ENDpoiNTs has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 825759, and is part of the EURION cluster

Epidemiology in Children as a basis for Test Development

Carl-Gustaf Bornehag
Karlstad University, Karlstad, Sweden
Icahn School of Medicine at Mount Sinai, NY, USA

Research for a healthier future

Swedish Environmental Longitudinal, Mother and child, Asthma and allergy study

Environmental factors in early life and the importance for health and development in children

SELMA

Research for a healthier future

Swedish Environmental Longitudinal, Mother and child, Asthma and allergy study

The developmental basis of adult health

RISK & RESILIENCE

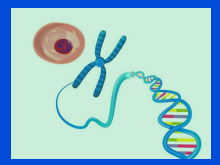
ENVIRONMENT
(N=+2,300)

26/41 EDCs
Pharmaceuticals
Etc.

*

Background
Stress
Mothers health
Diet nutrition
Life style
Etc.

GENETICS



7y

Epigenetics

14y

Epigenetics

Follow up II

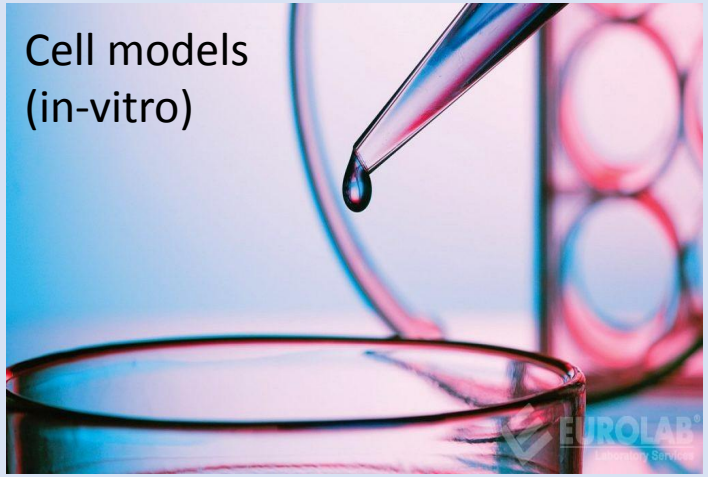
Neurodelopment

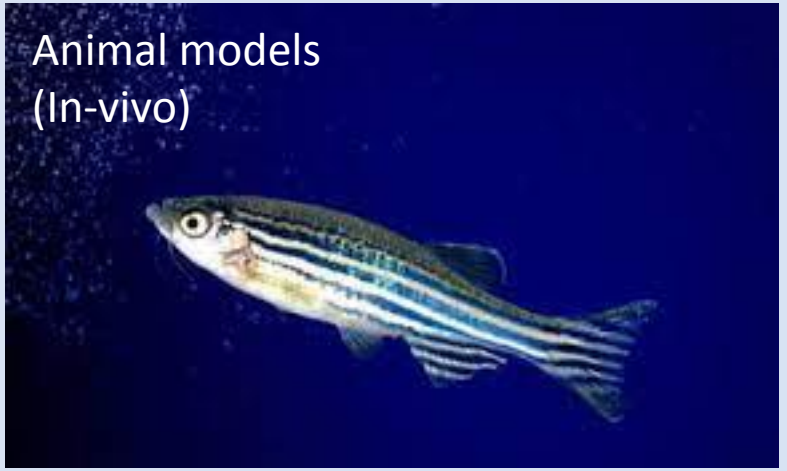
Sexual Development

Metabolic development

Immunogical development

Biosampling



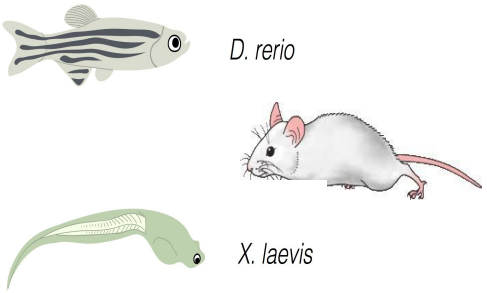


Computational
Toxicology
(In-silico)

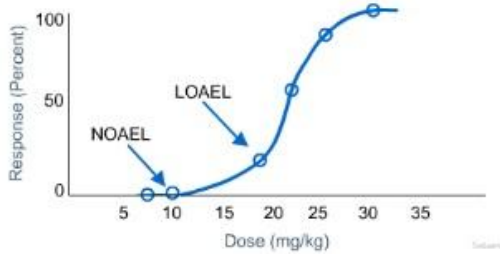
Single Compounds and Mixtures

Risk Assessment Metrics

Human Exposure:
External estimates;
Biomonitoring



Reference Dose (RfD)



$$\text{Regulatory Ratio} = \frac{\text{Human Exposure}}{\text{RfD}}$$

	Single Chemical
Regulatory Ratio	Hazard Quotient

However, we are never exposed to one chemical at a time!

Data from +2,300 pregnant women in SELMA

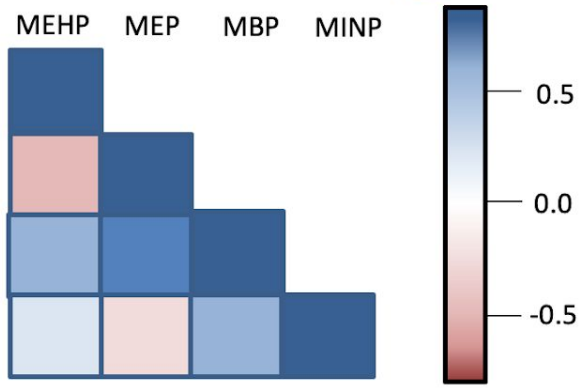
Research for a healthier future

Swedish Environmental Longitudinal, Mother and child, Asthma and allergy study

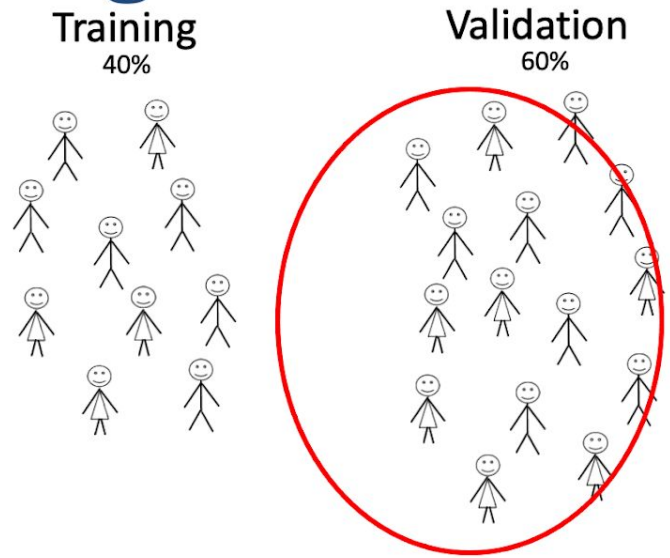
Matrix	Chemical Type	Compound *(further description)*	Abbreviation	LOD/ LOQ[a]	% ≥LOD	GM
Urine	Phenols	2,4,4′-trichloro-2′-hydroxydiphenyl ether	Triclosan	0.100	92	1.27
		bisphenol A	BPA	0.050	100	1.53
		4,4-bisphenol F *(BPA replacement analogue)*	BPF	0.024	92	0.16
		bisphenol S *(BPA replacement analogue)*	BPS	0.009	98	0.07
	Plasticizers *(Phthalate & non-phthalate)*	monoethyl phthalate	MEP	0.010	100	62.8
		monobutyl phthalate	MBP	0.100	100	67.5
		monobenzyl phthalate	MBzP	0.040	100	15.5
		mono(2-ethylhexyl) phthalate	MEHP	0.100	100	–
		mono(2-ethyl-5-hydroxyhexyl) phthalate	MEHHP	0.020	100	–
		mono(2-ethyl-5-oxohexyl) phthalate	MEOHP	0.030	100	–
		mono(2-ethyl-5-carboxypentyl) phthalate	MECPP	0.020	100	–
		di-(2-ethylhexyl) phthalate (*parent compound*)	DEHP[b]	-	-	63.8
		mono(hydroxy-iso-nonyl) phthalate	MHiNP	0.020	100	–
		mono(oxo-iso-nonyl) phthalate	MOiNP	0.010	100	–
		mono(carboxy-iso-octyl) phthalate	MCiOP	0.020	100	–
		diisononyl phthalate (*parent compound*)	DINP[c]	–	–	26.7
		monohydroxyisodecyl phthalate	MHiDP	0.031	100	1.25
		monocarboxyisononyl phthalate	MCiNP	0.031	100	0.68
		2-4-methyl-7-oxyooctyl-oxycarbonyl-cyclohexane carboxylic acid *(phthalate replacement)*	MOiNCH	0.023	99	0.31
		diphenylphosphate *(organophosphate flame retardant)*	DPHP[d]	0.042	100	1.33
	Other Short-Lived	3,5,6-trichloro-2-pyridinol *(organophosphate pesticide)*	TCP	0.035	100	1.25
		3-phenoxybenzoic acid *(pyrethroid pesticide)*	PBA	0.017	99	0.16
		2-hydroxyphenanthrene *(polycyclic aromatic hydrocarbon)*	2OHPH	0.003	100	0.20
Serum	Perfluoro-alkyl Substances (PFAS)	perfluorooctanoic acid	PFOA	0.020	100	1.55
		perfluorooctane sulfonate	PFOS	0.060	100	5.32
		perfluorononanoic acid	PFNA	0.010	100	0.53
		perfluorodecanoic acid	PFDA	0.020	100	0.26
		perfluoroundecanoic acid	PFUnDA	0.020	99	0.22
		perfluorohexanesulfonic acid	PFHxS	0.030	100	1.31
Plasma	Persistent Chlorinated	hexachlorobenzene	HCB	0.005	100	0.04
		trans-nonachlor	Nonachlor	0.005	78	0.01
		dichlorodiphenyltrichloroethane alone	DDTa	0.015	99	–
		dichlorodiphenyldichloroethylene	DDE	0.040	8	–
		total dichlorodiphenyltrichloroethane	DDT[e]	–	–	0.19
		polychlorinated biphenyl 74	PCB 74	0.005	73	–
		polychlorinated biphenyl 99	PCB 99	0.005	81	–
		polychlorinated biphenyl 118	PCB 118	0.005	99	–
		polychlorinated biphenyl 138	PCB 138	0.005	100	–
		polychlorinated biphenyl 153	PCB 153	0.005	100	–
		polychlorinated biphenyl 156	PCB 156	0.005	90	–
		polychlorinated biphenyl 170	PCB 170	0.005	100	–
		polychlorinated biphenyl 180	PCB 180	0.005	100	–
		polychlorinated biphenyl 183	PCB 183	0.005	76	–
		polychlorinated biphenyl 187	PCB 187	0.005	98	–
		total polychlorinated biphenyls	PCB[f]	–	–	0.37


Cleaning & Personal care products
Electronical devices
Building materials
Toys
Dietary, cook wares and packages
skin resurfacing cleanser
CANNED MEAT

Weighted Quantile Sum (WQS) Regression



Subject ID	Concentration of MEHP (ng/ml)	Rank (Q) of MEHP
1	3.4	4
2	1.2	2
3	10.3	9



Weights (W, %)

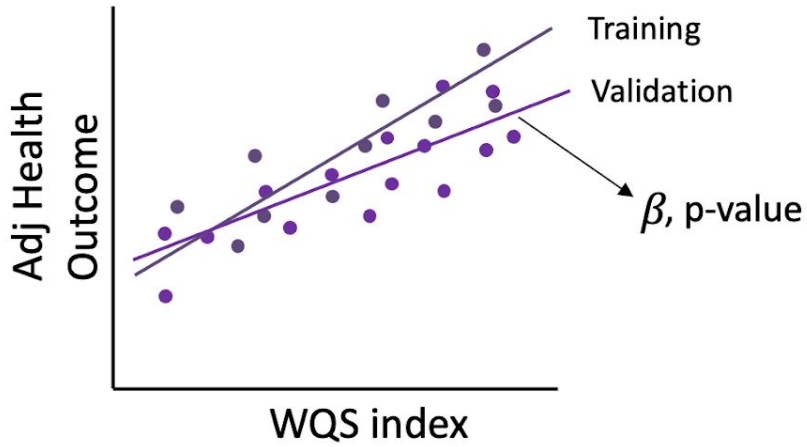
MEHP	62	58	67	59
MEP	34	36	31	35
MBP	3	3	1	2
MINP	1	3	1	4

100 times

Average of 100 weights (%)

61
34
3
2

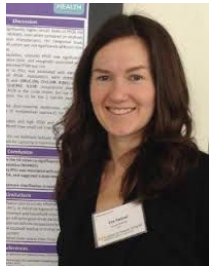
$$WQS = \sum_{k=1}^{K} W_k Q_k$$



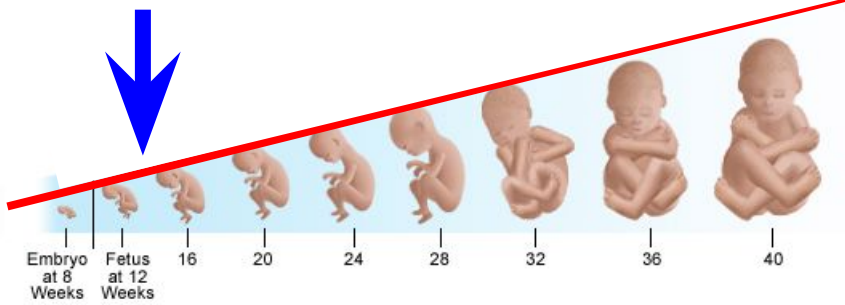
Research for a healthier future
Swedish Environmental Longitudinal, Mother and child, Asthma and allergy study

Natural hormones

Estrogen
Testosterone
Thyroids. etc.



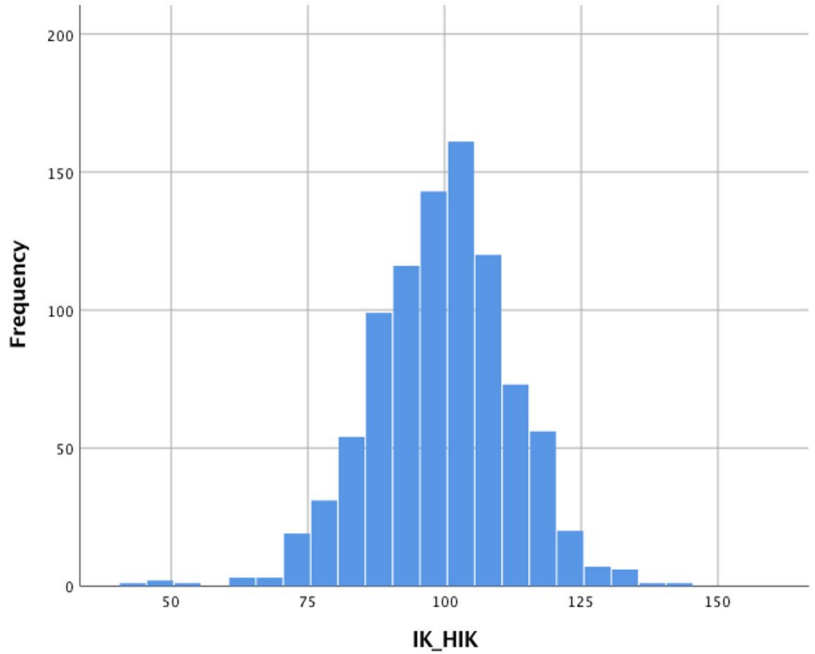
Eva Tanner
Mount Sinai, NY



26 EDCs (>40 analytes)
(N=917)
Mixture approach (WQS)

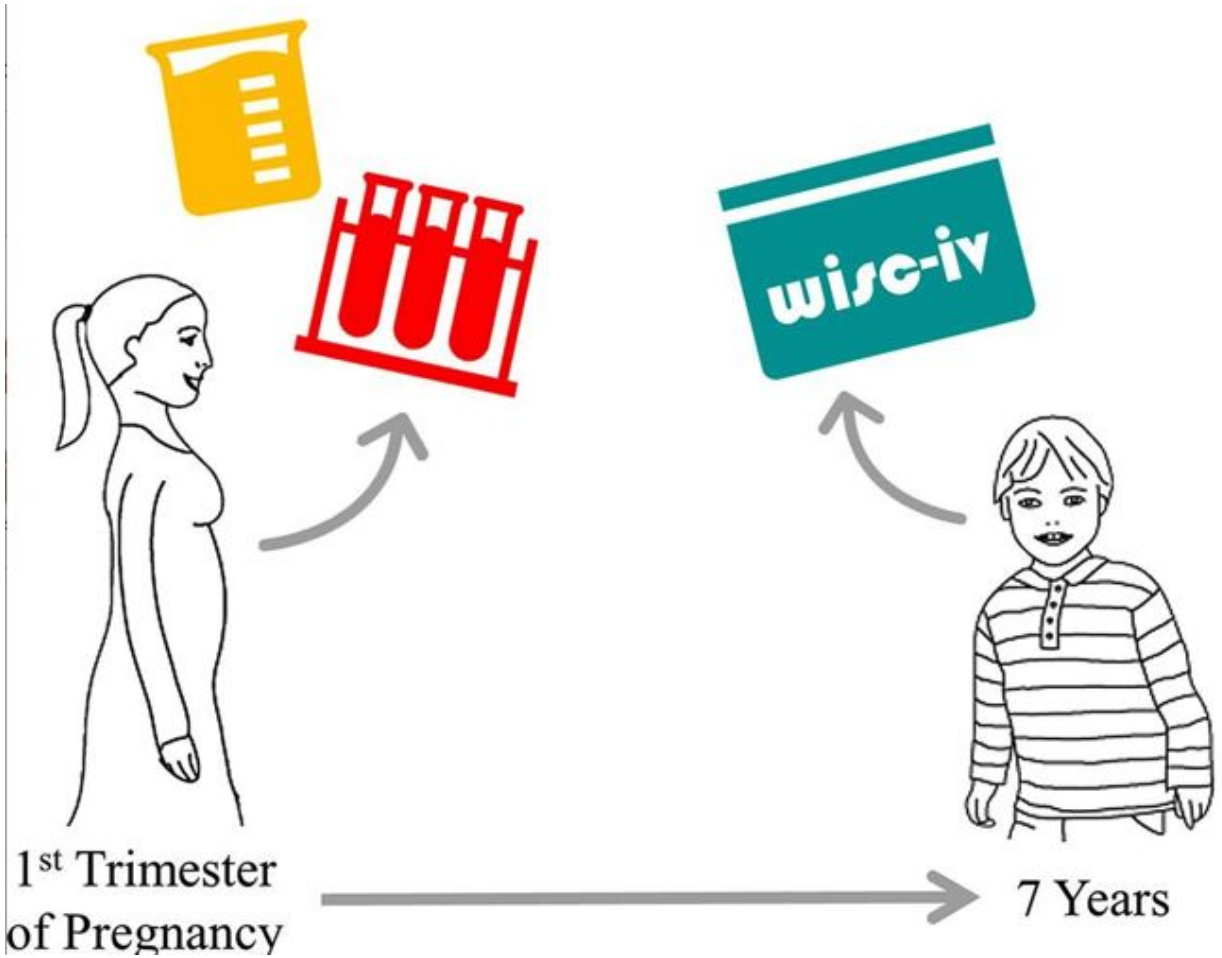
Co-factors
creatinine, sex, prematurity, mothers age, weight, IQ, and education, parity, and breastfeeding at 6 months of age

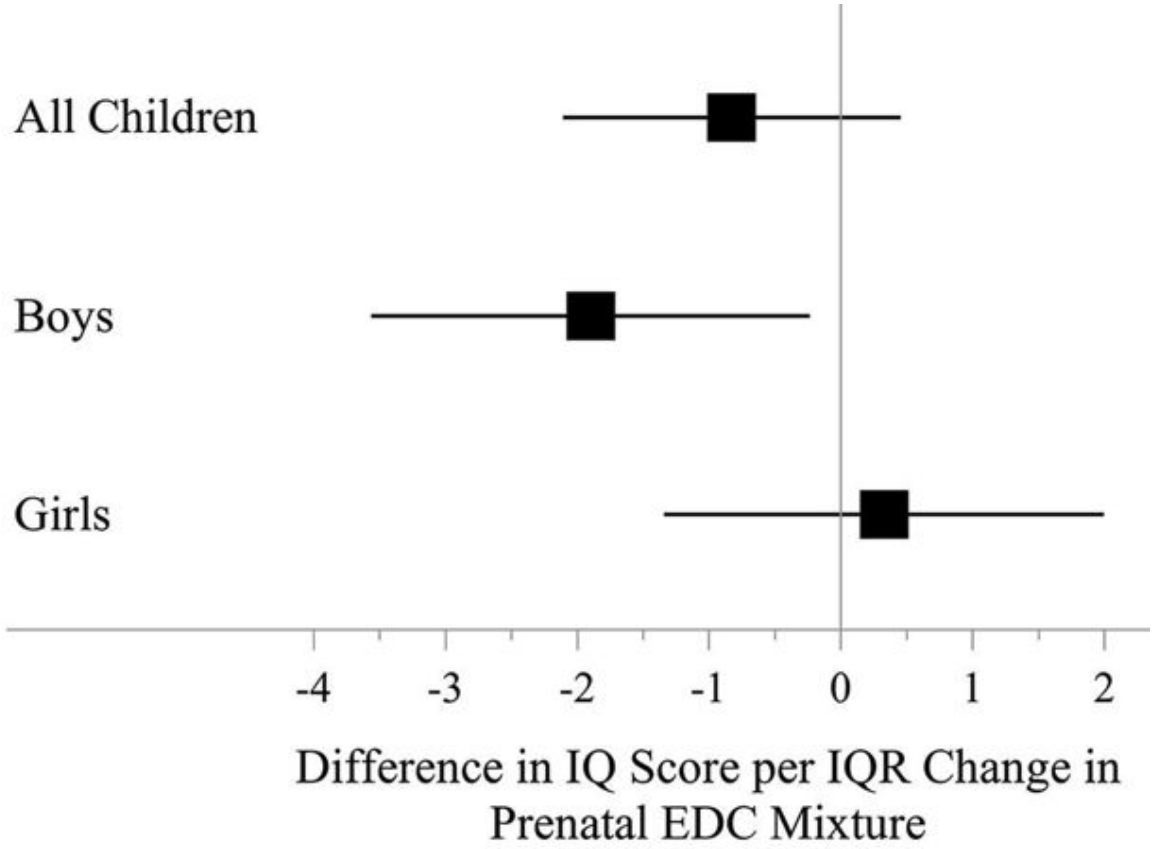
Cognitive function (IQ, WISC-IV) at 7y



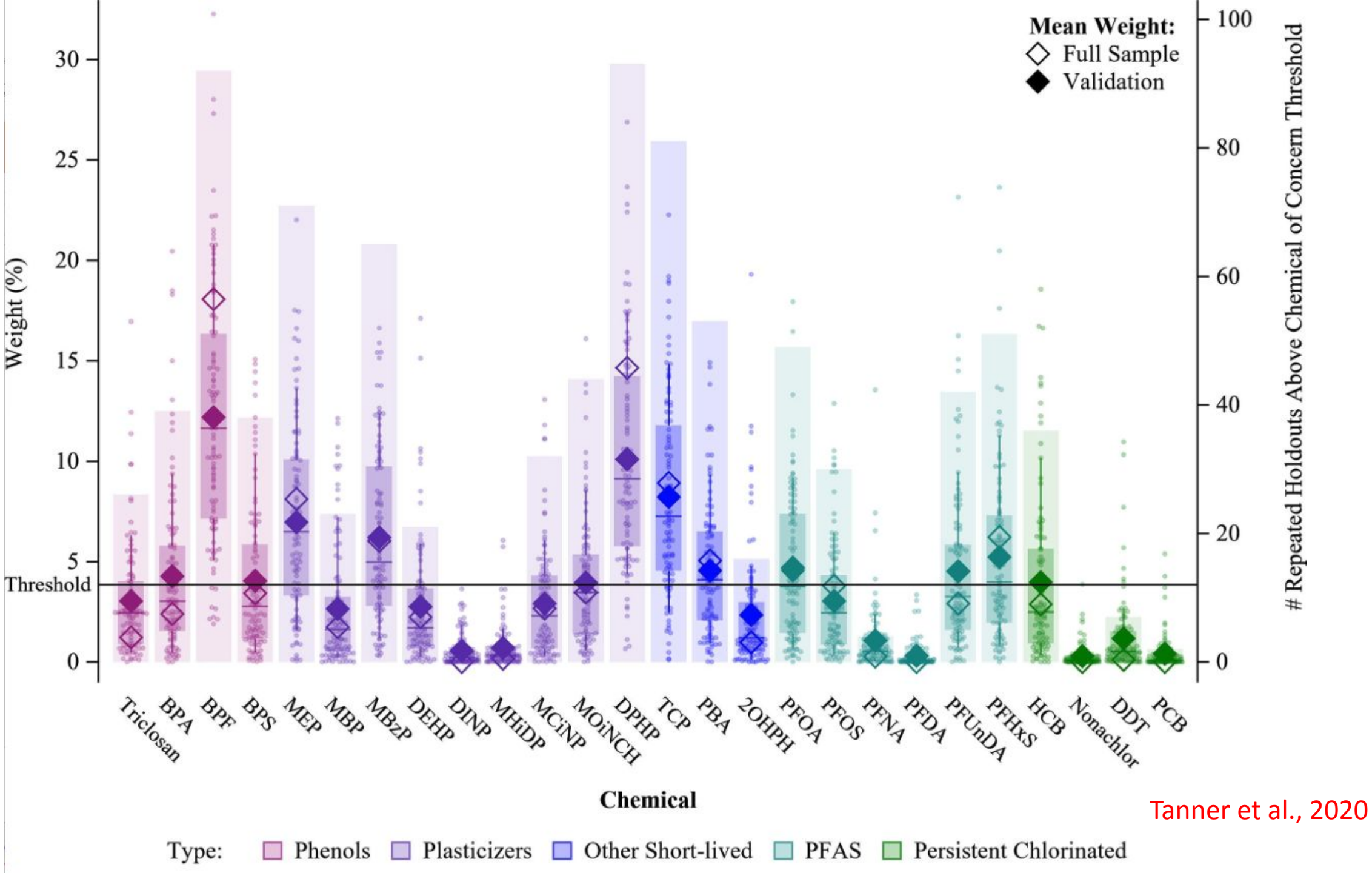
Tanner et al., 2020

Prenatal EDC mixture exposure and Childrens IQ at 7y (N=718) by WQS regression





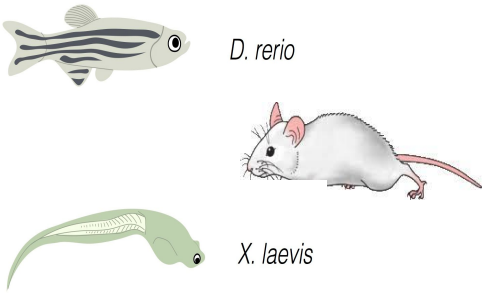
Tanner et al., 2020



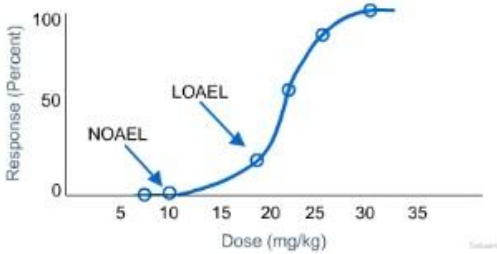
Tanner et al., 2020

Risk Assessment Metrics

Human Exposure:
External estimates;
Biomonitoring



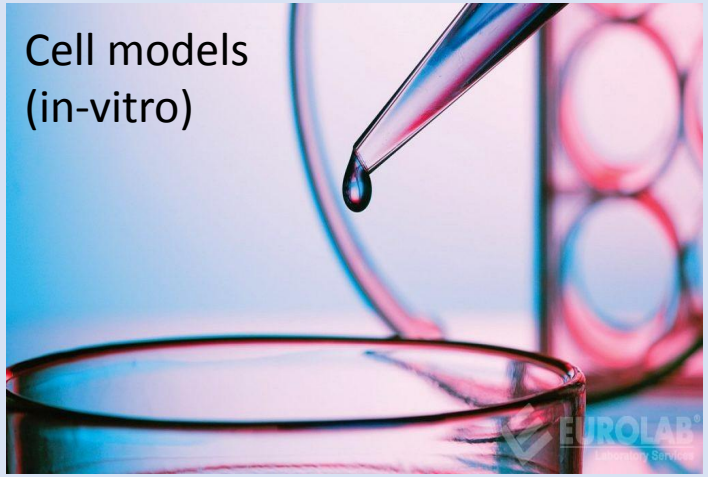
Reference Dose (RfD)



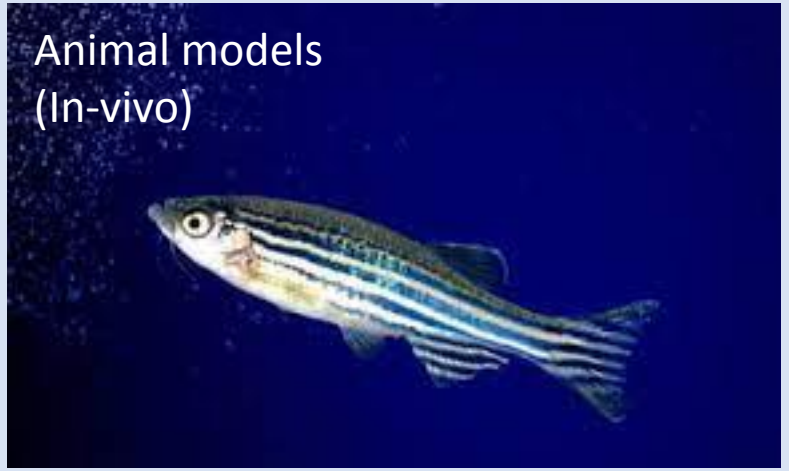
$$\text{Regulatory Ratio} = \frac{\text{Human Exposure}}{\text{RfD}}$$

	Single Chemical	Additive Mixture	General Mixture
Regulatory Ratio	Hazard Quotient	Hazard Index	Similar Mixture Risk Indicator *

* Calculated for those "sufficiently similar" to the reference mixture



EVIDENCE



Molecular mechanisms of action
(explaining associations in epidemiology)

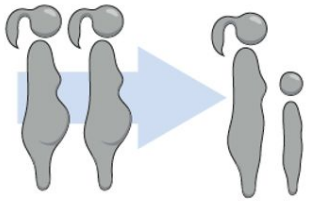
Dose-response relationships
(PODs for risk assessment)

Similar Mixture Approach (SMACH)

1. Identification of bad actors (mixtures) for health effects in epidemiological data

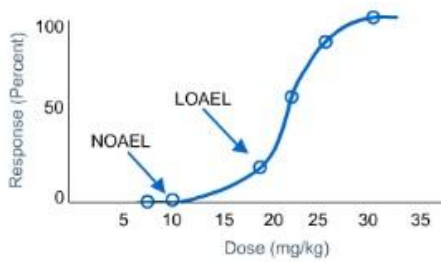
Epidemiology
EDC levels in urine, blood and clinical data

SELMA cohort

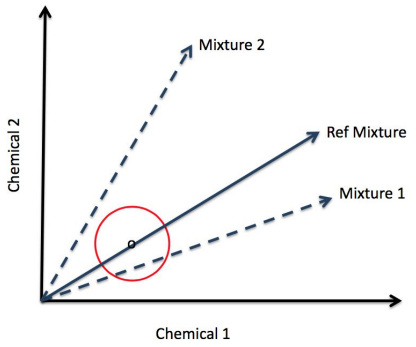


2. Composition of reference mixtures from population data for experimental evaluations

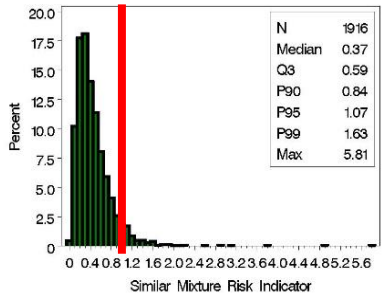
3. Experimental tests (in cells and animals) of reference mixtures for dose-response



4a Test for sufficient similarity with the reference mixture (%)



4b For sufficient similar subgroups; test for extreme mixing proportions, SMRI>1 (%)



EDC-MixRisk

From cohorts to molecules: Adverse impacts of endocrine disrupting mixtures

Science February 2022

RESEARCH ARTICLE

ENVIRONMENTAL TOXINS

From cohorts to molecules: Adverse impacts of endocrine disrupting mixtures

Nicolò Caporale[1,2,3]†, Michelle Leemans[4]†‡, Lina Birgersson[5]†, Pierre-Luc Germain[1]†§, Cristina Cheroni[1,2,3]†, Gábor Borbély[6]¶, Elin Engdahl[6,7]¶, Christian Lindh[8], Raul Bardini Bressan[9], Francesca Cavallo[1], Nadav Even Chorev[1], Giuseppe Alessandro D'Agostino[1]#, Steven M. Pollard[9], Marco Tullio Rigoli[1,2], Erika Tenderini[1], Alejandro Lopez Tobon[1], Sebastiano Trattaro[1,2], Flavia Troglio[1], Matteo Zanella[1]**, Åke Bergman[6,10,11]¶, Pauliina Damdimopoulou[6,12]¶, Maria Jönsson[7], Wieland Kiess[13], Efthymia Kitraki[14], Hannu Kiviranta[15], Eewa Nånberg[16], Mattias Öberg[6,17]¶, Panu Rantakokko[15], Christina Rudén[10], Olle Söder[18], Carl-Gustaf Bornehag[19,20]*††, Barbara Demeneix[4]*††, Jean-Baptiste Fini[4]††, Chris Gennings[20]††, Joëlle Rüegg[6,7]*¶††, Joachim Sturve[5]††, Giuseppe Testa[1,2,3]*††

***Corresponding author: Carl-Gustaf Bornehag, Barbara Demeneix, Joëlle Rüegg, Giuseppe Testa**

READ THE FULL ARTICLE AT
Science February 2022
https://doi.org/10.1126/science.abe8244

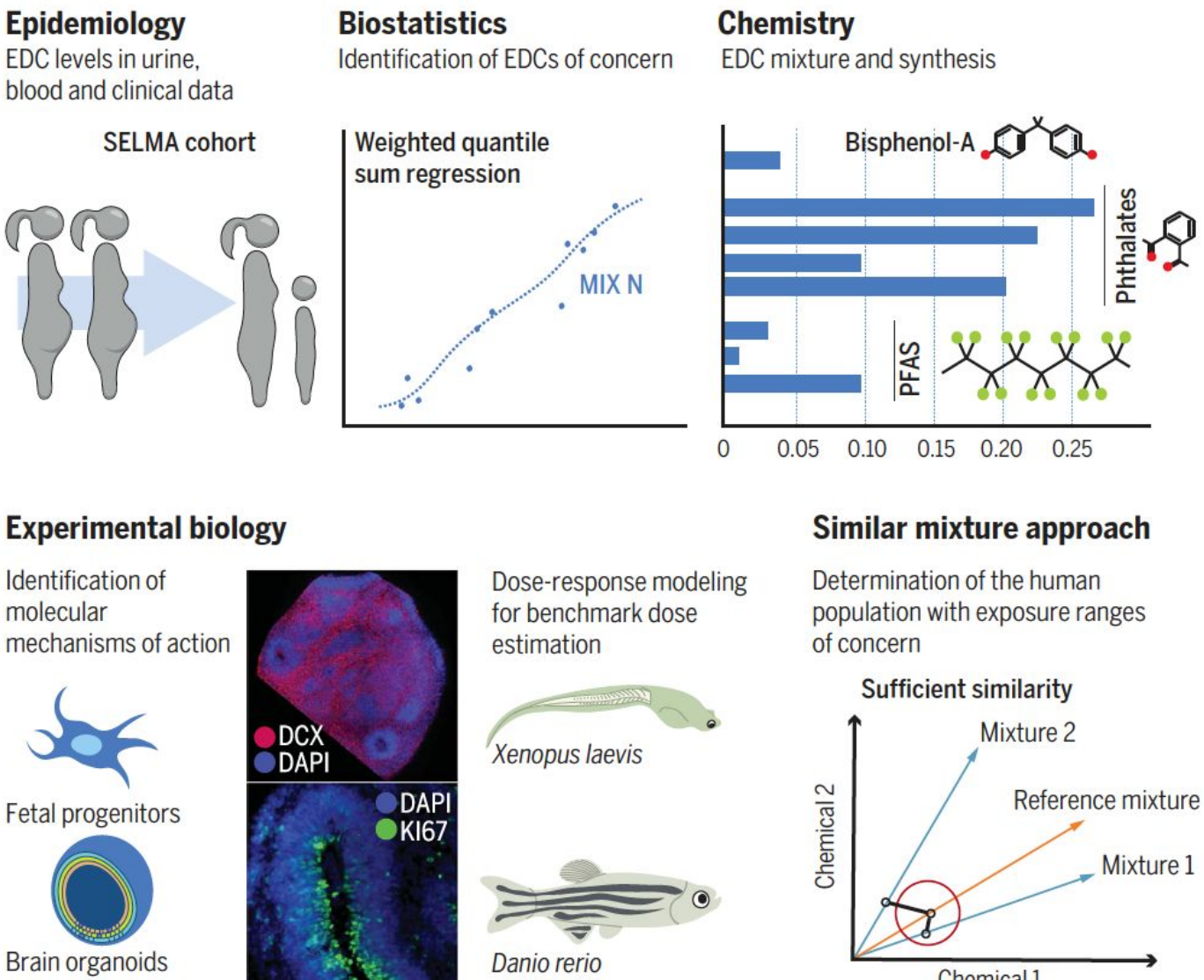


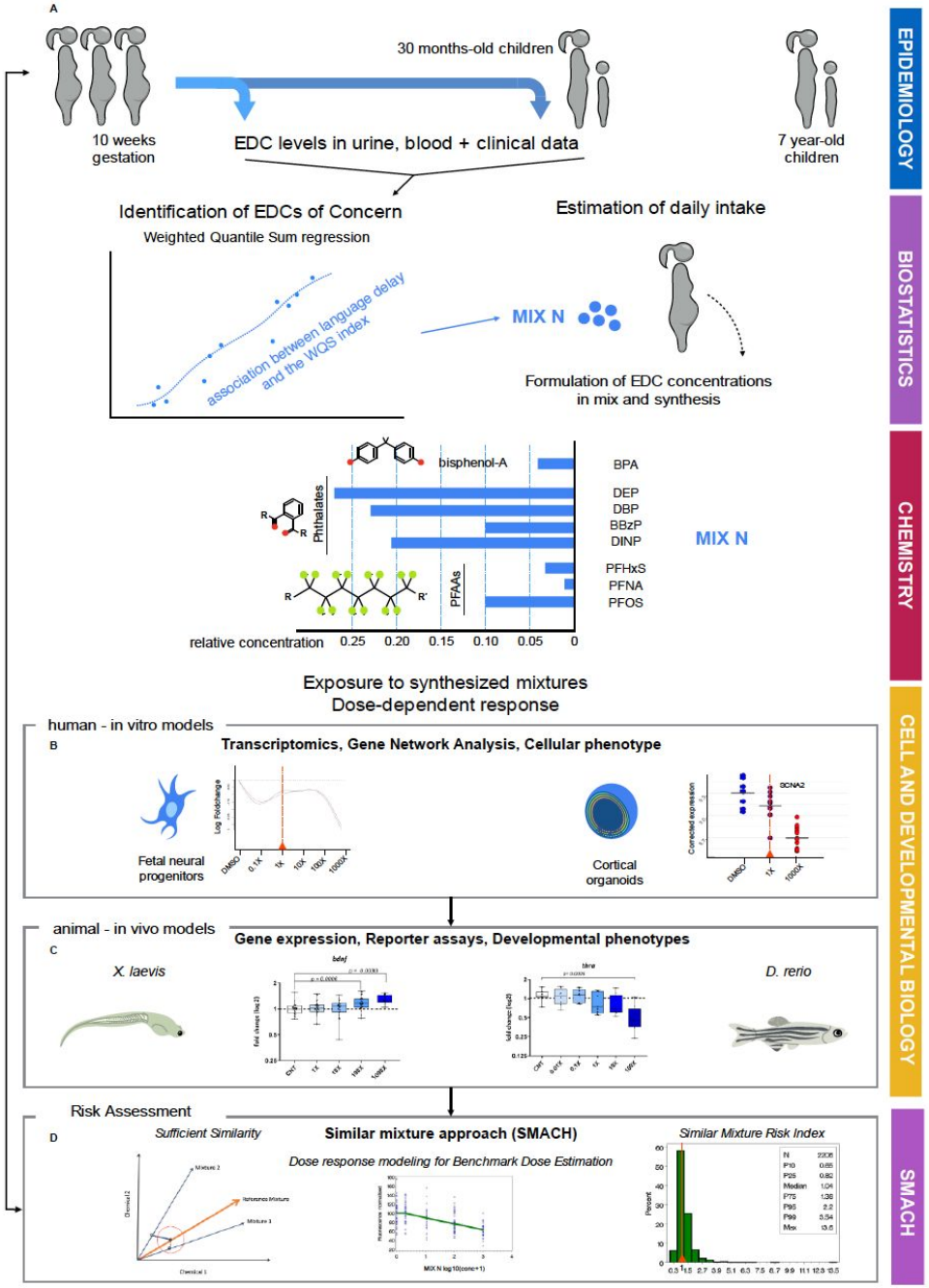
Diagram showing the integrative framework of the study. A mixture of EDCs was associated with adverse neurodevelopmental outcomes in the SELMA pregnancy cohort and was tested in human in vitro and in in vivo models to elucidate the molecular and functional impact of exposure. Experimental data were finally referred back to the cohort for risk assessment by a similar mixture approach. PFAS, perfluoroalkyl substance.

Conclusions

Exposure to a widespread chemical mixture of endocrine disruptors during pregnancy is associated with language delay in their children (reference mixture)

At human-relevant concentrations, the reference mixture disrupted hormone-regulated autism and intellectual disability genes in *human brain organoids* and altered behavioral responses in *in vivo models*

More than 90% of the pregnant women were determined to have *sufficiently similar exposures* to the reference mixture, and 54% of the pregnant women were found to be exposed to *experimentally defined levels of concerns* (SMRI>1)



One important question!

How relevant is the mixture of chemicals identified in a Swedish cohort – and tested experimentally for PODs – in other populations?

For this question we only need biomonitoring data for the compounds in the reference mixture

We used the US. NHANES data!

Table 2. Summary statistics (median, 95th percentile) for MIX N compounds for levels (µg/L) in urine (phthalates and BPA) and plasma (PFAS) as documented by NHANES (2011-16). Biomonitoring guidance values (µg/L) are provided for comparison. The minimum guidance value is displayed if more than one is given (https://biomonitoring.shinyapps.io/guidance/).

Compound	Biomonitoring guidance values	NHANES N=241,771,564[1]		NHANES Women (15-46 years old) N=57,748,243[1]	
		Median	95th percentile	Median	95th percentile
MEP	18,000 (age 6 and up)	30.3	531.4	31.0	504.8
MBP	200 (age 6 and up)	9.5	46.7	10.0	50.6
MBzP	3800 (age 6 and up)	4.1	32.5	4.8	41.7
MINP	390 (age 6 and up)	0.6	12.5	0.6	17.5
BPA	100 (children) 200 (adults)	1.2	7.8	1.2	7.3
PFHxS	-	1.3	5.4	0.7	3.2
PFNA	-	0.7	2.2	0.5	1.8
PFOS	5	5.5	19.4	3.3	10.4

[1] With sampling weights.

toxics

MDPI

Article

From cohort to cohort: a Similar Mixture Approach (SMACH) to evaluate exposures to a mixture leading to thyroid-mediated neurodevelopmental effects using NHANES data

Maria Sapounidou[1], Patrik L. Andersson[1], Michelle Leemans[2], Jean-Baptiste Fini[2], Barbara Demeneix[2], Joelle Ruegg[3], Carl-Gustaf Bornehag[4, 5], and Chris Gennings [5,*]

[1] Department of Chemistry, Umea University, SE-901 87 Umea, Sweden;

[2] UMR 7221, Phyma, CNRS–Muséum National d'Histoire Naturelle, Sorbonne Université, 75005 Paris, France;

[3] Department of Organismal Biology, Environmental Toxicology, Uppsala University, SE-752 36 Uppsala, Sweden;

[4] Faculty of Health, Science and Technology, Department of Health Sciences, Karlstad University, SE- 651 88 Karlstad, Sweden;

[5] Department of Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

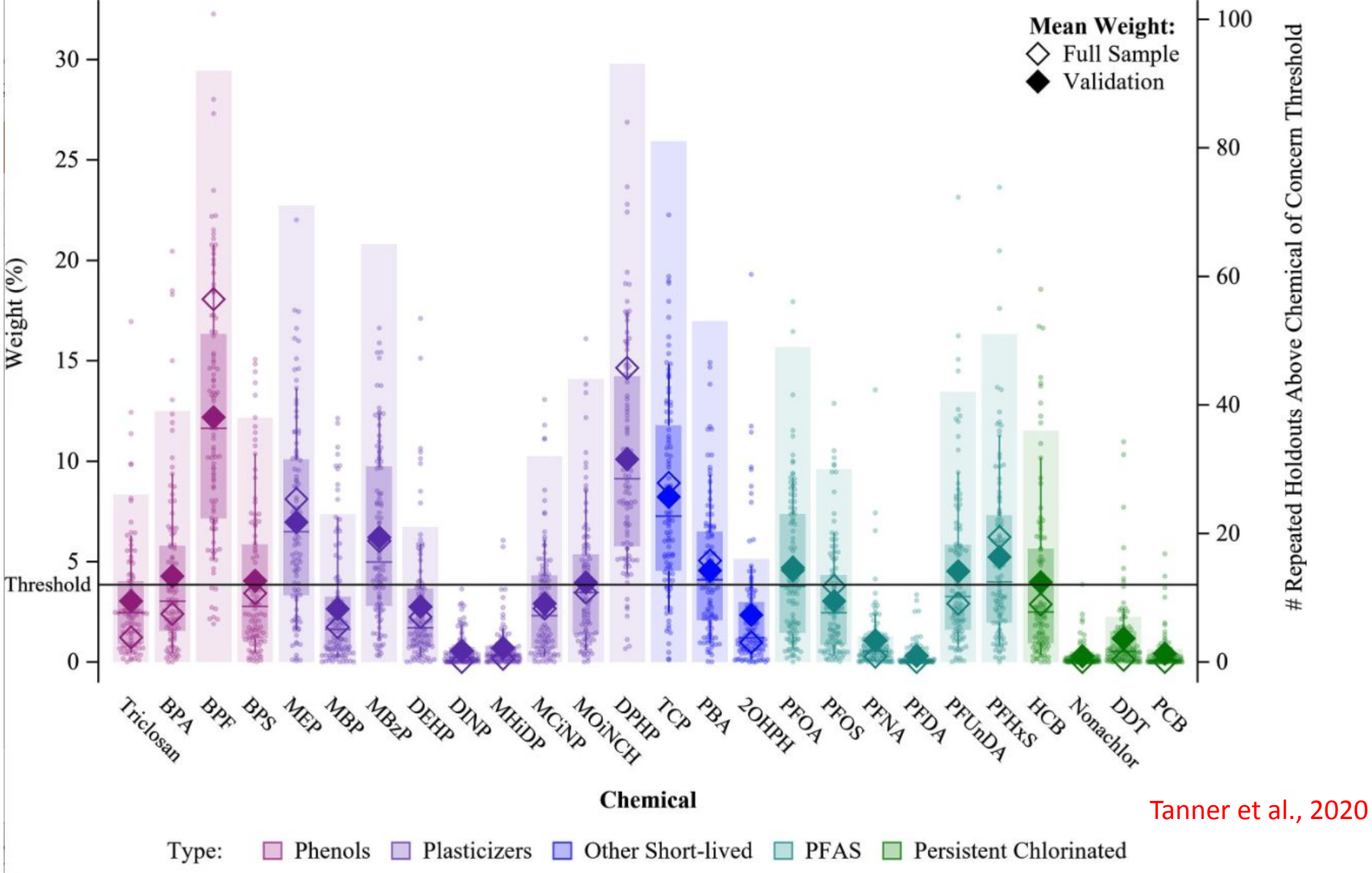
* Correspondence: chris.gennings@mssm.edu

Abstract: Prenatal exposure to a mixture (MIX N) of eight endocrine disrupting chemicals has been associated with language delay in children in a Swedish pregnancy cohort. A novel approach was proposed linking this epidemiological association with experimental evidence, where the effect of MIX N on thyroid hormone signaling was assessed using the *Xenopus* eleuthero-embryonic thyroid assay (XETA OECD TG248). From this experimental data, a point of departure (PoD) was derived based on OECD guidance. Our aim in the current study was to use updated toxicokinetic models to compare exposures of women of reproductive age in the US population to MIX N using a Similar Mixture Approach (SMACH). Based on our findings, 66% of women of reproductive age in the US (roughly 38 million women) had exposures sufficiently similar to MIX N. For this subset, a Similar Mixture Risk Index ($SMRI_{HI}$) was calculated comparing their exposures to the PoD. Women with $SMRI_{HI}>1$ represent 1.1 million women of reproductive age. Older women, Mexican American and Other/Multi race women were less likely to have high $SMRI_{HI}$ values compared to Non-Hispanic White women. These findings indicate that a reference mixture of chemicals identified in a Swedish cohort - and tested in an experimental model for establishment of (PoDs) - is of health relevance also in an US population.

Citation: To be added by editorial staff during production.

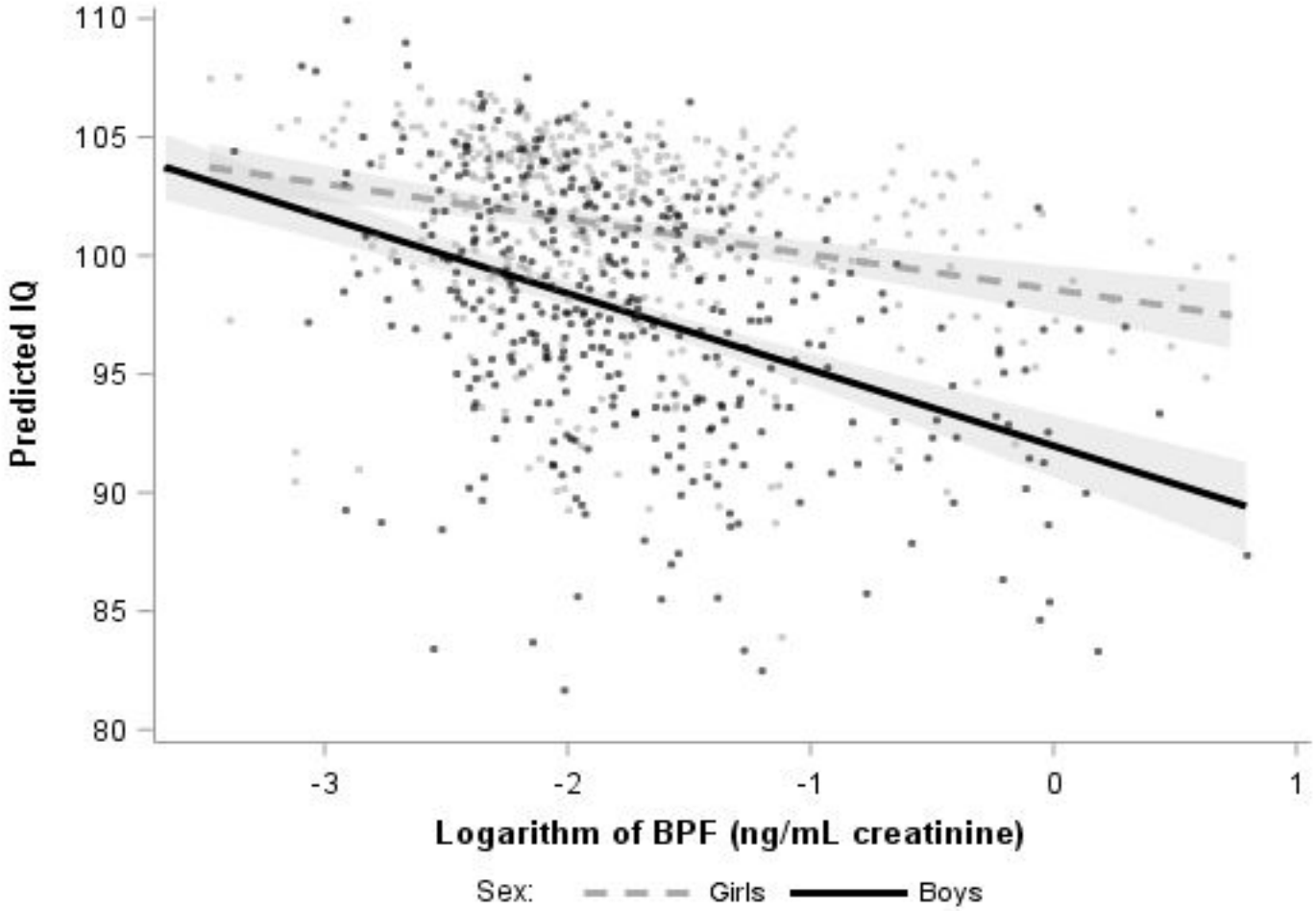
Abstract: Prenatal exposure to a mixture (MIX N) of eight endocrine disrupting chemicals has been associated with language delay in children in a Swedish pregnancy cohort. A novel approach was proposed linking this epidemiological association with experimental evidence, where the effect of MIX N on thyroid hormone signaling was assessed using the *Xenopus* eleuthero-embryonic thyroid assay (XETA OECD TG248). From this experimental data, a point of departure (PoD) was derived based on OECD guidance. Our aim in the current study was to use updated toxicokinetic models to compare exposures of women of reproductive age in the US population to MIX N using a Similar Mixture Approach (SMACH). Based on our findings, 66% of women of reproductive age in the US (roughly 38 million women) had exposures sufficiently similar to MIX N. For this subset, a Similar Mixture Risk Index ($SMRI_{HI}$) was calculated comparing their exposures to the PoD. Women with $SMRI_{HI}>1$ represent 1.1 million women of reproductive age. Older women, Mexican American and Other/Multi race women were less likely to have high $SMRI_{HI}$ values compared to Non-Hispanic White women. These findings indicate that a reference mixture of chemicals identified in a Swedish cohort - and tested in an experimental model for establishment of (PoDs) - is of health relevance also in an US population.

Thank you!



Tanner et al., 2020

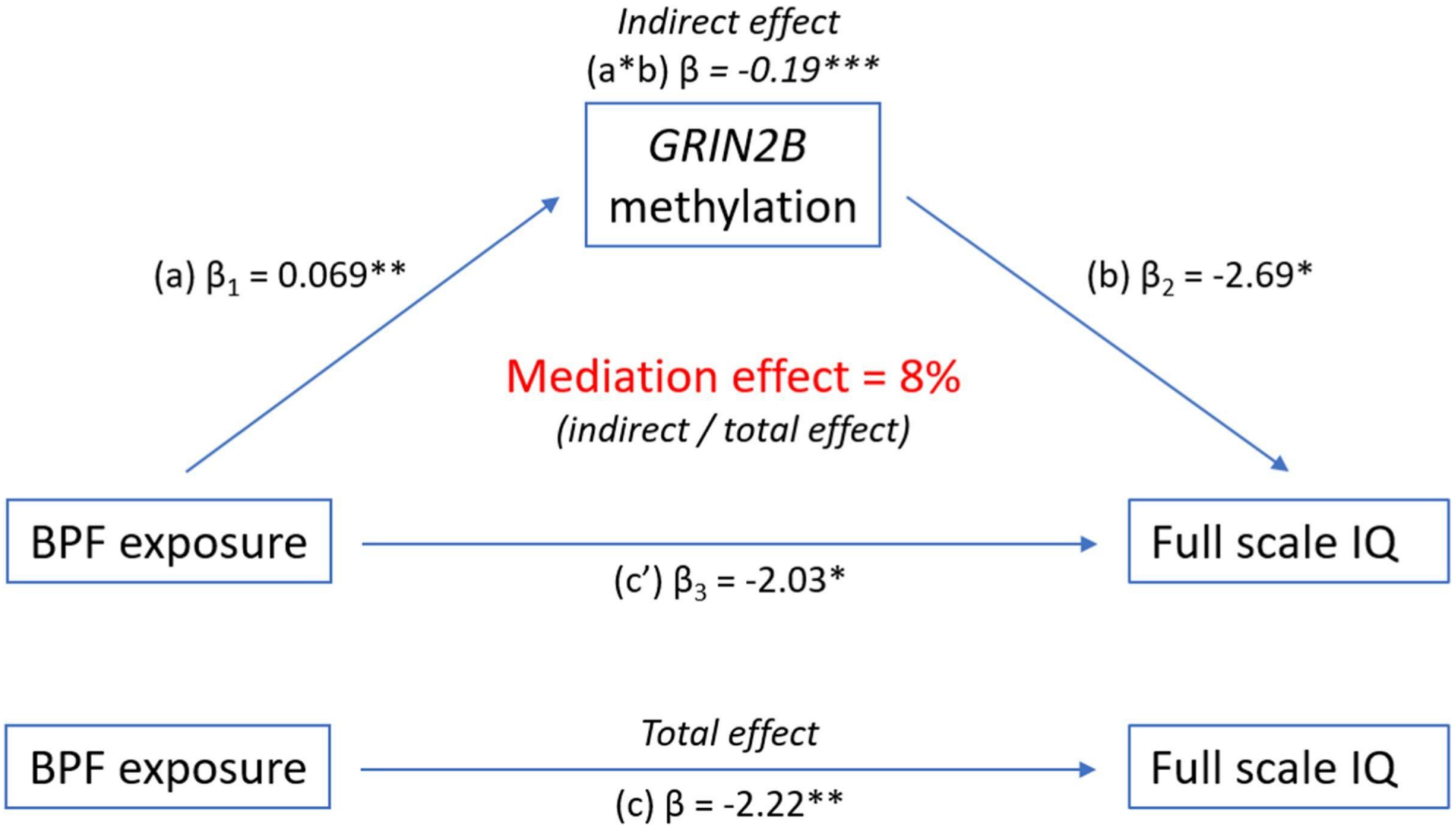
Prenatal exposure for BPF and IQ in children



Bornehag et al., *2021*

II BIOLOGICAL MECHANISMS

Biological mechanism, DNA methylation



Engdahl et al., 2021