3rd NORMAN workshop: New tools for biomonitoring of emerging pollutants, IVM Amsterdam, The Netherlands

A bioassay-directed monitoring strategy to assess the risks of complex pollutant mixtures in drinking water

Ron van der Oost & Minne Heringa

Waternet: water cycle management

Surface and ground water

Drinking water

kiwa

Partner for progress

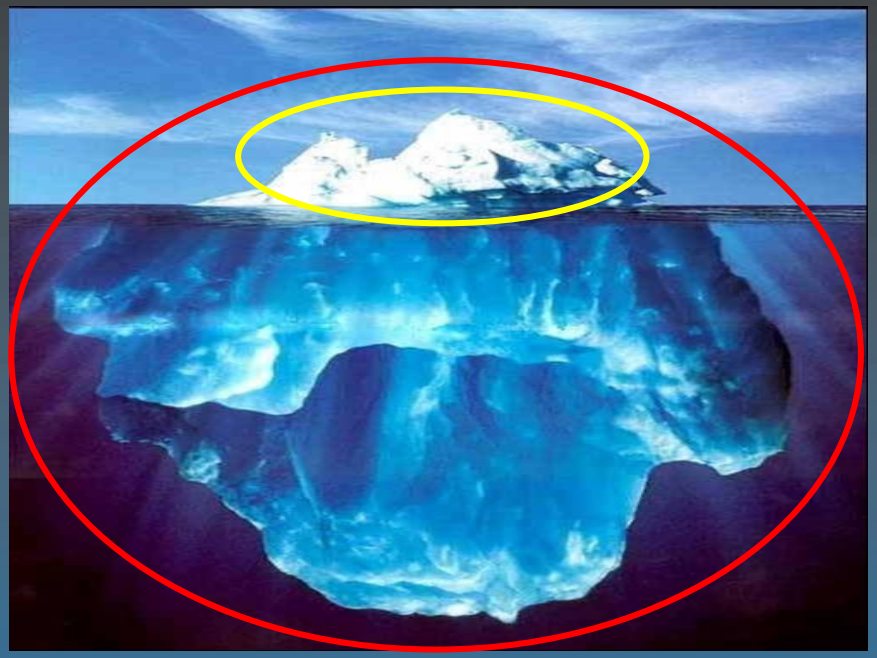
Waste water

waternet

Outline

- Introduction
- Biomonitoring in drinking water production
  - In vivo bioalarm system of sources (surface water)
  - Overview of in vitro bioassay applications from source to tap
- Interpretation of biomonitoring data
  - Limitations of in vitro assays
  - Proposal for guidelines and monitoring strategy
- Case study: endocrine disruption

Monitoring effects or substances?



- **Substances**:
  - selected priority pollutants (e.g. 33 for EU WFD)
- **Effects**:
  - General toxicity: effects of total mixture of pollutants
  - Specific toxicity: effects of substances with a similar mechanism of toxic action; high sensitivity!
  - Unknown cause of effect (TIE needed)

More reliable risk assessment by use of toxic screening prior to relevant chemical analyses

Monitoring effects or substances?

Toxicity:

- ☺ Limited amount of assays can give a cost-effective and reliable risk assessment
- ☹ Low substance specificity
- ☺ Bioavailability included
- ☺ Mixture toxicity included
- ☺ Metabolites included
- ☺ Unknown substances included
- ☹ Chronic exposure is difficult and expensive
- ☹ No accepted classification available
- ☹ Biomagnification not included
- ☺ No *in vivo* effects ☞ no worries

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Chemistry:

- ☹ Search for a needle in a haystack: obligatory analysis of more then 200 substances in drinking water
- ☹ Many analyses are yet impossible (e.g. matrix effects)
- ☹ Not enough toxicity data available for risk assessment (ERA)
- ☹ No information on bioavailability
- ☹ No information on mixture toxicity
- ☺ Direct comparison to substance-directed legal guidelines
- ☹ Low concentrations ☞ still worries
- ☹ Surrogate security and accuracy

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D. De Zwart (RIVM, Netherlands)

Mixture toxicity

	interaction	no interaction
same mode of action	complex similar	simple similar
different mode of action	dependent	independent

(Plackett & Hewlett, 1952)

- No interaction (synergism & antagonism) at low dose
- Response-addition not relevant at low dose
- Dose-addition can be relevant at low doses (TEF concept)

For drinking water dose-addition is most relevant!

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Risk assessment of mixtures in drinking water

Current biomonitoring applications:

- In vivo bio alarm for general toxicity of source water

Proposed strategy for additional biomonitoring:

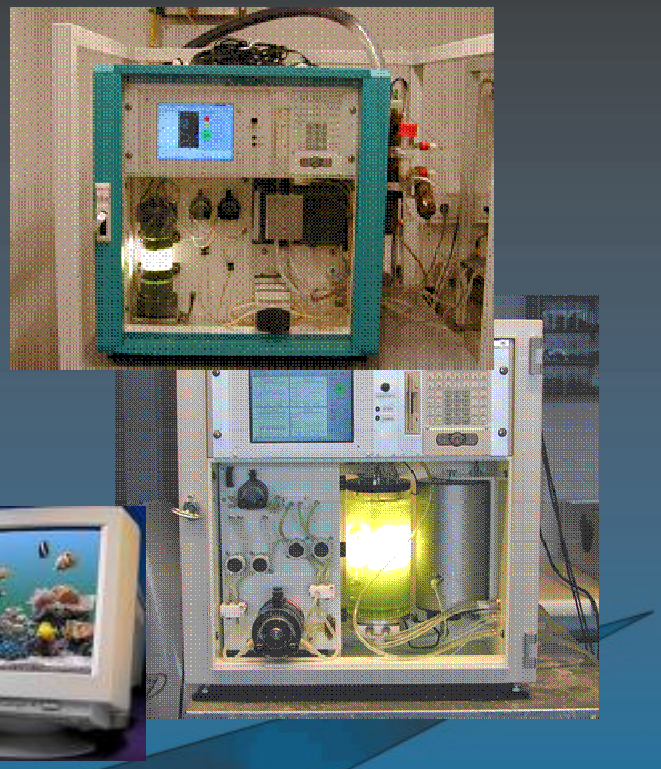
- In vitro screening for specific toxicity from source to tap
- Evaluation of 'suspicious' in vitro effects with in vivo assays or ADME tests
- Identification of effects by chemical analyses

Bioassay requirements

- Reliable and robust:
  - Quality assurance, reproducible (inter and intra lab comparisons)
  - International acceptance and validation
- Simple and cheap:
  - Easy and fast to perform (incl. sample preparation)
  - No expensive equipment, materials or lab facilities needed
- High throughput:
  - Fast screening of large series of samples possible
  - Possibility for on-line biosensor development
- Animal friendly:
  - Validated *in vitro* assay preferred

In vivo bioalarm system Waternet

- Online monitoring of inlet water for production of drinking water:
  - Fish behaviour
  - Daphnia movement & survival
  - Algal fluorescense & growth
  - Bacterial luminescence



After significant deviations water uptake is shut down!

Potential in vitro assays for drinking water testing

- Genotoxicity
- Carcinogenicity
- Endocrine disruption
- Teratogenicity
- Neurotoxicity
- Immunotoxicity
- Detoxification
  - Phase I: metabolism
  - Phase II: conjugation
  - Phase III: excretion

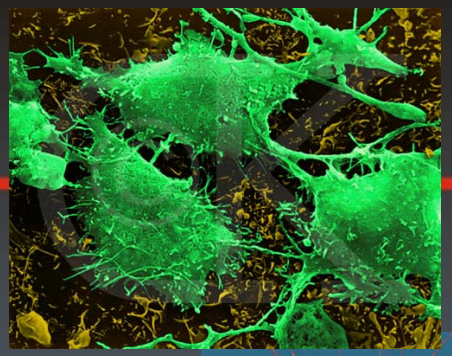
Genotoxicity

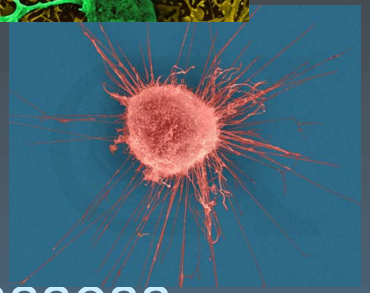
Low doses of genotoxic substances may damage human DNA

Evaluation made by Minne Heringa (KIWA):

- Ames II and high throughput comet or micronucleus assays are the most promising assays to assess DNA mutations and chromosomal abbreviations
- Results of Ames II application in drinking water production presented by Minne Heringa in next talk

Carcinogenicity



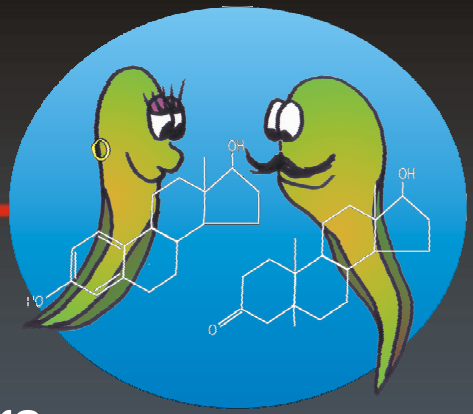


Cancer can be caused by genotoxic and non-genotoxic (tumor promoter) substances

Relevant assays for carcinogenicity:

- DNA microarrays seem to be most relevant to assess risks of non-genotoxic carcinogens (Minne Heringa)
- Chemically activated luciferase gene expression (CALUX) assays relevant for tumor promoting activity through AhR or E2 receptor mediated bioactivation
- Gap junction intercellular communication (GJIC) assay may be indicative for tumor promotion

Endocrine disruption

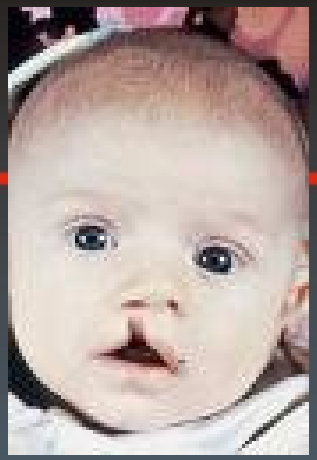


Many chemicals are able to disrupt hormonal systems through binding to endocrine receptors

Relevant assays for endocrine disruption:

- CALUX assays (α and β-ER, AR & pipelines) relevant for endocrine disruption at various hormonal receptors; initial results will be presented
- YES & YAS assays, E-screen, MCF-7, comparable but less sensitive and reproducible compared to CALUX

Teratogenicity

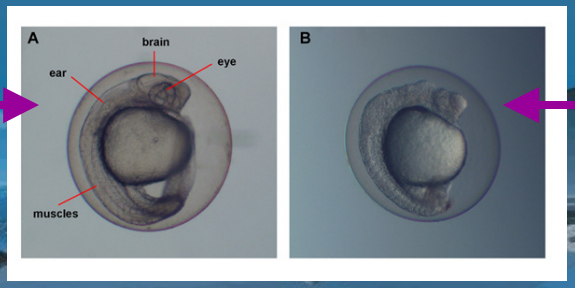


Certain chemicals are able to cause dramatic effects on fetal development

Relevant in assays for teratogenicity:

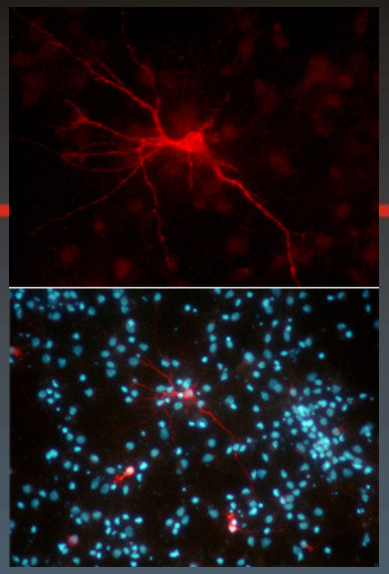
- Embryonic stem cell test (EST) is a relevant in vitro assay for teratogenic activity
- Most teratogenic research is performed with in vivo assays, such as the zebra fish embryo assay (Juliette Legler, IVM); verification of in vitro effects

Normal embryo



Methyl mercury exposure

Neurotoxicity

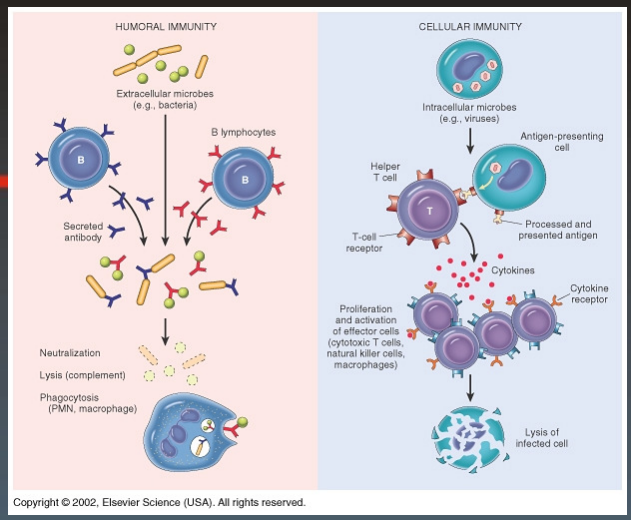


Certain water soluble chemicals cause effects on the central nerves system at very low doses

Relevant in assays for neurotoxicity:

- Acetyl cholinesterase (ACHE) inhibition is a relevant in vitro assay for neurotoxic activity
- Cell culture or yeast assays with human neurotransmitters
- Development of assays based on signal transmission (Tinca Murk, WUR)
- Development of biosensors to detect terrorist actions at drinking water distribution?

Immunotoxicity



Certain water soluble chemicals cause a Variety of effects on the immune system

Relevant in assays for immunotoxicity:

- Complication: the immune system can be disrupted in many ways, so a single assay is virtually impossible (microarray!)
- Development of NF-KB CALUX for assessment of anti-inflammatory effects (Bram Brouwer, BDS)
- Development of in vitro B lymphocyte proliferation assay (Raymond Pieters, IRAS)

Detoxification

Many chemicals are able to affect detoxification processes at higher doses

Phase I: metabolism (bioactivation)

- e.g. Cytochrome P450 (DR CALUX): relevance for polar compounds?

Phase II: conjugation

- e.g. Glutathione S transferase: low sensitivity

Phase III: excretion

- e.g. ABC transporter proteins (MXR): low sensitivity

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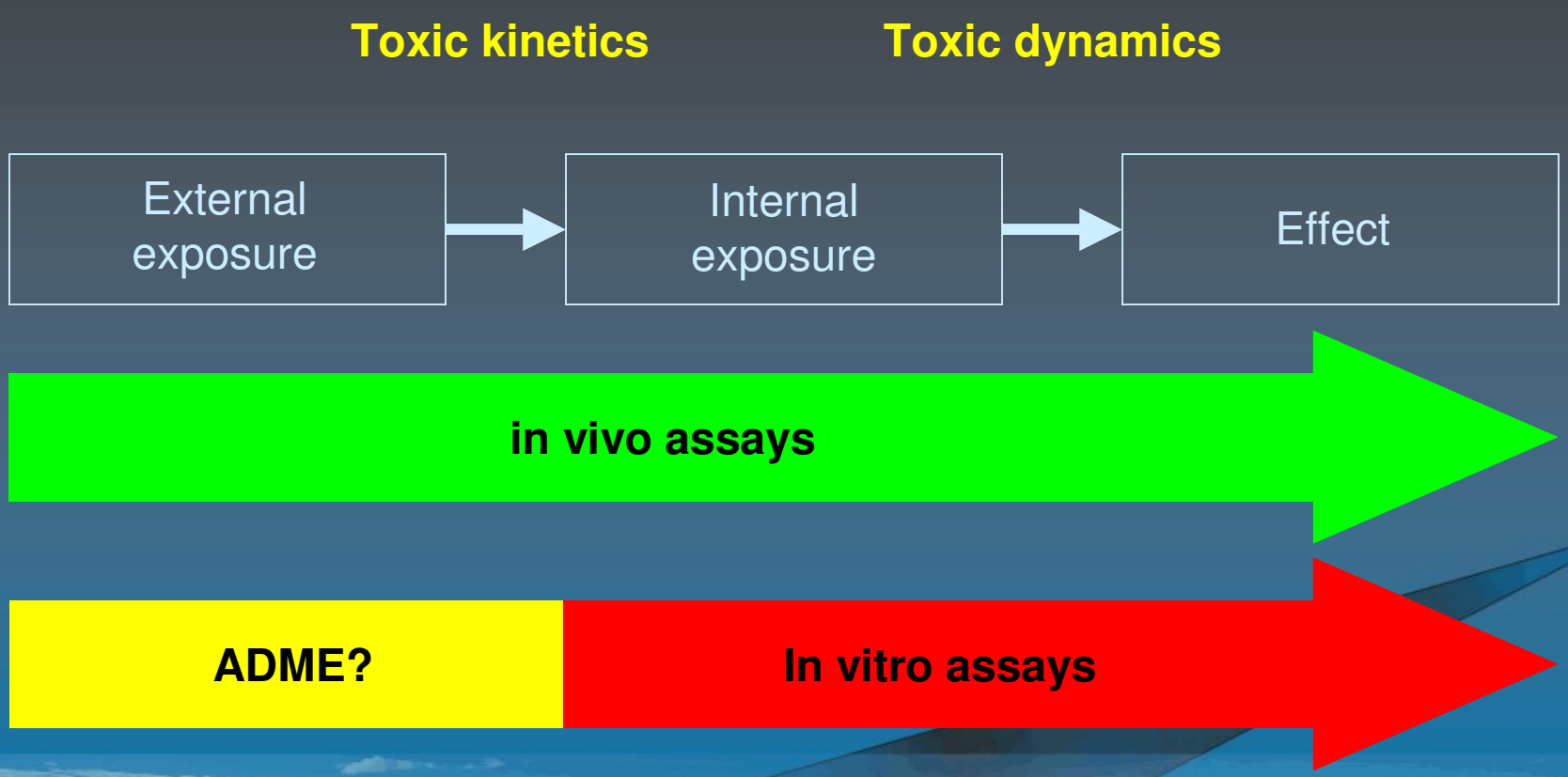
Relevance of observed toxicity



ADME testing in vitro extracts

Uptake

- Passage through a CaCo cell monolayer can be applied to assess the potential oral uptake of toxic substances

Metabolism

- Addition of an S9 mix in order to bioactivate substances to reactive metabolites that may be more toxic than the parent compounds
- Routinely used for genotoxicity assays, but also feasible for other assays, such as teratogenicity and endocrine disruption

Proposal for effect directed guidelines

- Select a relevant refecence substance for each assay
- Acceptable daily uptake (ADI) of reference is A µg/day/kg
- Person with body weight B can take up A*B µg of the reference substance per day
- Assumed that 10% of the uptake is through drinking water, then the allowed uptake will be A*B/10 µg per day
- If assumed that an average person drinks 2 liters of tapwater per day, then the maximal concentration in drinking water is A*B/20 µg/L
- The guideline for all substances in the mixture causing the same effect can be expressed as equivalents of the reference compound: A*B/20 µg REQ/L

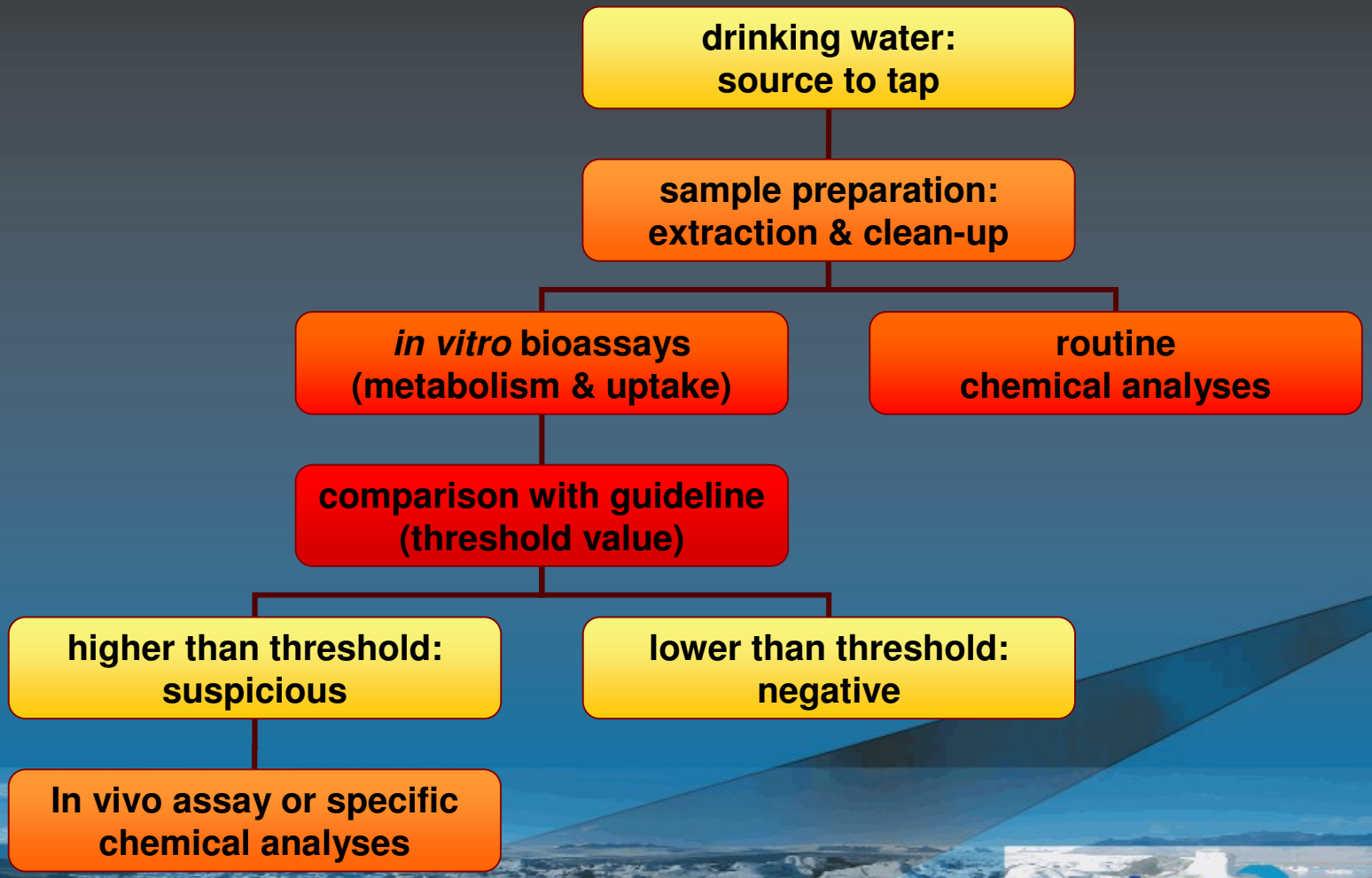
Limitations of effect-directed guidelines

- Influence of uptake, distribution, metabolism and excretion in vivo is generally unknown
- Toxic impact may be higher in young children
- Relative uptake by drinking water may vary for different substances
- Other uptake routes (inhalation and skin contact) may have an additional impact

Guidelines for in vitro assays should be regarded as treshold values for further research:

- Specific chemical analyses
- In vivo or ADME verification of effects

Proposed strategy for future risk assessment



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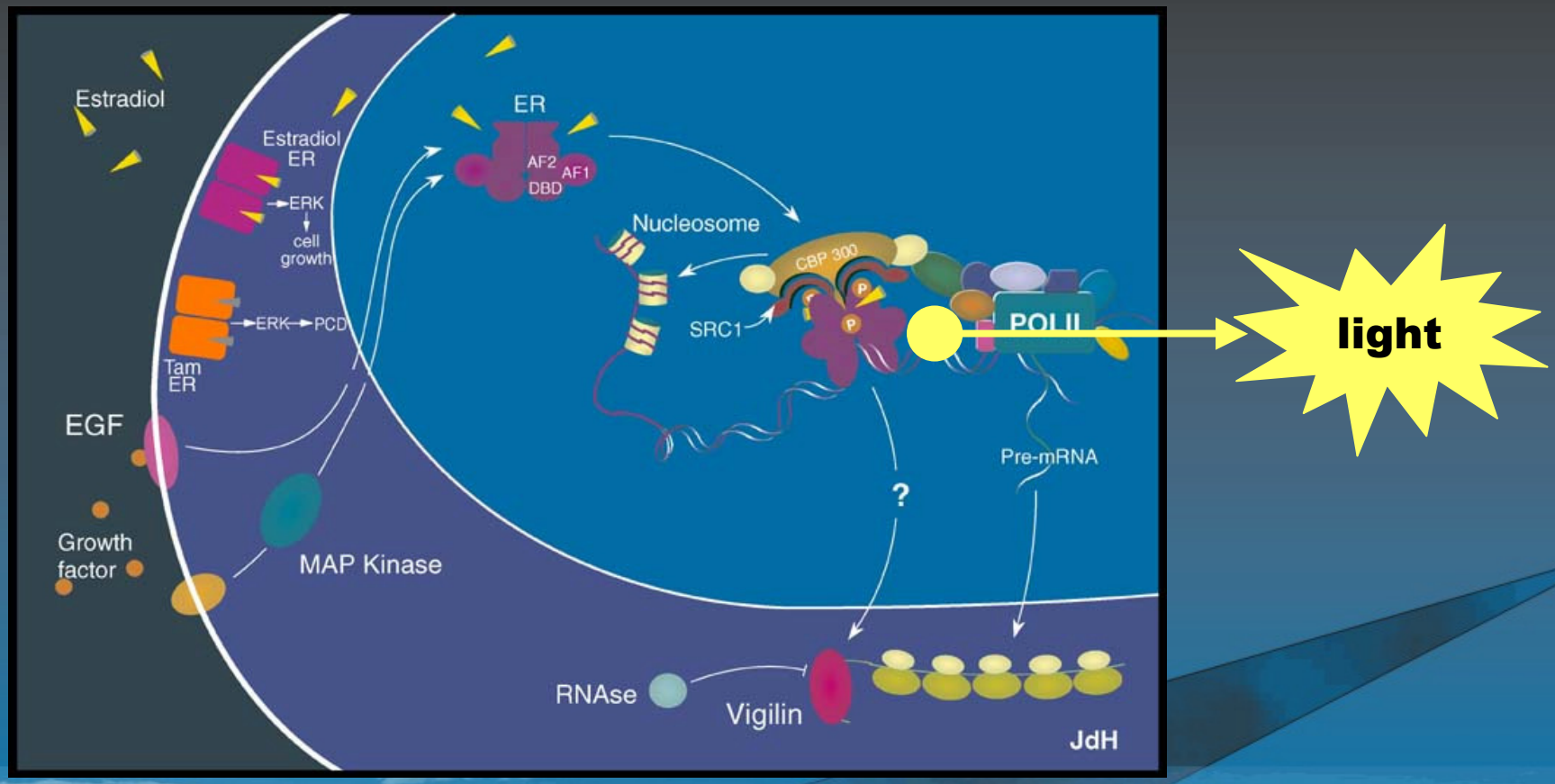
Endocrine disruptive effects from source to tap

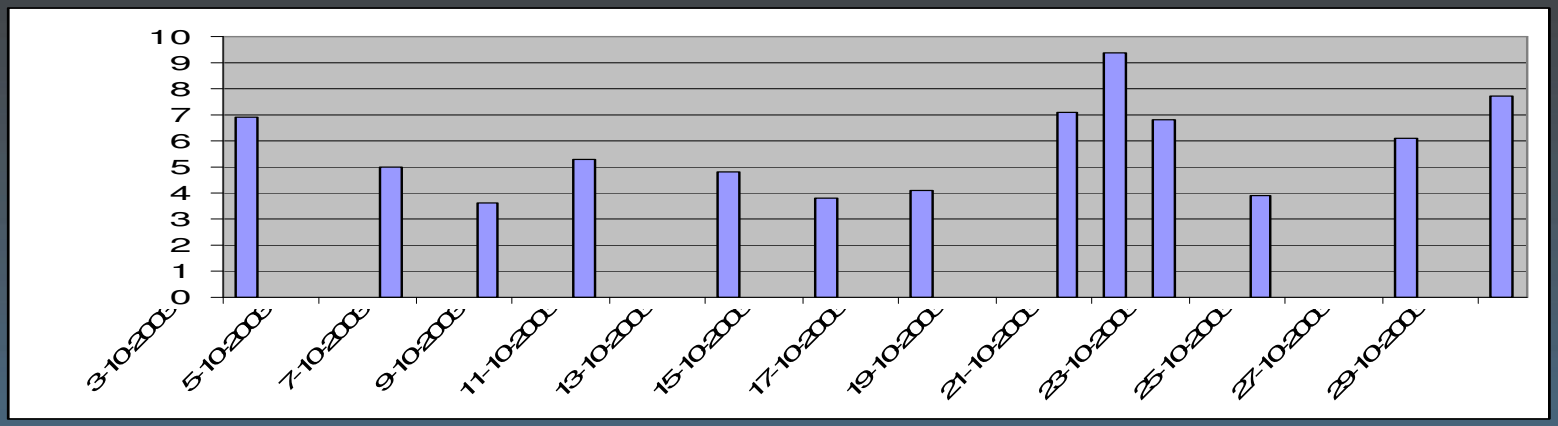
- Dutch drinking water is prepared from ground water and surface water
- Total effect of mixtures is unknown and many endocrine disrupting compounds can be missed ⟶ effect monitoring required
- Application of ER CALUX assay to study water contamination in the Netherlands (KIWA Water Research)

ER CALUX assay

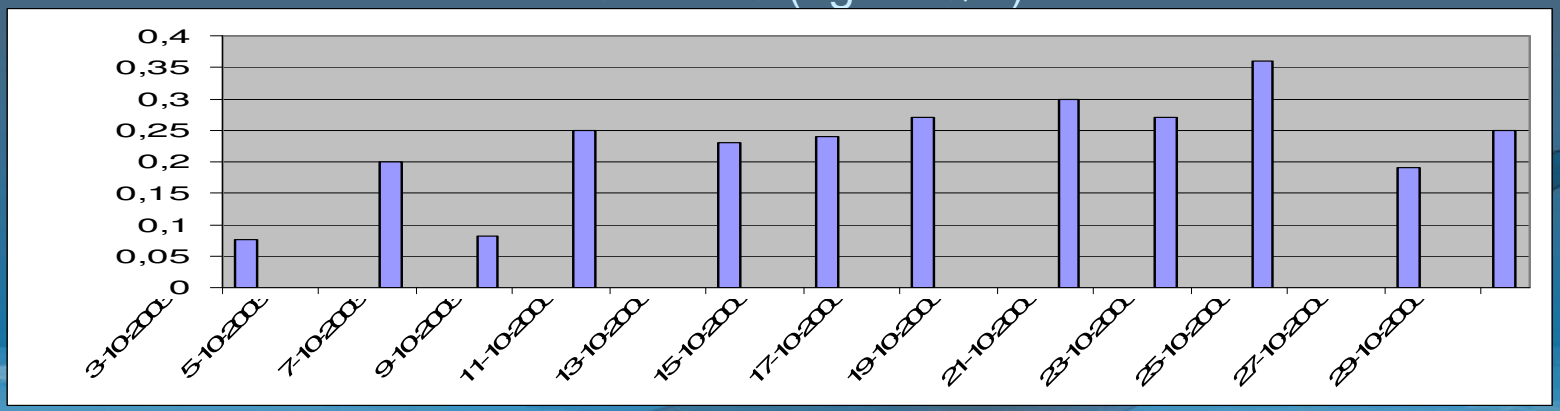


ER CALUX in sources

Bogers et al., 2007 River Meuse (ng EEQ/L)



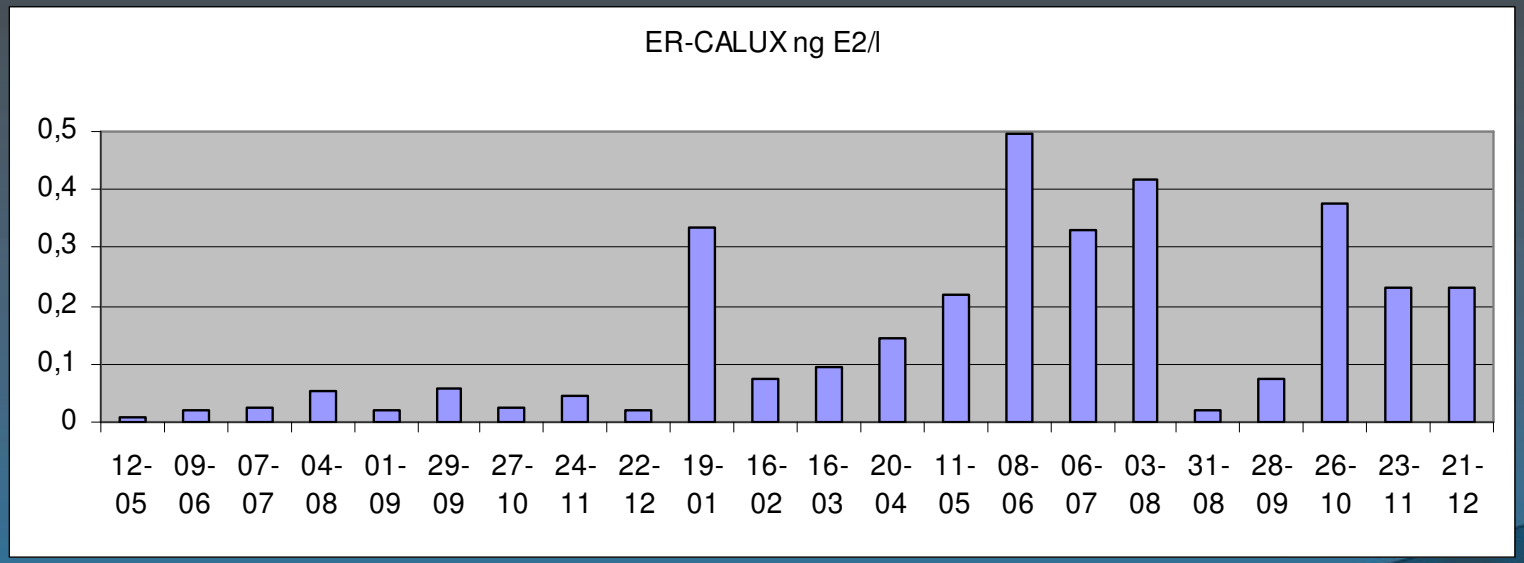
River Rhine (ng EEQ/L)



Trigger value human health: 7 ng EEQ/L (RIVM)

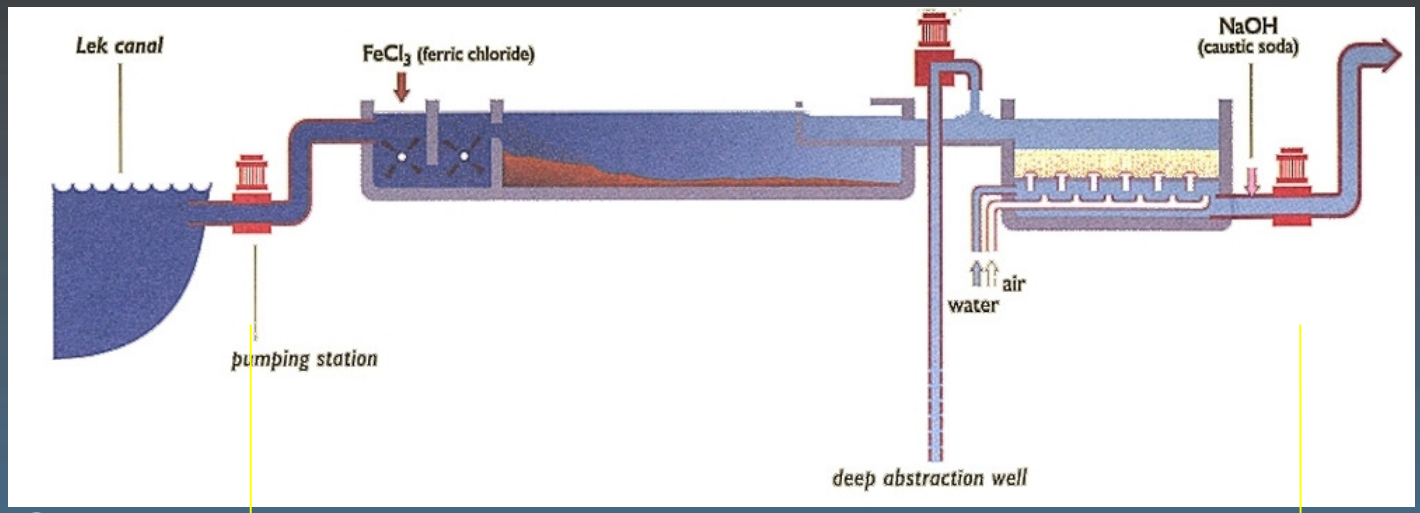
ER CALUX in river Rhine: seasonal variance

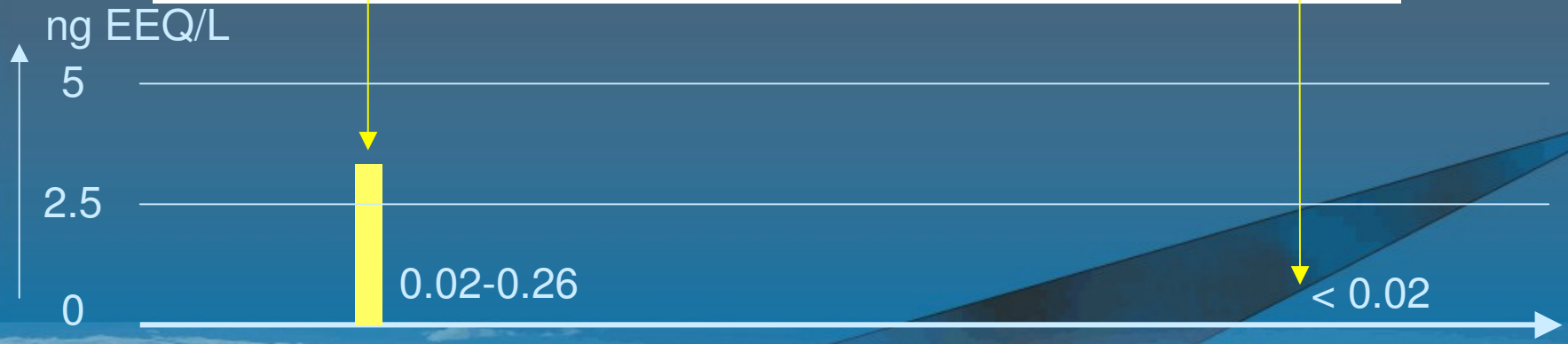
River Rhine (Lek canal)



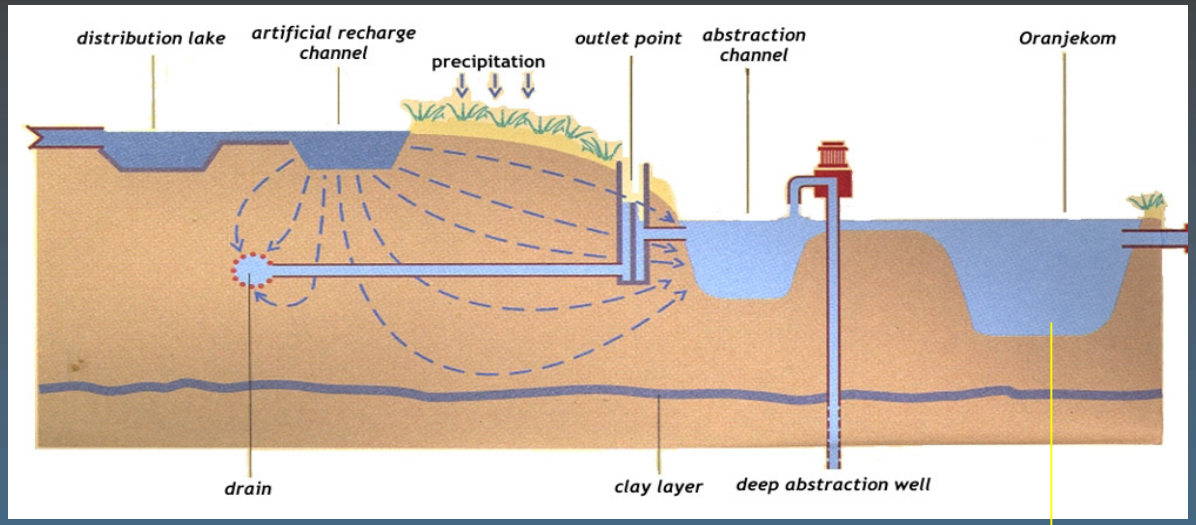
Bogers et al., 2007

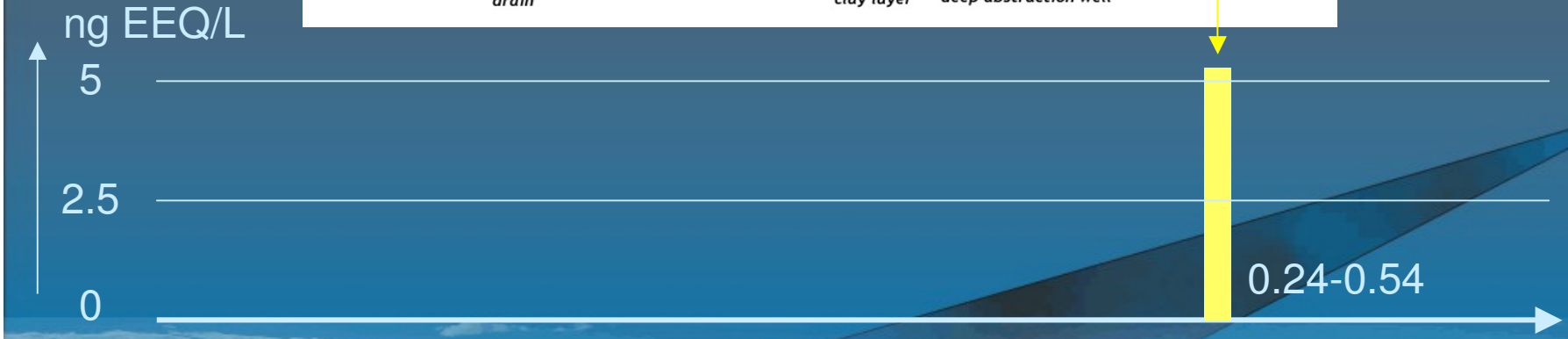
ER CALUX in source and after pre-treatment



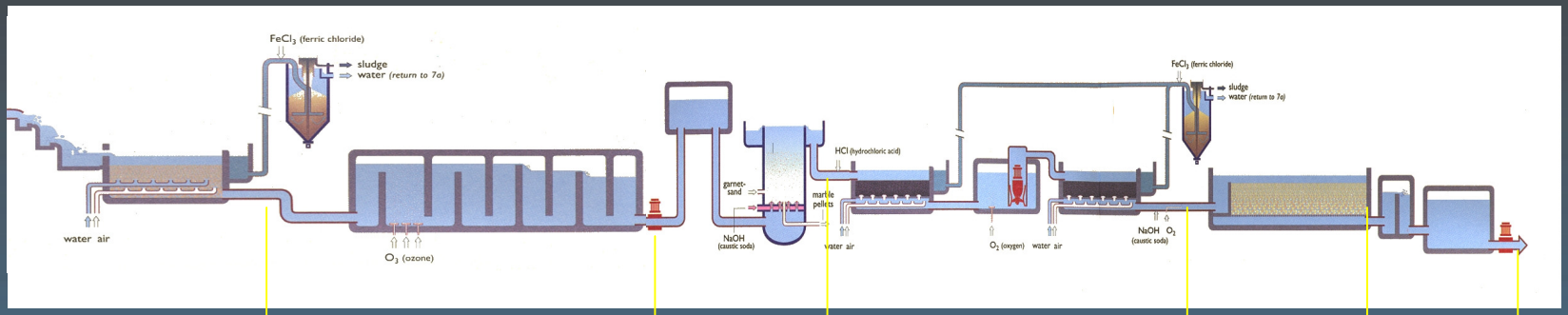


ER CALUX after dune filtration





ER CALUX in drinking water treatment plant



ng EEQ/L

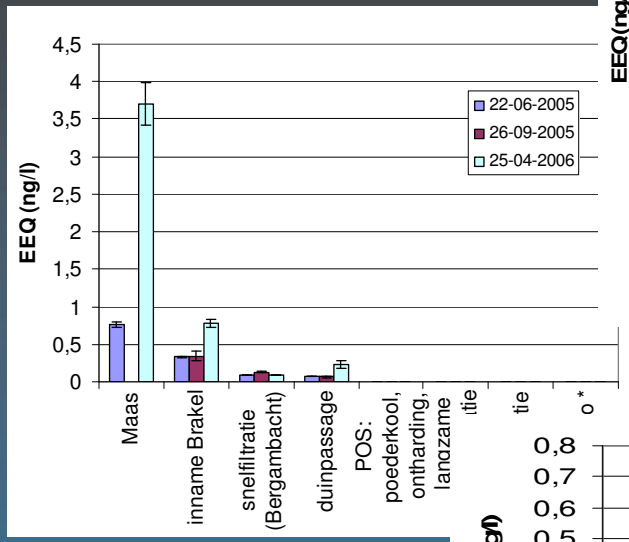
5

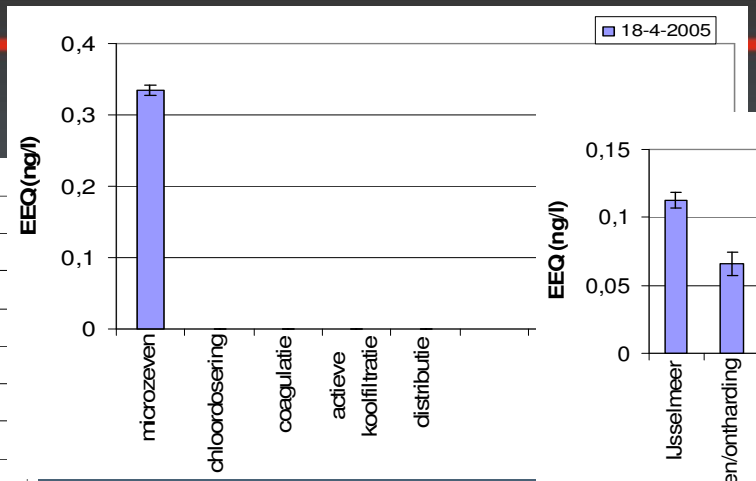
2.5

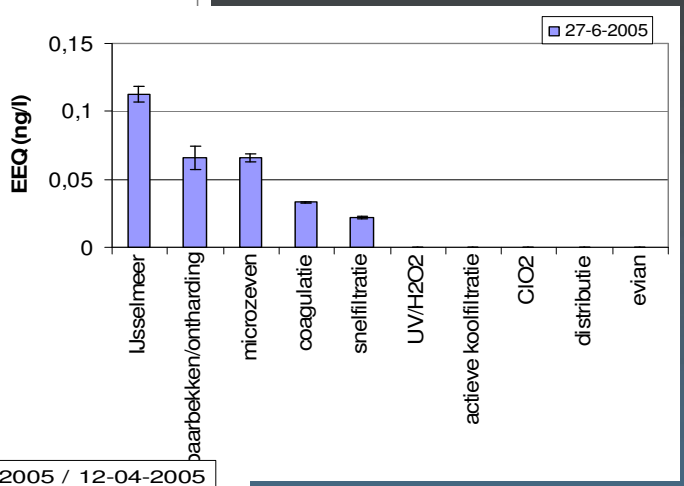
0

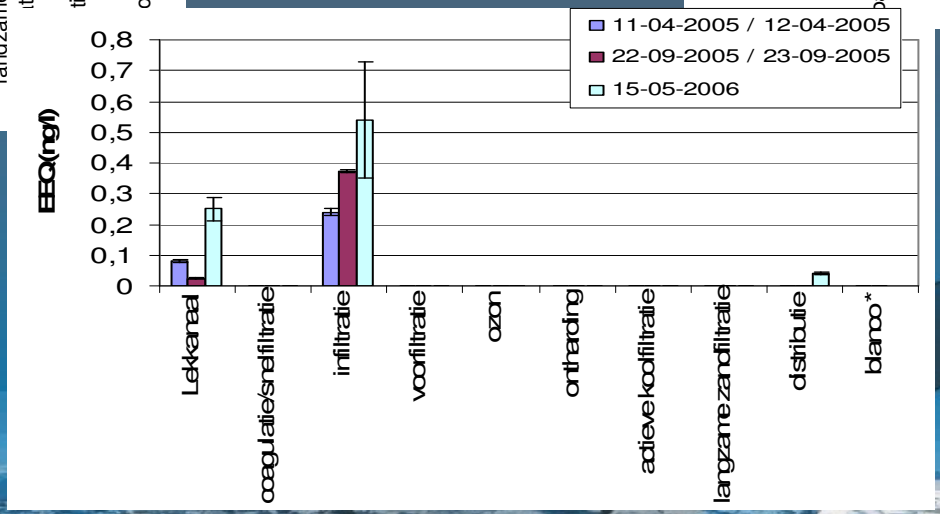
< 0.02 < 0.02 < 0.02 < 0.02 < 0.02 <0.02-0.04

ER CALUX in other drinking water companies









Conclusions on EDC effects in drinkingwater

- River Meuse water contains estrogenic activity above the trigger value for drinking water (7 ng EEQ/L, RIVM)
- Estrogenic activity in River Rhine water and ground water is below the trigger value for drinking water
- Very low estrogenic activity detected in drinking water distribution
- Androgenic activity was detected in none of the samples
- Robustness of Dutch water treatment plants seems to be sufficient for removal of endocrine disrupting chemicals

Thanks!