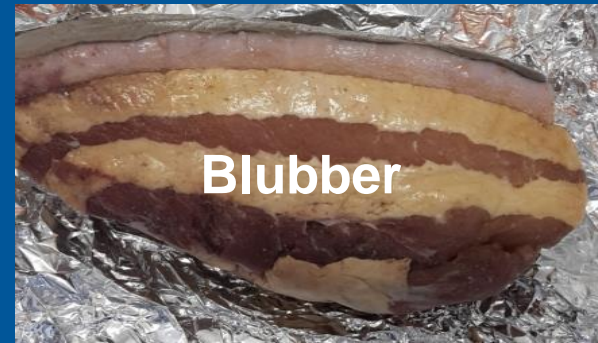


equilibrium
←→



Mixture effects of environmental pollutants in passive sampling extracts of blubber tissue of marine mammals

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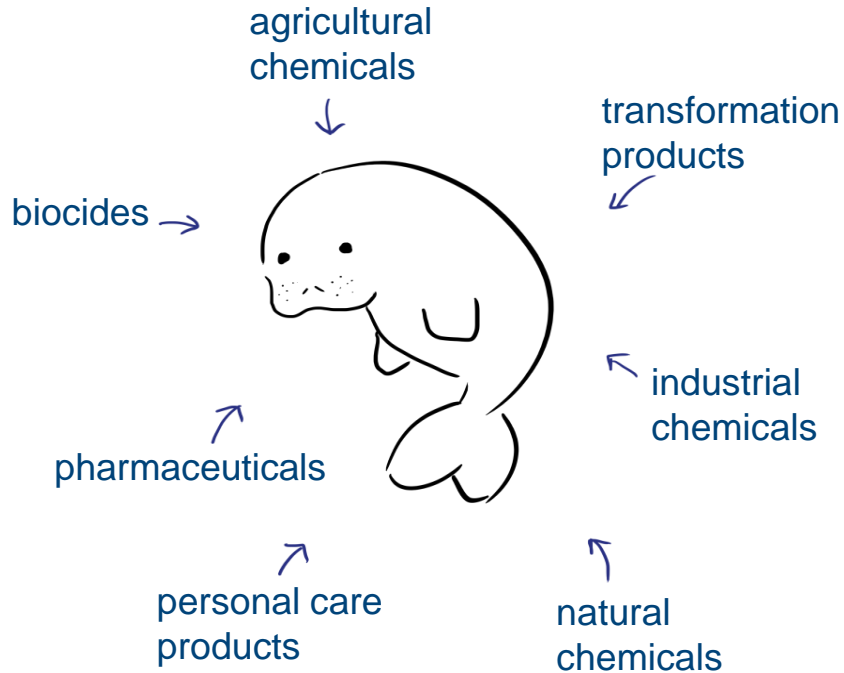
³Environmental Toxicology, Center for Applied Geoscience, Eberhard Karls University Tübingen, 72074 Tübingen, Germany



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11.05.2018

Background



→ **Mixture effect:**
Combined effect of all chemicals in a sample

blubber tissue

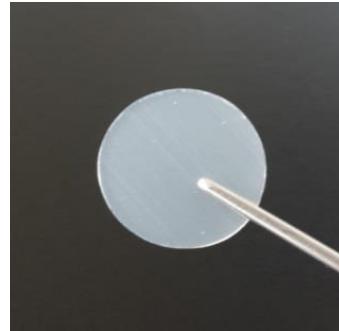
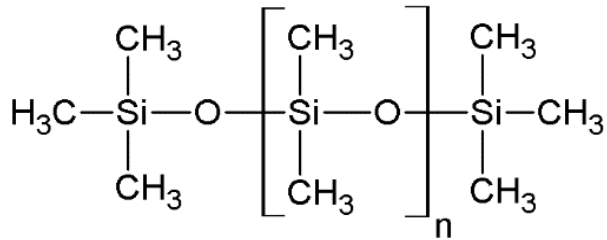


→ What types of effects are caused by the chemical cocktail?

Equilibrium passive sampling

- Conventional exhaustive extraction requires a clean-up to minimize bioanalytical interferences (matrix effects)
 - limiting spectrum of chemicals

PDMS
(Polydimethylsiloxane)



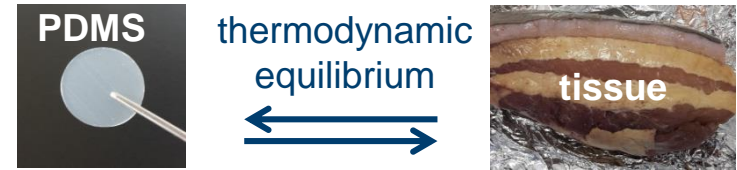
thermodynamic
equilibrium

←→



- Examination of mixture effects with bioanalytical tools

Equilibration kinetics



→ Equilibration kinetics:

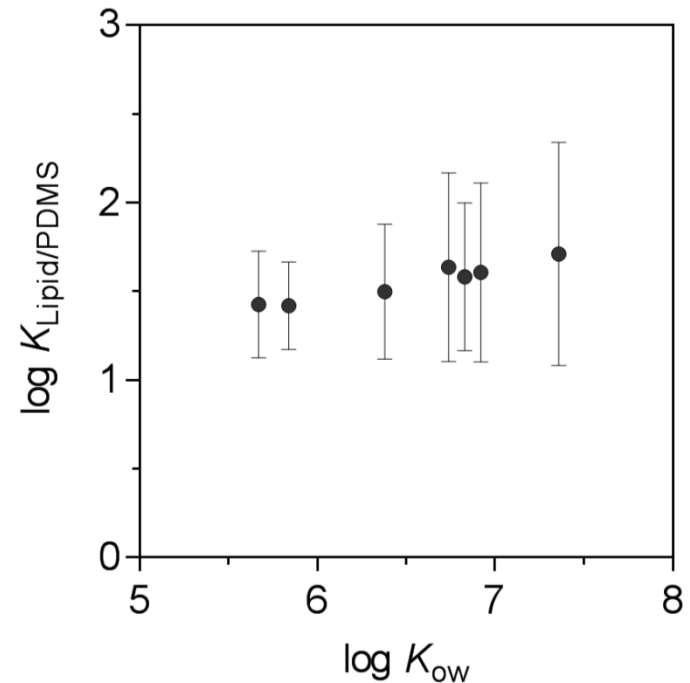
When will the thermodynamic equilibrium be reached?

- Blubber spiked with seven indicator PCBs (PCB 28, 52, 101, 118, 153, 138, 180)
- Samples collected at 8 time points between 10 min and 96 h

→ Equilibrium was reached within 1.5 (PCB 118 and 180) to 2.6 (PCB 28) hours

→ $K_{\text{Lipid/PDMS}} = 36.7 \pm 8.5$

→ Sampling for 24 h



mean \pm SD, $n=7$

Bioanalytical tools to assess cellular effects

Cellular toxicity pathway

Metabolism

Interaction with target

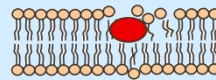
Defense

Cell death

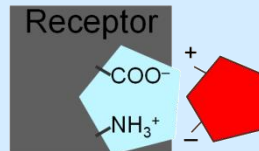
What can be measured?

Induction of xenobiotic metabolism pathways

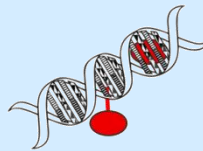
Non-specific toxicity



specific, receptor-mediated toxicity



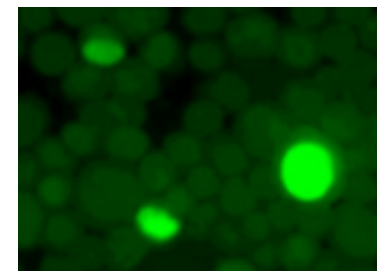
reactive toxicity



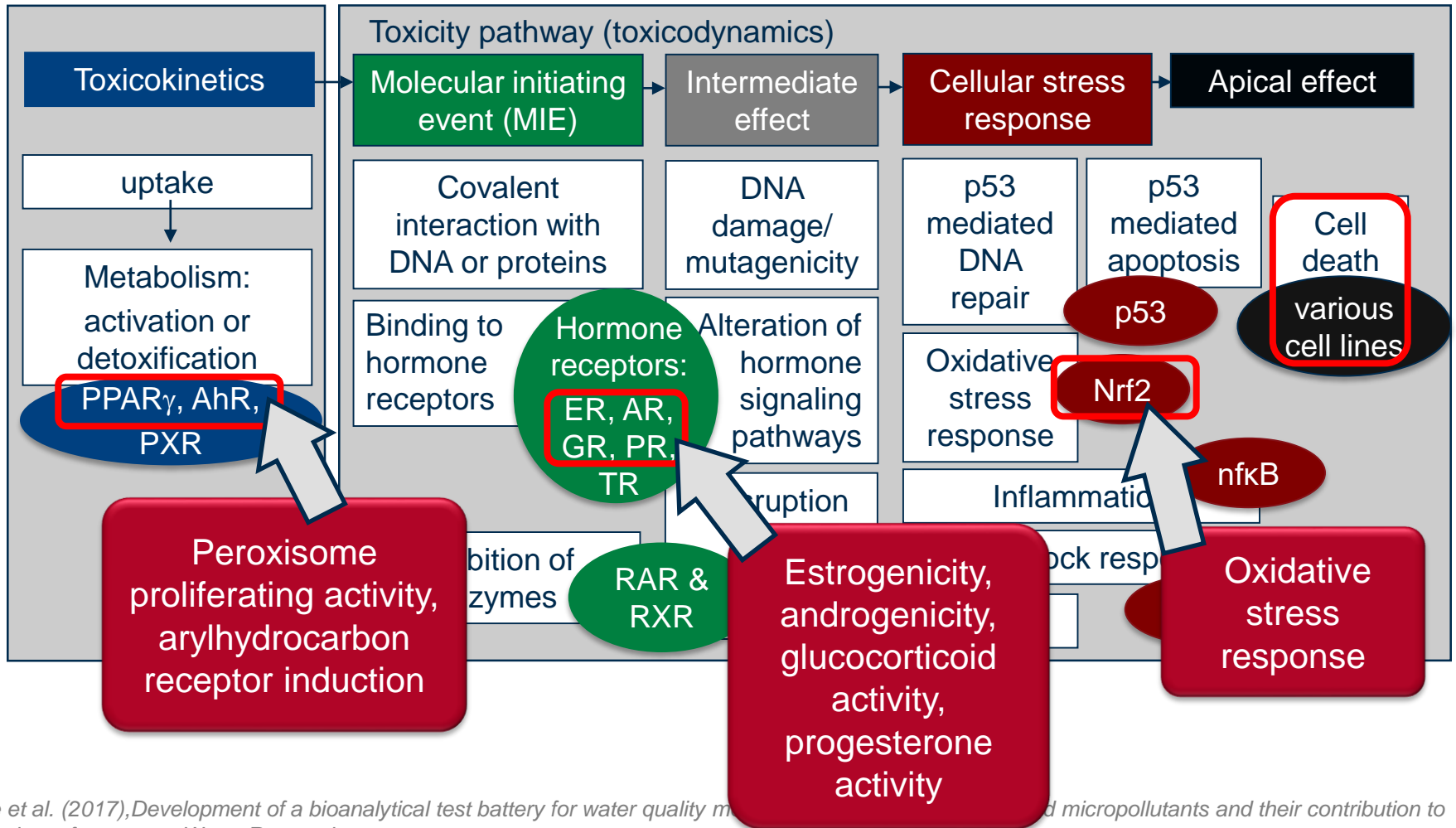
Induction of general stress response pathways

Cell viability (cells representative for system response)

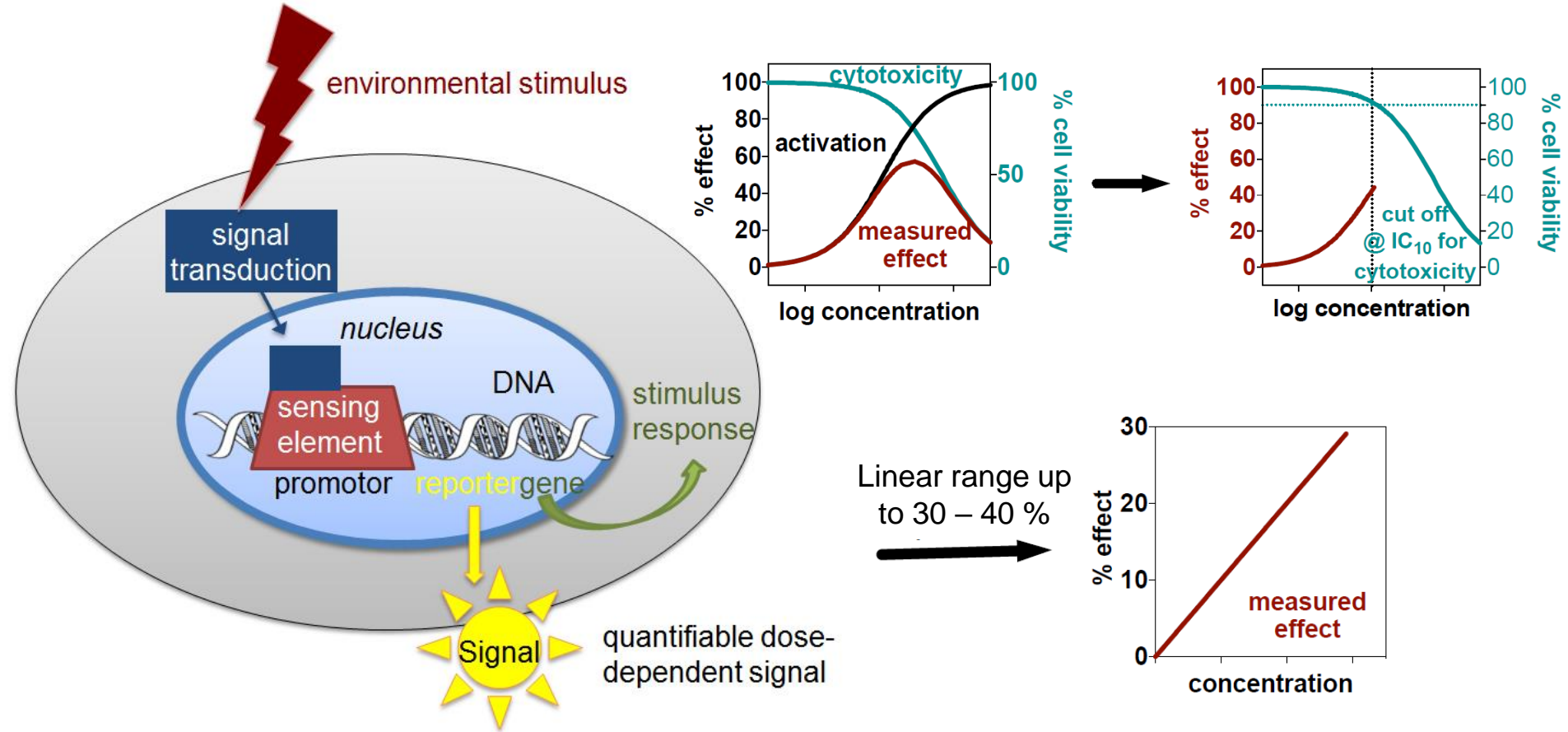
How can it be measured?
- *in vitro* cell-based assays
→ reporter gene assays



Bioanalytical tools to assess cellular effects



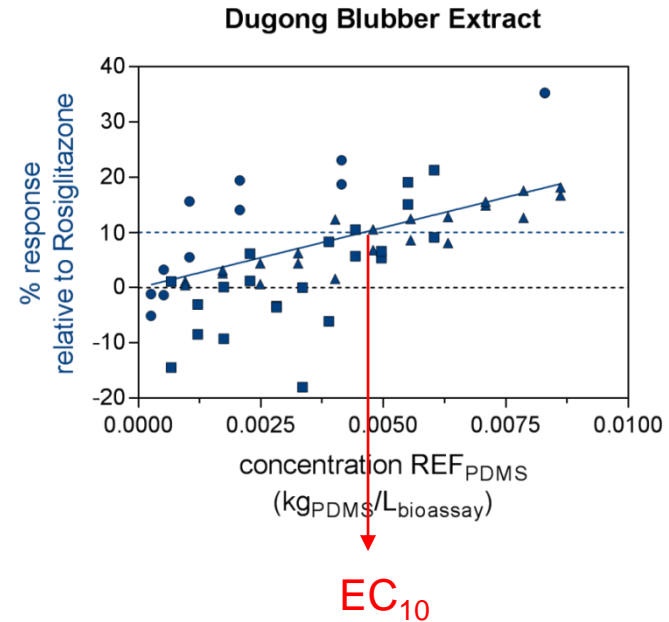
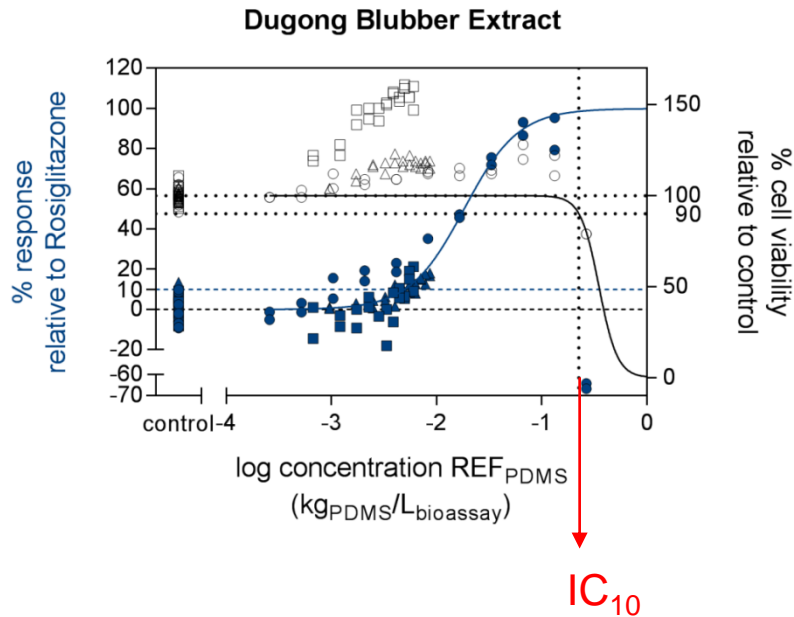
Cell-based bioassays



Dose-response in cell-based bioassays

1. dosed at high levels with serial dilution (> cytotoxicity)
2. repeated with linear dilution in the target range

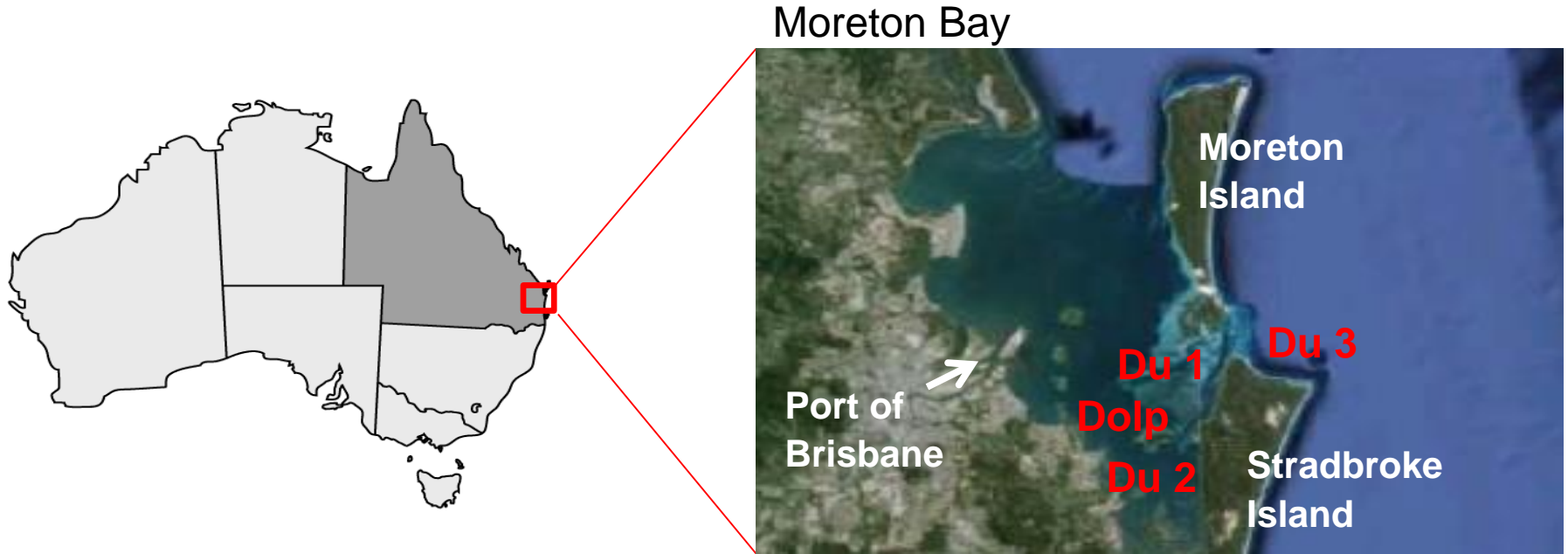
→ derive EC_{10} data



For oxidative stress
response assay : $EC_{IR1.5}$

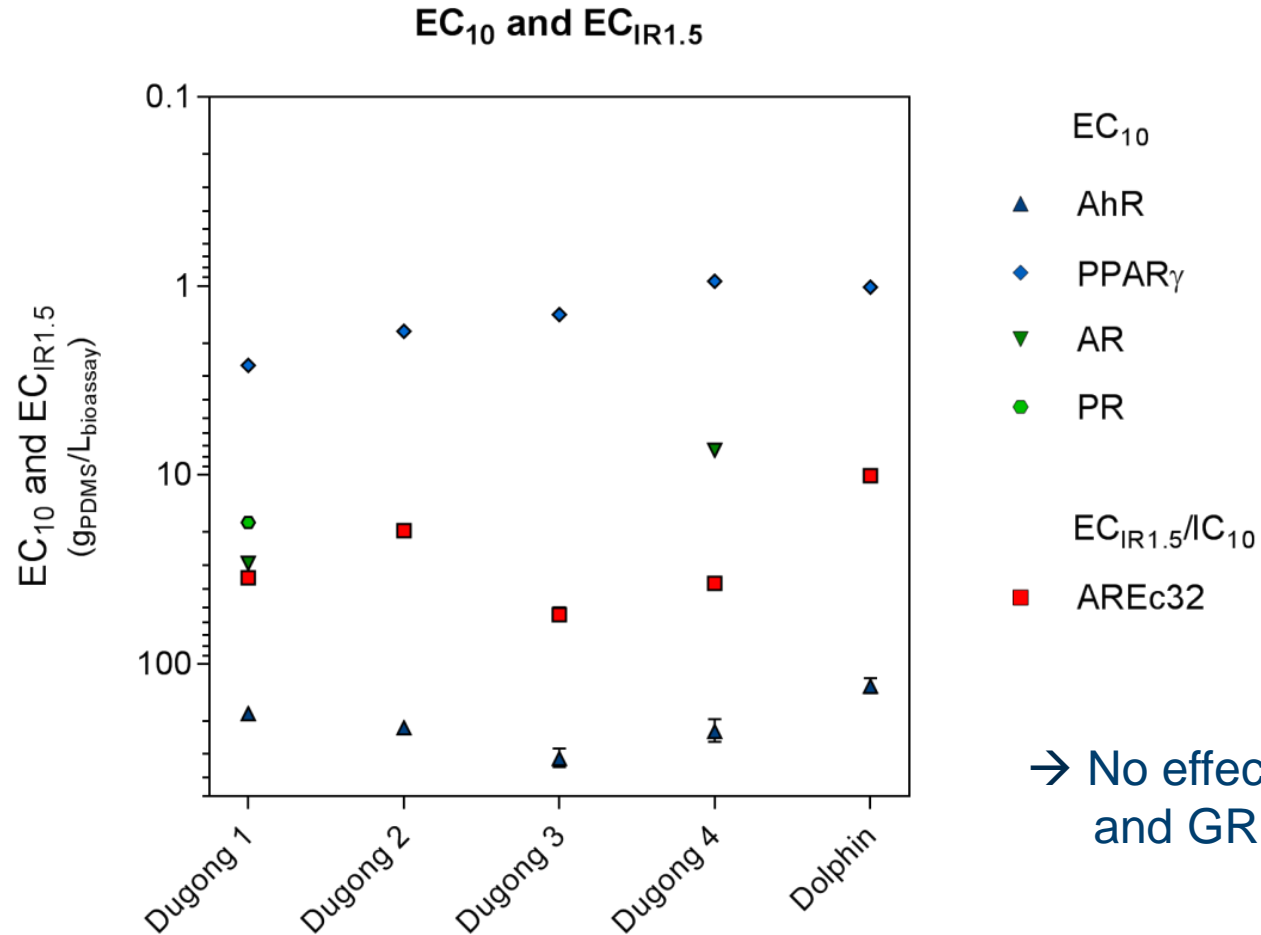
Samples: 4 dugongs (*Dugong dugon*) and 1 dolphin (*Sousa saahulensis*)

pilot study



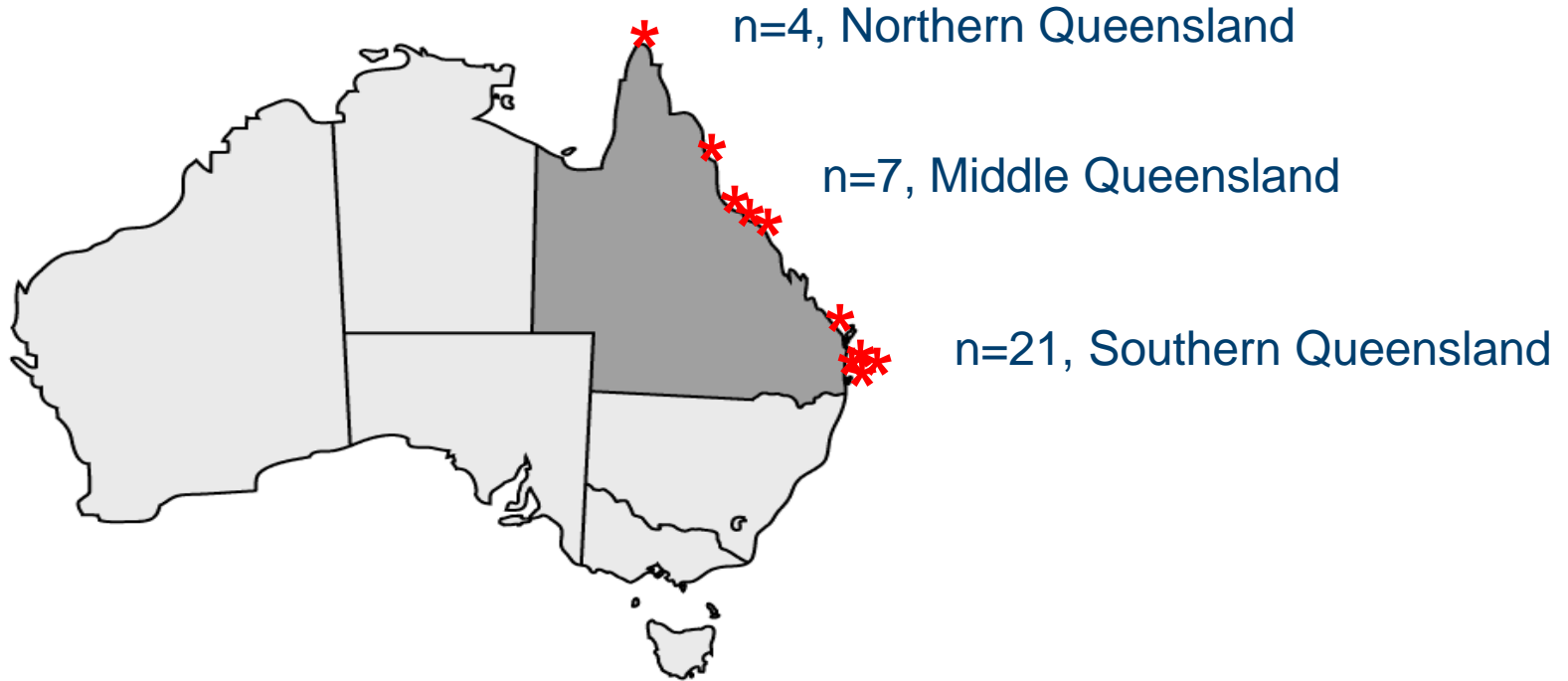
adapted from Weijs et al. (2016), Screening of organic and metal contaminants in Australian humpback dolphins (*Sousa saahulensis*) inhabiting an urbanised embayment, *Chemosphere*.

Specific responses of mixtures extracted from blubber



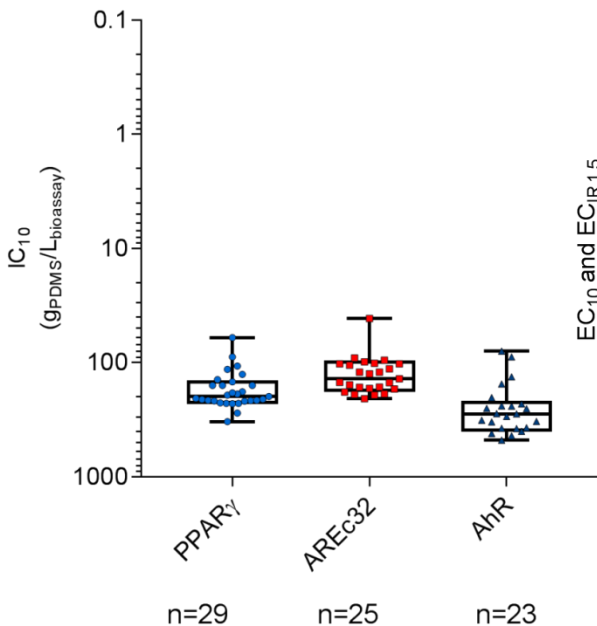
→ No effects detected with ER α and GR assays

Samples: 33 dugongs (*Dugong dugon*)

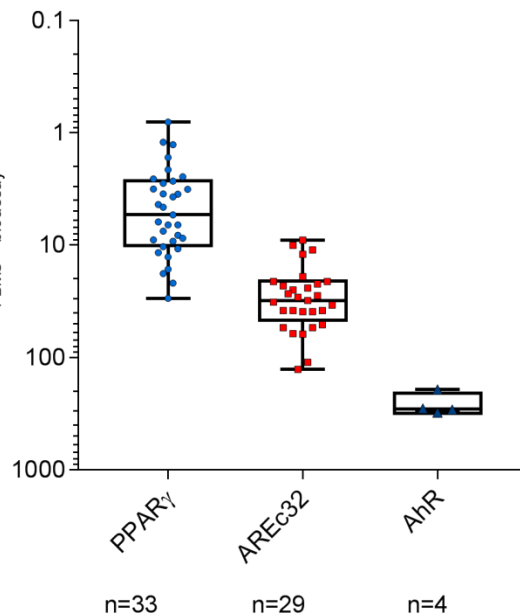


Cytotoxicity and activation of specific mode of action

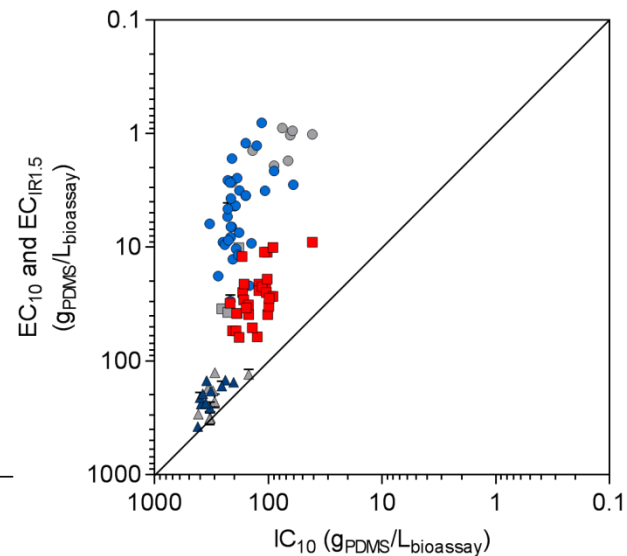
Cytotoxicity: IC_{10}



Specific response:
 EC_{10} or $EC_{IR1.5}$



How specific are the responses of mixtures extracted from blubber?

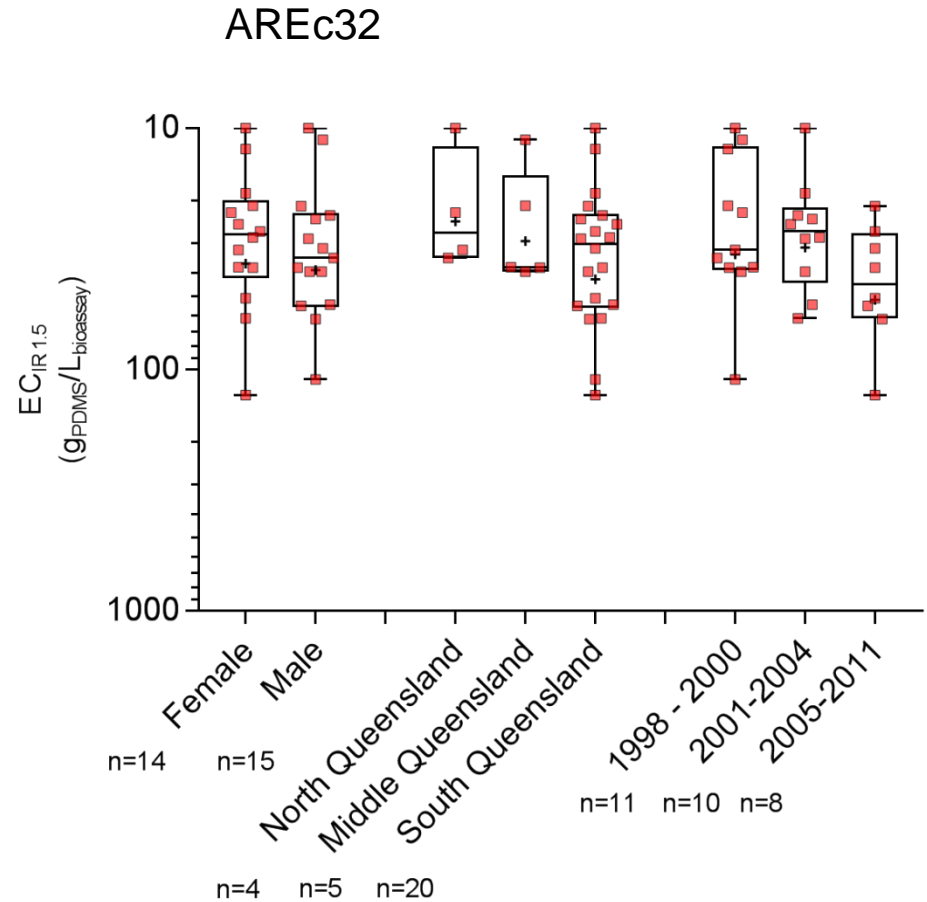
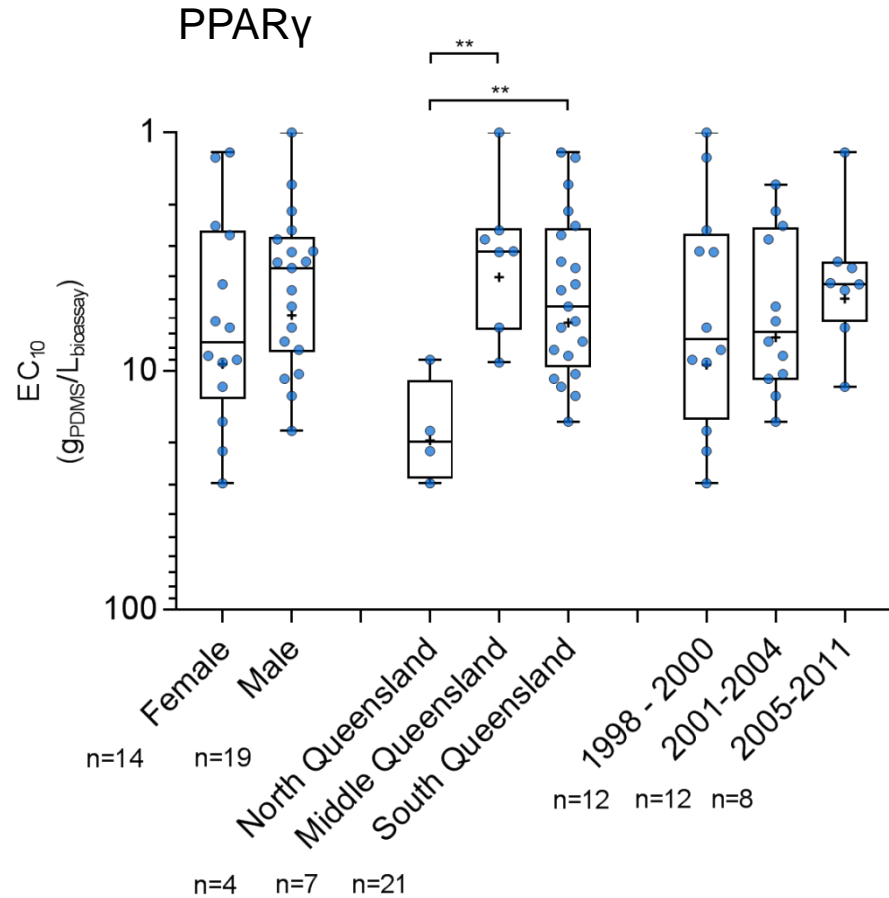


EC_{10} / IC_{10}

$EC_{IR1.5} / IC_{10}$

- ▲ AhR
- PPAR γ
- ▲ AhR (pilot study)
- PPAR γ (pilot study)
- AREc32
- AREc32 (pilot study)

Are there any sex, spatial or temporal trends?



1-way ANOVA:
 $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***)

From mixtures in PDMS to mixtures in blubber

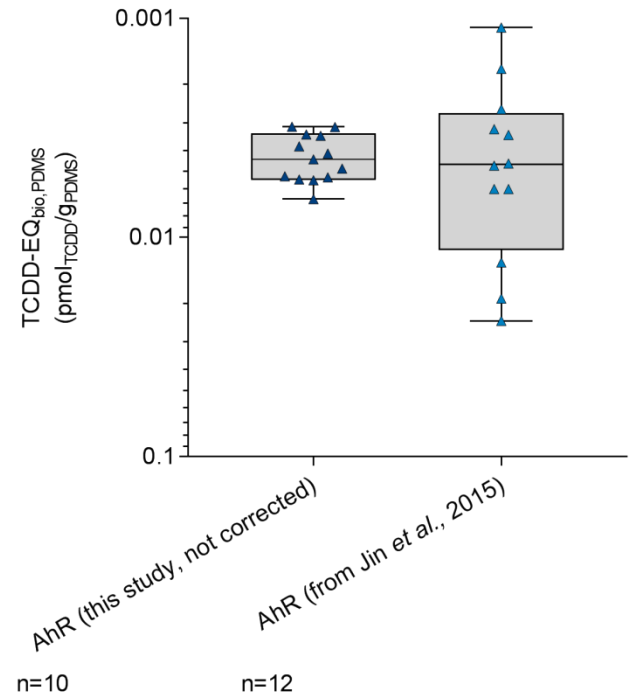
- Thermodynamic equilibrium was reached within 1.5 (PCB 118, 180) to 2.6 (PCB 28) hours
- $K_{\text{Lipid/PDMS}} = 36.7 \pm 8.5$
- No significant differences between sex or strand year
 - Dugong has a long life span
 - no information of age was available
- PPAR γ activity lower in animals from tropical North Queensland

Further steps:

- Chemical analysis of the extracts
- Total extraction of samples

Bioanalytical equivalent - BEQ:

$$\text{BEQ}_{\text{bio, PDMS}} = \frac{\text{EC}_{\text{reference compound}}}{\text{EC}_{\text{PDMS}}}$$



Acknowledgements



- Department Cell Toxicology at UFZ Leipzig
- Bioassay team

