The German Environmental Specimen Bank as a Tool for the Retrospective Monitoring and Assessment of Emerging Chemicals

Fraunhofer Institut Molekularbiologie und Angewandte Oekologie

Heinz Rüdel

Fraunhofer IME

Umwelt Bundes Amt
Für Mensch und Umwelt

Christa Schröter-Kermani

Federal Environment Agency

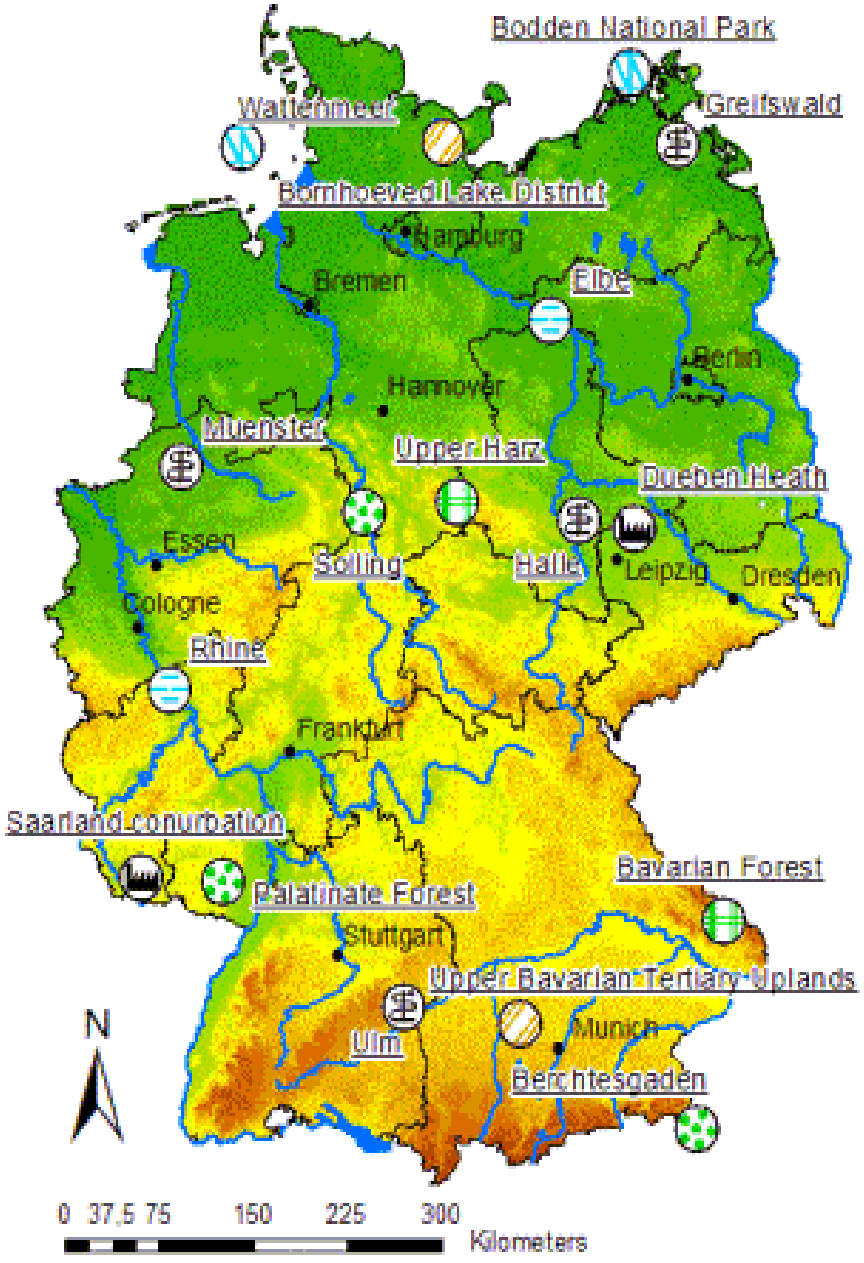
Structure

- The German environmental specimen bank program
- Aquatic specimens and sampling sites
- Emerging pollutants
  - examples from retrospective monitoring studies
  - indirect assessment of effects of chemicals
- Assessment of effects of chemicals - direct approaches
- Conclusions

Definition

An environmental specimen bank is an archive of representative environmental samples which are collected in regular intervals

The German environmental specimen bank program



The German **Environmental Specimen Bank** (ESB) provides a framework within which environmental specimens are **systematically drawn** from **representative ecosystems** and stored under conditions which will prevent the chemical decomposition over a **period of decades**.

The sampling **started in 1985.**

Types of sampling areas:

- **Ecosystem close to conurbation**
- **Riverine ecosystem**
- **Marine ecosystem**
- **Forestry ecosystem**
- **Nearly natural terrestrial ecosystem**
- **Agrarian ecosystem**

Marine ecosystems

- **North Sea**: Wadden Sea Biosphere Reserve and National Park Schleswig-Holstein / Lower Saxony
- **Baltic Sea**: Bodden National Park Mecklenburg-Vorpommern

Organisms sampled for the ESB:

Fucus vesiculosus	**bladder wrack (thallus)**
Mytilus edulis	**blue mussel (soft body)**
Zoarces viviparus	**eelpout (muscle and liver)**
Larus argentatus	**herring gull (eggs)**

Freshwater ecosystems

- **Rivers**: Elbe with tributaries Saale and Mulde, Rhine, Saar, Danube (16 sampling sites in total)
- **Lake**: Lake Belau

Organisms sampled for the ESB:

Abramis brama **common bream (muscle, liver, blood plasma)**

Dreissena polymorpha **zebra mussel (soft body)**

Aquatic specimens and sampling sites

Storage conditions

- From **each sample 2-3 kg** are collected; after homogenisation **200 sub-samples of approx. 10 g each** are prepared
- Storage **temperature below -150°C**; **inert gas atmosphere** from evaporating nitrogen
- Currently **approx. 1,300 different samples** with more than 200,000 sub-samples are archived

The environmental specimen bank as an archive for the retrospective monitoring of emerging pollutants

Examples from monitoring studies for

- **organotin compounds**
- **triclosan and methyl-triclosan**
- **polycyclic musk fragrances**

Organotin compounds

$R_{(n)}SnX_{(4-n)}$

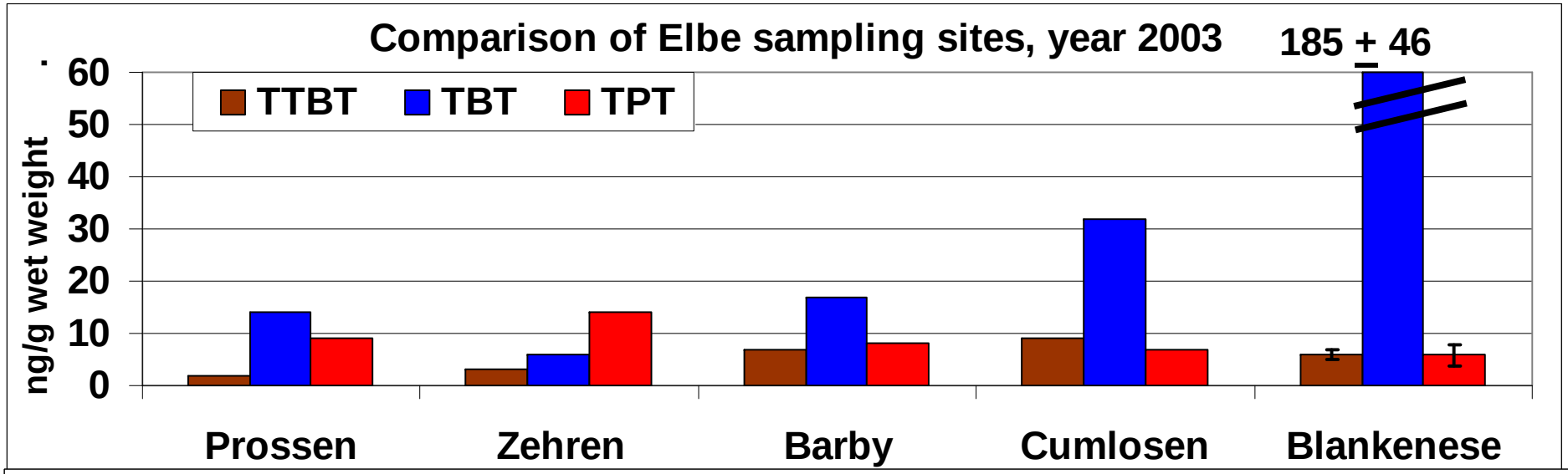
- most important **tributyltin – TBT** and **triphenyltin – TPT**
- **proof for endocrine disruption** in mollusks and snails: sterility (imposex effect), impaired reproduction **at ng/L-levels**

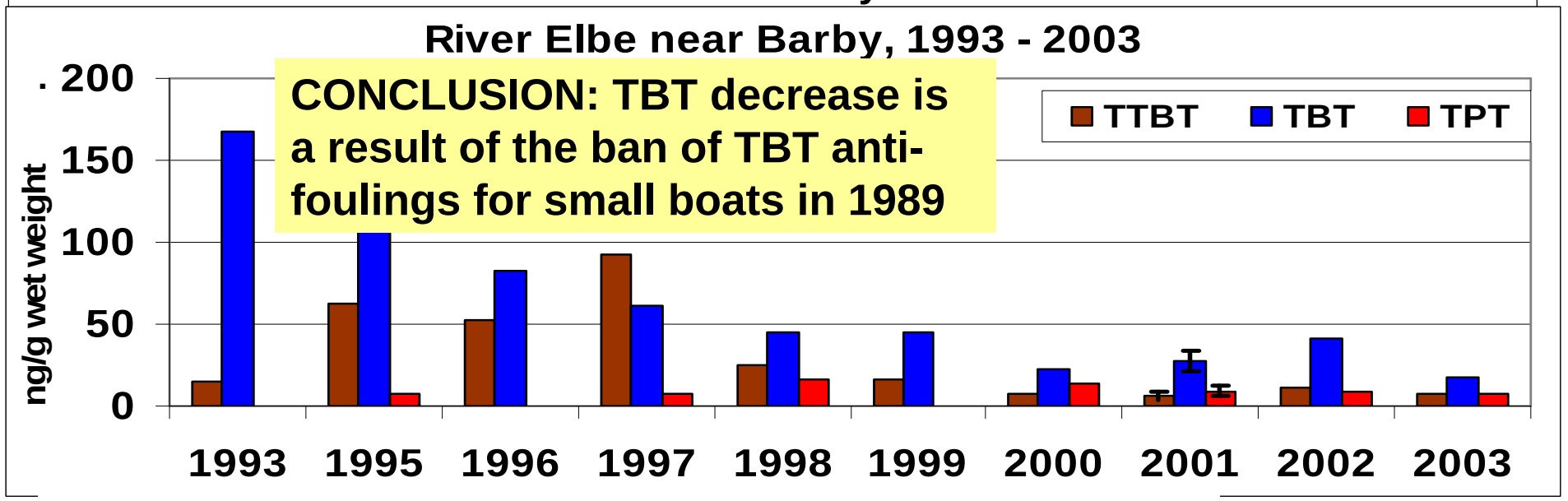
Annual consumption in the 1990s: 6,000 – 8,000 t (Graf 1996)

- **TBT** in **antifouling coatings** (since 2003 prohibited in the EU)
- **TBT** in **wood and material preservatives** (textiles, leather…)
- **TPT** in **fungicides**, as **co-toxicant in antifouling coatings**

Emerging pollutants - retrospective monitoring studies

Organotin compounds in bream (muscle tissue)



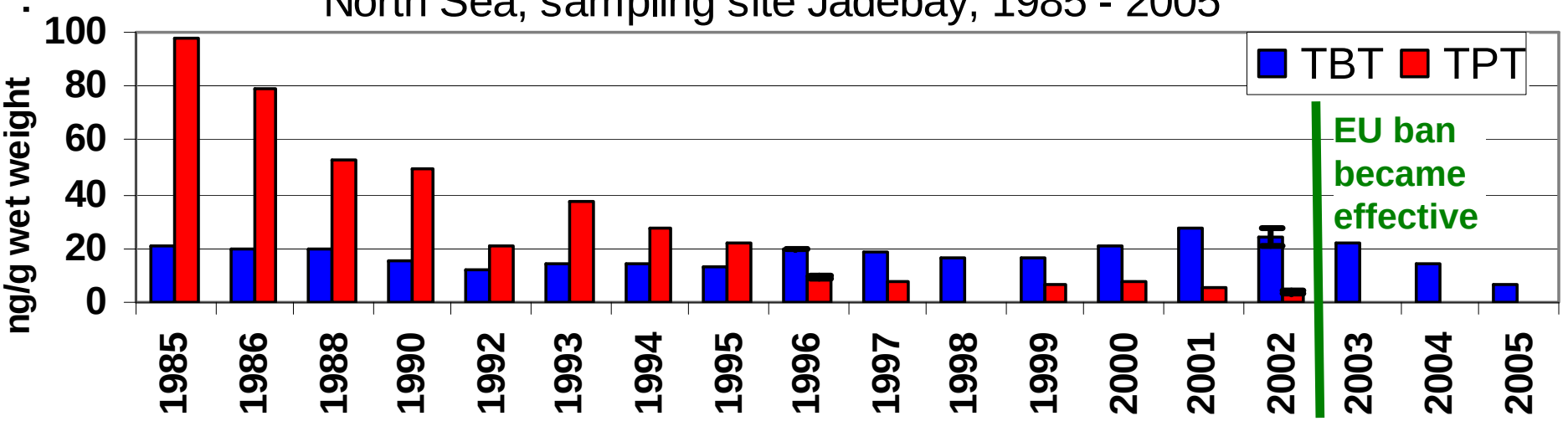


Data from: Ruedel et al. 2007, Chemosphere 66, 1884-1894

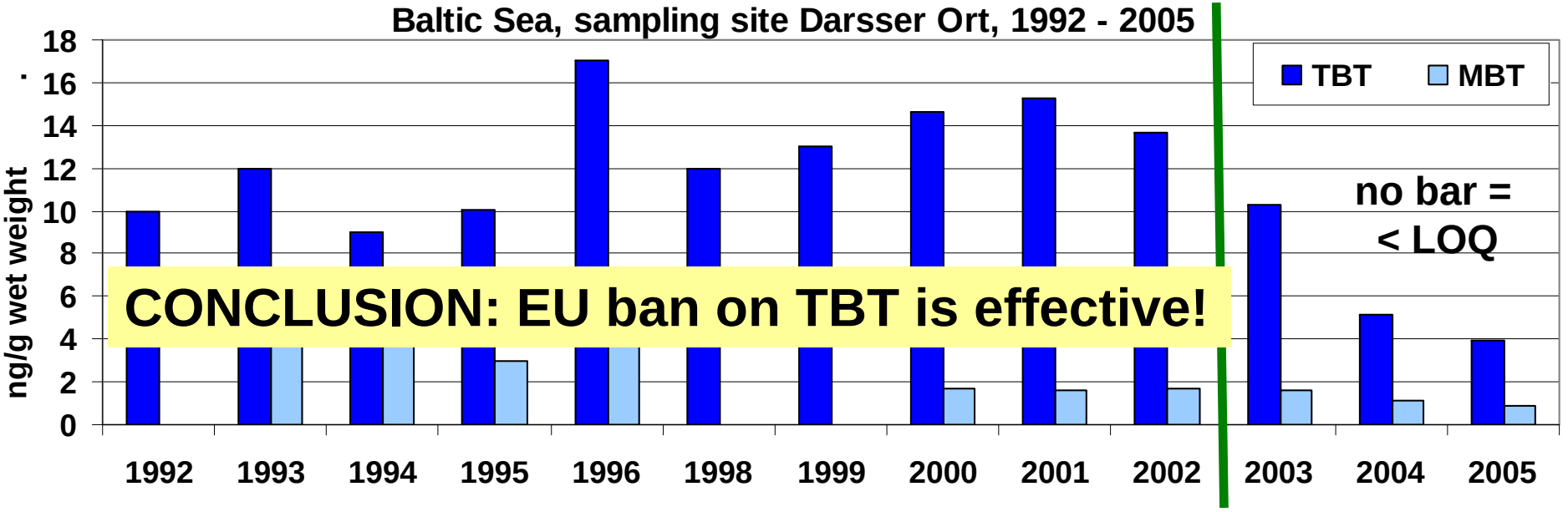
Emerging pollutants - retrospective monitoring studies

Organotin compounds in blue mussels

North Sea, sampling site Jadebay, 1985 - 2005



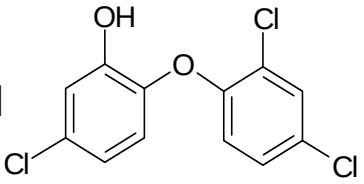
Baltic Sea, sampling site Darsser Ort, 1992 - 2005



CONCLUSION: EU ban on TBT is effective!

Triclosan

5-chloro-2-(2,4-dichlorophenoxy)phenol

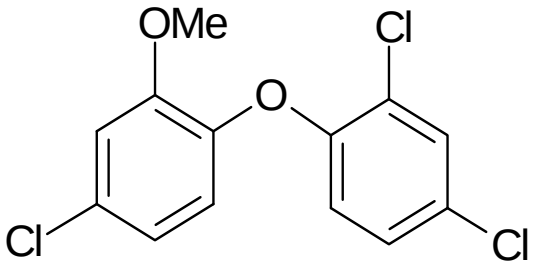


- **Triclosan is widely used as biocide in cosmetics and textiles. Annual consumption in Germany: 0.5 g/person = 40 t/a; estimated (Wind et al. 2004)**

- **Products which may contain triclosan: tooth paste, household cleaners, textiles (e.g. socks, sports wear), shoes**

- **Triclosan is eliminated in WWTP (95 %). It is degradable by photolysis: half-lives in summer are in the range of hours**

- **Predicted no effect concentration (PNEC) of triclosan: 0.05 µg/L (Danish EPA)**



5-chloro-2-(2,4-dichlorophenoxy)anisole

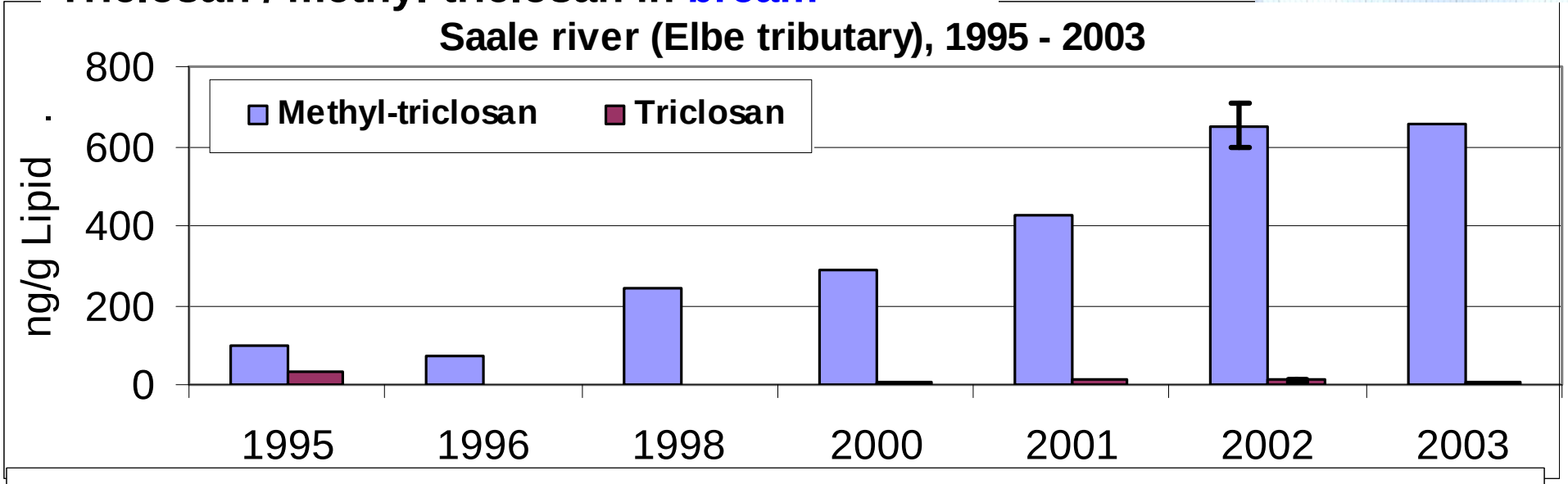
Methyl-triclosan

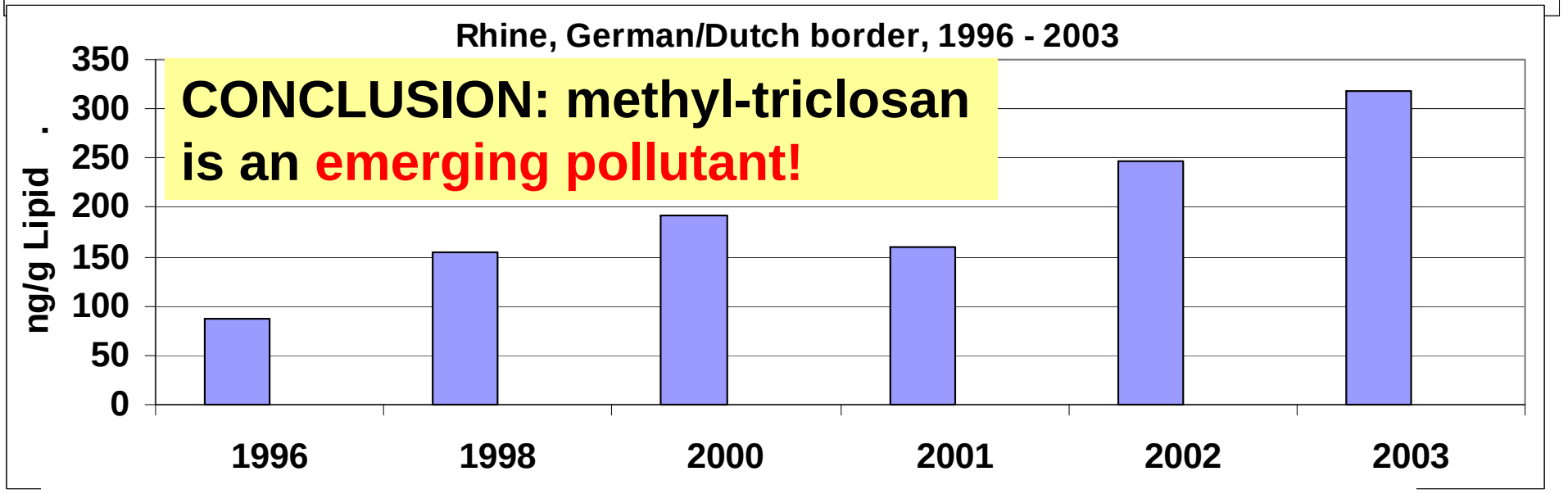
- By microbial transformation **methyl-triclosan** is formed. **Methyl-triclosan** is relatively **stable against photolytic degradation** and **more lipophilic than the parent compound**

- Currently there are no data on the ecotoxicity of **methyl-triclosan**; QSAR estimation of PNEC: 15 ng/L

Emerging pollutants - retrospective monitoring studies

Triclosan / methyl-triclosan in bream





CONCLUSION: methyl-triclosan is an emerging pollutant!

Data base: Boehmer et al. 2004, Organohalogen Comp. 66, 1516-1521

Estimation of water levels of methyl-triclosan (MTCS)

- When the bioconcentration factor (BCF) is known water concentration to which the fishes had been exposed can be estimated from tissue concentrations:

maximum MTCS tissue concentration: 650 ng/g lipid

BCF (lipid-based; Balmer et al. 2004): 100,000 – 260,000 L/kg

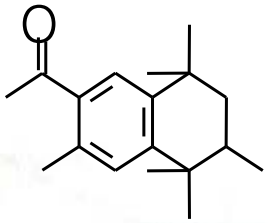
estimated water concentration: 2.5 - 6.5 ng/L MTCS

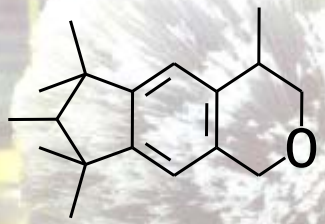
CONCLUSION: estimated water levels of MTCS are in the range of the QSAR-derived PNEC

Synthetic musk fragrances

Most relevant compounds due to broad application in personal care products and household cleaners:

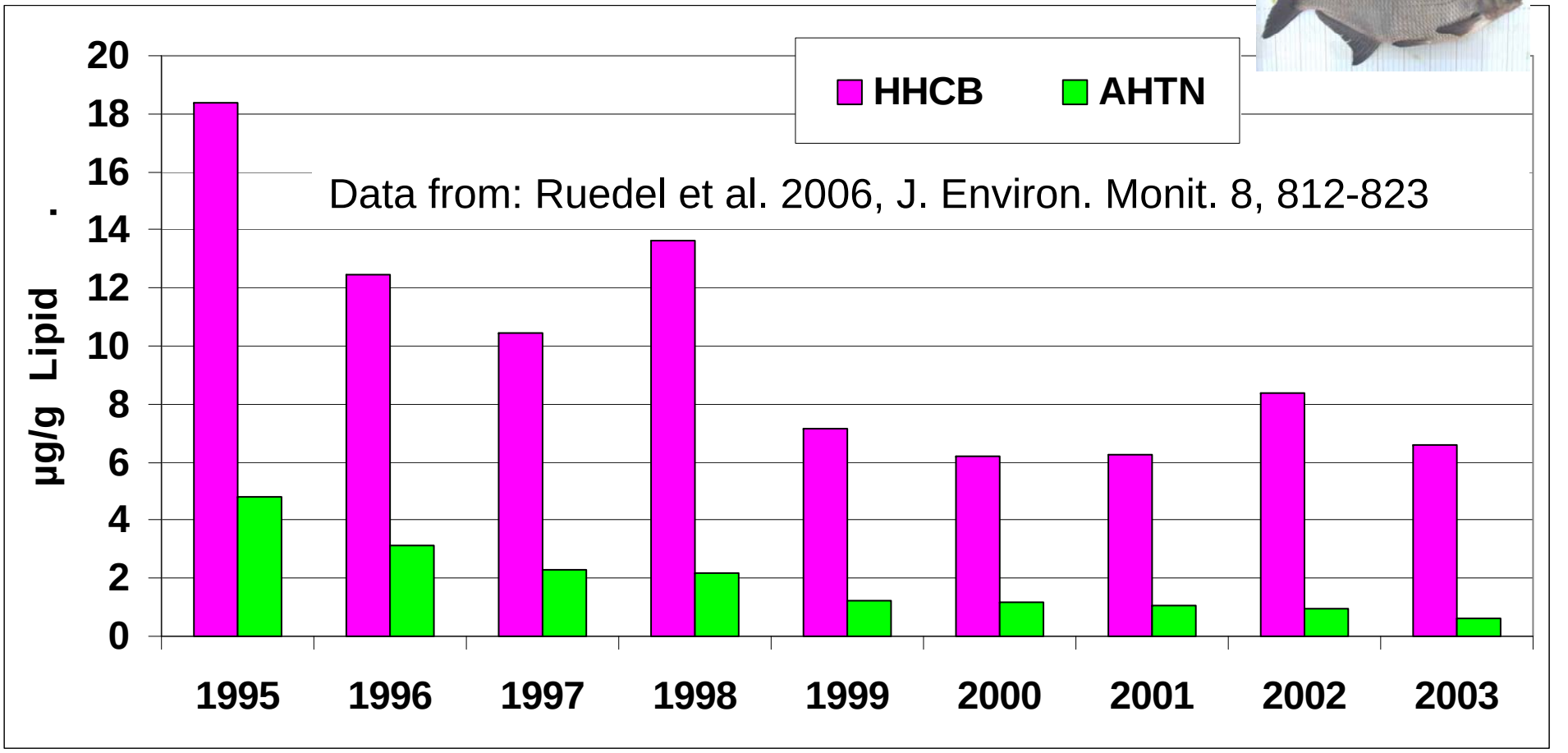
AHTN – tonalide and HHCB – galaxolide





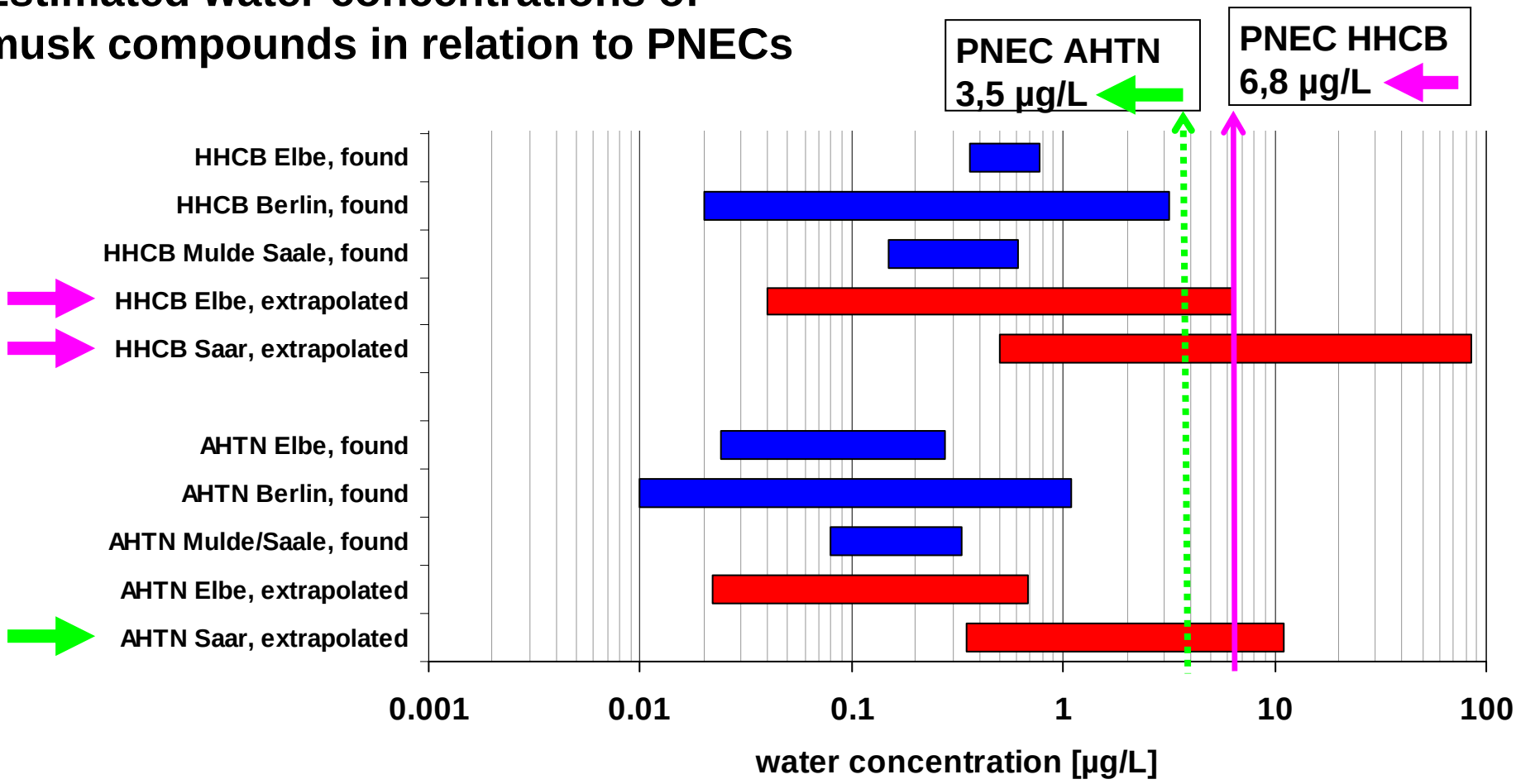
- **persistent**
- **high environmental concentrations - like PCB or PAH**
- **lipophilic log P_{ow} approx. 6 for HHCB, AHTN**
- **bioaccumulating BCF 600 – 1,600 for HHCB, AHTN**
- **hints for possible endocrine disruption effects**

Polycyclic musk compounds in bream (muscle): river Saale (Elbe tributary), 1995 - 2003



Emerging pollutants - indirect assessment of possible effects

Estimated water concentrations of musk compounds in relation to PNECs



CONCLUSIONS:

- good correlation between water analysis data from other studies (blue bars) and levels estimated with BCFs from ESB tissue data (red bars)
- at some sampling sites estimated water concentration exceeded PNEC values

The environmental specimen bank as a tool for the assessment of effects of (emerging) chemicals

Examples

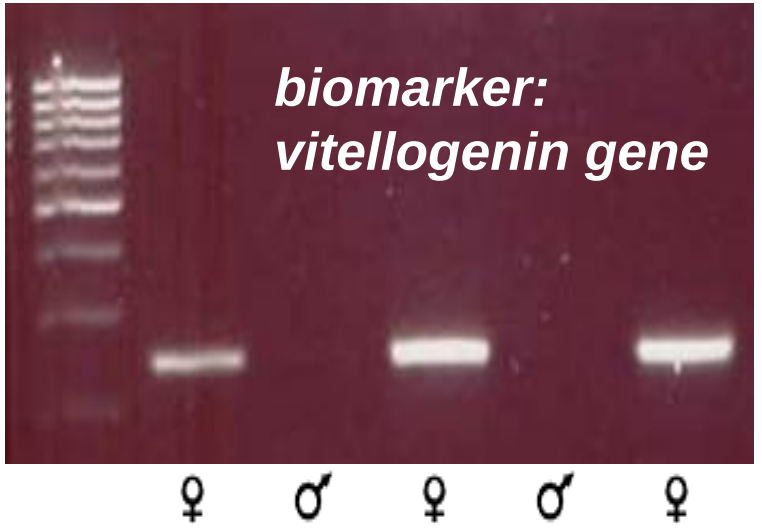
- usage of archived samples for ‘omics’ studies
- usage of routine specimens for additional studies
- additional samples taken for specific questions (problem-oriented)

Direct effect assessment - application of biomarkers

B. Seidel et al. 2007 (Poster, NORMAN workshop, Amsterdam)

aim: quantification of effects caused by pollutants on a molecular level in archived ESB samples

- **early detection of effects on DNA/RNA level**
- **detecting of effects of non-persistent pollutants**
- **Examples: vitellogenin, cytochrome P450 monooxygenases, metallothioneins, stress proteins**
- **usage of zebra fish (*Danio rerio*) genom (related to bream)**
- **the main result of this pilot study:**
  RNA and DNA could be extracted from archived ESB tissue samples and analyzed with standard molecular-biological methods



Download: http://www.umweltbundesamt.de/umweltproben/publikat/UPB-Biomarker_fin.pdf

Direct effect studies - eelpout reproductive impairment

Eelpout (*Zoarces viviparus*) is a common sentinel species for marine monitoring. It is used by the German ESB as a bioaccumulation indicator.

This study investigated the **prevalence of gonadal disorders** in eelpout from North Sea and Baltic Sea - **intersex and atresia.**

Sampling: spring 2006 (**ESB routine specimens**)

Study for the German ESB by:

J. Gercken and M. Sundt

Institut für Angewandte Ökologie

Forschungsgesellschaft mbH Broderstorf / Rostock

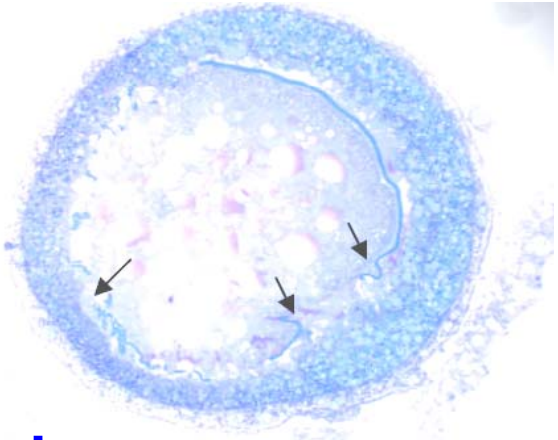
Download: http://www.umweltbundesamt.de/umweltproben/publikat/Aalmutter_Effektmon_06_Abstr.pdf

Direct effect studies - eelpout reproductive impairment

Ovaries from **female eelpout** were examined microscopically and histologically revealing typical characteristics of follicular **atresia** (severe degeneration / resorption of follicle and oocyte).

North Sea: 100% both at Varel (n=27) and Meldorf Bay (n=22)

Baltic Sea: 93% (n=27)



follicle with heavily swollen follicle cell layer, destroyed Zona radiata (arrows) and beginning resorption of the vitellogenic oocyte

Download: http://www.umweltbundesamt.de/umweltproben/publikat/Aalmutter_Effektmon_06_Abstr.pdf

Direct effect studies - linkage with exposure

Roland Klein et al. (2005)
Environ. Res. 98, 55-63
Institute for Biogeography,
University of Trier

Example: is the bioaccumulation of alkylphenol compounds linked to the effects on male fish?

A previous ESB monitoring study revealed that

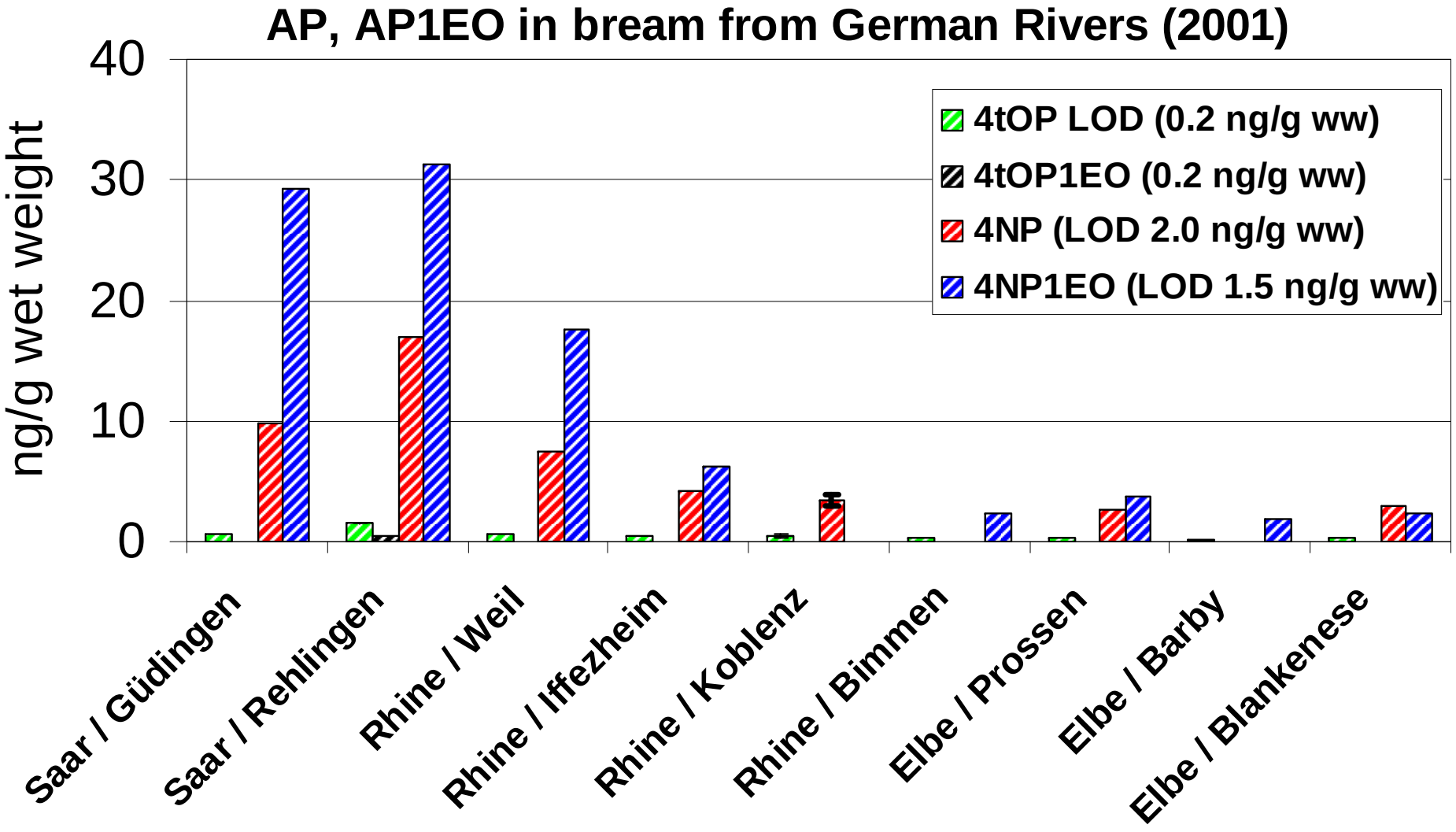
alkylphenols and their ethoxylates (AP, APEO)

were present in German freshwater fish with

high levels in bream from the river Saar

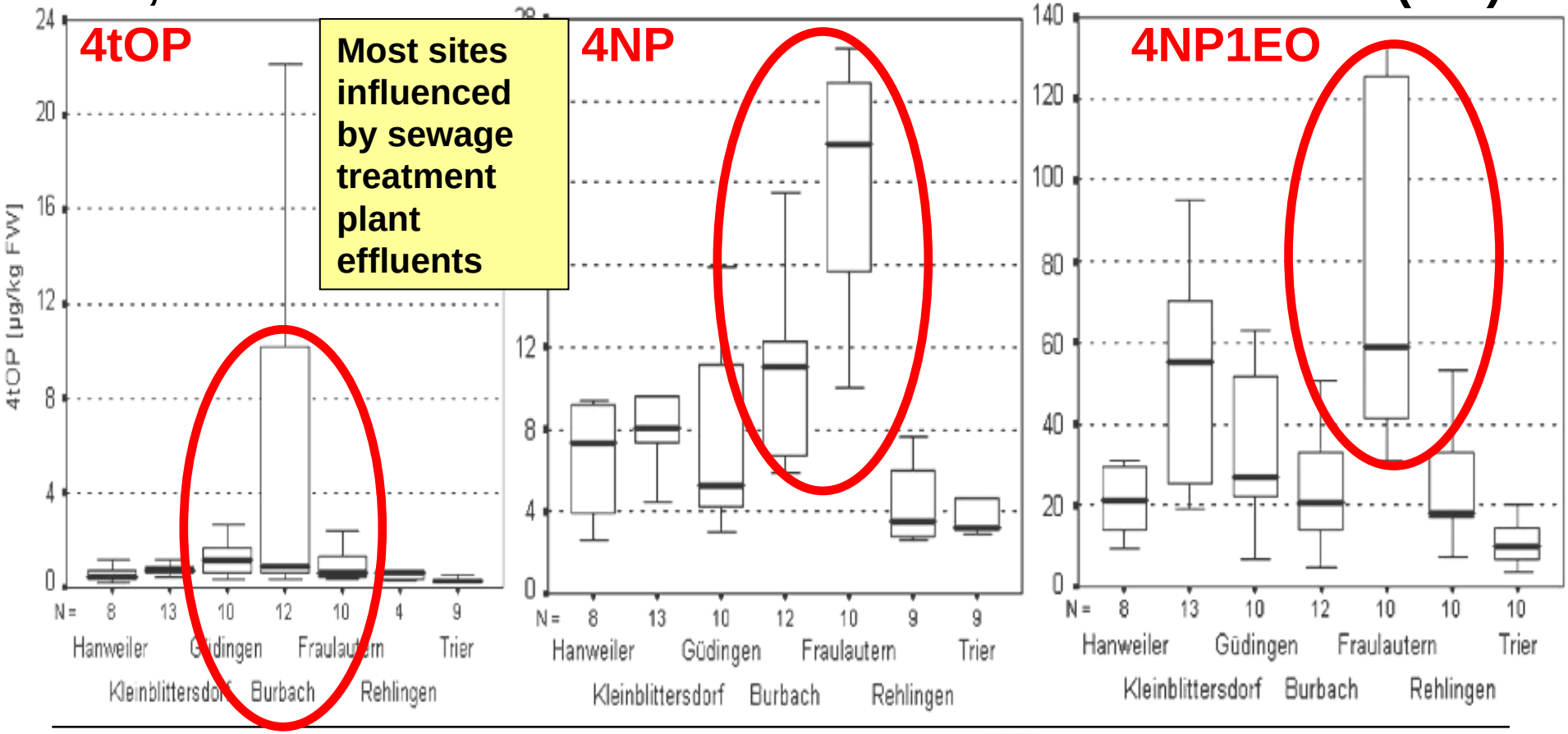
Direct effect studies - linkage with exposure

(monitoring data from Wenzel et al. 2004, Environ. Sci. Technol. 38, 1654-1661)



Example: is the bioaccumulation of alkylphenol compounds linked to effects on male fish?

AP, APEO in male bream from the Rivers Saar and Mosel (ww)



Klein et al. (2005)
Environ. Res. 98, 55-63

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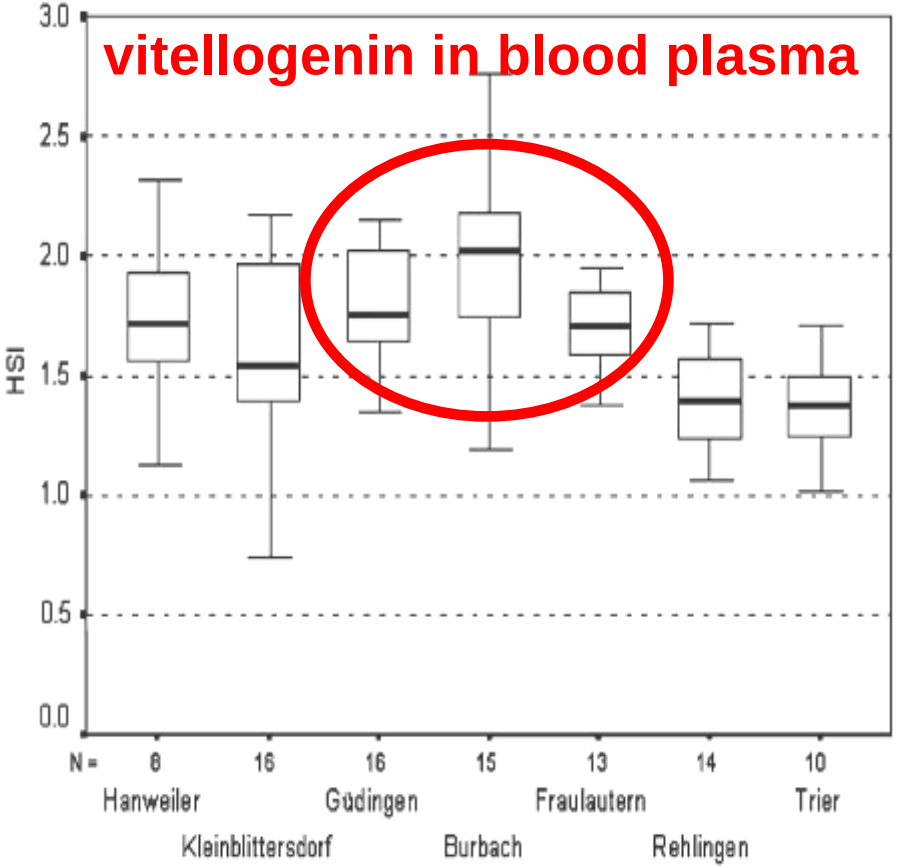
Example: is the bioaccumulation of alkylphenol compounds linked to effects on male fish?

effect data for male bream from the Rivers Saar and Mosel



CONCLUSION: no direct linkage between tissue levels and effects of AP/APEO, but relationship between elevated vitellogenin concentrations and sites influenced by effluents of large sewage treatment plant (sites Güdingen, Burbach, Fraulautern) was evident

Klein et al. (2005)
Environ. Res. 98, 55-63

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Conclusions - application of the ESB for exposure measurements

- An environmental specimen bank is an excellent tool for the retrospective monitoring of pollutants in environmental samples
- Concentration trends can be identified by analyses of appropriate biota samples from different levels of the trophic system allowing the identification of emerging pollutants (e.g. methyltriclosan)
- Even small temporal changes or slight regional differences of concentrations become obvious due to standardized samples
- The monitoring data can be used as basis for the justification of political measures (e.g. banning of pollutants with accumulation potential)
- Monitoring results allow the assessment of results of political measures taken in the past (e.g. use restrictions for TBT)

Conclusions - application of the ESB for effect assessment

- An indirect assessment of effects is feasible by extrapolation of body burdens to water concentrations using the BCF and comparison to the aquatic no effect level (PNEC)
- DNA and RNA analysis of homogenized standard ESB samples is possible allowing investigations of temporal trends and spatial comparisons (e.g. anthropogenic influenced vs. pristine regions)
- Effect data can be assessed together with data from exposure monitoring of samples from the same site and sampling period
- Special effect studies can be performed with routine specimens or with specimens sampled additionally to the normal ESB sampling campaigns

more information at www.umweltprobenbank.de