

**NORMAN Workshop NIVA 1-2 March 2012**

“How do we identify the next generation of emerging contaminants?”



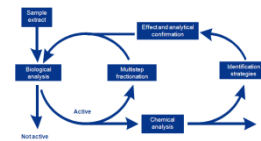
## Accurate mass screening for known unknowns

Jana Weiss, Dept. Chemistry & Biology, IVM VU University Amsterdam

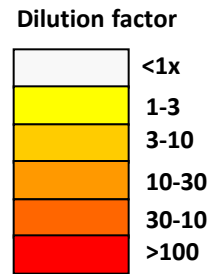
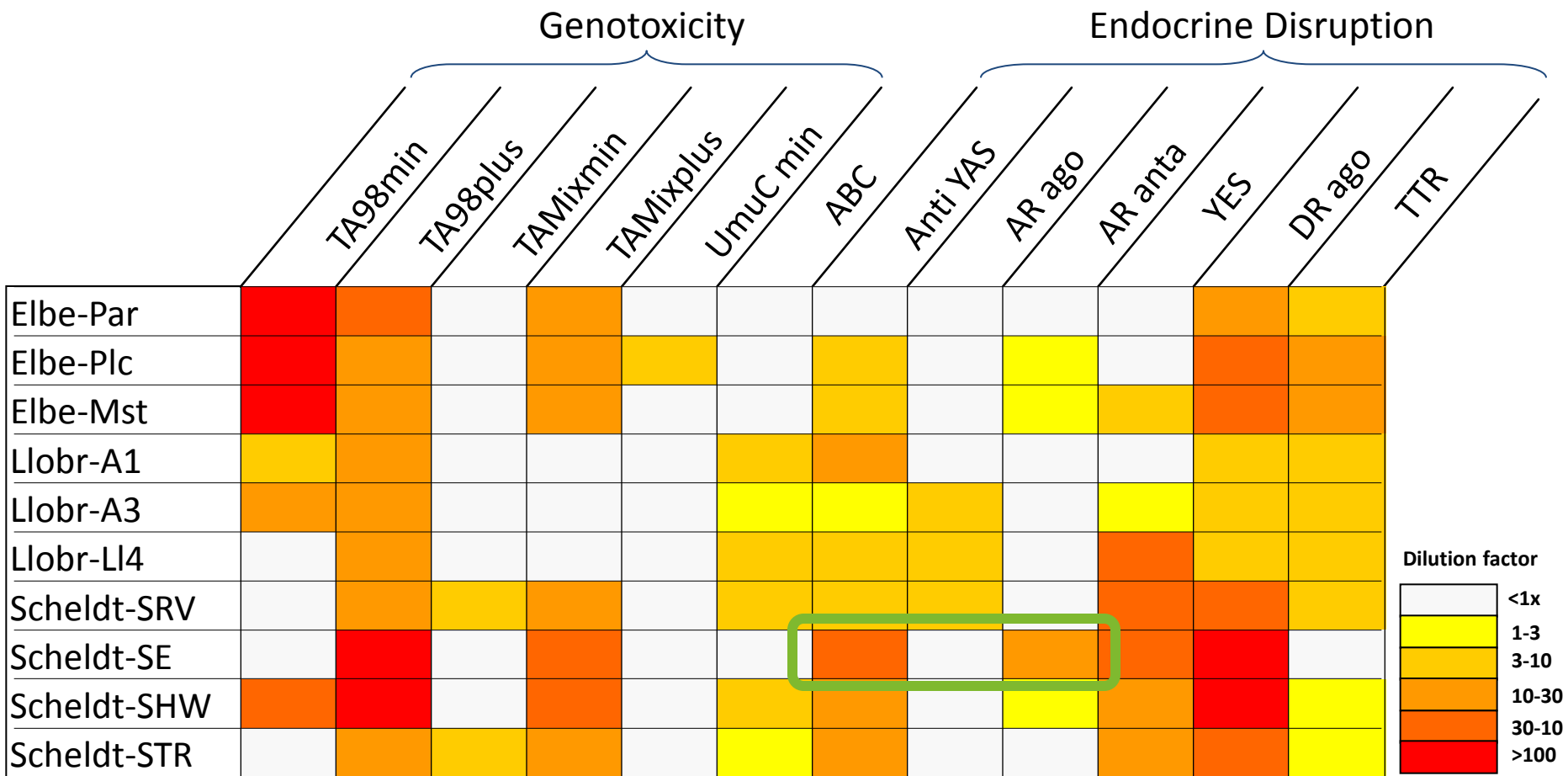
# Identification strategies

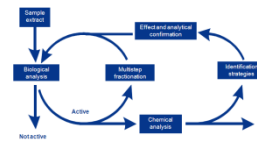
1. Target analysis of priority pollutants
2. Prediction of emerging contaminants – target analysis
3. Effect-directed analysis (EDA) to determine the key-toxicants
4. Accurate mass screening of known unknowns

| Nr | Sensitive | Time-efficient | Inclusive | Comment                          |
|----|-----------|----------------|-----------|----------------------------------|
| 1  | +++       | +++            | ---       | 33 +8 priority cmpd's in WFD     |
| 2  | ++        | --             | -         | Different prediction strategies  |
| 3  | -         | ---            | +++       | Identifies key-toxicants         |
| 4  | +         | ++             | +         | Limitation is existing databases |

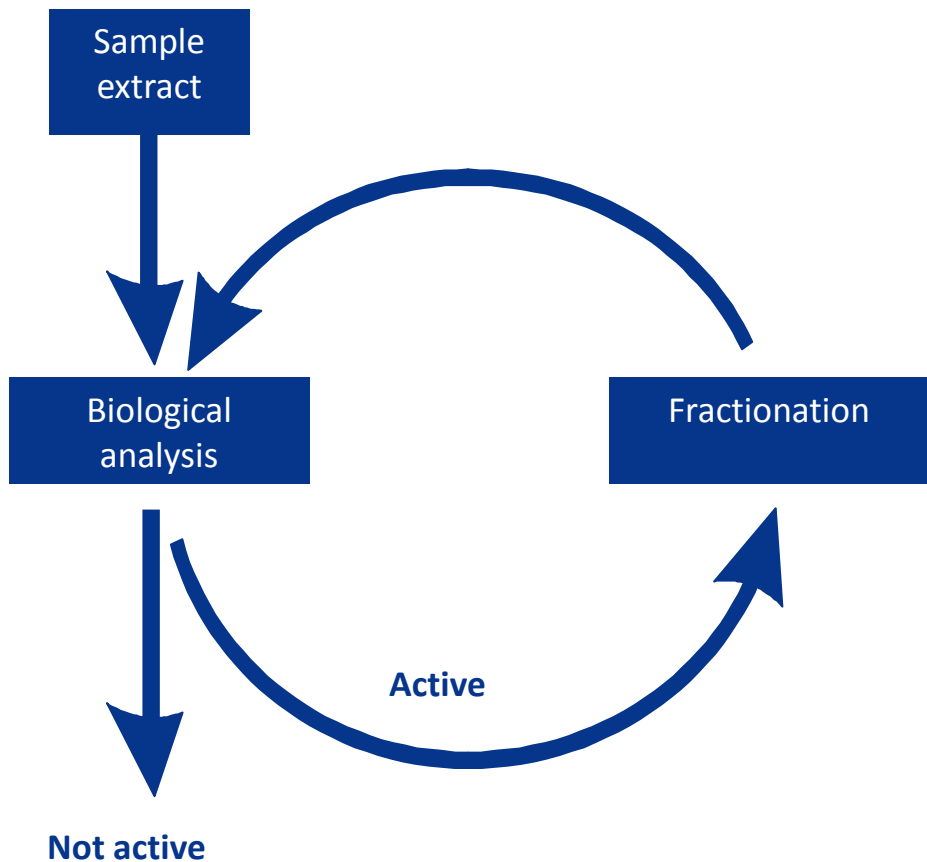


# Toxicity profile of sediment samples – Modelkey





# Effect-Directed Analysis (EDA) scheme

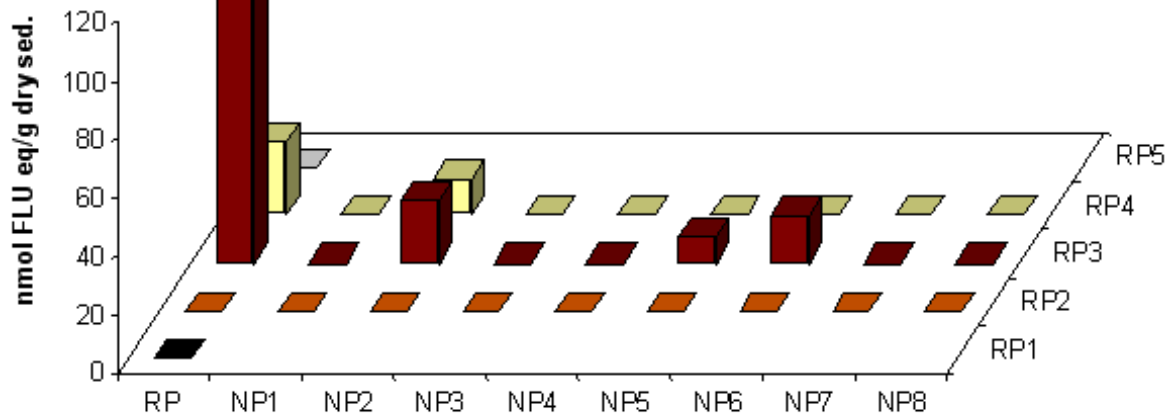


- Simplifies the matrix
- Single fractionation into several fractions
- Multiple serial fractionations based on different chemical/physical properties

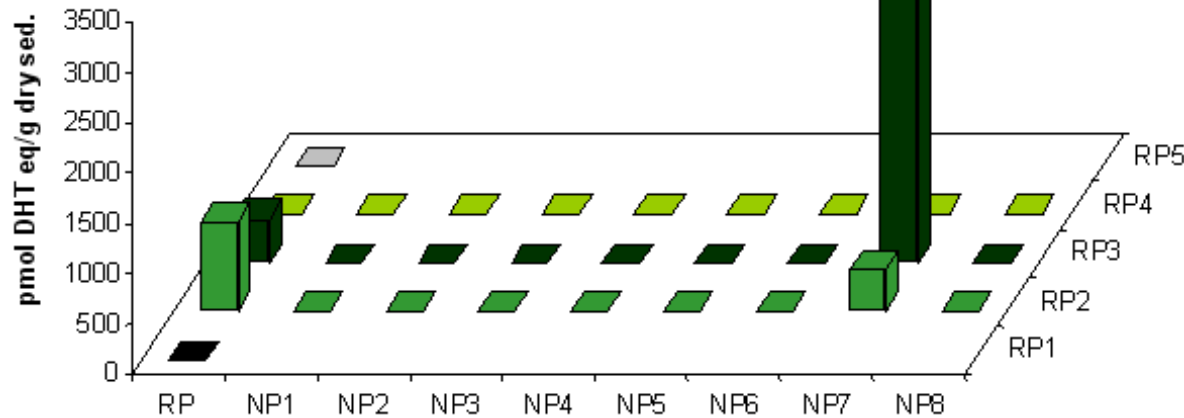
# Androgenicity in sediment extracts



**A. Anti-androgenic activity**



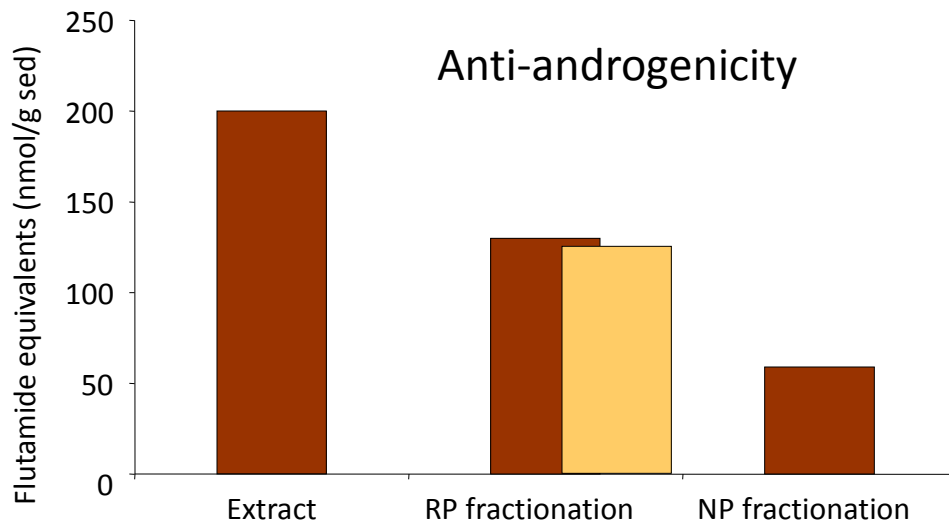
**B. Androgenic activity**



Active fractions:  
3 distinct groups of compounds with different polarity

(Weiss et al. 2009, ABC 394:1385-1397)

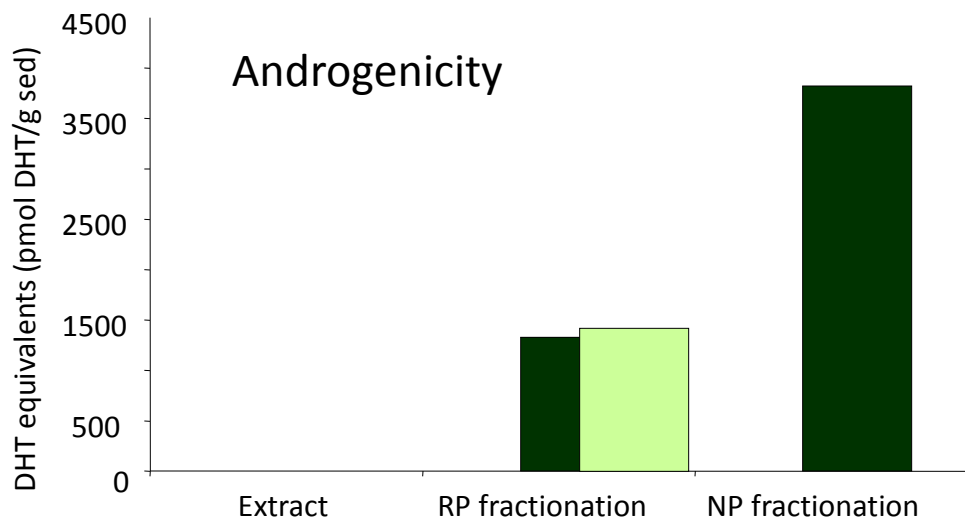
# Androgenicity in sediment extracts



Anti-androgenicity decrease after fractionation

Not due to low recovery

Presence of partial agonists, or low sensitivity in the bioassay



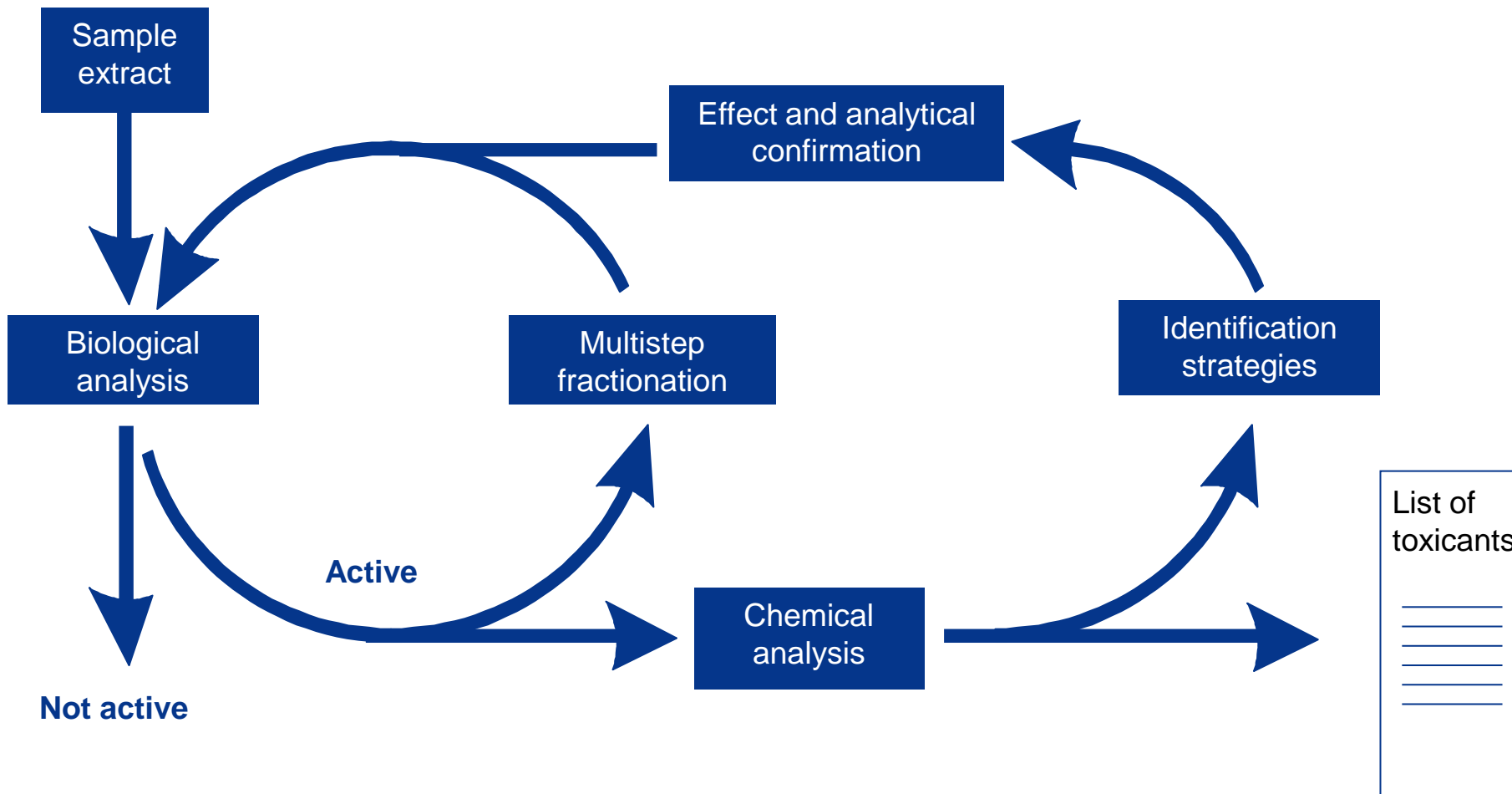
Androgenicity increase after fractionation

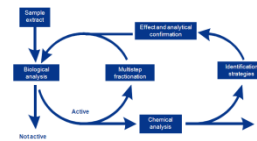
Separation of disrupting antagonistic compounds

Screening of extract do not reveal the full androgenic potency



# Effect-directed analysis





# Chemical analysis

## GC-MS

DB5 column, full scan mode (m/z 50-650)

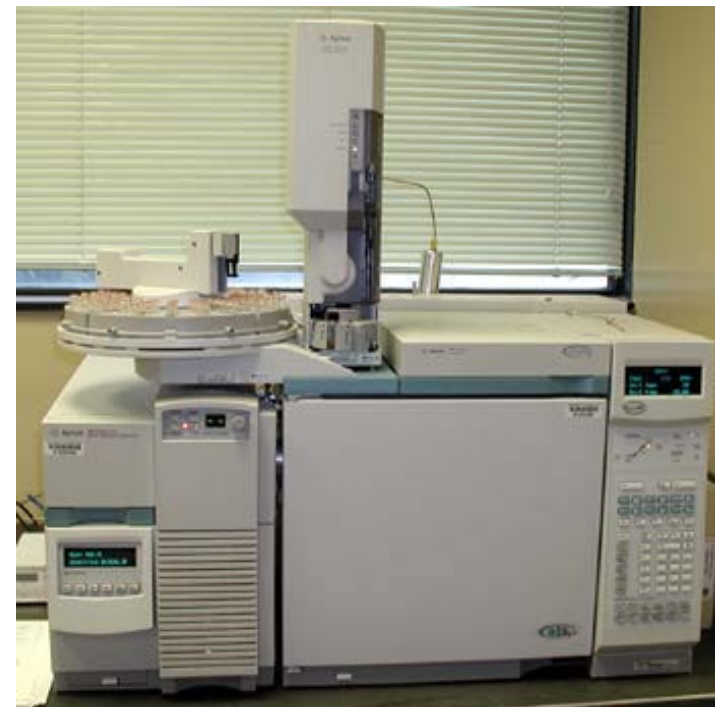
Electron impact ionisation (EI)

Mass spectra deconvoluted using AMDIS

NIST searched, match factor  $\geq 80\%$

The Kovats Retention Indices (KRI) values were used to identify the compounds to Quality Peak Identification Database (QPID)

Background subtraction with QPID



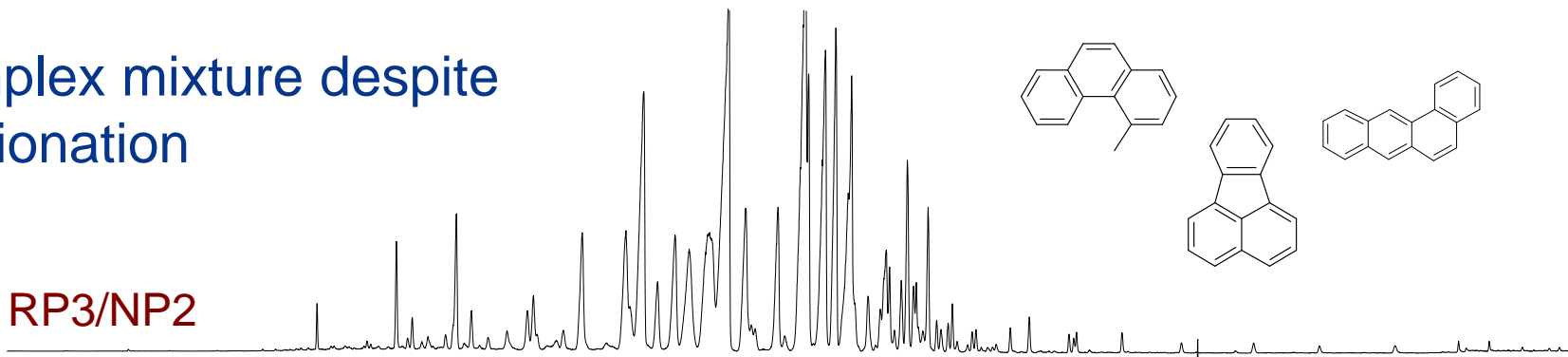
*Weiss et al 2009 (ABC 394:1385-1397)*



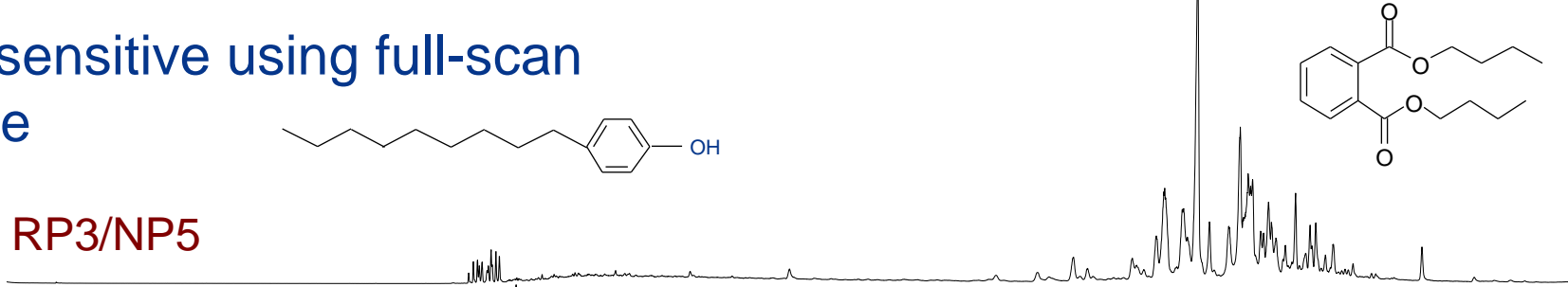


# Chemical analysis

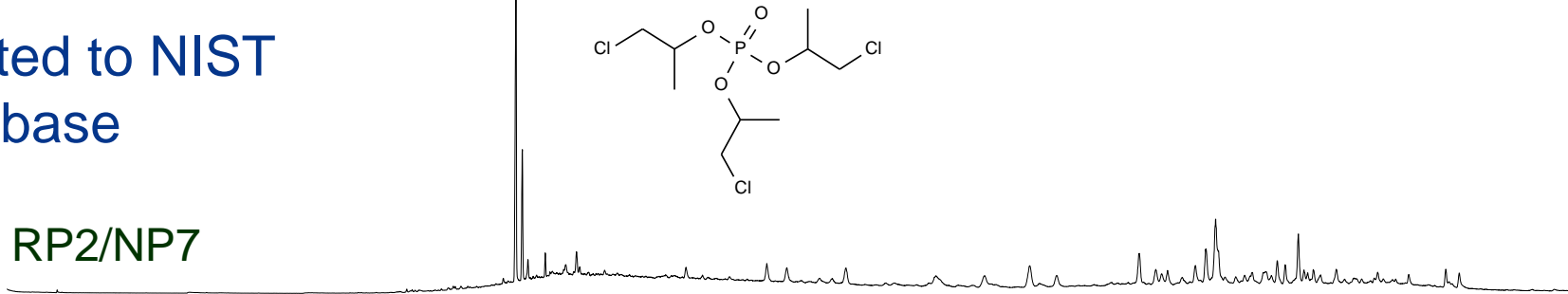
Complex mixture despite fractionation

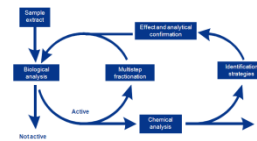


Not sensitive using full-scan mode



Limited to NIST database





# Chemical analysis

## LTQ-Orbitrap

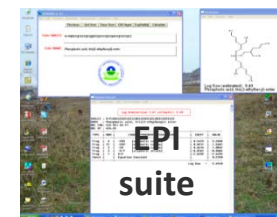
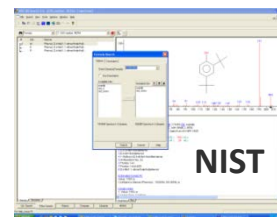
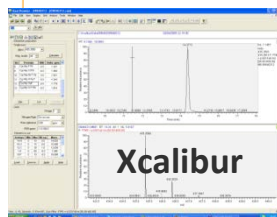
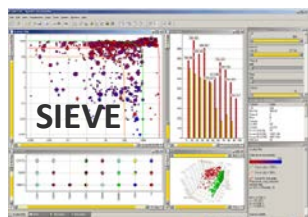
C<sub>18</sub> HPLC column, full scan mode (m/z 50-600)

Accurate Mass Capabilities

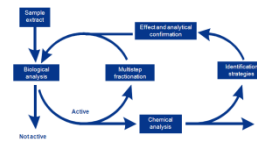
Resolution 30 000

Data Dependent™ acquisition with Dynamic Exclusion™

Background subtraction with SIEVE

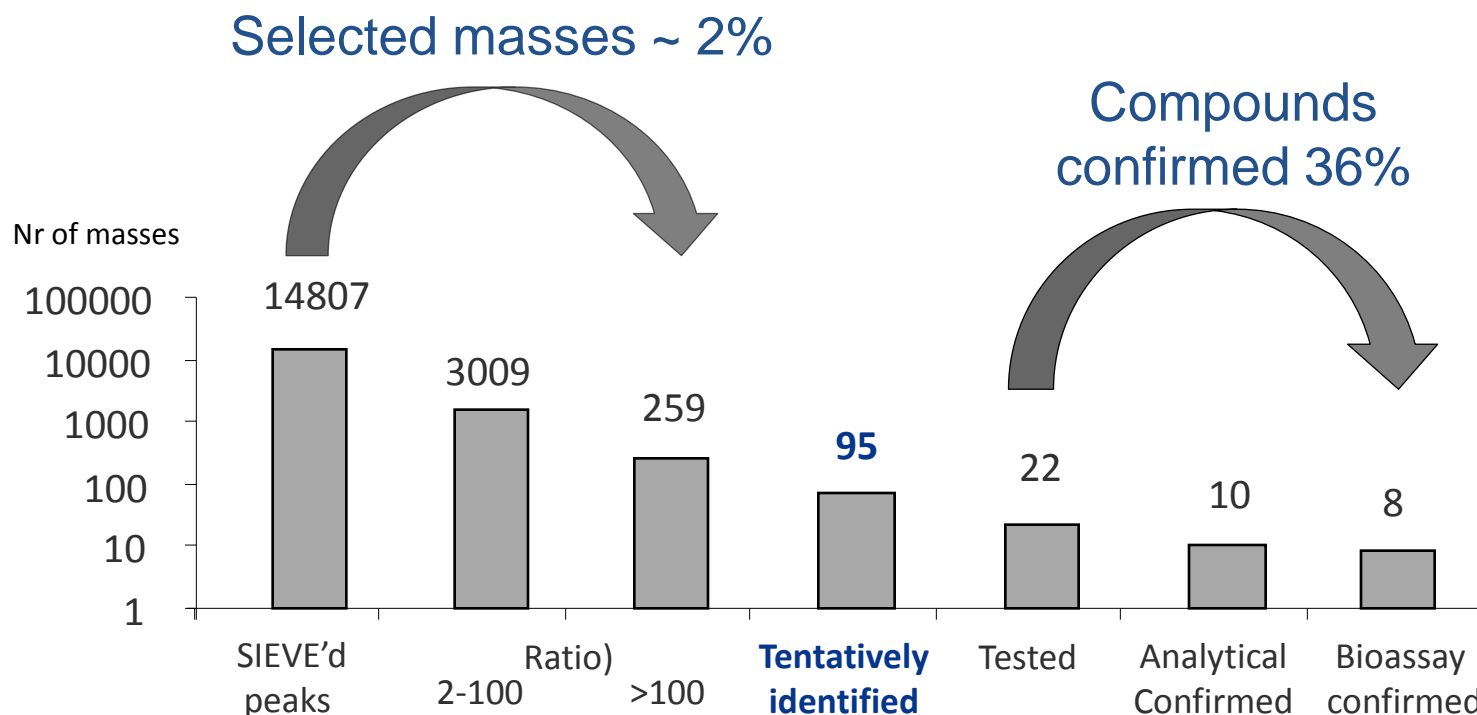


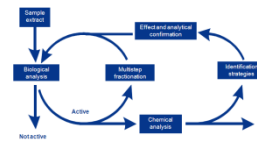
Weiss et al 2011 (ABC 400:3141-3149)



# Chemical analysis

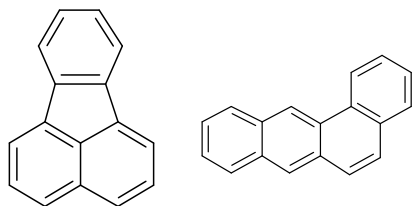
1. Ratio >100 between mass in active and non-active fractions
2. Need to exist in NIST (~200 000 compounds)
3. Possible to purchase (high purity)



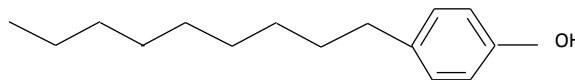


# Identified compounds GC/MS and LC/MS

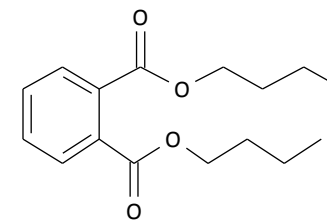
**PAH's**



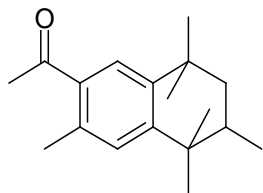
**Nonylphenol (technical mixture)**



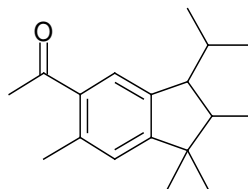
**Phthalate's**



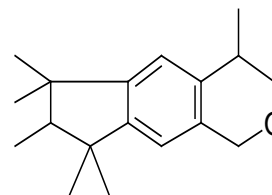
**Tonalide**



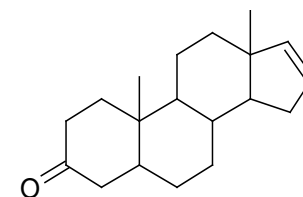
**Traseolide**



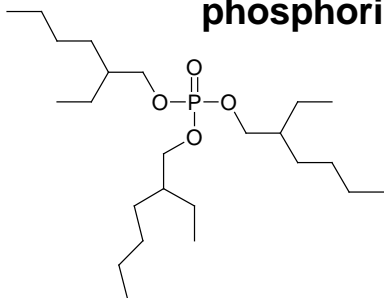
**Galoxolide**



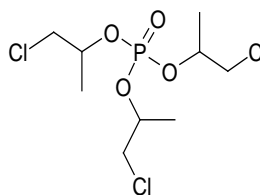
**Androstenone**



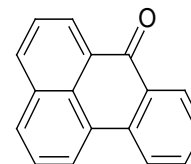
**Tris(2-ethylhexyl) phosphoric acid**



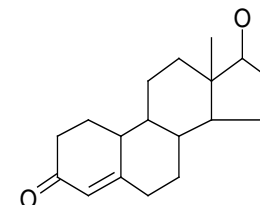
**Tris(2-chloroisopropyl) phosphoric acid**

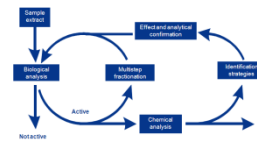


**Benzanthrone**



**Nandrolone**





# Effect confirmation by GC-MS Target analysis

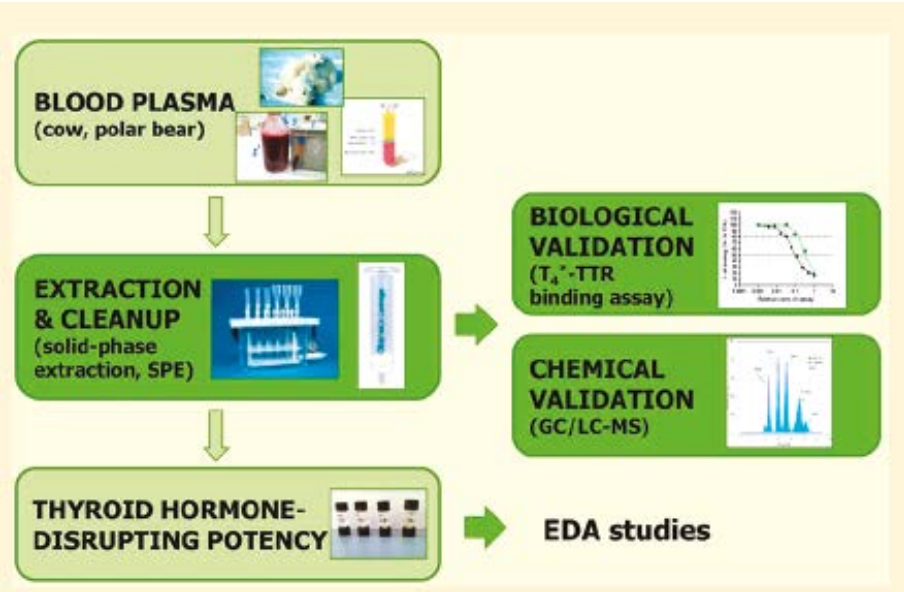
Danube sediment average OCP levels in between 0.005-2.5 ng/g dw  
 The sum of the AR activity in the fractions was 60 nmol/g sed, 3% explained.



# EDA in polar bear plasma

## Blood Plasma Sample Preparation Method for the Assessment of Thyroid Hormone-Disrupting Potency in Effect-Directed Analysis

Eszter Simon,<sup>†</sup> Jenny Bytingsvik,<sup>†</sup> Willem Jonker,<sup>†</sup> Pim E. G. Leonards,<sup>†</sup> Jacob de Boer,<sup>†</sup> Bjørn M. Jenssen,<sup>‡</sup> Elisabeth Lie,<sup>§</sup> Jon Aars,<sup>||</sup> Timo Hamers,<sup>†</sup> and Marja H. Lamoree<sup>\*†</sup>



## Target analysis of known thyroid hormone disrupters, *i.e.* OH-PCBs and OH-PBDEs by GC-MS

| Polar bears (n=2)           | T <sub>4</sub> -Eq (nM) |
|-----------------------------|-------------------------|
| Calculated TH-like activity | ~1000                   |
| Measured TH-like activity   | ~1400                   |

~70 % explained activity in the bears

~40 % explained activity in the cubs





# EDA in polar bear cubs plasma

Clean chromatograms of the cub samples  
–fractionation (?)



**LC-microTOF  
(Bruker, Bremen)**

**Positive and  
negative mode**



# Accurate mass and library search

|   | Library   | Nr. of compounds in library  | Nr. of matches "Suspects" |
|---|---|------------------------------|---------------------------|
| 1 | Compounds analyzed in polar bear + AMDIS identified compounds                               | 31                           | 7                         |
| 2 | High production volume pharmaceuticals (P and/or B) ( <i>Howard and Muir 2011, EST 45</i> ) | 106                          | 5                         |
| 3 | High production volume halogenated chemicals ( <i>Howard and Muir 2010, EST 44</i> )        | 594                          | 25                        |
| 4 | TTR-binding compounds (based on literature studies)   | 175                          | 20                        |
| 5 | Bruker library with dyes, pharmaceuticals, pesticides                                       | 225                          | 20                        |
|   |   | <b>Total tentatively id.</b> | <b>70</b>                 |

**33 compounds selected** for confirmation study (based on use, physical-chemical properties and chemical structure)

17 compounds tested, 15 wrong RT and 2 need to be confirmed

8 standards are not available or too expensive

8 compounds not yet tested

# Experience from EDA – the long way

## ■ Research for researchers

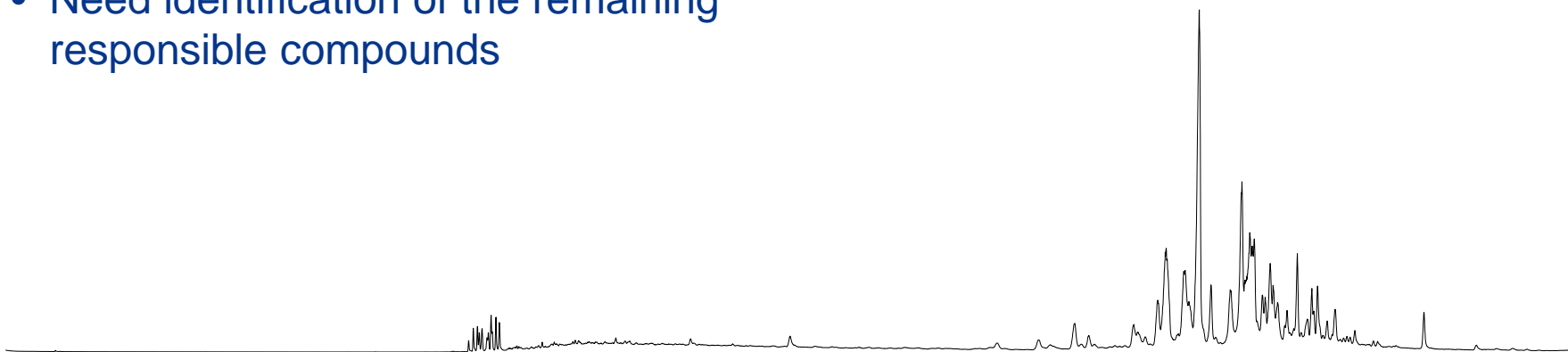
- Laborious
- Time consuming
- Demands experienced chemists for the identification

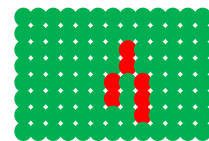
## ■ Low output

- A fraction of measured effects can be explained
- Need identification of the remaining responsible compounds

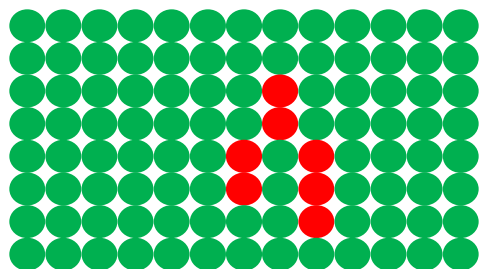
## ■ Need improvements

- Bioassay sensitivity
- Chemical analysis
- Fractionation strategies
- Identification strategy
- Database sharing





# Improved EDA- 96-well plate fractionation



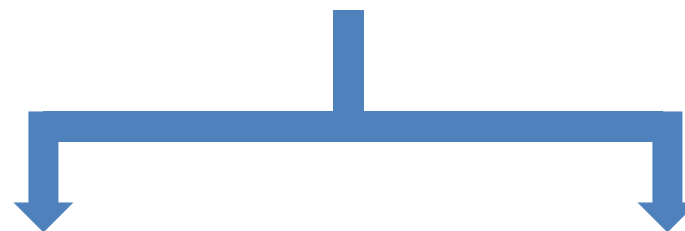
**Water samples**  
(active and passive sampling)



**Extraction**

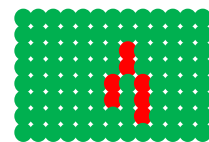


**Fractionation**  
96 wellplate (20 sec fractions)



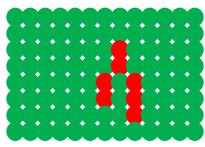
**Bioassay**  
(Pulse Amplified  
Modulation fluorometry)

**Chemical analysis**  
LC/GC-microTOF-MS  
pos and neg mode



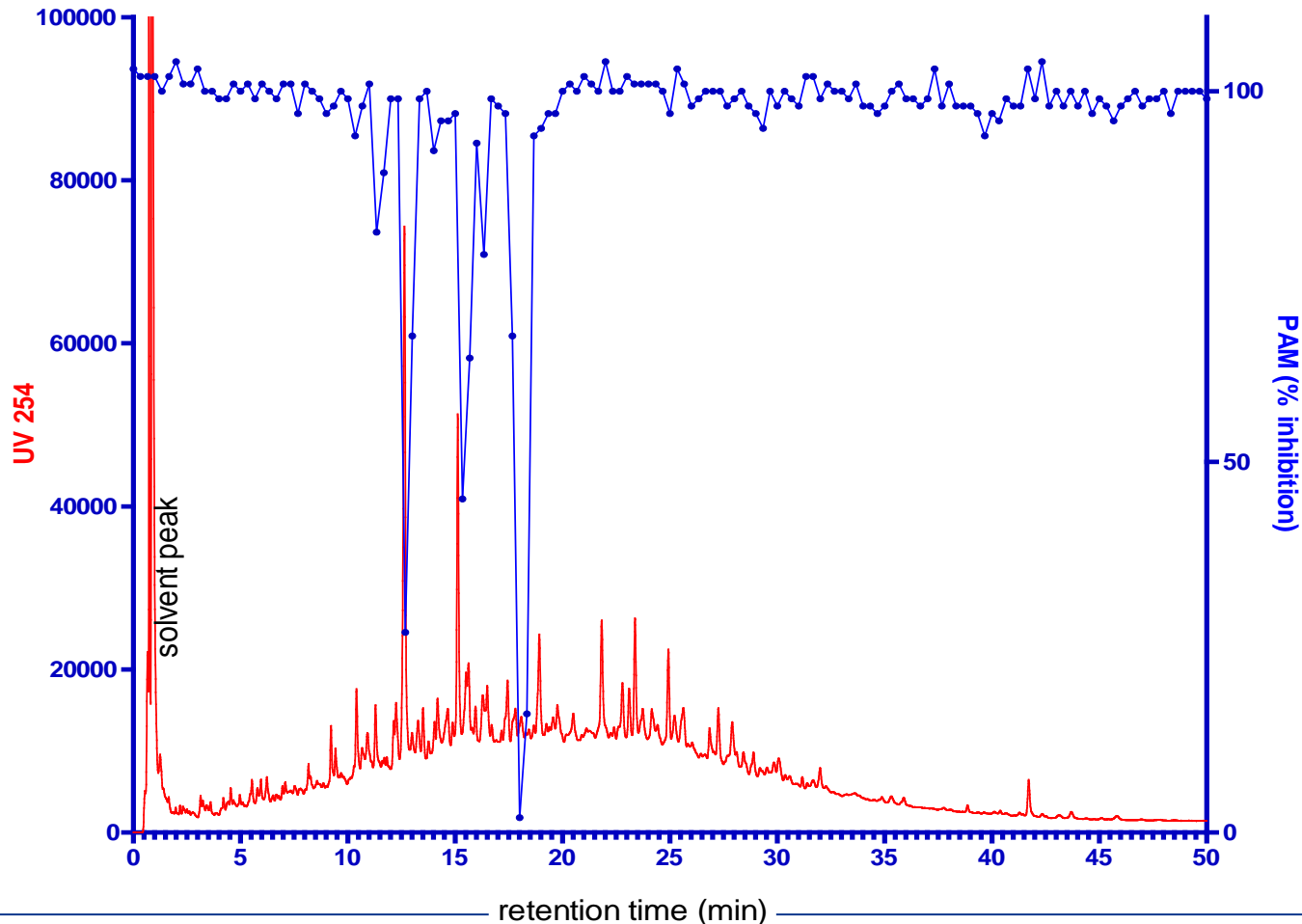
# Improved EDA - 96-well plate fractionation

- Bioassay - PAM determines the effect on Photosystem II efficiency as a direct and quick measure for toxicity in algae
- Chemical analysis with complementary methodologies:
  - LC-microTOF-MS (pos and neg mode)
  - GC-microTOF-MS (pos and neg mode)
- Chemical formula screen for possible effect-causing compounds
- Using library search (NIST/plus available databases)

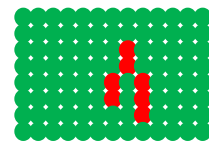


# Improved EDA - 96-well plate fractionation

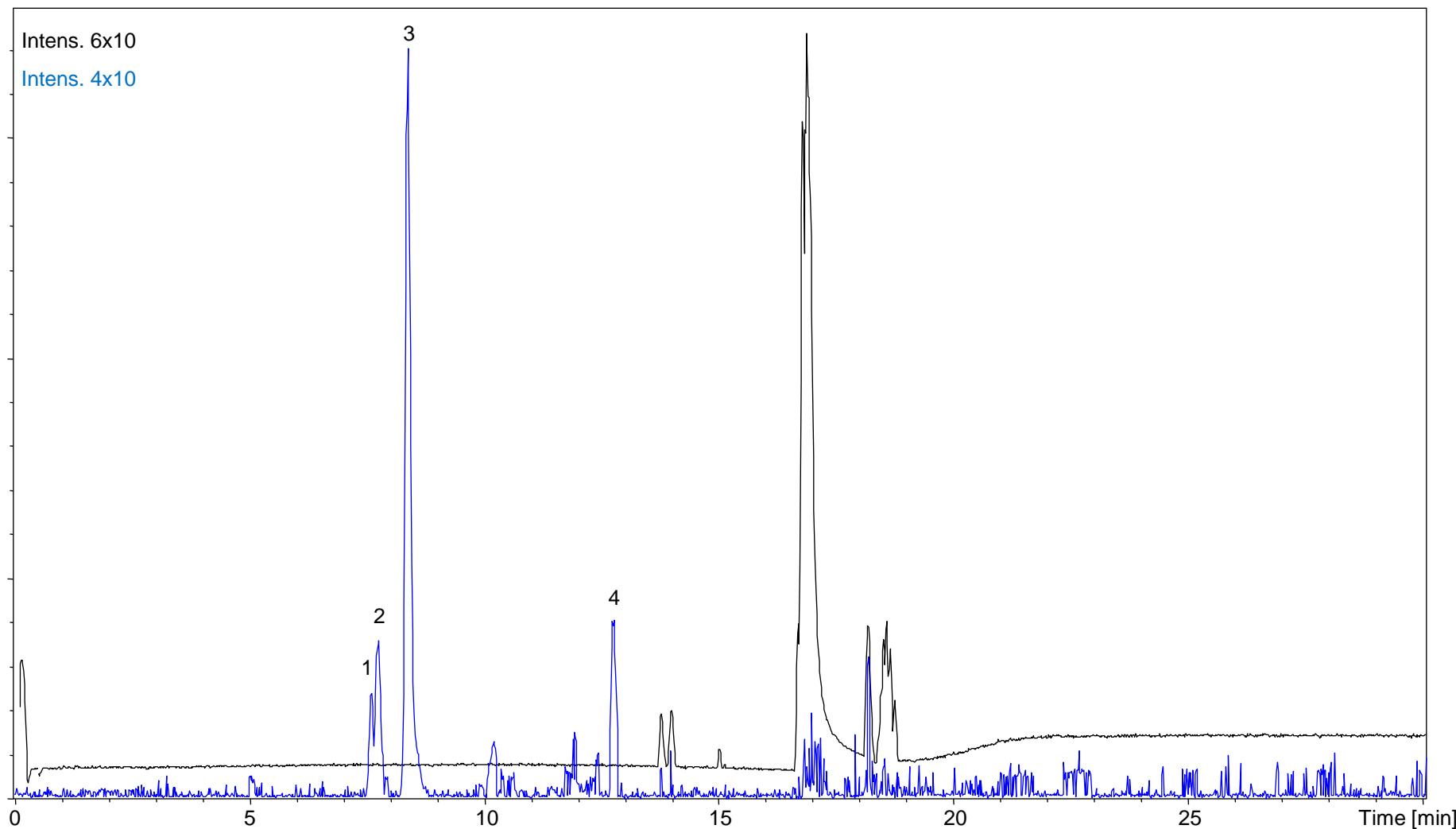
10/1058 sheets Hansweert 2-7-2010 t/m 8-9-2010

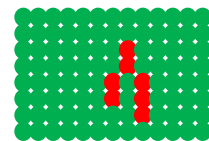




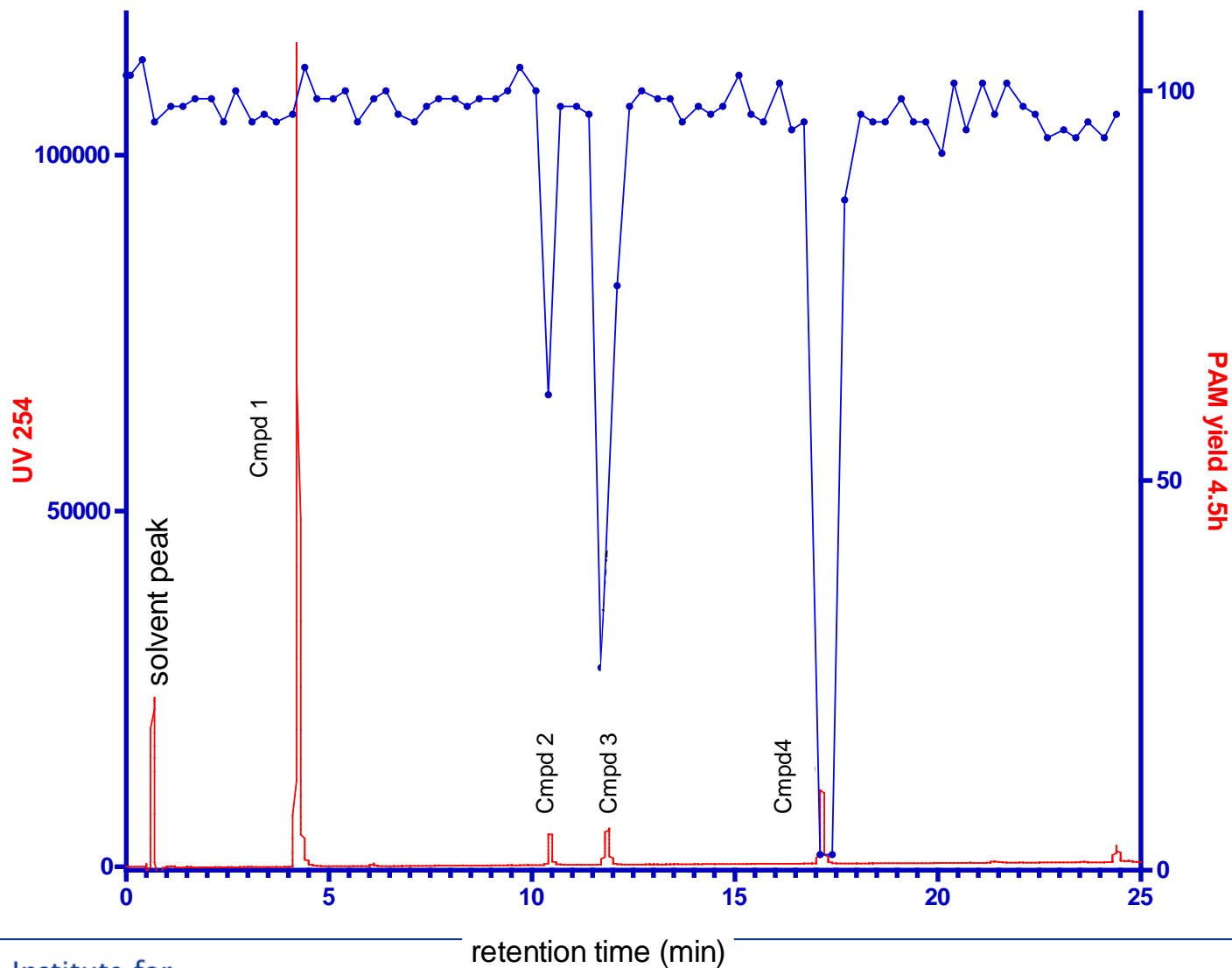


# Improved EDA – 96-well plate fractionation





# Improved EDA - 96-well plate fractionation



# In conclusion

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- An expanded fractionation before identification is promising
- Accurate mass determination is promising
  - Is non-exclusive
  - Chromatograms can be saved for future evaluation
  - Complementary analysis available (pos/neg and LC/GC)
- Prediction lists need to be evaluated for their accuracy
- Database sharing is encouraged

# Acknowledgement



Marja Lamoree  
*Modelkey*



Eszter Simon,  
*Keybioeffects*



Petra Booijs  
*Stress Alg*



Modelkey and the participants  
Contract-No. SSPI-CT-2003-511237-2



Keybioeffects and the participants  
Contract-No. MRTN-CT-2006-035695



Stress alg project and the  
participants (Deltares)

# Examples of priority lists

- WFD – 33 + 8 compounds
- Norman priority list – 744 compound based on Scientific discussion
- Van der Ohe 2011 – 500 compounds based on occurrence (Elbe, Danube, Scheldt and Llobregat)
- Diamond et al 2011 – 517 compounds based on occurrence (U.S. Waters)
- Diamond and Howard 2011 – pharmaceuticals, 275 occurrence, 106 P and/or B not yet analyzed in the environment.
- Weiss et al (unpublished) – ca. 180 compounds tested for their thyroid hormone disrupting potency
- Andersson et al 2011 – ca. 100 drugs predicted to be of concern based on their similarities to known compounds in PCA