Endocrine-Disrupting Chemicals: how to track the fear factors?

Dr. D. Gruson
Pharm. Biol. - EurClinChem
Division of Endocrine Biology
UCL St-Luc

Agenda

1) **Background**

2) **How to evidence a potential endocrine disrupting action?**

3) **How to detect and monitor EDCs?**

4) **Which perspectives for exposure indicators?**

5) **Take home messages**

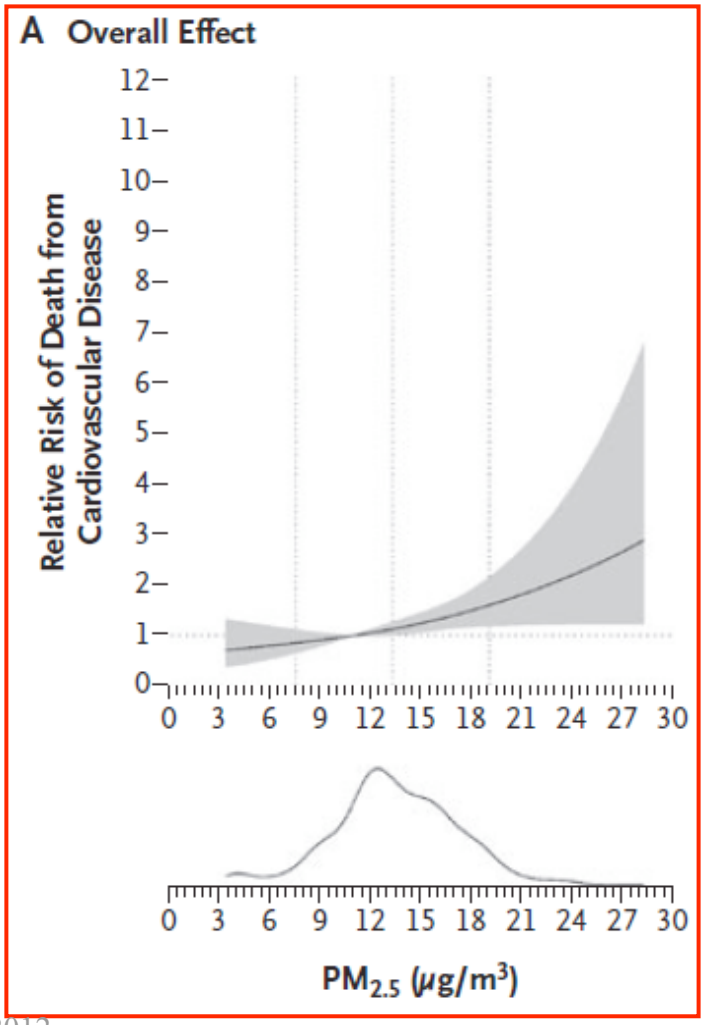
Background (1)

Environmental Biology, Toxicology, Endocrinology…

EDITORIALS

Cardiovascular Risks from Fine Particulate Air Pollution

Douglas W. Dockery, Sc.D., and Peter H. Stone, M.D.



Background (2)

Endocrine-Disrupting Chemicals (EDCs)

« An exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction and developmental process »

Health / Social / Economical Burden

OECD
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

THE ENDOCRINE SOCIETY®

The International Programme on Chemical Safety (IPCS)
WHO ILO UNEP

Background (3)

Background (5)

Synthetic origin

Hormones

17α-Ethinylestradiol

Diethylstilbesterol

17β-Trenbolone

Insecticides

DDT

Dieldrin

Endosulfan

Lindane

Industrial Chemicals

Phtalates

Bisphenol A

P-Nonylphenol

PCBs

Herbicides

Atrazine

Simazine

Methoxychlor

Personnal care products…

Biological origin

Hormones

17β-Estradiol

Estriol

Estrone

Progesterone

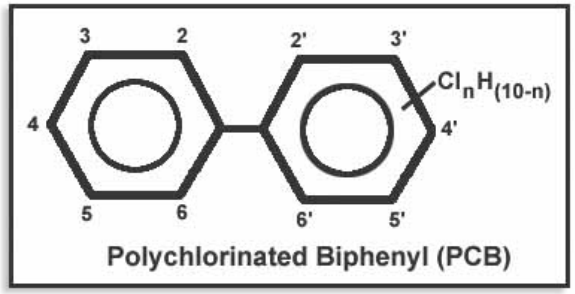
Testosterone

Phytoestrogens

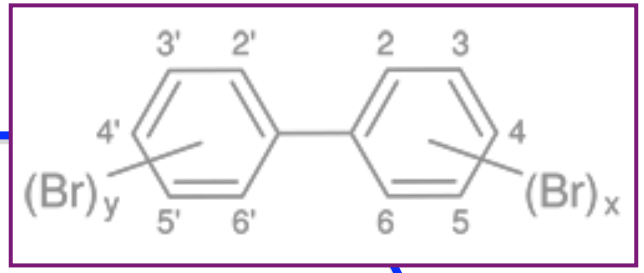
Sesquiterpenes

Phytosterols

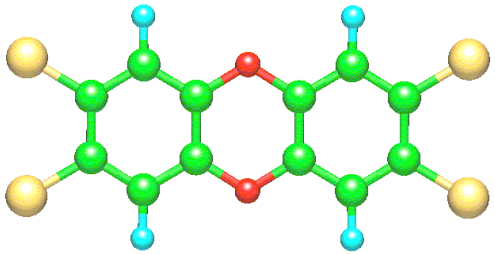
Background (6)

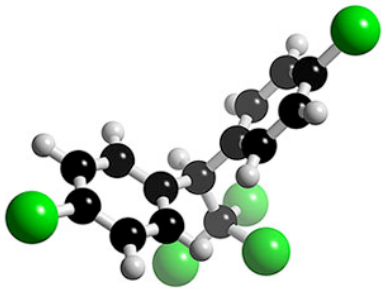


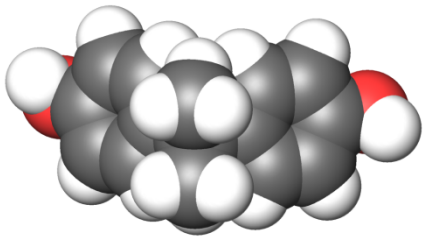
Polychlorinated Biphenyl (PCB)



A large diversity of chemical and biological properties



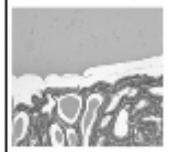
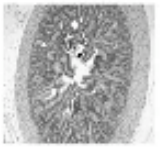
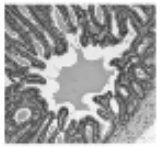


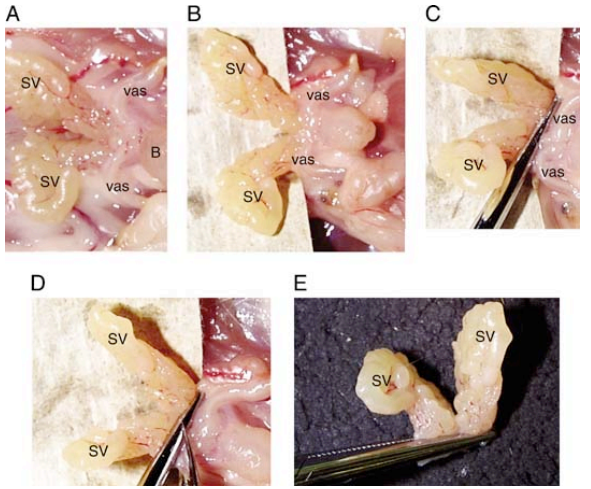


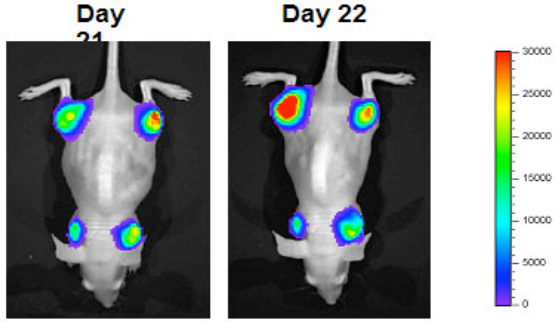
How to evidence a potential endocrine disrupting action?

« *In Vivo* »

- **Uterotrophic screen**
- **Hersberger screen**
- **Enhanced subacute test (TG 407)**
- **Rodent pubertal female screen**
- **Rodent pubertal male screen**
- **Fish reproduction screen**
- **Frog metamorphosis screen**

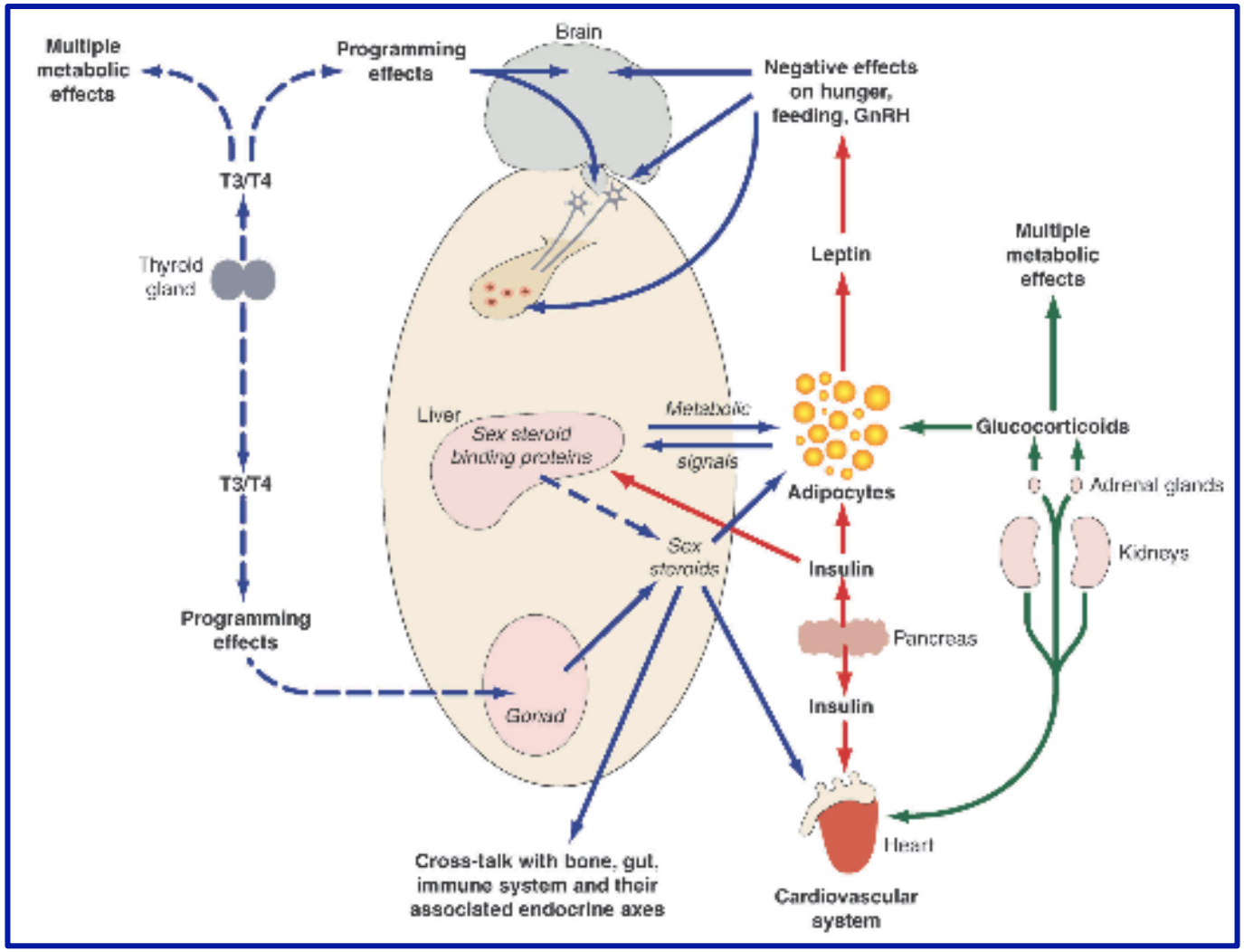
Group	Control	TP	TP + 3mg/kg/d FM	TP +160mg/kg/d DDE	TP + 100mg/kg/d LIN
A. SEMINAL VESICLES 100x magnification					
	Normal morphology; immature glands (minimal growth), little secretion	Induced glandular growth, increased secretion	Similar to controls	Similar to controls	Similar to TP





How to evidence a potential endocrine disrupting action?

« *Cross-Talks* »



How to evidence a potential endocrine disrupting action?

« *In Vitro* »

Competitive Estrogen Receptor Binding Assays

Estrogen Receptor Activation and Cellular Proliferation Assays

Estrogen and androgen receptor reporter gene screens

Vitellogenin assay

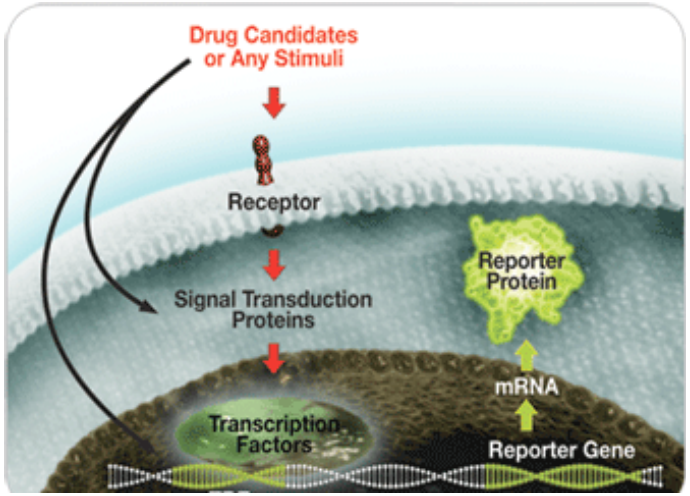


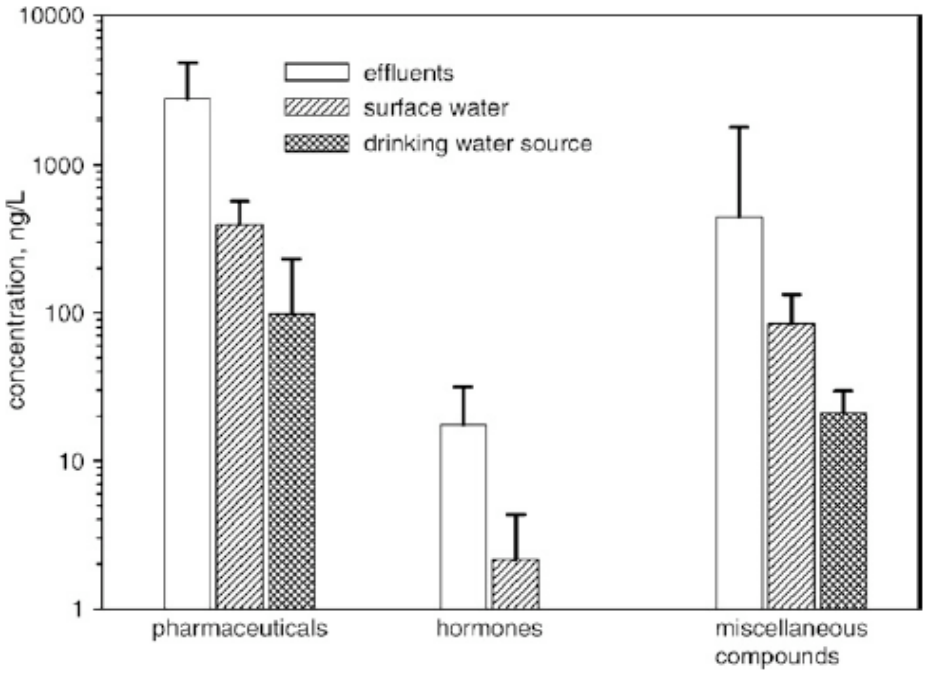
TABLE IV. Estrogenic equivalents (EEQs) of EDCs compared to 17β-estradiol using E-screen cell proliferation assay

Compound	EEQ[a]	Reference
Diethyl stilbestrol	10	Soto et al. (1992)
17β-estradiol	1	Soto et al. (1992)
Genistein	0.00020	Fang et al. (2000) and Koerner et al. (2001)[b]
4-*tert*-Octylphenol	0.000065	Fang et al. (2000) and Koerner et al. (2001)[b]
Nonylphenol	0.000003	Soto et al. (1992)
o,p′-DDT	0.000001	Soto et al. (1992)

[a] Quantification of estrogenicity: EEQ (estrogen equivalent) = $EC_{50\,ESTRADIOL}/EC_{50\,SAMPLE}$.
[b] Mean of values reported in Fang et al. (2000) and Koerner et al. (2001).

How to detect and monitor EDCs?

How to detect and monitor EDCs?



ng/L

ppb / ppt

TABLE III. Concentrations of estrogens (ng/L) in sewage effluent in Europe, North America, and Australia

Treatment type	County of origin	17 β-Estradiol	17 α-Ethinyl estradiol	Estrone
Activated sludge system[a]	Netherlands	0.09	< lor	4.5
Activated sludge system[a]	Netherlands	< lor	< lor	0.4
Clarification and aeration[a]	Germany	< lor	1	9
Clarification and aeration[a]	Canada	6	9	3
Treated effluent[a]	Britain	11	0.73	17.3
Activated sludge[b]	Australia	< 5	NA	< 1–27

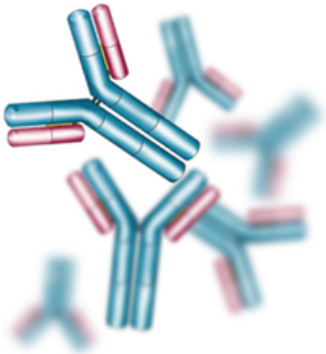
[a] Desbrow et al. (1998, Table 1): lor - limit of reporting, < 1 ng/L
[b] Leusch et al. (2005c, Table 3).

How to detect and monitor EDCs?

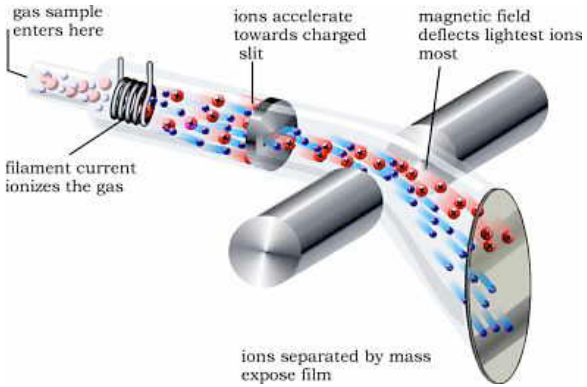
ELRA

ELISA

PORTABLE BIOSENSOR
(Endotect™ / RIANA)



MASS-BASED ANALYSIS PROCESS



How to detect and monitor EDCs?

The use of enzyme-linked immunosorbent assays (ELISA) for the determination of pollutants in environmental and industrial wastes.

Hirobe et al. 2006

A new competitive enzyme-linked immunosorbent assay (ELISA) for determination of estrogenic bisphenols.

Zhao et al. 2002

Comparison of Elisa- and LC-MS-Based Methodologies for the Exposure Assessment of Bisphenol A .

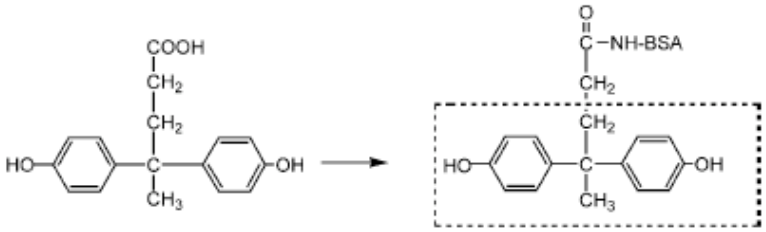
Fukata H et al. 2006

Maternal serum and amniotic fluid bisphenol A concentrations in the early second trimester.

Yamada H et al. 2002

How to detect and monitor EDCs?

A new competitive enzyme-linked immunosorbent assay (ELISA) for determination of estrogenic bisphenols



4,4-Bis(4-hydroxyphenyl) Valeric Acid — BHPVA-BSA Conjugate

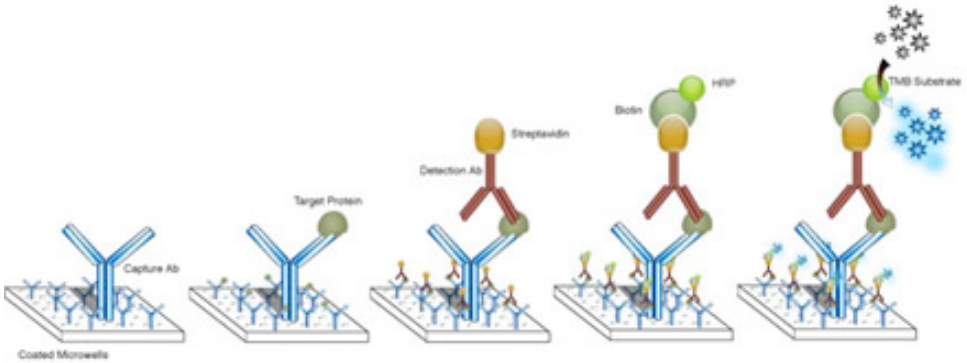
Table 1
Recovery of BPA in the real water and serum samples by the established ELISA

BPA pg per well	Recovery (%)[a]	
	Water samples	Serum samples
10	103 ± 3	–
100	105 ± 4	104 ± 7
1000	92 ± 7	96 ± 5

LOQ = 0.1 ng/mL in real water

LOQ = 2 ng/mL for serum samples

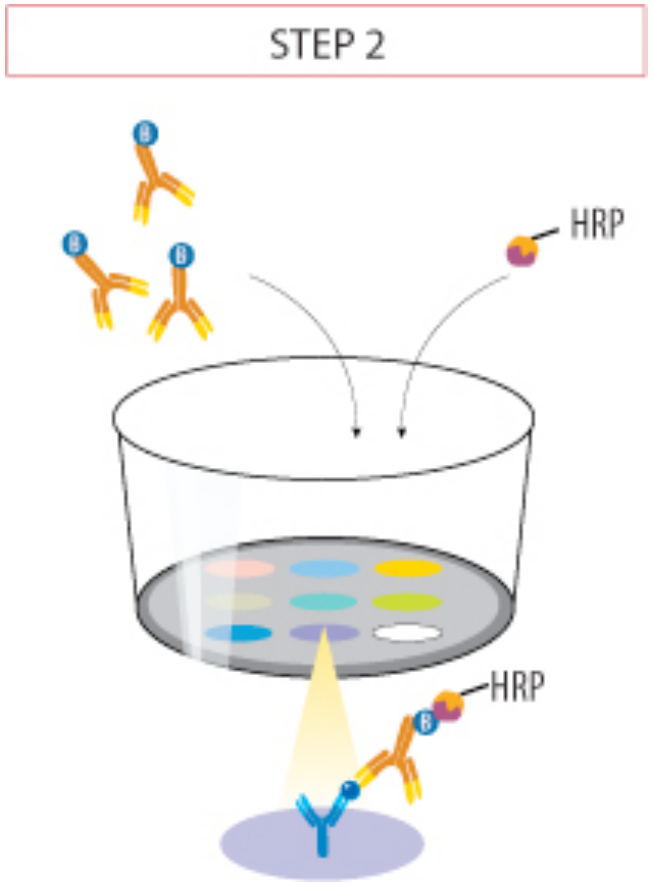
- **LOQ**
- **Functional sensitivity**
- **Linearity**
- **Precision**
- **Cross-reactivities**
- **Matrix effect - Stability**



How to detect and monitor EDCs?

MULTIPLEXING

Mosaic Elisa



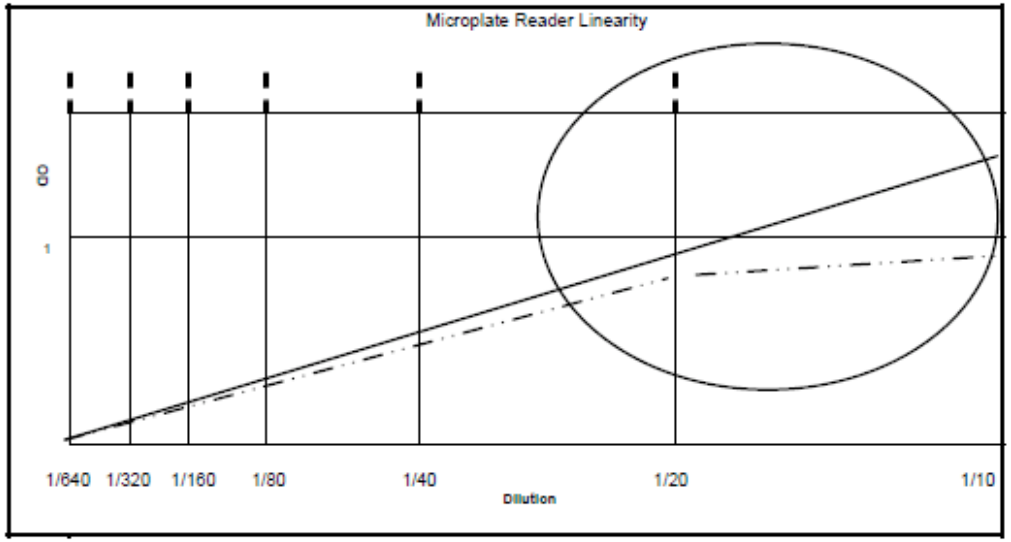
How to detect and monitor EDCs?

How to detect and monitor EDCs?

	TEST		
	LINEARITY	PRECISION / WASHER	PIPETTORS
PERIOD	Once a Month	Once a Week	Once every 2 Weeks (bi-weekly)
MATERIALS		2 Strips	
			1 Strip
	2 Strips		
TOTAL	2 Strips / Month	8 Strips / Month	2 Strips / Month

Microplate reader linearity

Well	Dye Dilution	
	Replicate 1	Replicate 2
	Column 1	Column 2
A	Blank (No Dye)	Blank (No Dye)
B	1/10	1/10
C	1/20	1/20
D	1/40	1/40
E	1/80	1/80
F	1/160	1/160
G	1/320	1/320
H	1/640	1/640



How to detect and monitor EDCs?

Microplate reader precision test

	OD			
Row	Reader A		Reader B	
A	0.431	0.417	0.430	0.424
B	0.435	0.427	0.613	0.625
C	0.425	0.420	0.620	0.613
D	0.418	0.421	0.426	0.419
E	0.429	0.418	0.419	0.416
F	0.415	0.422	0.422	0.429
G	0.414	0.430	0.418	0.412
H	0.425	0.433	0.427	0.417

Microplate washer test

	OD			
Row	Washer A		Washer B	
A	0.045	0.042	0.043	0.039
B	0.042	0.040	0.041	0.042
C	0.047	0.040	0.041	0.045
D	0.045	0.043	0.145	0.139
E	0.044	0.045	0.140	0.142
F	0.042	0.041	0.042	0.039
G	0.044	0.047	0.039	0.040
H	0.047	0.044	0.044	0.043

Pipeting device Test

How to detect and monitor EDCs?

Globalization

We are not alone !!!!

How to detect and monitor EDCs?

MASS-BASED ANALYSIS PROCESS

Mass spectrometry: the key player

Method	Detection limit (ng/L)
ELISA	20-40
LC-MS/MS	0.08-33
SPE-GC/MS	12-32
GC-MS/MS	0.05-2.4
SPME-HPLC	0.064-1.2
SPE-HPLC/ESI-MS/MS	3.5-44

High sensitivity
High specificity
Multiplexing
Automation

Capital investment
Personnel training

How to detect and monitor EDCs?

Analysis of Endocrine Disruptors, Pharmaceuticals, and Personal Care Products in Water Using Liquid Chromatography/Tandem Mass Spectrometry

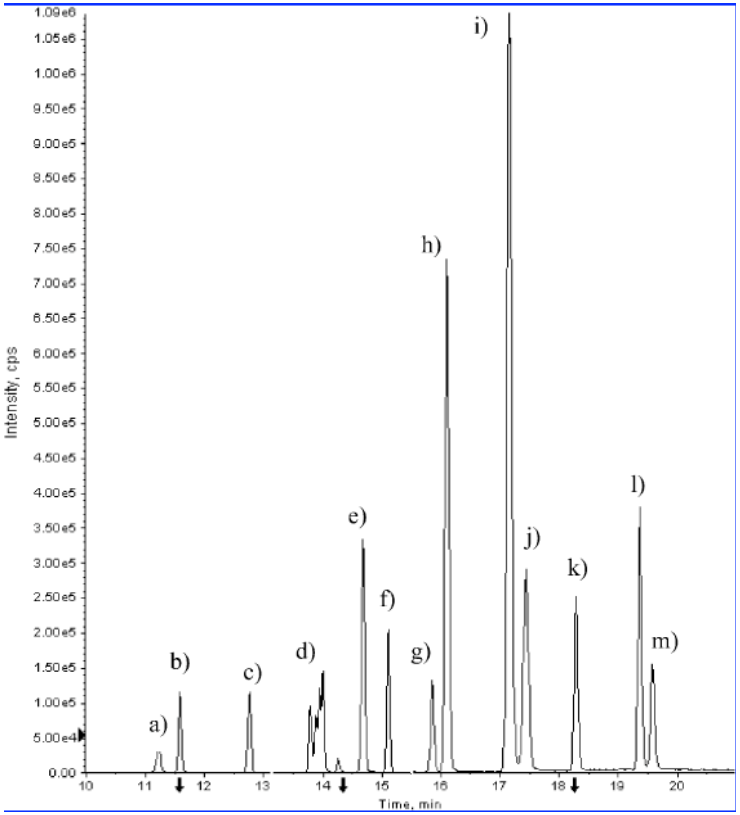


Table 6. Sulfuric Acid Preservation Study

compound	mean surface water recovery (%) unpreserved	sulfuric acid preserved
Degradation Observed		
acetaminophen	10	26
trimethoprim	70	101
fluoxetine	49	76
androstenedione	56	97
testosterone	50	97
progesterone	22	93
Unaffected Compounds		
caffeine	71	72
atrazine	70	73
ibuprofen	65	64
diazepam	60	62
ethynylestradiol	98	100

Vanderford et al.

How to detect and monitor EDCs?

Lower Serum Testosterone Associated with Elevated Polychlorinated Biphenyl Concentrations in Native American Men

Alexey Goncharov,[1] Robert Rej,[2,3] Serban Negoita,[4,5] Maria Schymura,[4,5] Azara Santiago-Rivera,[6,] Gayle Morse,[6,**] the Akwesasne Task Force on the Environment,[7] and David O. Carpenter[1,2,8]*

Associations of Serum Concentrations of Organochlorine Pesticides with Breast Cancer and Prostate Cancer in U.S. Adults

Xiaohui Xu,[1] Amy B. Dailey,[1] Evelyn O. Talbott,[2] Vito A. Ilacqua,[3] Greg Kearney,[4] and Nabih R. Asal[1]

Association between Serum Perfluorooctanoic Acid (PFOA) and Thyroid Disease in the U.S. National Health and Nutrition Examination Survey

David Melzer,[1] Neil Rice,[1] Michael H. Depledge,[2] William E. Henley,[3] and Tamara S. Galloway[4]

Association of phthalate esters with endometriosis in Indian women

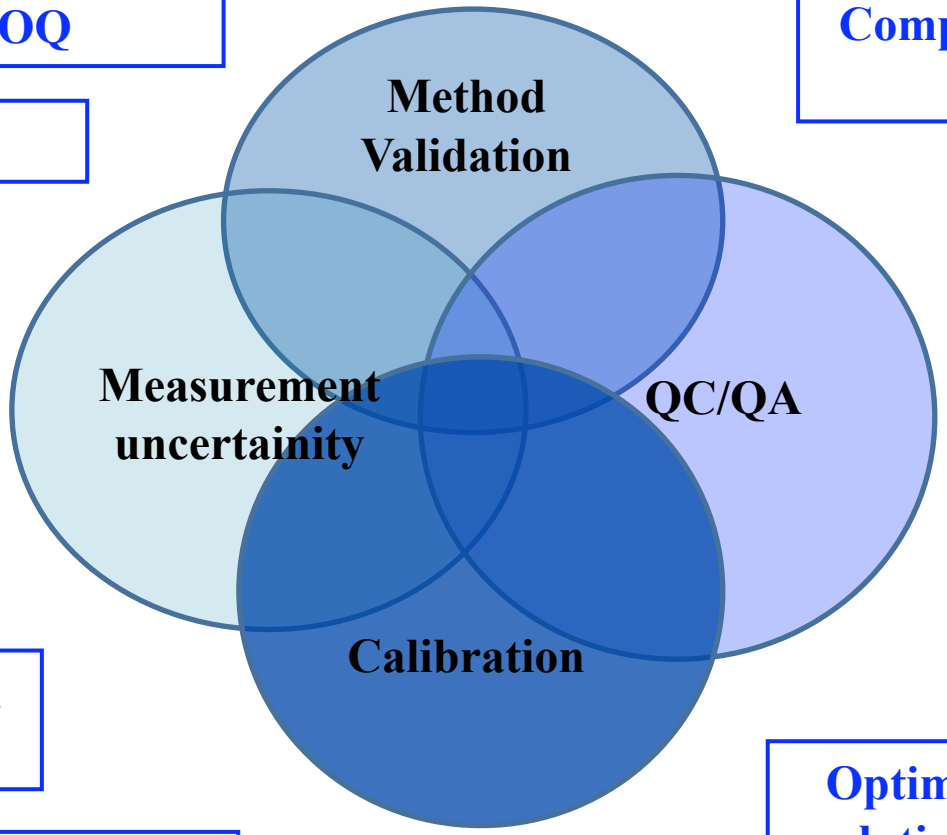
BS Reddy,[a] R Rozati,[b] BVR Reddy,[c] NVVSS Raman[c]

Urinary Concentrations of Bisphenol A and 4-Nonylphenol in a Human Reference Population

Antonia M. Calafat, Zsuzsanna Kuklenyik, John A. Reidy, Samuel P. Caudill, John Ekong, and Larry L. Needham

How to detect and monitor EDCs?

Validation process



- LOQ
- Range
- Precision and accuracy
- Specificity and selectivity
- Calibration and linearity
- Matrix effect
- Optimizing the washing step
- Optimizing the elution solvent
- Conditioning and loading steps
- Ionization method
- Source dependent parameters
- Compound dependent parameters

How to detect and monitor EDCs?

Extraction procedure

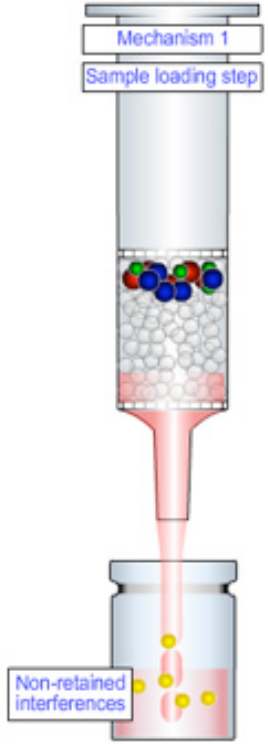
LLE

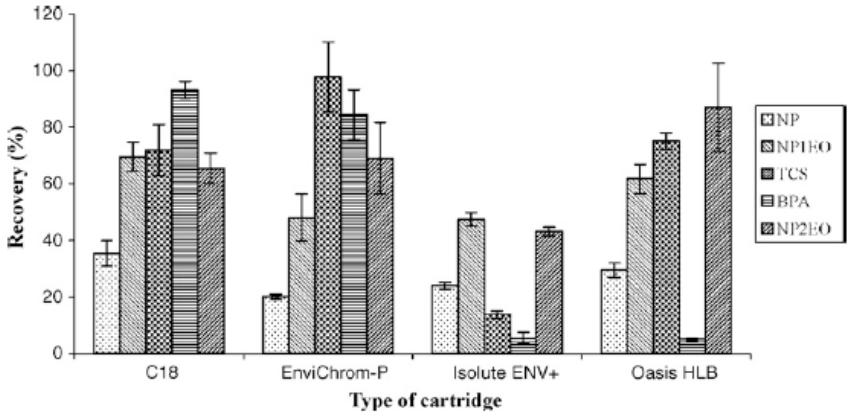
SPE

SPME

MISPE

LPME

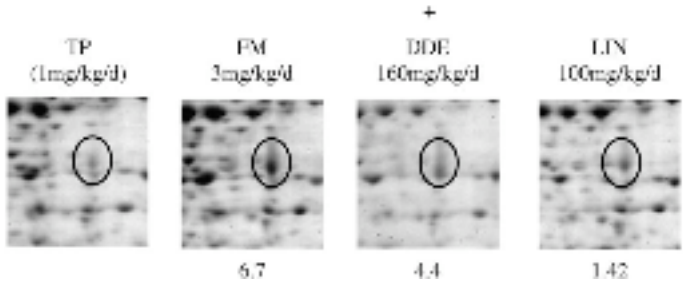




Which perspectives for exposure indicators?

Proteomic approach

Transcriptomic and metabolomic responses



Dynamic epigenomic / DNA methylation

miRNome approach

EXPOSURE → **RISK STRATIFICATION**

Take Home Messages

- Ability to assess the endocrine disrupting power
- Implementation of accurate assays for detection and monitoring of EDCs
- Development of reliable markers of exposure and risk stratification
- Need of multidisciplinary approaches

Thank you for your attention

Questions / Discussion

damien.gruson@uclouvain.be