Monitoring of Dutch waters by CALUX panel

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Biodetection Systems

Why monitor surface waters with bioassays?

- Compounds are present – as a complex mixture – that can influence biological pathways
- Some compounds can do this at relatively low concentrations
- We cannot determine all compounds chemically
- …. and if we could we would most of the time not know their biological effect(s)

Bioassay monitoring - past

- **Bioanalysis of endocrine disruptors**
  - Equals estrogens in many cases
  - Screening projects (e.g. LOES)

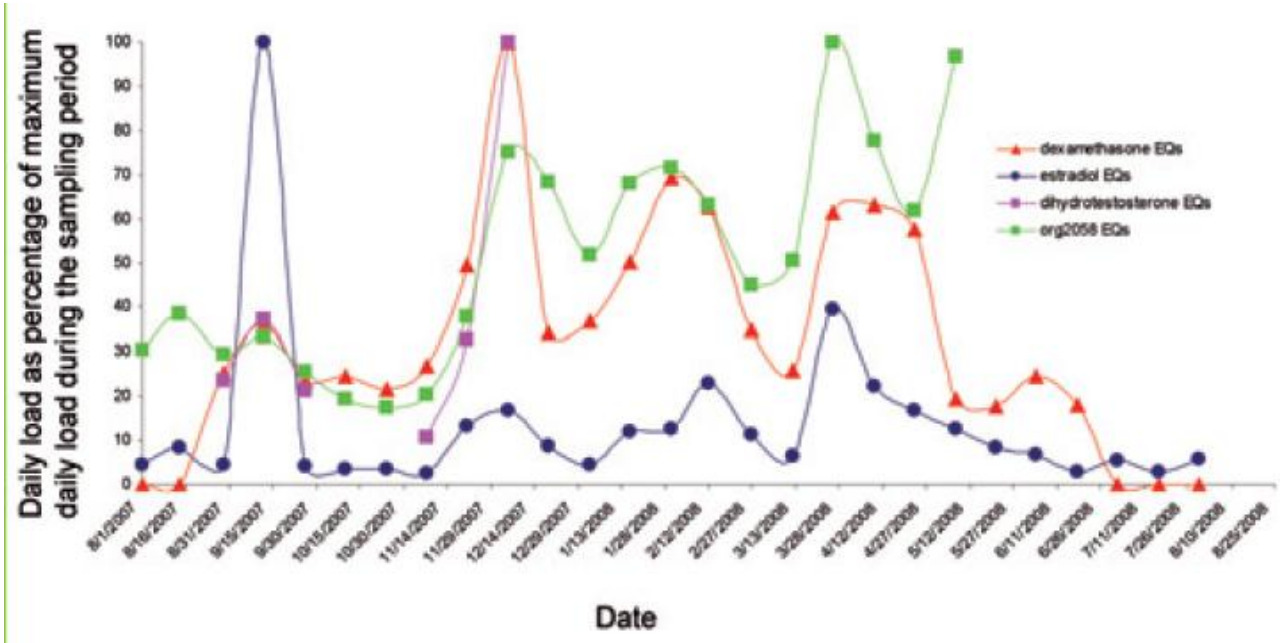
- **Chemical analysis**
  - TIE in combination with assays
  - Natural hormones, synthetic hormones, industrial chemicals, pesticides

What classes of compounds can be expected?

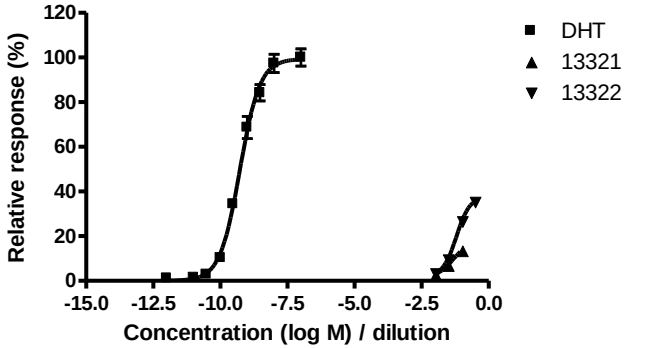
- **Pharmaceuticals**
  - potent compounds, biological activity intended for humans/animals, low concentration?
- **Pesticides**
  - Biological activity intended for plants/insect/mammals, non-human targets, possibly potent compounds
- **Personal Care Products**
  - Activity non-intended, low potency
- **Excretion products**
  - (Degradation products of) natural ligands, possibly potent
- **Industrial chemicals**
  - High volume, low potency?, biological activity generally not intended

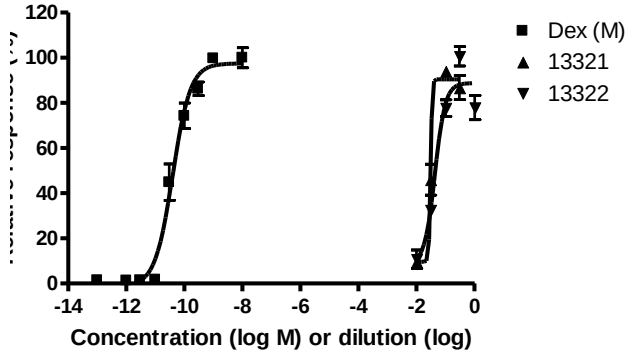
Bioassay monitoring - present

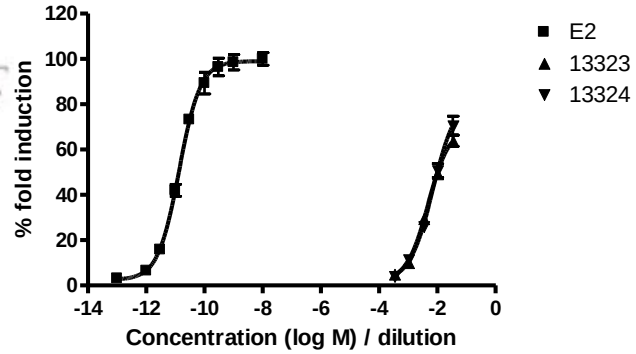
• CALUX panel for four different types of hormonal activity (AR, PR, GR, ER)



RIWA (2009)

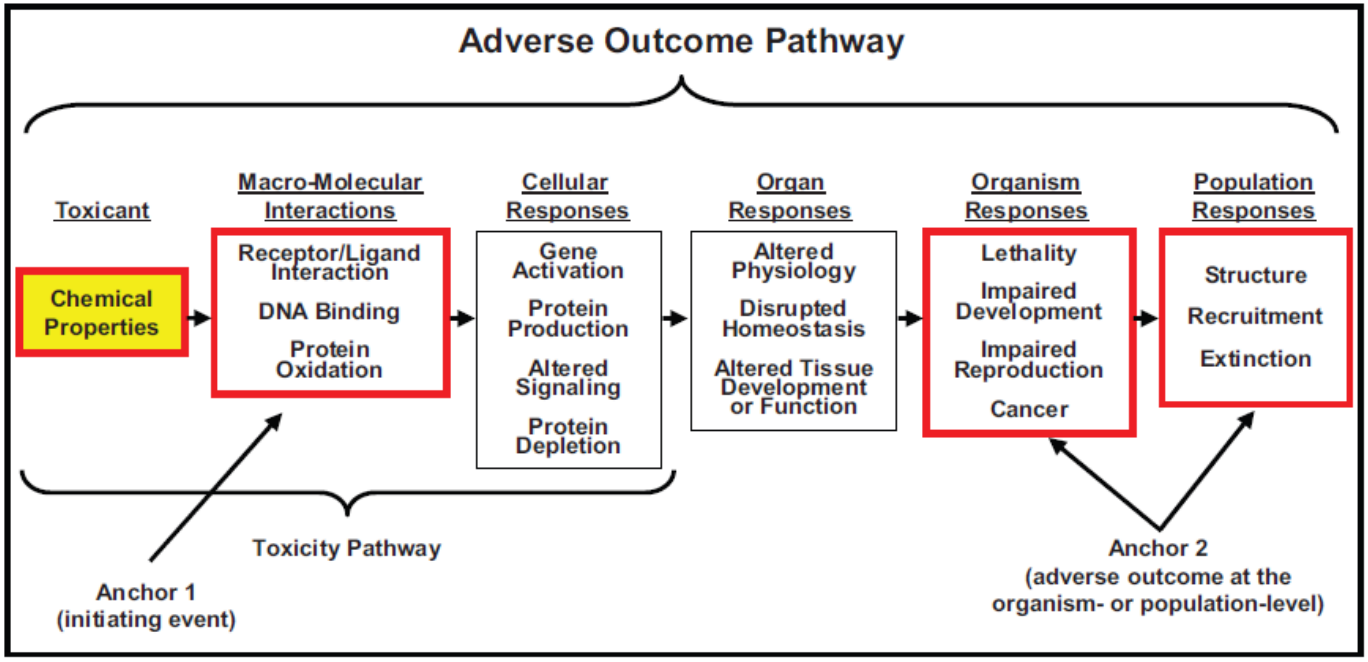






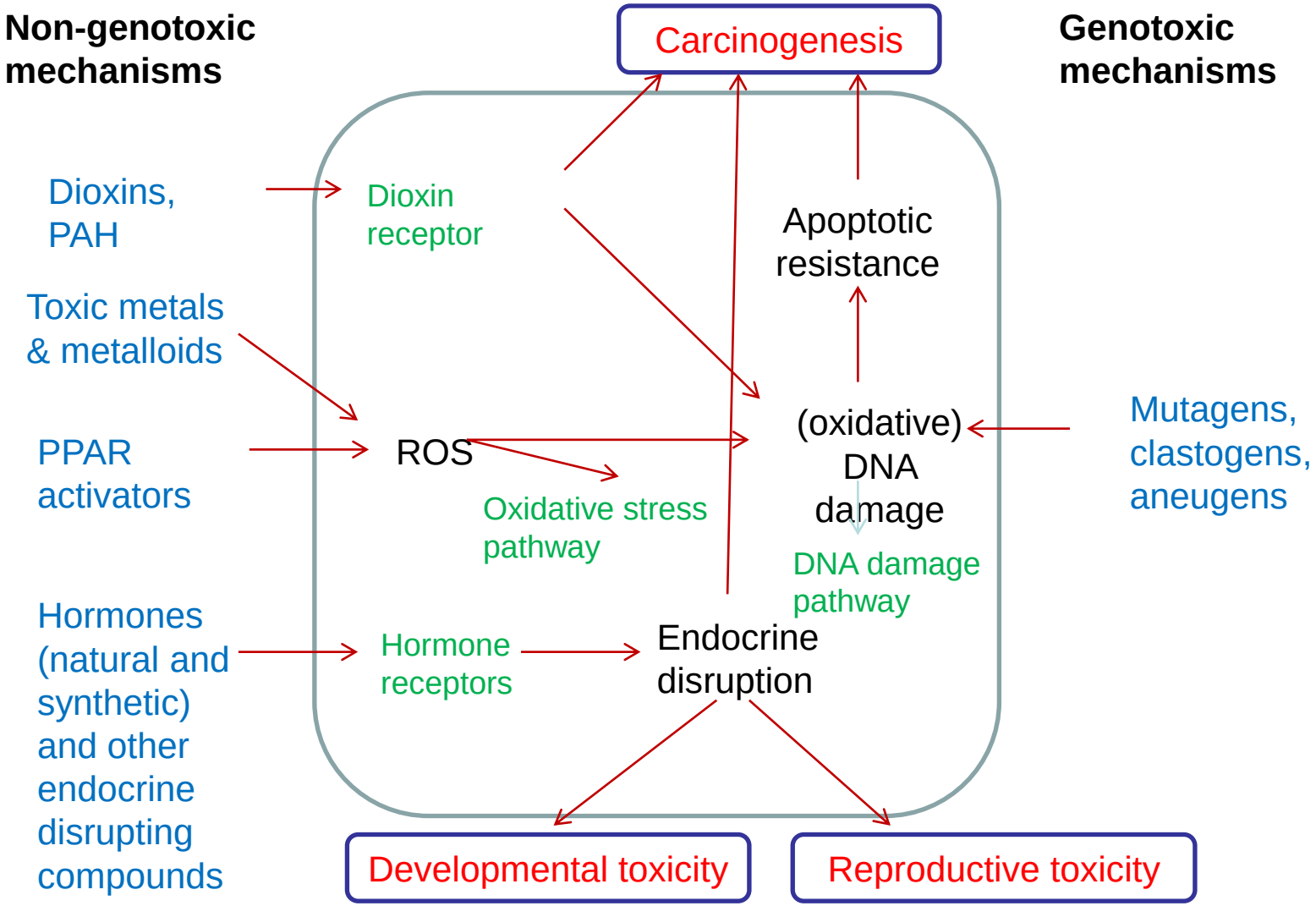
Bioassay monitoring - future

- **Screen for activity on multiple pathways**
  - Adverse outcome pathways (Ankley et al (2010), NRC, Tox21)

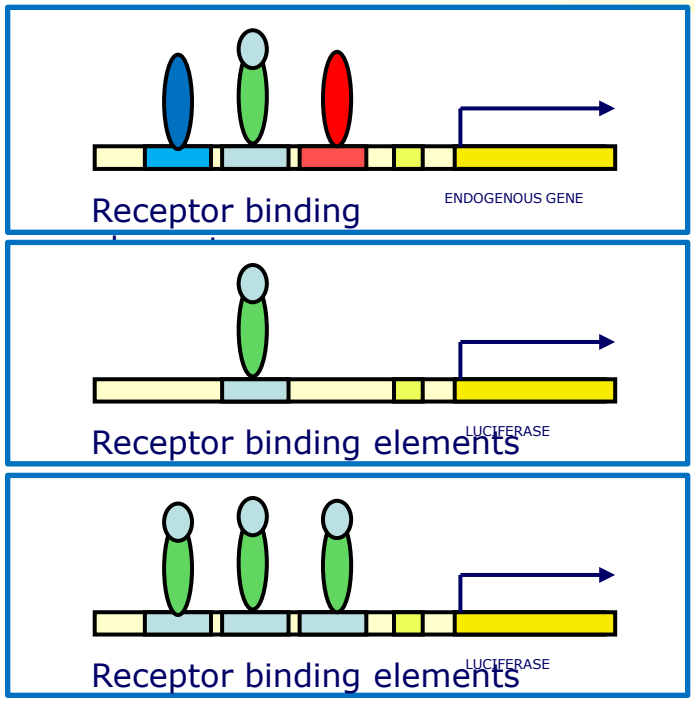


- **But how many pathways?**
  - Focus on endocrine disruption, reproductive toxicity, genotoxicity

Which pathways should be monitored?



Which assays are available?



endogenous situation

selective

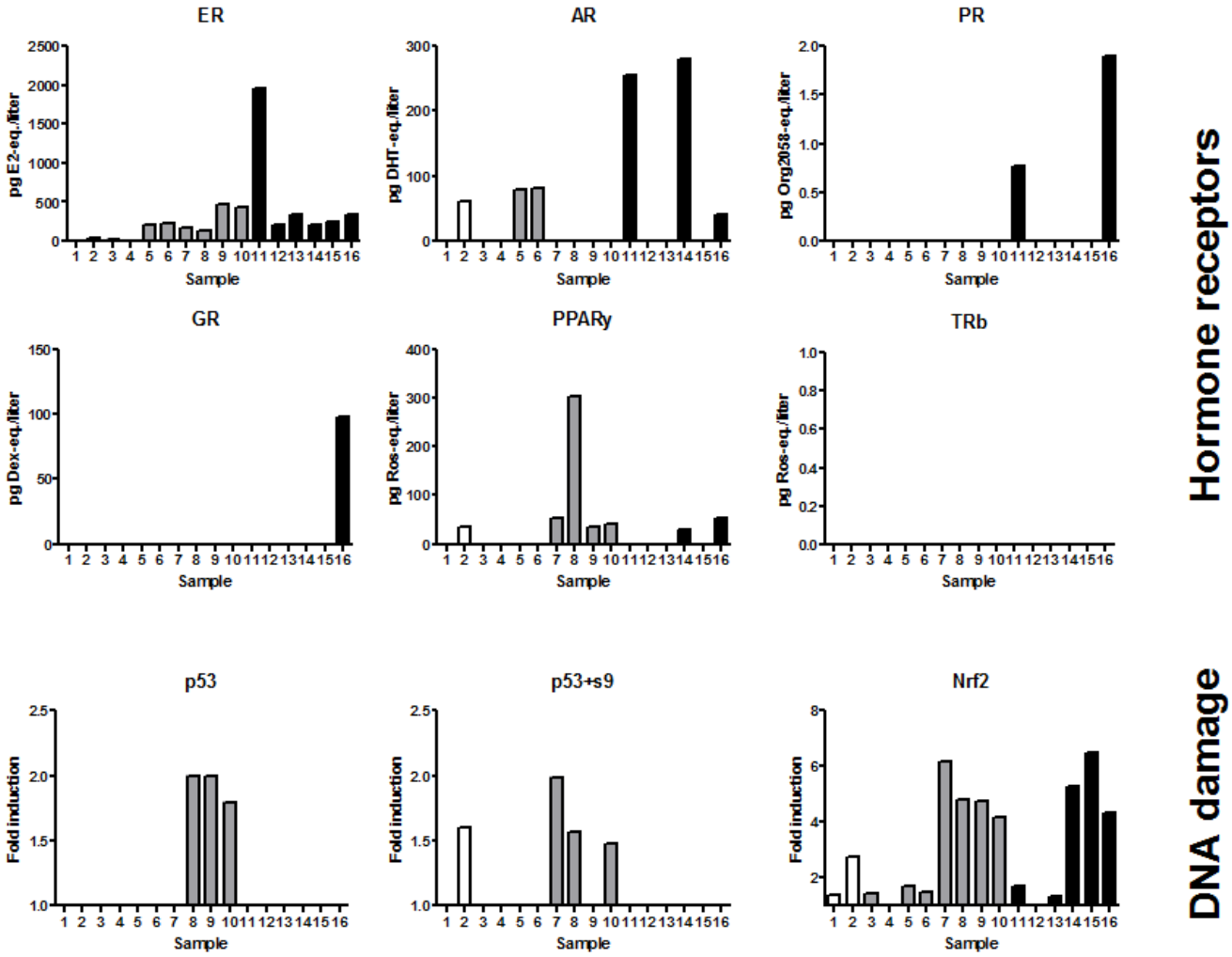
selective and responsive

- **Nucleur receptors**
  - DR, PAH, ER(α,β), AR, PR, GR, TRβ, RAR, PPAR(α, γ), LXR
- **Signaling pathways**
  - NFκB, Nrf2, TCF, ESRE, AP-1, p21, p53, Hif1α

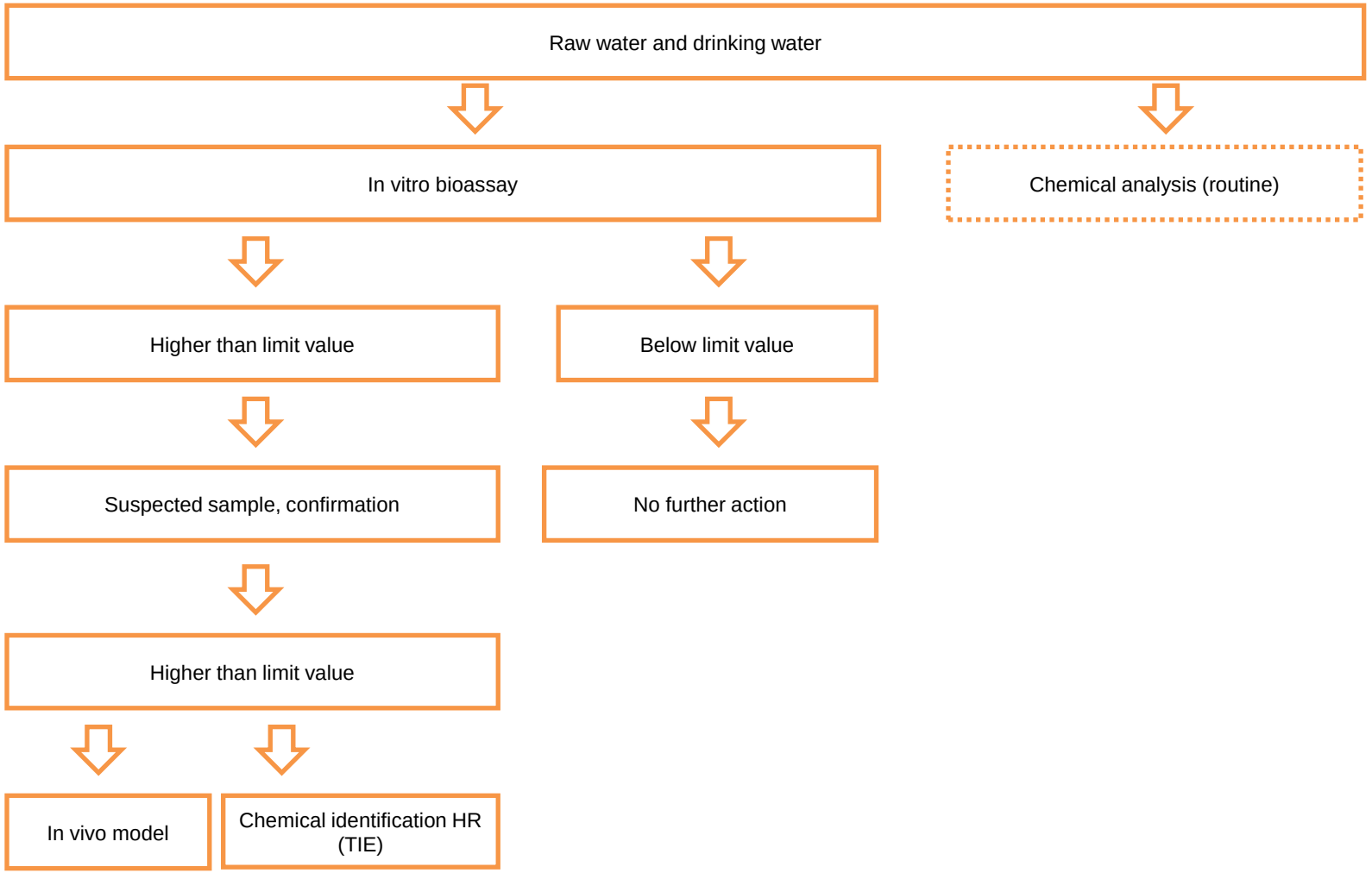
Expand panel with additional pathways

- **P53**
  - marker for genotoxic stress
  - with and without metablic activation
- **Nrf2**
  - involved in oxidative stress response
- **PPARgamma**
  - involved in cellular differentation and metabolism
  - part of PPARgamma receptor family (under development)
- **And additional assays….**

Which types of activity are detected?



How to incorporate bioassays in monitoring?



Trigger values for bioassays

- **To use bioassay results for screening, trigger values are needed!**

  Trigger values

  > more detailed examination warrented

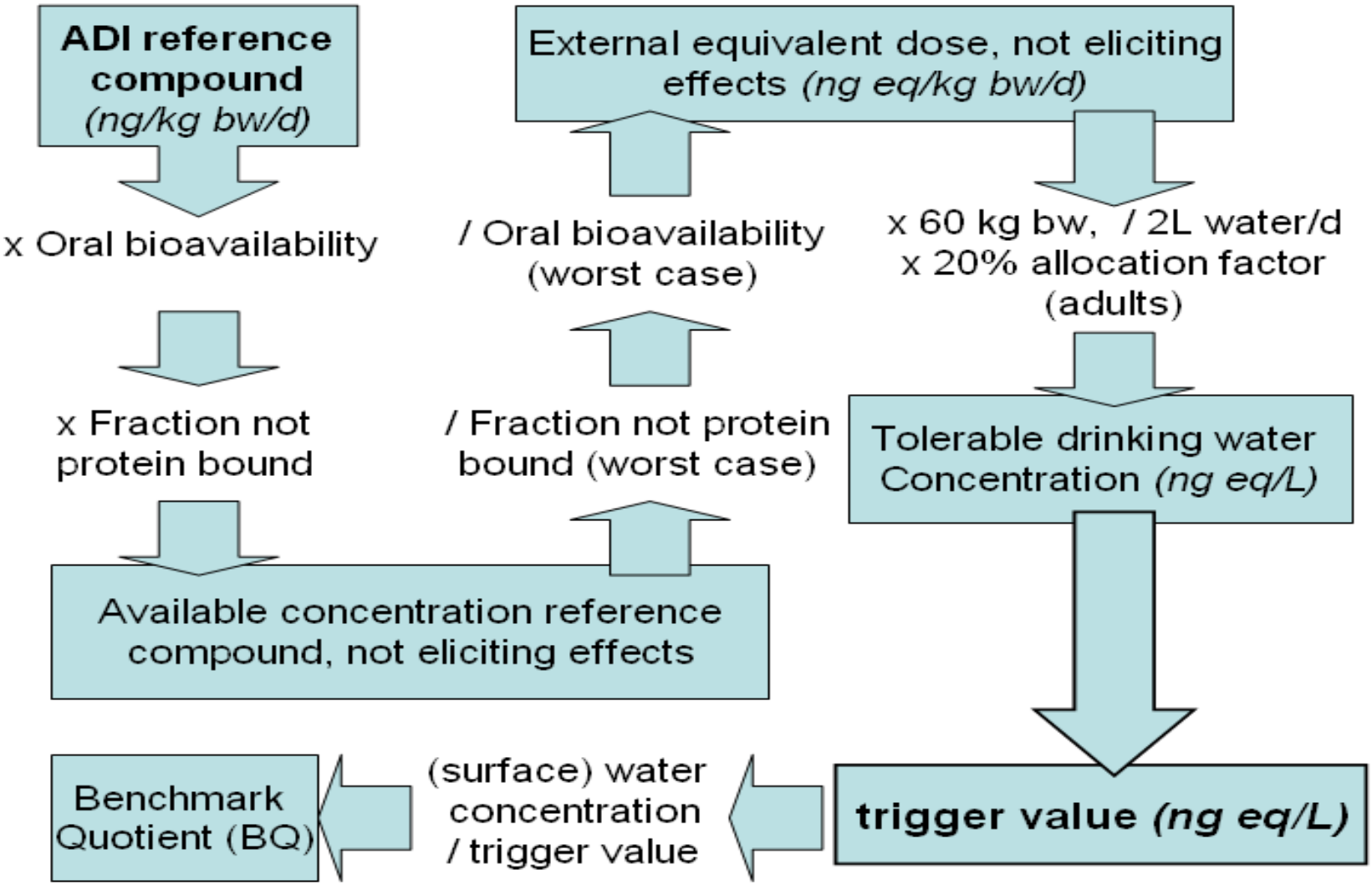
  < health risks can be waived

- **Bioassay results indicative for total amount of active compounds…**
- **…but compound identity is unknown!**

Point of departure for trigger values

- **Provisional Acceptable or Tolerable Daily Intake (ADI/TDI) of reference compound (WHO/JECFA)**
- **Pharmacokinetic factors for bioavailability reference compound**
- **“Worst-case” assumptions pharmacokinetic factors other compounds**
- **WHO body weight (60 kg), volume drinking water (2L)**
- **WHO allocation factor for drinking water (20%)**

How to derive trigger values?



(Brand *et al, in prep*.)

Trigger value for estrogens (as example)

ADI estradiol (E2)	50 ng/kg bw/d *(WHO/JECFA)*
Oral bioavailability	5%
Free fraction	2%
Available concentration	50 x 0.05 x 0.02 = 0.05 ng/kw/bw
External equivalent dose	0.05 / 0.50 / 0.50 = 0.2 ng E2-eq/kg bw/d
Trigger value	0.2 x 60 kg bw / 2 L x 20% = **1.2 ng E2-eq/L**

With the help of….

BioDetection Systems

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Thank you for your attention!