Can flood events affect rainbow trout? The biomarker-cascade after exposure to PAHs in sediment suspensions

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OVERVIEW

• Ecotoxicological relevance of flood events
• The project FLOODSEARCH: Results and open questions
• Static exposure experiments with suspended particles
  – Materials and methods
  – Concentration dynamics of PAHs
  – PAH metabolites in bile liquid
  – Hepatic EROD activity
  – Oxidative stress (lipid peroxidation)
  – Micronuclei in peripheral erythrocytes
• Summary & conclusions
ECOTOXICOLOGICAL RELEVANCE OF FLOOD EVENTS

Fig. 1: From Power & Chapman (1992), modified.

Sediment

Sedimentation/Re-suspension = Sink/Source
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Sediment/Re-suspension = Sink/Source
The project FLOODSEARCH: Results and open questions of the proof-of-concept study
Figure 2: Annular flume at the Institute of Hydraulic Engineering and Water Resources Management, RWTH Aachen University (Photograph: Catrina Cofalla).
• Simulation of flood events in the annular flume
• Exposure of rainbow trout at 12°C (n=15)
  – No sediment
  – Formulated sediment (OECD 218)
    ▪ 20 % clay
    ▪ 5 % peat
    ▪ 75 % sand
  – OECD 218 sediment spiked with PAHs
    ▪ 4.1 mg/kg pyrene
    ▪ 5.0 mg/kg phenanthrene
    ▪ 3.3 mg/kg chrysene
    ▪ 8.3 mg/kg benzo[a]pyrene
• Investigation of biomarker responses in exposed animals
Figure 3: Concentration of the biliary metabolite 3-Hydroxybenzo[a]pyren (mean ± range) in bile fluid of exposed rainbow trout \((n=15)\). Groups with different letters are significantly different (Kruskal-Wallis ANOVA & Dunn’s Test, \(p\leq0.01\)).
Figure 4: Micronucleus frequency (mean ± SD) in 4000 erythrocytes each relative to the median of the control group (n=15). Groups with different letters are significantly different (Kruskal-Wallis ANOVA & Dunn’s Test, \( p \leq 0.01 \).
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Figure 5: Linear regression and 95% confidence intervals between micronucleus frequency and 3-hydroxybenzo[a]pyrene concentration in bile fluid of exposed fish (Spearman’s rank correlation coefficient, $r=0.64$, $p=0.01$).

• The annular flume is suitable for simulation of flood events under laboratory conditions
• The formulated sediment according to OECD 218 is erodible, but has substantial drawbacks: Behaviour of natural sediments?
• Biomarker study underlines the relevance of short flood events: Dynamics of the reactions?
• „Classic biomarkers“ (EROD, GST) did not respond after exposure: Mechanism?
• Can we derive an optimal exposure period for experiments in the annular flume?
• The lid of the flume can currently not be opened during running experiments
  ✓ Static exposure experiments are needed
FLOODSEARCH II: Static exposure experiments with suspended sediments
Pyrene (4.1 mg/kg)  
Phenanthrene (5.0 mg/kg)  
Chrysene (3.3 mg/kg)  
Benzo[a]pyrene (8.3 mg/kg)

Sediment surface sample from the Rhine River (harbour Ehrenbreitstein)

Exposure with/without PAH-spiking at 12/24°C,  
Sampling of water and fish (n=10) after 0, 1, 2, 4, 6, 8, 12 days of exposure:

- **Dissipation**: Concentration dynamics of PAHs (GC-MS)
- **Metabolism**: PAH metabolites in bile liquid (HPLC-F)
- **Aryl Hydrocarbon Receptor**: Hepatic EROD activity
- **Oxidative stress**: Lipid peroxidation (TBARS)
- **Genotoxicity**: Micronuclei in peripheral erythrocytes

750 L glass-fibre reinforced suspension tanks

10 g/L
**Figure 6**: Concentration dynamics of the PAHs at 12 and 24°C were evaluated in the spiked and un-spiked treatments by GC-MS following PLE.
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Figure 7: Concentration dynamics of PAH metabolites in bile liquid at 12 und 24°C were measured in the spiked and un-spiked treatments using HPLC-F.
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Differences in sediment concentrations spiked/un-spiked:
- Phenanthrene: 7-fold
- Pyrene: 4.5-fold

Differences in biliary metabolite concentrations spiked/un-spiked:
- Phenanthrene: 120-fold (12°C)
- Pyrene: 29-fold (12°C)

✓ Drastic differences in bioavailability between spiked and naturally aged sediments!
Figure 8: Dynamics of EROD activity during exposure to spiked and un-spiked sediment at 12 and 24°C, respectively.
SAME RESULTS WERE OBSERVED FOR HEPATIC GENE EXPRESSION (REAL-TIME RT-PCR)

Figure 8: Dynamics of EROD activity during exposure to spiked and un-spiked sediment at 12 and 24°C, respectively.
Figure 9: Oxidative stress (TBARS) during exposure to spiked and un-spiked sediment at 12 and 24°C, respectively.
**Figure 10:** Micronucleus frequency during exposure to spiked and un-spiked sediment at 12°C.
**Figure 11**: Conceptual model of the observed biomarker cascade. A potential adverse effect following the transient biomarker responses cannot be excluded.
• Again, an elevated micronucleus frequency was observed
  ✓ Relevance of short flood events was confirmed
• Even substantial uptake of PAHs from un-spiked sediments
  ✓ Relevance of sediment re-suspension was confirmed
• Oxidative stress during PAH exposure only in combination with temperature stress
  ✓ Differential response under different temperature regimes was observed
• EROD activity was (again) no conclusive biomarker of PAH exposure
  ✓ Set of biomarkers is crucial for suspension experiments
• Investigations of native and spiked sediments are currently conducted in the annular flume
• Silicon passive-samplers are currently evaluated as a tool to predict the bioavailability of sediment-bound PAHs
Thanks for your kind attention!

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