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# Anticoagulant Rodenticides in Fish: Spatial and Temporal Distribution in German Freshwater Aquatic Systems

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# Rodenticides

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Rodent control relies almost exclusively on anticoagulant rodenticides (ARs):  
8 active substances have been approved for use in biocides

- 1st Generation (FGARs)

- Warfarin, Chlorophacinone, Coumatetralyl

- 2nd Generation (SGARs)

- Bromadiolone, Difenacoum, Brodifacoum, Difethialon, Flocooumafen
- PBT/vPvB-substances

- Concern: release to environment

- High risks of primary & secondary poisoning
- Detected in many terrestrial non-target animals → biomagnification
- Concerns for aquatic food chain due to use in sewer systems?



# Example for Oslo rodent control



# German Environmental Specimen Bank

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Retrospective analysis to uncover temporal and spatial trends



- Biota samples
- Soil samples
- Suspended particulate matter (SPM)
  
- Samples are very valuable
- Samples are limited
- Matrix is limited
  
- ~ 5 g per sample



# Method Development

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Excess  $\text{Na}_2\text{SO}_4$  Homogenization and Solvent Extraction

Liquid Chromatography

ES<sup>-</sup>

High Resolution MS/MS (Orbitrap)

- High Specificity
- High Sensitivity



Similar Analyte Characteristics allow Application of a Single Sample Preparation Method

# Three stages of an environmental monitoring study

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## 1. Identify suitable Matrix

Bream liver vs. muscle

Suspended Particulate Matter

## 2. Identify suitable locations

- Spatial analysis

## 3. Retrospective Monitoring

- Temporal analysis

# Method Validation

Analysis of fortified Matrix samples, n = 6

Matrix matched calibration from 0.02 to 20  $\mu\text{g kg}^{-1}$  wet weight

Substance	Generation	Bream liver			SPM		
		Validated LOQ level	Recovery	RSD	Validated LOQ level	Recovery	RSD
		[ $\mu\text{g kg}^{-1}$ ]	[%]	[%]	[ $\mu\text{g kg}^{-1}$ ]	[%]	[%]
<b>Flocoumafen</b>	2	0.2	99.5	5.4	1.0	97.9	6.9
<b>Bromadiolone</b>	2	2.0	95.2	8.1	1.0	96.1	4.8
<b>Brodifacoum</b>	2	1.0	92.5	6.6	2.0	98.2	5.9
<b>Difenacoum</b>	2	0.2	95.8	9.7	1.0	97.9	10.3
<b>Warfarin</b>	1	0.2	103.2	6.9	0.2	101.5	7.5
<b>Chlorophacinone</b>	1	1.0	93.4	7.2	2.0	116.0	26.3
<b>Coumatetralyl</b>	1	0.2	109.5	4.1	0.2	106.0	5.4
<b>Difethialone</b>	2	1.0	94.5	3.3	1.4	102.7	4.6

# Sampling map of German ESB

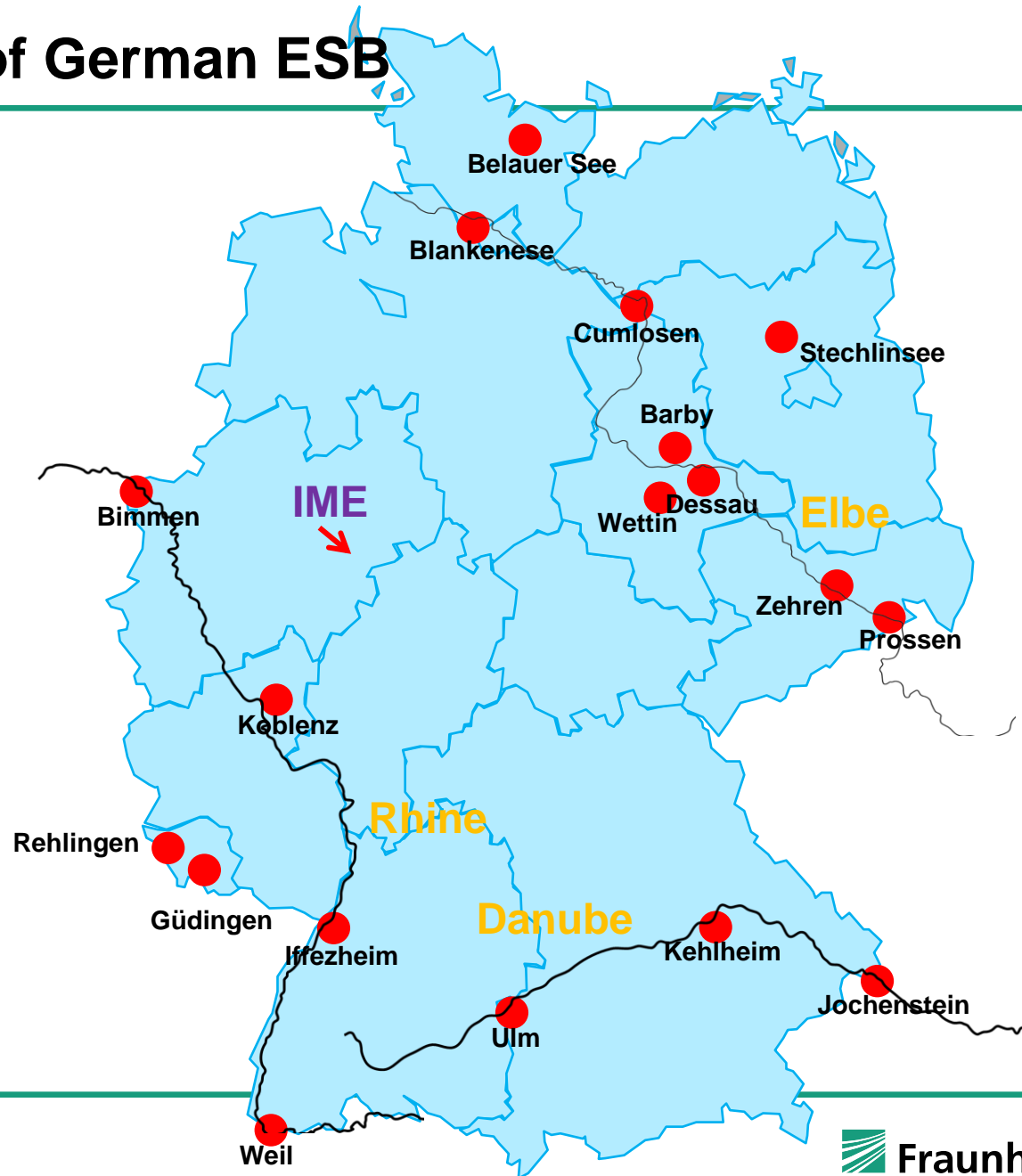
Bream:

Sampling Year 2015

Sampling Year 2011

SPM (riverine sites):

Sampling Year 2015;  
except Bimmen: 2014



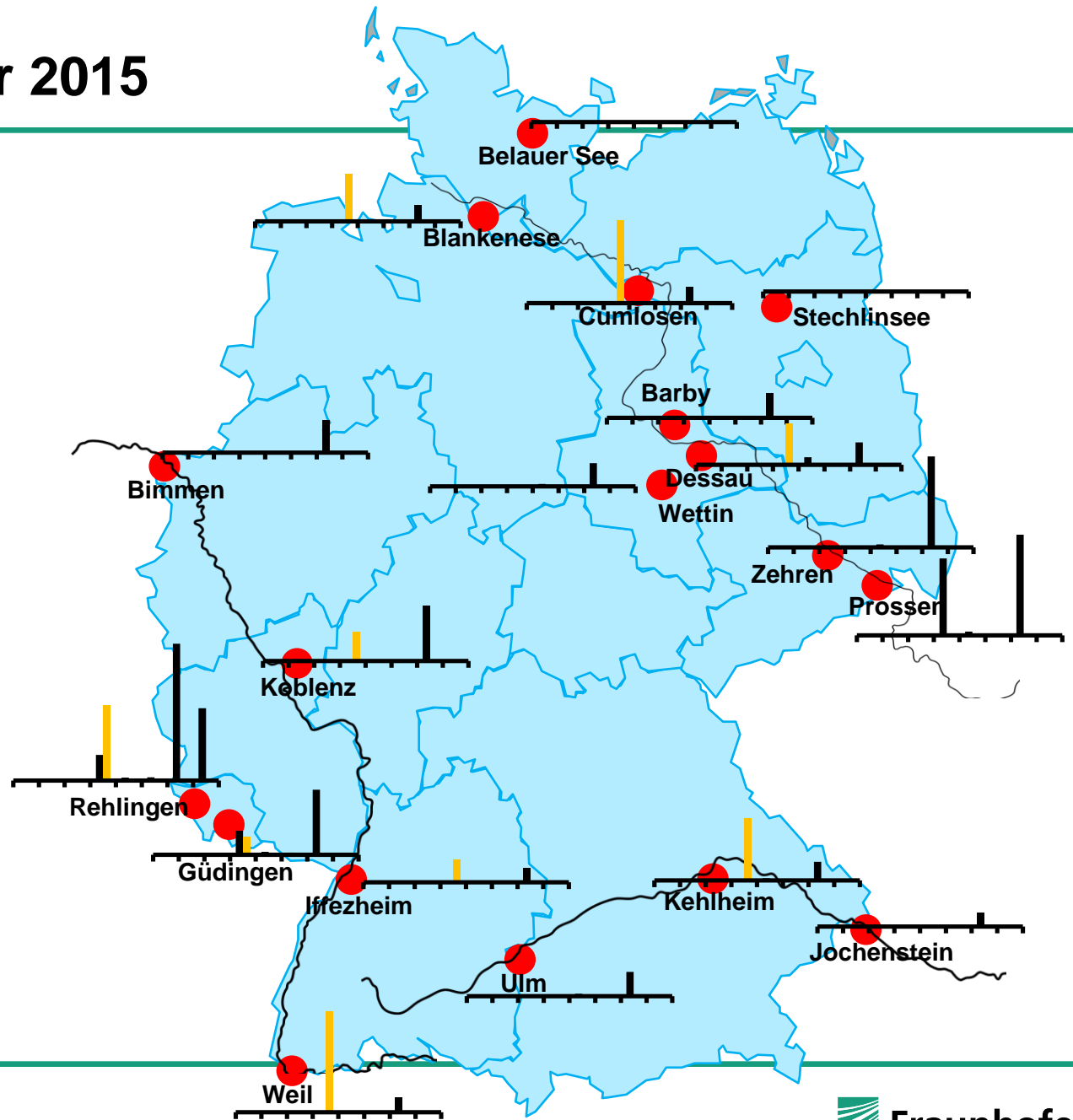


# Spatial data for 2015

█ SPM (in rivers)  
█ Bream liver

█ 10  $\mu\text{g}/\text{kg}$  wet weight

Coumatetralyl  
 Warfarin  
 Chlorophacinone  
 Bromadiolone  
 Difenacoum  
 Flocoumaten  
 Brodifacoum  
 Difethialone



# Key Findings Bream

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Major Rodenticides identified in bream liver:

- Brodifacoum

- 88 %; max 12.5 µg/kg; Ø 3.4 µg/kg; med: 2.1 µg/kg

- Difenacoum

- 44 %; max 0.7 µg/kg; Ø 0.1 µg/kg

- Bromadiolone

- 17 %; max 7.1 µg/kg; Ø 0.6 µg/kg

- Difethialon

- 6 %; 6.3 µg/kg

- Flocoumafen

- 12 %; 0.29 µg/kg



# Key Findings SPM

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Exclusive rodenticide identified in SPM:

- Bromadiolone

- 56 %; max 9.2 µg/kg; Ø 4.9 µg/kg; med: 4.3 µg/kg

## Additional analyses

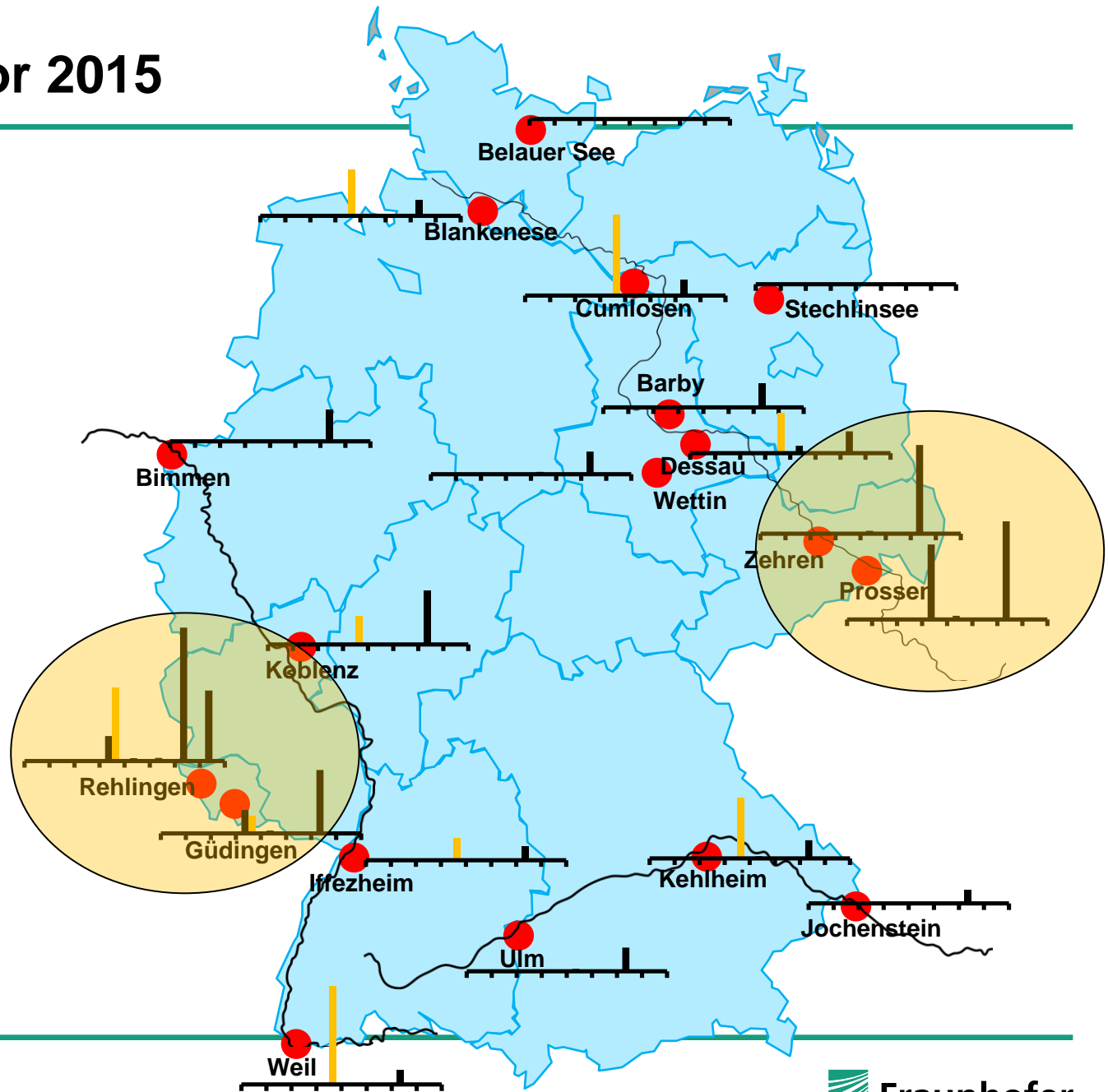
No findings in five otter liver samples

# Spatial Data for 2015

█ SPM (in rivers)  
█ Bream liver

10 µg/kg

Coumatetralyl  
 Warfarin  
 Chlorophacinone  
 Bromadiolone  
 Difenacoum  
 Flocoumaten  
 Brodifacoum  
 Difethialone

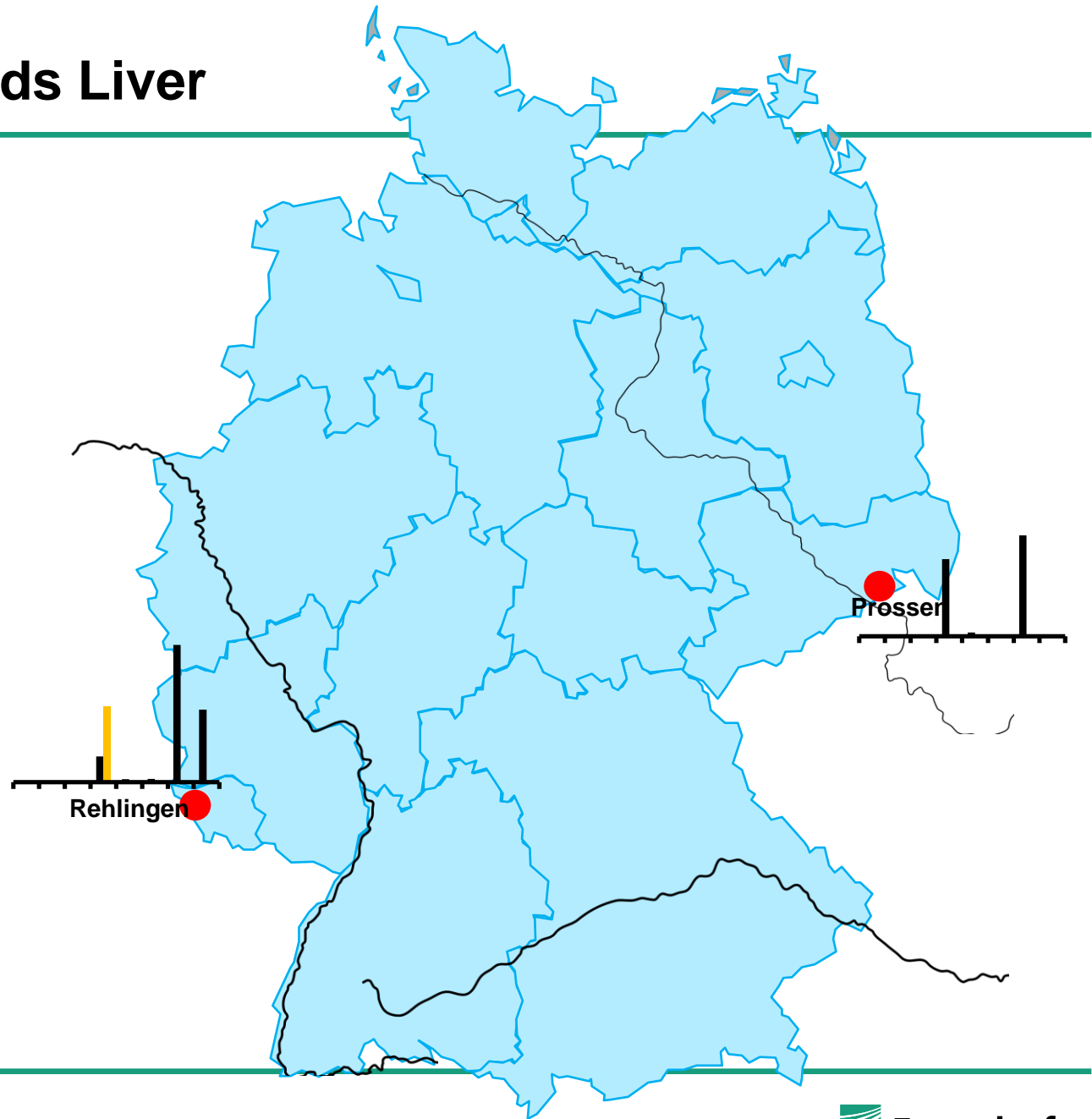


# Temporal Trends Liver

SPM (in rivers)  
Bream liver

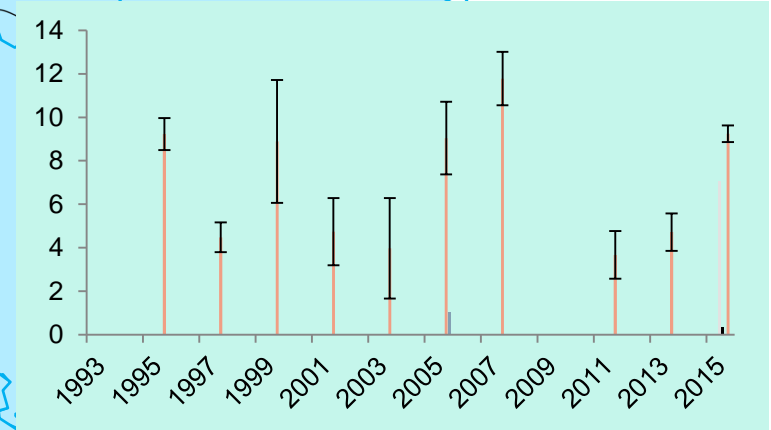
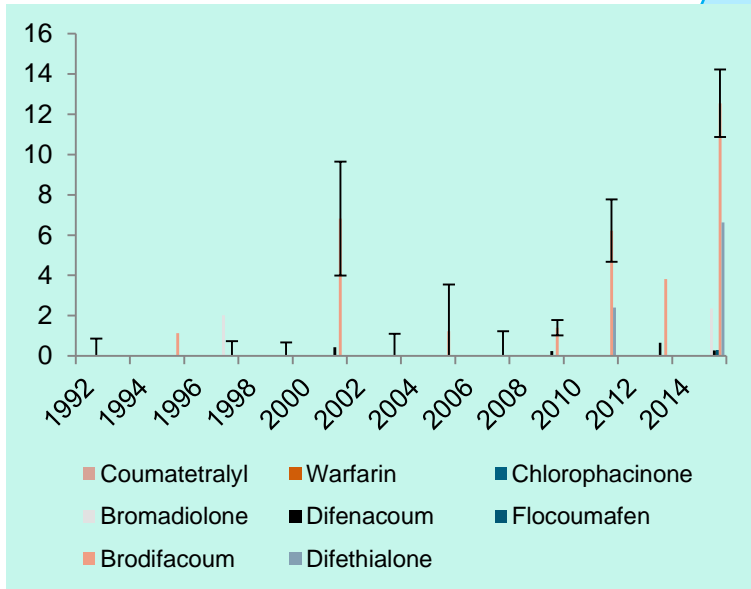
10  $\mu\text{g}/\text{kg}$

Coumatetralyl  
Warfarin  
Chlorophacinone  
Bromadiolone  
Difenacoum  
Flocoumaten  
Brodifacoum  
Difethialone



# Temporal Trends Liver

## Brodifacoum

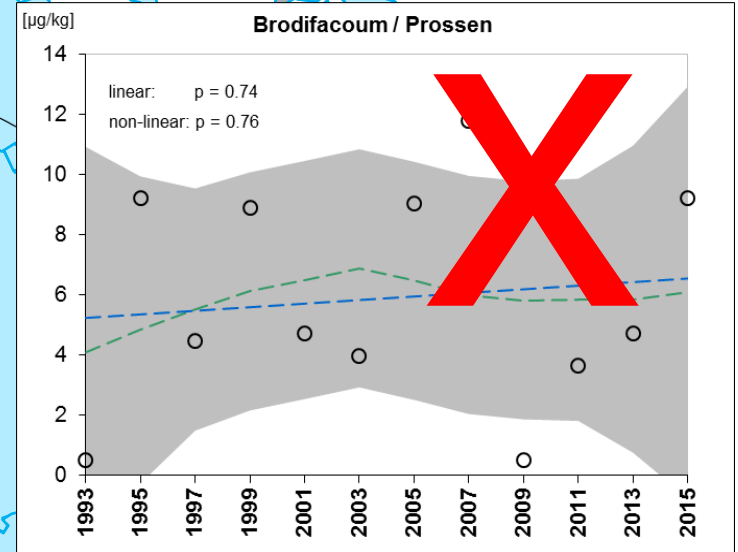
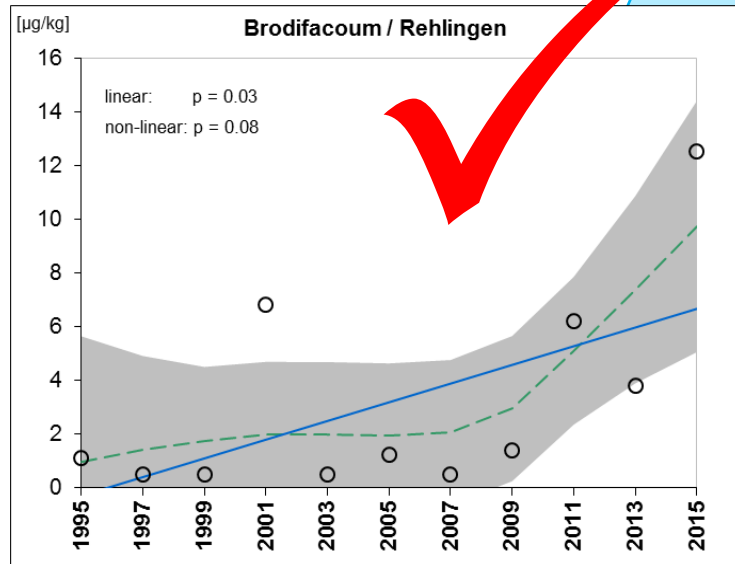


Rehlingen

Prossen

# Temporal Trends Liver

## Brodifacoum



Rehlingen

Prossen

LOESSTrend, Version 1.1, based on Microsoft Excel  
Data < LOQ treated as ½ LOQ

# Summary

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- Rodenticide-residues were found in fish samples from all riverine sites in Germany – no findings in samples from lakes
- Only SGARs with brodifacoum being most abundant have been detected at concentrations of up to 13 µg/kg ww – no FGARs have been found
- A significantly increasing time trend was observed for brodifacoum at the sampling location Rehlingen/Saar – role of bioaccumulation
- A different substance pattern (only bromadiolone) was found in SPM
- Further monitoring is warranted
- Sources of environmental exposure need to be identified or verified – e.g. sewer baiting
- Risk mitigation measures for the use of ARs should then be reviewed with stronger focus on the protection of aquatic species