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EXPO 2015

Managing Water Quality for Public Health

October 14th 2015

Aquatic effect-based monitoring tools.

UNIVERSITÀ DEGLI STUDI DI TORINO
ALMA UNIVERSITAS TAURINENSIS

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The transition of health risks

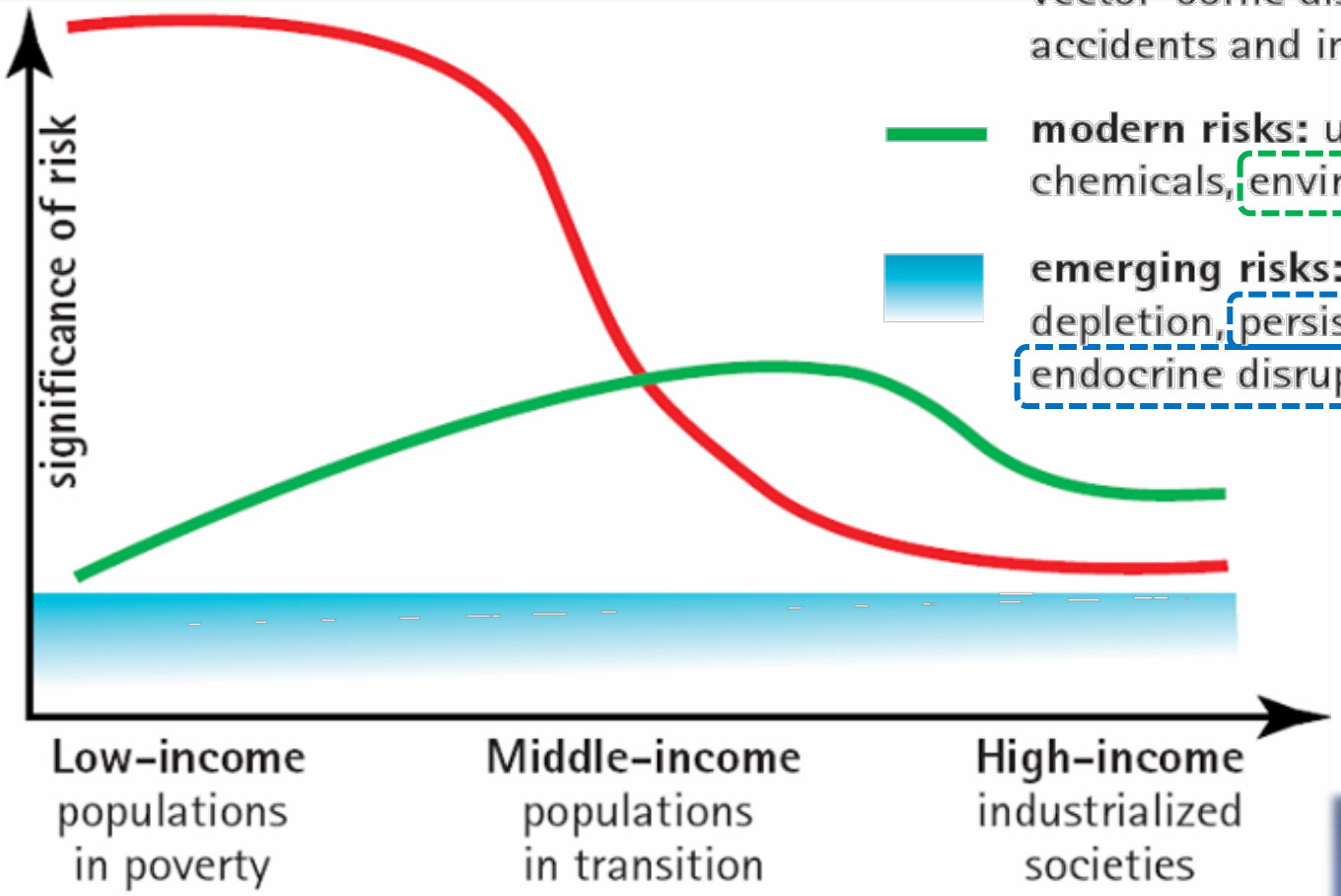
http://www.ircwash.org/news/elusive-health-impact-wash

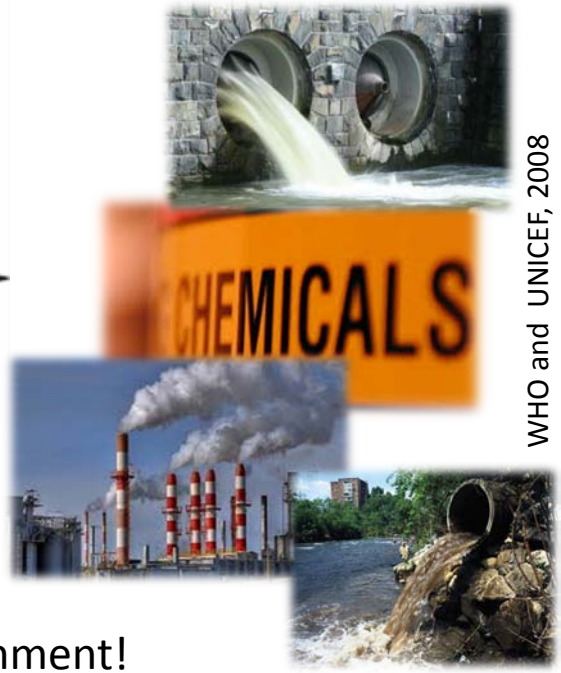
The transition of health risks

basic risks: lack of safe water, sanitation and hygiene, indoor air pollution, vector-borne diseases, hazards that cause accidents and injuries

modern risks: unsafe use of chemicals, environmental degradation

emerging risks: climate change, ozone depletion, persistent organic pollutants, endocrine disruptors





WHO and UNICEF, 2008

Human activities = thousands of compounds released in the Environment!

Water contamination

Thousands of molecules

Use
Spill

Uncomplete degradation - Spill

Presence
Impact?
Need to monitor water quality!

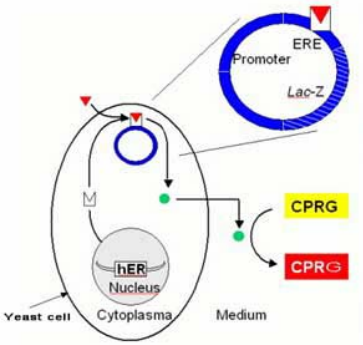
Water monitoring

Chemical Approach

- ✓ **Detection of targeted compounds**
- ✓ **New and Unknown compounds?**
- ✓ **Transformation products?**
- ✓ **Mixture effect?**
- ✓ **Low levels** (ng/L => sensitivity – traces)**?**
- ✓ **Emerging contaminants?**
  (no regulation => not monitored)

We analyze what we know?

Biological Approach

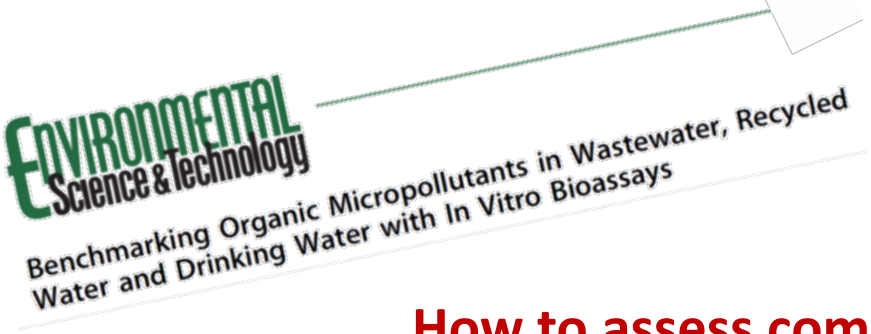


- ✓ **Detection of active compounds**
- ✓ **No *a priori* knowledge on or selection of compounds required**

➔ **Effect-based monitoring tools**

Contamination ⟷ **Effects**

Effect-base monitoring tools



Future water quality monitoring – Adapting tools to deal with mixtures of pollutants in water resource management

How to assess complex contamination?

Effect-based tools are described in three main groups:

- ✓ **Bioassays:** *in vitro* and *in vivo* bioassays that measure the toxicity of environmental samples (toxicity, genotoxicity, mutagenicity, estrogenicity…)
- ✓ **Biomarkers:** biological responses at individual level (or below) observed in field exposed organisms.
- ✓ **Ecological indicators:** biological responses at higher organisation levels, e.g. population and community.

➔ tools for cumulative assessment of contamination!

Wernersson et al. *Environmental Sciences Europe* (2015)
Esher et al. *Environmental Science & Technology* (2015)
Altenburger et al., Science of the Total Environment (2015)

Effect-base monitoring tools

***Toxicity Identification Evaluation* (TIE)**

***Effect-Directed Analysis* (EDA)**

Integration of **BIOASSAYS** and CHEMICAL ANALYSIS in sequential procedures **1 – 2 – 3** could allow to identify the unknown COMPOUNDS in a sample that cause the specific bioassay response.

1st step ➔ Bioassay

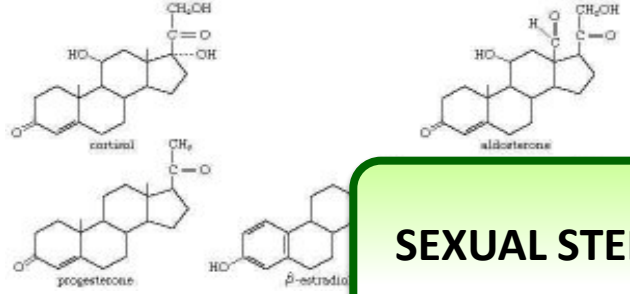
Back W. (2003) *Anal Bioanal Chem.*
Petrovich M. (2004)) *Anal Bioanal Chem.*

Endocrine Disruptors Compounds

Definition of EDCs (IPCS, 2002)

*"An **endocrine disruptor** is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations."*

… one of the Top Five most significant scientific developments in the last century…



SEXUAL STEROIDS

PHITOESTROGENS

Naturally-occurring

Endocrine Disruptors

… a long list of substances …

Synthetic (man-made)

PERSISTENT ORGANIC CONTAMINANTS

COSMETICS

HERBICIDES
PESTICIDES
PESTICIDES

SUBSTANCES FOR INDUSTRIAL USE

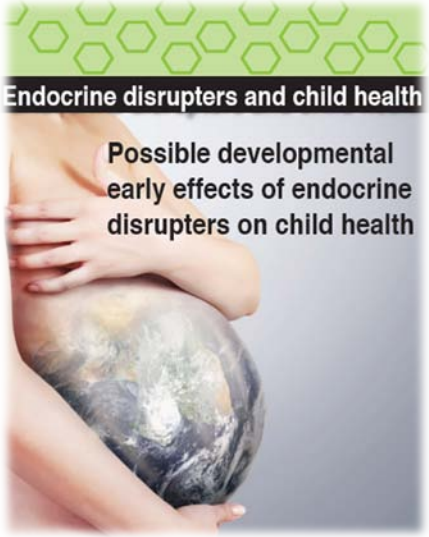
DRUGS

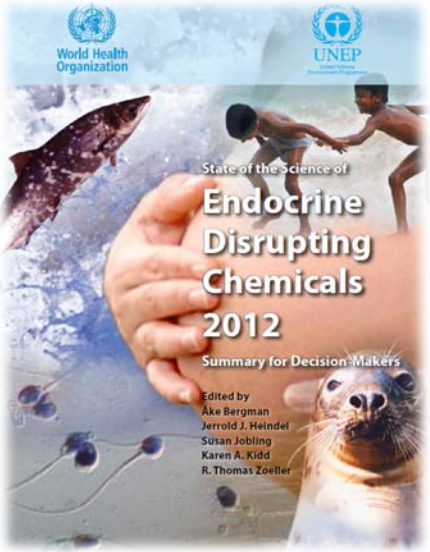
Endocrine Disruptors Compounds

"Key concerns"

- ✓ High and increasing incidence of endocrine-related disorders;
- ✓ Early incidence of breast cancer in young women;
- ✓ Increased incidence of cryptorchidism and hypospadias in childhood;
- ✓ Neurobehavioral disorders associated with thyroid disease in childhood;
- ✓ Increase prevalence of obesity and metabolic diseases.

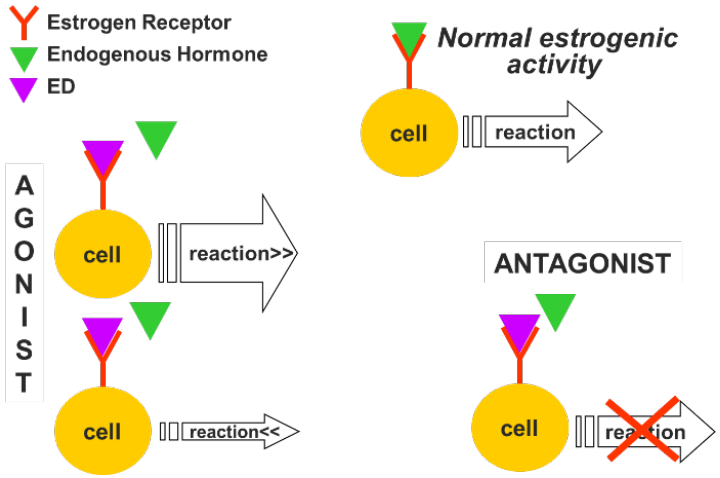
Reproductive health and Children are more at risk!





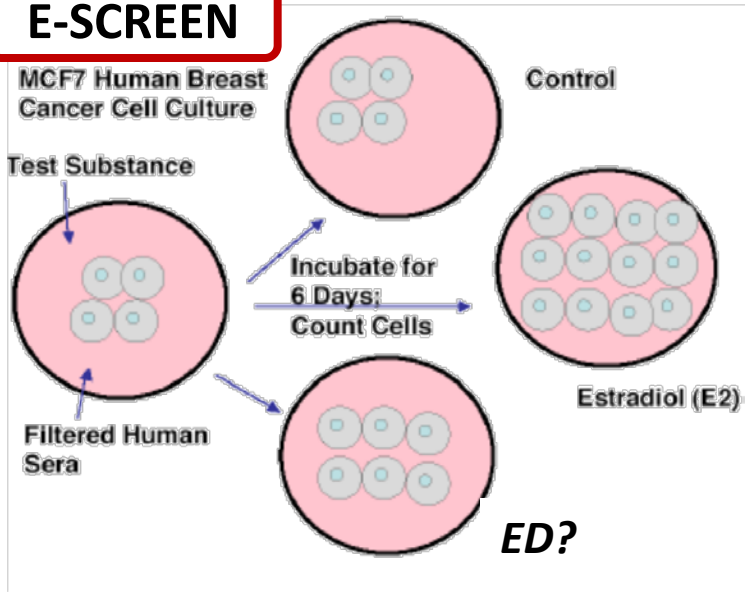
WHO 2012, State of the Science of Endocrine Disrupting Chemicals
WHO, Regional office for Europe, European health for all database, 2010

Endocrine Disruptors Compounds

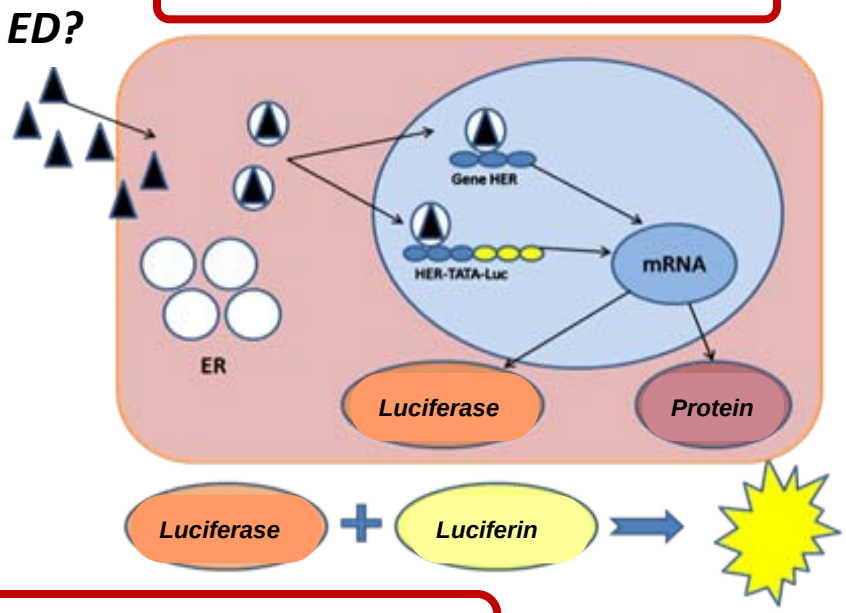


The main *biological tests* for the evaluation of EDCs exploit the ability to link with hormone receptor ...

E-SCREEN



MELN GENE REPORTER



EEQ (ng/L) = EC50 control E2 / EC50 sample

The estrogenic activity of the sample is expressed in **EEQ equivalent concentration of estradiol** without knowing the chemical nature of all the substances involved.

Endocrine Disruptors & effect-based monitoring tools

The estrogenic activity of aquatic ecosystems is monitored to assess:

- the impact of wastewater treatment plants;
- the risk for wildlife living in water bodies;
- the human exposure risk because, increasingly,
  the surface water is used as a source of drinking water ...

Average values of estrogenic activity in effluent of wastewater treatment plants (WWTP) by different *in vitro* tests in several regions of the world.

1-10 ng/L is a concentrations at which chronic exposure has been reported to affect the endocrine system of living organisms….

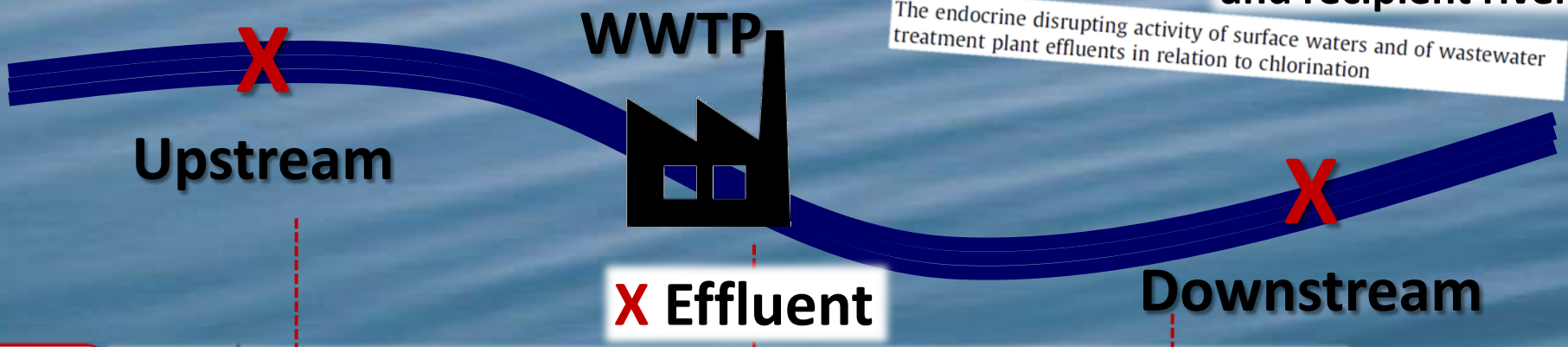
EEQ (ng/L)	Country	*In vitro* assay
<1–4.1	Australia and New Zealand	Estrogen receptor binding assay
<1–16	Netherlands	ERCALUX
4–35	Japan	Yeast assay
2–25	Germany	E-screen
<1–7.8	Germany	E-screen
<3–13	United Kingdom	Yeast assay
1–15	USA	Yeast assay
1–67.8	Australia	E-screen
4.8–5.6	Finland	Yeast assay
30–80	Canada	Yeast assay

Bicchi et al. *Science of the Total Environment* (2009)

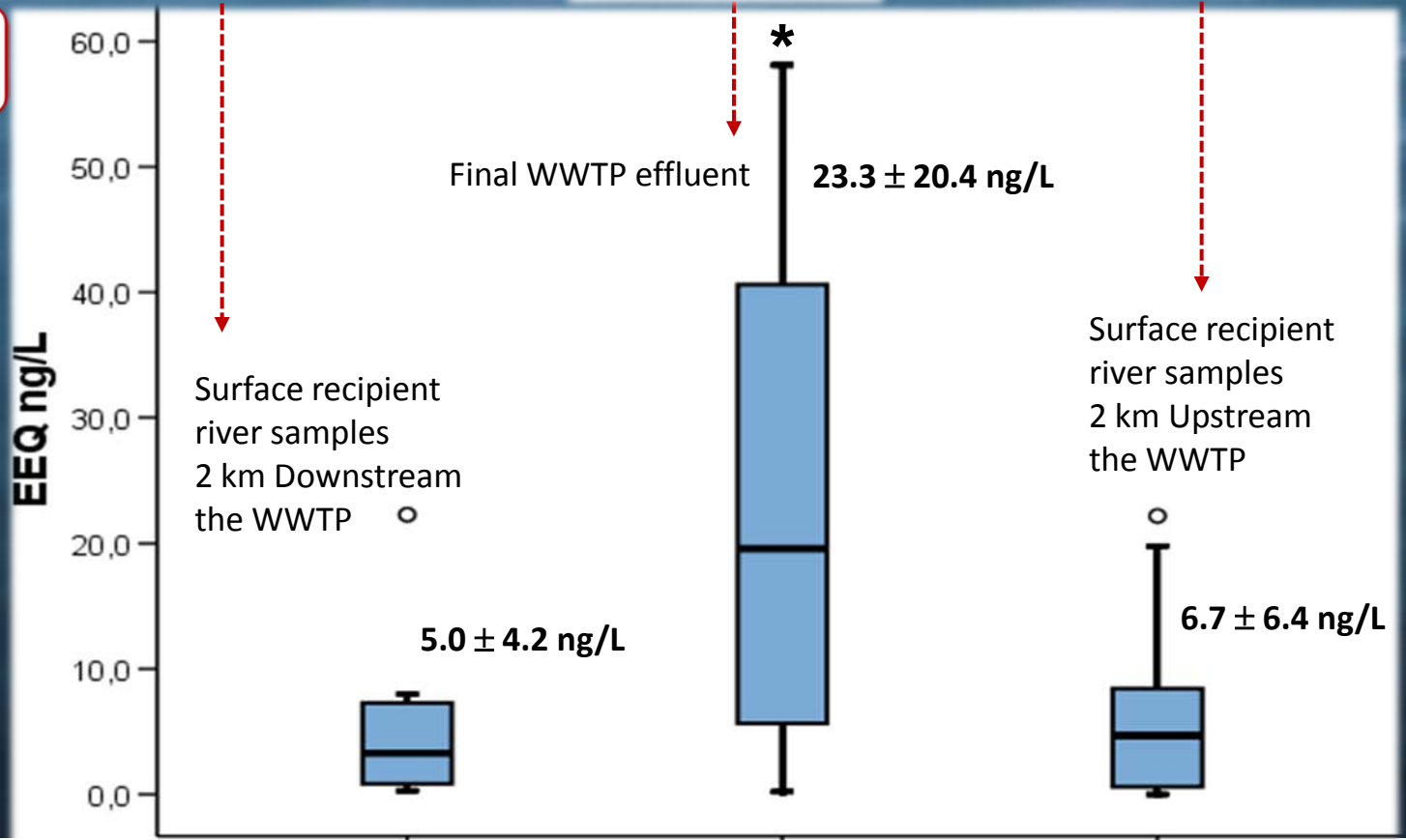
Endocrine Disruptors & effect-based monitoring tools

Estrogenic activity in WWTP effluent and recipient river

The endocrine disrupting activity of surface waters and of wastewater treatment plant effluents in relation to chlorination



E-screen assay



ANOVA
F = 7.22
p<0.01

Chemosphere 75 (2009) 335–340

Schilirò et al., (2009)

Endocrine Disruptors & effect-based monitoring tools

Analysis of environmental endocrine disrupting chemicals using the E-screen method and stir bar sorptive extraction in wastewater treatment plant effluents

Table 3 – Selected analytes present in the different sampling sites (ng/L): the final effluent of the WWTP (OUT), upstream (US) and downstream (DS) the WWTP, using SBSE with *in situ* derivatization, followed by thermal desorption (TD)–GC–MS

Samplings	2.4-DCP	4-t-BP	4-n-NP	4-n-OP	BPA	DEP	DBP	DEHP	E1	E2	EE
US											
1	nd	44.5	3874.8	nd	88.0	29.3	43.5	40.0	65.2	nd	nd
2	nd	53.2	704.7	888.7	72.1	2821.0	21,209.5	2402.4	141.6	nd	nd
3	nd	46.5	nd	nd	22.5	194.9	28.9	82.1	81.2	nd	nd
4	nd	nd	nd	nd	42.0	126.9	150.9	64.5	13.8	nd	nd
5	nd	48.0	nd	nd	3.7	313.5	276.6	63.1	1.8	nd	nd
6	nd	8953.0	nd	nd	3.6	239.4	160.4	42.4	5.2	nd	nd
OUT											
1	nd	nd	nd	nd	992.8	2059.7	40.0	105.0	125.8	nd	nd
2	nd	nd	nd	nd	71.2	69.8	62.3	47.7	200.0	nd	nd
3	nd	nd	nd	nd	30.3	41.5	35.1	48.6	121.8	nd	nd
4	nd	237.0	nd	nd	6.2	372.3	128.7	45.9	nd	nd	nd
5	nd	34.5	nd	nd	40.3	260.7	311.1	82.1	nd	nd	nd
6	nd	nd	nd	nd	201.1	158.6	76.3	33.5	95.9	nd	nd
DS											
1	nd	12.1	nd	nd	26.8	54.1	127.6	386.9	84.5	nd	4381.6
2	nd	nd	nd	nd	48.9	83.3	694.1	120.3	592.7	nd	nd
3	nd	41.0	nd	nd	21.2	53.2	949.2	291.4	109.1	nd	nd
4	nd	286.6	nd	nd	2.6	73.6	348.5	80.6	nd	nd	nd
5	nd	34.2	nd	nd	6056.5	75.0	31.4	124.8	nd	nd	nd
6	nd	186.0	nd	nd	186.0	150.9	79.8	19.3	41.6	nd	nd

nd = below detection limit; 2.4-DCP = 2.4-dichlorophenol; 4-t-BP = 4-t-butylphenol; 4-n-NP = 4-n-nonylphenol; 4-n-OP = 4-n-octylphenol; BPA = bisphenol A; DEP = diethyl phthalate. DBP = dibutyl phthalate; DEHP = di-(2-ethylhexyl) phthalate; E1 = estrone; E2 = 17β-estradiol; EE = ethynilestradiol.

SCIENCE OF THE TOTAL ENVIRONMENT 407 (2009) 1842–1851

Chemcal analyis SBSE➔ quantification of some selected chemicals:
phenols, phtalates and natural hormones…

Bicchi et al., (2009)

Endocrine Disruptors & effect-based monitoring tools

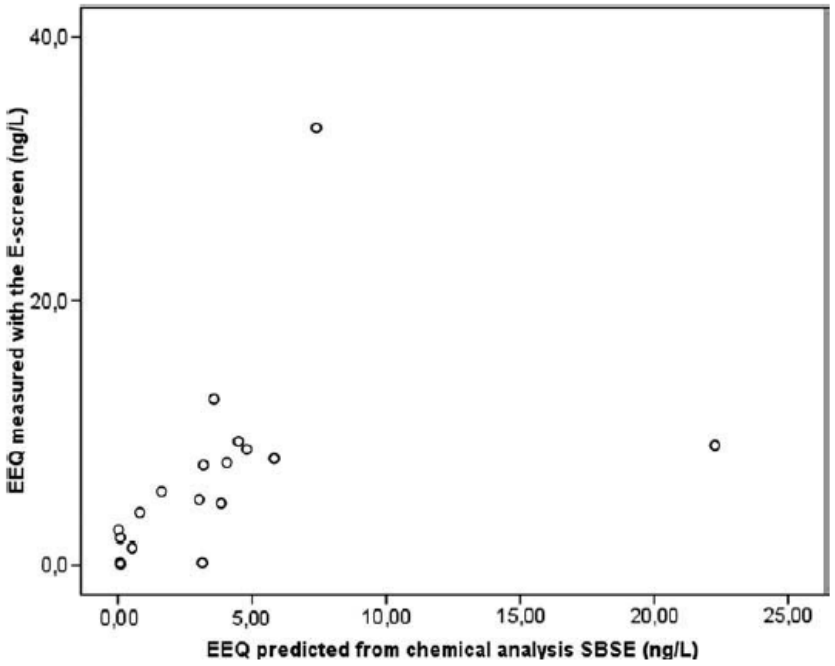


Fig. 3 – Correlation between the measured E-screen assay EEQ and the predicted EEQ from the results of the water samples from all the sampling sites.

EEQ predicted from chemical analyses

EEQ of the *E-screen assay*

(34 ± 20 %)

Bicchi et al., (2009)

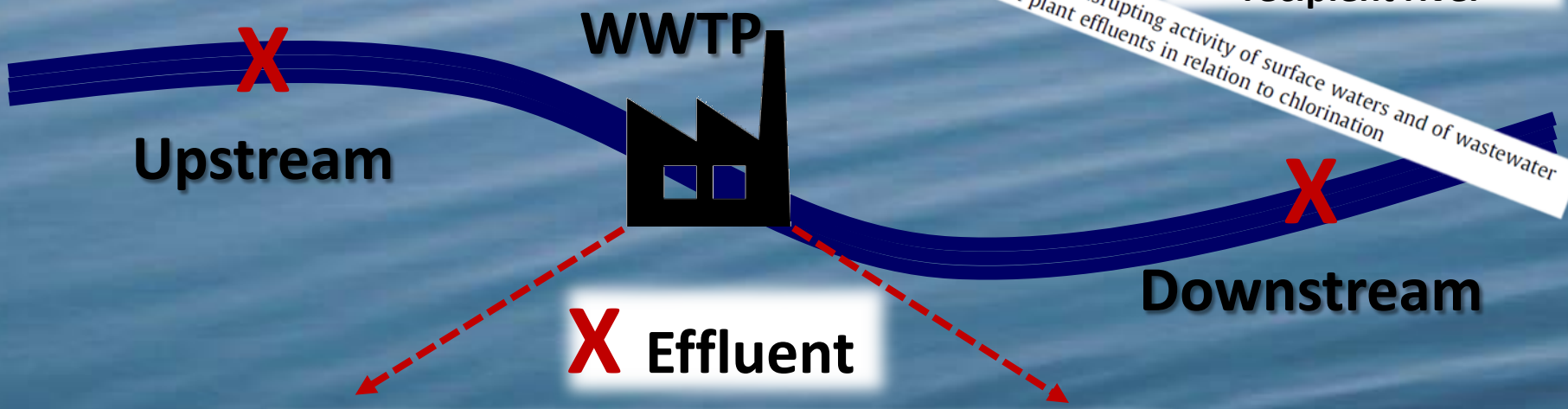
Table 1 – Mean estrogenic activity of the sample extracts from the different sampling sites: the final effluent of the WWTP (OUT), upstream (US) and downstream (DS) the WWTP, in MCF-7 BUS breast cancer cell and comparison between the E-screen test and chemical analysis

Samplings	EEQ (ng/L) Measured with the E-screen	RPE % Measured with the E-screen	EEQ (ng/L) Predicted from the chemical analysis	EEQ % Predicted/ measured
US				
1	4.6	35	3.9	84.2
2	8.0	75	5.8	72.8
3	4.9	35	3.0	62.5
4	1.3	127	0.5	39.4
5	2.0	25	0.1	4.5
6	7.5	81	3.2	42.3
OUT				
1	8.7	41	4.8	55.4
2	33.6	61	7.4	22.0
3	9.3	28	4.5	48.5
4	0.2	166	0.1	47.1
5	2.6	33	0.02	0.8
6	12.5	72	3.6	28.7
DS				
1	0.2	52	3.1	1495.2
2	9.0	107	22.3	247.3
3	7.7	41	4.1	52.9
4	0.1	130	0.1	76.9
5	3.9	34	0.8	20.6
6	5.5	63	1.6	29.6

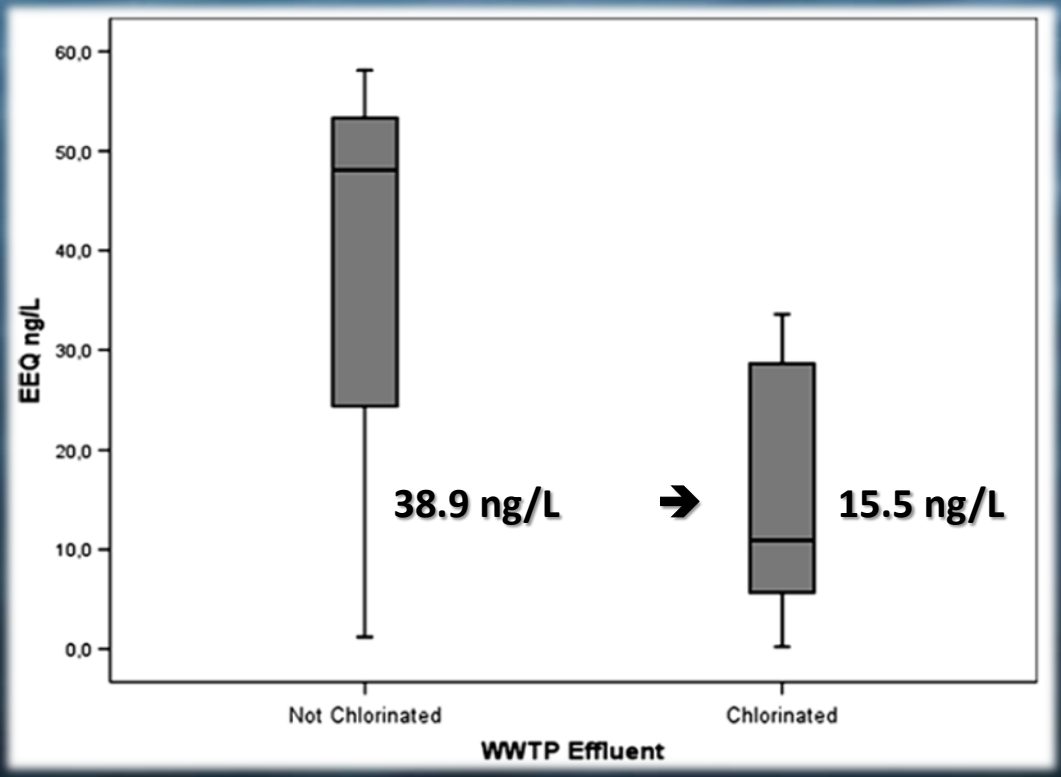
Endocrine Disruptors & effect-based tools

Estrogenic activity in WWTP effluent and recipient river

The endocrine disrupting activity of surface waters and of wastewater treatment plant effluents in relation to chlorination



E-screen assay



Chlorination

EEQ - 60%
E. coli -90%
Toxicity + 100%
THM + 89%

Chemosphere 75 (2009) 335–340

Schilirò et al., (2009)

Endocrine Disruptors & effect-based tools

Removal of micropollutants by fungal laccases in model solution and municipal wastewater: evaluation of estrogenic activity and ecotoxicity

Enzymatic treatment
LACCASES of *Trametes pubescens*

Collection of **municipal WWTP effluent**

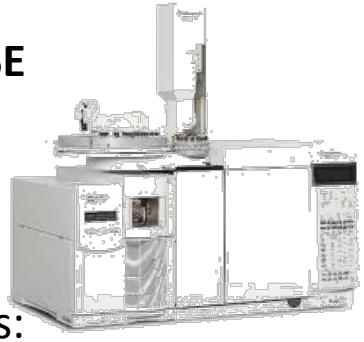
Incoming waters (municipal, agricultural, rivers, etc.)

Primary sedimentation

denitrification, biological oxidation, filtration, sedimentation, etc.

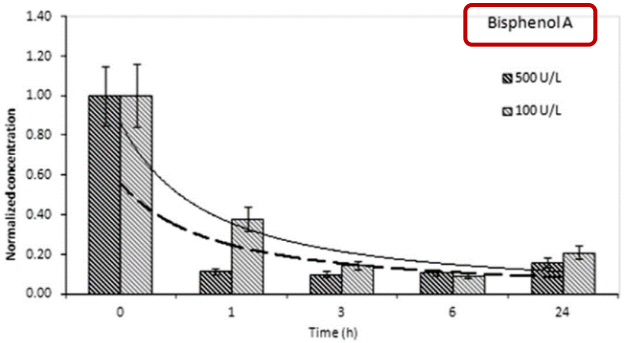
WWTP final effluent

Target EDCs were detected with **chemical analyses SBSE** in the **final effluent**….



Laccase treatments were active towards all the detected compounds: **phenols, phtalates, estrogens, etc…**

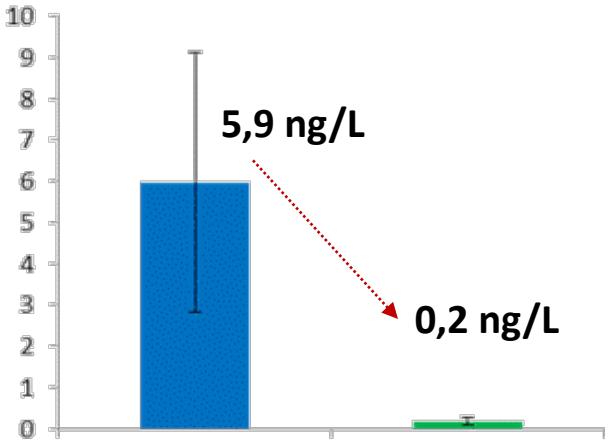
The mean percentage of **chemical removal in the final effluent was 61 %.**



Spina et al., *Journal of Cleaner Production* (2015)

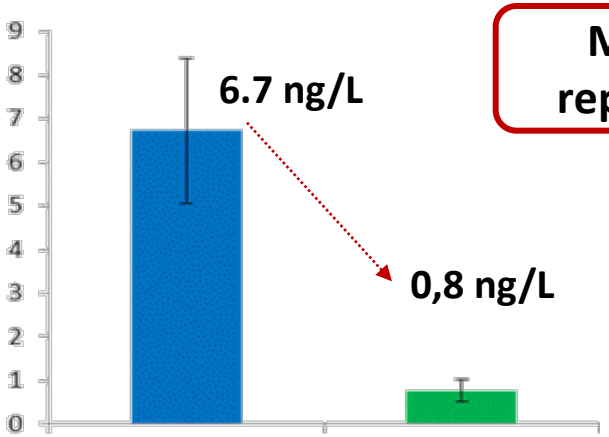
Endocrine Disruptors & effect-based tools

E-screen assay



WWTP final effluent

MELN gene reporter assay



laccase treatment

There is a significant **reduction of the estrogenic activity** of the effluent after the **enzimatic treatment**…

By this estrogenic bio-removal, EDCs are deposited into the river via WWTP at concentrations lower than <1 ng/L…

In future….validation and optimization of the enzymatic process in order to improve its stability, making it applicable on an industrial scale.

Effect-based monitoring tools

- What kind and how many *in vitro tests* for the assessment?

***In vitro* tests** are specific systems to detect chemicals interacting with a specific cellular patway…

- How to standardize *in vitro tests?*

- It is possible to develop guidelines based on the results of bioassay?

Wernersson et al. *Environmental Sciences Europe* (2015)
Jarosova et al., *Environment International* (2014)

…to be continued…

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Thank you for your attention!

Thanks to proff.

Cristina Varese, *Dep. Life Sciences and Systems Biology*

Chiara Cordero*, Dep. Science and Technology of Drug*