EB$_{\&}$T - Ecophysiology, Biochemistry and Toxicology
www.ecotox.be

University of Antwerp

In vitro mechanistic toxicology: what can it offer to ecotoxicology?

Ingrid Nobels, Caroline Vanparys, Tine Hectors, Karlijn van der Ven, Johan Robbens, Wim De Coen, Ronny Blust

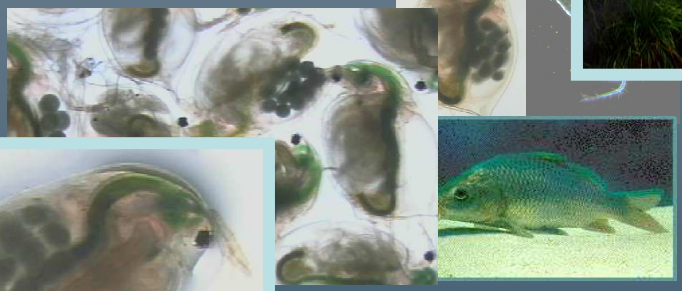
I-sup 22-25 April 2008

Protecting the ecosystem

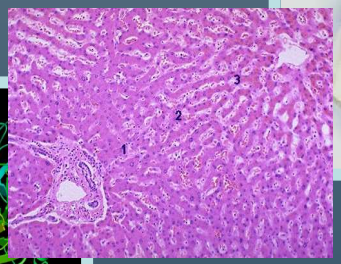
?

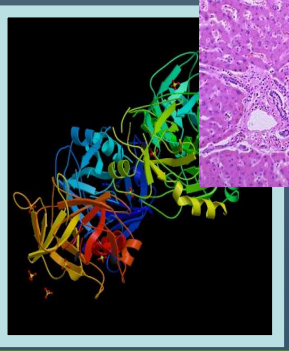
Ecotoxicology

Ecological relevance



Early warning system provides mechanistic information





"Black box"

Time

In vitro assays in ecotoxicology what can it add?

- **Mechanistic information**

Providing mechanistic information for pre-emptive risk identification

Case study

- **Specific in vitro systems for biomonitoring**

The presence of certain classes of compounds can be observed through specifically designed in vitro methods.

Case study

Case study: providing mechanistic information Glyphosate

- Broad spectrum herbicide frequently used and sold under different tradenames
- Environmental monitoring indicates that the levels in surface water increase each year!
- Allowed levels are based on the active ingredients but what is the effect of the formulations?

?

Are the formulations of glyphosate provoking more/less or other effects than the active ingredient alone

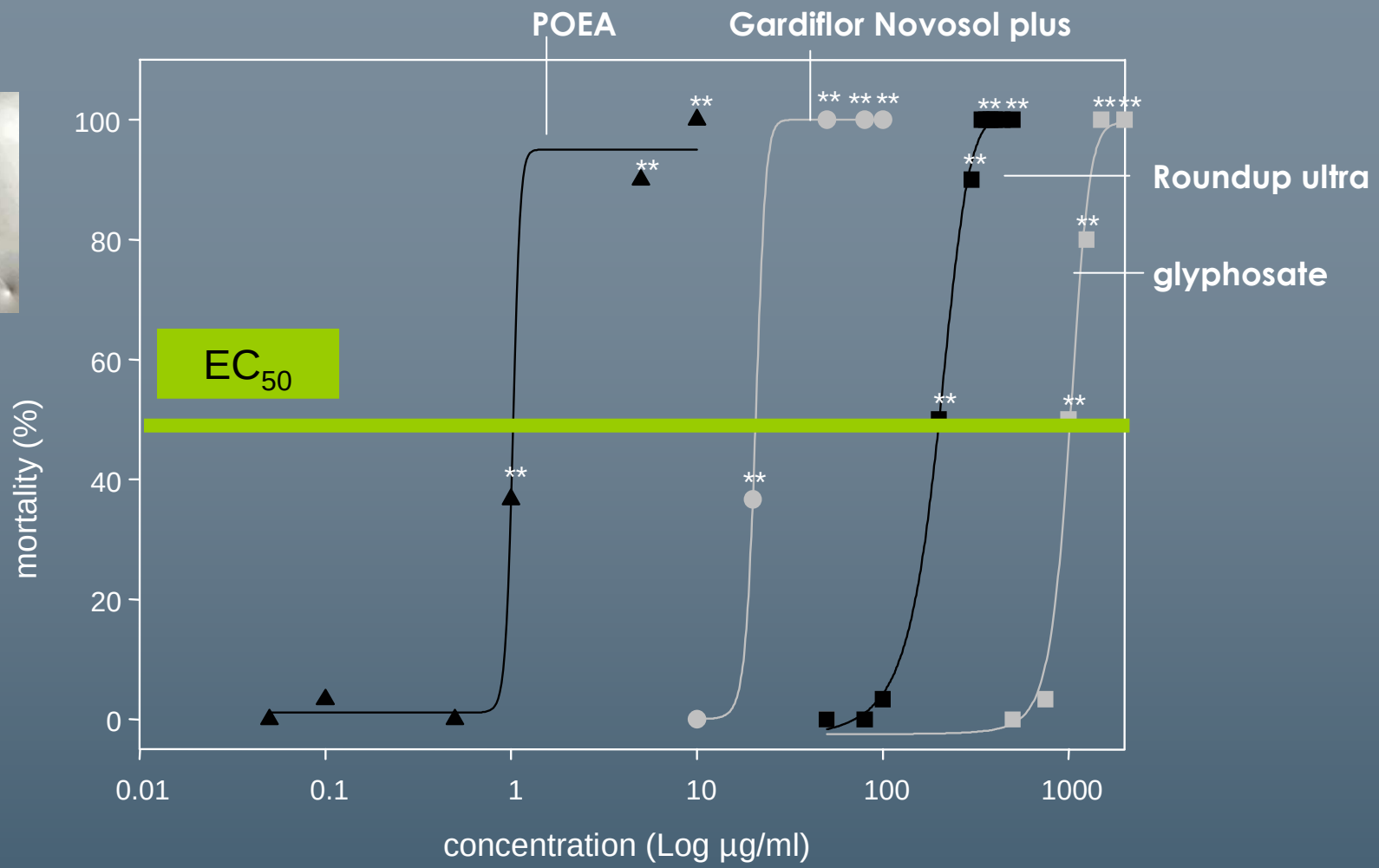
?

Gobio gobio LC_{50}

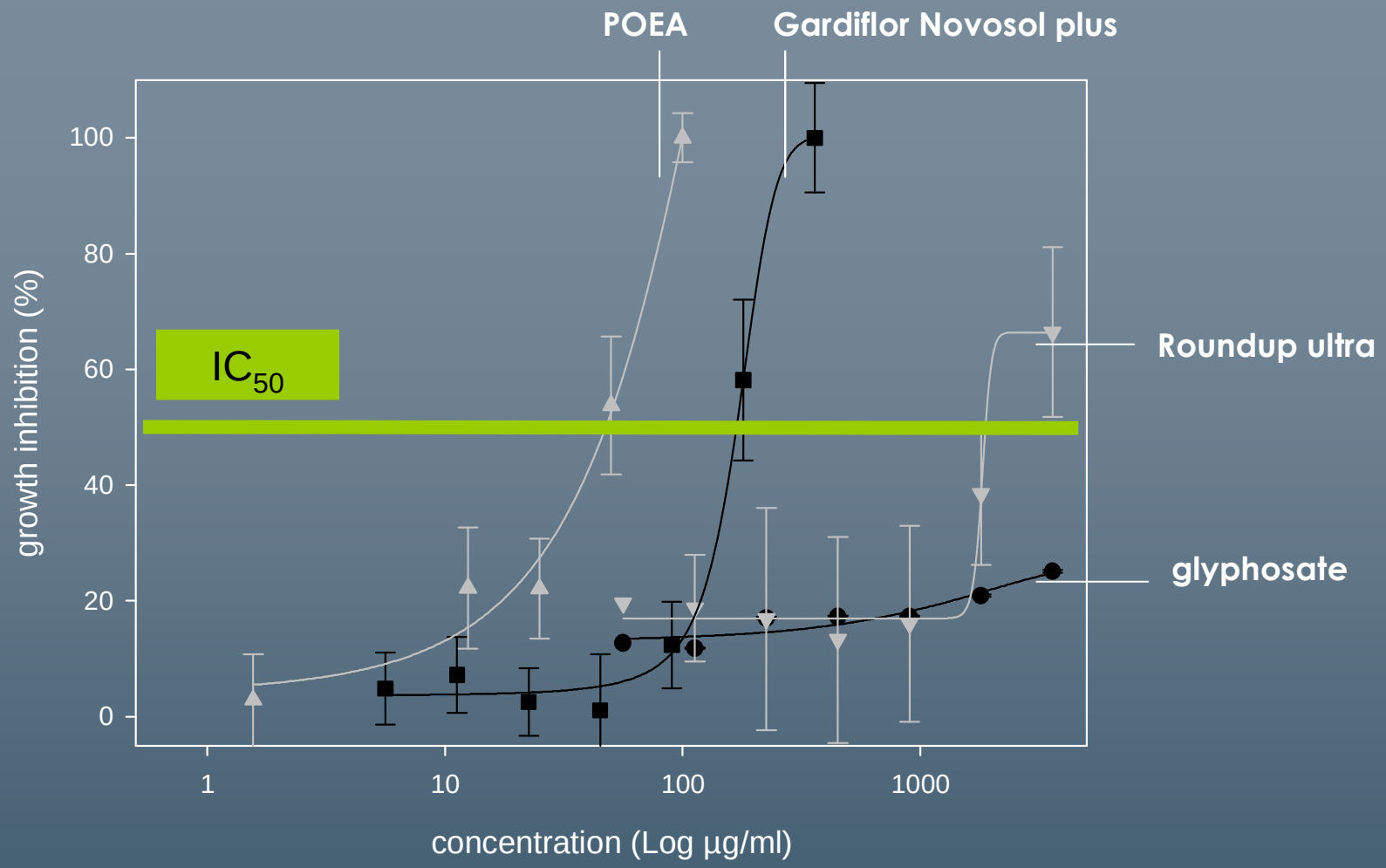
Gammarus pulex LC_{50}

Daphnia magna EC_{50}

Bacterial (E. coli) multiple endpoint in vitro assay: mechanistic information and IC_{50}



	µg/ml	Glyphosate	Roundup Ultra	Gardiflor Novosol plus	POEA
IC_{50}, $EC_{50}$and LC_{50}	$EC_{50-48\,h}$ *D. magna*	1020[a] (960 – 1080)	190[b] (170 – 210)	20[c] (20 – 30)	1.58[c] (1.23 - 2.03)
	LC_{50-48h} *G. gobio*	2770[a] (2370 – 3380)	1330[b] (1150 – 1550)	< 5	-
	LC_{50-48h} *G. pulex*	780[a] (680 – 910)	> 100	40[b] (13 – 55)	6.72[b] (4.17 – 10.83)

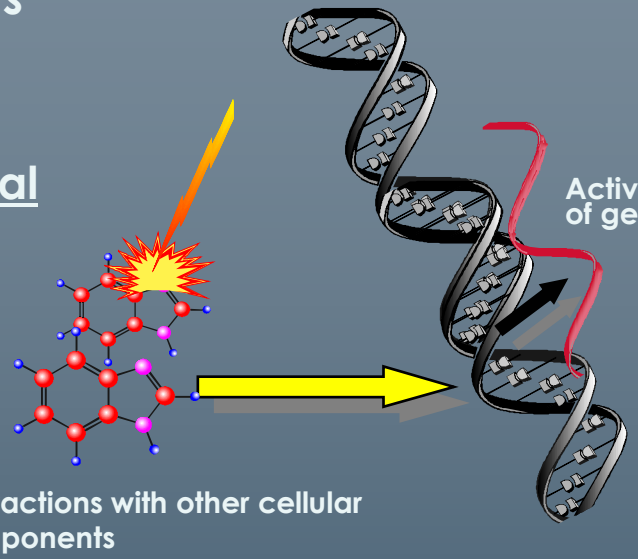


IC_{50} values with E. coli show the same order of increasing toxicity than EC_{50} and LC_{50} for *D. magna*, *G. pulex* and *G. gobio*

? Mechanistic information?

Gene Profile Assay Technology

Measure gene activity after exposure to stressors



Bacterial
- E. coli

Promoter	LacZ Reporter Gene

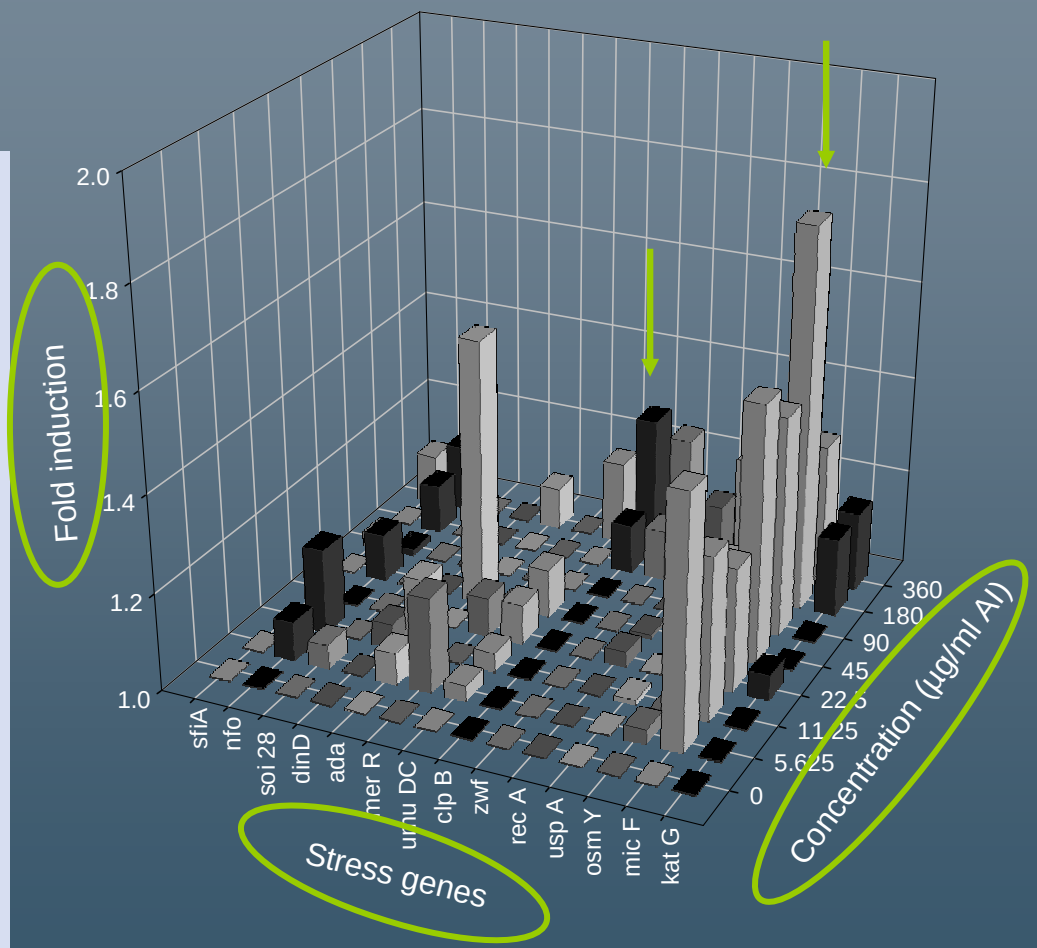
β-galactosidase activity determined spectrophotometrically on bases of ONPG

$$\text{Fold Induction} = \frac{\text{GAL activity at Dose}_x}{\text{GAL activity at Dose}_0}$$

General endpoint classes

Oxidative stress
Protein perturbation
DNA damage
Metal ion presence
Membrane effects
Osmolarity

Glyphosate

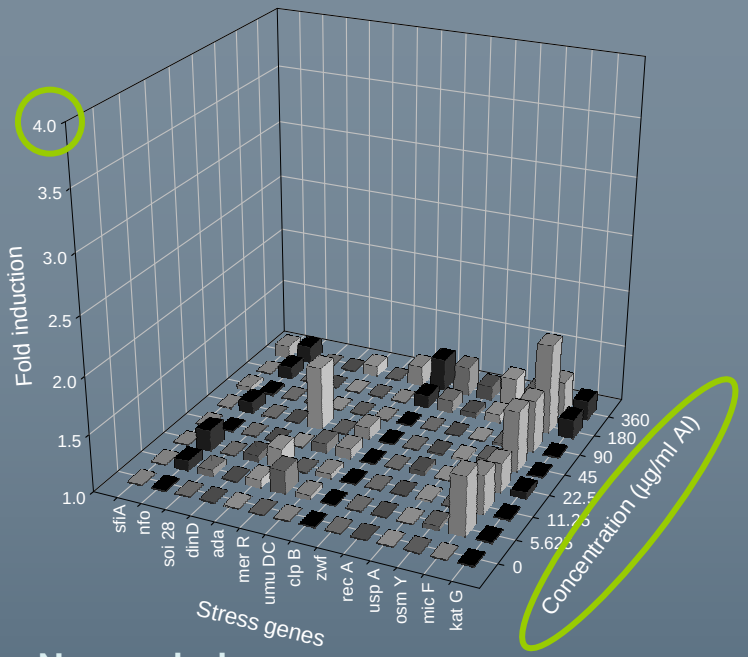


FI are considered significant if:

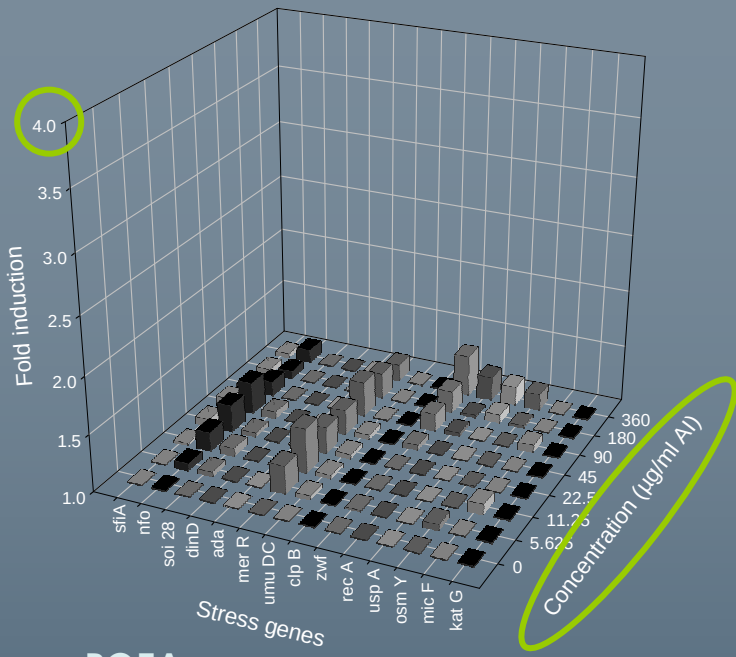
-clear positive dose response relationship is observed ($R^2 > 0.5$, $p<0.05$)

- Inductions are statistically different from the blanc (ANOVA, Dunett's test $p < 0.05$)

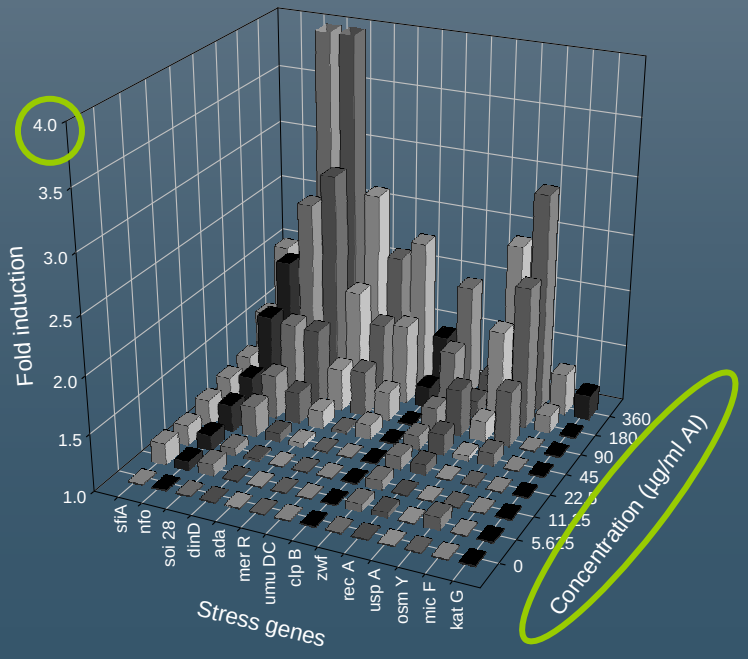
Glyphosate



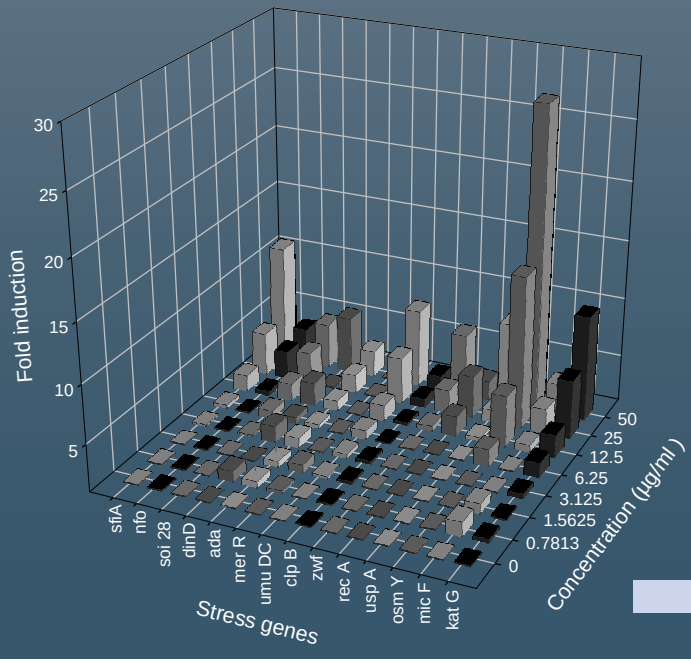
Roundup Ultra



Novosol plus



POEA



		Glyphosate	Roundup Ultra	Gardiflor Novosol Plus	POEA
Cellular and osmotic stress	MicF	-	-	1,35 ± 0,11	3,58 ± 0,50
	OsmY	1,16 ± 0.09	1,13 ± 0,07	2,91 ± 0,33	26,82 ± 4,44
	UspA	1,23 ± 0.08	1,16 ± 0.04	2,43 ± 0,01	8,01 ± 4,82
Oxidative damage	KatG	1,16 ± 0,04	-	-	9,94 ± 3,07
	Zwf	-	1,36 ± 0,12	1,98 ± 0,07	6,25 ± 1,96
	Soi28	-	-	4,05 ± 0,17	4,76 ± 1,64
	Nfo	-	-	1,91 ± 0,01	3,88 ± 2,81
DNA damage	RecA	1,12 ± 0,04	1,20 ± 0,07	-	4,54 ± 1,76
	UmuDC	-	-	2,30 ± 0,14	7,58 ± 1,88
	Ada	-	-	2,65 ± 0,07	-
	DinD	-	-	4,55 ± 0,38	-
	SfiA	-	-	2,03 ± 0,13	10,89 ± 6,12
Protein perturbation	ClpB	-	-	1,48 ± 0,02	2,11 ± 0,43
Heavy metal stress	MerR	-	-	2,13 ± 0,04	-

# of genes level of induction

Case study: environmental monitoring of endocrine disrupting chemicals

Case study:

→ Detection of ovotestis in gudgeon in different rivers in Wallonia (Prof. Kestemont, FUNDP)

- The presence of endocrine disrupting chemicals in surface water.
- MCF-7 human breast carcinoma cells (flow-cytometry detection system)

→ 5 water samples examined:

2 control river samples (Lesse and Ourthe)

2 sewage treatment plants (STPs)effluents

1 industrial effluent

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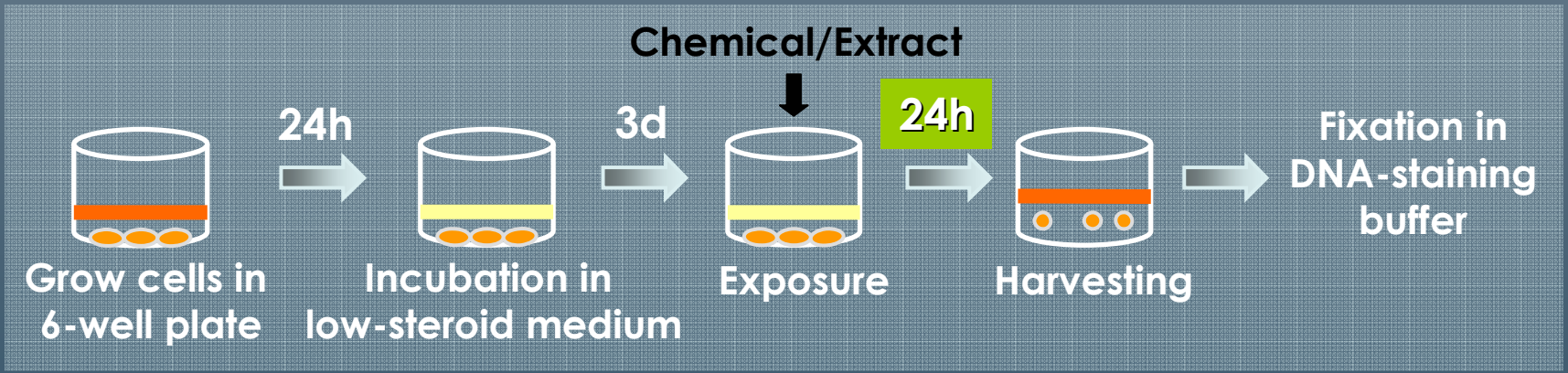
MCF-7 cell proliferation assay

MCF-7 breast cancer cells → estrogen-depended growth

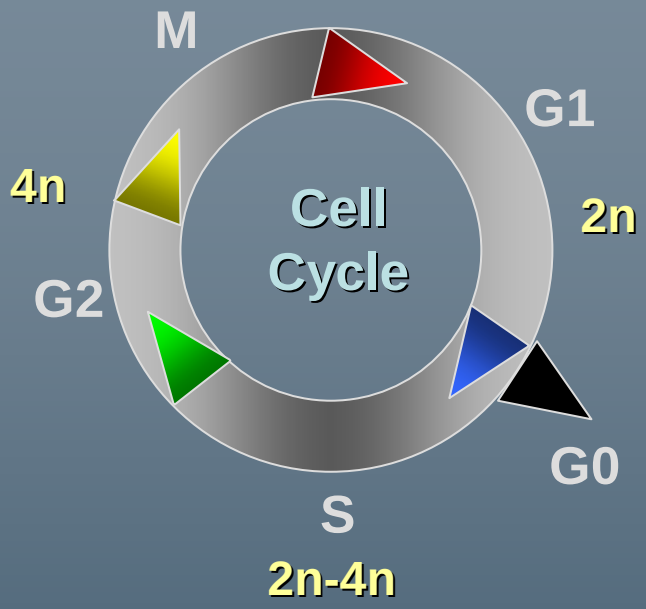
Basic principle

no estrogens → no growth

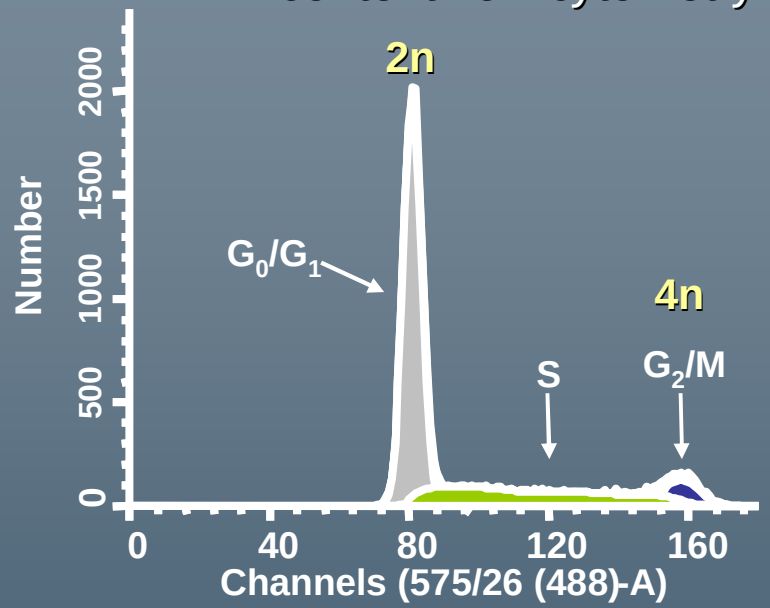


Flow cytometric cell cycle analysis

Flow-cytometric cell cycle analysis



DNA content flow cytometry



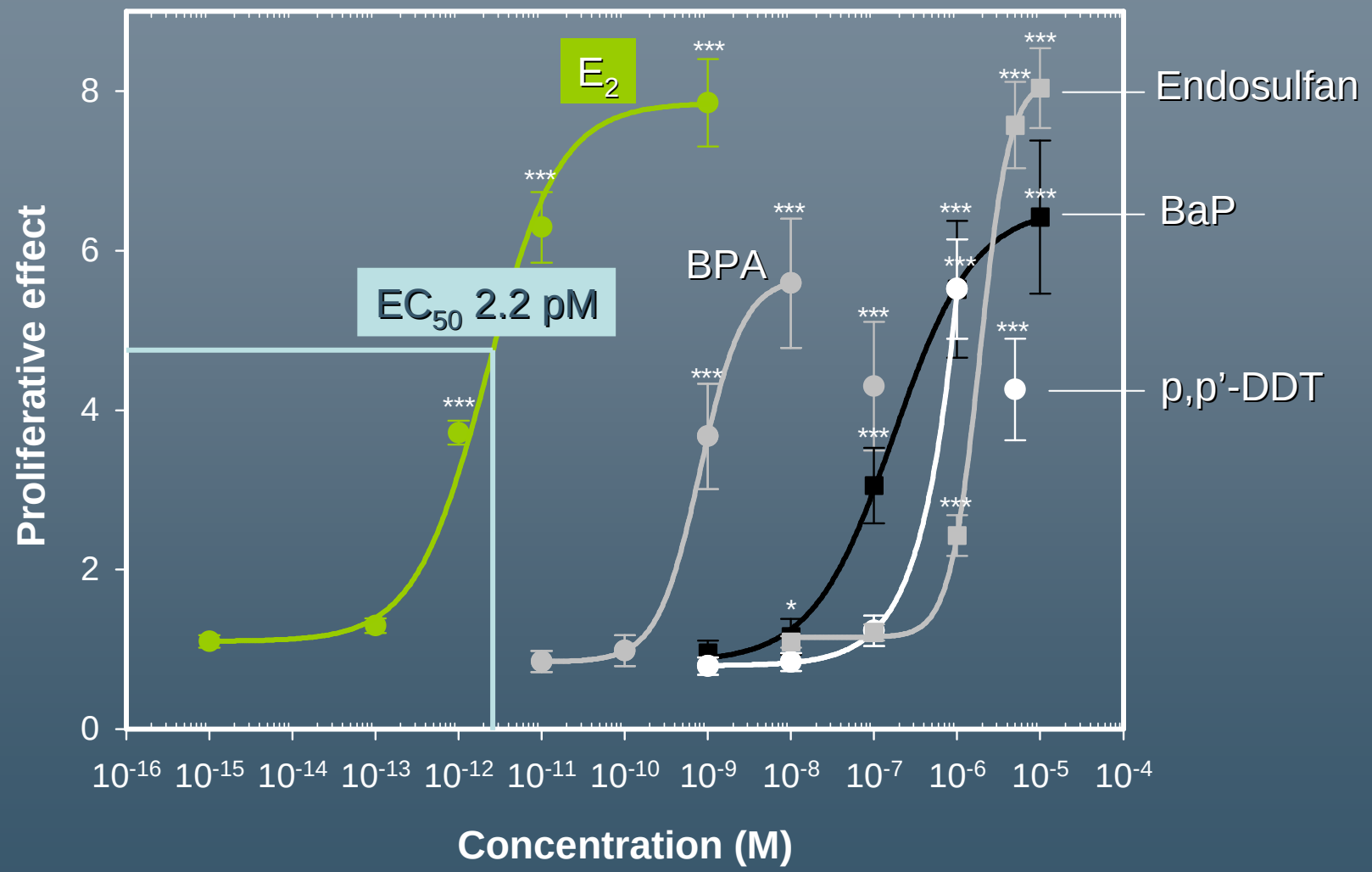
Estrogenicity

% cells in S(ynthesis)-phase
=
proliferative effect

% of cells in each phase of the cell cycle

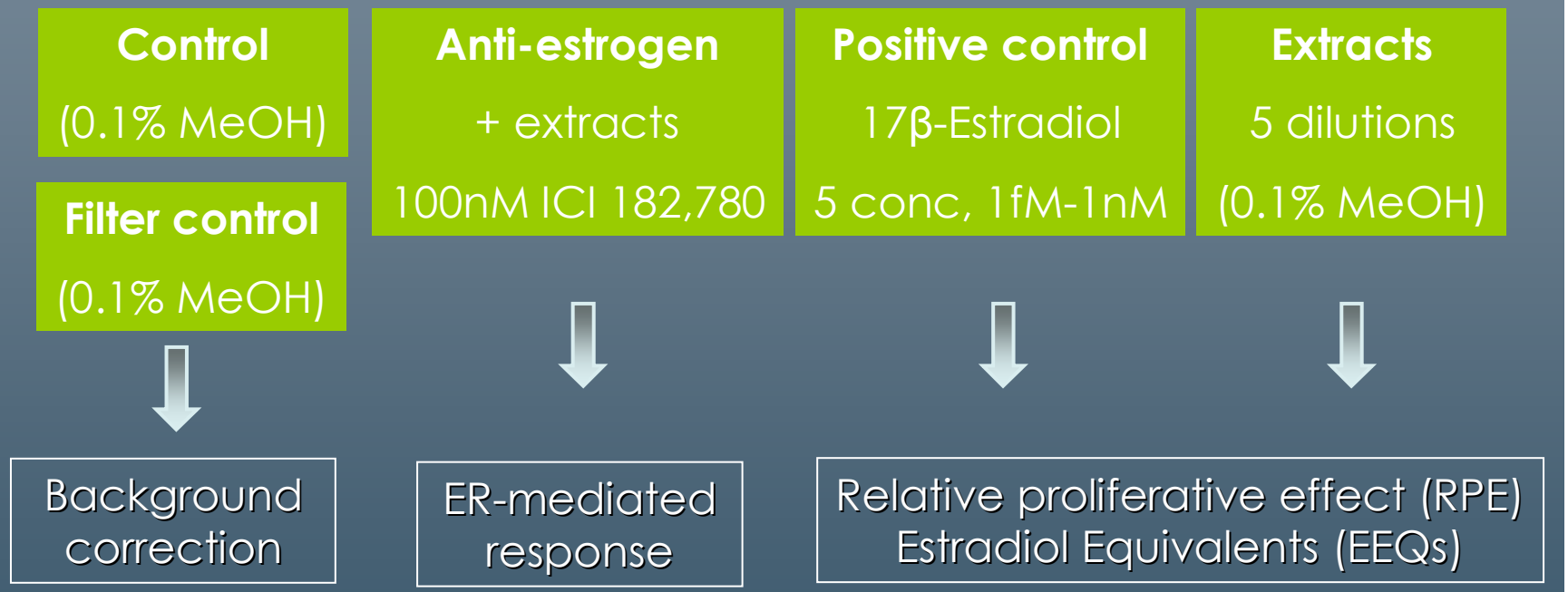
	G_0/G_1	S	G_2M
control	83%	**10%**	7%
E_2	37%	**47%**	15%

Chemicals


E2
EC50 2.2 pM
BPA
Endosulfan
BaP
p,p'-DDT
Proliferative effect
0
2
4
6
8
10-16 10-15 10-14 10-13 10-12 10-11 10-10 10-9 10-8 10-7 10-6 10-5 10-4
Concentration (M)

Environmental extracts

Experimental set-up

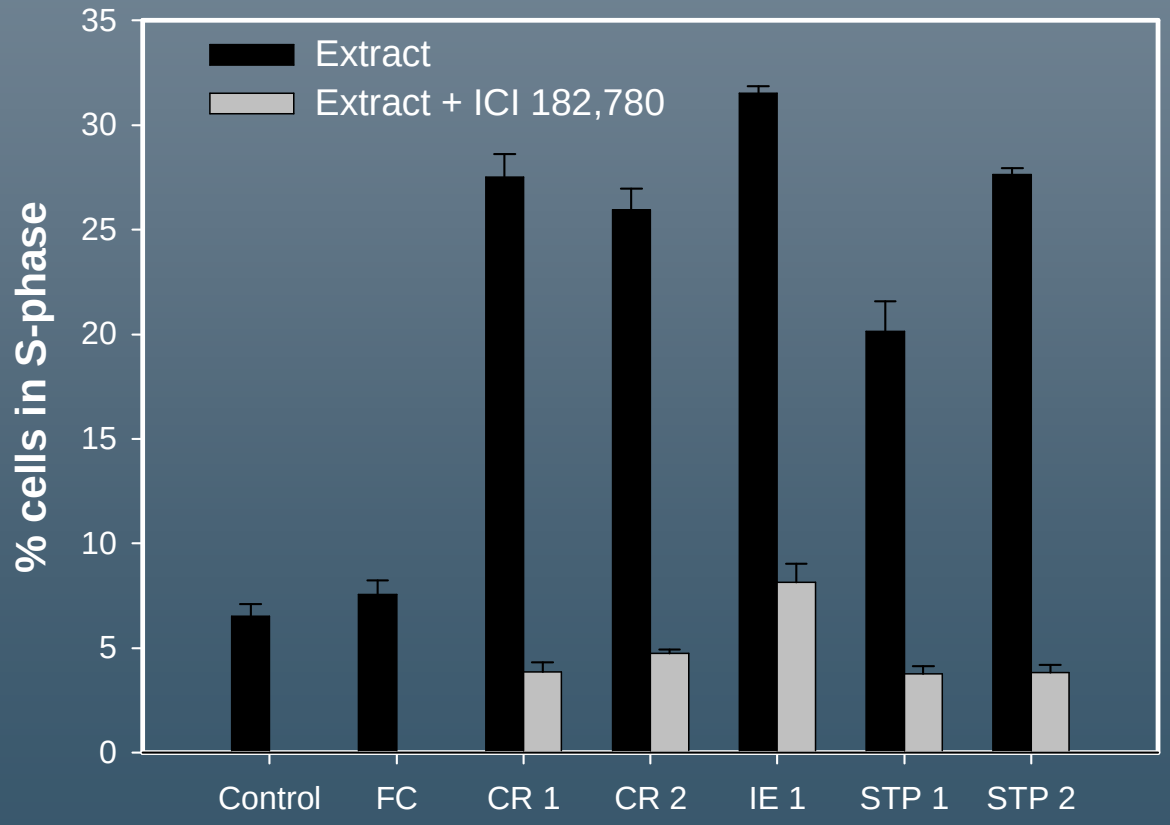


Environmental extracts

Anti-estrogen

+ extracts

100nM ICI 182,780

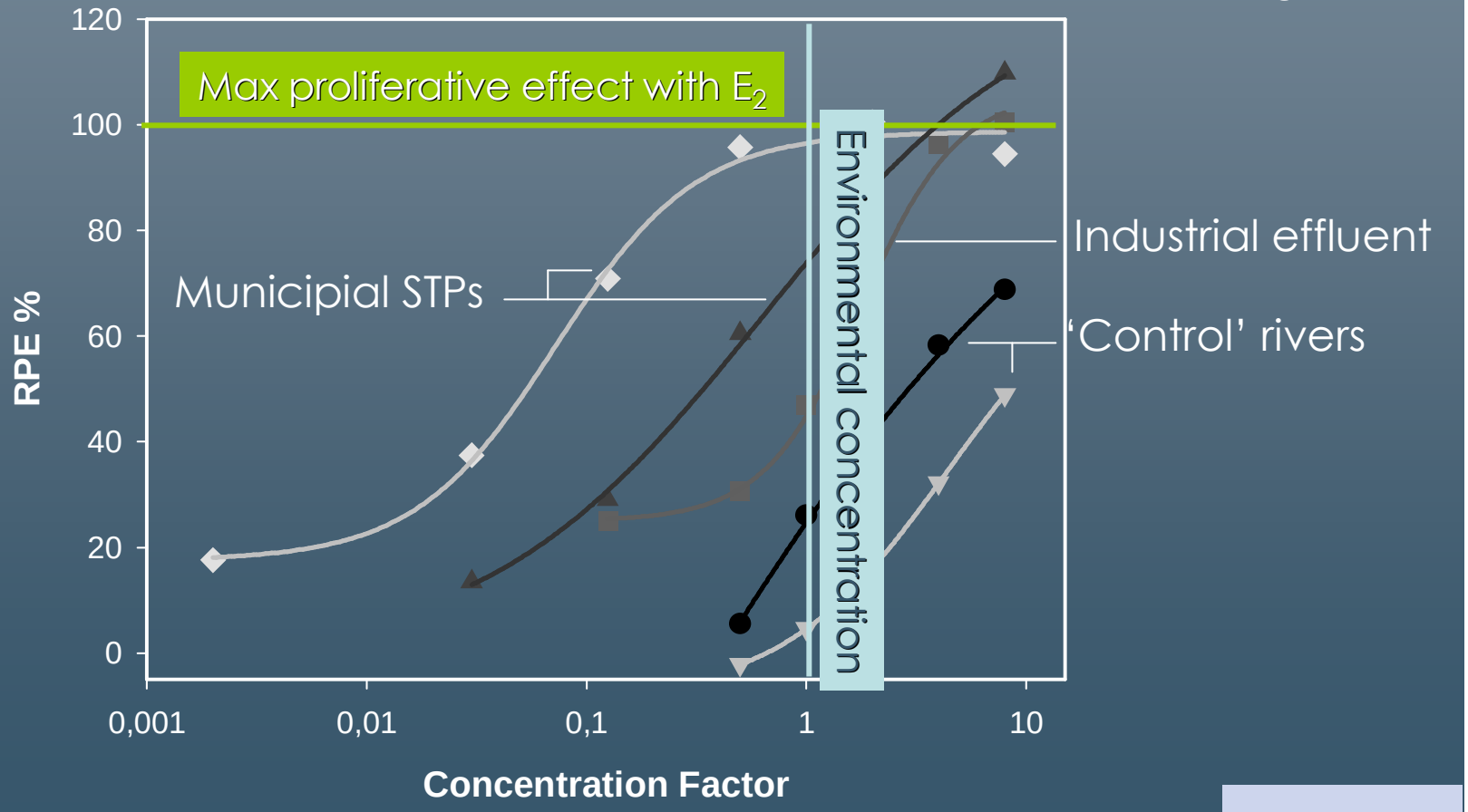


Environmental extracts

Positive control
17β-Estradiol
5 conc, 1fM-1nM

Extracts
5 dilutions
(0.1% MeOH)

EEQ
CR2: 0.362 ng/L
CR1: 0.215 ng/L
IE: 0.363 ng/L
STP: 8.011 ng/L
STP: 1.035 ng/L



General Conclusions

- In vitro assays provide mechanistic information, which can be used for a better understanding of in vivo results.
- In vitro based biomonitoring offers a rapid activity screening that can be used as a ranking system for quality of surface water and/or sewage treatments plants