Det Sundhedsvidenskabelige Fakultet

CALUX tests as human biomarker of Exposure/effect

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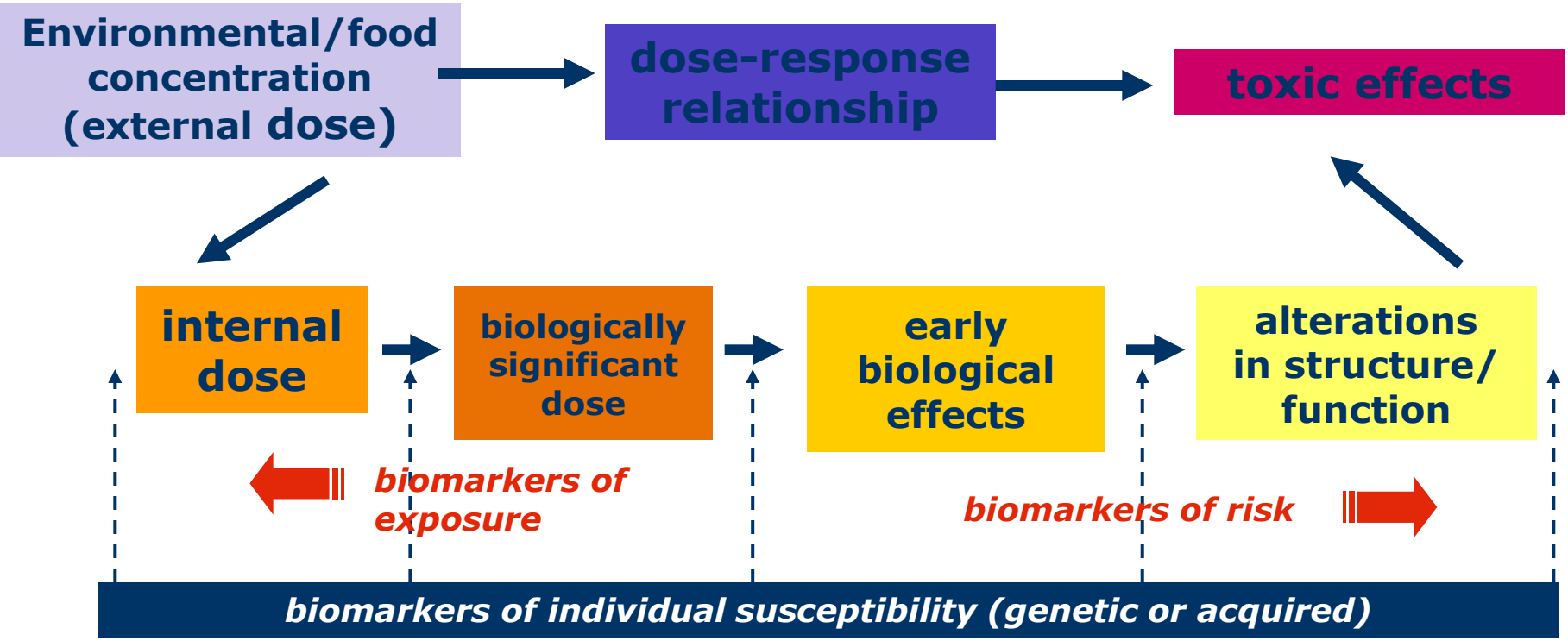
Biomarkers in environmental/food health research



Biomarkers of exposure (internal dose or biologically significant dose)

Biomarkers of risk (early biological effects or alterations in structure and function of cellular macromolecules)

Adverse effects from fetal exposures

Drugs
Alcohol
Diabetes
Nutrition
Air pollution
Medicine

Premature birth

Low birth weight

Fetal programming

Genetic damage
Reproductive damage

Neurodev
Diabetes
etc...

Fetus & Infant vulnerability

Fetuses & infants → especially vulnerable to the effects of environmental risk factors due to :

a) critical windows of vulnerability that occur during the rapid growth and development of organs and systems

b) immaturities in metabolism

c) greater intake and absorption of noxious agents in children relative to their body weight

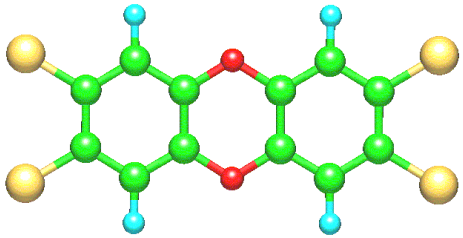
Dioxins and dioxin-like compounds

POLYCHLORINATED

- dibenzodioxins (PCDDs) 75 congeners, 7 toxic
- dibenzofurans (PCDFs) 135 congeners, 10 toxic
- biphenyls (PCBs) 209 congeners, 12 toxic (dl-PCBs)

The most toxic

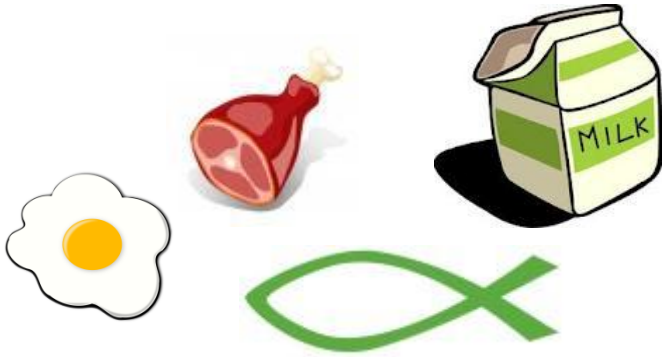
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

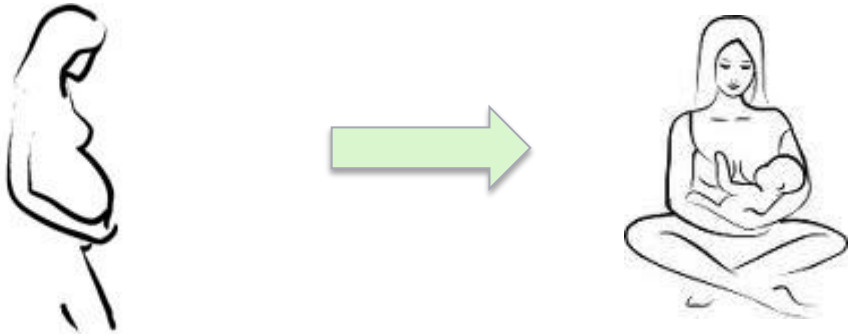


TCDD has been classified as a known human carcinogen (IARC 1997)

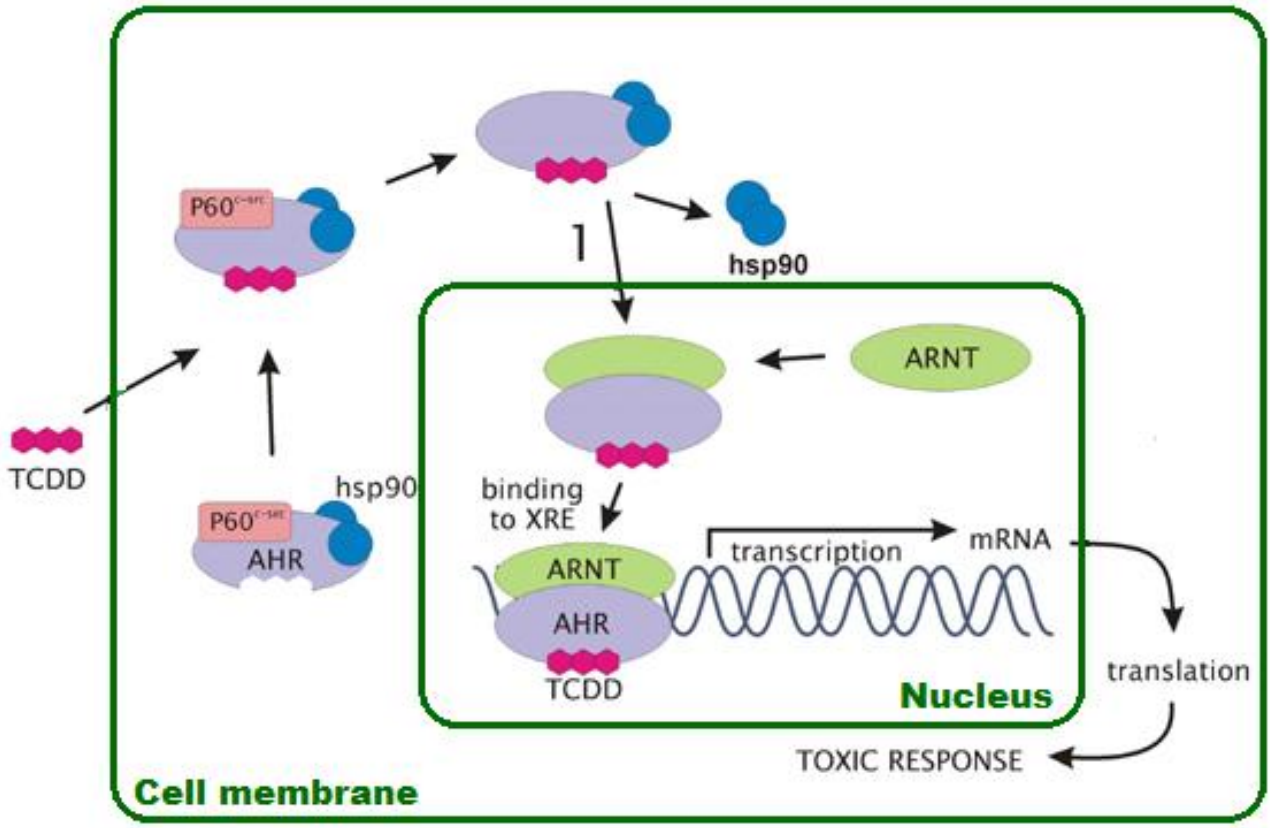
Dioxin & dioxin like compounds

- Long half-lives : ≈ 7 yrs TCDD, variation among and within individuals, accumulation
- TCDD has been shown to disrupt multiple endocrine pathways
- 95% of exposure through diet





Dioxin toxicity-AH receptor



Picture adapted http://www.helsinki.fi/science/denteny/dioxin.html

Maternal exposure to dioxins can affect fetal growth and infant development

- Altered infant sex ratio in the offspring of the residents of Seveso, Italy
- Increase in the incidence of birth defects in the offspring of Vietnamese and U.S. veterans exposed to Agent Orange
- Low birth weight in Yusho and Yu-cheng children

Dioxins and birth outcomes: Low-level exposure

Low-level exposure to dioxins and dioxin-like compounds during pregnancy →adverse birth outcomes in infants

Studies	Conclusion
Konishi K. et al, 2009	**lower birth weight, only in boys**
Tawara K. et al, 2009	in breast milk → inversely correlated to **length** & **head circumference**
Sonneborn et al., 2008	**lower birth weight, only in boys**
Nishijo M. et al, 2008	smaller **head circumference /** no associations with height, weight and chest circumference
Sagiv et al., 2007	lower **birth weight , length & head circumference**
Lin CM et al, 2006	**Ambient dioxin** exposure → might pose small effects on **gestational age**

Dioxins and birth outcomes: Low-level exposure

However, these associations have not been demonstrated in other studies

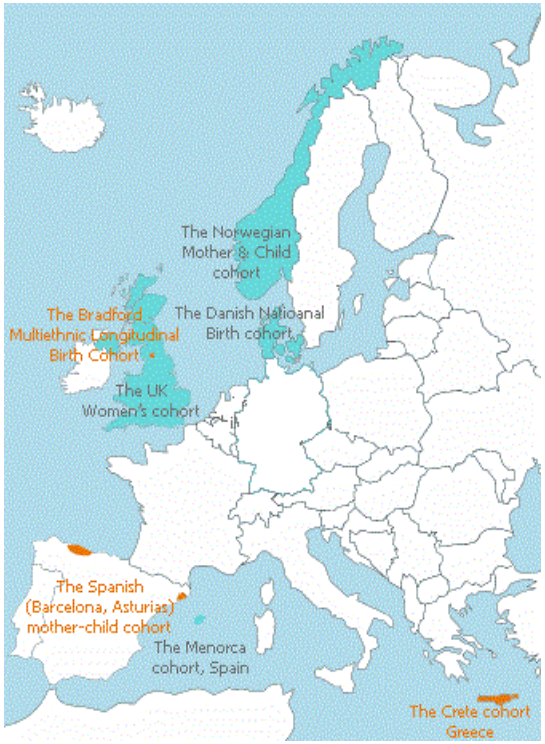
Studies	Conclusion
Weisskopf et al., 2005	no association **with birth weight**
Longnecker et al., 2005	no association **with birth weight, gestational age and prematurity**
Rogan et al., 1986	no association **with birth weight & head circumference**

Thus, the controversy over whether maternal exposure to polychlorinated organic compounds is associated with decreased birth weight in humans continues

Aim

Evaluate the association between in-utero exposure to dioxins and dioxin like compounds and birth outcomes in the newborns of the NewGeneris project

Dioxins and birth outcomes



- Population: Newborns and Genotoxic exposure risks (NEWGENERIS) project mother-child pairs

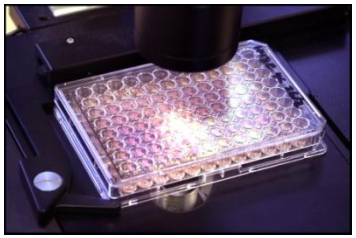
NewGeneris
Newborns and Genotoxic exposure risks

- 5 European mother-child birth cohorts: Greece (Rhea), Spain (HMAR), UK (BiB), Norway (Moba) and Denmark (Danish samples for Newgeneris)

Information on birth outcomes such as birth weight, length and head circumference were gathered from clinical records by each cohort

Exposure assessment-DR CALUX ®

- Maternal and cord blood samples collected at delivery
- Dioxin-like activity→ measured in plasma→ DR-CALUX ® bioassay
- Reporter gene assay→ detects all compounds that activate the AhR
- Rat hepatoma cell line containing the luciferase gene under transcriptional control of AhR



- Luciferase is produced *in vitro* after exposure
- + luciferine for the luciferase enzyme, light is emitted

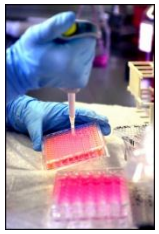
DR CALUX ® vs HRGC/HRMS→ less expensive, quicker, biologically relevant, total toxic equivalence ≠ individual congeners

DR CALUX procedure

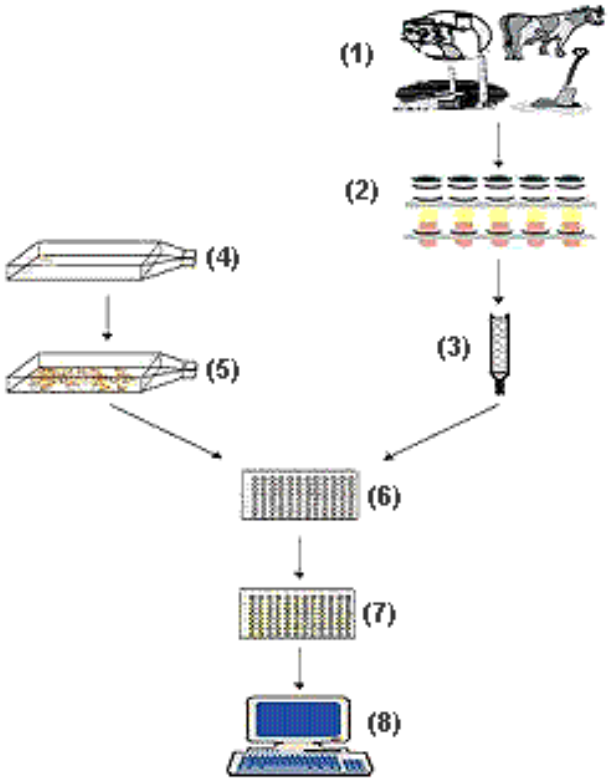
1,2,3 : Sample collection , clean

4,5: Cell culture,6: 96 well plates

7: Exposure



8,9: Lysis, luciferine, quantification



Luminance is calibrated with respect to TCDD in TEQs

Results : pg CALUX-TEQ /g lipid or pg CALUX-TEQ /ml of plasma

Birth outcomes

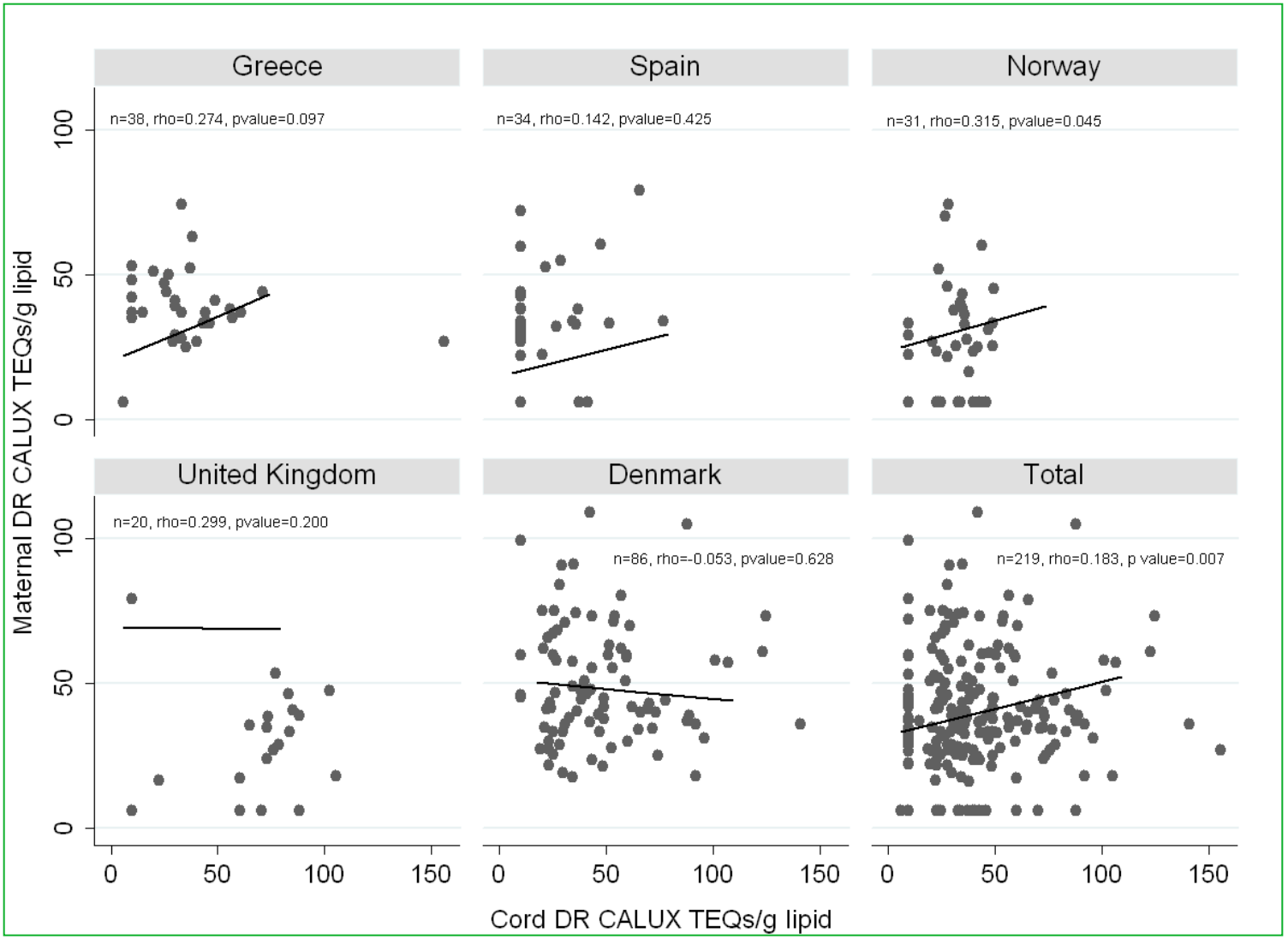
	With fetal DR CALUX pg TEQ/g lipid n=269					With maternal DR CALUX pg TEQ/g lipid n=791				
			Percentile					Percentile		
Outcome variables	n	Mean±SD	25th	50th	75th	n	Mean±SD	25th	50th	75th
Birth weight (g)	269	3476±450	3170	3452	3782	791	3447±456	3130	3430	3750
Head circumference (cm)	211	35.15±1.51	34	35	36	697	34.89±1.5	34	35	36
Gestational age (weeks)	269	39.07±1.44	38	39	40	791	39.07±1.43	38	39	40

Exposure

Maternal and fetal dioxin-like activity, overall and in boys and girls separately.

	Maternal pg TEQ/g lipid		Fetal pg TEQ/g lipid	
	n	Median (IQR)	n	Median (IQR)
All	791	38.3(29.3)	269	34.2(28.9)
Boys	392	38.9(29.0)	128	33.0(34.6)
Girls	399	38.0(29.5)	141	35.0(26.9)

Correlations between maternal and fetal dioxin-like activity, overall and by country.



- **The estimated change in birth weight per 10 pg fetal CALUX-TEQ/g lipid was -5.9 g (95%CI: -27.8 to 16.0) although this decrease was not statistically significant. A lower birth weight was observed comparing medium (β:-57.3g, -176.0 to 61.3) and high (β:-82.0g, -215.6 to 51.6) to low fetal DR CALUX tertiles, after adjusting for potential confounders.**
- **An association with a shorter gestational age of approximately half a week was observed when comparing the highest to the lowest fetal DR CALUX tertile (β: -0.44 weeks, -0.84 to -0.05) and remained significant only for boys (β: -0.77 weeks, -1.33 to -0.22).**

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Dioxin-like exposures and effects on estrogenic and androgenic exposures and micronuclei frequency in mother–newborn pairs

Marie Pedersen [a,*], Thorhallur I. Halldorsson [b,c], Line Mathiesen [a], Tina Mose [a], Abraham Brouwer [d], Morten Hedegaard [e], Steffen Loft [a], Jos C.S. Kleinjans [f], Harrie Besselink [d], Lisbeth E. Knudsen [a]

Dioxin-like exposures and Spearman Rank Correlations between fetal and maternal levels

Dioxin-responsive (DR) Chemical-activated luciferase expression (CALUX)

	Cord blood Median (range)	**DR CALUX (pg TEQ/g lipid)** **33 (15–141)**
Maternal Median (range)		**DR (pg TEQ/mL)** **0.08 (0.01–0.38)** N=71 (52>LOD)
DR CALUX (pg TEQ/g lipid) **37 (6–118)**		**0.3**
DR (pg TEQ/mL) **0.33 (0.03–1.18)** * 98 (97>LOD)		

** P<0.05 from Wilcoxon Signed Ranks Test (Two-tailed)*

● *p<0.05*

Human placenta *ex vivo* perfusions

Placenta collected immediately after birth and informed consent

Intact tissue mimics physiological *in vivo* conditions

Placental transfer, accumulation, metabolism, acute toxicity etc.

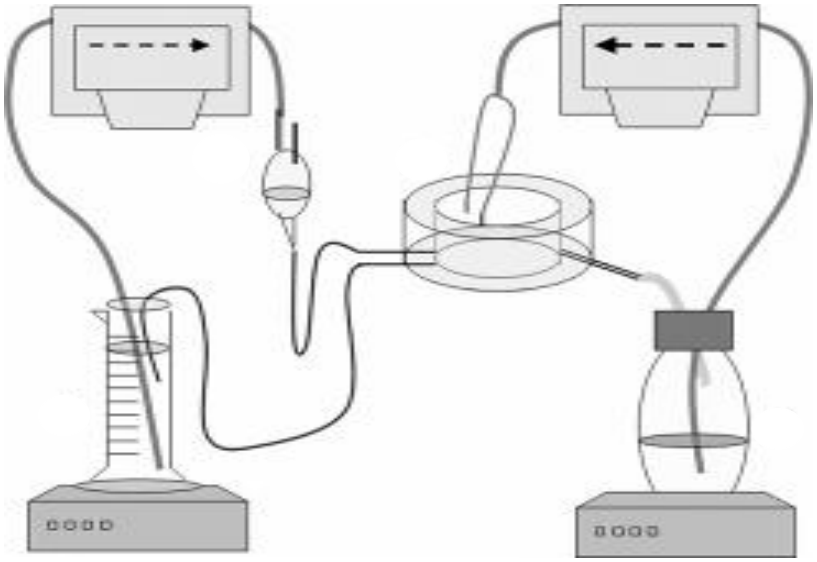
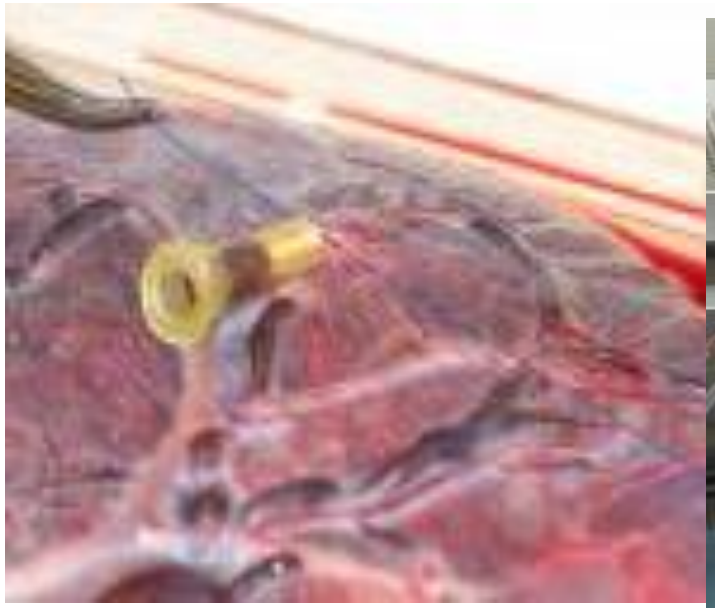


Figure from (Mathiesen et al. 2010)



Photos from Tina Mose

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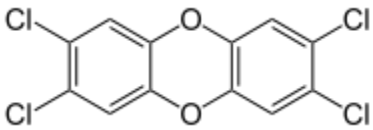

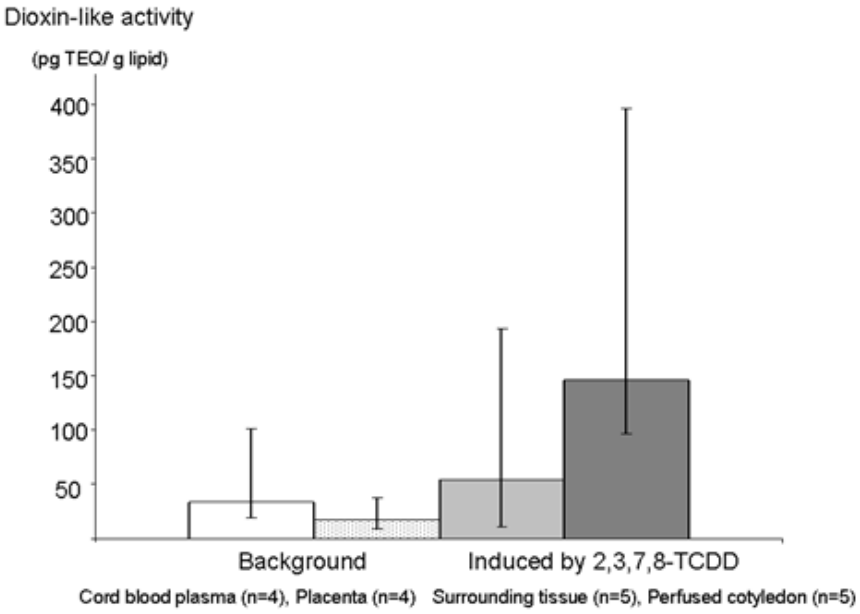

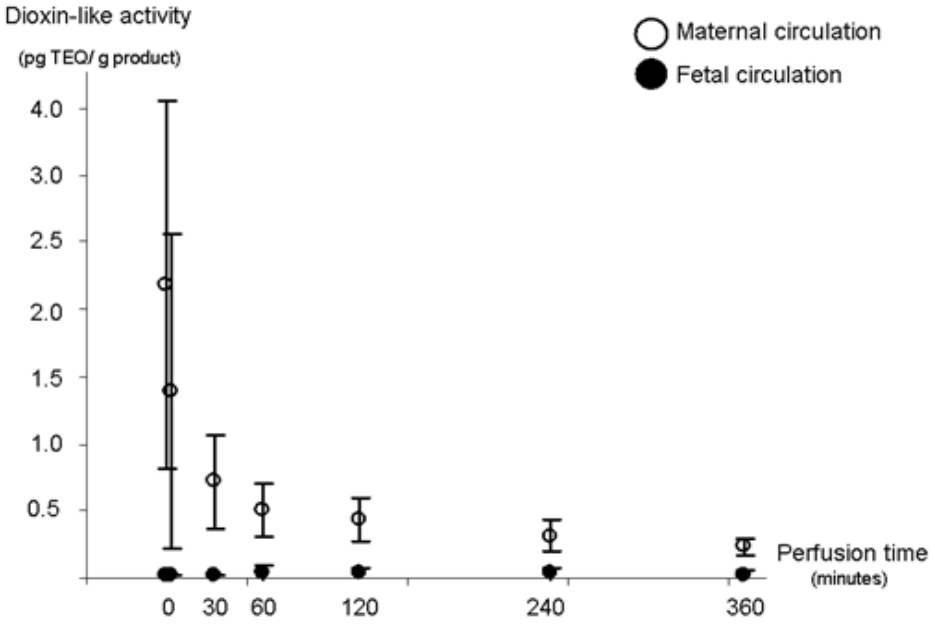

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Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

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

Community address: www.elsevier.com/locate/mutres

Maternal diet and dioxin-like activity, bulky DNA adducts and micronuclei in mother–newborns

Marie Pedersen[a,*], Thorhallur Ingi Halldorsson[b,c], Herman Autrup[d], Abraham Brouwer[e], Harrie Besselink[e], Steffen Loft[a], Lisbeth E. Knudsen[a]

	Dioxin-like plasma activity[a]	
	Mothers ($n = 92$)	Newborns ($n = 67$)
Median (95%CI)	38 (19–78)	33 (17–107)
Regression estimates (β) and the 95% CI for categorical parameters and hig		
Blackness[d]	2.4 (−6.4 to 11.3)	12.0 (−4.2 to 28.1)
Supplements[e]	−8.8 (−21.7 to 4.1)	18.7 (−5.0 to 42.4)
Fruits	−1.0 (−13.2 to 11.2)	13.4 (−9.3 to 36.2)
Vegetables	6.1 (−6.0 to 18.3)	−16.2 (−38.1 to 5.7)
Fish	−1.6 (−13.7 to 10.6)	−4.3 (−26.0 to 17.5)
Fish oil[f]	6.5 (−5.1 to 18.0)	−2.6 (−31.3 to 26.1)
Red meats	−4.6 (−16.8 to 7.6)	−11.3 (−33.9 to 11.2)
Poultry	2.6 (−9.4 to 14.7)	−3.6 (−25.1 to 17.9)
Dairy products	6.8 (−5.1 to 18.6)	12.4 (−9.3 to 34.2)
Eggs	−8.3 (−20.4 to 3.7)	−2.0 (−25.6 to 21.6)
Bread and cereals	−4.0 (−16.2 to 8.1)	8.5 (−13.9 to 30.9)
Alcohol[g]	2.7 (−5.9 to 11.3)	7.0 (−8.9 to 22.8)
Coffee[g]	−9.1 (−17.7 to −0.4)*	6.8 (−9.0 to 22.6)
Fats	−7.9 (−20.0 to 4.2)	11.5 (−9.3 to 32.3)
Proteins	1.8 (−10.3 to 13.9)	−9.1 (−14.6 to 32.7)
Carbohydrates	6.6 (−5.6 to 18.7)	12.1 (−11.0 to 35.2)
Fibres	11.9 (−0.1 to 23.8)	−2.9 (−25.5 to 19.8)
Energy	3.5 (−8.6 to 15.6)	16.5 (−6.1 to 39.0)

[a] Linear regression model with dioxin-like plasma activity per gram lipid (pg CALUX®-TEQ/g lipid) as dependent variable.

Conclusions

- Biomarkers integrated in carefully designed and conducted epidemiological studies, enable studies of exposures and effects in utero
- Biomarkers of genotoxic exposure and effects in cord blood are indicators of the actual fetal exposure
- Cord blood can be obtained in a non-invasive way upon informed consent from tissue otherwise discarded
- Study material collected and prepared can be used for further research on the potentially disproportionate impact that intrauterine exposure to environmental pollutants might have on the health of exposed children

The Phd-project of Thit Aarøe Mørck-

- Supplement to a large biomonitoring project in EU
  - COPHES/DEMOCOPHES
  - School children and their mothers

- Opened possibilities to measure environmental chemicals in children, which was not measured in Denmark before and to compare with other European countries

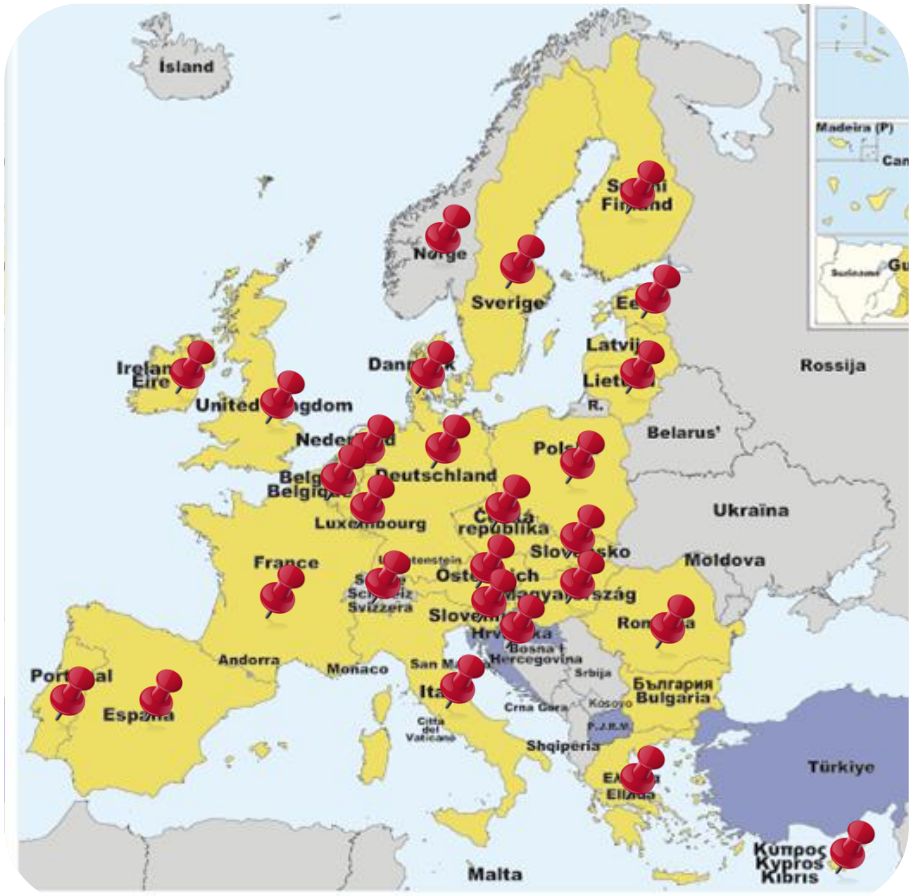
The EU project: COPHES-DEMOCOPHES

- COPHES:

  Consortium to Perform Human biomonitoring on a European Scale

  - 35 partners in 27 countries

The main objective of this coordination action is to:
develop a functional framework that contributes to the definition, organisation and management of a coherent approach towards human biomonitoring (HBM) in Europe.



DEMOCOPHES

DEMOnstration of a study to COordinate and Perform Human biomonitoring on a European Scale

- Pilot study which involves 17 european countries
- Running from: 1.9.1010 til 30.11.2012
- Involves young school children and their mothers
- Urine and hair samples
  - Cadmiun, Cotinine, Phthalates and Bisphenol A (urine)
  - Mercury (hair)
- Questionnaire (mothers)
- Sampling in september-november 2011

Sampling design (DEMOCOPHES):

Target group	Children aged 6 to 11 years
Add. group	Accompanying mothers < 45 years of age
Sample size	120 children and 120 mothers
Region	2 sampling locations (upper and lower end of the degree of urbanisation)

- 60 children from 6-11 in each area
- 10 children per year of age and area.
- 5 boys and 5 girls

Substances – DEMOCOPHES in DK

Extended scenario including blood samples:

- cadmium, phthalates, cotinine in urine (basic)
- mercury in hair (basic)
- BPA in urine (7 countries) – phd
- parabenes, triclosan and (Benzophenon-3) in urine (DK extended)
- PCBs, PFCs and BFRs in blood samples (DK extended scenario)
- Micronucleus and dioxin-like activity in blood – phd

My PhD..

Bisphenol A (BPA)

- Polycarbonat plastic, cans, thermal paper
  - In DK (and Canada) BPA is abandoned in products directed at young children (0-3 years of age)
    - (babybottles, containers for baby food and milk etc.)

- Measurable in the urine (in >90%, Calafat et al. 2008)
    - Highest among young children

Bisphenol A

BPA is an endocrine disrupter

- Binds to and activates the estrogen receptor (ER), androgen (AR) and thyroid receptor (TR)
  (Wetherill et al. 2007)

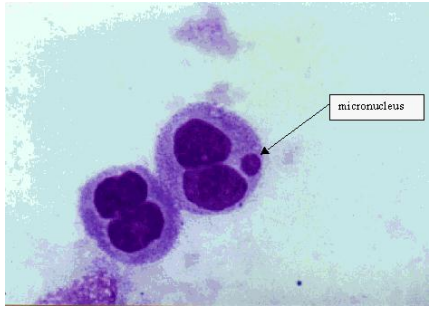
- Associations have been found between BPA og PolyCystic Ovarian Syndrome (PCOS) and lifestyle diseases (Takeuchi et al. 2004; Melzer et al. 2010)

- Animal studies show
  - early puberty
  - disturbance of development of reproductive organs
  - change in levels of sex-related hormones
  - changes en mental development

  (Howdeshell et al. 1999; Vandenberg 2007)

My PhD..

Micronucleus (MN)



- Biomarker of cytogenetic effekts
- Association between high exposure to trafic and MN frequency
  (Pedersen et al., 2009)

- Increased risk of cancer development with increased MN frequency
  (Bonassi et al. 2009)
- Induction of MN by BPA in mussels
  (Barsiene et al. 2006)

My PhD..

Dioxin-like activity

- Biomarker of hormonal effects
  - Dioxins have an endocrine disrupting potential
    - Disturbance of hormone receptor functions and inhibition of the production and metabolism of estrogen (Long et al. 2007).
  - Primarily via the aryl hydrocarbon receptor (AhR).
    - Transcription factor mediating and regulating gene expression of important genes in xenobiotic metabolism and immune supression and female reproduction.

Dioxin-like activity

- Dixon like activity is measured in serum
  - AhR activity in tranfected cells (AhR-luciferase reporter gene)
  - Results indicate total disturbance by dioxin and dioxin-like substances

- BPA has been shown to inhibit the AhR function (Bonefeldt-Jørgensen et al. 2007)

QUESTIONNAIRE (DEMOCOPHES)

The basic questionnaire consists of 6 modules:

1. Residential environment and residence
2. Nutrition
3. Smoking behaviour
4. Exposure-relevant behaviour (non-food)
5. Occupation of the mother
6. Socio-demography

Additional questionnaire in DK

1. Exposure relevant behaviour related to substances in the extended scenario
2. Interview with children on illness and view on illness perception of pain and self-rated health (Phd by Janne F. Jensen)

The recruitment and collection in Denmark

Urban vs Rural: < 150 inhabitants/km^2 → rural
(NUTS classification, OECD)

- Urban area – Gentofte: Gentofte skole (2733 inhabitants/km$^{2)}$
- Rural Area – Dåstrup: Dåstrup skole and Peder Syv skole (139 inhabitants/km^2)
- Substance analysis:
  - University of Southern Denmark: Cotinine, Cadmium, Mercury, and several POPs
  - Rigshospitalet, vækst og reprodruktion: Phthalater, BPA. Triclosan, parabenes
  - Here: MN
  - University of Århus, Eva Bonefeldt-Jørgensen: Dioxin-like activity

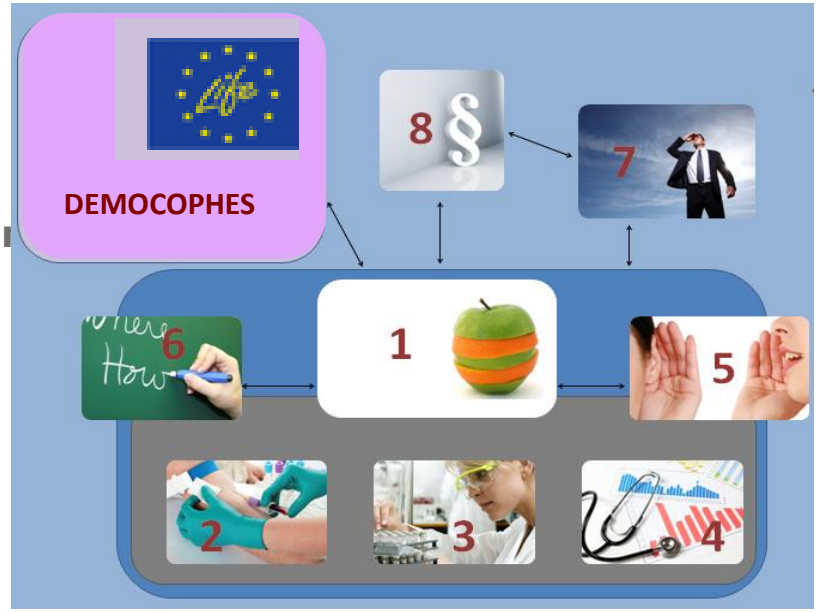
Det Sundhedsvidenskabelige Fakultet

http://www.eu-hbm.info/cophes

COPHES
Consortium to Perform Human Biomonitoring on a European Scale

FP7 funded by the European Commissio

→Provides framework
→Provides guidance
→Analyses results on a EU level
→Recommendations & Conclusions



DEMOCOPHES
Demonstration of a study to coordinate and perform human biomonitoring on a European Scale

Life+, funded by European Commission and Member States

→children and their mothers
→at least 3600 study subjects
→cadmium, phthalates, cotinine in urine
→mercury in hair
→(bisphenol A in urine)

Harmonised approach for HBM in Europe

Acknowledgements

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NewGeneris
Jos Kleinjans et al
Mischeline Kirch-Volders et al
Kirsi Vähänkangas et al
Päivi Myllynen et al
Harrie Besselink et al
Margareta Törnquist et al

NanoTEST
Maria Dusinska et al
Margaret Saunders et al

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