Effects-directed identification of emerging compounds

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Are there unknowns?

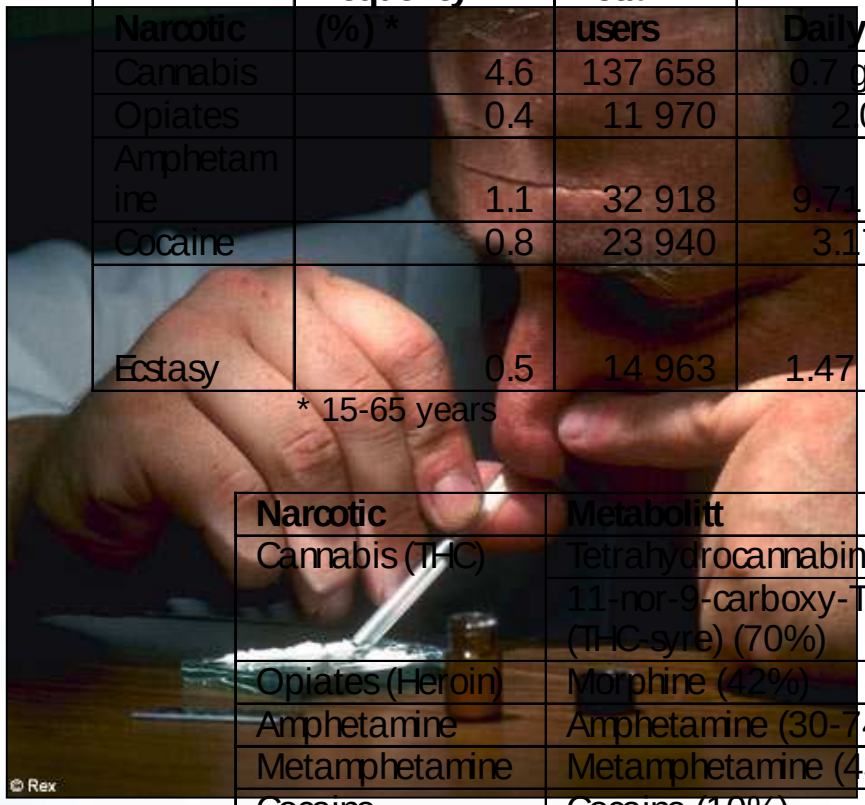
- The list of ’new’ contaminants is continually growing.

Brominated dibenzo-*p*-dioxins (Haglund et al., 2007 ES&T)

Approach 1: Prioritise & target

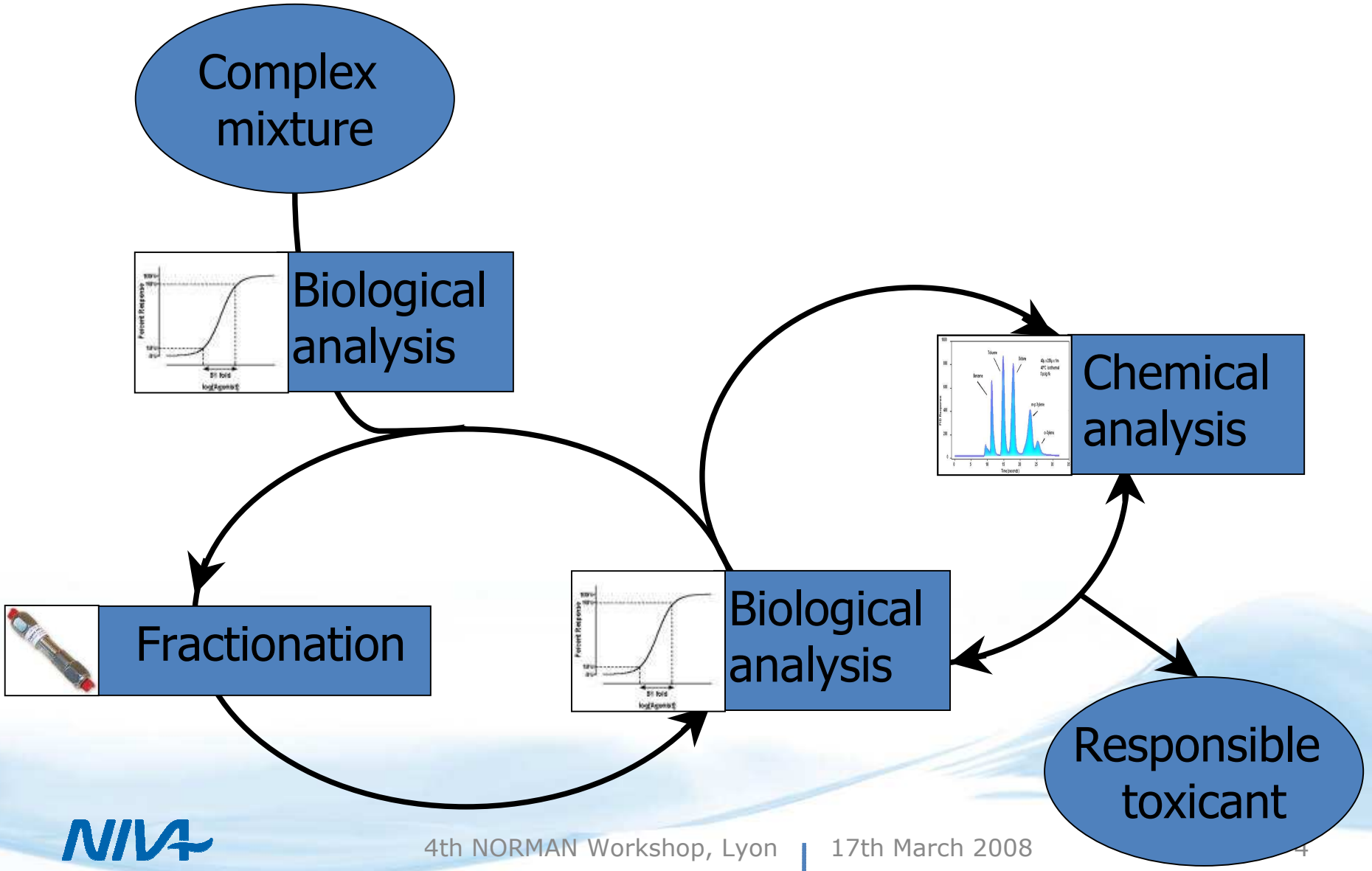
Narcotic	Use frequency (%) *	Total users	Daily usage	User dose(g)	Purity	Estimated output (kg/yr)	PEC (µg/L)
Cannabis	4.6	137 658	0.7 g (THC)			35 172	10.29
Opiates	0.4	11 970	2.09g	0.25	0.29	662	0.19
Amphetamine	1.1	32 918	9.71 points	0.1	0.43	4 871	1.43
Cocaine	0.8	23 940	3.17 hits	0.1	0.38	1 053	0.31
Ecstasy	0.5	14 963	1.47 tablets	0.1	1	803	0.23

* 15-65 years



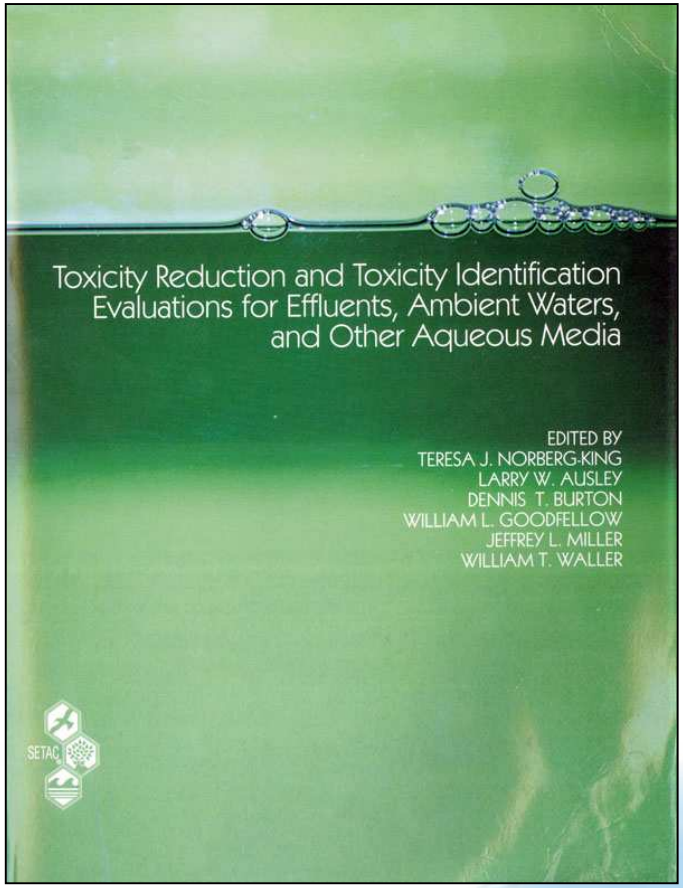
Narcotic	Metabolitt	PEC (µg/L)	MEC (µg/L)
Cannabis (THC)	Tetrahydrocannabinol (30%)	3.1	
	11-nor-9-carboxy-THC (THC-syre) (70%)	7.2	
Opiates (Heroin)	Morphine (42%)	0.08	
Amphetamine	Amphetamine (30-74%)	0.33-0.80 *	
Metamphetamine	Metamphetamine (43%)	0.15 *	
Cocaine	Cocaine (10%)	0.03	0.15†
	Benzoylecgonin (90%)	0.28	0.02†
Ecstasy (MDMA)	MDMA (65%)	0.15	

Approach 2: Effects-directed



A little history….

- First reported in 1979 (Parkhurst et al.)
- 1988 adopted by the USEPA (as TIE)
- > 300 publications since
- 50+ bioassays



Successful applications

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Effect-Directed Identification of Oxygen and Sulfur Heterocycles as Major Polycyclic Aromatic Cytochrome P4501A-Inducers in a Contaminated Sediment

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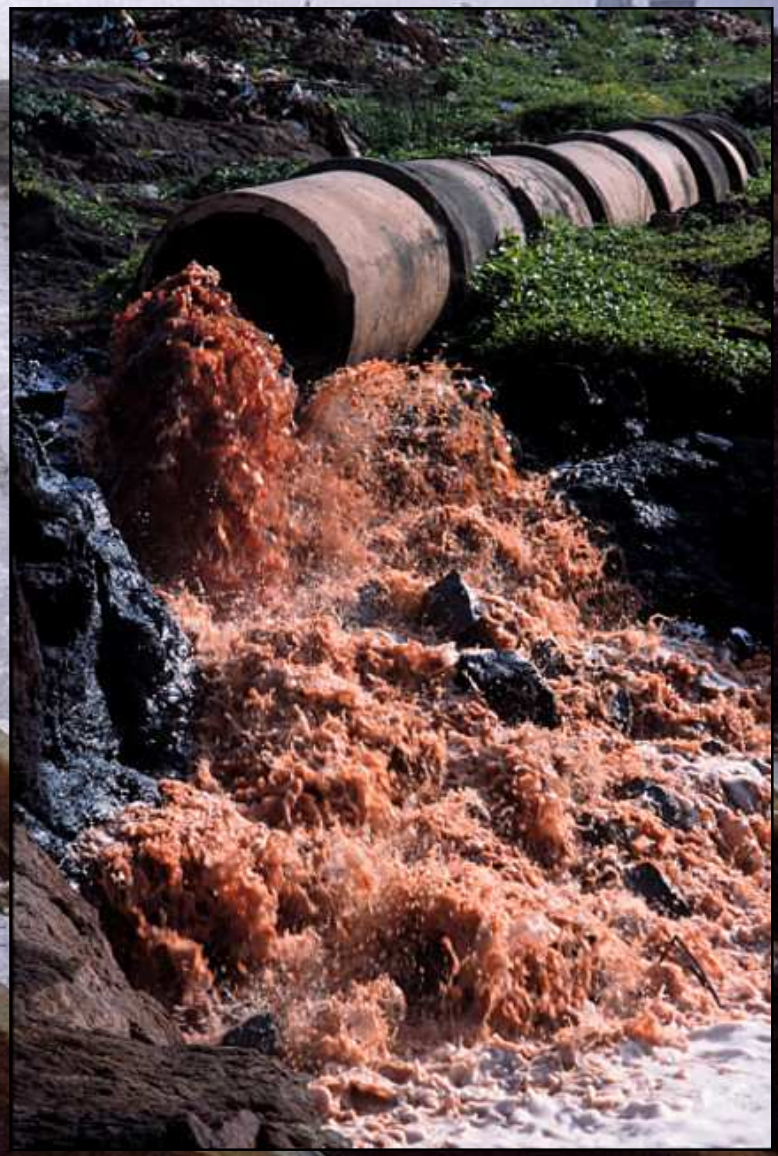
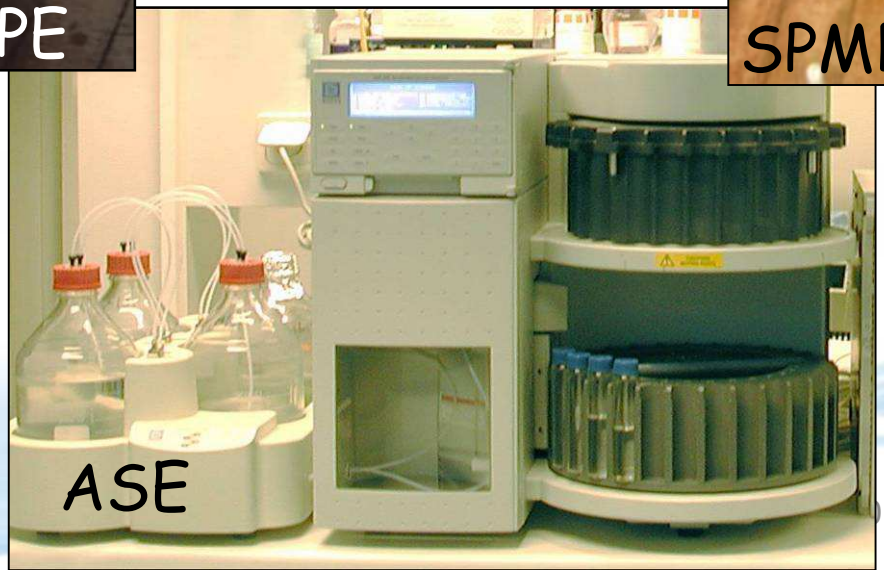
Heterocyclic polyaromatic compounds, including dinaphthofurans, 2-(2-naphthalenyl)benzothiophene, methylated chrysene, and benz[*a*]anthracene, were identified and confirmed as major cytochrome P4501A (CYP1A)-inducing compounds in a contaminated sediment close to the industrial site of Bitterfeld (Germany). Identification was achieved by the application of an effect-directed fractionation and analysis approach. This approach comprised the combination of a rainbow trout liver cell line (RTL-W1) bioassay to select for CYP1A-inducing effects by measuring 7-ethoxyresorufin-*O*-deethylase activity, a multistep fractionation procedure, and various methods of chemical characterization. The identified nonpriority pollutants

and the subsequent induction of cytochrome P4501A-(CYP1A-)dependent monooxygenases, commonly measured as 7-ethoxyresorufin-*O*-deethylase (EROD) activity, is closely related to the dioxin-like toxicity of these compounds (*3*). As for HAHs, PAHs have been found to bind to the AhR and to elicit CYP1A induction (e.g., in fish (*4*) and cultured fish cells (*5*)). Although PAHs are commonly metabolized through CYP1A activity, prolonged exposure may yield typical dioxin-like effects. For example, EROD induction, yolk sac edema, subcutaneous hemorrhaging, reduced growth, and craniofacial malformations have been observed for the AhR-binding PAH, retene, in early life stages of zebrafish (*Danio rerio*) (*6*). This is in agreement with former investigations using chick embryos for which a close relationship between EROD-inducing potency of PAHs and mortality as well as malformation has been found (*7*). These results suggest that at least some of the PAH toxicity is mediated via binding to the AhR and related to EROD induction. For indirect carcinogens, such as PAHs, which form reactive intermediates upon metabolism because of monooxygenase activity, CYP1A induction is also thought to be related to their carcinogenic activity (*8*). In addition, cell proliferation and promotion of mutated cells are thought to be mediated through the AhR (*9*). Thus, even if EROD induction is a surrogate measure and does not necessarily result in toxicity (*2*), it has been found to be a sensitive marker of exposure to those PACs that are expected to exhibit harmful effects to biota. Therefore, throughout the past years considerable efforts have been directed to investigate EROD induction potencies of single PACs and complex environmental mixtures applying mammalian (*10*−*13*) and fish liver cell lines (*13*−*18*).

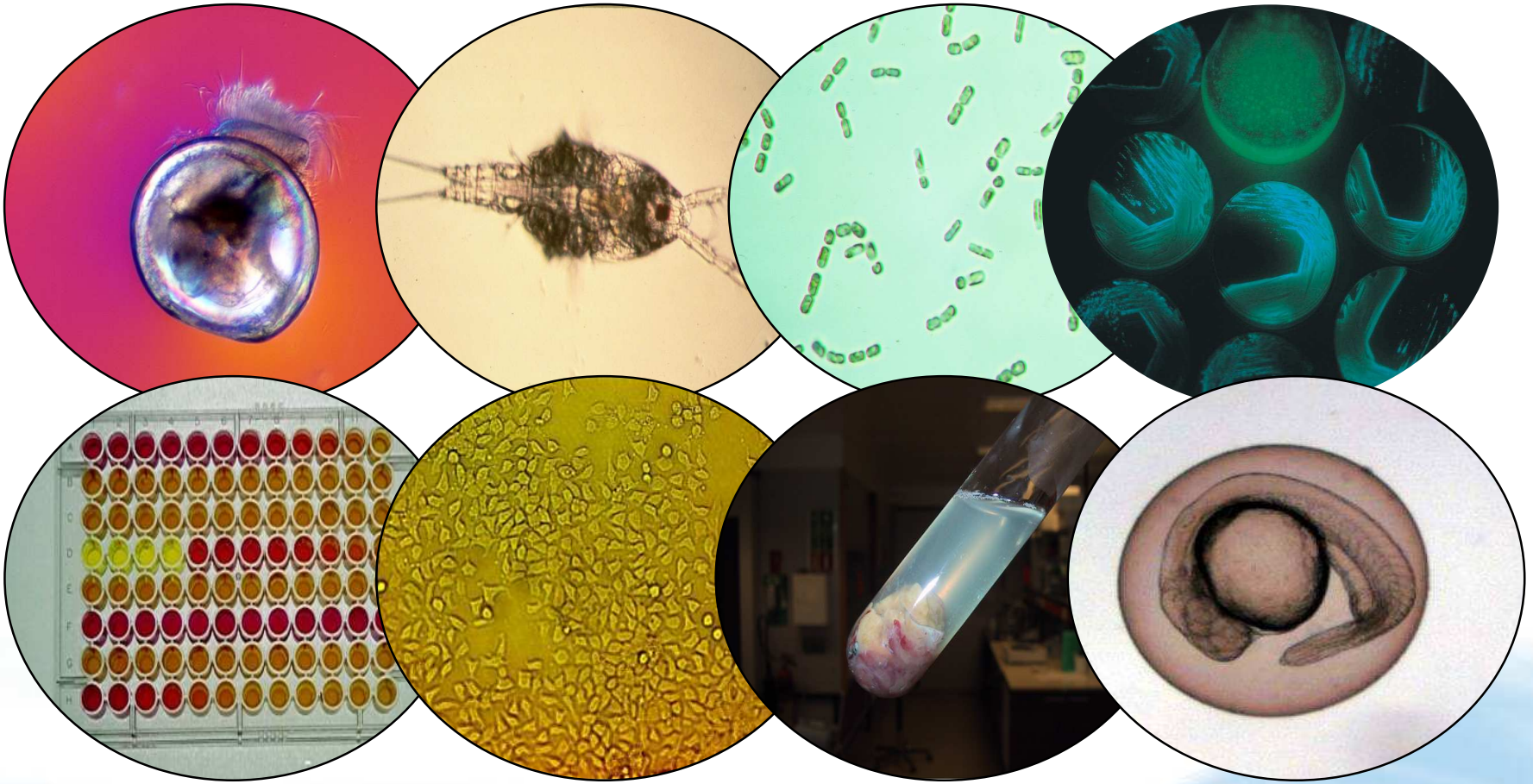
Chemical analysis of PACs in environmental samples is



Extraction techniques

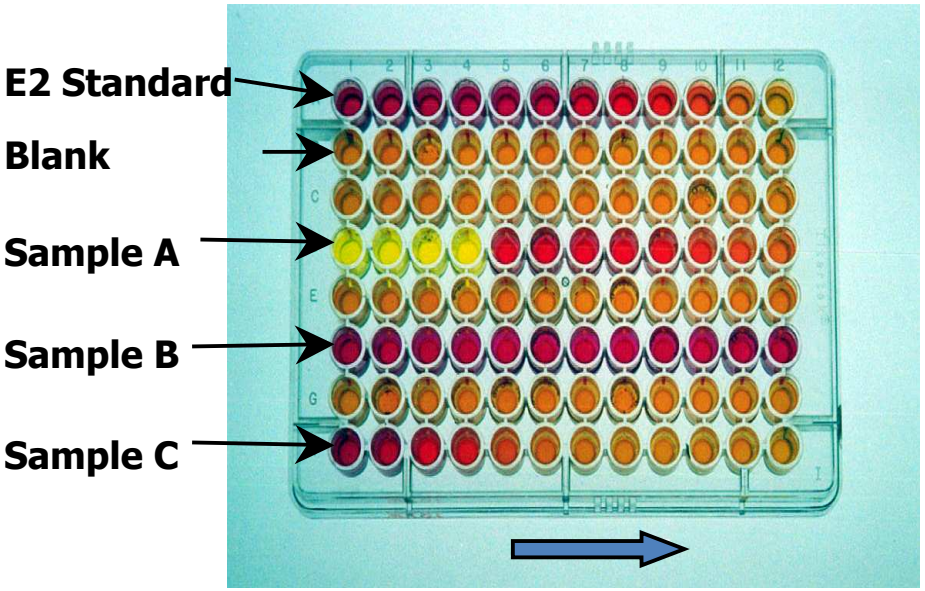


Good EDA bioassays



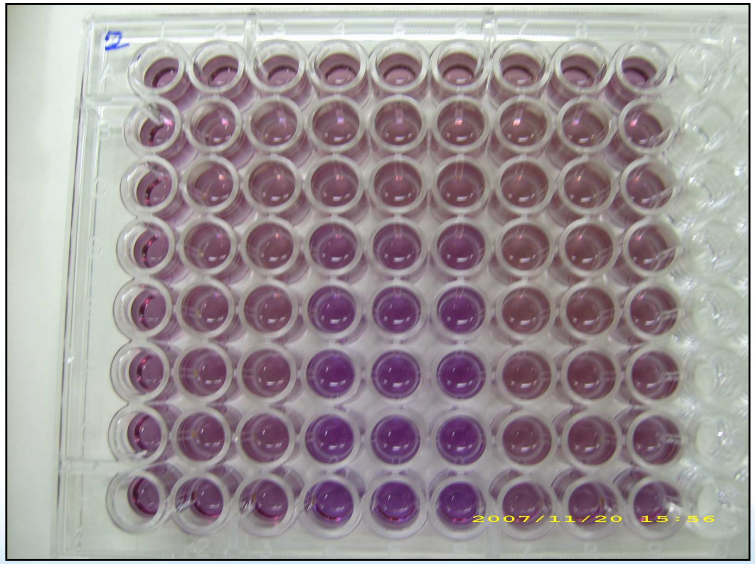
In vitro bioassays

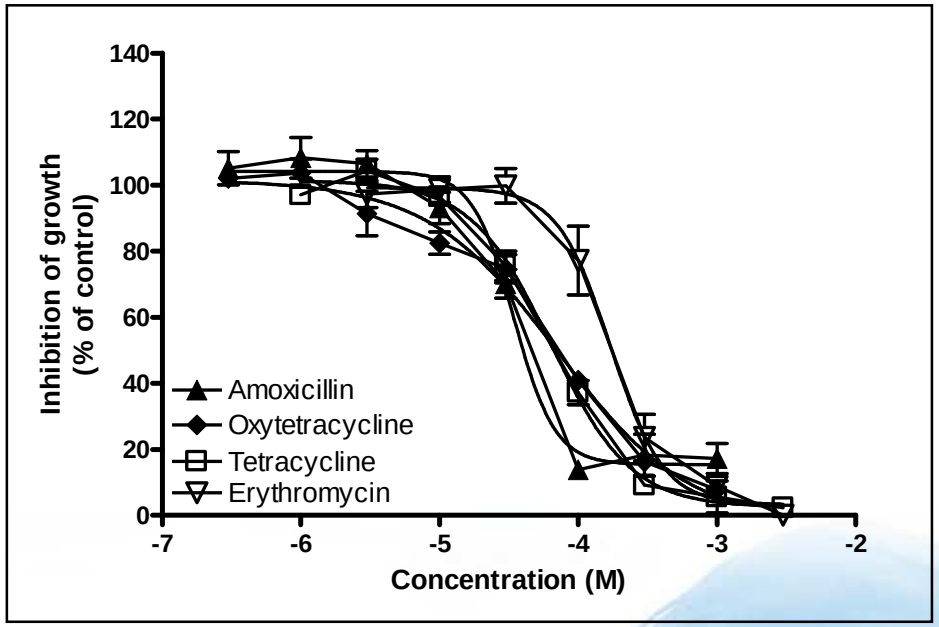
- *In vitro* cell bioassays: sensitive, specific, and rapid bio-analytical tools that are useful in characterising responses in complex environmental mixtures.
- Examples:
  - Yeast estrogen screen (YES)
  - Yeast androgen screen (YAS)
  - CALUX
  - T-screen
  - Mutatox
  - Fish hepatocyte assays



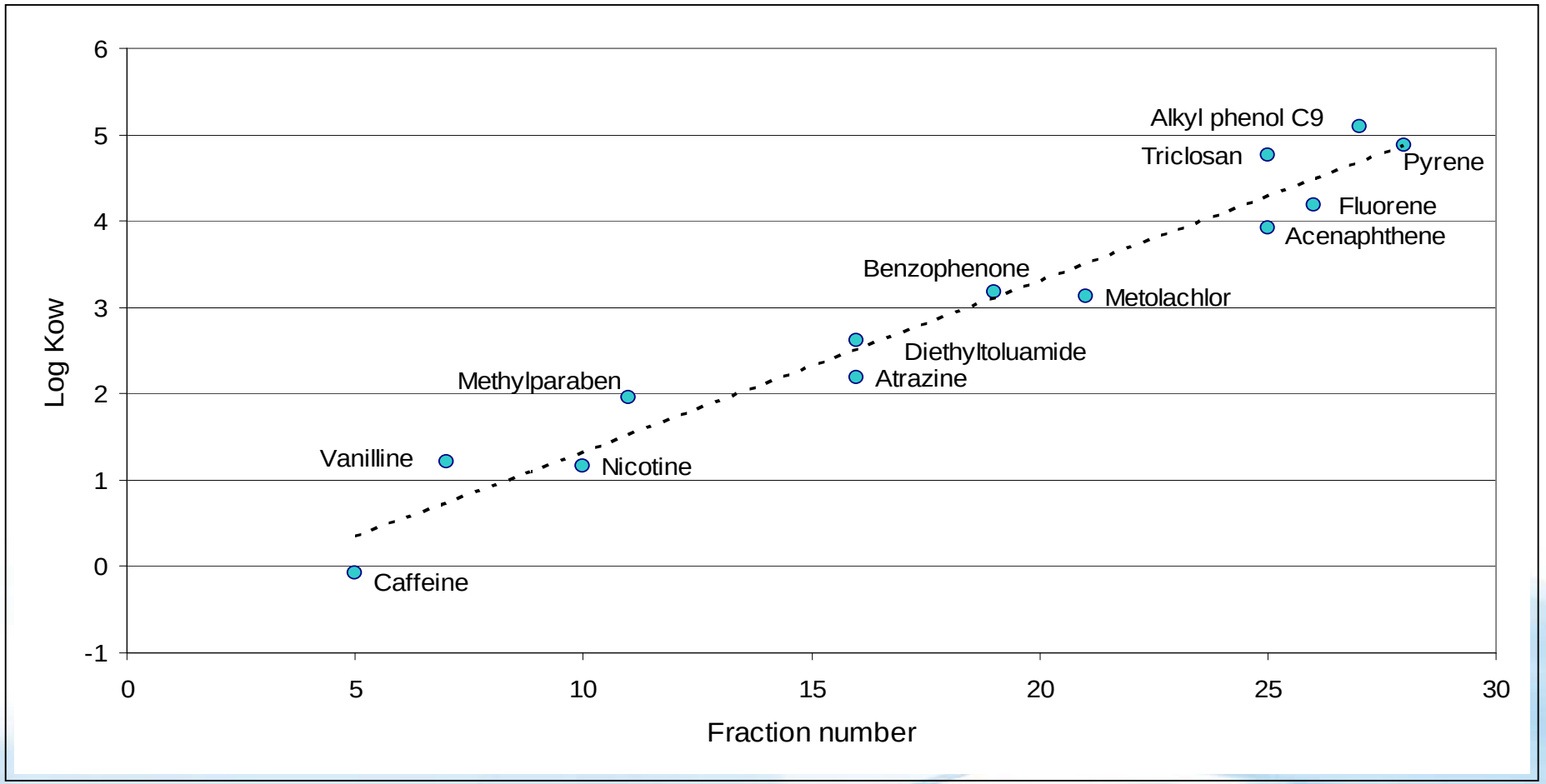
New bioassays: ABC

- Rapid screening bioassay for antibiotics.
- Termophil bacteria, *Geobacillus stearothemophilus.*

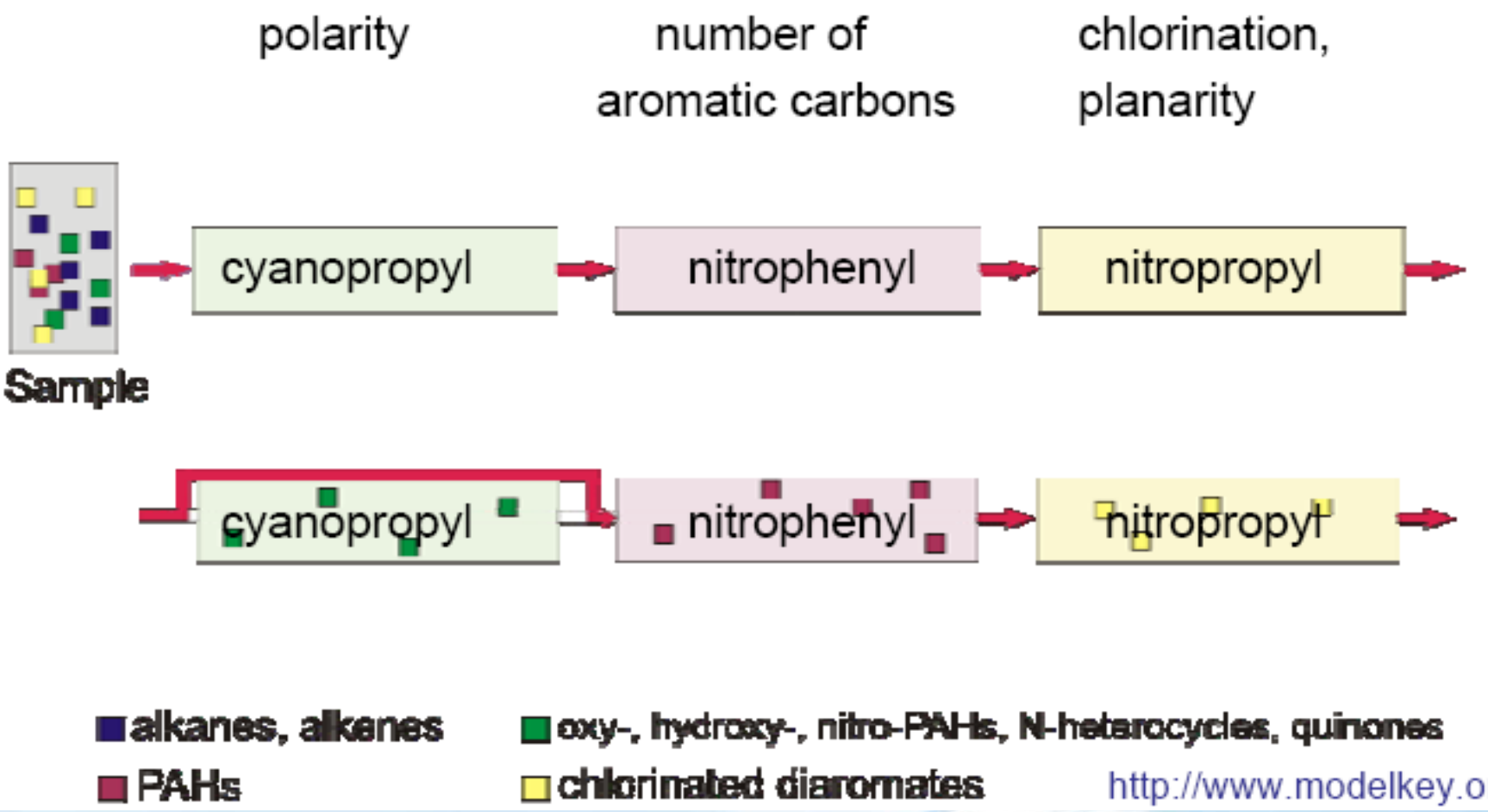




Fractionation: Simple (RP)



Fractionation: Multistep



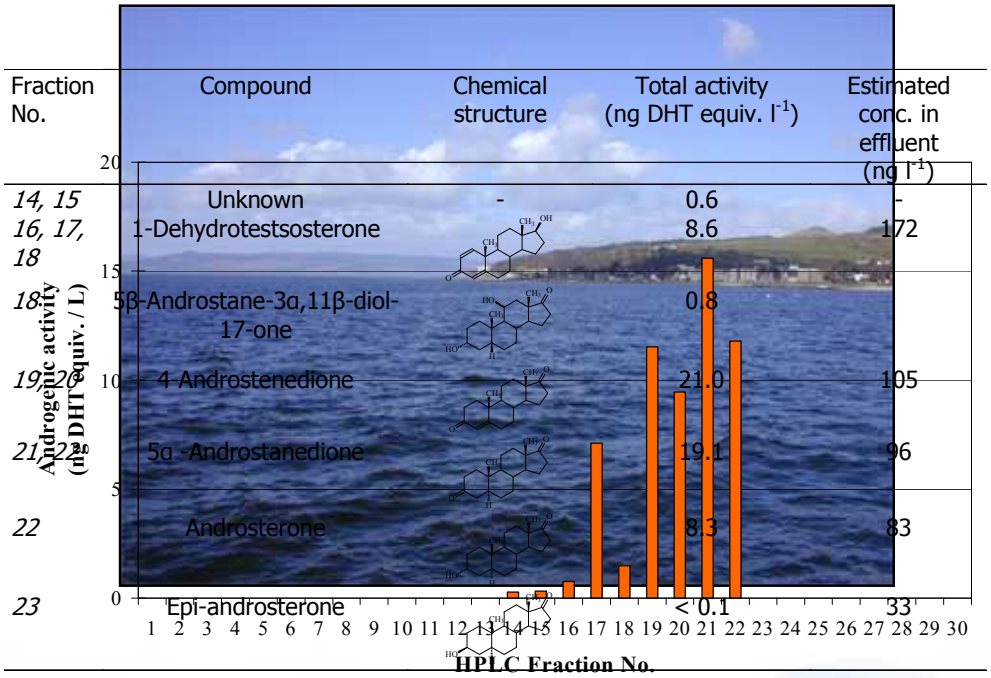
Identification

- Conventional techniques:
  - GC-MS (EI&CI) in full scan.
  - Automated Mass Spectral Deconvolution System (AMDIS) to process files.
  - Identified compounds checked against reference databases (e.g. HSDB, RTECS).
  - Authentic reference compound obtained, Kovats RI and biological activity determined.

- Developing/advanced
  - GC(xGC)-TOF
  - LC-MS^n
    - QTOF
    - Orbitrap

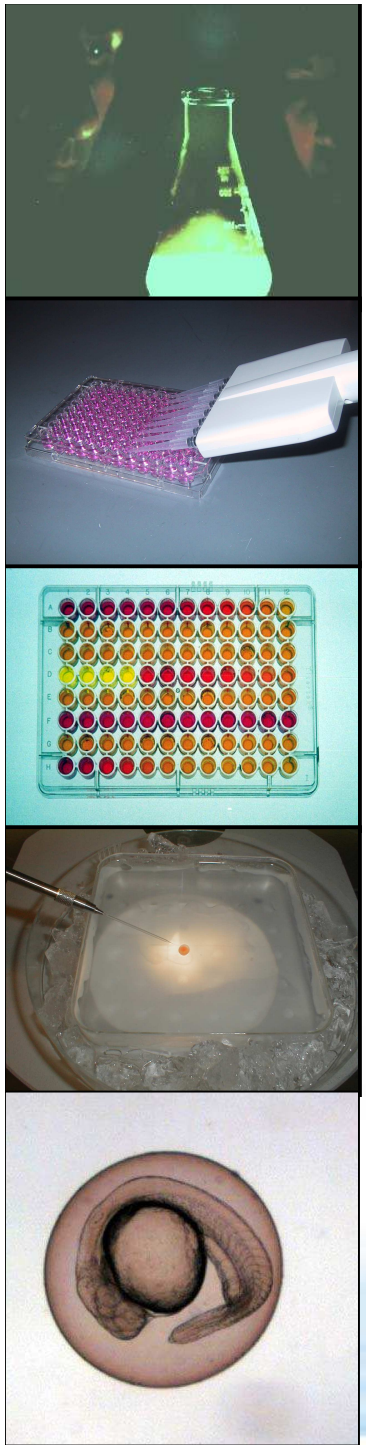
Example 1: Environmental androgens

- Environmental androgens are a group of compounds that to date have received very little attention.
- *In vitro* activity of pulp mill effluent.
- Masculinisation of female eelpout (Sweden).
- Since ER agonists in STW effluents- possibility that androgen receptor (AR) agonists also present.



Example 2: Produced water discharges

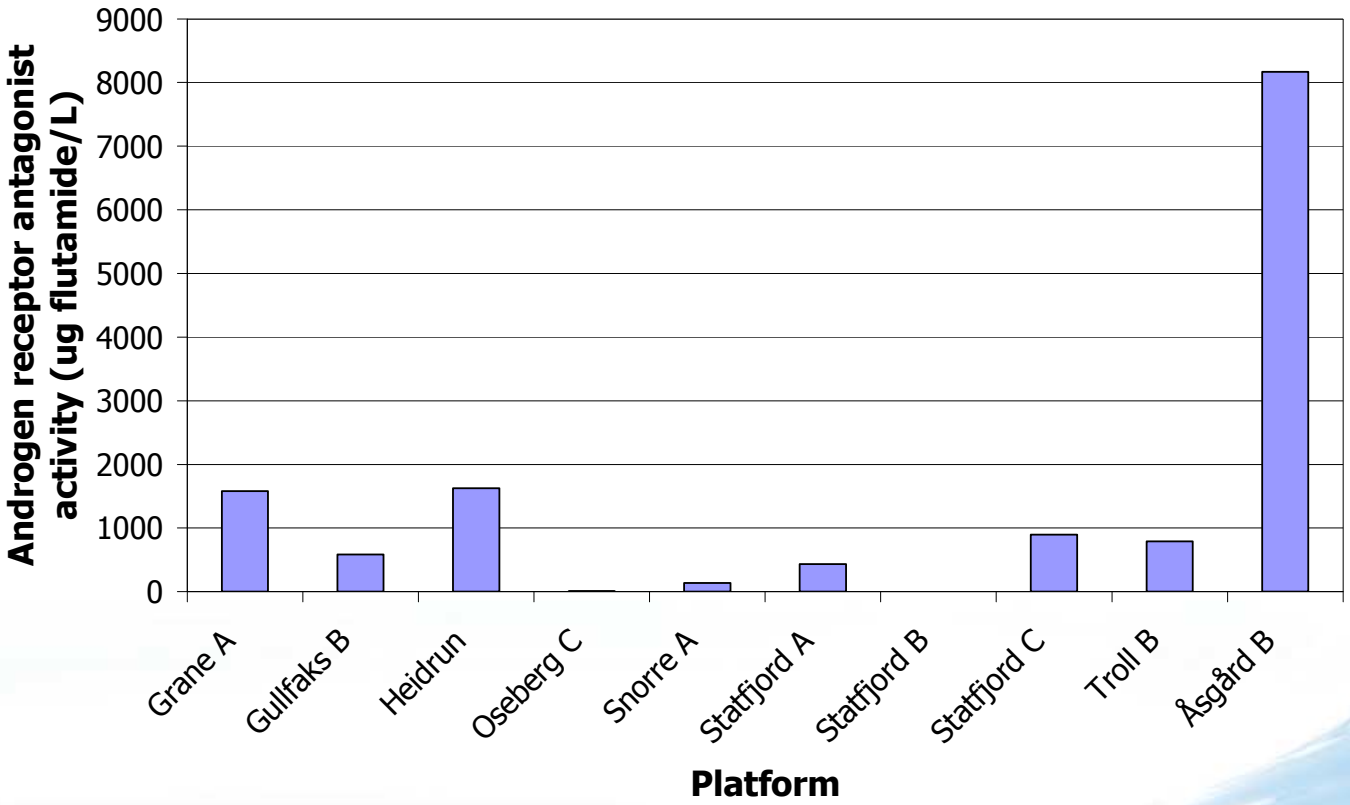
- Largest wastewater stream in offshore oil & gas production (~ 340 x 10^6 m^3 yr^{-1}).
- Complex composition:
  - small amounts of dispersed oil
  - dissolved organic compounds (including h/c)
  - organic acids
  - phenols
  - production chemicals
  - inorganic compounds



Toxicity: what to expect?

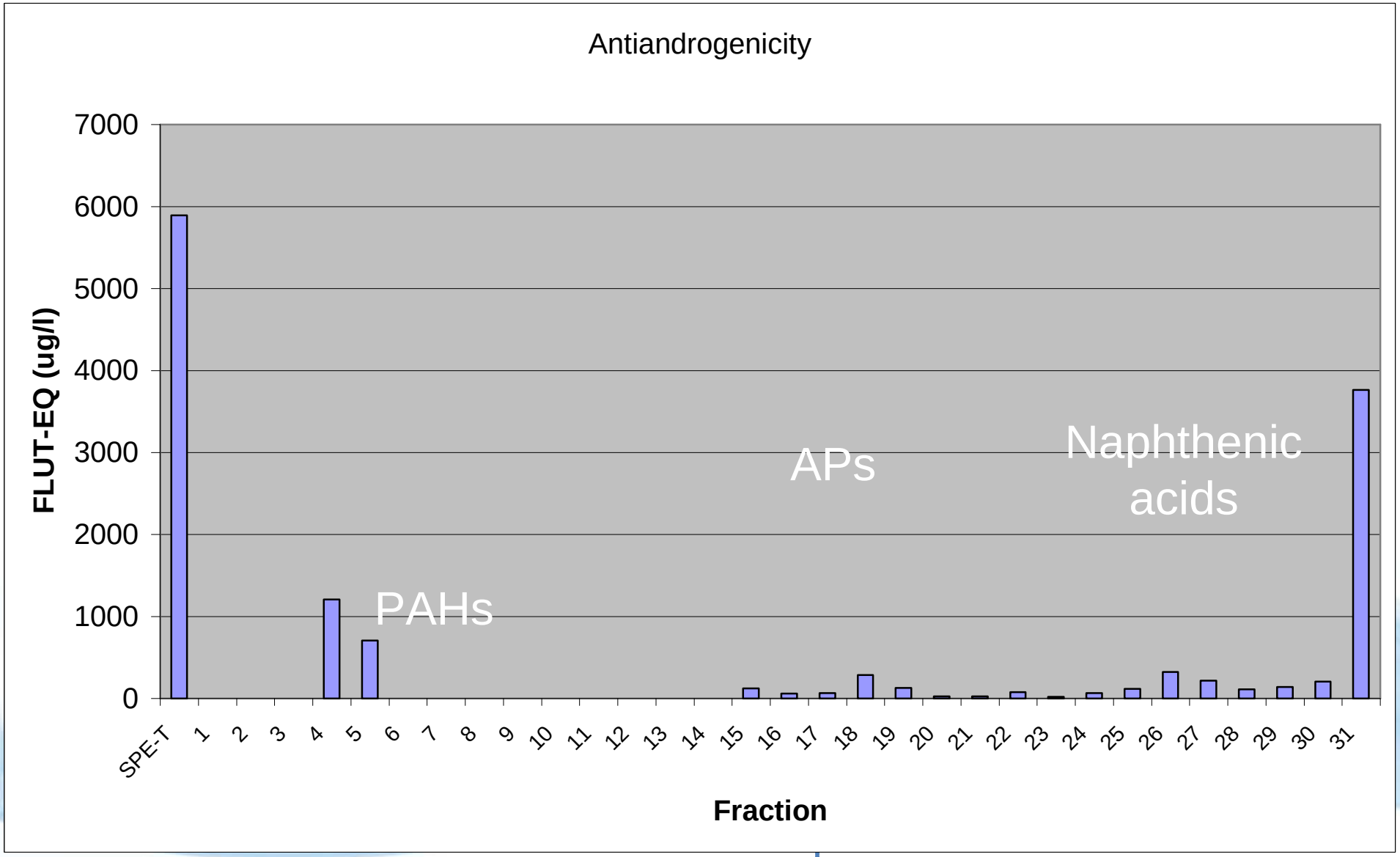
Oil	WSF	Toxic endpoint
+	++	**Acute toxicity (Microtox & hepatocytes)**
++	+	**AhR-toxicity (EROD - hepatocytes)**
+	++	**Estrogenicity (YES & Vtg - hepatocytes)**
-	-	**Androgenicity (YAS)**
+	++	**Oxidative stress (hepatocytes)**
+	++	**Direct mutagenicity (Mutatox)**
n.a.	+	**Embryotoxicity (Fish egg exposure)**

Androgen receptor antagonists

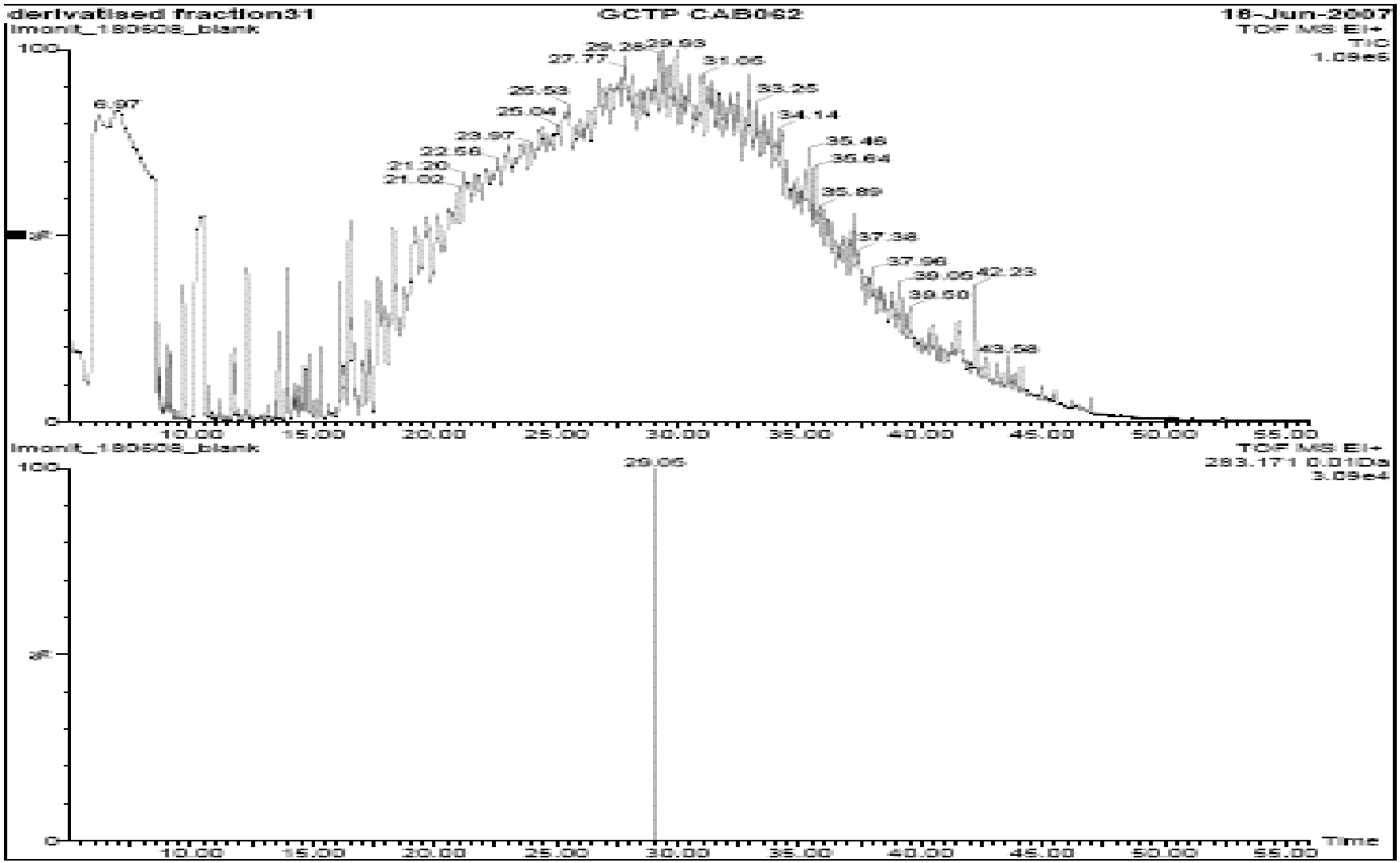


Estimated discharge to Norwegian sector: 10 000 kg Flutamide!

AR-antagonists

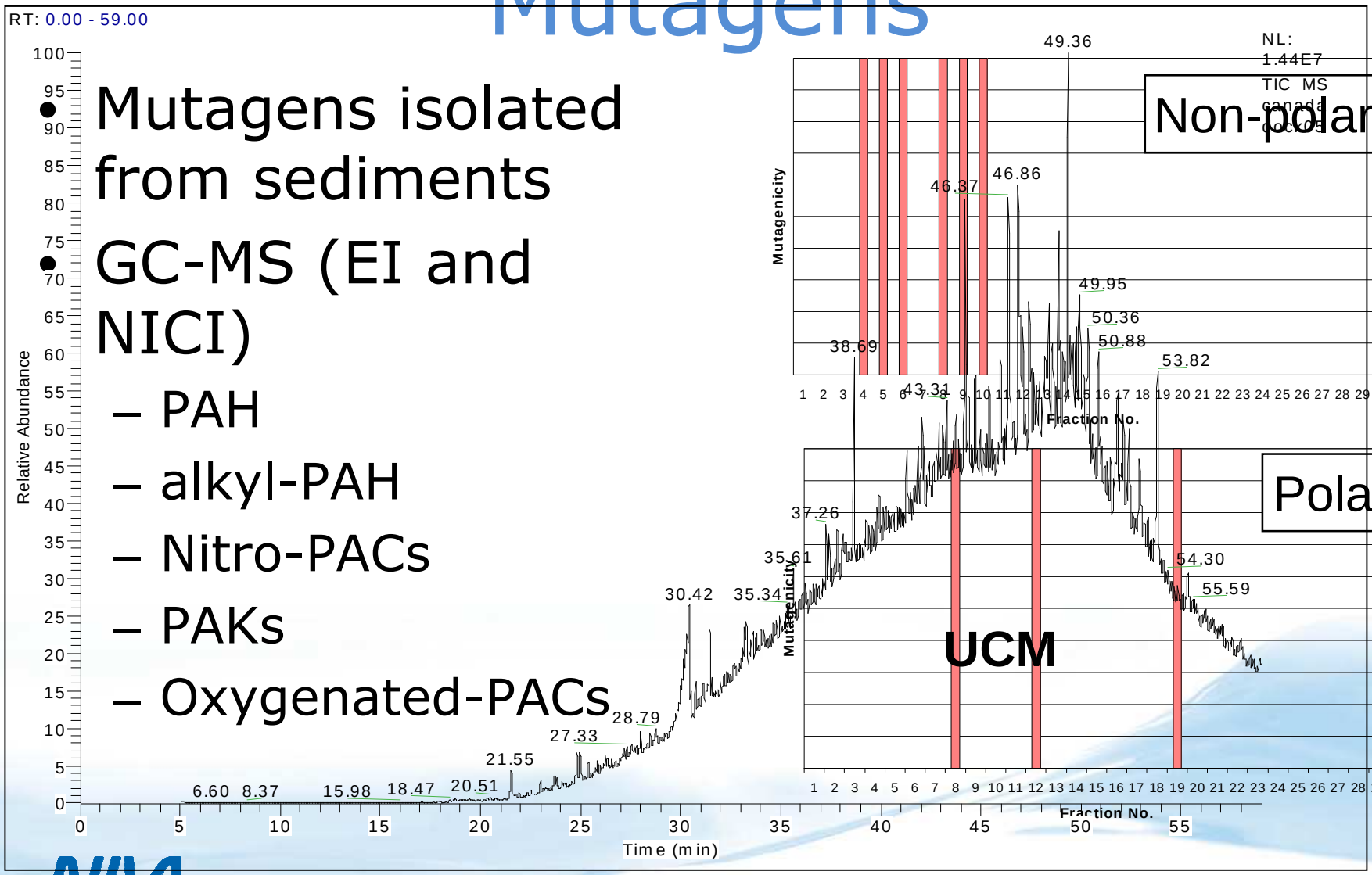


Accurate mass: useful!

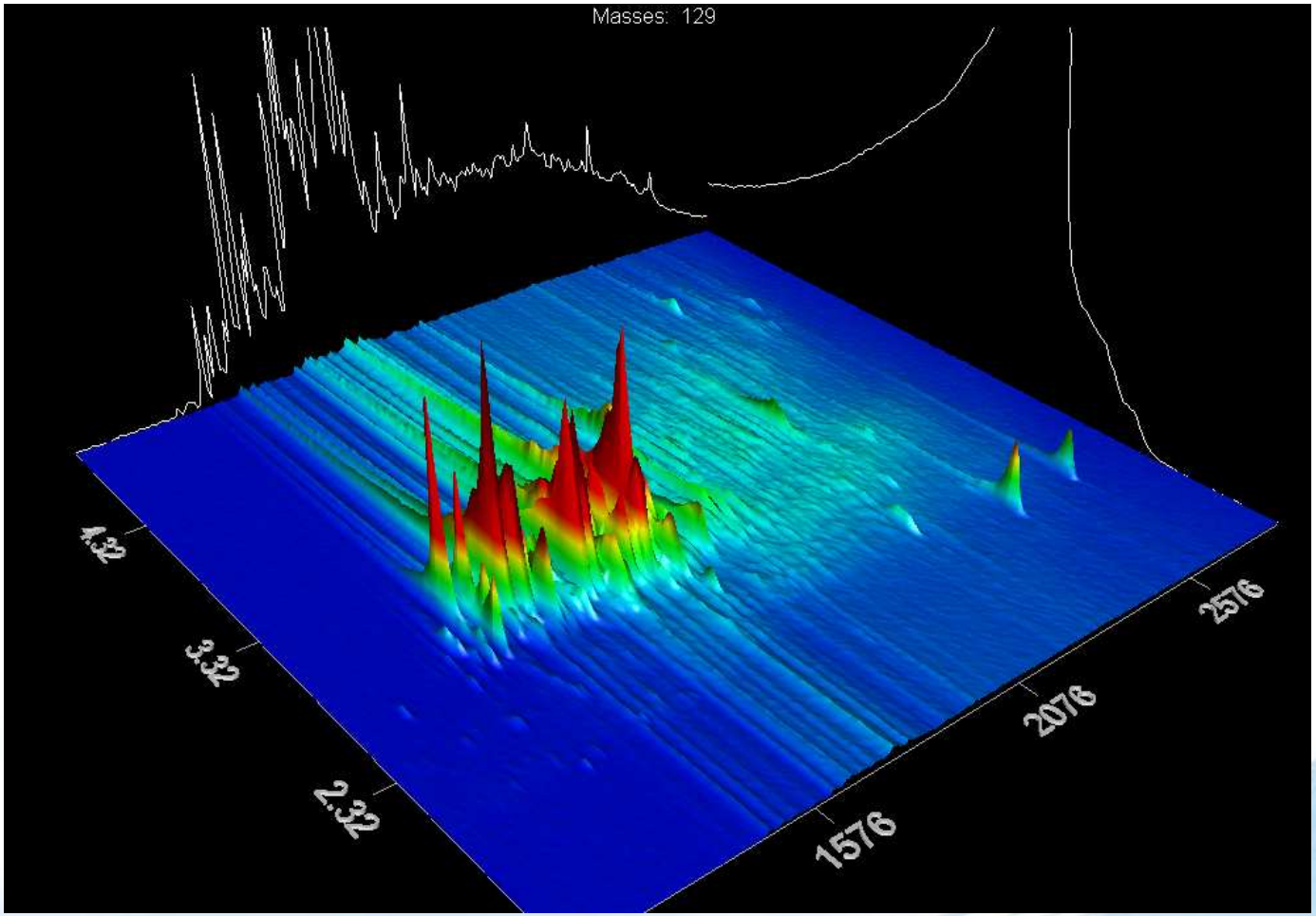


Unresolved components: Mutagens

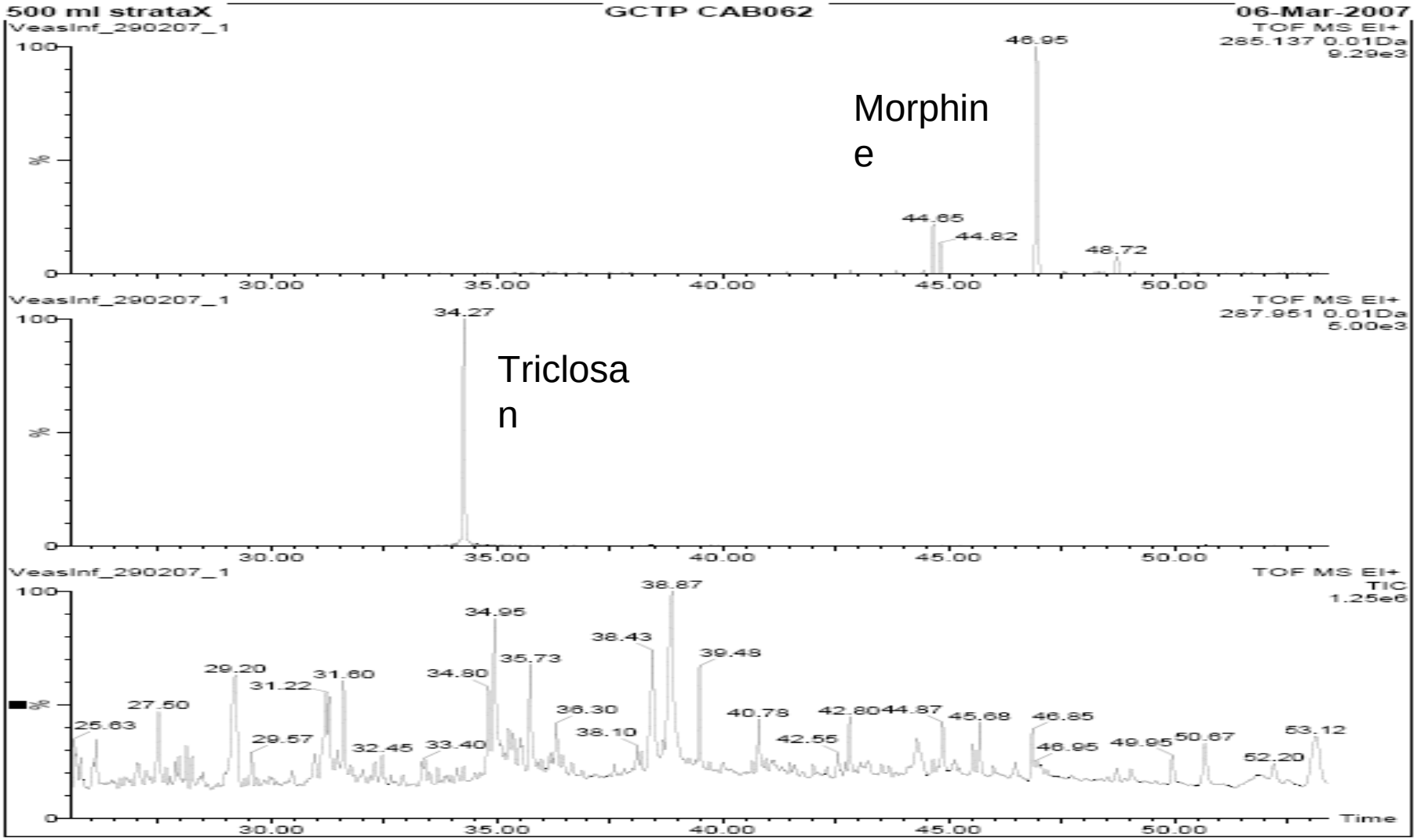
- Mutagens isolated from sediments
- GC-MS (EI and NICI)
  - PAH
  - alkyl-PAH
  - Nitro-PACs
  - PAKs
  - Oxygenated-PACs



GCxGC of sulf-oxides



What else might we find?



Where next?

- 'Known-unknowns':
  - Mutagens
    - Polar fractions
  - AhR
    - Sediments
    - Biota
    - Effluents
- ER
    - Sediments
- Anti-AR
    - Effluents
    - Sediments

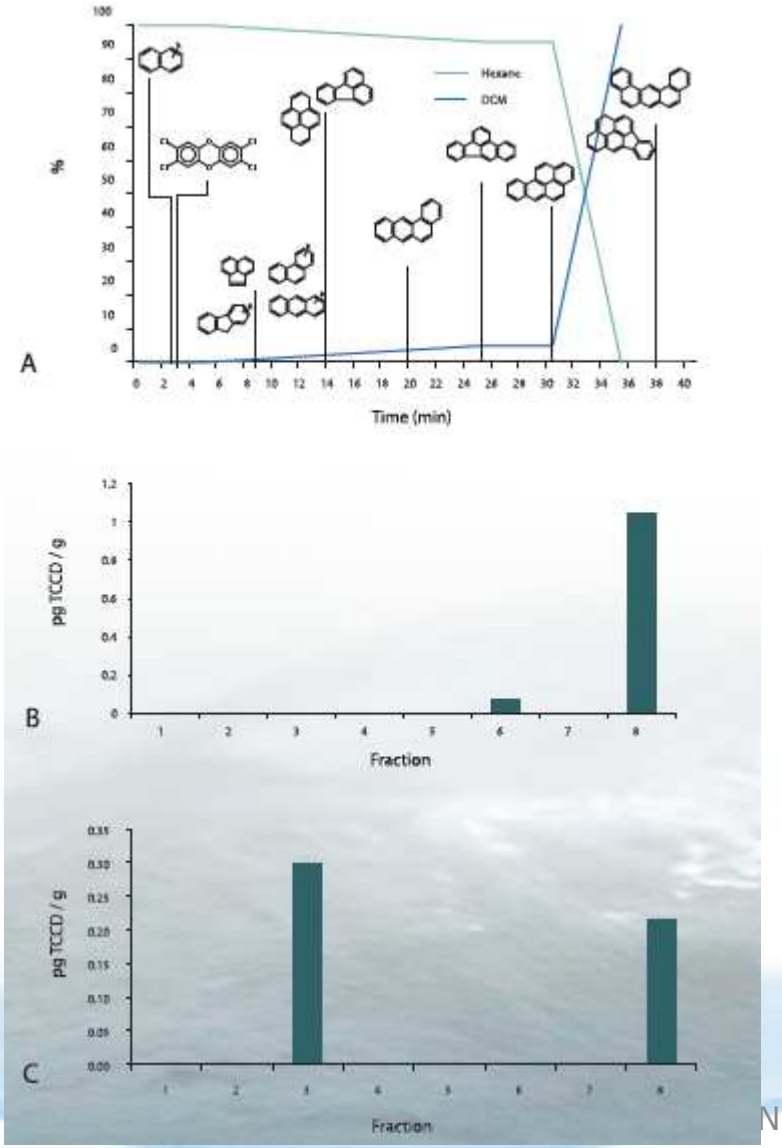
- New areas:
  - End-points:
    Antibiotics
    Thyroxins
  - Locations:
    Artic
    Mountains
    Lakes
  - Matrices
    Biota
    Mammals
    Plants

Will the techniques work?

Polar cod bile

- Bioassays:
  - YES, YAS and DR-CALUX
- Compounds identified:
  - Hydroxy-PCBs
  - PCP, PBP
  - hydroxy-chlorinated diphenyl ethers
  - Hydroxy-brominated biphenyls
  - Hydroxy-PBDEs
  - Hydroxy-PAHs

Dolphin blubber: AhR agonists



Summary

- Unknown causes of effect occur within the environment
  - Possibly caused by emerging contaminants
- EDA is one suitable approach for their I.D.
  - Complementary to compound-specific approaches
- Developments are required and are happening

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- Cooperation and sharing of data is imperative
- Future actitivities will expand the current scope.

Contributors

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