"The Human Early-Life Exposome novel tools for integrating early-life environmental exposures and child health across Europe"

ENV-FP7-2012-308333

Report of HELIX Final Scientific Symposium

Barcelona, 30-31 October 2017

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Introduction

The final scientific symposium of the HELIX project took place on 30-31 October 2017 in Barcelona, Spain. This was the final meeting where the project consortium and stakeholders came together to discuss the key successes of the project studies, and translation of the results into future action at scientific and policy level. The meeting was structured around the different work packages of the project. External experts from other projects presented their views on Exposome research.

This report provides a summary of the presentations and discussions that took place over the course of the meeting.

"New Horizons for Early Life Exposome Research"

Scientific Symposium - Programme

Barcelona – 30-31 October 2017

Organising committee:

- HELIX: Martine Vrijheid, Lea Maitre, Diana van Gent, Iolanda Molina (ISGlobal), Cathrine Thomsen (NIPH Norway), Muireann Coen (Imperial College London), John Wright (Bradford), Rémy Slama (Inserm, Grenoble), Peter van den Hazel (VGGM, Netherlands)
- EXPOSOMICS: Paolo Vineis (ICL), Roel Vermeulen (IRAS, Utrecht)

Day 1

9.00-9.15 Welcome

9.15-11.00 Session 1. Setting the scene - the Exposome projects (chair: Mark Nieuwenhuijsen)

- The global, societal and policy challenge - Manolis Kogevinas
- Research on Environment and Health and the Exposome – EU Perspectives - Tuomo Karjalainen
- What have we learnt in HELIX and why does it matter? Martine Vrijheid
- What have we learnt in Exposomics and why does it matter? Paolo Vineis
- What have we learnt in HEALS and why does it matter? Spyros Karakitsios
- Update on US exposome efforts – Hercules. Gary Miller
- Update on US exposome efforts – NIEHS. Gwen Collman
- Discussion

11.00-11.30 Coffee

11.30-13.00 Session 2. Describing the early life Exposome and its determinants and variability across Europe (chair: Martine Vrijheid) – part 1

- **Keynote:** The In-utero and early life chemical exposome – Cathrine Thomsen (NIPH)
- Estimating the early-life exposure to two perfluorinated compounds (PFOS and PFOA) using PBPK modeling and biomarker measurements – Celine Brochot
- Diet as a source of exposure to environmental contaminants for pregnant women and children - Eleni Papadopoulou
- **Keynote:** The urban exposome, determinants & variability – Mark Nieuwenhuijsen (ISGlobal)
- ExpoApp: the integrative system to assess the external exposome & Personal exposure to the external exposome in pregnancy and childhood in Europe – David Donaire

13.00-14.00 Lunch & Posters

14.00-14.30 Session 2. Describing the early life Exposome and its determinants and variability across Europe (chair: Martine Vrijheid) – part 2

- Early life exposome patterns in 6 countries – Xavier Basagaña
- ➢ Discussion of summary statement: "achievements and challenges"

14.30-15.45 Session 3. Omics signatures related to multiple early-life environmental exposures (chair: Muireann Coen) Part 1

- **Keynote** – Cataloguing omics signatures of many environmental exposures – HELIX results (Mariona Bustamente / Lea Maitre ISGlobal)
- The urinary and serum metabolome in children from six European populations – Alexandros Siskos
- Epigenetic marks of early life exposure to particulate matter – Michelle Plusquin
- Smoking and methylation – Marta Vives
- The exposome and telomere length – Diana Clemente

15.45-16.15 Coffee

16.15-17.30 Session 3. Omics signatures related to multiple early life environmental exposures (chair: Hector Keun) Part 2

- **Keynote** - Biostatistical/bioinformatics challenges – Marc Chadeau (ICL)
- Human epigenome, transcriptome, metabolome and proteome variability explained by the exposome - Juan Ramon Gonzalez
- Molecular signatures time variability in HELIX panel paired-samples - Carles Hernandez

➢ Discussion of summary statement: "achievements and challenges"

Day 2

9.00-10.45 Session 4. Assessing health effects of the early life Exposome (chair: Rémy Slama)

- **Keynote** - Systematically associating multiple exposures with child health: Methodological challenges & HELIX results – Rémy Slama (INSERM)
- Selection of short presentations with HELIX results
    - Exposome and cognitive and behavioural development – Léa Maitre
    - Exposome and allergies – Berit Granum
    - Exposome and lung function – Lydiane Agier
    - Exposome and blood pressure - Charline Warembourg
    - Exposome and obesity - Martine Vrijheid
    - Discussion
- Beyond bioinformatics: contributions from health informatics to exposome research – Fernando Martin–Sanchez (ISCIII)

➢ Discussion of summary statement: "achievements and challenges"

10.45-11.15 Coffee

11.15-12.30 Session 5. Translation of Exposome results to policy (chair: John Wright)

- **Keynote** - Health impact assessments related to the early life exposome - David Rojas (ISGlobal)
- Development of Exposome App – John Wright

- Discussion of policy and societal implications of results (based on last slide of each presentation) – John Wright / Peter van den Hazel

12.30-13.30 Session 6. Networking between Exposome-related projects in and outside Europe – "building an exposome toolbox" (chair: Cathrine Thomsen)

- Short presentation on each project's match with "exposome toolbox": HBM4EU (Marike Kolossa), HELIX, LifeCycle (Martine Vrijheid), EXPOsOMICS, LifePath (Paolo Vineis), DynaHealth (Marjo-Riitta Jarvelin), US projects (Gary Miller, Gwen Collman), HEALS (Spyros Karakitsios)
- Project 'Speed-date' (Diana van Gent)
- Discussion on networking, complementarities, gaps, future ...

13.30-14.30 LUNCH – networking continued + posters

14.30-16.00 Session 7. Reflections on the Future of (early life) Exposome Research (chair: Remy Slama)

- Toxicologist's view: Thomas Hartung (Human Toxome project)
- Epidemiologist's view: Roel Vermeulen (IRAS)
- SAB statement (SAB members)
- Closure

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Posters

No	Abstract Title	Presenting Author
1	**Environmental exposures and the development of asthma in children**	Pippa Bird, Noortje Uphoff
2	**Use of high-resolution metabolomics to identify potential metabolic pathways associated with traffic-related air pollutants**	Rachel Golan
3	**The early life full exposome: associations with child cardiometabolic risk and proatherogenic lipid profile**	Vafeiadi M
4	**The exposure to NO_2 eliminates the short-term positive effects of physical activity on children's lung function**	David Donaire-Gonzalez
5	**Exposure to endocrine-disrupting chemicals and blood pressure during pregnancy**	Maribel Casas
6	**Exposure assessment of pregnant women to Di(2-ethylhexyl) phthalate by reverse dosimetry: Variability in repeated spot sample**	Florence Zeman
7	**Characterizing the cognitive and behavioral outcome in HELIX sub-cohort children - HELIX PSYCHOMETRIC DATA**	Kristine Bjerve Gutzkow
8	**Personal exposure of different fractions of particulate matter in children from INMA cohort in Valencia**	Ferran Ballester
9	**Assessing the associations between persistent organic pollutants and endometriosis: single versus multi-pollutant approaches applied to a first case-control study**	German Cano-Sancho
10	**Environmental Contaminants in Breast Milk in Israel**	Elkana Kohn
11	**Exposome research in Flemish cohorts**	Sylvie Remy
12	**Linkage between methylation probes and expression transcripts**	Carles
13		Eliana Ein Mor
14	**Urban Exposome during pregnancy and its socio-economic determinants**	Oliver Robinson

Session 1 Setting the scene

- *Background and Objectives of the HELIX symposium*

The final meeting of the HELIX project "New Horizons for Early Life Exposome Research" took place in Barcelona, Spain on 30-31 October 2017. The meeting programme was structured around the main research topics in HELIX and networking across the Exposome-focused projects. The presentations fell into four main themes: presentations on parallel research initiatives on the study of the exposome in Europe and in the United States, presentation of the main results and the advances in exposome research achieved through the HELIX project and its translation to policy, networking between Exposome-related projects in and outside Europe – "building an exposome toolbox" and reflections on the future of (early life) exposome research.

This paper summarizes the presentations and discussions at this meeting.
What have we learnt in HELIX and other Exposome projects?
Why does exposome research matter?
Establishing a network of experts in Exposome research - How can we reinforce links within and outside Europe?

Exposome research has flourished across the world and the main actors were invited to present their project highlights and why does this research matter.

- *The global, societal and policy challenge - Manolis Kogevinas*

Manolis Kogevinas from ISGlobal put environmental health research in the context of science and its general benefit for society. The argument was based around the quote from Francis Bacon, 1605 "Science discovery should be driven not just by the quest for intellectual enlightenment, but also for the relief of man's estate". In the case of environmental health research, benefit society by improving health and wellbeing and promoting sustainable development and wealth. The general public concerns about sustainability and science integrity was highlighted by the "March for science" movement across the world. The March for Science was a series of rallies and marches held in Washington, D.C., and more than 600 other cities across the world on Earth Day, April 22, 2017. Although this movement was led by scientists it emphasized society support for government funding for scientific research, government transparency, and government acceptance of the scientific consensus on climate change and evolution.

There may also be an economic benefit of the exposome research. Based on the example of the human genome project, each dollar invested (USD 3.8 billion) has generated economic activity worth USD 140 (see Tripp S & Grueber M. Economic Impact of the Human Genome Project. Battelle Technology Partnership Practice. 2011. Available at https://www.battelle.org/). However, the economic benefits may not be distributed down through society and may increase inequities. For example in the case of the human genome project driving the healthcare cost up through expensive drugs only affordable by a few.

Science research has to benefit society and be perceived as so. To ensure that research reaches this goal, the following criteria were proposed (Kogevinas, Environ Epi 2017):

- Novelty: Will research in a specific area produce new knowledge?

- Importance to People: Will the life and well-being of many populations be positively affected?
- Impact on Policy: Will research in a specific area produce
- knowledge that meaningfully informs evidence-based health policies and prevention?
- Technical Innovation and Development: Will research produce new technologies and help economic development?

Another challenge, beyond research needing to be designed for societal impact, there is a lack of governance for major health/environment issues. There issues that need global action but encountered difficulties such as the climate change action and pesticides e.g. glyphosate. To improve the uptake of health/environment evidence into policy, researchers should take into account the political agenda and their timescale (CJM Whitty, BMC Med 2015).

Finally, communication to a wide audience is key, for example the use of new media to communicate science. New communication channels such as Twitter may be as important as the citation index of scientific communication.

- *Research on Environment and Health and the Exposome – EU Perspectives - Tuomo Karjalainen*

Tuomo Karjalainen from the Research Programme Officer DG Research and Innovation Health Directorate gave the EU perspective on Exposome research. The EU was pioneer in 2010, funding the first large exposome projects while at the time only one publication from Christopher Wild (2010) was describing the exposome. Around 360 projects have received over a billion euros from FP5/FP6/FP7/H2020 between 1998 and 2016. 1190 institutions from 68 countries worldwide benefitted from the FP7 programme which shows a wide-reaching outreach. However, in 2010 the health and environment directorates were separated which meant less knowledge about the environment component. This was rectified with the 7th Environment Action Programme 2013-2020. Currently, the exposome concept has gained popularity among the scientific sphere with numerous publications but also at the policy level. The French government was the first to include the word “exposome” in its political agenda in the 2015-2019 national health plan.

The different strategies of the EU-funded research on environment and health can be found in a series of publication (Karjalainen T. 2015, 2016, 2017).

The EU Exposome Cluster projects include:

- HEALS, Health and environment-wide associations based on large population surveys 2013-2018
- HELIX The human early-life exposome – novel tools for integrating early-life environmental exposures and child health across Europe - 2013-2017
- Exposomics: Enhanced exposure assessment and omic profiling for high priority environmental exposures in Europe 2012-2017

And the Projects under H2020:

- LifeCycle: Early-life stressors and LifeCycle health 2017-2021
- Lifepath: Lifecourse biological pathways underlying social differences in healthy ageing 2015-2019
- HBM4EU – The European Human Biomonitoring Initiative 2017-2021

- HBM4EU which is coordinated by the German Environment Agency (UBA) is a joint European programme for monitoring and scientific assessment of human exposures to chemicals and the potential health impacts. It is particularly designed to answer open policy relevant questions. It also aims to give policymakers a fast and easy access to results and data through the IpCheM (https://ipchem.jrc.ec.europa.eu/).

Visions for the future of the exposome were collected via an online survey in 2016 mainly answered by academics but from multiple disciplines. The key elements of the future exposome are:

- Establish causality between environmental/lifestyle factors and major chronic diseases
- Technology push for exposure assessments in two domains: (1) External exposure: personal portable sensors and the (2) Internal exposome: high-throughput analytical methods
- Pan-European (international) and multidisciplinary
- Open access to data through data warehouse of exposome data

The future approaches should both be holistic by not focusing on pre-defined exposures and health outcomes to understand complex interactions between biology/disease/exposures but at the time it was also envisaged to have a more targeted approach for health outcomes. For example to focus on highly exposed population or sensitive period of life. It was suggested on the same line that participants from low and middle income countries should be included to broaden the exposure and interaction range. The leading actors in the future exposome projects should still be academic research centers but also involve regulators for example EU agencies such as ECHA and the private sector for providing better technology (sensors, omics platforms, services aiming at (personalised) disease prevention). The private sector might also be key in providing data (e.g. google maps).
The public health benefits of the future exposome lies in improved science-based risk assessment and management and personalized risk assessment. The main expected impact are:

- Stimulation of innovation in environmental health science and technology, in ubiquitous sensing, in high throughput biological analysis technologies and in big data analytics
- Promotion of transdisciplinary education and training to create a new breed of environmental health scientists who would be science “integrators” and “translators” into prevention
- Enhancement of diagnosis and treatment of multi-factorial diseases and the efficacy of public health programmes via inclusion of environmental factors and their health impact
- Support precise prevention policies rendering them much more cost-effective and thus enhancing their outreach

Several upcoming calls for proposals 2018-2019 – Health, Demographic Change and Wellbeing Societal Challenge are relevant for future exposome research, in particular “The Human Exposome Project: a toolbox for assessing and addressing the impact of environment on health (2019)”. Drafts are available at https://ec.europa.eu/programmes/horizon2020/en/what-work-programme

With the deadline for submission for 2018 calls: 18 April 2018.

- *What have we learnt in HELIX and why does it matter? Martine Vrijheid*

The HELIX project focuses on (1) early life, the starting point for a life-course exposome and on a (2) wide-coverage of the exposome.

- The following policy relevant questions within the exposome framework were formulated:
- How do environmental exposures from a wide range of sources correlate in different populations across Europe?
- Can we identify groups exposed to many environmental “hazards”?
- Which factors determine multiple exposure patterns?
- Which are the exposures or exposure patterns or exposure interactions most strongly associated with child health outcomes (in order to set priorities)?
- Can we identify molecular imprints of external exposures (in order to improve risk assessments and knowledge on mechanisms and pathways)?
- Can we estimate the health impacts related to many exposures?

The HELIX project is a nested multi-level design across six existing birth cohort studies in Europe. HELIX estimated prenatal and postnatal exposure to a broad range of chemical and outdoor urban exposures. Exposure models were developed for the full birth cohorts totaling 32,000 mother-child pairs, and biomarkers were measured in a subset of 1,300 mother-child pairs. Nested repeat-sampling panel studies (n=150) collected data on biomarker variability, used smartphones to assess mobility and physical activity, and perform personal exposure monitoring. Harmonised and comparable outcomes data were archived for neurodevelopment, respiratory health/allergy, growth/obesity in six countries. Omics techniques provided molecular profiles (metabolome, proteome, transcriptome, epigenome) associated with exposures. An exposome database (www.projecthelix.eu/index.php/es/data-inventory) has been built with comparable biomonitoring data, geospatial data, sensor data, health outcomes and omics signature during pregnancy and childhood. This will be available to external collaborators and researchers. As part of the description of exposome patterns across Europe, sources and determinants and high risk groups can be identified. The health associations with multiple exposures allowed a systematic evaluation of child health risk and may allow ranking of important exposures to target prevention. Advancement of methods and tools for the analysis of the Exposome was achieved through testing different approaches including regression-type approaches (Agier, Portengen et al., EHP, 2016), multiple testing correction, interaction (Barrera, et al. Environ Health 2017). A health impact assessment exercise evaluated risks and benefits of combined exposures through different scenarios. Some work has already been published (6 publications) and more than 70 are underway for end of 2017-2018.

- *What have we learnt in EXPOsOMICS and why does it matter? Paolo Vineis*

Paolo Vineis from Imperial College London reflected on the lessons learnt through the European Exposomics project that ended in June 2017. The focus of the project was on water contaminants and air pollution, both at the population and individual levels (external and internal exposome approaches) and the use of novel technologies to improve exposure assessment and effect. The highlights of the project included:

(1) the use of metabolomics to capture perturbation pathways in short term exposure experiments to swimming (water contaminant) and air pollution;
(2) the meet-in-the-middle approach to reinforce causal assessment. This approach was applied to identify potential mediating effects of exposure to air pollution on cardio-vascular disease risk through OMICs signals (proteome and methylome). Key inflammatory markers were found as mediating effects (Fiorito et al, Environmental Molecular Mutagenesis, in press).

(3) The use of new technologies such as adductomics that may serve the purpose of increasing sensitivity and specificity in identifying relevant chemicals in mixtures, low-dose effects and dose-response.
(4) Effects of components in a mixture of air and separately water contaminants. Mixture effects were analysed through Ven diagrams of OMICs signals and statistical modelling (Marc Chadeau presentation).

Limitations were observed, in particular with metabolomics and difficulties in annotation and pathway reconstruction. Metabolomics was also revealed to be instructive on detecting signals in population with high exposures for air pollution.

- *What have we learnt in HEALS and why does it matter? Dennis Sarigiannis*

- Exposures occur in clusters and thus pathway network analysis is needed to grasp the complexity.
- Need to bridge epidemiology and toxicology with mechanistic understanding of adverse outcome networks.
- Need to embrace complexity – use mechanistic information to arrive to simple interpretations giving rise to the concept of simplexity in environmental health science and risk assessment.
- We need to understand how multiple stressors interact with human physiology and identify simple strategies to address adverse outcomes targeting the critical nodes in the biological/metabolic regulatory networks.

- *Update on US exposome efforts – Hercules. Gary Miller*

Gary Miller from the University of Emory, Atlanta, USA, is the director of the HERCULES Exposome Research Center (https://emoryhercules.com/) which was funded in May 2013 by a Core Center Grant from the National Institute of Environmental Health Science. After Chris Wild coined the term Exposome 12 years ago (C.P. Wild Cancer Epidemiol Biomarkers Prev 2005) - the work to apply this concept in new projects seemed colossal but the HERCULES center took the challenge and started the different labours. It worked on promoting the concept of the Exposome through online and conference channels, educational programmes and community engagement in Atlanta (Community mini-grant program to address issue of environmental concerns, Exposome Roadshow). The center assesses both external exposures through satellite remote sensing and internal through high resolution metabolomics (HRM). The HRM effort, led by Dean Jones, is based on a new gas-chromatography- Orbitrap system that allows to quantify hundreds of environmental chemicals and capture thousands of untargeted features. The HERCULES center hopes that this platform will develop to be the most effective way to measure the exposome. It has the capacity to measure both polar (representing mainly the biological response) and the volatile fraction of metabolites present in biospecimens (mainly environmental exposures). The center is equipped with a strong bioinformatics group developing pathway analysis tools and databases. Further developments include analyte detection in microneedle patches and C. Elegans modeling for assessment of complex exposures and G x E analyses. New collaborators such as the Mayo clinic are interested to include environmental aspect in their studies.

- *Update on US exposome efforts – NIEHS. Gwen Collman*

Gwen Collman from the National Institute of Environmental Health Science (NIEHS) presented specific examples of ongoing exposome-related projects in the U.S in the three areas of exposomics:

Tools and resources:

a) Sensors: Funding the development of wearable tools for measuring chemical exposures and applying them in large epidemiological studies to improve exposure assessment and estimation of exposure-health outcome relationships.

b) Big Data Knowledge (BD2K): This program was launched in 2004 to increase the use of biomedical big data, develop and disseminate analysis methods and software, enhance training and establish centers specialized in biomedical big data.

c) The Children's Health Exposure Analysis Resource (CHEAR): They support researchers with biological samples to measure environmental exposures. The data generated can be found in the CHEAR data repository. The CHEAR data center will develop community-based data standards and ontologies and metadata standards to promote broader data sharing. This requires researchers to use certain terminology for comparison purposes.

Mixtures:

a. National Toxicology Program Mixtures Research (NTP): To estimate the combined effects of mixtures through component-based and whole mixtures approaches. The component-based use individual chemical dose-response data to predict mixture effects, one example is the PAC Program, which assess whether the toxicity of a PAC mixture can be predicted using information about the toxicity of individual PACs. NTP will generate data on PAC mixtures that could predict the toxicity of untested mixtures. The whole mixtures approach used similar reference mixtures to estimate the toxicity of related mixtures.
b. Powering research through Innovative Methods for mixtures in Epidemiology (PRIME): Development of innovative statistical, data science and other quantitative approaches to studying the health effects of complex chemical mixtures.

Exposure and Biology:

a. Biomarkers signatures: Funding of research linking exposures to biological responses, to improve the understanding of the mechanistic connections (such as telomere length changes, epigenetic modifications…).
b. TaRGET II Consortium: Comparing epigenetic changes from environmental exposures in target tissues. In order to determine whether epigenetic changes in easily obtained samples (eg. Blood) can simulate changes in other tissues.
c. NIH Common Fund Metabolomics Program: Development of technologies of metabolomics, and provide training and increase the inventory of chemically identifiable metabolites by data sharing.
d. Small Business Innovation Research and Small Business Technology Transfer Programs: NIEHS supports small businesses to develop innovative applications to bring environmental health research products to market.
The NIEHS SBIR / STTR Programs focused on development of novel approaches using state-of-the-art technologies for environmental health sciences. The NIEHS Exposomics priority areas in the U.S. and

beyond are the technology and infrastructure support, mixtures approach, data standards, biological response, international and interdisciplinary collaboration and training.

NIH common fund metabolomics program

Next phase peak identification

- *Plenary Discussion*

Tuomo Karjalainen from the EU emphasised the existence of databases such as IPchem to upload exposome data. However, the current exposome projects responded that the existing platforms cannot handle the type of data produced in exposome projects such as longitudinal data or health outcomes with strict data privacy policies. There are sustainability and ethical issues to be solved for exposome projects. For instance, the need for a budget dedicated to maintaining exposome databases after the project's end. In addition, intellectual property should be discussed as part of a public database. The databases of HELIX and Exposomics are maintained currently in the different institutes and HELIX is granting access to its database to external researchers under specific approvals.

John Wright from the BiB cohort (UK) highlighted the potential room for collaboration across cities with personal monitoring of exposure assessment, also across projects HELIX and Exposomics. Results could be replicated across the cities.

Question about air pollution omics results from exposomics
For overlap across exposures, what threshold to use for multiple testing, BN too stringent for Ven diagrams? Correlation across exposures taken into account? No because univariate analyses.

Session 2 Describing the early life exposome and its determinants and variability across Europe

- *Keynote: The in-utero and early-life chemical exposome - Cathrine Thompsen*

This study presents harmonized and completely comparable biomonitoring data for a broad range of environmental contaminants in children from several European countries.
Close to 20,000 chemical analyses were performed. Among the persistent chemicals measured, PFOS dominate, whereas among the non-persistent compounds two phthalates dominated (MEP or MEHHP depending on the cohort). In addition comparison between mother and children (HELIX subcohort, approximately n=1300) and between cohort centers were presented. Variability and reliability of exposure biomarkers within the panel studies were also presented.

:

- *Estimating the early-life exposure to PFOS and PFOA using PBPK modeling and biomarker measurement - Céline Brochot*

PBPK modeling was applied (1) to estimate the early life exposure of the children of the HELIX subcohort (in utero to the age of 10) and (2) to compute indicators of internal exposure related to the perfluorinated compound (PFC) toxicity.

A reverse dosimetry approach was used: from biomonitoring data to exposure level (daily intake estimated).
Data used included maternal (weight and weight gain during pregnancy, maternal age, parity, duration of previous breastfeeding period), newborn (birth weight, breastfeeding period and formula milk) and child (age and body weight) characteristics.

Indicators of internal exposure in target organs were calculated for the brain, kidney, and liver (the AUC increases, respectively).

For the future:

1. This approach was applied to two chemicals with sufficient toxicological and toxicokinetic data available but how to proceed with thousand of chemicals?
2. The models presented for PFOS only because PFOA is highly variable between mothers. Need to understand sources of variability.

Question: What about diet as a predictor of PFOS and PFOA exposures? → → The data on food consumption collected via the questionnaire were not included in our approach, but the intake from diet was integrated in the estimated daily intakes.

- *Diet as a source of exposure to environmental contaminants for pregnant women and children - Eleni Papadopoulou*

Eleni Papadopoulou presented preliminary results on the associations between food intake and the levels of measured contaminants (biomarkers) in mothers and children. Several associations have been observed and she focused on the associations observed among both the mothers and the children. Another very interesting part of this analysis was to study the levels of biomarkers that were studied according to a healthy diet (international recommendations, mediterranean diet) and frequency of organic food consumption. The results show the need to potentially reassess the risk and the benefits balance of major food groups by taking into account their contamination. Climate change impact could also have an impact of food contamination and availability.

Question: To go further, possibility to cross these data with data on food contamination (e.g. Total Diet Study).

- *Keynote: The urban exposome, determinants and variability - Mark Nieuwenhuijsen*

Mark Nieuwenhuijsen presented advances in building the urban exposome in the HELIX project. One of the goals of the project was to develop and apply novel tools and methods to obtain robust estimates of chemical and physical exposures in the outdoor environment ("the urban exposome"), focusing on key built environment measures (e.g. walkability, facility density) and outdoor exposures (outdoor air pollutants, noise, temperature, green space, UV radiation). The urban exposome made of 34 indicators is available for the entire HELIX cohort (around 32,000 subjects) in nine cities. In the subcohort study, additional personal information from questionnaire (e.g. physical activity) and geocoding data were integrated. In the panel studies, personal monitoring data were obtained from smartphones and sensors and integrated to ambient data on air pollution and other indicators.

- *ExpoApp: the integrative system to assess external exposome and personal exposure to the external exposome in pregnancy and childhood in Europe - David Donaire*

David Donaire presented descriptive results on personal exposure to the external exposome in pregnancy and childhood assess by sensors and smartphones. The aim was to characterize exposure levels to air pollution, noise, green spaces, UV-B radiation and physical activity and to assess the variability within and between participants and cities from the panel studies. He reported that pregnant women and children experienced environmental exposures above the international recommendations.

- *Early life exposome patterns in 6 countries - Xavier basagaña*

Here we aimed to describe the early-life exposome using data from the Human Early-Life Exposome (HELIX) project, in which 212 environmental exposures were measured in pregnant mothers and their children at 6-11 years in 6 European birth cohorts. Specifically, we focused on the correlations between multiple environmental exposures, their patterns and their variability across European regions and across time (pregnancy and childhood periods).

Completeness of the data: 98% of the participants have at least 70% of non-missing data.

Statistical challenges: missing data, collinearity, co-exposure, longitudinal changes, measurement errors.

- *Discussion of summary statement: "achievements and challenges"*

Chemical contaminants.

Key messages

- children are exposed to a wide range of chemicals
- almost all exposures vary substantially by cohort - reflect different sources, habits, etc
- recent exposure to banned pesticides
- measurement error estimates - reliability of measures can be taken into account
- PBPK modelling to reconstruct exposure in between biomonitoring points → understanding long-term exposure scenarios
- similar biomarkers levels can reflect large differences in exposure
- sources of higher contaminants: vegetables, fish, fruits (OP pesticides)
- organic food consumption related to lower concentrations of OP pesticides

Outdoor/Urban exposome.

Key messages

- new tools to collect data on commuting routes - qGIS → better data on exposure during commuting
- cohort determines your exposure pattern
- social patterns in the urban exposome vary by cohort
- tools to build the "urban exposome"

Patterns

- cohort effects
- changes over time
- 13% exposed to more or equal to 10 exposures in the highest quartile

Challenges

- efficient platforms for chemical analytics and GIS are key (20,000 biomarkers measurement, 1.6 milion GIS estimates)
- missing data and harmonization
- statistical
    - high dimensional data → not easy to reduce to a few principal components
    - cohort effect

Future?

- taking variability and measurement error into account in dose-response estimation
- using HRM techniques to measure many exogenous exposures? advantages?
- sensors development and downsize the equipment

Discussion/question:

- Add scaling up to urban exposome
- Transferability of methodology?
- Error in measurement might influence the PCA and patterns and make it more complex than what it looks? Comment from John - Harvard
- Bioinformatics challenges
- Drugs absent in helix exposome pic
- Data storage and sustainability
- Pooling biomonitoring data? Tuomo

Session 3 Omics signatures related to multiple early life environmental exposures

- *Keynote – Cataloguing omics signatures of many environmental exposures – HELIX results (Mariona Bustamente / Lea Maitre ISGlobal)*

As part of HELIX, the aim of omics applications is to describe the influence of the environmental exposures (i.e. the external exposome) on molecular phenotypes (i.e. the internal exposome) in children. Omics in exposome research can both improve exposure assessment through identifying biomarker of exposure (e.g. smoking during pregnancy and DNA methylation score) and to can allow to better understand molecular mechanisms perturbed by exposures and identify early signs of damage ("biomarkers of effect"). The molecular phenotypes, i.e. methylation, gene expression (including mRNA and miRNA), proteins, and metabolites (urine and serum) were acquired in 1300 children in HELIX from six different cohorts. Different approaches were applied across the omics technologies: non-targeted versus targeted approaches and different biological systems were covered, e.g. immune system (genetic content in white blood cells) versus systemic regulation (free metabolites in serum). The analysis pipeline include the description of the (1) main patterns in HELIX children molecular signatures, with two special focuses on the determinants of metabolic profiles and the temporal variability of omics data, (2) the main analysis in HELIX which provides a catalogue of the exposure-omics associations via univariate and multivariate approaches, both describing the prenatal and postnatal exposome associations.
In this keynote talk, Léa Maitre and Mariona Bustamante described the Exposome-wide association analysis (ExWAS) with the full omics datasets in an agnostic approach. These results reveal the

different nature of information extracted from five types of molecular profiles and how different exposures may be associated only with certain molecular strata/omics.

- *The urinary and serum metabolome in children from six European populations - Alexis Siskos and Chung-Ho E. Lau*

The HELIX metabolomics study has generated unique data defining the metabolome and its dynamics in European children. 1202 urine and serum samples from children were analysed by ^{1}H NMR and targeted LC-MS/MS analyses (Biocrates AbsoluteIDQTM p180 Kit) respectively. The main suspected determinants of the metabolome accounted in average for less than 10% of the variability in the profiles, including the analytical batch (technical), the time to last meal before sample collection, demographic parameters (age, gender, BMI, ethnicity), dietary food group frequency intake and cohort. Child BMI was mostly associated with already published markers of obesity in adult population. Associations were consistent across different adiposity measures and cardio-metabolic outcomes. Dietary food groups provided further insights into the origins of some urinary and serum metabolites, in particular related to fruit, dairy products and fish intake.

- *Epigenetic marks of early life exposure to particulate matter – Michelle Plusquin*

As a part of EXPOsOMICS project, the aim is to study longitudinal DNA methylation to discover signals associated to early life exposure to PM10. An epigenome-wide study of DNA mthylation in relation to PM10 exposure has been done at birth, childhood and adolescence and no CpGs were significantly associated after corrections for multiple testing in utero exposure and early life to particulate air pollution. Comparing ranks of methylation in childhood and adolescence in relation to in utero exposure vs current exposure it has been found differences in BCL7C, F12, PPP2R2C, EXOC2.
Several novel CpG sites and mechanisms, that may create a molecular basis for the association between air pollution and health outcomes, have been detected. The identified targets point toward neurological, cell division control and coagulation mechanisms, though they have to be further tested in future studies.

- *Smoking and methylation – Marta Vives*

As part of HELIX project, the aim is to study the association of prenatal maternal smoking and prenatal and postnatal environmental tobacco smoking with molecular signatures in children. Two different variables at a prenatal level were modeled (one self-reported based on questionnaires about mother smoking status and the other one based on cotinine levels of the mother during pregnancy) and two variables at a postnatal level (one about exposure of the children to tobacco smoking based on questionnaires and the other one based on cotinine levels in children). It was found that these postnatal smoking exposures are cohort dependant and that the two variables are correlated but not perfectly.
Linear regression models were performed between each exposure and each omic adjusting for cohort, sex and age and also for surrogate variables in the case of methylation, transcriptomics and miRNA.

- *The exposome and telomere length – Diana Clemente*

As part of HELIX project, the aim is to assess the effect of exposure to environmental stressors (air pollution, obesity, tobacco smoke and endocrine disruptors) on telomere length measured in the HELIX subcohort. Telomeres are repetitive DNA sequences at the end of the chromosomes that protect them from degradation and from end-to-end fusion. It has been shown that shorter telomeres are associated with age-related diseases and that telomere length is affected by genetic

determinants and environmental factors, including inflammation and oxidative stress. Regression models adjusted for cohort, child's sex, child's age, maternal ethnicity, maternal age at birth and maternal education were performed.
The results highlight the importance of intervention that may impact the future life by decreasing comorbidities in adulthood.Standards should be based on vulnerable time periods

- *Keynote - Biostatistical and bioinformatics challenges, Marc Chadeau*

Statistical challenges for:

- Screening models 'OMICs & Exposure profiling': identify within each OMICs platforms & (sets of) exposures, relevant signatures of exposures health outcomes. The challenge is to take into account mixture and complex study design
- Interpretation, functional and biological characterisation of identified OMICs biomarkers
    - OMICs mechanism exploration: identify sets of markers jointly responding to exposures
    - OMICs integration: integrate results arising from several OMIC platforms and explore their interplay

OMICs and Exposome profiling:
Compare to the internal exposome, the external exposome has some specificities since exposome data are lower dimensional, highly heterogeneous and present complex correlation structures within and across groups of exposures. When the number of predictors (that could be OMICs or exposures data) is larger than the number of observations, three main statistical approaches are available: (1) univariate ExWAS that correct for multiple testing, (2) dimension reduction techniques, and (3) variable selection approach. A simulation study for identifying the best approach to analyse exposome data tested various methods and identified the clear advantage of using variable selection methods such as DSA algorithm to reduce the false discovery rate and maintain a good sensitivity (Agier et al. 2016).

Experimental studies can also be useful to evaluate changes in OMIC signatures related to one or several exposures which are controlled by the investigator. Statistical methods are available to deal with this complex study design and to account for the repeated characters of these measures (multiple exposures and multiple OMIC profiles per participants). The first one is a naive approach ignoring the natural correlation within individuals and the experimental conditions (not recommended, loss of power), the second is the use of mixed models that take into account the correlation within individuals but independently of the experimental conditions, and the last is the use of multivariate normal models which in addition take into account the experimental conditions in the variance-covariance matrix construction. To illustrate this, the results of three experimental studies were presented: the PISCINA, the Oxford Street and the TAPAS studies. From the PISCINA study, effect of the experiment was independently detected at all 3 molecular levels studied (van Veldhoven et al., Espín Pérez et al., Vlaanderen et al). To investigate the effect of multivariate exposures on one OMIC feature, sparse PLS can be applied and extended for multi-level to take into account the repeated measure design (Jain et al., Liquet et al.). Accounting for the correlation across OMIC (eg, across proteins) is improving the model's fit compared to linear mixed model or PLS and this is a useful approach for exposure prioritisation.

Another issue is the integration of interaction between exposures. A comparison study of the existing methods has been published and reported that GLINTERNET (group LASSO) and DSA provide the best balance between sensitivity and specificity (Barrera-Gómez et al 2017). However, to date, these methods only allow testing two-way and parametric interactions.

Looking at more than one OMIC feature at a time is still a challenge. The relationships within and across exposure-related OMIC markers need to be explore as perform in a study evaluating the effect of smoking on 2 OMIC features (methylation changes and gene expression) by regressing candidate CpG sites on all transcripts and comparing the CpG-transcript pairs correlation according to the smoking status (Guida et al. 2015). The method presented used differential network approach which could also be combined with Bayesian Variant Selection (BVS).

Conclusions and perspectives

- OMICs and Exposome profiling:
  - Non-linear models are being reviewed
  - Methods accommodate complex designs
  - Some developments allow the investigation of joint effects, interaction

  Methods are established and have successfully been applied

  OMICs-integration:
  - Classical regression based approach can integrate OMICs
  - Network inference is a powerful tool: beyond visualization
  - Possibility to include group structure via prior knowledge

  Need to improve interpretability

  Longitudinal models:
  - Multistate models: the role of exposure history and its increments
  - Inclusion of molecular signals provides hints on functional role
  - Need for repeated measurements

  Ongoing method's development

Upcoming courses on exposome:

- Exposome basis: former molecular epidemiology course, Utrecht (July 2018)
- Exposome advances: follow-up from Stat-XP, London (April 2018)
- Exposome practice: Surf64 summer school, France (June 2018)

Discussion

- Inflammation in serum metabolome? https://en.wikipedia.org/wiki/Phospholipase_A2
- Expansion of tools and shared
- Challenges in methylation and annotation of genes, trans patterns? Further away genes affected, histone modification?
- Paolo vineis adaptive response versus early effect/"response", allostatic load.
- "Normal" exposures have measurable imprits
- Tissue specificity markers missing, we can use other models, c.elegans to test? GTEX dataset tissue level info
- (markers form pharmacology of liver, renal damage etc? note from me)
- Longitdunial data?

- *Human epigenome, transcriptome, metabolome and proteome variability explained by the exposome, Juan Ramon Gonzalez*

OMICs analysis - multivariate approach - background:

- Several methods exist to analyze two omics datasets at the same (PLS, CCA, CIA, O2PLS) and can be extended to multiple omics (OnPLS, GCCA, MCIA, RGCCA/SGCCA)
- Example with O2PLS to decompose the variability into two components (variability in the metabolome and variability in the transcriptome) and estimate the proportion of the variability of the intersection of them (joint part) explained by the exposome

- Extension to OnPLS: allow to decompose the variability of each omic features explained by the exposome (Global join part, local joint part and unique part, residual)
- Generalized Canonical Correlation can be used to visualize all OMICs

O2PLS applied to HELIX data - results:

- Number of latent variables needed to capture 90% of variance varies according to omics (from 2 axes for the proteome to 32 for the epigenome)
- OMICs variability explained (R^2) by the exposome ranged from around 5% to 20%: urine metabolome<miRNA<epigenome<transcriptome<serum metabolome<proteome
- Lifestyles and organochlorine compounds are the two components of the exposome that explained the more omics' variability

Deliverables - R package development: MultiDataSet, rexposome, CTDquerier

- *Molecular signatures time variability in HELIX panel paired-samples, Carles Hernandez*

Aim: to partition the variance of omic markers among inter-cohort, inter-individual and intra-individual variability

- *Discussion of summary statement: "Achievements and challenges"*

Achievements

1. Unique datasets generated, integrated to identify novel associations
2. Better understanding of sources variability at different time scale/between cohorts
3. Better understanding of variable selection methods (e.g. DSA) and value of multivariate models
4. Expansion of available tools
5. Exposure at current low levels lead to omic signature changes
6. Increased opportunities for training

Challenges

1. Still underpowered?
2. Challenges in methylation and annotation of genes:
    a. What do *trans* patterns of methylation mean?
    b. Genes affected by CpG sites methylation might be further away than the ones will currently assign
    c. What about other epigenetic mechanisms such as histone modification?

Problems of annotating metabolomic signals. Even in targeted serum metabolomics, as performed in HELIX, chemical structures cannot always be linked to a specific metabolite in particular in the case of lipids. For example for poly-unsaturated fatty acids (PUFAs), we know the number of carbons and double bonds of both chains but we don't know the position of these, which could indicate if they are omegas 3 for example.

Do we always test all relevant assumptions when modelling data?

Qualitative vs quantitative prediction

How OMICs may help to refine exposure assessment?

What is an adaptive response and start of a pathological process? We don't know the distinction of these different stages. This is the idea of the allostatic load.

Are we ready to influence risk assessment with these data? Yes, we are already doing this (evidence of causality), depending of the exposure that you are testing.

New approaches (pathways) of risk assessment is still to come.

Tissue specificity. Can the translation be made to the different tissues? We should be looking at other data (maybe experimental) to get to a better inference. For example with the use of C. Elegans (*in vivo* model) or GTEX dataset tissue level information (public database).

Session 4 Assessing health effects of the early life Exposome

- *Keynote - Systematically associating multiple exposures with child health: Methodological challenges & HELIX results – Rémy Slama (INSERM)*

Typical example of early studies of environmental exposures: London smog, Doll & Hill doctor's study
Progress in exposure assessment was marked by:

- Maps and questionnaires (1850)
- Outdoor monitors (1950-2000)
- The biomarker revolution (1982)
- Fine-scale environment models (LUR) (2000)
- Refinement of the biomarkers with metabolomics, sample pooling approaches, lower limit of detections (2015-)
- Exposome

Progress in study design: 3 generations of birth cohorts:

- 1st generation - 1990s: recruiting literally participants from birth, paper-based
- 2nd generation - from 2000s: recruitment during pregnancy with collection of prenatal exposure information and with collection of biospecimens
- HELIX (between 2nd and 3rd generation)
- 3rd generation: Early pregnancy, recruitment at preconception with "wired" participants, paperless

Issues with single exposure studies :

- Selective reporting of associations, publication bias, chance finding through multiple-testing…
- Not corrected for multiple testing
- Confounded by co-exposures
- Lack of consideration of mixture effects
- Exposure misclassification (for short half-lived biomarkers)

Early Exposome studies (2010-2015):

- Early "Exposome" (mixtures) studies relied either on biomarkers only (Patel C, PlosOne, 2010) and was cross-sectional or on outdoor models only (Dadvand, Epidemiol, 2014) to assess exposures
- ExWAS-type approach or independent testing of each exposure without correction for multiple testing
- Other statistical approaches (data reduction and other variable selection techniques) have been meanwhile developed (reviewed e.g. by Chadeau-Hyam et al, EMM, 2013) and recently considered in Exposome studies (e.g., Lenters, OEM, 2015)

ExWAS versus single exposure studies:

- All tests are reported, no selective reporting
- Correction for multiple comparisons not as efficient as we want

- Mixture effects not considered
- Variability in exposure misclassification

DSA (Deletion/Substitution/Addition):

- Reduces false positives
- Detect interactions between exposures (Paper Jose Barrera)
- Fewer hits than ExWAS
- Reduces confounding by co exposures
- Mixture effects (in progress)

HELIX achievements:
The joint HELIX and Exposomics statistical working group have published two papers on statistical analysis methods to deal with exposome data. The first one is a simulation study on the efficiency of various statistical methods to detect true exposure-health associations (Agier et al. 2016). In this study for the first time the false detection probability was noticed to be very high with ExWAS whereas variable selection methods, especially the DSA algorithm, performed better in terms of false detection probability, but has less sensitivity. The second paper aims to compare efficiency of statistical methods to detect interaction between exposures (mixture effect) and lead to similar conclusions: 2-step ExWAS has a good sensitivity but a very high false discovery proportion whereas variable selection methods, particularly GLMINTERNET and DSA2, performed better in term of false discovery but with a lower sensitivity (Barrera-Gomez 2017). Interaction in HELIX will be investigated following these results.

Against expected results, identification of interaction doesn't cost so much to identify single associations

Variability in exposure misclassification is expected in Exposome studies (including HELIX) when the amount of exposure misclassification will differ between exposures (Slama and Vrijheid, OEM, 2015). HELIX first take on this important issue was to use a posteriori disattenuation based on the Intra Class Coefficients of Correlation (ICCs), as described in single exposure studies by Perrier et al (2016) (Agier et al. in preparation). Calculation and integration of ICC is in progress.

Health and policies implications

- The first results point towards green space exposure being a predictor of birth weight
- Biological plausibility given that green space exposure is a predictor of air pollution (and noise) exposure, which is a plausible
- Confounding by SES variables cannot be totally discarded
- Greenspace exposure is controllable

Questions:
Jordi Sunyer raised the question whether or not we should abandon single exposure studies. However, these study have the advantage to go more in depth in understanding the mechanisms, etc.
Will ontologies allow to look at entities and relationship, hierarchy?

- *Summary of the Exposome-health outcome studies*

In HELIX all the exposome - health analyses followed the same statistical approach, as defined by the HELIX statistical working group. Briefly, two approaches were used: first, a regression-based approach (ExWAS, Exposome-Wide Association Study) correcting the threshold of significance according to the effective number of tests performed (Li et al. 2012), and secondly we applied the

DSA, a variable selection algorithm. The consistency of the results was assessed by various sensitivity analyses including complete case analysis, meta-analysis by cohort, evaluation of non linear dose-response, additional covariate adjustment, co-adjustment for pre- and postnatal exposures and more.

The prenatal urban exposome was used for the analysis on birth weight and covered 63 exposures in 31,326 pregnancies. The full exposome was composed of 177-214 exposures, 88-92 from the prenatal period and 126-130 from the postnatal period, encompassing five domains (lifestyle, urban environment, biomarkers, smoking and diet). Analyses using the full exposome were performed on 20 imputed datasets for 1,033-1,301 subjects. The analyses looked at the association of the full exposome with birth weight, neurodevelopment outcomes, allergy-related outcomes, lung function, blood pressure and obesity outcomes.

- *Beyond bioinformatics: contributions from health informatics to exposome research – Fernando Martin–Sanchez (ISCIII)*

Fernando Martin–Sanchez is a Research Professor in Health Informatics at the National Institute of Health "Carlos III" of Spain in the Environmental and Participatory Health Informatics Research Group (ENaPHI) and a Professorial Fellow at the University of Melbourne. The ENaPHI research group works with Electronic Health Records (EHRs); digital health data management (individual exposome sensors); digital component of the exposome; and ontologies and Social Determinants of Health (SDoH). Prof. Martin-Sanchez gave a talk about the contribution from health informatics to the Exposome research. Using data from different levels (population, individual, tissue, cell, molecule, atom) biomedical informatics must "provide a coherent framework for dealing with multi-scale population data including the phenome, the genome, the exposome, and their interconnections" (Martin-Sanchez et al. JAMIA 2014). Exposome data are collected using differents methods (Surveys, GIS, biomarkers, smartphones, sensors, wearables, Electronic Health Records) and at different levels (e.g. social media, integrated health record, research repositories). and knowledge representation (for artificial intelligence) is crucial. Bioinformatics can build ontologies that allow relationships between entities (e.g. creating high level conceptual models that represent the environmental factors included in the diseases of interest). Beside other determinants of health (e.g. genetics and biology, medical care and physical environment) Prof. Martin-Sanchez suggest to include in the exposome the SDoH and the individual/behavioral determinants. (Paolo Vineis commented that SDoH are collected in Lifepath). From health records we can have access to other exposome components: infections (generally looked at as clinical outcomes) and medications history. Also, he highlighted the digital component of the exposome, i.e. the whole set of tools and platforms that an individual use and the activities and processes that an individual engage with as part of his/her digital life (Lopez, Merolli & Martin-Sanchez, MEDINFO 2017 Proceedings,in press). Prof. Martin-Sanchez emphasized the potential key role of Exposome research in precision medicine, "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person" (Precision Medicine Initiative definition. Further, he presented the open working group from the International Medical Informatics Association on Exposome informatics (https://exposomeinformatics.wordpress.com). The webpage has several data resources (databases, ontologies, and analytics and visualization) and a curated news section.

- *Discussion of summary statement: "achievements and challenges"*

Achievements:

- HELIX is a multi-exposure (Exposome), multi-outcome (Diseasome), cross-omics harmonized multi-cohort study
- HELIX is a unique project in which information on several layers of omics data and a large number of exposures have been collected and harmonized for 1,200 mother-child pairs
- All information are available for all participants (with the exception of the temporal variability panel studies)
- Helix is one of the first Exposome projects to simultaneously estimate the associations of exposure biomarkers and of "outdoor exposures" (the urban Exposome) on health
- Does HELIX do better than former ExWAS-type Exposome studies?
    - As in ExWAS-type Exposome studies, in HELIX all test performed are reported and multiple testing is corrected for (the expected FDR should be lower than for ExWAS, but also sensitivity).
    - Confounding by co-exposure is still possibly an issue in ExWAS-type Exposome studies, while HELIX rely on multiple regression models (DSA).
    - Also, mixture effects cannot be considered in ExWAS-type studies, while the approach used in HELIX allows to account for this.
    - The statistical approach used in HELIX allows for correction for variability in exposure misclassification, although this needs to be fully implemented, while this cannot be considered in ExWAS-type Exposome studies.

Challenges:

- More stringent p-values correction vs abandon of reliance on statistical testing (in Exposome studies we are testing exposures that have gone through some toxicological study, not random exposures)
- Multicentric vs monocentric studies (theoretical issue not specific to exposome research)
- Investigating multiple exposure is desirable, but we should take into account their causal (hierarchical) mutual relations (e.g. SES, green spaces and air pollution have complex interrelations). We go beyond a flat representation of the exposome, there are hierarchy in particular in the social exposome, SES is not solely a cofounder. (Comments from Paolo Vineis that Lifepath is a project dealing with this)
- No statistical model is ideal (high sensitivity vs low false detection rate)
- Variability in exposure misclassification (e.g. bigger effort in exposure assessment for persistent compounds vs smaller effort in exposure assessment for non-persistent compounds)
- Also, we should keep in mind the longitudinal changes in the Exposome
- Probably many associations we found in HELIX can be linked to socio-economic status. Is it confounding by SES and/or we are deconstructing the social inequalities in health?
- Consistency and comparability with previous studies, although tempting, should not be used as a validation of the exposome approach. In fact, as a new tool, the exposome is supposed to bring new (and unexpected?) insights.
- The exposome is a new tool and we still have to think how (and if it's time to) convey results to the society.

Future directions (2020) (What the next generation of Exposome studies should or could look like?)

- Equal effort to assess exposure to short- and long-lived compounds: *repeated biospecimens collection - e.g. using the within-subject pooling approach if the budget is small, as suggested

by Perrier. 2016) and reliance on personal dosimeters; *alternatively: omics based assessment of exposures (mid- or long-term solution?)
- Correction for multiple testing and consideration of mixture effects implies to be able to rely on (many) more subjects
- Longer-term follow-up is warranted, given the results of long-term toxicological studies
- Cross-omics analyses incorporating biological knowledge

Other things we can highlight in the summary:

- Challenges:
  - Given the cross-sectional nature of the postnatal exposome analyses, reverse causality cannot be excluded in some cases.

Session 5 Translation of Exposome results to policy

- *Keynote - Health impact assessments related to the early life Exposome - David Rojas (ISGlobal)*

Health impact assessment is a tool to combine evidence, policy opportunities and stakeholder visions.

HELIX is in the process to provide extensive evidence on multiple exposures (exposure assessment) across Europe and the relationship between multiple health outcomes.

The work on health impact assessment in HELIX was focusing to use available data on environmental exposures across Europe to estimate (based on evidence) the burden of disease of environmental risk factors in children, and the health impact assessment of transport interventions in children.

The environmental burden of diseases in children in Europe show the relevance of the impact of seven environmental risk factors in several health outcomes. Quantifying 210,000 disability adjusted life years loosed in children less than 18 years old in the European Union of the 28 countries(EU28). This burden is five times bigger than the burden produced by traffic injuries and fatalities in the same age group in EU28. Between the pollutants included in the analysis, air pollution particulate matter and ozone) was the one producing more burden (70%). The environmental burden of disease help to identify priorities for policies in environmental health, also to do comparisons between countries and risk factors. Finally the environmental burden of disease also provide general recommendation to researchers and public health practitioners to increase and reinforce their work in provide evidence of the exposure levels of different environmental risk factors in children across Europe and produce more epidemiological evidence of the relationship of those risk factors and health.

The health impact assessment (HIA) of walking to school in Barcelona, showed the health implication of a non-health policy with influence in several health determinants and the health outcomes. The HIA presented assessed the health risk and benefits of an specific scenario in Barcelona: “what if all the children between 6-10 years old who live less than 1 km from school walk to school?”. The HIA provide the first quantitative HIA of walking to school, identifying different health determinants (air

pollution, traffic incidents and physical activity) related to transport policies in children. The HIA results showend a larger health benefits related to walking to school activities than risk, compared to travel to school by motorized transport.

Q (Joel): non collapsibility of OR/RR
Q/C (Manolis): we should do an economic assessment of the benefits for stakeholders

- *Development of Mobile App – John Wright*

- App in BiB
  - → dynamic data collection → reverse flow direction → from people to researchers
  - Policy implications
    - tragedy of the commons (air pollution)
    - power to the people → personalized health
    - potential to harness technology

  "Our Voice"
  - Activate Residents to Create Healthier Communities → citizen science effort is empowering communities to advance health equity

- *Discussion of policy and societal implications of results (based on last slide of each presentation) – John Wright / Peter van den Hazel*

Policy relevant questions within the exposome framework

- How do exposures (from a wide range of sources) correlate in different population across Europe?
- Can we identify groups exposed to many environmental "hazards"
- Which factors determine multiple exposure patterns?
- Which are the exposures or exposure patterns or exposure interactions most strongly associated with child health outcomes (in order to set priorities)?
- Can we identify molecular imprints of external exposures (in order to improve risk assessment and knowledge on mechanisms and pathways)?
- How the exposome can be translated in policy recommendations?
- An exposome index is needed for policy?

Session on health effects

- Molecular longevity is determined by early life exposure
- The importance of intervention that may impact the future life by decreasing comorbidities in adulthood
- Simultaneously exposure to high levels of several pollutants can be an issue in European populations
- Greenspace exposure being a predictor of birthweight
- OCs, lead, air pollution may also affect ADHD, social competence, behaviours → these impairments are related to increased rates of global aggressiveness and impulsivity, increased crime rates in society
- The placental transfer of PFOA was estimated to be highly variable between mothers. this points to potentially very sensitive individuals. understanding the sources of this variability is important
- Endocrine disruptors are most likely influencing lung function, both pre- and postnatally, while outdoor exposures were not highlighted in the study

Expectations

- Ultimately, results would help guide public health efforts by allowing us to intervene on those chemical agents or urban exposures that are most likely to be associated with childhood obesity. results would thus help prioritization of interventions
- Our multi-omic data integrations analysis will allow us to determine biomarkers of exposures
- Perform personalized assessment of exposure biomarkers
- Feasible to collect large amounts of exposure data on large populations.

Implications for health:

- Governmental policies on environmental risk factors
- Early prevention in children should be effective (children with elevated BP are more at risk to be hypertensive in adulthood)
- Improve quality of life and decrease health costs

the road ahead

- Compile findings and recommendations for visualization questions to partners
- From science to practice
- From article to factsheet
- → make findings interpretable for the general population

Comments:

- Rising awareness in communities in order to push local politicians to make changes to local policies
- Service for dissemination of the EU (deadline november 2017)
- Educate communities (NGOs, environmental justice groups) → partnership with communities
- Small interventions in existing cohorts to decrease exposure → rather than more, more, more
- When we think about stakeholders, we should look to specific ones (e.g. for traffic reduction and endocrine disruptors, they are obvious). other stakeholders might be more general (e.g. for social inequalities)

Session 6 Networking between exposome-related projects in and outside Europe - "*Building an exposome toolbox*"

Name of the project	Coordinating institution	Objectives	Part of the exposome covered	Exposome toolbox
HELIX *www.projecthelix.eu*	ISGlobal, Barcelona	To implement novel exposure assessment and biomarker methods to characterize early-life exposure to multiple environmental factors and associate these with omics biomarkers and child health outcomes	Early life Urban, chemical and lifestyle exposome Internal exposome	▪ Accessible database ▪ Statistical methods ▪ Sensors and apps ▪ Biobank ▪ GIS platform ▪ Multi-disciplinary expertise
LifeCycle *www.lifecycle-project.eu*	Erasmus University Medical Centre, Rotterdam	To establish a european child cohort network To study early life stressors affecting health across the life cycle	Early life Urban, social and lifestyle exposome Internal exposome Life course disease trajectories	▪ Harmonised data platform ▪ Harmonised exposome variables in EU child cohorts ▪ DNA methylation and gene expression ▪ Longitudinal modelling of exposome
DynaHealth *www.dynahealth.eu*	Centre for Life Course Health Research, Oulu	To understand the dynamic determinants of glucose homeostasis and social capability to promote healthy and active aging. To identify and validate indicators of gluco-psychosocial axis across the lifecourse	Psychosocial Internal exposome	▪ Longitudinal assessment of the exposome ▪ e-tool for health practitioners to predict glycemic health later in life

Exposomics www.exposomicsproject.eu	Imperial College, London	To develop a new approach to assess environmental exposures and to use OMICs to link biochemical and molecular changes in our body	Air pollution and water contaminants Internal exposome	▪ Personal exposure monitoring ▪ Advanced land use regression model and satellite data ▪ Multiple 'omics across the life course, and cross-omics analyses ▪ Causal methods for the exposome (MITM)
LifePath www.lifepathproject.eu	Imperial College, London	To investigate the biological pathways underlying social differences in healthy ageing. To provide updated, relevant and innovative evidence for healthy ageing policies	External exposome associated with socioeconomic position Internal exposome	▪ Better definition of socioeconomic position and its components ▪ Epigenetic and metabolomic ageing ▪ Life-course allostatic load
Hercules www.emoryhercules.com	Emory University, Atlanta	To development technologies to measure the exposome (known environmental chemicals, metabolites, biological responses, and untargeted features for future discovery)	Chemicals External monitoring from personal to satellite Internal exposome	▪ High resolution metabolomics and exposomics ▪ Mummichog-pathway integration for metabolomics ▪ xMWAS-multiomic integrator, network analysis ▪ Comprehensive biomonitoring ▪ Develop of microneedle patch to collect interstitial fluid

HEALS www.heals-eu.eu	UPMC, Paris and AUTH, Thessaloniki	To improve the refinement of an integrated methodology and the application of the corresponding analytical and computational tools	External exposome Internal exposome	▪ Agent based modelling ▪ Internal dosimetry modelling ▪ Exposure biology pipeline
HBM4EU www.hbm4eu.eu	German Environmental Agency	To answer open policy relevant questions as defined by EU services & partners countries To generate human biomonitoring data representing Europe To assess toxicological impact and mixtures To improve chemical policy To bridge the gap between science and policy	Chemicals	▪ Harmonizing procedures for human biomonitoring ▪ Chemical risk assessment ▪ Exposure pathways and upstream sources
CHEAR	NIEHS, Durham	To provide the extramural research community access to laboratory and data analyses that add or expand the inclusion of environmental exposures in children's health research.	Chemical and physical stressors Lifestyle and social environments Internal exposome Early life (from conception through adolescence)	▪ Data repository ▪ Analytical and statistical support ▪ Network of laboratories providing access to state-of-the-art infrastructure for analysis of biological samples

Session 7 Reflections on the Future of (early life) Exposome Research

- *Toxicologist's view: Thomas Hartung (Human Toxome project)*

1. The exposome includes:
    - radiations
    - drugs
    - lifestyle
    - stress
    - diet
    - pollution
    - infections

2. Pollutants and their metabolites
    - Toxicological ignorance: 80 millions of chemicals synthesized → 140,000 in consumer products but only 10% tested whose 3% were extensively tested.
    - From REACH registration, natural language processing found 10,000 chemicals/800,000 toxicological studies
    - Lack of public animal data and evaluation of long-term effect
    - Alternative to animal experimentations:
        - See ALTEX journal (alternative to animal experimentation)
        - Reach-across tool → organizes REACH data (ECHA reports) to be easily accessed (payment service; ECHA reports are freely available, BUT not machine readable) → 70+ million structures; 300,000 with biological data; 20,000 with animal data → artificial intelligence: 0.5 billion calculations per prediction → it finds more than 80% of toxic substances and it has predictions for ⅔ of the chemical universe

3. Endogenous metabolites
    - Metabolomics is closest to phenotype
    - Targeted VS untargeted metabolomics : either miss most of metabolites or have no identity for most
    - No reference control for quality assurance of metabolomic → need to be developed
    - Untargeted metabolomics:
        - pros: very sensitive, not expensive, close to phenotype, little species differences, only thousand of parameters, knowledge on molecular pathway
        - cons: small effect strength, flux more important, fast changes, incomplete extraction and measurements, metabolic identification is difficult, QA incomplete

4. Epigenetics, RNA, microRNA
    - Around 2,500 microRNA in humans (conserved across species)
    - 60% of mRNA are targets of miRNA
    - One miRNA may regulate 100s mRNA
    - miRNA targets are twice as likely to be affected by chemicals than not miRNA targets
    - Organ-specific and development-specific expression
    - circulating miRNAs are biomarkers for : cancer, liver toxicity/injury, heart injury, nephrotoxicity, CNS disease;
    - microRNA are more stable than mRNA (can be found in biofluids, formalin-fixed paraffin-embedded tissues, in archived samples; also in degraded samples, e.g. mummies (A. Keller *Molecular* Biology and Evolution, 2017))

→ Inferring causation from correlation

→ Need to making sense to data by studying mechanisms
→ the human toxome project (altex)
→ Move toward AOP (Adverse Outcome Pathway)

AOP: Narrative, low level of detail, existing info; Biased by existing knowledge; Not quantitative, no flux, no dynamics; No QA / validation yet

Pathways of toxicity (PoT) (Toxome): Molecular, high level of detail, emerging info; Untargeted identification, causality; Aiming for quantitative relations, fluxes; Causality (to be shown)

Problems with OMICs: too many variables, small n, plus noise → Favor omics close to phenotype and with less variables; Combine omics (cluster) to reduce noise; Reduce dimensionality by mapping to pathways

Lessons learnt from the human toxome:

- Any untargeted approach necessarily has some noise in the data owing to artifacts, outliers, and misidentified metabolites.
- Depending on the chemical analytical choices (sample extraction, chromatography, instrument and settings etc.) a largely different and only partial representation of all metabolites will be achieved, very much biased by the largely putative identification of metabolites.
- Using a data analysis approach based exclusively on pathway annotations will at best miss much what is of interest and at worst will produce perturbed pathways that are statistically significant yet uninformative for the biological sample at hand.

The basis for making big sense from big data is good big data

- Biological samples
- Measurements

Start untargeted, follow-up targeted

- Need for reference data
- Reduction of dimensionality via mechanism leading to biomarkers
- Networks – correlation is more important than significance of individual

Cells model can help to understand/identify mechanisms

- Culture quality
- Opportunity stem cells for (early life) human models
- Microphysiological systems

Not all health outcomes can be assessed with animal tests (eg, autism)

Mini brains:

Opportunities for human mini-brain research

- Drug development
- Map the neurotoxic chemical universe
- Characterization of medical countermeasures
- Neurotoxic and DNToxic side effects
- Brain trauma, infectious disease and neurodegenerative disease research
- Individual susceptibility using patient iPSC – genetic risk factors
- Long-term culture and co-culture with other organs

The opportunity of combining Toxome (AOP) and Exposome (from untargeted to targeted)

Take home message:

- Tox big data to fill gaps
- Omics quality & combination
- Sense by AOP
- Synergy Toxome & Exposome

- *Epidemiologist view: Roel Vermeulen (IRAS)*
  Accordingly to Wild, the exposome "is the science of the impact of the environment (broadly defined) on health and disease and refers to the "totality of exposures from conception to death." It is based on technical developments in external measurements (such as sensors) - the "external" exposome - and in internal omics measurements, the "internal" exposome.

The general external exposures include education and financial status, climate, urban-rural environment, social capital, and stress. The specific external exposures include physical activity, lifestyle factors, infectious agents, diet, air pollution, chemical contaminants.

The internal exposome: biomonitoring, biological resilience, exposure-specific or broader biological imprints → OMICs

Functional trajectories throughout the life-course depend on exposure to risk factors that occur both early in life and adulthood. Early life exposure to risk factors influences the overall functional reserve, and therefore the likelihood that limitations occurs during adulthood.

The pollutome is defined as the totality of all forms of pollution that have the potential to harm human health. The pollutome can be viewed as a fully contained (nested) subset of the exposome. "A high proportion of the 140,000 chemicals and pesticides in commerce have never been adequately tested for safety" (Landrigan et al., Lancet, 2017), not to mention their combination.

There is no single technology that can measure the entire exposome. The external exposome assessment can be done through:

- GIS based exposure surfaces;
- pictures
- omics
- exposure markers in biological media
- big data
- wearable
- questionnaires

In HELIX and EXPOSOMICS big data (e.g. health record, public database) but pictures are missing

Why the exposome?

- enhance exposure assessment
- complement studies of disease etiology by evaluating biological plausibility and mechanisms of suspected hazards and their combinations (hazard identification)
- can aid in a more refined derivation of the exposure-response association (risk quantification)
- can evaluate potential disease risks of new exposures before diseases begin to occur (early screening)

For the implementation of the external exposome new investigative tools are being developed (e.g. use of satellite data; TROPOMI is the first satellite that will specifically monitor the troposphere; use of mobile platforms; air quality data from extensive mobile monitoring with Google street view cars → data from the few to the many; citizen science, distributed networks with on the fly calibration; wearable)

Reflections on the external exposome:

- exposome projects have successfully piloted the use of GIS, mobile platforms, wearables
- new technology is still developing and will result in higher resolution data in space and time
- will require computational solutions
    - we are still looking at univariate marginal effects

  scaling will require public/private partnership

Implementation of the internal exposome: OMICs (Adductomics; Epigenomics; Transcriptomics; Proteomics; Metabolomics; Microbiomics).

In the last two decades the number of number of KB per day (in genomics) and analytes (for the other OMICs) has steadily increased.

OMICs markers in environmental epidemiology can help i) increasing exposure assessment (e.g. smoking and Aryl-Hydrocarbon Receptor Repressor - AHRR - methylation as a biomarker of smoking exposure; ii) understanding biological mechanisms (e.g. PM2.5 and UFPs, linoleate metabolisms and diseases - cardiovascular disease and asthma); iii) in a more refined derivation of the exposure-response association (e.g. supervised hierarchical clustering to generate a heat map to allow visualization of patterns of gene expression across exposure categories).

Reflections on the internal exposome:

- exposome projects have successfully piloted the use of new omic platforms
- variability in response (lab, intra-, inter-individual)
- high-dimensional data with partial coverage and annotation and often unknown inter-relations)
- will require bio-informatical and computational solutions
- it is still a step from associations to causal inference
    - replication/population stratification
    - replication on single exposures

Complexity (Dimension) Reduction

- agnostic filtering (mainly driven by correlation patterns)
- informed reduction
    - biological inferred filtering (e.g. prioritized pathways, AOP)
    - Prior empirical observations (HUman, in vivo, in vitro) → The exposome meets the Toxome

Historical reflection between the Genome and the Exposome. We developed the methods, and we developed “exposome globes” (equivalent of HapMap for the Genome). We ran ExWAS studies with N>X000. We still have to reach the point we can run ExWAS N>XXX000).
For the genome the effect size is small and the volatility is null. For the exposome we do not know the effect size, and we know the volatility is high.

Questions: Is larger always better? Is more always better?

Will invest on the exposome pay-off? Previous examples would indicate yes (air pollution mitigation and lead in gasoline: for each 1 dollar invested in research, 30 dollars were saved). But this is true only if results can be transferred to effective policy interventions in the future. And effective evidence based policies can only be made if the complex system is sufficiently understood.

We are at the dawn of the exposome research era.

- *SAB statement (SAB members)*

1. Jean-Pierre Cravedi

Positive points:

- Harmonized data (especially postnatal exposome)
- Development of different tools in different areas (biomonitoring, statistics, modelling…)
- High dimension of data produced - Richness of data
- HELIX as a unique project
- Exposures and OMIC data
- Successful to estimate exposure-outcome associations
- Better understanding of variability between individual and source of variability
- Improvement in monitoring tools and statistics
- Link between internal and external exposome → PBPK modelling

The challenges in HELIX included the production data and the policy impact.

There are still pending questions:

- We need to fill the gap between new data and risk assessment.
- There is concern about the sustainability of the database, and accessibility to data should be improved.
- Sampling strategies regarding non persistent contaminants
- Handle mixture
- Importance one vs other exposures

Recommendations:

- To improve the exposome approach it should better incorporate existing toxicological knowledge.
- Food contaminants → need to know the type of food and use data on food contamination (see as example the French Total Diet Study)

2. Joel Schwartz

a. Using bio-impedance data and blood concentrations we can calculate the body burden using PBPK models
b. Hierarchy in exposures should be taken into account
c. Do not include NDVI and NO2 in a model (when the outcome is affected by both)
d. Food frequency questionnaires
e. Control for omega 3 in mercury analyses
f. Marginal structural models could be used for causal inference
g. Interactions social environment/social stress*chemicals
   I. Supportive vector machine
   II. Least square … machine
   III. Bayesian methods (large number of interactions, selection, shrinkage)

IV. For the repeated biospecimens collection → mixed models with random intercept for individuals rather than pooling the samples

3. Matthew Longnecker: brief intervention

Extra thoughts
In HELIX the following dimensions of the external exposome are missing or investigated less in depth:

- infections during pregnancy
- medications during pregnancy
- stress
- radiation?

- *Closure*

Acknowledgment to all participants.
Particular acknowledgment to Oliver Robinson and Léa Maitre (co- project coordinators), Jose Urquiza (data manager) and Diana van Gent (project manager).
Martine Vrijheid proposed to organize annual meeting to join actors of the differents exposome projects to discuss methodological advances and keep the databases growing.

HELIX Scientific Publications

2014
The Human Early-Life Exposome (HELIX): Project Rationale and Design Martin Vrijheid et al. Environ Health Perspect. June 2014; 122:6. DOI:10.1289/ehp.1307204
2015
Some challenges of studies aiming to relate the Exposome to human health. (Commentary) Rémy Slama, Martine Vrijheid. Occup Environ Med 2015.
The Pregnancy Exposome: Multiple Environmental Exposures in the INMA-Sabadell Birth Cohort. Oliver Robinson et al. Environmental Science & Technology 49(17) · July 2015. DOI: 10.1021/acs.est.5b01782
2016
The Pregnancy Exposome. Oliver Robinson et al. Current Environmental Health Reports. DOI: 10.1007/s40572-015-0043-2
The exposome concept: a challenge and a potential driver for environmental health research. Siroux V, Agier L, Slama R. Eur Respir Rev 2016;25(140):124-9. DOI:10.1183/16000617.0034-*2016*.
A Systematic Comparison of Linear Regression–Based Statistical Methods to Assess Exposome-Health Associations. Lydiane Agier et al. Environ Health Perspect. Environ Health Perspect 124:1848–1856; http://dx.doi. org/10.1289/EHP172
2017
A systematic comparison of statistical methods to detect interactions in exposome-health associations. Barrera-Gómez J et al. Environ Health. 2017 Jul 14;16(1):74. doi: 10.1186/s12940-017-0277-6.
MultiDataSet: an R package for encapsulating multiple data sets with application to omic data integration. Carles Hernandez-Ferrer, Carlos Ruiz-Arenas, Alba Beltran-Gomila, and Juan R. González. BMC Bioinformatics. 2017; 18: 36. doi: 10.1186/s12859-016-1455-1
Assessment of metabolic phenotypic variability in children's urine using 1H NMR spectroscopy. Léa Maitre et al. Nature Scientific Reports 7:46082 | DOI: 10.1038/srep46082
2018 – under review
Cohort Profile: the Human Early Life Exposome (HELIX) study - A European Population-Based Exposome Cohort. Léa Maitre et al. Submitted to BMJ Open.
Environmental Burden of Childhood Disease in Europe. David Rojas et al. Submitted to EHP.

Report from Science Advisory Board

The program committee scheduled an excellent overview of exposome projects in Europe and the U.S. as well as many high-quality presentations of preliminary results from HELIX addressing the goals of the project. The well-attended meeting was held in the auditorium of the Barcelona Biomedical Research Park ("PRBB").

The HELIX investigators proposed an ambitious, complex project and achieved their goals with respect to data collection, methods development, specimen analysis, database management, and statistical analysis. They are now about 4.5 years into the project, and funding will end soon. The majority of manuscripts reporting results are forthcoming, which was felt to represent the normal course of progress in projects of this sort.

The results that were presented at the meeting were preliminary, thus an assessment of the scientific yield of this exposome project would be premature. Nonetheless, the preliminary results suggested confirmation of well-accepted risk factors as well as identification of a few somewhat unexpected new risk factors. Analyses of associations with exposure mixtures are forthcoming.

All key presenters clearly were thinking in terms of potential public health messages stemming from their findings, though the preliminary nature of the findings precluded any such statements at this time.

Dr. Vrijheid outlined her plan for making the data publically available, though noted the support for this in the longer term was in question.

Andrea Baccarelli, HELIX SAB member who was unable to attend, adds:

While I regret not having been an active participant of the HELIX advisory board, mostly due to my recent transition to Columbia University and my new responsibilities, I would like to take the time to tell you how much I have appreciated your leadership and work with the HELIX consortium.

HELIX has become—in a very short time—a house brand name in environmental health and this has to do with the work that you and all of your team members have done.

I would like to congratulate all of the HELIX team members for amazing work and incredible service to environmental health worldwide.

Addendum: HELIX data is not publically available but access to HELIX data is regulated through external data access procedures (http://www.projecthelix.eu/index.php/es/data-inventory).