Challenges and promises of the Exposome concept for environmental health research

First lessons of HELIX early-life exposome project

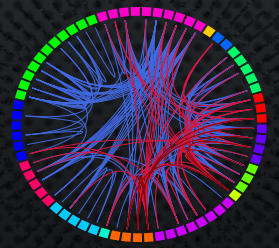
Rémy Slama

Inserm (National Institute of Health and Medical Research)

Team of environmental epidemiology

IAB (Institute for advanced Biosciences) –Inserm-CNRS-University Grenoble-Alpes, France

***On behalf of* HELIX early-life Exposome project consortium**



Conférence Santé Globale et nouveaux flux de risques

15 December 2020

Outline

I. The exposome concept. Motivation

II. Characterization of the exposome in human populations

- Issues related to *within-subject variability*
- The exposome as a way to inform *environmental justice*

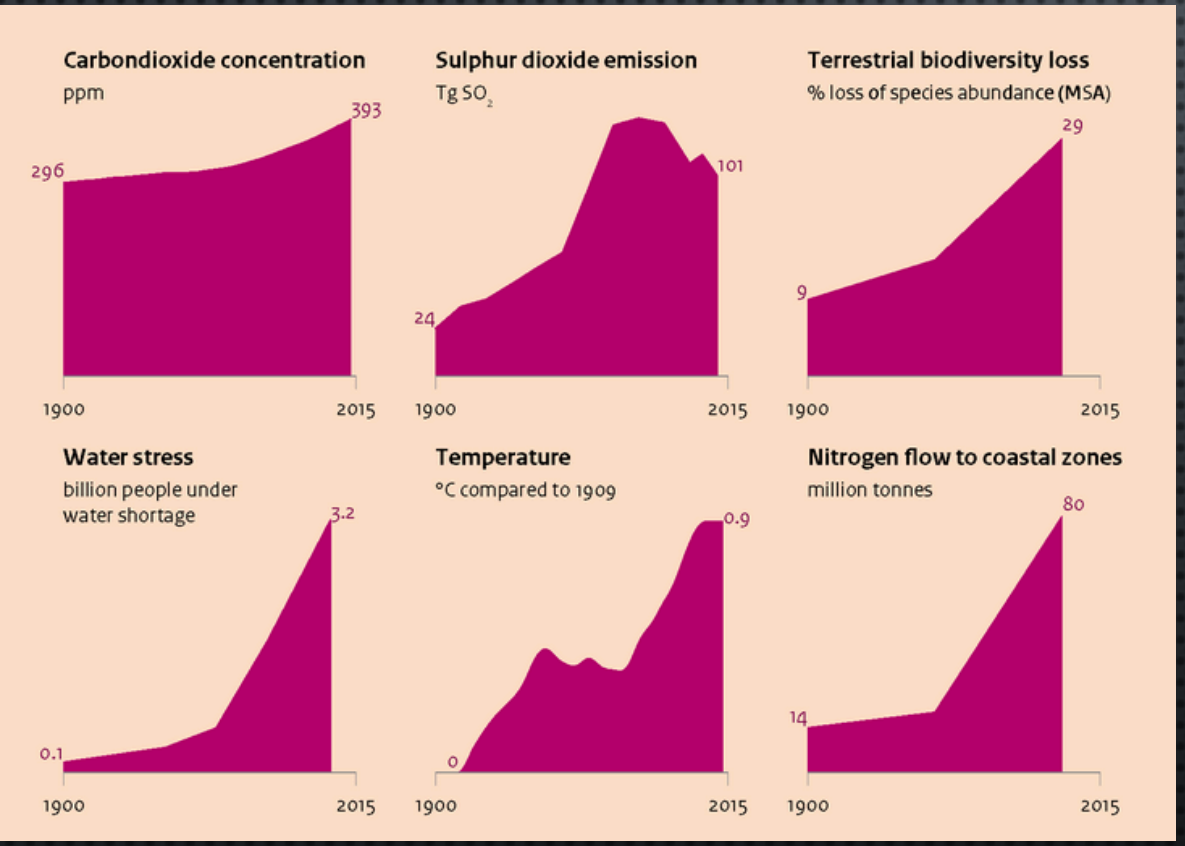
III. Studies relating the exposome to health - *First results of Helix project*

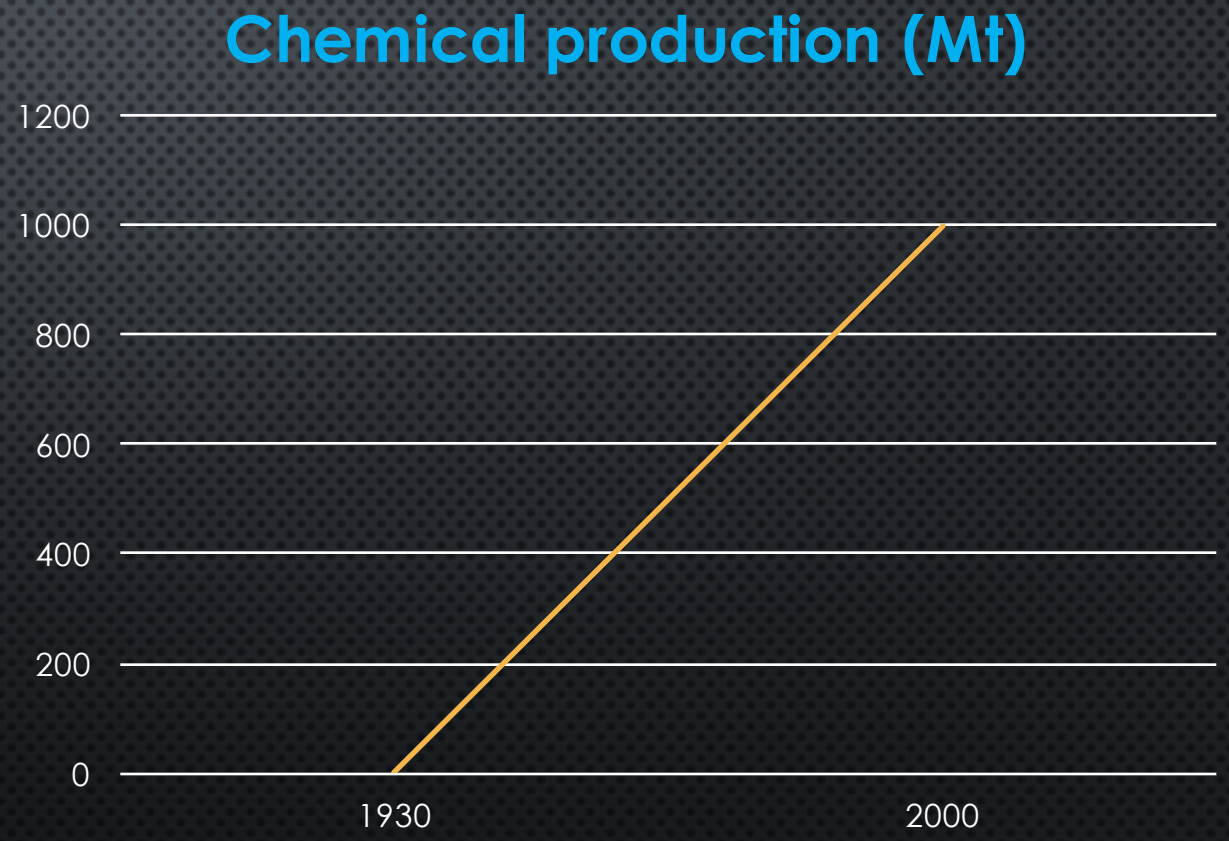
IV. Studies relating the exposome to health – *Methodological considerations*

V. First lessons for future studies

I. The exposome concept. Motivation

Changes in our environment





>23,000 chemicals currently marketed in the EU

5,000 high volume chemicals

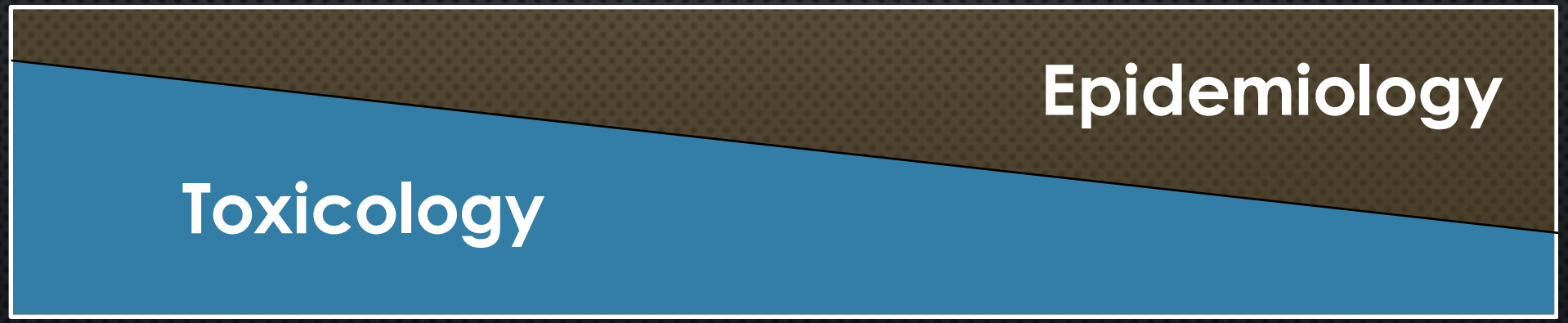
Thousands of exogenous chemicals detected in the human body

Pre-marketing test requirements are too limited to identify all harmful exposures

Hazard identification → **Risk assessment**

Dose-response characterization

Assessment of human exposure



TOXICOLOGY

Human experiments (1-5/year)

(Rodent) in vivo studies (10-100/year)

In vivo studies on « alternative » models, Ex-vivo studies…

***in vitro* cell-based or biochemical assays** *(>10,000/day)*

Throughput

Relevance for humans, level of evidence, cost

EPIDEMIOLOGY

Randomized studies

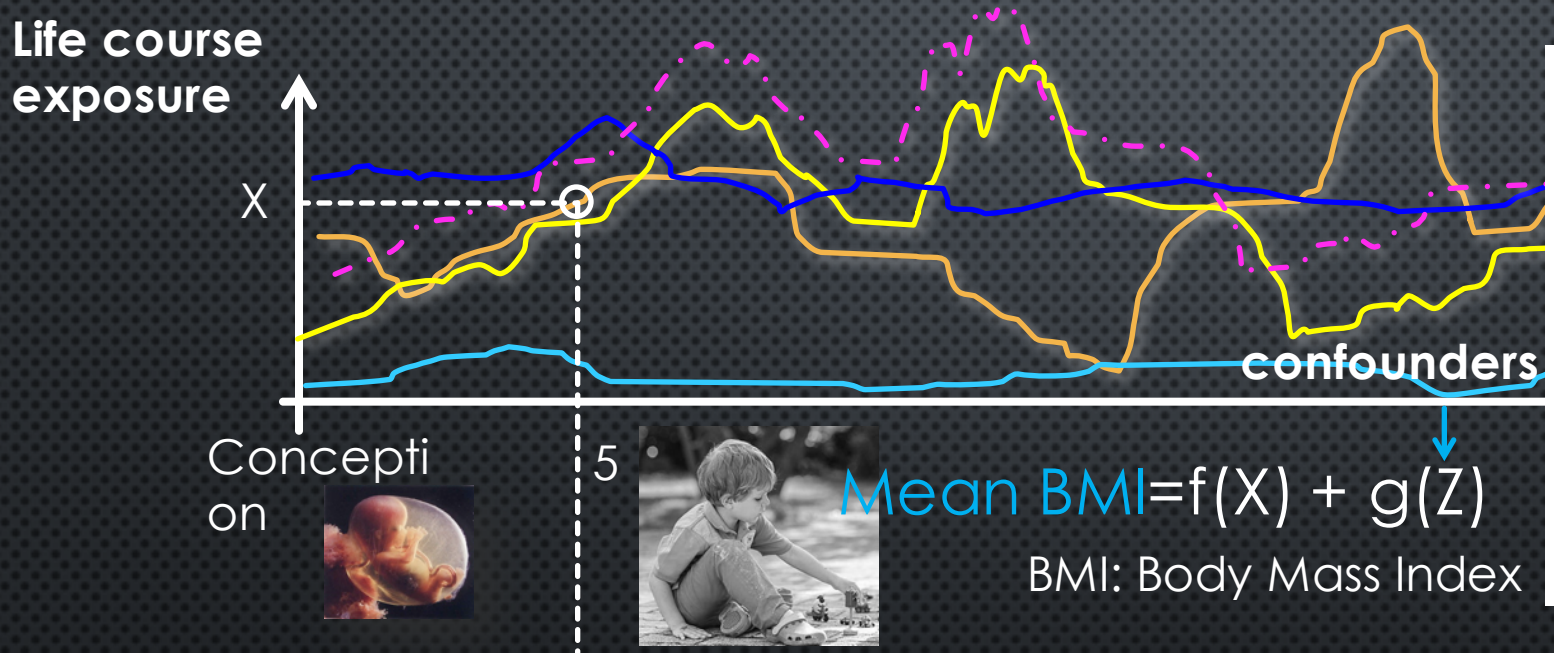
Cohorts

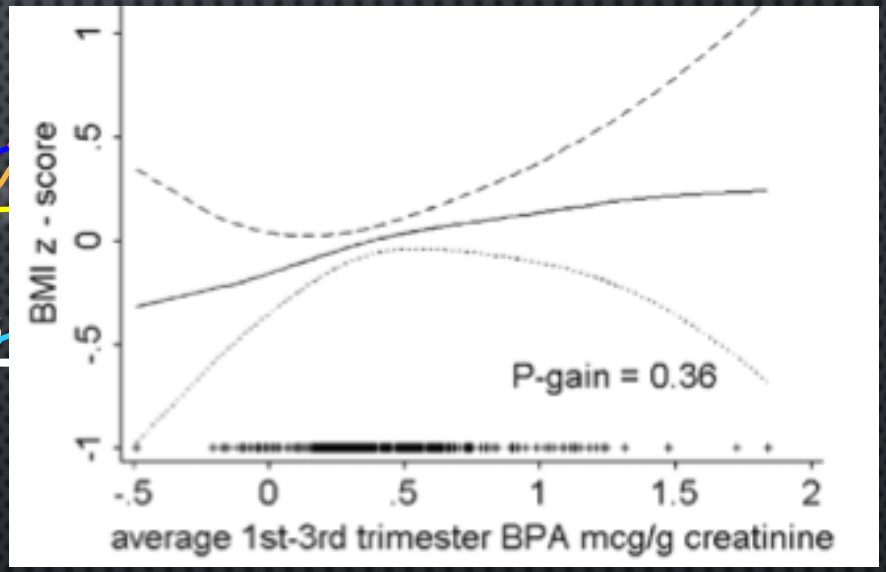
Case-control studies

"High-Throughput" **exposome cohorts**?

Ecological studies

***Exposome* vision:** Continuous exposure to a large number of exogenous factors at varying doses throughout life





(Valvi, *Epidemiology*, 2013)

Epidemiology, until 2010 (generally): Spot exposure to a single (or a few) factor(s) at a single time point

"At age t, subject i was exposed to factor A to a level X"
(known for a large number of subjects)

Issues with (repeated) *single exposure* studies

Cannot discard **confounding by co-exposures**

Selective reporting of associations (by authors and journal) / **Publication bias**

No correction for **multiple testing** (one can debate if/when this is needed, see Rothman, Epidemiology 1990)

Low throughput, given the number of substances to test

Lack of consideration of "**mixture effects**"

Exposure misclassification (for short half-lived biomarkers) (see e.g. Perrier, *Epidemiology*, 2016, Vernet, 2018)

Not very easy to rank exposures (e.g. in terms of health impact), although possible (GBD, *Lancet*, 2018)

Deciphering the exposome: Motivation

There is a desperate need to develop methods with the same precision for an individual's environmental exposure as we have for the individual's genome. I would like to suggest that there is need for an ''exposome'' to match the ''genome.''

At its most complete, **the exposome encompasses life-course environmental exposures (including lifestyle factors), from the prenatal period onwards**.

(Wild CP, *Cancer Epid Biom Prev*, 2005)

Promises of the exposome concept

Selective reporting of associations (by authors and journal) / **Publication bias**

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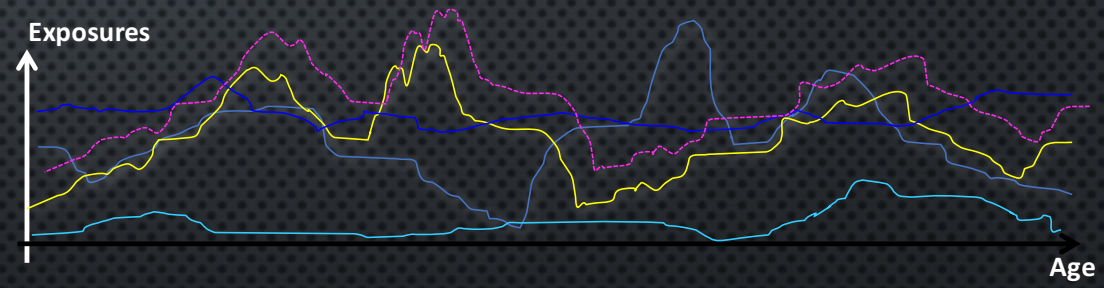
Not very easy to rank exposures (e.g. in terms of health impact), although possible (GBD, *Lancet*, 2018)

Allow identification of population subgroups cumulating several hazardous exposures ("*environmental justice*")

Can (could) be taken care of in an "exposome" approach



?

II. Issues related to exposure assessment

To next section

Metrologic issue

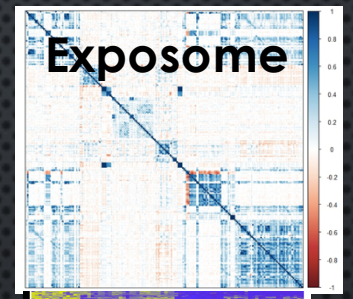
Increasing the number of (exposure) factors considered should not be done at the cost of a decrease in the quality of their assessment.

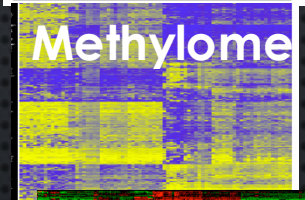
(cf. *curse of dimensionality* data science concept)

More exposures, better characterized

Assessment of the exposome of European children Helix early-life exposome project (Vrijheid, *EHP*, 2014, Maitre, *BMJ Open*, 2018)

- Aim: to describe the early-life exposome and characterise its impact on specific health outcomes in childhood.
- Design: assessment of a wide range of external, internal exposures and 'omics markers in **1300 children from 6 European countries**.

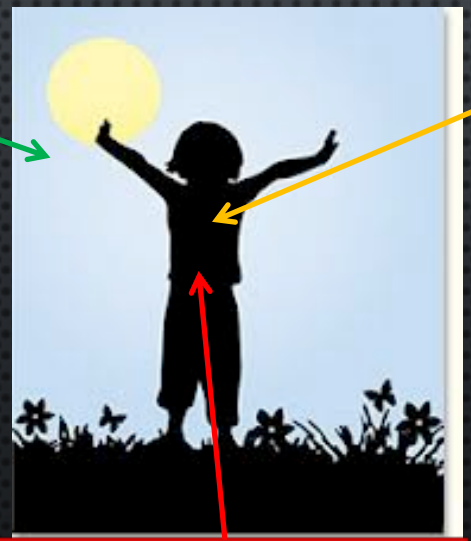




Metabolome

External exposome

Air pollutants (LUR models)
Passive smoking
Water pollutants
Greenspace exposure (GIS data)
Noise
UV radiation
Diet
Temperature



'omics markers

Methylome (Infinium 450k chip)
Transcriptome (mRNA, miRNA)
Metabolome (Lau, *BMC Med*, 2018)

Internal exposome

Phenols, phthalates, Persistent Organic Pollutants, Heavy metals, organophospate pesticides, perfluorinated compounds…

(Haug, *Env Int*, 2018)

Health (growth, neuro-dev., respiratory…)

European Union
SEVENTH FRAMEWORK PROGRAMME

Commentary

(wileyonlinelibrary.com) DOI: 10.1002/sim.5499 Published online in Wi

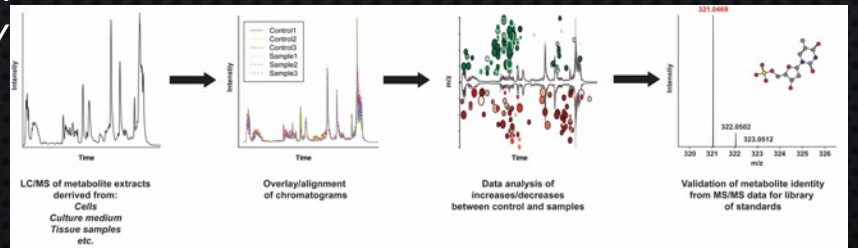
The biomarker revolution

Enrique F. Schisterman[a*†] and Paul S. Albert[b]

Targeted analyses (hundreds of chemicals), *very sensitive, annotated*

5 ml

Untargeted analysis (thousands of chemicals, *not always quantitative, partly annotated*)



Compound		Maternal samples P25	P50	P75	Max	n analyzed	% quantifiable samples
PCB 118	ng/g lipid	1.57	2.64	4.82	39.0	829	79.1
PCB 138	ng/g lipid	5.54	9.10	16.1	132	1048	96.5
PCB 153	ng/g lipid	10.4	17.6	30.5	214	1048	99.6
PCB 170	ng/g lipid	1.84	3.69	7.06	84.5	826	99.5
PCB 180	ng/g lipid	5.78	10.4	18.6	201	1048	97.6
DDT	ng/g lipid	0.82	1.33	3.06	94.1	826	65.6
DDE	ng/g lipid	25.9	52.3	111	1903	1048	99.9
HCB	ng/g lipid	5.59	8.16	13.0	164	1048	99.1
PBDE 47	ng/g lipid	0.27	0.43	0.75	34.74	684	80.8
PBDE 153	ng/g lipid	0.03	0.45	0.66	198	648	72.9
PFOA	µg/L	1.38	2.30	3.34	31.6	1240	99.7
PFNA	µg/L	0.43	0.69	1.08	5.92	1240	97.9
PFUnDA	µg/L	0.10	0.19	0.29	2.80	1032	95.4
PFHxS	µg/L	0.31	0.55	0.91	21.0	1240	97.5
PFOS	µg/L	4.12	6.41	9.63	48.0	1240	100
As	µg/L	0.28	1.19	2.27	90.1	833	58.5
Cd	µg/L	0.15	0.22	0.33	27.9	833	99.6
Cs	µg/L	1.19	1.56	2.15	10.9	833	100
Hg	µg/L	0.98	1.90	3.45	43.5	1020	98.9
Pb	µg/L	7.14	9.66	13.20	187	833	100
Tl	µg/L					833	1.1
MEP	µg/g creat.	72.0	179	469	17,733	1080	99.0
MiBP	µg/g creat.	23.3	38.7	60.7	705	1088	99.9
MnBP	µg/g creat.	18.3	29.6	47.3	6445	1089	100
MBzP	µg/g creat.	3.63	7.33	15.3	775.1	1088	99.7
MEHP	µg/g creat.	4.42	8.73	15.3	417	1085	99.5
MEHHP	µg/g creat.	10.5	18.2	31.2	967	1089	100
MEOHP	µg/g creat.	8.29	14.1	23.7	783	1089	100
MECPP	µg/g creat.	22.4	33.6	52.3	1361	913	99.9
oh-MiNP	µg/g creat.	0.61	0.91	1.47	66.5	914	92.6
oxo-MiNP	µg/g creat.	0.62	1.03	1.75	75.1	914	95.7
MEPA	µg/g creat.	39.5	167	389	39,241	815	99.8
ETPA	µg/g creat.	1.14	6.26	26.72	6774	817	97.4
PRPA	µg/g creat.	8.87	44.2	134	12,463	1083	97.3
BUPA	µg/g creat.	0.36	3.37	14.4	371	1083	97.0
BPA	µg/g creat.	1.55	2.82	6.60	107	1084	99.4
OXBE	µg/g creat.	1.46	4.90	27.5	12,837	1085	99.3
TCS	µg/g creat.	1.50	6.28	79.9	1653	1085	98.5
DMP	µg/g creat.	4.13	8.37	16.4	321	1080	90.8
DMTP	µg/g creat.	2.05	4.96	12.4	220	1084	88.9
DMDTP	µg/g creat.	0.05	0.19	1.54	134	969	41.6
DEP	µg/g creat.	1.86	3.33	6.44	198	1082	97.8
DETP	µg/g creat.	0.12	0.58	2.56	44.3	1037	50
DEDTP	µg/g creat.					1084	1.7
Cotinine	µg/g creat.	1.49	5.25	23.7	15,410	1093	43.7

(Haug, *Env Int*, 2018)

Characterization of the chemical "exposome" in EU children (Helix project, n=1300)

(only results for non-persistent compounds displayed)

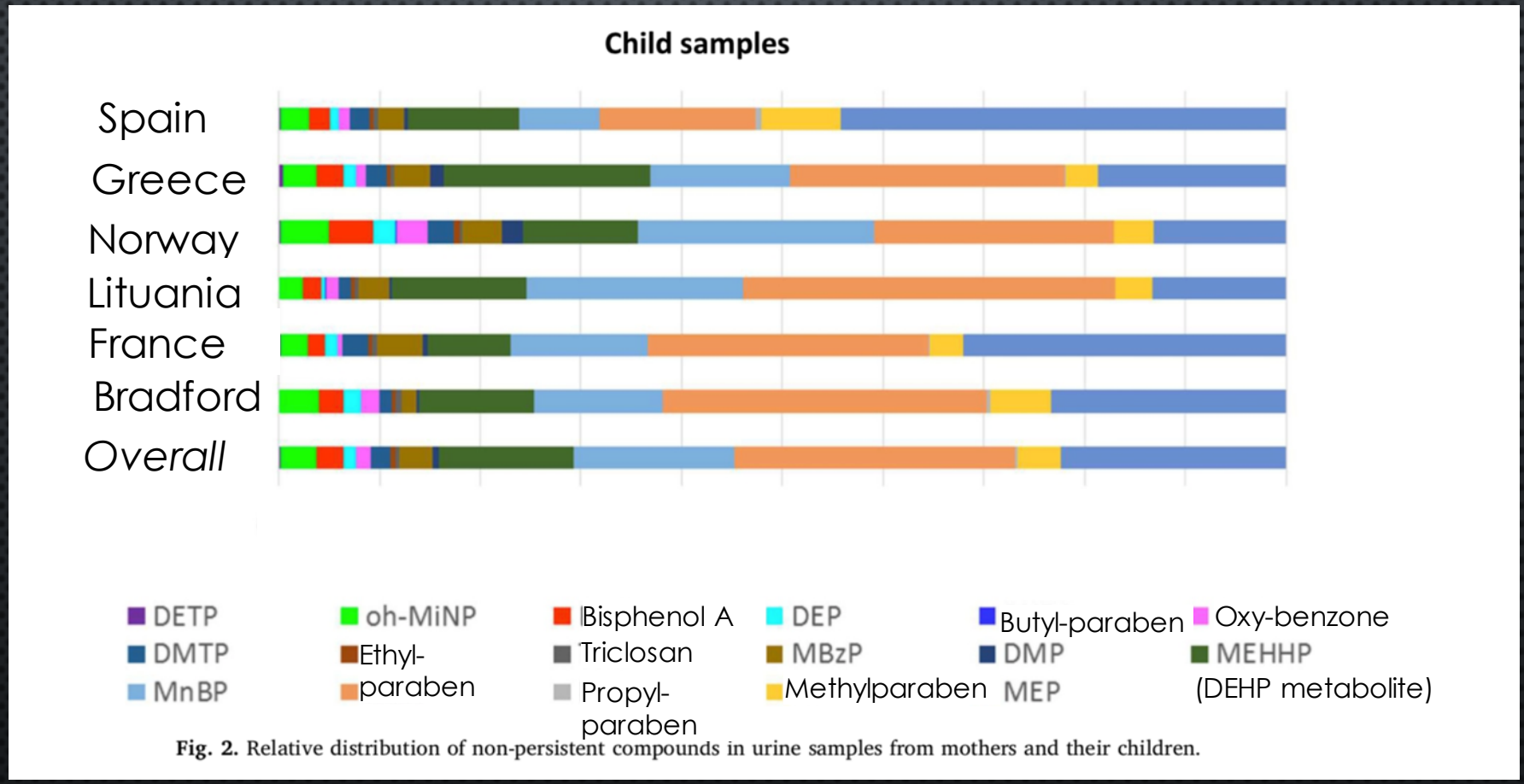


Fig. 2. Relative distribution of non-persistent compounds in urine samples from mothers and their children.

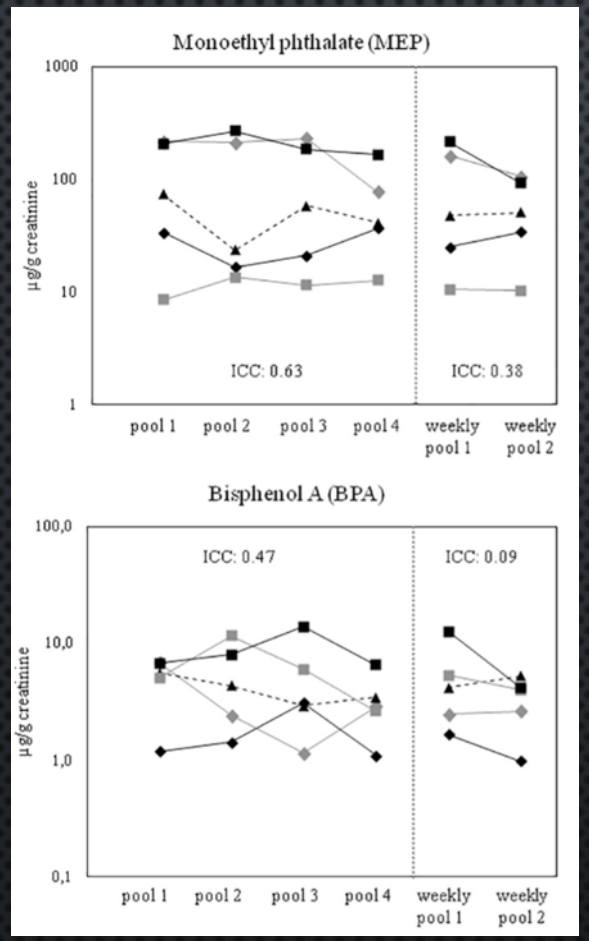
33 out of 45 biomarkers detected in 90% of the population

(Haug et al, *Env Int*, 2018)

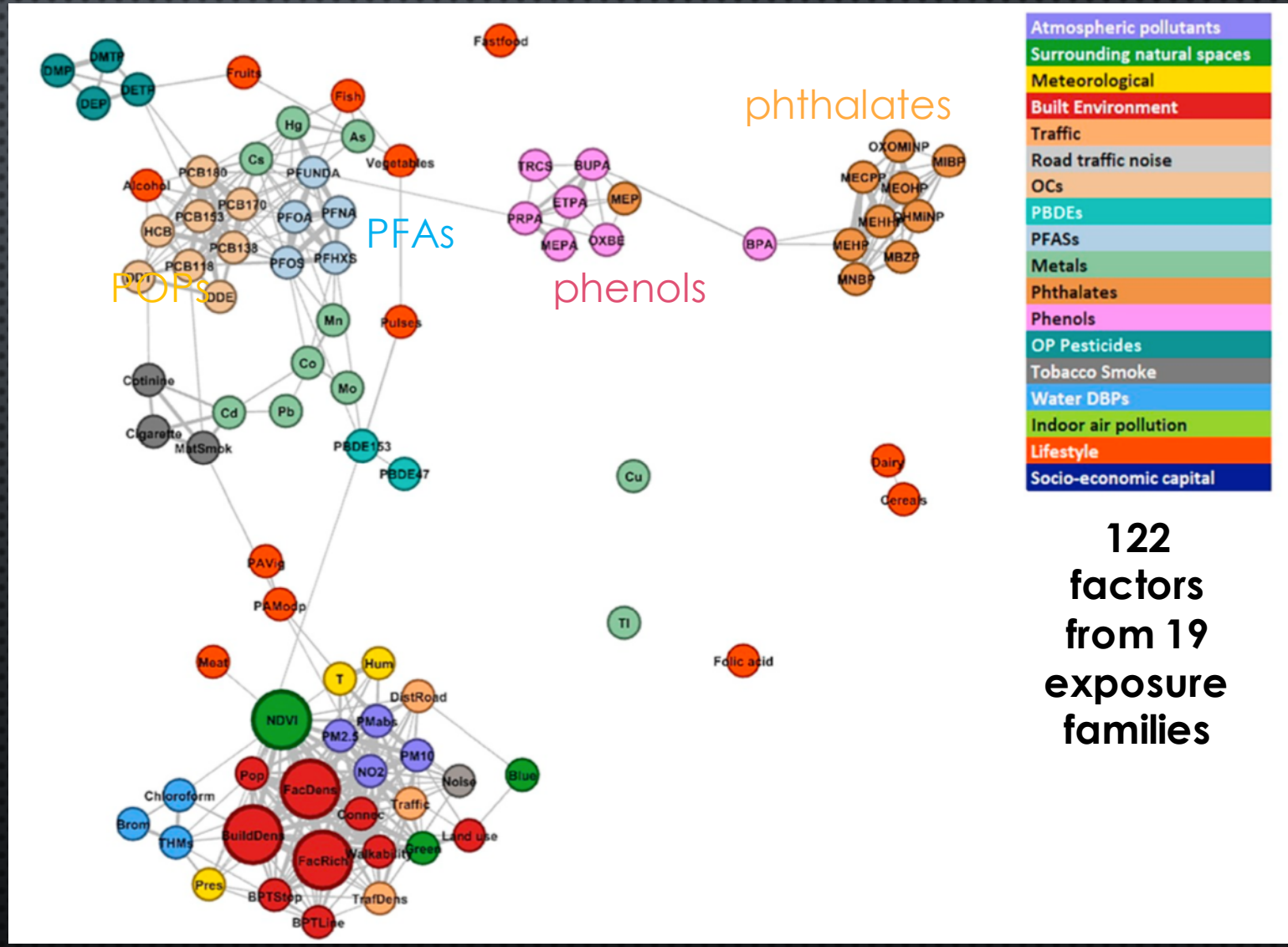
HELIX BUILDING THE EARLY-LIFE EXPOSOME

Characterization of ***correlations*** in the exposome

Within-subject (temporal variability) or *between exposures*



(Casas, *Env Int*, 2018)
See also (Vernet, *EHP*, 2018)



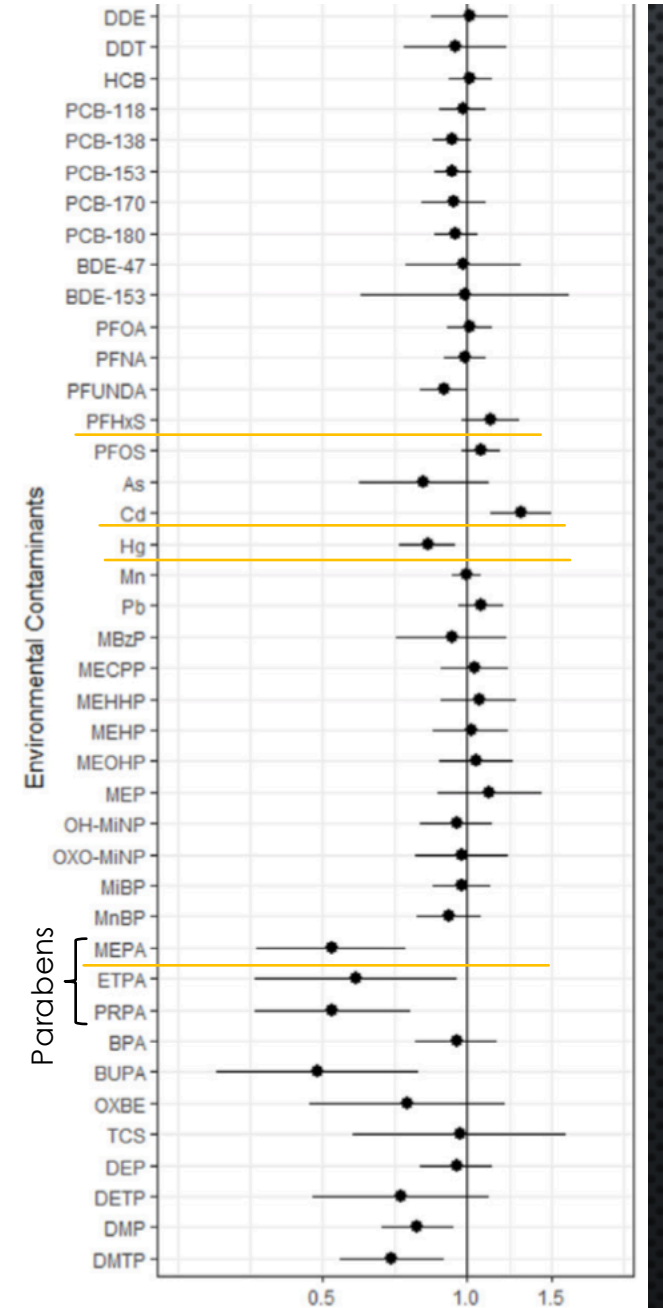
122 factors from 19 exposure families

(Tamayo, *Env Int*, 2019)

Exposome studies as a way to inform **environmental justice**

Mean pregnancy levels according to maternal education (n=1301)

Exposure change in lower Education women (ref: higher education)

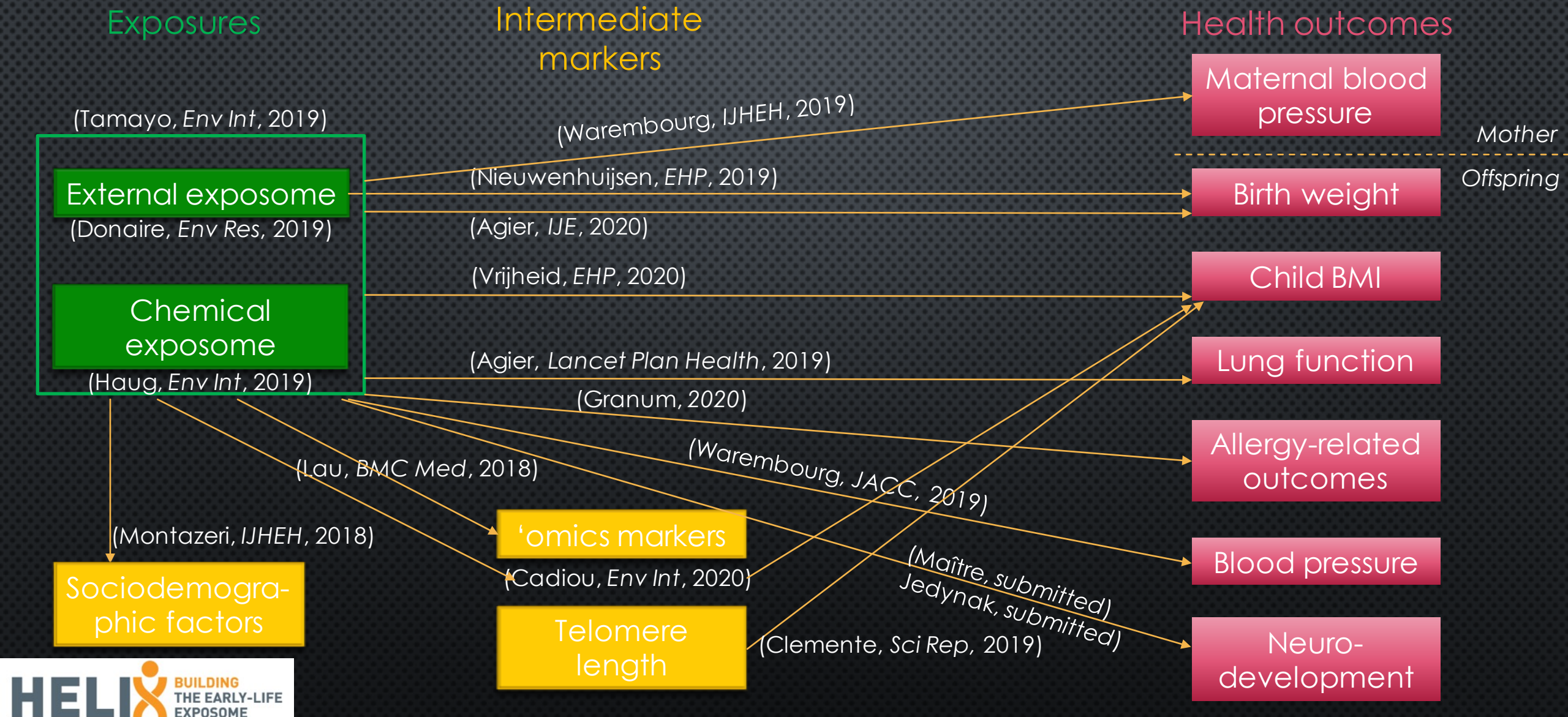


(Montazeri, *Int J Hyg Env Heal*, 2019)

III. Studies relating the exposome to health

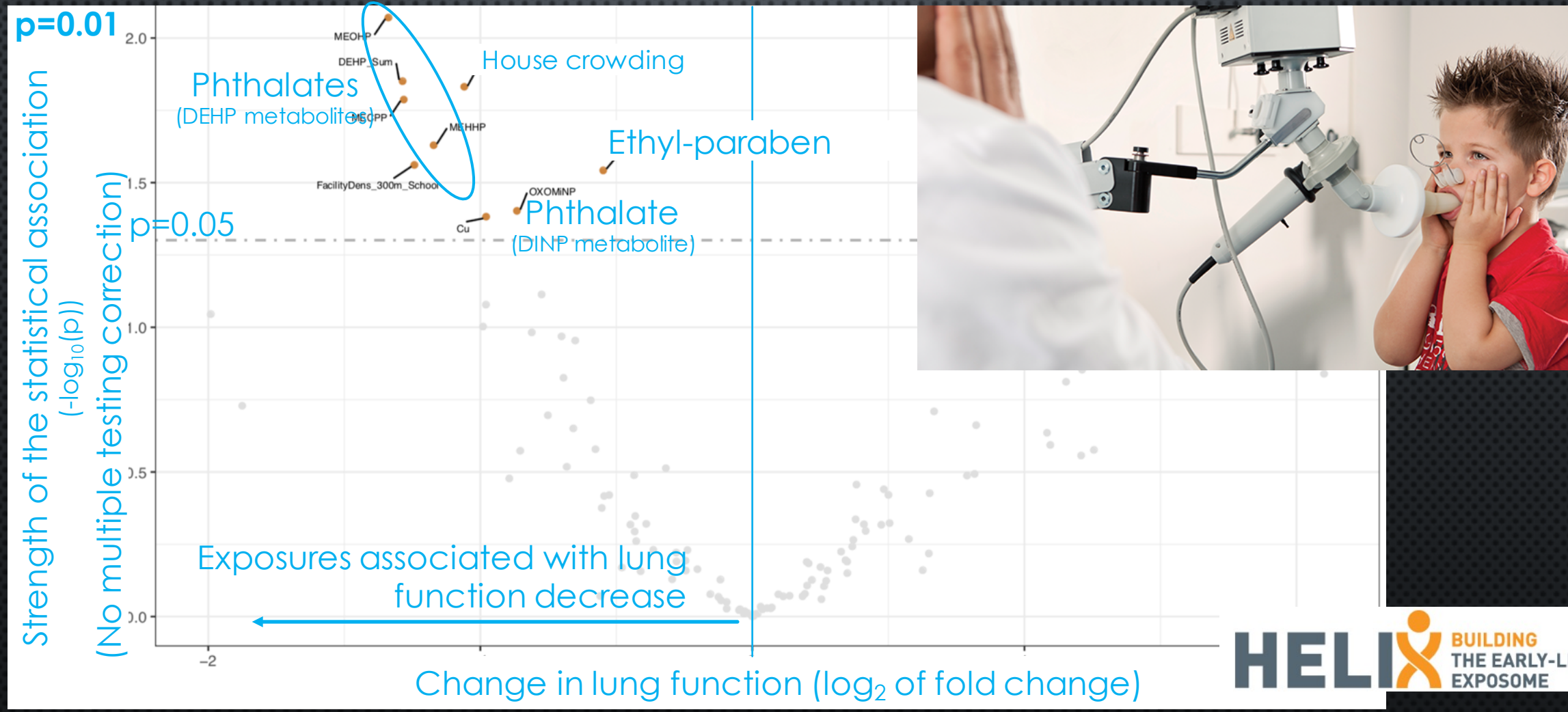
To next section

Relating the exposome to health: HELIX studies *(so far)*



HELIX BUILDING THE EARLY-LIFE EXPOSOME

Linking the child *postnatal* exposome (125 exposures) with children lung function (FEV1 – Forced Expiratory Volume in 1s; 1033 children)

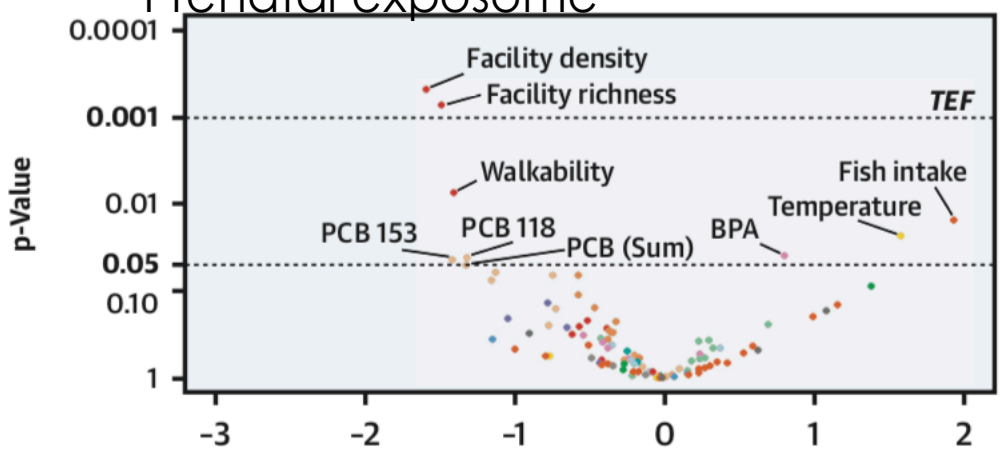


HELIX BUILDING THE EARLY-LIFE EXPOSOME

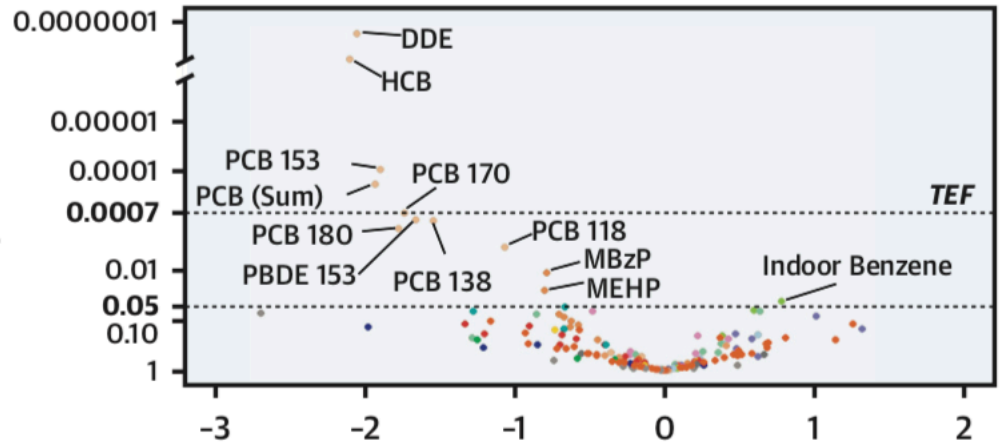
(Agier… Siroux, *Lancet Plan Health*, 2019)

Linking the exposome with child blood pressure

Prenatal exposome

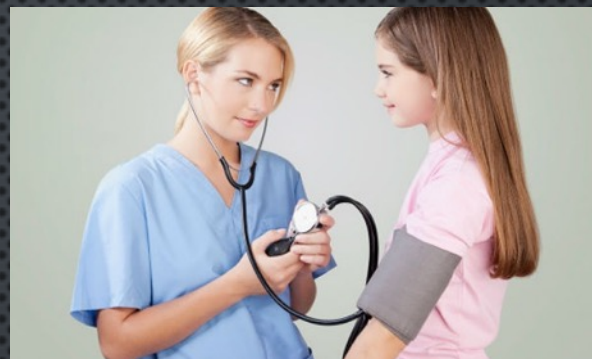


Postnatal exposome



Mean change in blood pressure (mmHg) for an interquartile increase in exposure

EWAS approach

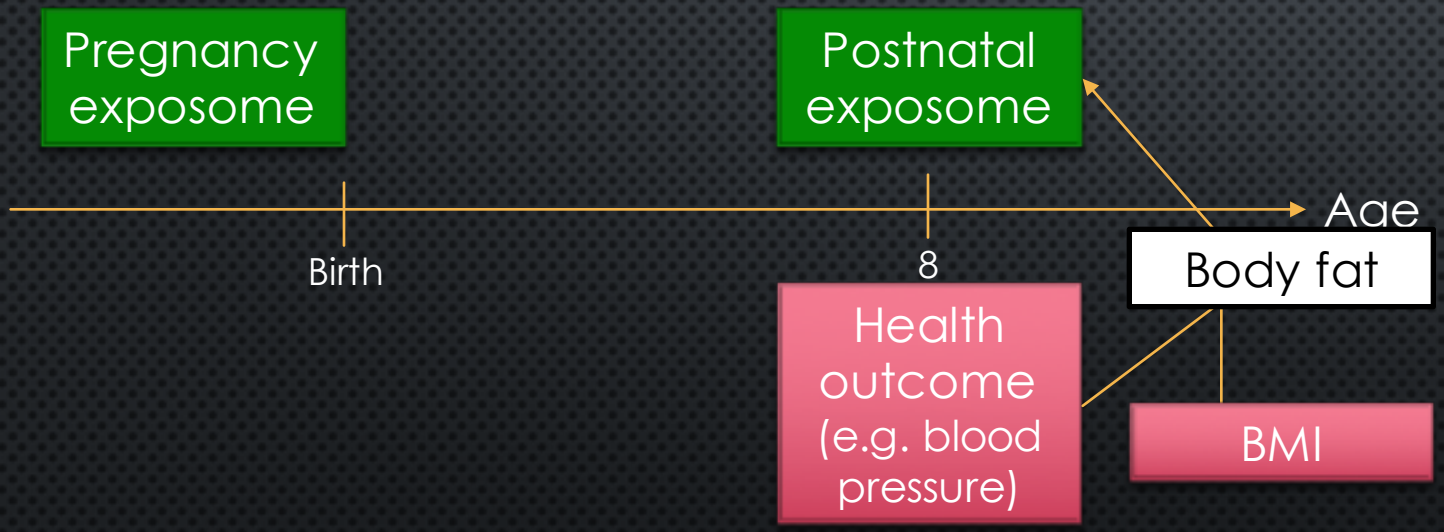


Exposures		Systolic Blood Pressure		
	Interquartile Range	Frequency (%) of Selection†	Adjusted Beta (95% CI)‡	p Value
Pregnancy period				
Facility density (300 m)	49.5 U/km^2	98	−1.7 (−2.5 to −0.8)	0.0003
Polychlorobiphenyl 118	2.8 ng/g lipids	56	−1.4 (−2.6 to −0.2)	0.0262
Fish and seafood intake	–	50	–	0.0414
<2 times/week vs. 2-4 times/week	–	–	1.0 (−0.5 to 2.5)	0.1799
>4 times/week vs. 2-4 times/week	–	–	2.0 (0.4 to 3.5)	0.0121
Temperature (average pregnancy)	5.8°C	34	1.6 (0.2 to 2.9)	0.0240
Cotinine (µg/l)	–	8	–	0.1211
18.4-50.0 vs. <18.4	–	–	−0.8 (−2.5 to 2.8)	0.3784
>50.0 vs. <18.4	–	–	1.2 (−0.3 to 2.8)	0.1138
Bisphenol-A	4.9 µg/g creatinine	2		
Childhood period				
Hexachlorobenzene	5.1 ng/g lipids	100	−1.5 (−2.4 to −0.6)	0.0018
Dichlorodiphenyldichloroethylene	34.0 ng/g lipids	18	−1.6 (−2.4 to −0.7)	0.0004
Mono benzyl phthalate	5.5 µg/g creatinine	6	−0.7 (−1.3 to −0.1)	0.0189
Perfluorooctanoate	0.8 µg/l	6	0.9 (0.1 to 1.6)	0.0213
Temperature (daily average)	10.8°C	0		
Copper	186 µg/l	0		

DSA approach

(Warembourg, *J Am Coll Cardiol*, 2019)

Issues related to reverse causality (lipophilic exposures)



Possible cures in future studies:

- Toxicokinetic modeling
- Assay lipophilic compounds from fat biopsies
- Increase follow-up time to limit cross-sectional analyses

Linking the exposome with birth weight (n=1287 newborns)

No association *after correction* for multiple testing.

Without correction for multiple testing:
Associations with lead,
$PM_{2.5}$ concentration
and $PM_{2.5}$ absorbance
levels during pregnancy

Associations reported for about 81 exposures from the urban and chemical exposomes (to be used e.g. in future meta-analyses)

EWAS approach

Association with lead (-98g change for each doubling in lead level; 95% CI: -182; -14 g)

(after correction for exposure measurement error)

DSA approach

(Agier, *Int J Epid*, in press)

IV. Relating the exposome to health: *Methodological issues*

To next section

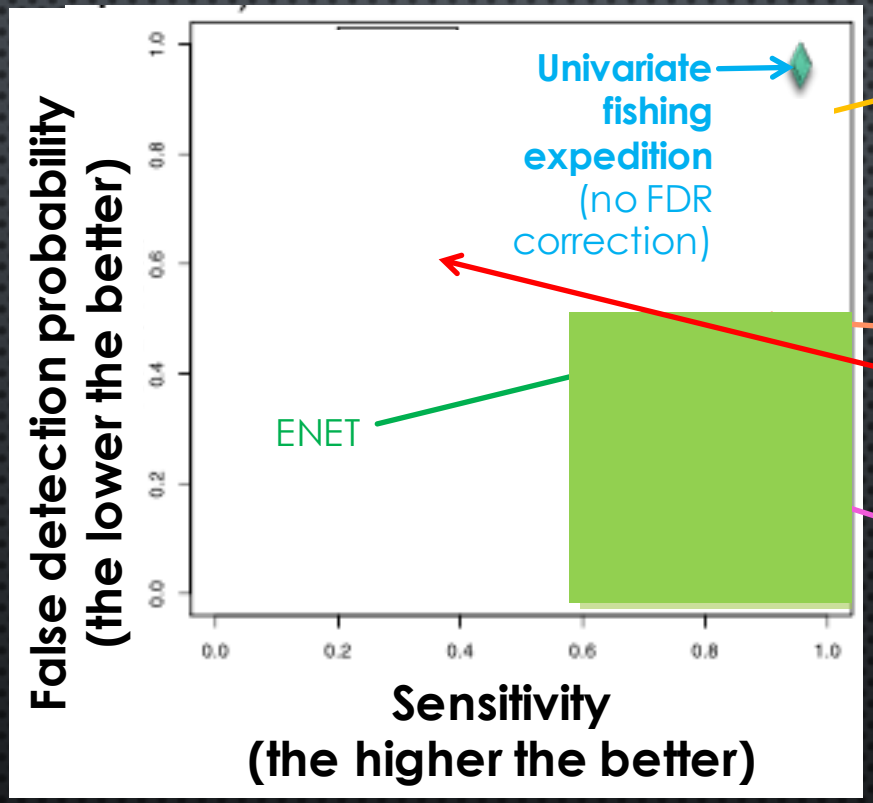
3 curses, one dream

- Correlation curse
- Mismeasurement curse
- Sample size curse

Tackling synergistic effects of mixtures

Efficiency of various statistical methods to relate the exposome to a health outcome (simulation study based on realistic hypotheses)

The GWAS approach used in genetic research cannot be applied in a straightforward way (EWAS) to the exposome **(correlation curse)**



EWAS: Exposome wide association study (similar to *GWAS* approach with FDR correction)

GUESS (Bayesian variable selection method)

EWAS-MLR: EWAS followed by multiple linear regression

DSA (Deletion/Substitution/Addition Algorithm)

Simulation study aiming at identifying k=1, 2, 10 or 25 real predictors out of 238 exposures (average results)

(Agier et al., *EHP*, 2016)

The curse of sample size: *possible answers*

1) Stop focusing on power alone and put it in the broader picture of the sensitivity-false detection rate (and stability) trade-off
2) Increase sample size (without increasing measurement error!)
3) Borrow information from toxicology

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Cumulative risk assessment of phthalates associated with birth outcomes in pregnant Chinese women: A prospective cohort study☆

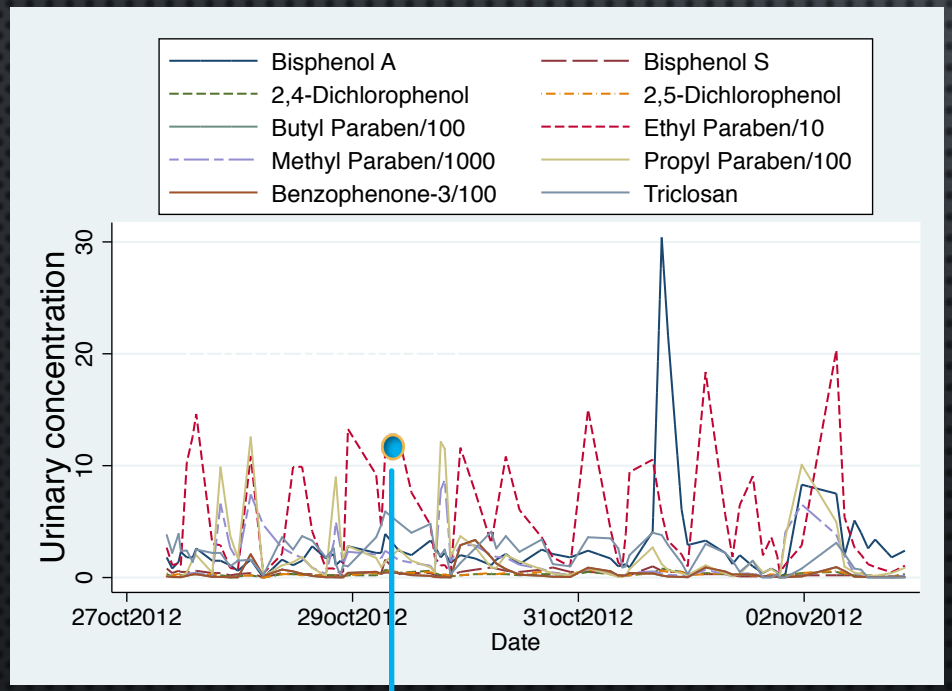
Hui Gao [a], Yuan-yuan Xu [a, c], Kun Huang [a], Xing Ge [a], Yun-wei Zhang [a], Hui-yuan Yao [a],

On the basis of DI estimations, we calculated HQs and HIs to assess risks from a single phthalate exposure and combined exposure, respectively. The formula was as follows:

$$HQ = \frac{DI(\mu g/kg\ bw/day)}{RfV(\mu g/kg\ bw/day)}; \quad HI = \sum HQ$$

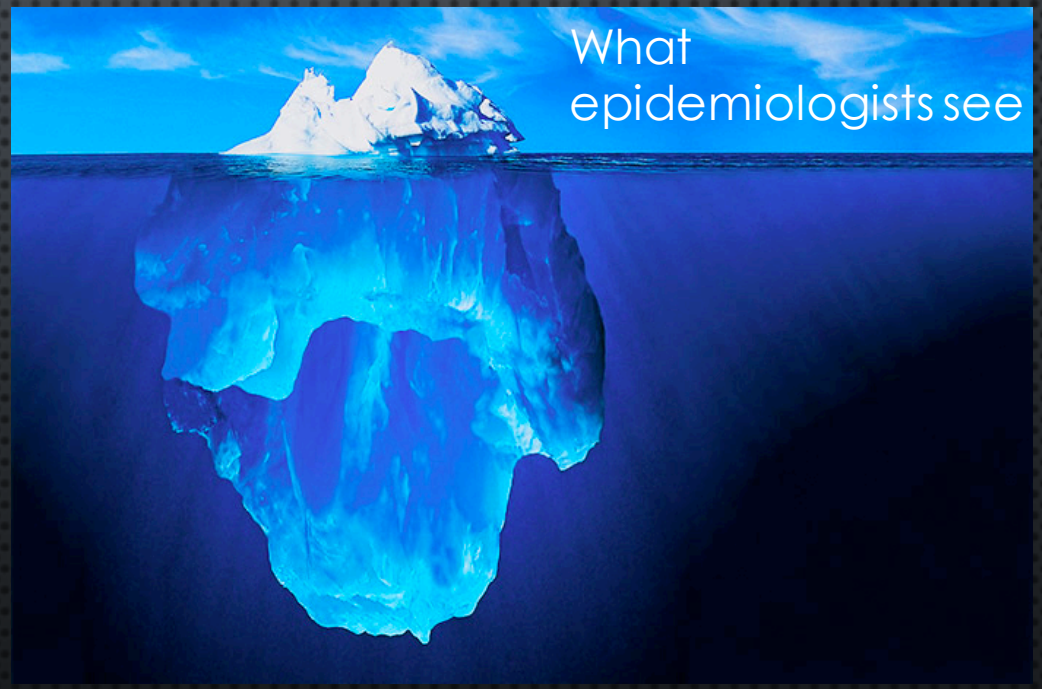
In addition, today's environmental contaminants are moving targets in the body

SEPAGES-feasibility study, sampling of all urine samples for 1 woman during a week (about 70 urine samples); A. Calafat's lab (CDC)



(Vernet, *EHP*, 2018)

…leading to attenuation bias in dose-response functions

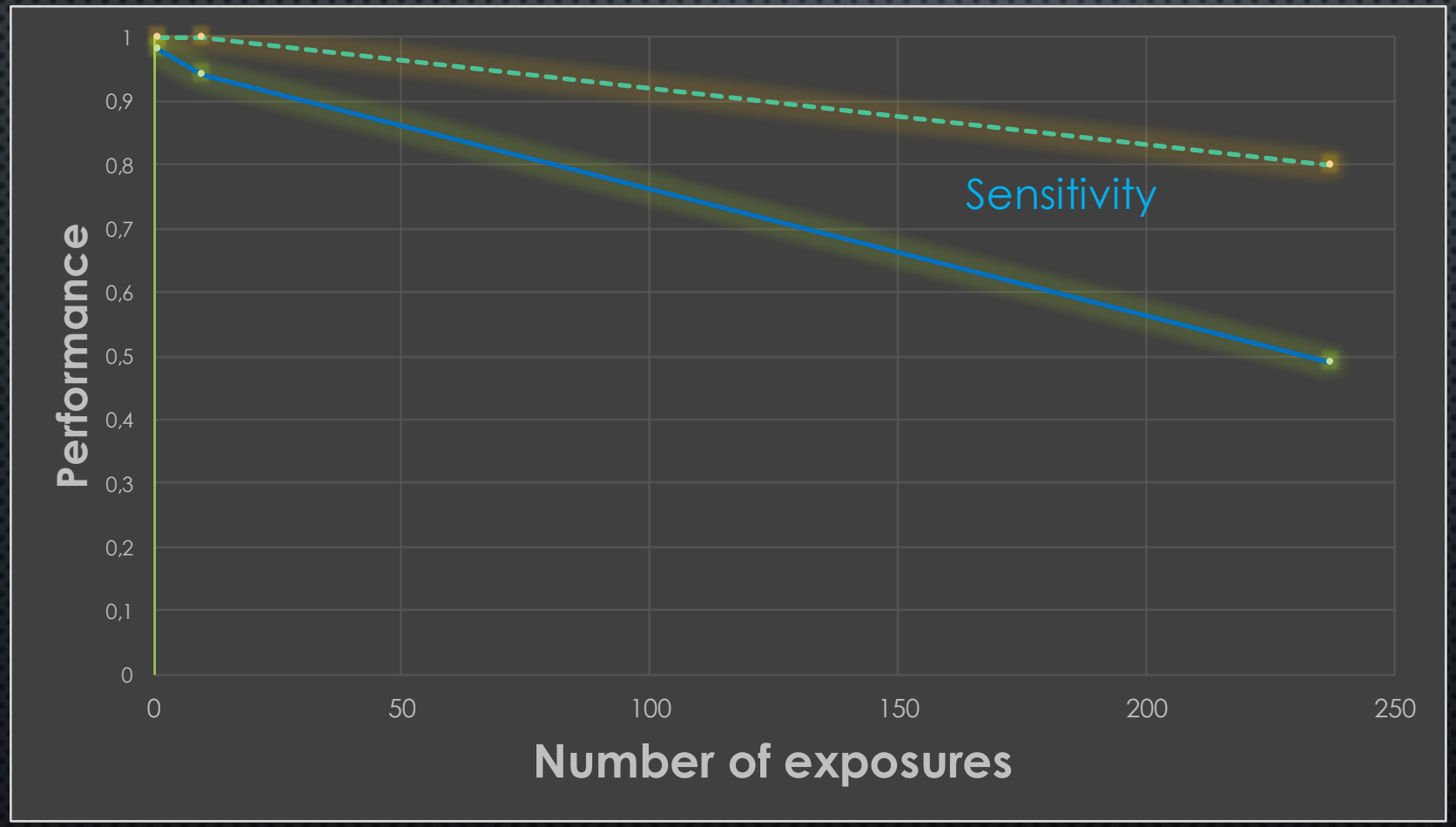


What may be the true effect

Mismeasurement curse

(Perrier, *Epidemiology*, 2016; Vernet, *Epidemiology*, 2019)

Impact of measurement error in exposome studies



No measurement error (T_j)

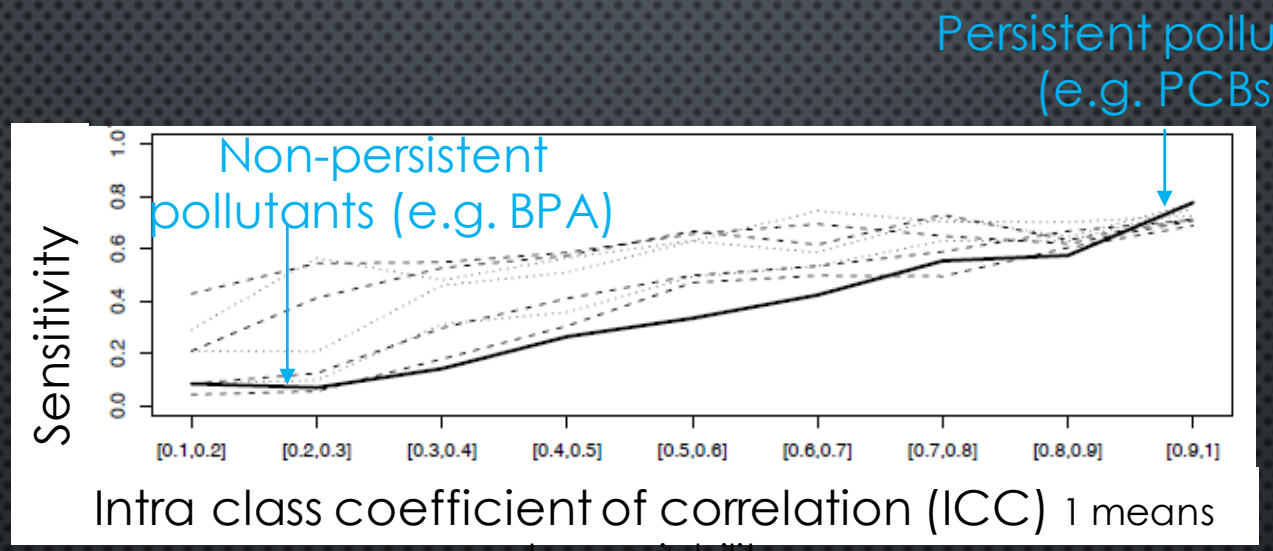
Error-prone exposures (X_j)

(Agier, Slama & Basagaña, *Env Res,* 2020)

Impact of measurement error in exposome studies

(Agier, Slama & Basagaña, *Env Res,* 2020)

Generalization: influence of the biomarker's variability on the sensitivity of ***exposome*** studies



The higher the temporal (within-subject) variability of a compound (low ICC), the lower the sensitivity of an exposome study to detect it.

Simulation study assuming 1200 participants and similar effect sizes for true exposures whatever their ICC.

(Agier, Slama & Basagaña, *Env Res,* 2020)

Statistical power = f (sample size (or number of cases), exposure distribution, measurement error…)

Related to the within-subject variability of the compound

Is there a cure?

>1 spot biospecimen / subject

Time

Δt: toxicologically relevant exposure window

Average (*true*) exposure: $(T_j)_{j \le d}$

Measured error-prone exposure: $(X_j)_{j \le d}$

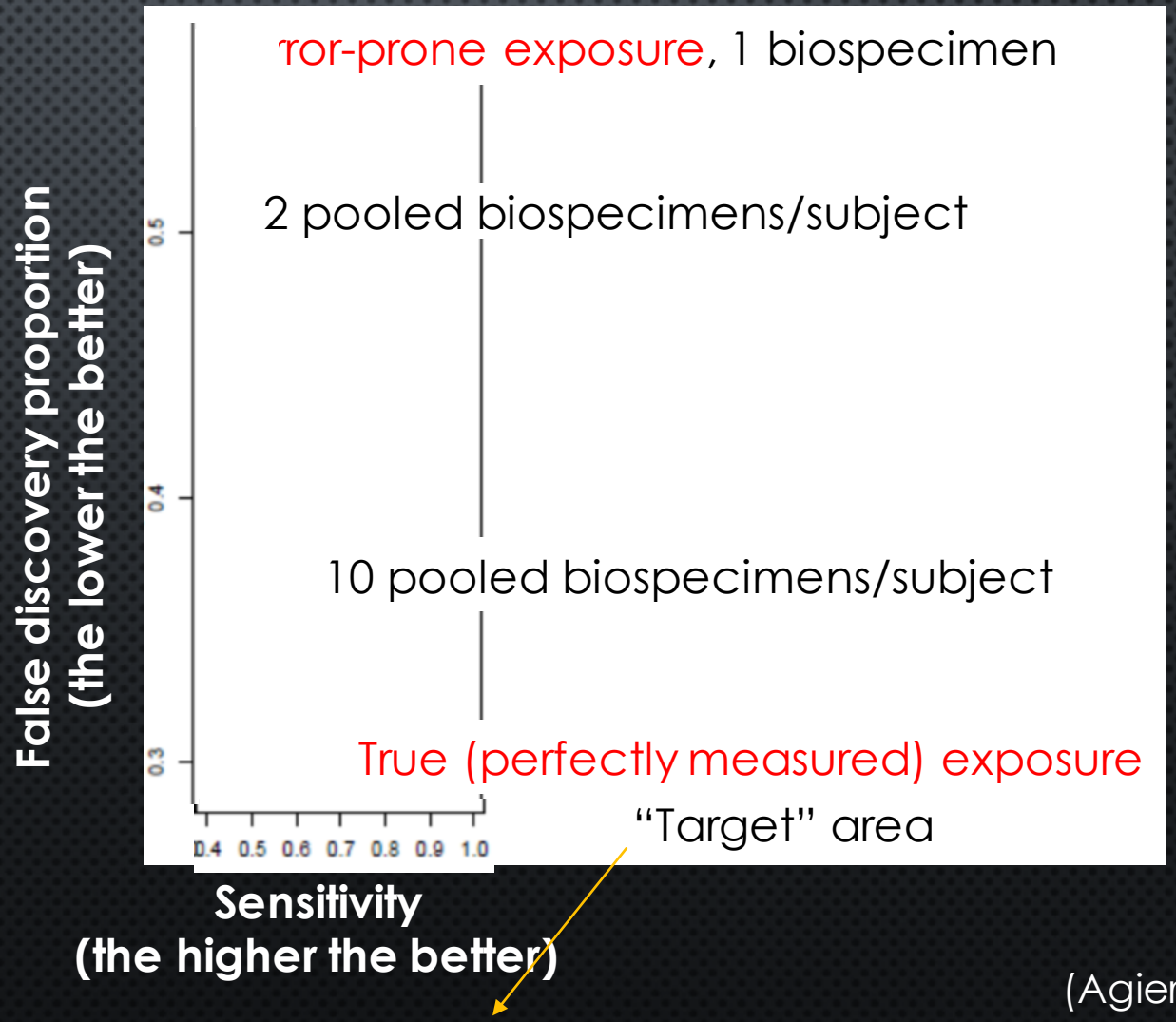
"Within-subject biospecimens pooling approach"

Validated in the single-exposure case (Perrier, *Epidemiology*, 2016)

Also considered in an exposome context (Agier, *submitted*)

Theoretical efficiency of exposome studies relying on repeated biospecimens (simulation)

Assumption: 10 true predictors truly affects the health outcome, out of 237 exposures



To be implemented in ATHLETE H2020 exposome project

(Agier et al., *Env Res*, 2020)

Achievements of the first early-life exposome studies (e.g. Helix) – ***Methodological achievements***

Single exposure studies

- Selective reporting of associations
- No correction for multiple testing
- Confounding by co-exposures
- Lack of consideration of mixture effects
- Exposure misclassification (short half-lived biomarkers)

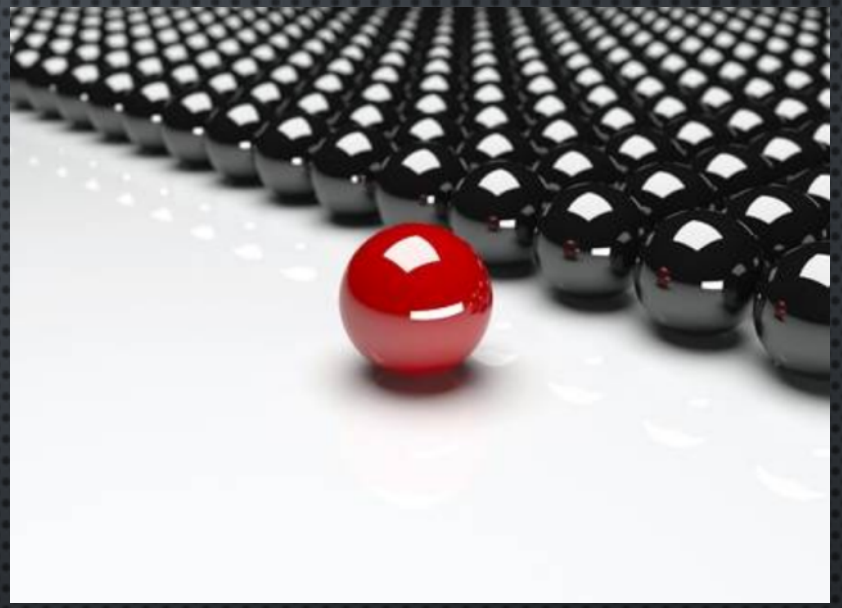
Limitations of the ExWAS approach, identification of more efficient regression-based techniques (Agier, *EHP*, 2016)

(Barrera-Gomez, *Env Health*, 2017)

HELIX BUILDING THE EARLY-LIFE EXPOSOME

- All tests performed are reported
- Explicit multiple testing (EWAS) or at least ability to a priori quantify the power/FDR trade-off
- Reliance on multiple regression models (e.g., DSA)
- Mixture effects considered (probably very low power)
- Correction for differential exposure misclassification *(in progress, see* Vernet, *Epidemiology* 2019; Agier, submitted)

Growing in number or drowning by numbers?



Not safe to increase the number of exposures considered…

-if you cannot simultaneously improve the quality of their assessment

(which can be done by increasing the number of biospecimens collected per subject; see Perrier, *Epidemiology*, 2016)

-if you cannot simultaneously increase sample size

What the next generation of Exposome studies could/*sh*ould look like

- Issues of exposure measurement error and power should be taken very seriously
- Short- and long-half-lived compounds should be given equal chances

  Collection of repeated biospecimens (within-subject biospecimens pooling approach, Perrier, 2016) and reliance on personal dosimeters

  If this is not possible, attempts to correct regression models for exposure measurement error should be undertaken (probably less efficient)
- 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 toxicological studies done e.g. in the context of endocrine disruptors and DOHaD research
- Toxicologists, epidemiologists and biostatisticians need to work together

Better – More – Longer - Multidisciplinary

ATHLETE

HELIX BUILDING THE EARLY-LIFE EXPOSOME

Collaborators and funding

Centre for Research in Environmental Epidemiology (**ISGlobal**), Spain	Lea Maitre, Xavier Basagaña, Mariona Bustamante, Maribel Casas, David Donaire, Diana van Gent, Juan Ramon Gonzalez, Mark Nieuwenhuijsen, Oliver Robinson, David Rojas, Jordi Sunyer, Ibon Tamayo, **Martine Vrijheid**
Norwegian Institute for Public Health (**NIPH**) Oslo	Catherine Thomsen, Berit Granum, Kristine Gutzkow, Helle Margrete Meltzer, Per Schwarze
University of Crete (**UoC**), Greece	Leda Chatzi, Vicky Lebentakou, Marina Vafeiadi
Inserm, France	Rémy Slama, Valérie Siroux, Lydiane Agier, Solène Cadiou, Lise Giorgis Allemand, Sarah Lyon-Caen, Joane Quentin
Bradford Teaching Hospitals NHS Foundation Trust (**BTHFT**), United Kingdom	John Wright, Lesley Fairley, Rosemary Dagmar Waiblinger, …
Vytauto Didziojo Universitetas (**VDU**) Lithuania	Regina Grazuleviciene, Sandra Andru
Imperial College of Science, Technology and Medicine (**ICL**), United Kingdom	Muireann Coen, Toby Athersuch, Hec Garcia
Centre for Genetic Regulation (**CRG**), Spain	Eva Borras, Xavier Estivill, Eduard Sabi
Veiligheids- en Gezondheidsregio Gelderland Midden (**VGGM**) Netherlands	Peter van den Hazel
Hylobates Consulting S.R.L. (**HYLO**), Italy	Francesca Bucchini, Luca Bucchini
Sensing & Control Systems S.L. (**S&C**), Spain	Alberto Fernandez, Antonella Guzzon, Elena Turco
Institut National de l'Environnement et des risques (**INERIS**), France	Céline Brochot, Florence Zeman
University Santiago de Compostela	Angel Carracedo, Manuela Gago

Funding:

SEVENTH FRAMEWORK PROGRAMME

Need more training on the exposome?

Come and join **ALEXS**

The Alpine Exposome Summer School

Online course (2021)

ALEXS
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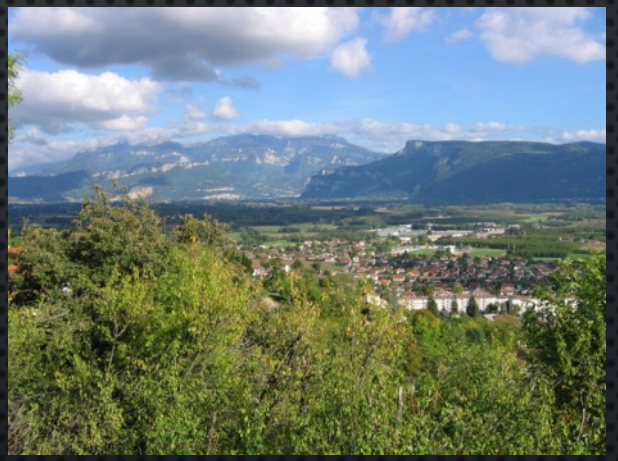
Summer School



With lectures from
B. Eskenazi, C. Kennedy (UC Berkeley), M. Vrijheid, X. Basagaña,
J.R. Gonzalez (ISGlobal), J. Lepeule, V. Siroux, R. Slama (Inserm)…

Contact and information: https://exposomesummerschool.com/ contact@exposomesummerschool.com