

Effect directed analysis of sediment samples from the Elbe basin



Department Effect-Directed Analysis

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Starting point

What are the chemicals of environmental concern ?

Bioanalysis / ecotoxicology:

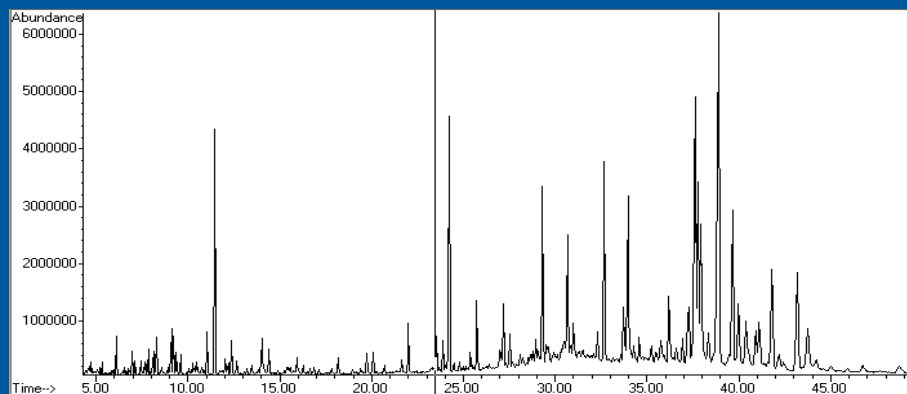
- in vivo / in vitro bioassays (with extracts) of environmental samples (water, sediment, soil...)

Analytical chemistry:

- target analysis, non-target analysis (screening)
 - monitoring programs of chemicals (e.g. priority pollutants) that
 - are well known,
 - caused problems in the past,
 - are representatives of other compounds
- **often those chemicals do not explain observed effects**

What are the chemicals of environmental concern ?

- not possible to analyse all compounds in the environment (> 50 Mill. compounds registered by CAS)

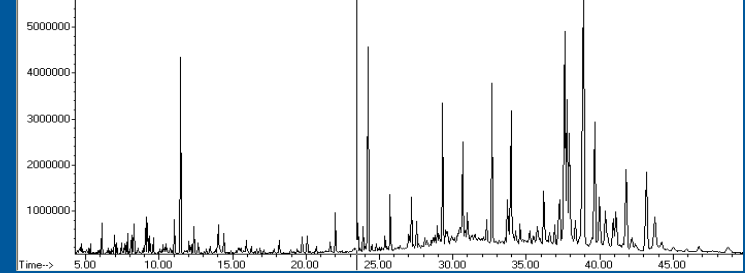
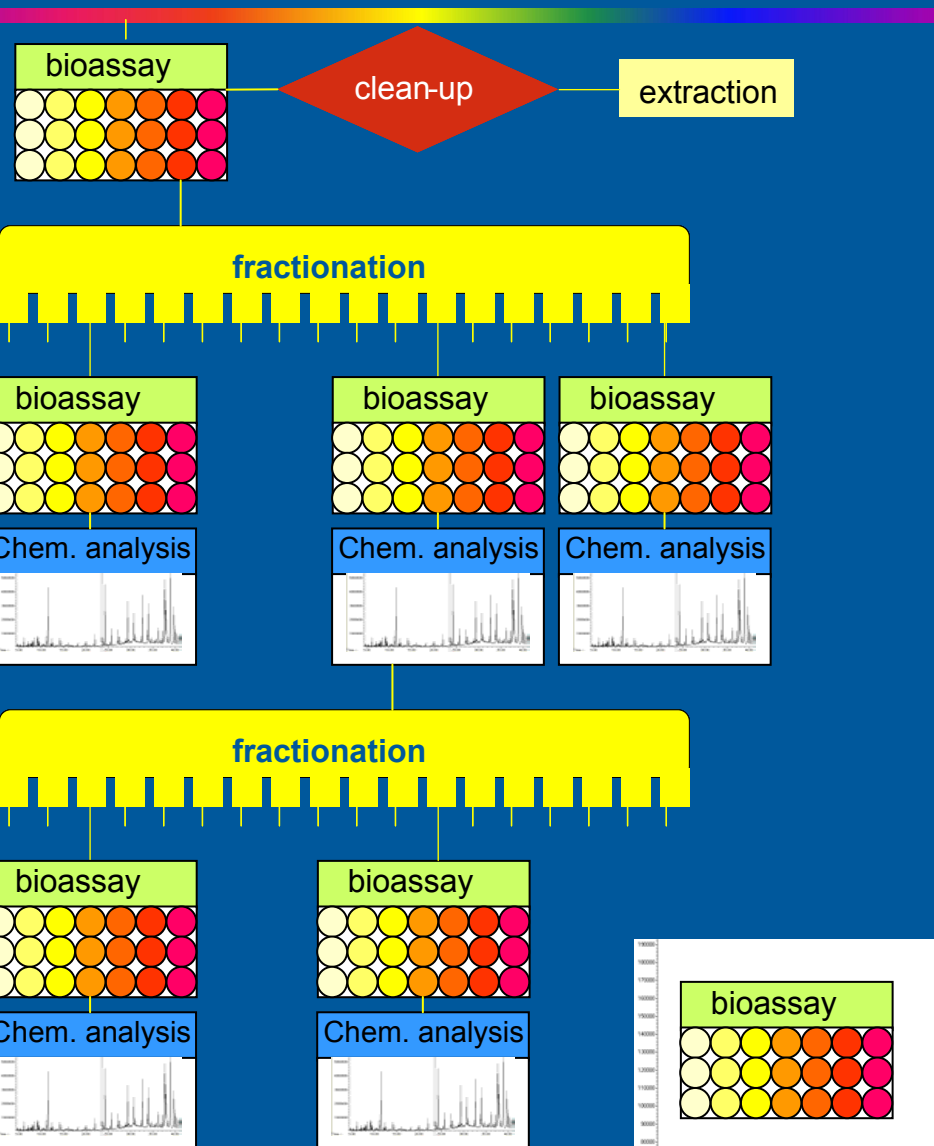


→ chemical analysis should be directed by effects (observed with bioassays)

What are the chemicals causing effects observed with bioassays ?

- Effect directed analysis / bioassay directed analysis as a tool to identify chemicals showing effects in the environment

Effect directed analysis



Identification possible?

no

reduce complexity

Select "hot" fraction

Identification possible?

no

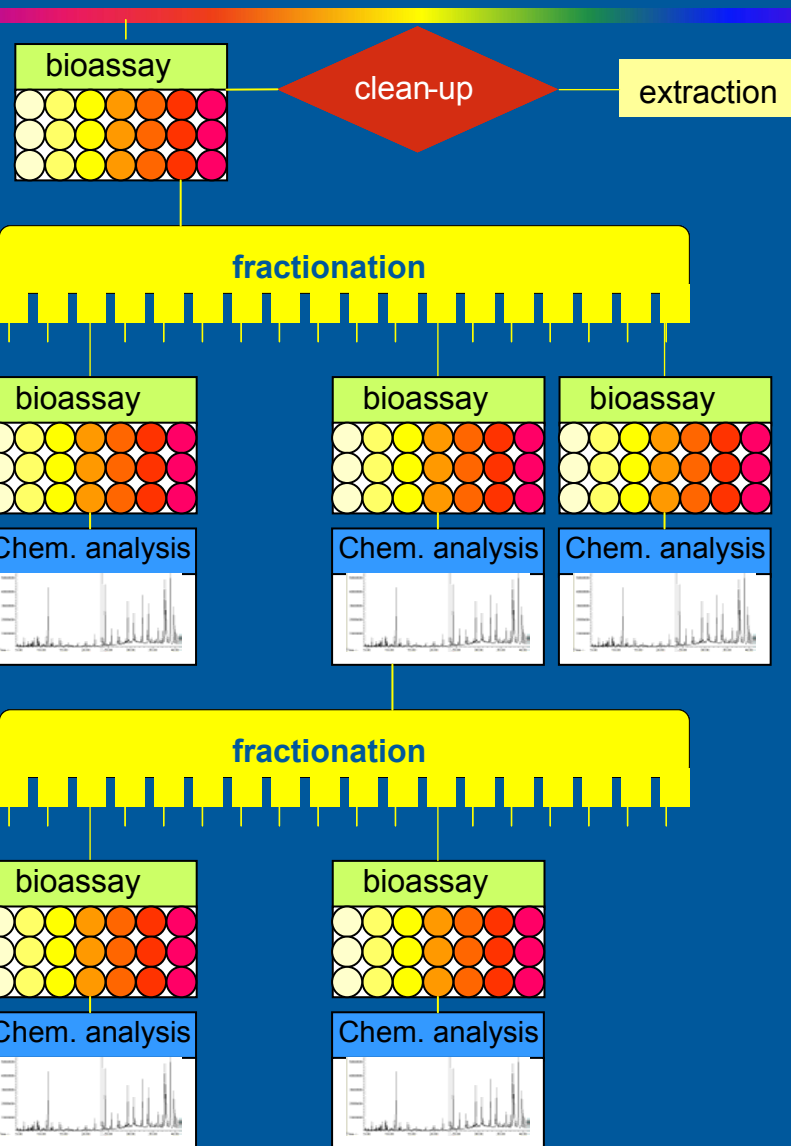
reduce complexity

Select "hot" fraction

Identification possible?

yes

Effect directed analysis



EDA:

- bioassay
 - fractionation
 - chemical identification
-
- Confirmation step
 - how much toxicity explained?
 - compounds lost?
 - mixture toxicity?

Sampling sites – Elbe basin

B Bitterfeld, Spittelwasser

- Chemical industry, dump sites



Germany

Poland

M Most, Bilina

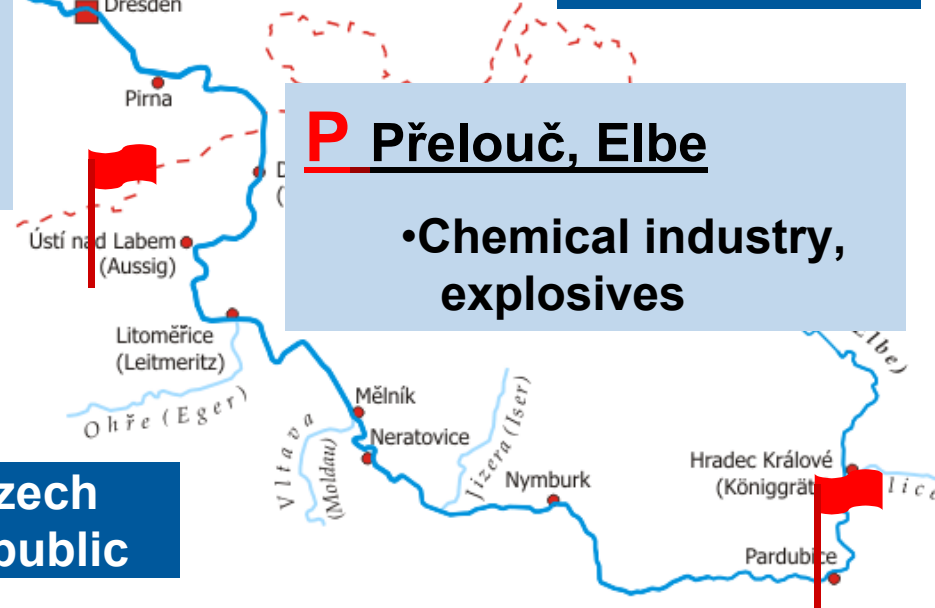
- refinery, chemical industry, municipal waste water



P Přelouč, Elbe

- Chemical industry, explosives

Czech Republic



Bioassays

Estrogenic activity	ER-CALUX* c=10 mg/mL	human breast carcinoma T47D.Luc cell line transfected with pEREtata-Luc reporter gene	[1]
Tumor promotion	Gap Junction c=100 mg/mL	WB-F344 rat liver epithelial cells	[2]
Ah-receptor mediated activity	DR-CALUX™* c=10 mg/mL	rat hepatoma H4IIEGud.Luc1.1 cell line transfected with a luciferase reporter gene under control of dioxin responsive element	[3]
Muta-genicity	Ames II c=200/400 mg/mL	<i>Salmonella typhimurium</i> , tester strain TA98, with/without metabolic activation by S9	[4]

[1] Legler et al., 1999. Toxicological Sciences 48, 55-66, [2] Blaha et al., 2002. Toxicological Sciences 65, 43-51, [3] Machala et al., 2001. Mutation Research 497, 49-62, [4] Reifferscheid et al., 2005. Env. Mol. Mut. 46, 126-139

*BioDetection Systems, Amsterdam, The Netherlands

Sample preparation/fractionation

Freeze-dried sieved (<63 μm) sediment



Accelerated Solvent Extraction (ASE)



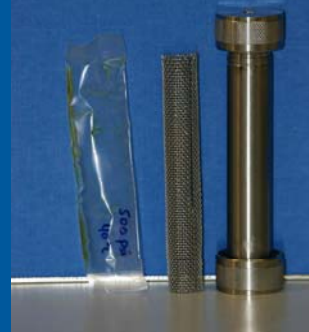
Accelerated Membrane Assisted
Clean-up (AMAC)



1st Fractionation on three coupled and automati-
cally switched normal-phase HPLC columns



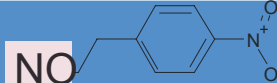
2nd Fractionation with C18-reversed
phase HPLC

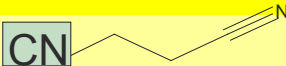


Normal-phase HPLC (first fractionation)

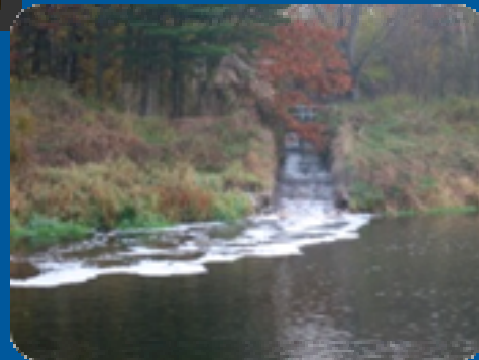


	Elution from porous graphitised carbon	
	PGC electron-rich surface	
Fraction	4	5
	co-planar PCBs, PCNs 3-6 Cl	PCDD/Fs, PCNs > 6 Cl

Elution from nitrophenyl column – PAHs and coeluting compounds						
6	7	8	9	10	11	12
Number of aromatic rings of eluting PAHs						
<3	3	4	4	5	6	7

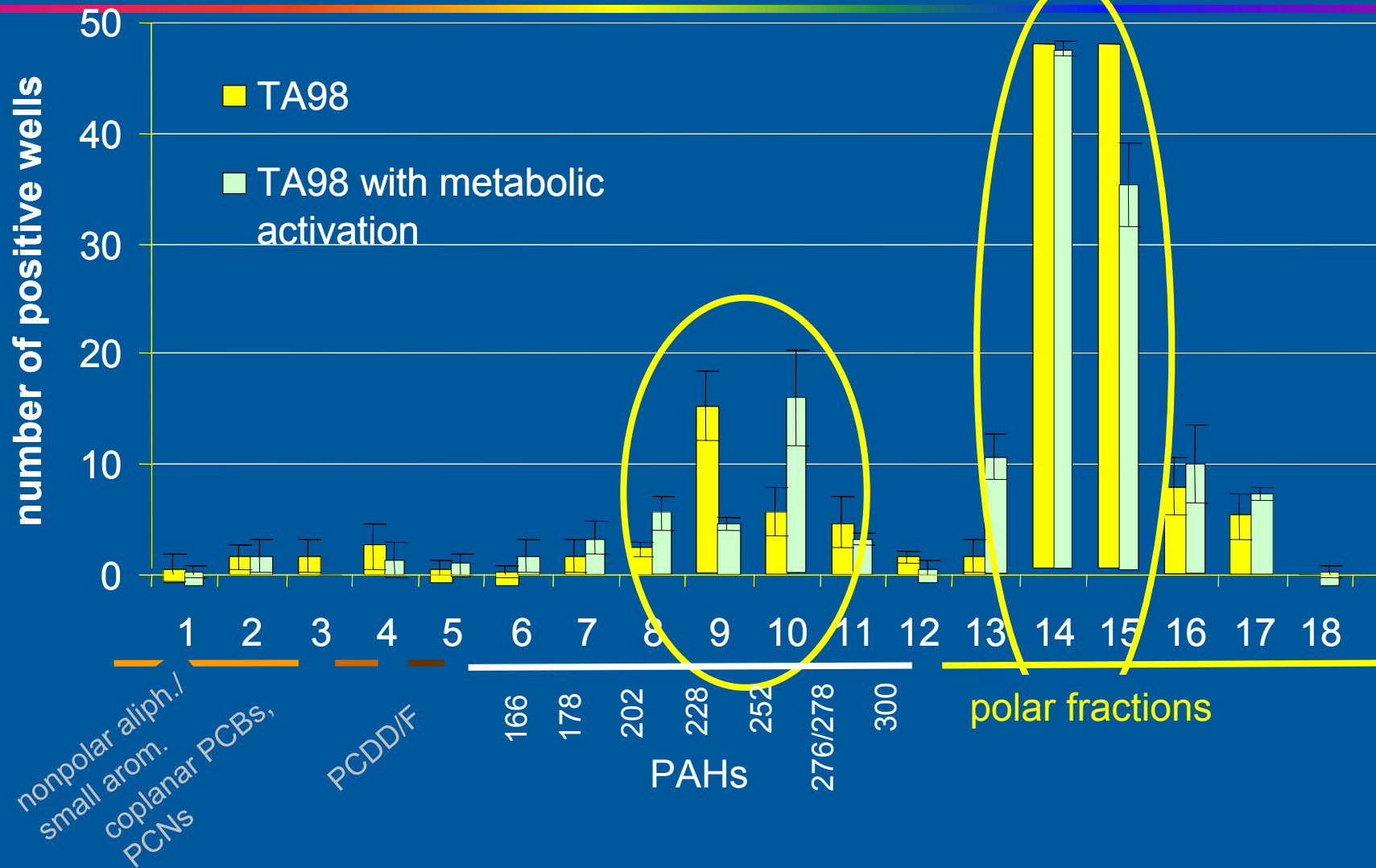
13	14	15	16	17	18
Elution from cyanopropyl column – (hydroxy-)quinones, keto-, (di-)nitro-, hydroxy-PAHs with rising polarity					
					

1. Example



Mutagenicity (Ames) – 1st fractionation

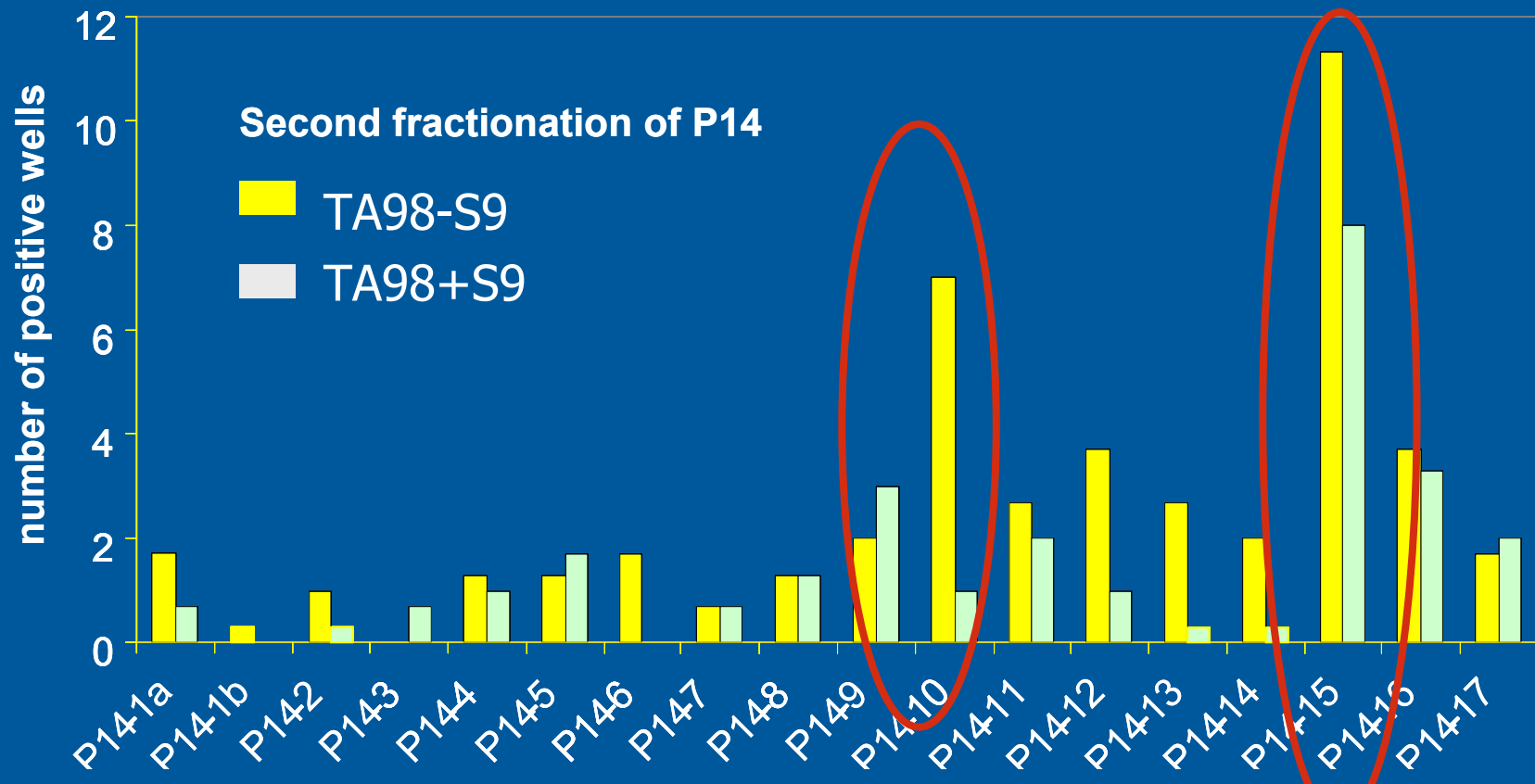
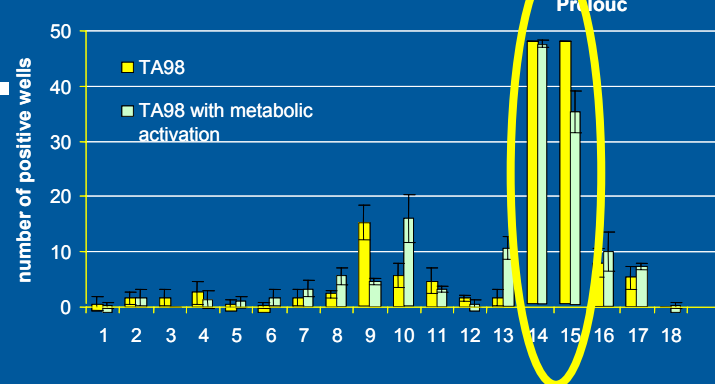
Preloc



- mutagenicity in PAH-fraction (F9 and F10)
- high mutagenicity in polar fractions (F14 and F15)

Mutagenicity (Ames) – 2nd fract.

- two fractions with higher direct mutagenicity or direct mutagenicity



Mutagenicity (Ames) – identification P14-10

Tentatively identified

■ methyl-11-benzo(a)fluorene-11-one

■ dimethyl-9H-fluoren-11-one

■ benzo(a)fluoren-11-on

■ benzanthrone

■ dichlorobenzophenon

■ dimethylantraquinone

■ methyl-acridine

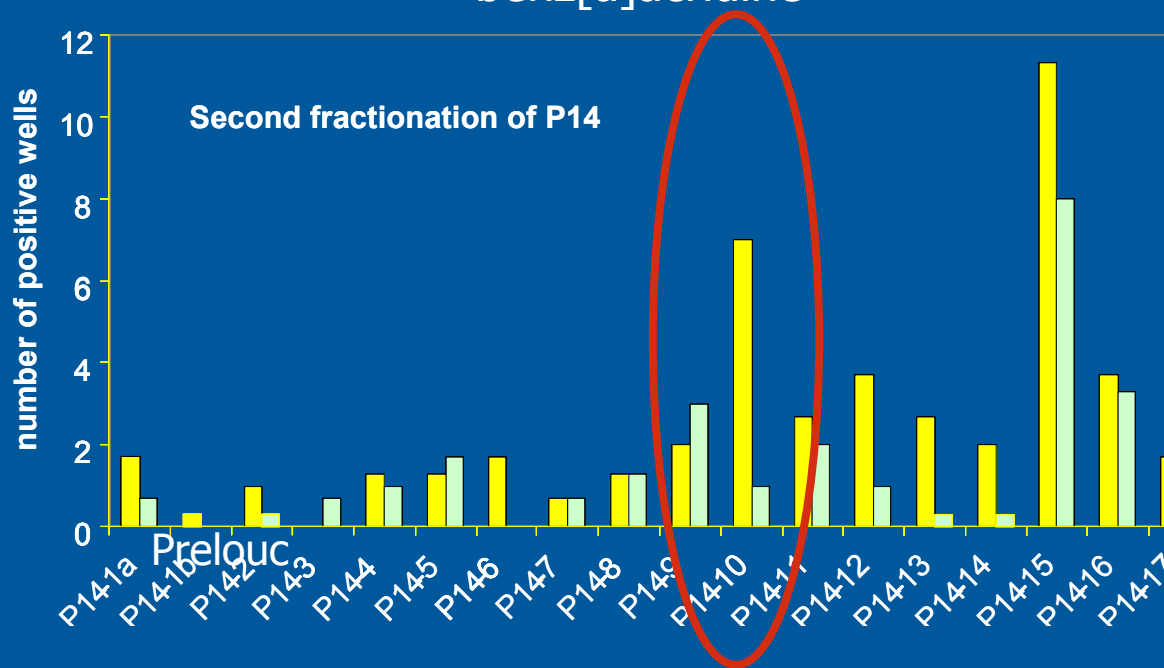
■ trimethylcarbazole

■ 7H-benz[de]anthracen-7-one

Confirmed identification

■ 9-fluorenone

■ benz[a]acridine

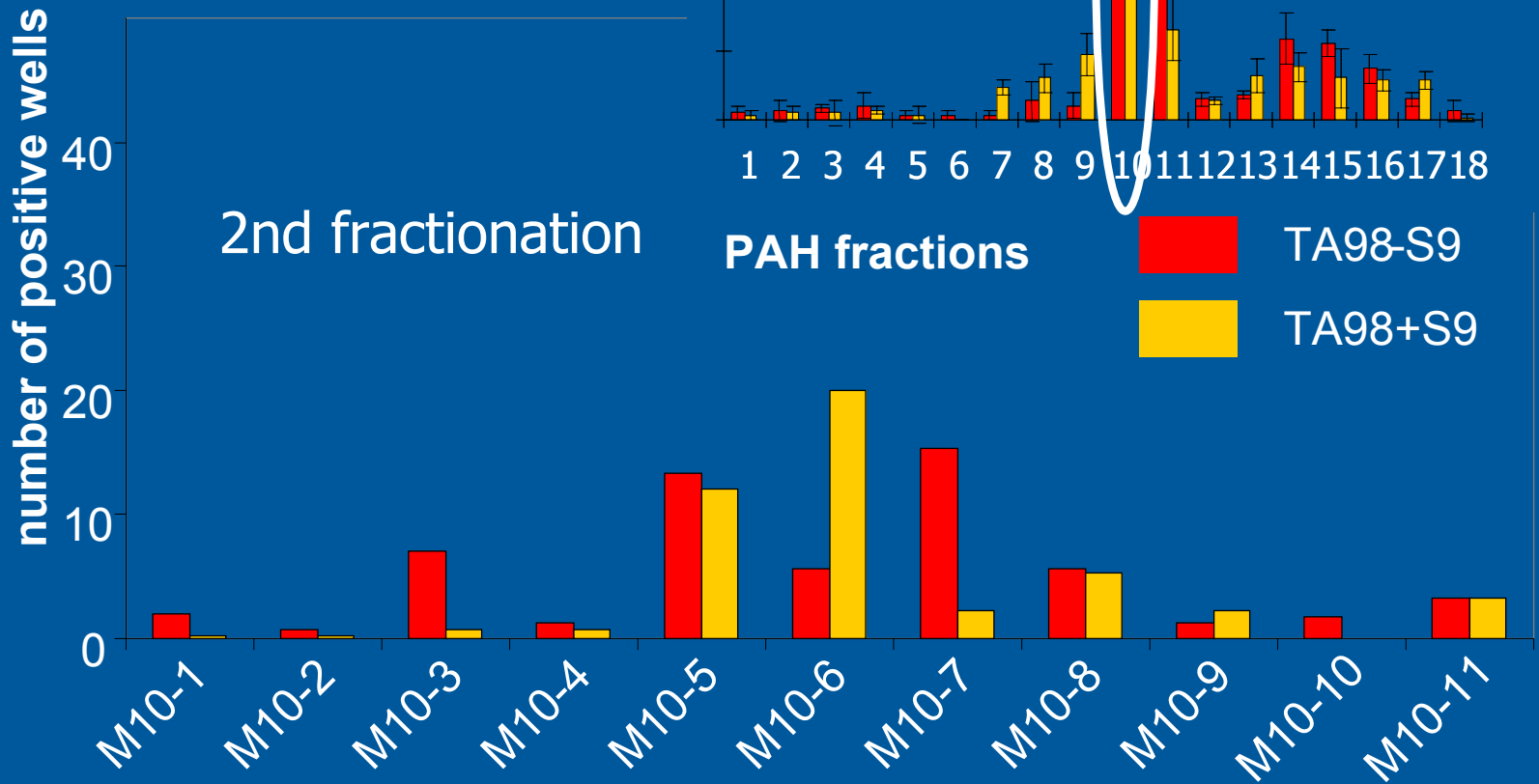


- Fractionation necessary to reduce complexity
- Mutagenicity also in other fractions than expected

2. Example



Mutagenicity (Ames)



- similar picture as with sediment Prelouc:
 - PAH-fractions and polar fraction contribute most to mutagenicity
 - direct mutagens in "PAH"-fraction

Mutagenicity (Ames)

number of positive wells

40
30
20
10
0

M10-1 M10-2 M10-3 M10-4 M10-5 M10-6 M10-7 M10-8 M10-9 M10-10 M10-11

Most

1st fractionation

2nd fractionation

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Compound

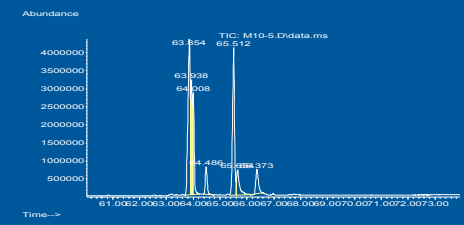
C (µg/ml)

M10-5

M10-6

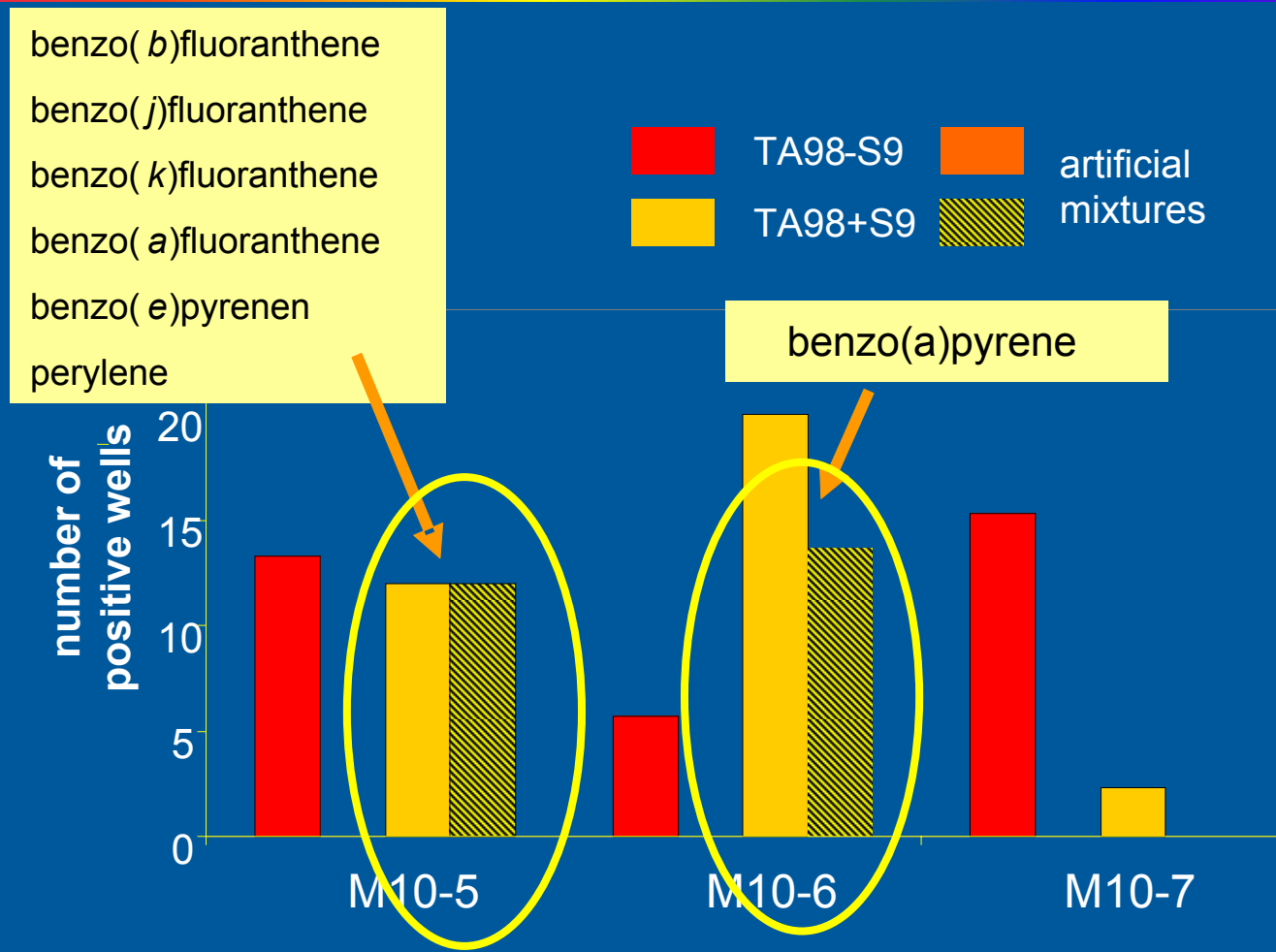
M-10-7

benzo[<i>b</i>]fluoranthene	1.12	0.02	-
benzo[<i>j</i>]fluoranthene	0.51	-	-
benzo[<i>k</i>]fluoranthene	0.59	0.04	-
benzo[<i>a</i>]fluoranthene	0.17	..-	-
benzo[<i>e</i>]pyrene	0.86	0.02	-
benzo[<i>a</i>]pyrene	..-	0.48	0.02
perylene	0.18	..-	-



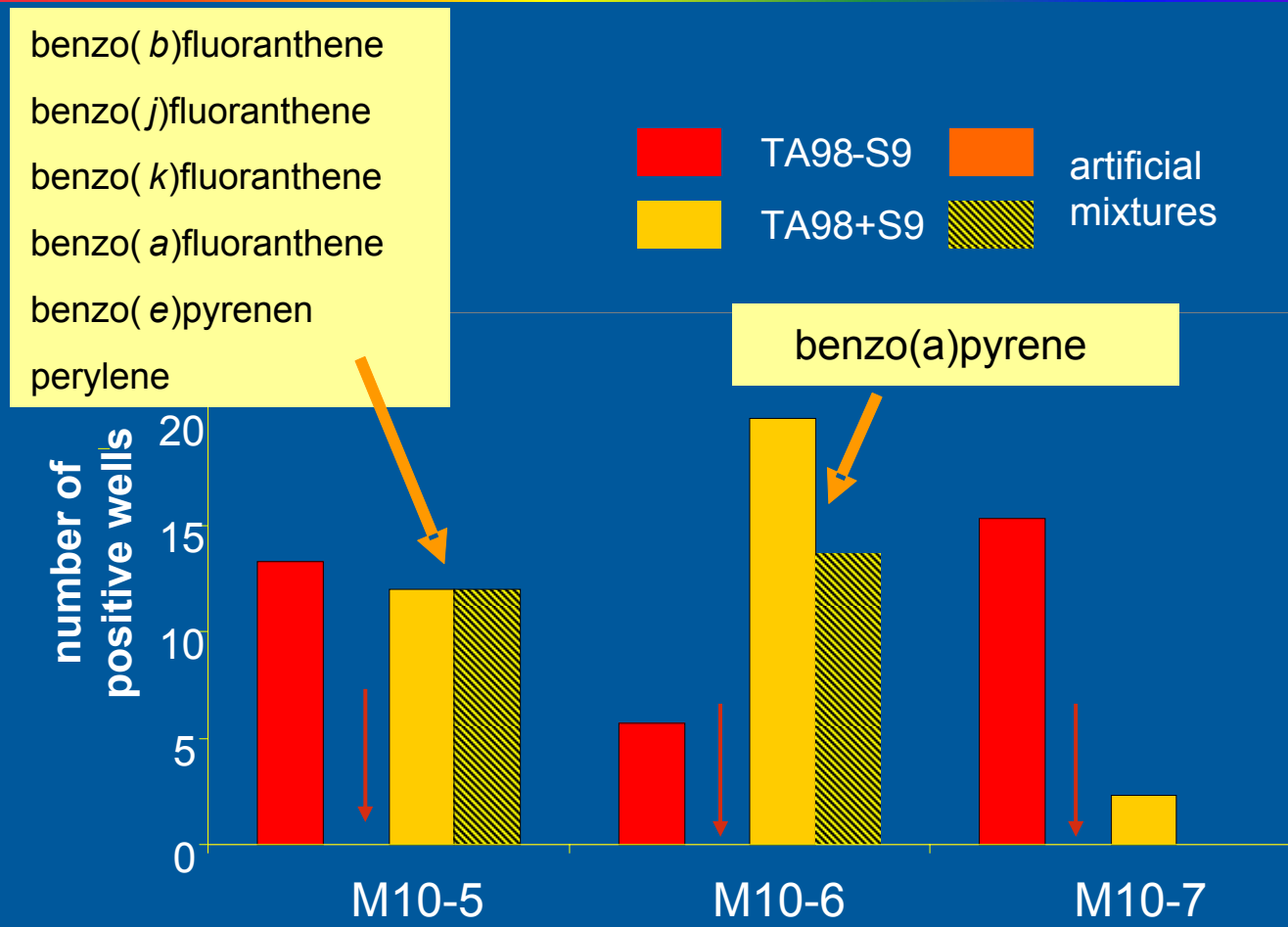
methylated benzofluoranthenes/-pyrenes

Confirming effective compounds with artificial mixtures - mutagenicity



indirect mutagenicity confirmed (M10-5 and M10-6)

Confirming effective compounds with artificial mixtures - mutagenicity

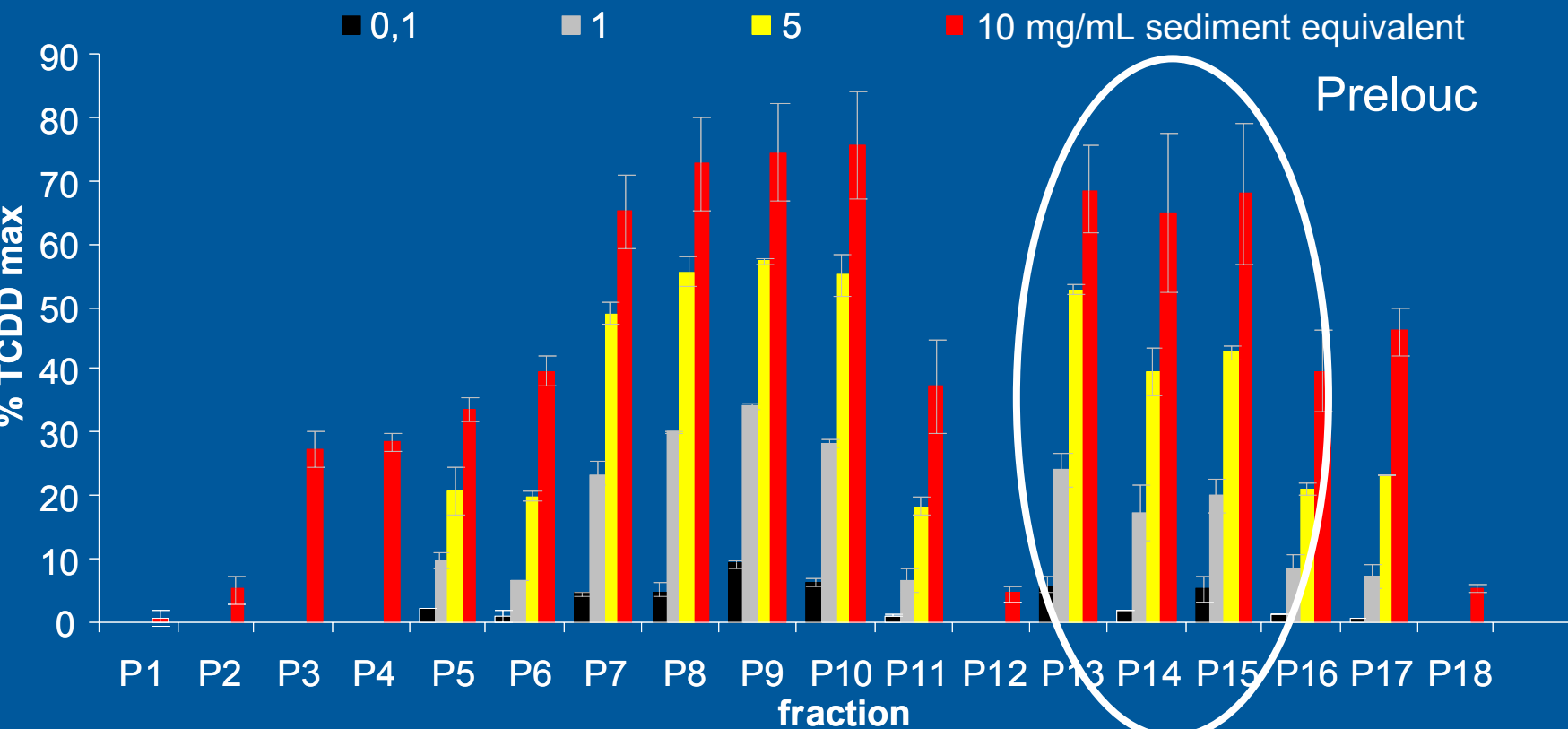


- Confirmation step important to recognize whether all effective compounds have been identified

3. Example

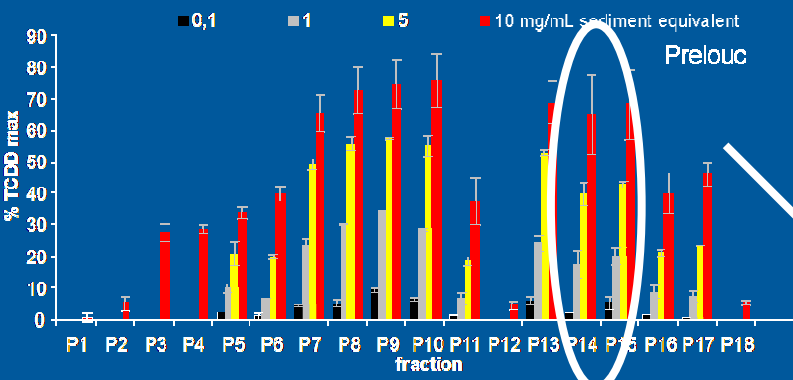


AhR-mediated activity

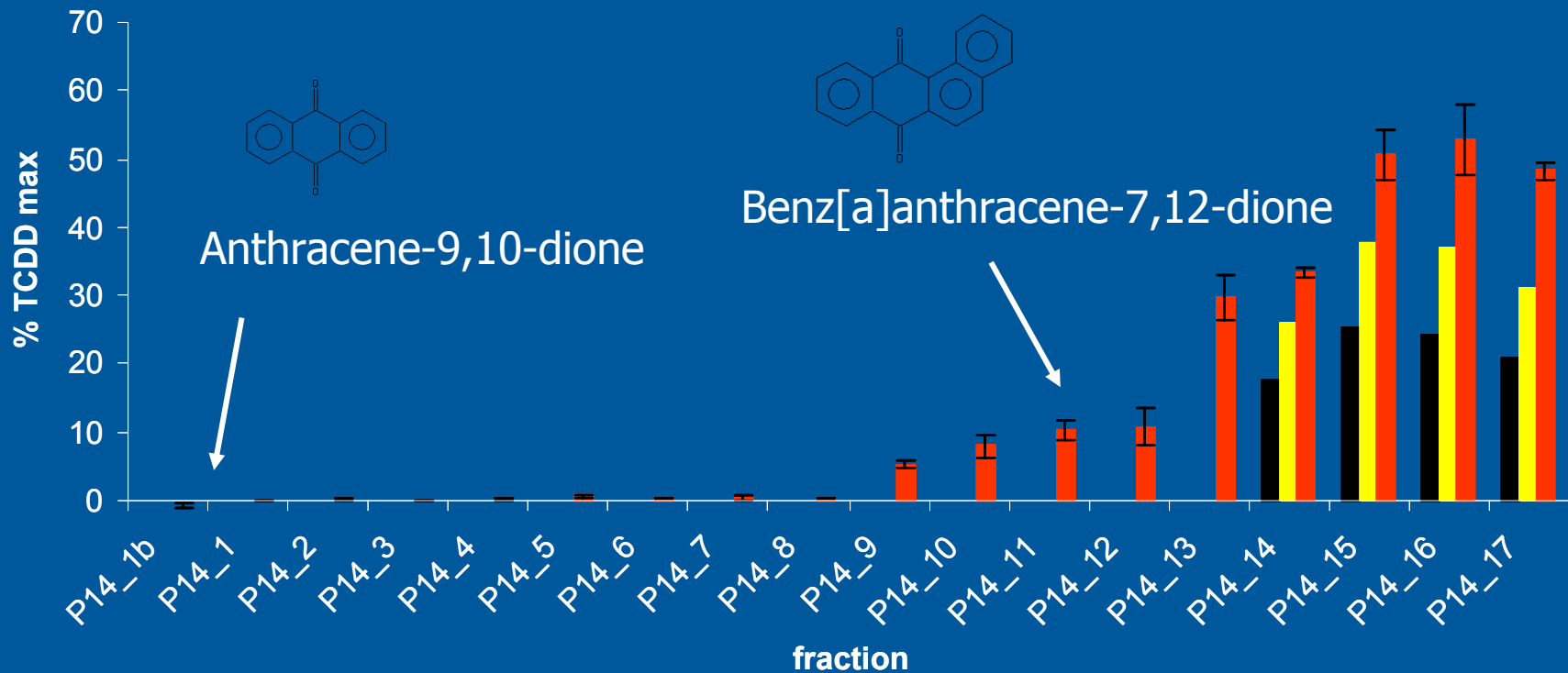


High AhR-mediated activity
also in polar fractions

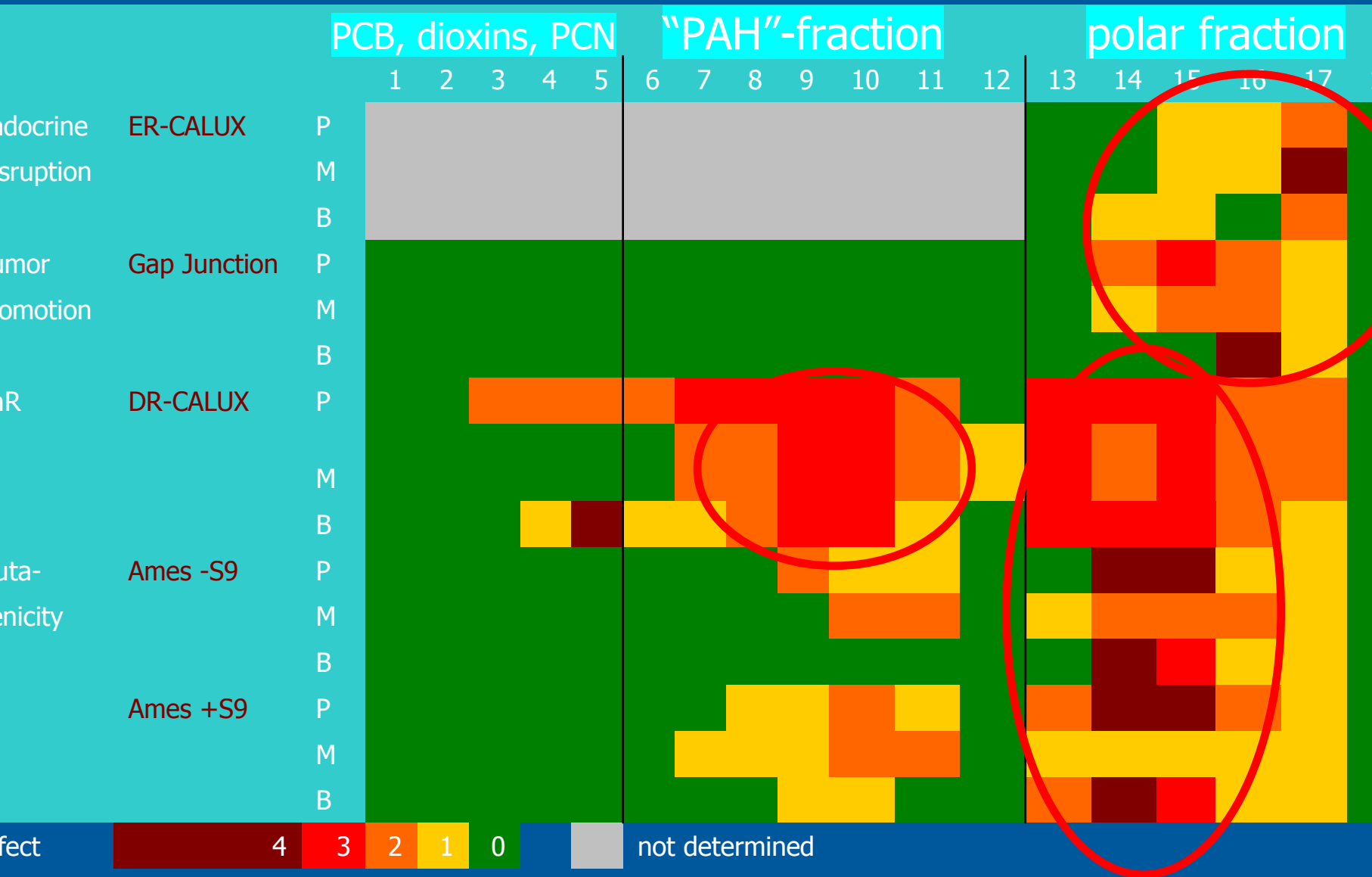
AhR-mediated activity



- most abundant compounds: not responsible for effects
- oxygenated or nitrated PAHs: not responsible for effects
- effective compounds not yet identified



Results all bioassays → Effects especially in polar fractions



Summary

- Effect directed analysis is a powerful and necessary tool for the identification of chemicals of environmental concern;
- successful identification only possible with combination of
 - bioassay
 - fractionation
 - chemical identification techniques
- Confirmation steps are important to demonstrate completeness of EDA or to recognize existence of unidentified effective compounds
- Polar compounds often contribute significantly to total toxicity

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...all other MODELKEY partners

