DEHP, DiNCH, BBzP, DnBP, DiNP, Metals - Cadmium, Flame Retardants - TCEP, Anilines - ortho-toluidine, Polycyclic Aromatic Hydrocarbons - Pyrene, Benzo[a] pyrene, Perfluorinated compounds - PFAS, PFOA) (Figure 11).




derived from a variety of European Cohorts. Furthermore, we started creating a PBBK model for copper.

**Fig 11:** General Framework of in-silico model

In addition, an effort to introduce **advanced machine learning algorithms** to create and optimize **quantitative structure-activity relationship models** for predicting the kinetic parameters of ADME processes for poor data chemicals has started (Fig 12).

As the project continues, we will use **bioinformatics algorithms** to perform pathway analysis in order to study the associations among chemicals, **activated metabolic pathways** and health outcomes.

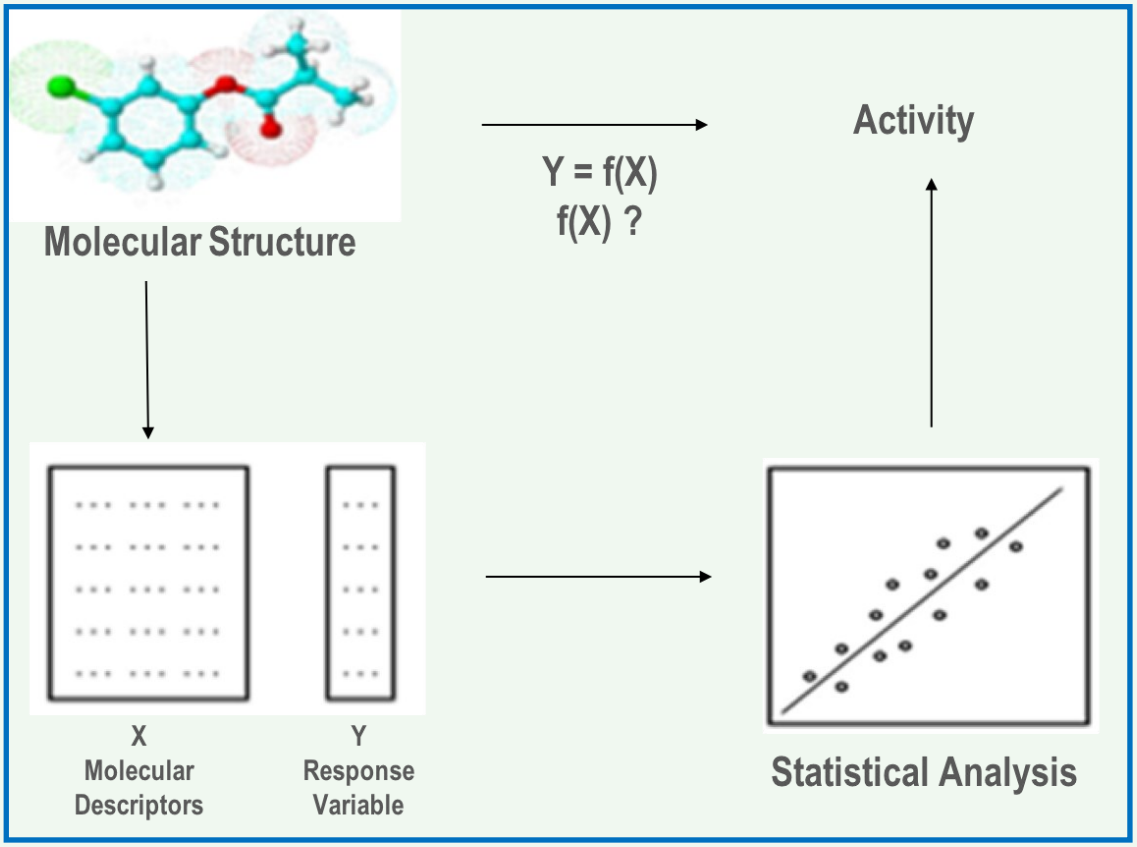



**Fig 12:** Quantitaive Sturctural Activity Relationship (QSAR) model using molecular descriptors and response variables

## ESR11: Advancing exposure assessment through environmental monitoring, human biomonitoring and use of personal sensors

Vazha Dzhedzheia, Prof. D. Sarigiannis, AUTH

The main objective of my research is to combine the information from **environmental exposure** and **personal sensors data** in order to calculate personal exposure and validate it against HBM data.

Associations among HBM and exposure data will be investigated, aiming to identify the way environmental data and HBM data should be sampled, in order to better reflect human exposure. This is going to be achieved by using several **analytical chemistry techniques** and instrumentation (GC-MS/MS, ICP-MS, HPLC-MS, NMR) (Fig 13).

Monitoring of the environmental contamination will be done in two ways - sample collection for further analysis with a method suited for each specific **environmental contaminant** (e.g. metals, POPs, pesticides and plasticizers)

and nonchemical – measuring the mass concentration of PM in ambient air via different techniques (based on Beta ray attenuation, magnitude of the frequency change or intensity of scattered light).

**Personal exposure monitoring** will be done by sampling and chemical analysis of particulate matter through gravimetric analysis for particle mass and chemical analysis for specific compounds, personal exposure sensors will measure CO, CO2, VOCs, PM2.5, O3, NO2, air pressure, temperature, humidity, combined with personal activity sensors measuring steps, active minutes, calories/energy, sleep, respiration, heart rate, GPS, temperature, light. In order to develop an exposure model, **INTEGRA modelling** environment will be used. The program offers a number of models such as multimedia environmental model, indoor air quality model and exposure models for the different espo-sure routes (inhalation, oral and dermal) to evaluate internal doses in target tissues, which will be compared to human HBM data.

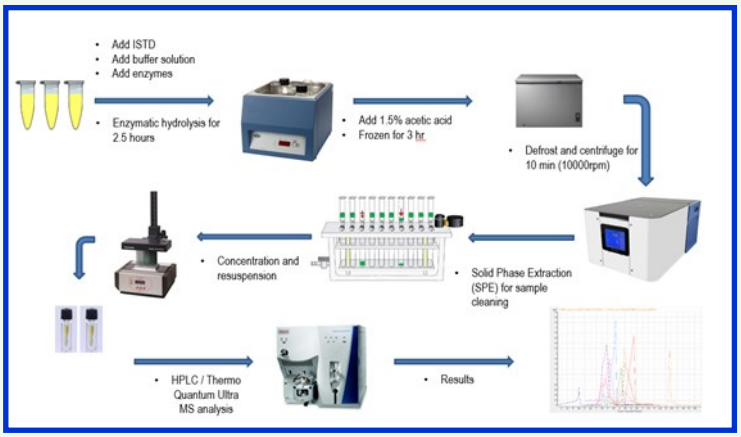



**Fig 13:** Human biomonitoring of phthalate exposure

## ESR13: Development of "big data" analytics approaches and their use to relate omics, exposure and health outcome data

Ourania Anesti, Aristides Tsatsakis, ToxPlusSA

My work consists of collating and organizing existing omics, exposure and health outcome data (related to neurodevelopmental disorders) and to applying advanced methods for multi-output classification and regression to the data in order to find genome-exposome-wide health associations.

To date, I have been working on analysis of data coming from the **Greek EXHES cohort**, and specifically sub-cohort related to exposure to waste contaminated sites. **HERACLES,** is a cohort study aiming at assessing the contribution of environmental contamination from heavy metals and how the latter are associated with child neurodevelopment (Fig 14).

Around 350 children aged 3 to 8 were enrolled in HERACLES, living at a distance between 0.5 to 12 km from the Fili landfill outside Athens. In order to derive **exposome-wide associations** with adverse health outcomes pertaining to neurological perturbations, several exposure factors have been investigated, including: exposure to heavy metals, such as Cd, Hg and As in urine, Pb in blood, Mn and Hg in hair, additional proxies of exposure, such as distance from the contaminated sites, concentration of heavy metals in the soil of the child address (Fig 15).

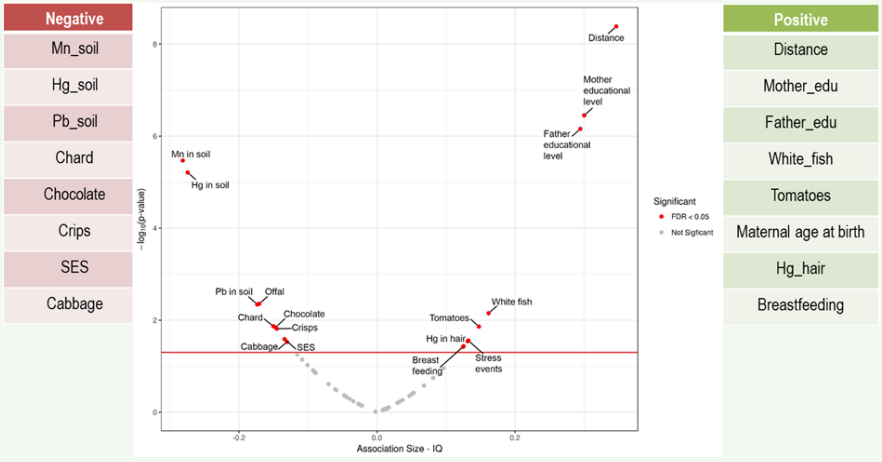



**Fig 14: Chemicals present in different samples**

**Neurodevelopmental progress** in children was estimated with the Wechsler Intelligence Scale for children (WISC-IV). In addition, analysis of **metabolomics profiles** from urinary samples and the subsequent pathway analysis were carried out to identify the pathways perturbed and associated with neurodevelopmental progress (Fig 15).

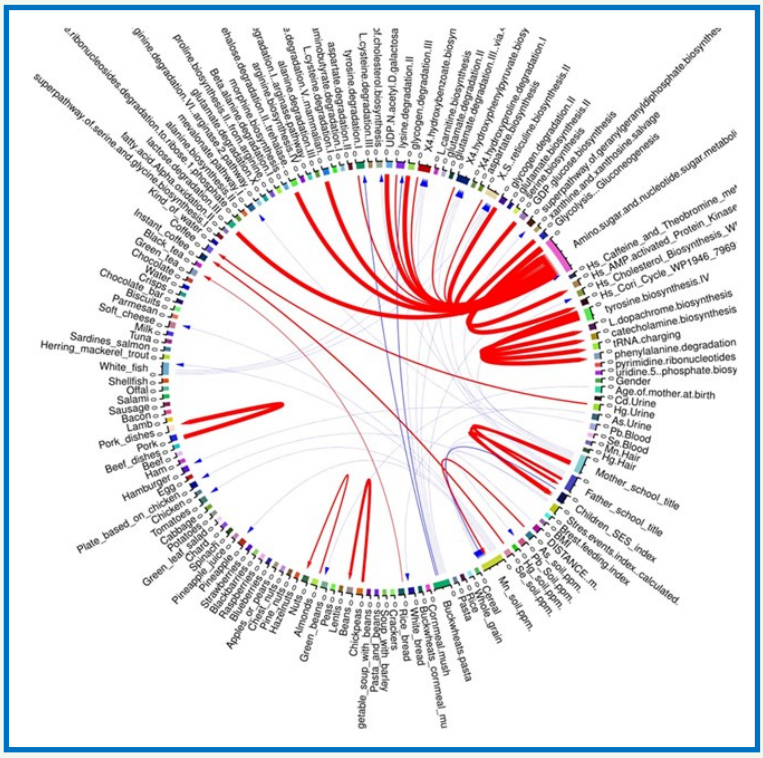



**Fig 15:** Correlation globe among external exposome and metabolic pathways

**EWAS analysis** showed that distance of the child residence from the waste management site is a key factor associated with almost all the indices of the WISC IV test, including the intelligence quotient (IQ). Among the several exposure parameters, it was found that soil contamination with Mn, Hg and Pb has a negative effect on intelligence quotient; the latter is also affected negatively by consumption of chard, chocolate and cabbage. Low SES has also been shown to be negatively associated with IQ. Other factors also affect the IQ.

Based on the **metabolomics analysis** and the subsequent **bioinformatics analysis**, several metabolic pathways were identified. Hg in child hair and maternal educational level are associated with perturbations in the mevalonate pathway. The mevalonate cascade is a key metabolic pathway that regulates a variety of cellular functions and is thereby involved in the pathophysiology of many brain diseases, including neurodevelopmental and neurodegenerative disorders. Further work will include analysis of datasets coming from other cohorts from the countries involved in NEUROSOME.

## ESR 14: Genome-wide profiling and identification of single nucleotide polymorphisms (SNPs) relevant to susceptibility to neurodevelopmental disorders

Irene Fragkiadoulaki, V. Pecile, BURLO

The study design (Fig 16) is based on the collection of **cord blood** and **cord tissue samples** obtained from **European population studies** such as PHIME cohort, in order to extract the DNA and apply **whole genome genotyping** using SNP arrays by Illumina.

Quality control of the raw data is required, using the Genome Studio and PLINK software before performing genome-wide association analysis with data obtained from the population studies.

So far, samples from the **Italian PHIME cohort** have been collected and analyzed. From 604 cord blood and tissue samples DNA has been extracted, quantified and genotyped with the Infinium global screening arrays by Illumina.

With these particular **SNP arrays ~700.000 SNPs** can be detected in each sample, the most common SNPs that have been

identified in the **human genome** and that occur within **genes** and **regulatory regions.**

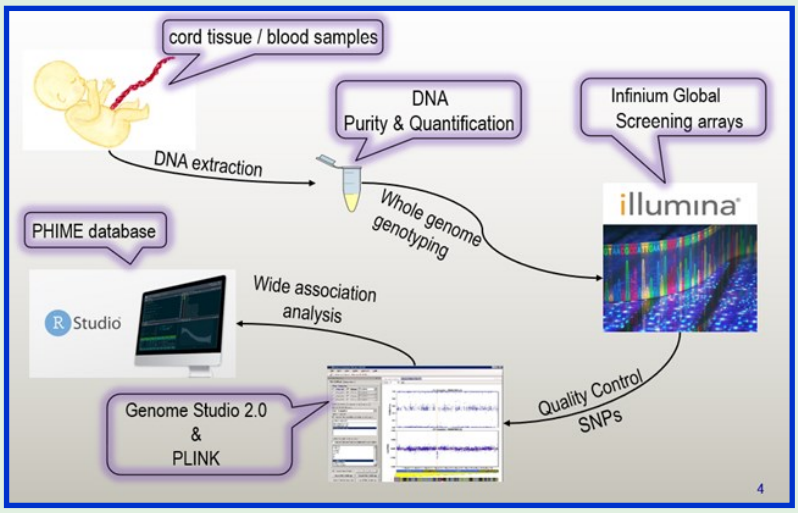



**Fig 16:** Study design for genome analysis

Quality control of the raw data was carried out in two stages. The first entailed using the Genome Studio software to detect the genotype rate of each sample and the possible contamination of the samples during the procedure. Fifty six samples with call rate less than 98% were excluded, as per manufacturer's specifications. In the rest of the samples, a second quality control was performed with the whole genome data analysis toolset PLINK. In this case, 148.168 variants with genotype call rate less than 90%, with minor allele frequency less than 1% and with p value less than 0.001 of deviation from Hardy-Weinberg Equilibrium were excluded.

# *Dissemination Activity*

## NEUROSOME: Interim Meeting

Neurosome Interim meeting took place on 19th December, 2019 at National Centre for scientific research, Athens, Greece.

Main agenda for the meeting was discussion about periodic report for each workpackage, Supervisory board report, ESRs periodic report and information package for MSCA fellows on its rights and obligations.

Meeting was attended by experts from Marie Sklodowska Curie actions: Dr. Sara Garcia-EU project officer and external expert (Monitor) Dr. Mariana Guergova-Kuras.

It started with discussion about progress on work package activity which was presented by different supervisors. Advanced environmental and exposure science (Prof. J. Grimalt), Analytical exposure biology (Dr. G. Calamandrei), High dimensional bioinformatics and in silico toxicology (Prof. D. Sarigiannis), Genome-exposome-wide health association studies (Prof. P. Stivaktakis).

Other work packages include Structured training courses (Prof. R. Barouki), Dissemination and communication (Prof. M. Schuhmacher) and Recruitment and career enhancement (Prof. D. Sarigiannis).

It was followed by 10 min presentation by each ESR on progress of their project and interaction with other ESRs for acquisition of data and training needed for running of the project.


Meeting also included discussion of ESRs with EU officer and external experts for discussing about the problems and possible solution for smooth working of ESRs.

It was followed by Supervisor meeting, management issues and final conclusion, outlook and next steps for the NEUROSOME project,

## NEUROSOME Training & ESR Networking

Neurosome Training and ESR networking meeting took place on 17-18 December 2019 in Athens, Greece.
Agenda of the training was to train the ESRs about different work packages and create networking among them.
It started with work package 4 which include discussion about multimedia models and personal sensor for exposure assessment.

Further, discussion was about analysis of high throughput data (metabolomics) and pre-processing of metabolomic data.
High dimensional bioinformatics and in silico toxicology was discussed which includes PBPK model, qAOP, analytical exposure biology and integration of different techniques for risk assessment.
Genome wide association studies which associates neurodevelopmental disorders at genetic level were also discussed.

ESR network meeting included progress about each ESRs work, discussion about ESR in relation to personal career development plan, dissemination activities and training.

It enhanced the understanding and improved communication within ESRs helpful for healthy and productive research.

# *FORTHCOMING EVENT*

1. Training course organized by BURLO on Genome Wide Association Study (GWAS) and will take place in ISS, Rome, Italy.

2. Training course on High throughput OMICS data (metabolomics, proteomics, genomics, transcriptomics) at IUSS, Pavia, Italy.

3. Bioinformatics and in silico toxicology training course at AUTH, Thessaloniki, Greece.

4. Summer school focused on Human bio-monitoring data (HBM) and OMICS in July at AUTH, Thessaloniki, Greece.

Training Courses will be held for 4-5 days.

## Editorial Board

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Prof. Denis Sarigiannis

**Editorial Information**

If you wish to share any information or contribute to the newsletter, please inform Prof. Marta Schuhmacher.
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