Masking effect of anti-androgens on androgenic activity in a European river sediment

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Objective and approach

Identification of compounds that are responsible for the observed biological activity at a so-called hot spot in the river Scheldt basin

We use a bioassay for (anti-)androgenic activity to direct or focus our chemical analysis and identification

Effect Directed Analysis

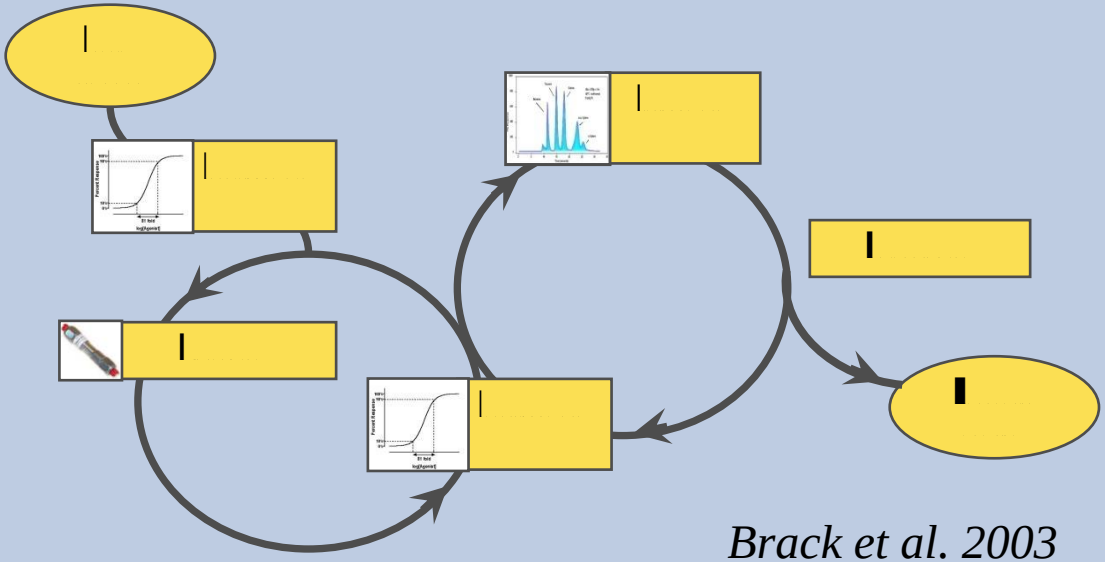
Toxicity profiling/screening of whole extract

Multiple fractionations to decrease the complexity of the extract

Bioassays to identify active fractions

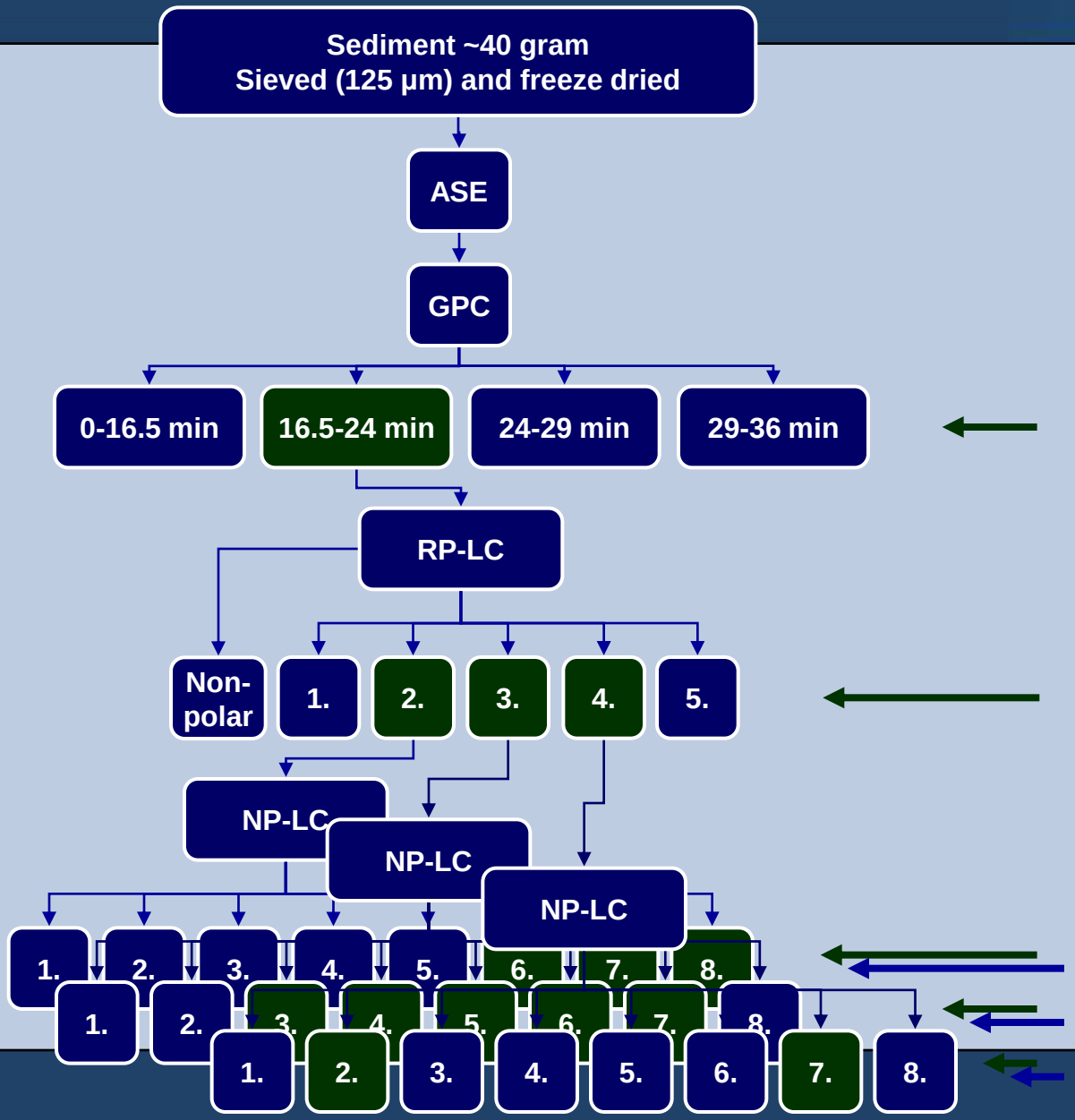
Chemical analysis to identify keytoxicants

Multiple confirmation steps - analytical and toxicological



Brack et al. 2003

Methodology



AR-CALUX

(Sonneveld *et al.* 2005)

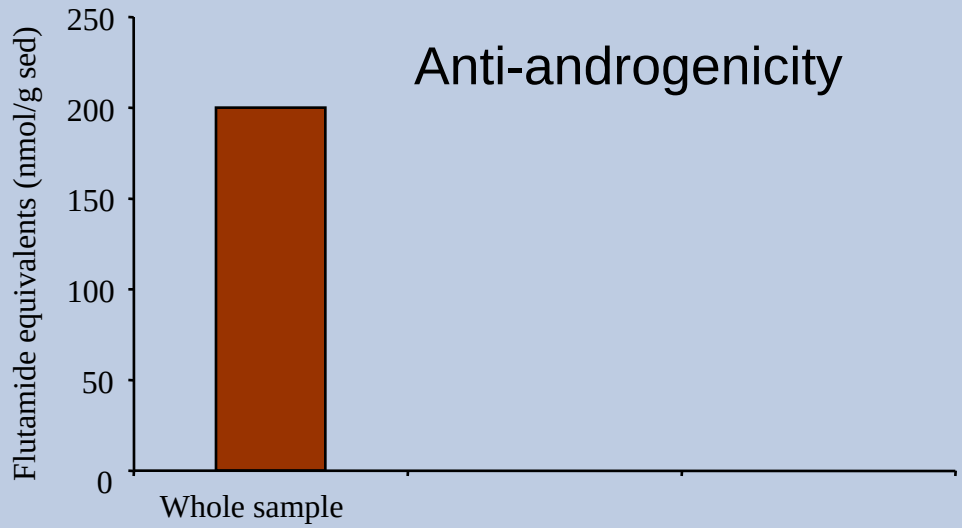
AR-CALUX

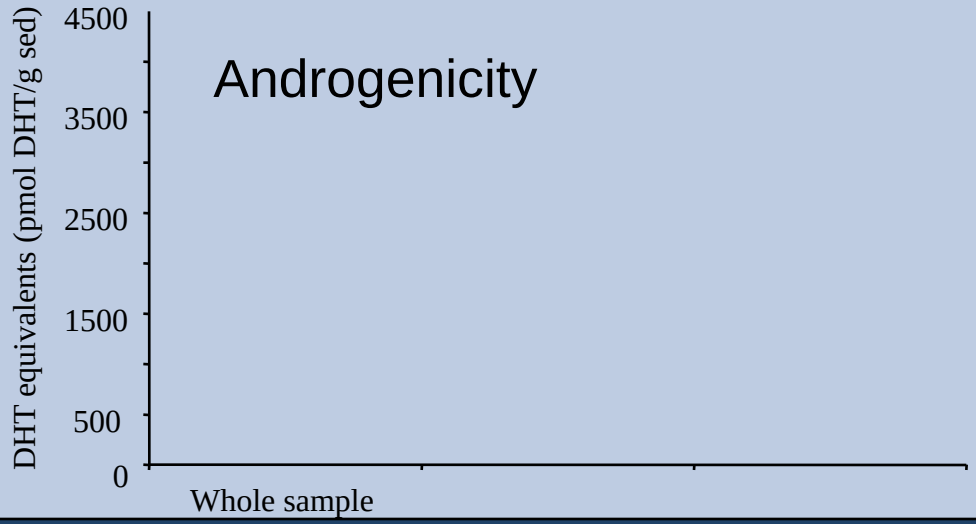
AR-CALUX

Chemical analysis

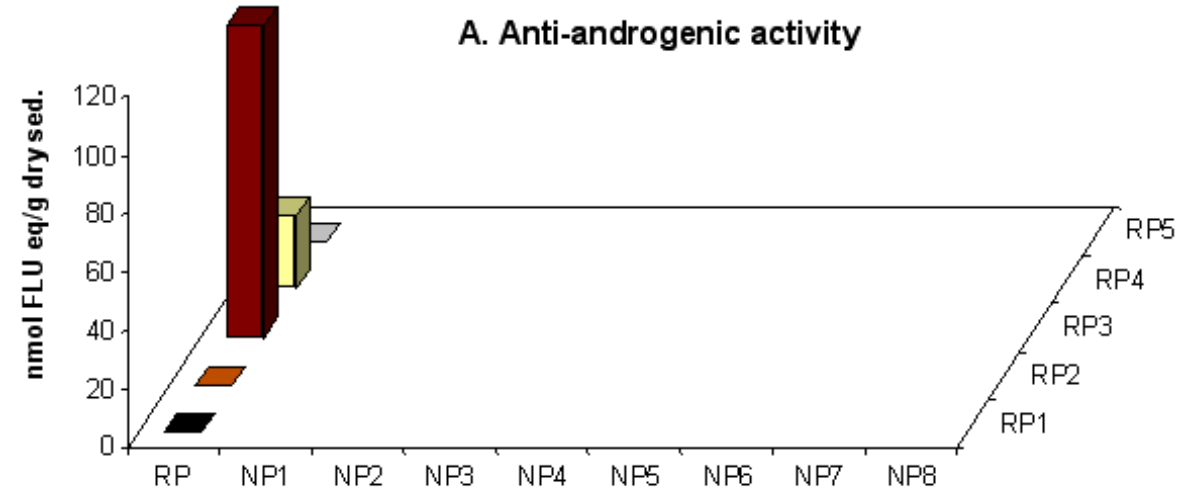
Study location

Androgenicity whole extract





(anti-)AR-CALUX results



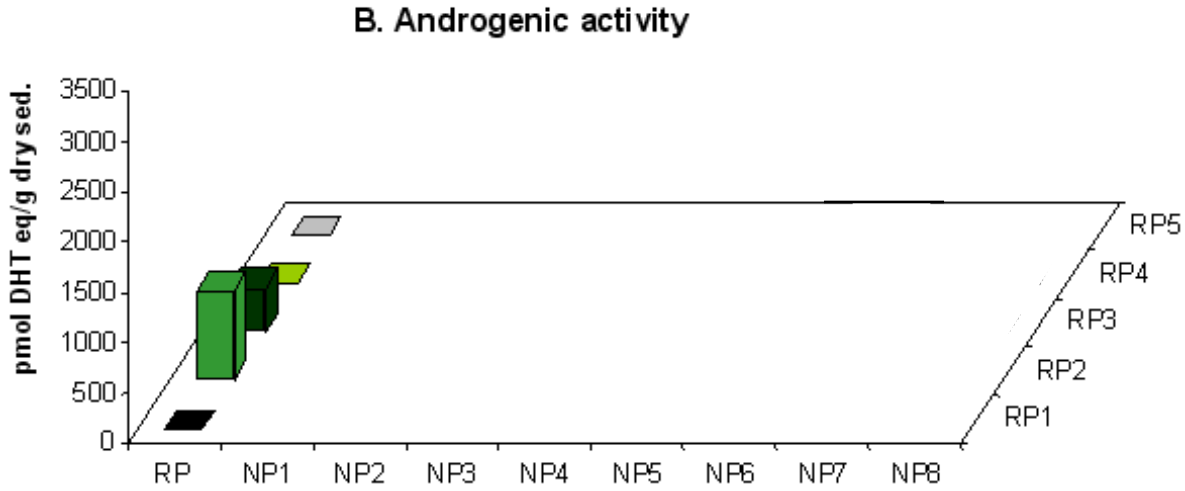


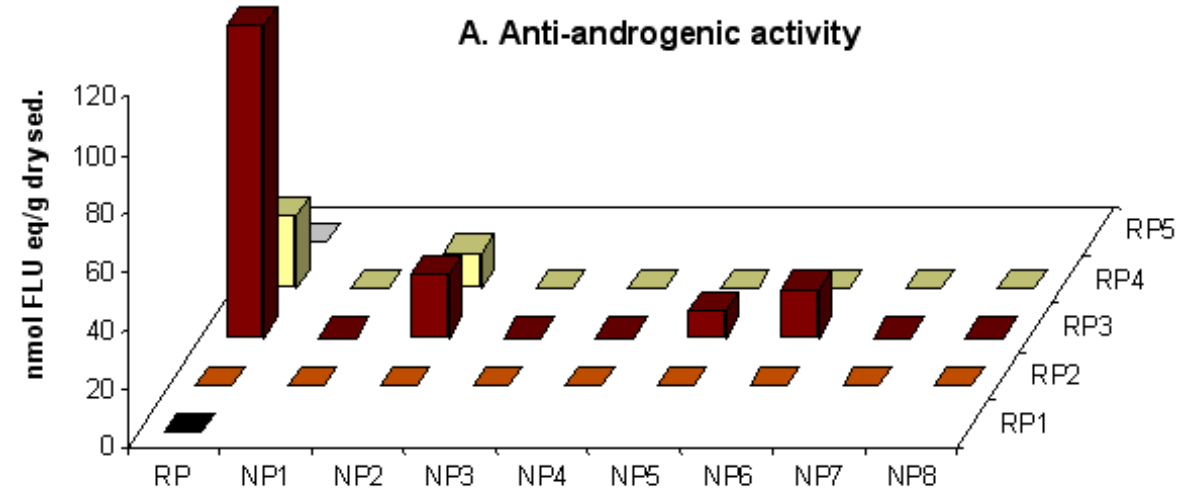
Figure 4A and B in

Weiss et al.

Analytical Bioanalytical Chemistry

Volume 394, 2009

(anti-)AR-CALUX results



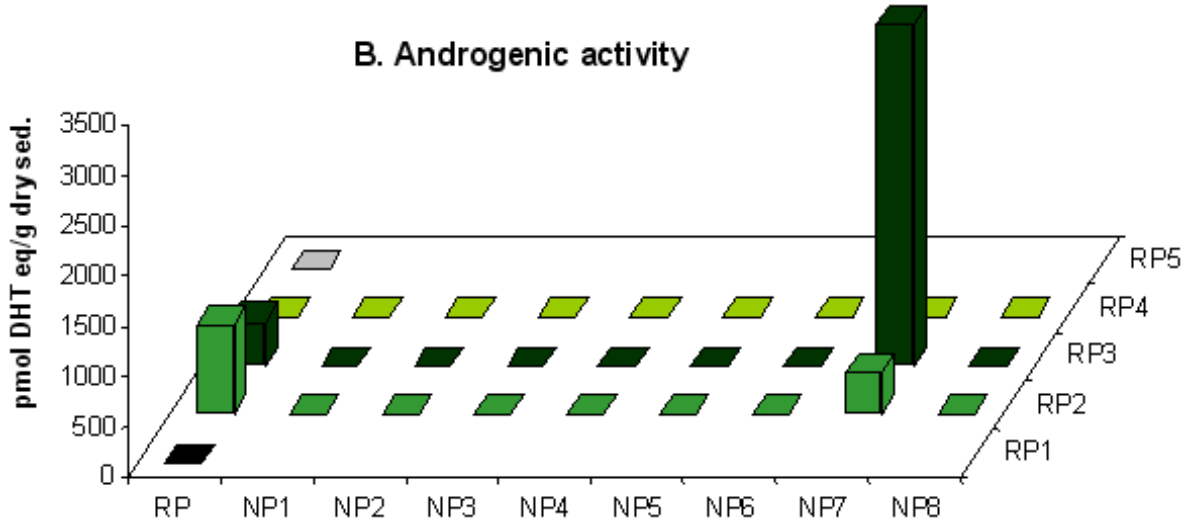


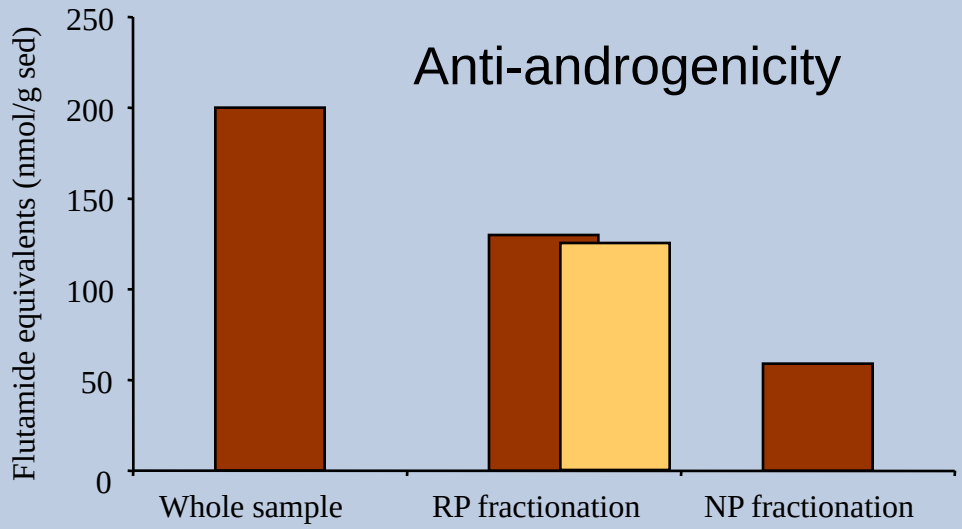
Figure 4A and B in

Weiss et al.

Analytical Bioanalytical Chemistry

Volume 394, 2009

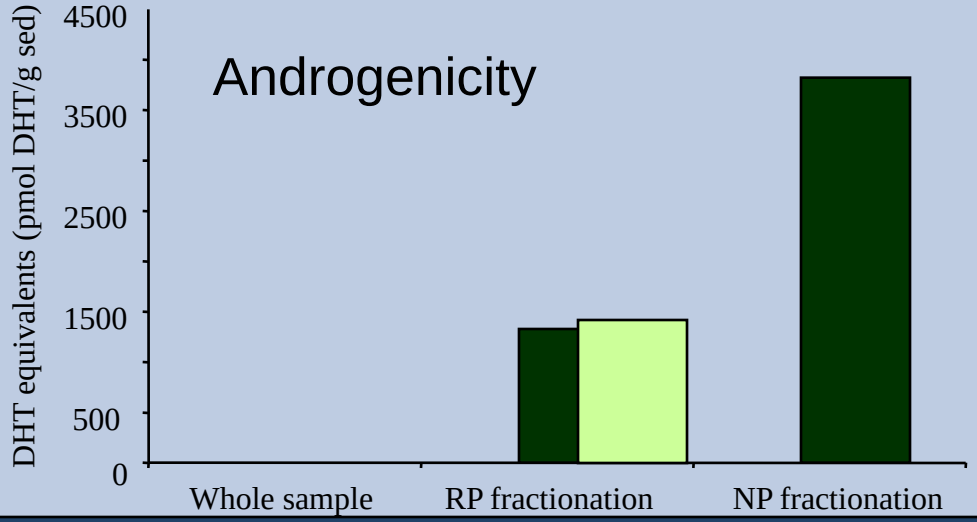
Masking of the ‘full’ androgenicity



Anti-androgenicity decreases after fractionation

Not due to low recovery

Probable presence of partial agonists



Androgenicity increases after fractionation

Androgenic activity separated from interfering antagonistic compounds

Shows that a first screening of samples does not reveal the true androgenic potency

Masking effect in the literature

- Two studies reported masking of androgenic effects by anti-androgenic compounds:
  - Svenson & Allard (2004) in pulp and paper mill effluents
  - Urbatzka et al. (2007) in water and sediment from the river Lambro, Italy
- Usually it deals with cytotoxicity masking other effects, like described by Hollert et al. (2005)
- Gagné et al. (1999) describe a mixed estrogenic/anti-estrogenic effect caused by PAHs

Lessons learned

- cts into account when

MIXTURES!

- Realize that the proc
  influence on the bioas

CHEMISTRY!

Chemical analysis

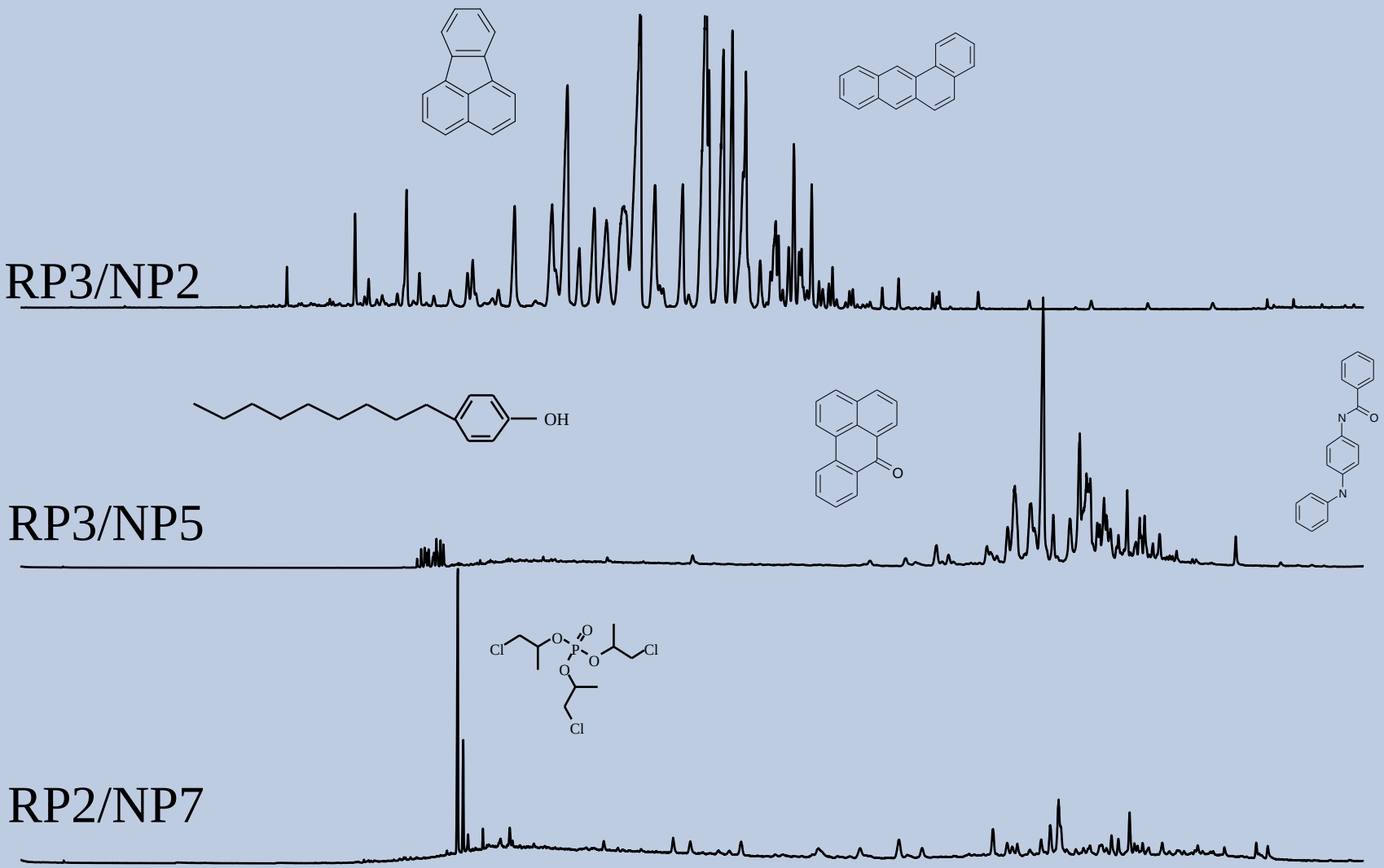
GC-MS

- DB5 column, full scan mode (m/z 50-650)
- Electron impact ionisation (EI)
- Mass spectra deconvoluted using AMDIS
- NIST searched, match factor ≥80%
- The Kovats Retention Indices (KRI) values were used to identify the compounds to Quality Peak Identification Database (QPID)
- Background subtraction with QPID

LTQ-Orbitrap at Rijkswaterstaat, Waterdienst

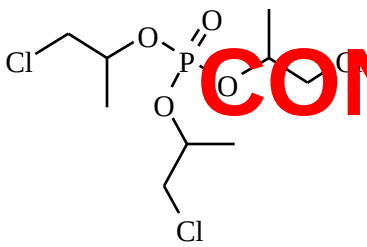
- C_{18} HPLC column, full scan mode (m/z 50-600)
- Accurate Mass Capabilities
- Resolution 30 000
- Data Dependent™ acquisition with Dynamic Exclusion™
- Background subtraction with SIEVE

Chemical analysis/ GC-MS

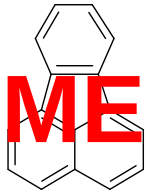


anti-androgens tentatively identified by GC-MS

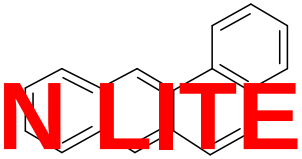
Tris(2-chloroisopropyl) phosphate
(13674-84-5)



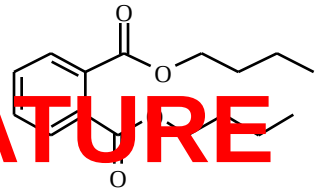
Fluoranthene
(206-44-0)



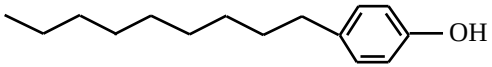
Benz(a)anthracene
(56-66-3)



Dibutylphtalate
(84-74-2)

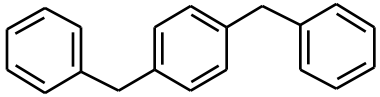


CONFIRMED IN LITERATURE

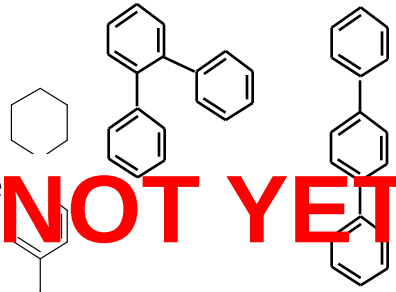


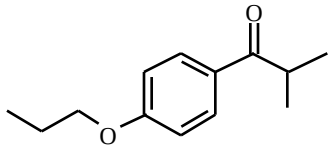
***p*-Nonylphenol, technical mixture, 8 identified peaks** (104-40-5)

1,4-bis(phenylmethyl) benzene
(793-23-7)



***o*-Terphenyl**
(92-94-4)



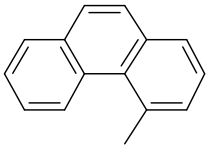


N-[4-(Phenylamino)phenyl]-benzamide
(no cas)

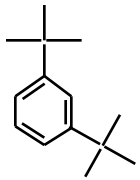
***p*-Dicyclohexylbenzene**
(1087-02-1)

NOT YET CONFIRMED

4’Propoxy-2-methylbiphenol
(64456-60-3)

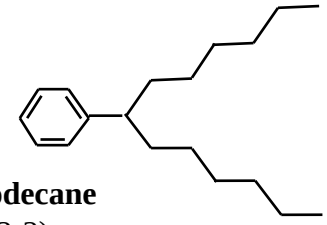


4-Methyl-phenanthrene
(832-64-4)

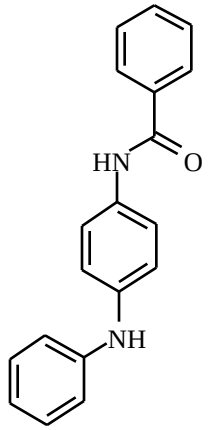


***p*-Terphenyl**
(92-94-4)

1,3-bis(1,1-dimethylethyl)-benzene
(1014-60-4)



6-Phenyldodecane
(2719-62-2)

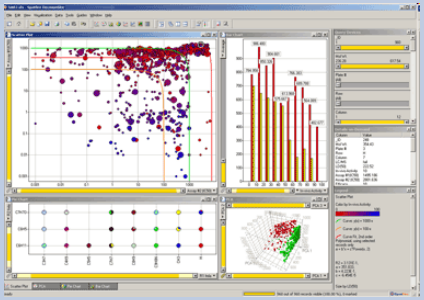


SIEVE strategy

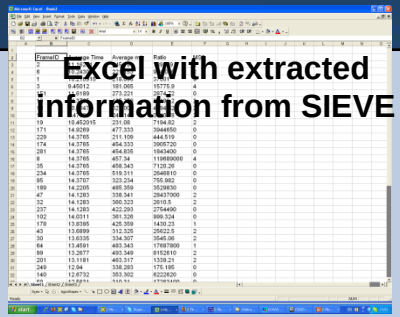
LTQ-Orbitrap

1. Sample 20090405.raw file
   Control 20090406.raw file

SIEVE aligns and frames peaks

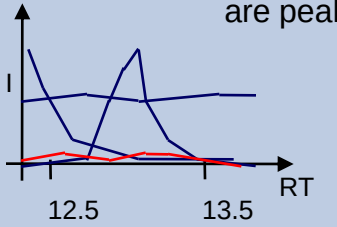


2.

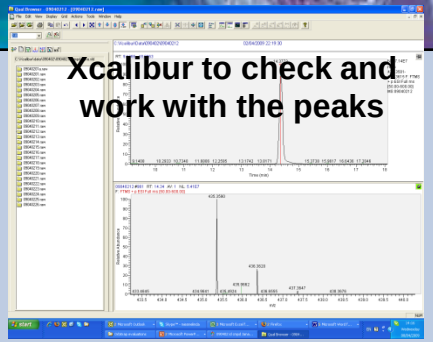


Excel with extracted information from SIEVE

3. Check each m/z that the frames are peaks indeed

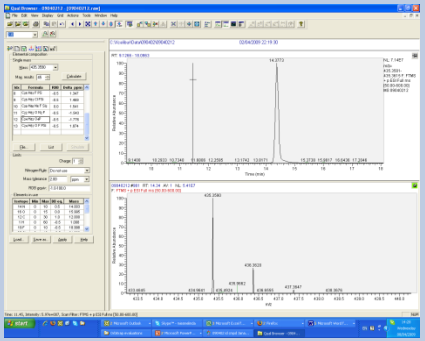


4. Check each m/z that it is the molecular mass in Xcalibur

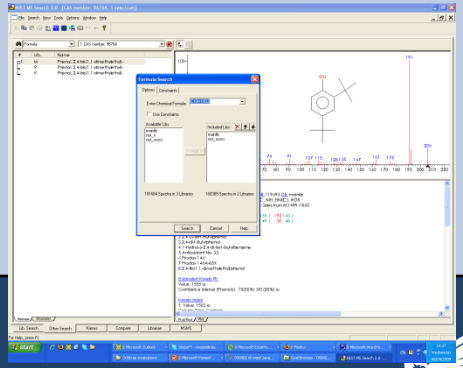


Xcalibur to check and work with the peaks

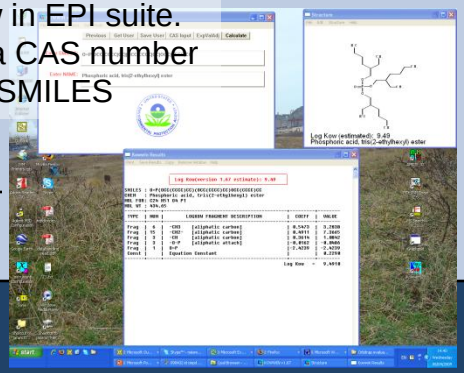
5. Check the m/z in the elemental composition tool in Xcalibur



6. Check the formulas if they are present in NIST (Formula -H)



7. Check the ,compounds log Kow in EPI suite. Either via CAS number or SMILES



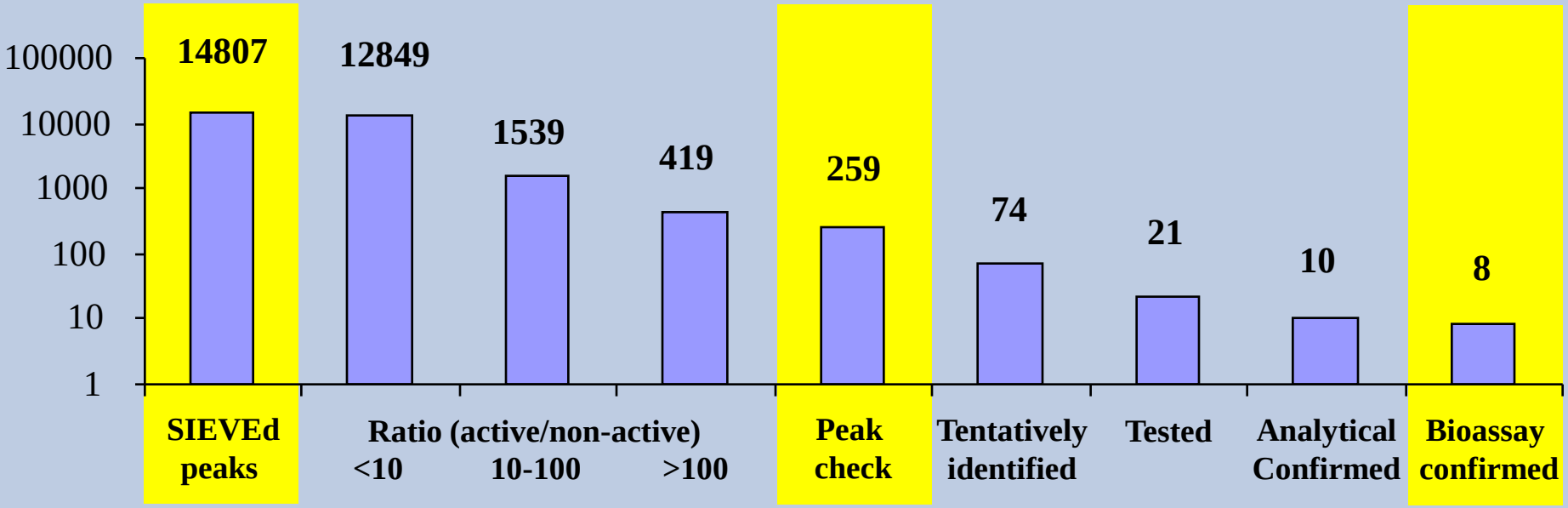
8. **Tentative identification**

vrije Universiteit amsterdam

LTQ Orbitrap data evaluation

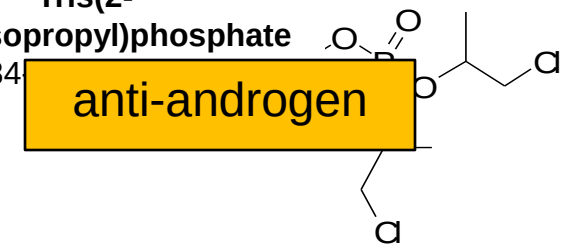
Selected masses~ 2%

1/3 of tested compounds confirmed



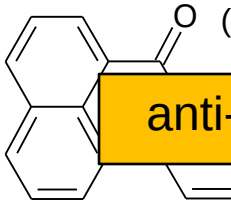
Confirmed (anti-)androgenic compounds

Tris(2-chloroisopropyl)phosphate (13674-84-



anti-androgen

7H-Benz[de]anthracen-7-one (82-05-3) PAH found in urban airborne particulate



anti-androgen

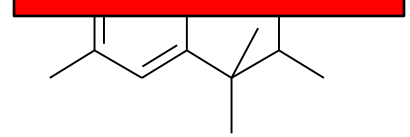
Androstenone (18339-16-7)
A steroid found in both male and female sweat and urine

weak androgen

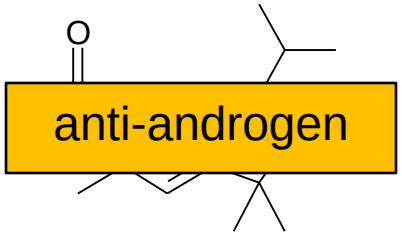
to test sows for timing of artificial insemination.

Tonalide (1506-02-1)
Tetralin derivate musk

strong anti-androgen

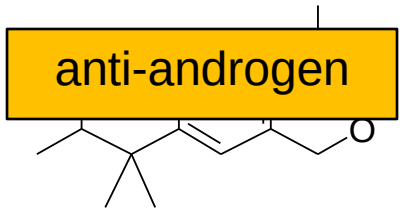


Traseolide (68140-48-7)
Indane derivate musk

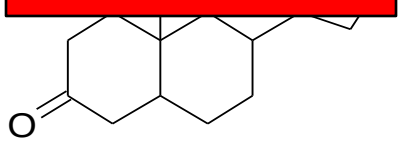


anti-androgen

Galoxolide (1222-05-5)
Isochroman derivate musk

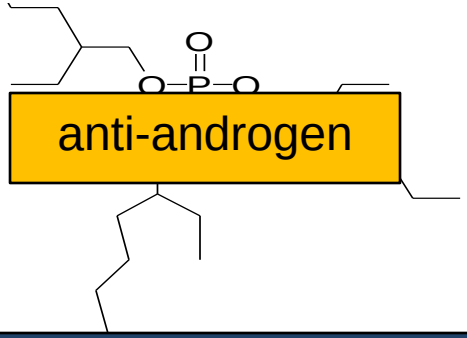


anti-androgen



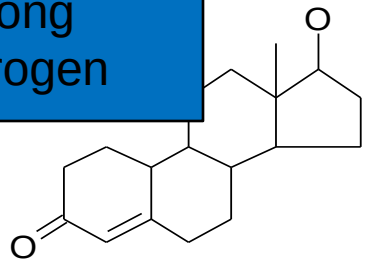
strong anti-androgen

Phosphoric acid, tris(2-ethylhexyl) ester (78-42-2)
Industrial high production volume chemical. lubricant additives etc.



anti-androgen

Nandrolone (434-22-0)
An anabolic steroid, present naturally in the human bod
commercially
(Deca-Duraboli
a phenylpropi



strong androgen

Summary of identified compounds

GC/MS

- Presence of PAHs, phtalates and organophosphates, all of which are documented anti-androgens
- Target analysis on whole sample reported ~5ug/g sediment of PAHs
- No potent androgenic compounds identified
  - Literature shows that it is mainly natural and synthetic steroid that can cause androgenic responses
  - GC/MS technique not optimal for steroid analysis

LTQ-Orbitrap

- Two steroids were identified as potent androgenic compounds
- Musks, organophosphates, and a PAH identified as potent anti-androgenic compound

Advanced confirmation

- AR-Calux testing of unconfirmed compounds from the GC-MS analysis
- Target analysis of identified compounds to establish concentration in the fractions and recovery of the clean up
- Target analysis of additional musk compounds in the sediment
- In vivo confirmation of identified potent androgen compounds

Acknowledgements

Collection of samples

Eric de Deckere and Chris van Liefferinge (University of Antwerp)
Sander van Vliet (RWS)
Bert van Hattum (IVM)

RWS, Waterdienst

Joan Staeb
Gerard Stroomberg
Ronald de Boer

NIVA, Norway

Kevin Thomas

... and the IVM colleagues

Modelkey Contract-No.
SSPI-CT-2003-511237-2

Keybioeffects Contract-No.
MRTN-CT-2006-035695

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