FH CAMPUS WIEN
UNIVERSITY OF APPLIED SCIENCES

Migratox

Christian Kirchnawy:

Sample Preparation:
The long journey from the packaging to the bioassay

Sample preparation

Recommendations aligned with parallel ILSI Expert Group recommendations

Food Additives & Contaminants: Part A

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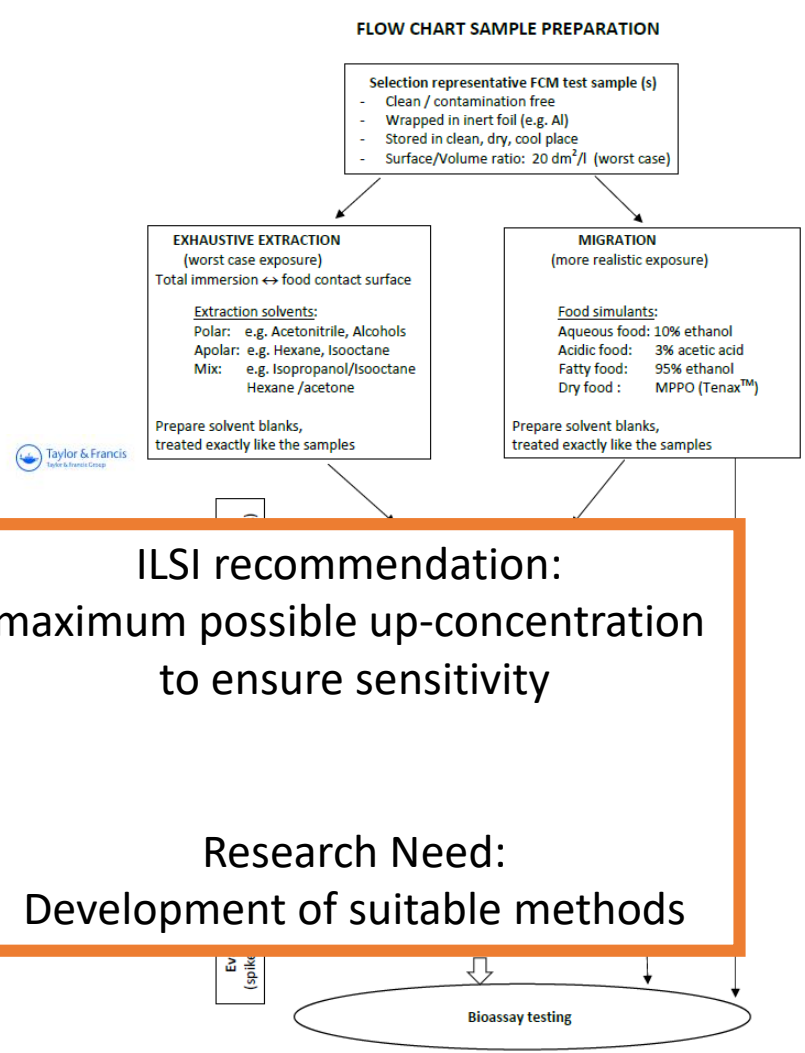
Value and limitation of *in vitro* bioassays to support the application of the threshold of toxicological concern to prioritise unidentified chemicals in food contact materials

Benoit Schilter, Karin Burnett, Chantra Eskes, Lucie Geurts, Mélanie Jac
Christian Kirchnawy, Peter Oldring, Gabriele Pieper, Elisabeth Pinter,
Manfred Tacker, Heinz Traussnig, Peter Van Herwijnen & Alan Boobis



ILSI recommendation:
maximum possible up-concentration to ensure sensitivity

Research Need:
Development of suitable methods

ILSI Europe

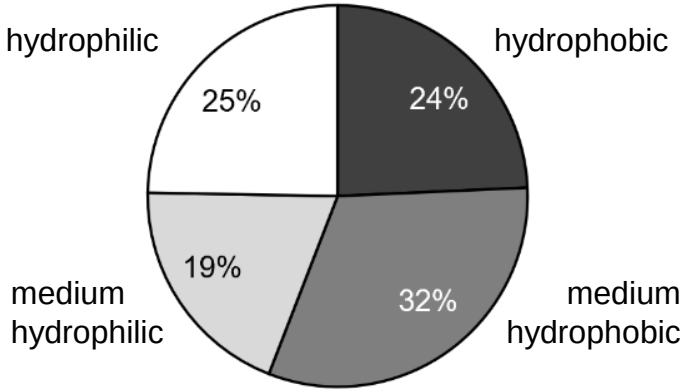
A broad spectrum of physico-chemical properties

Evaluation of ~ 700 AMES POSITIVE substances for their:

Evaluation based on EURL ECVAM Genotoxicity and Carcinogenicity Consolidated Database (Corvi and Madia, 2018)

Hydrophobicity

n = 712



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Hydrophilic	<0	Medium hydrophobic	1 to 3
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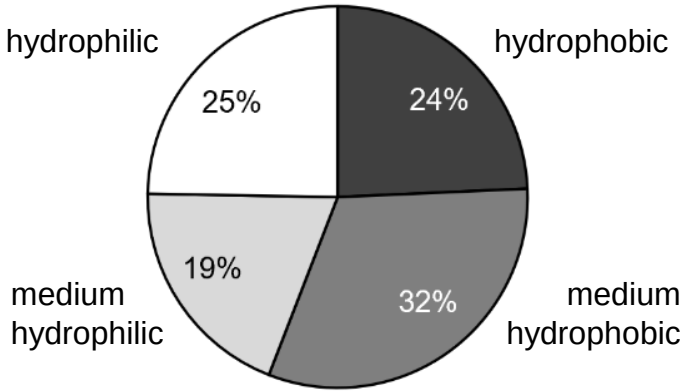

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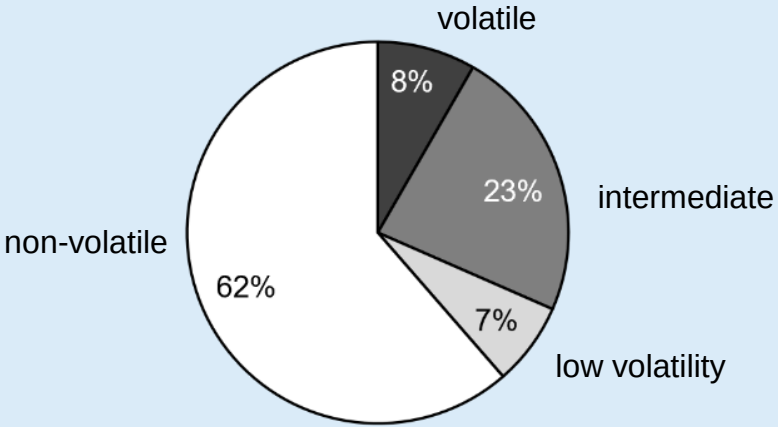

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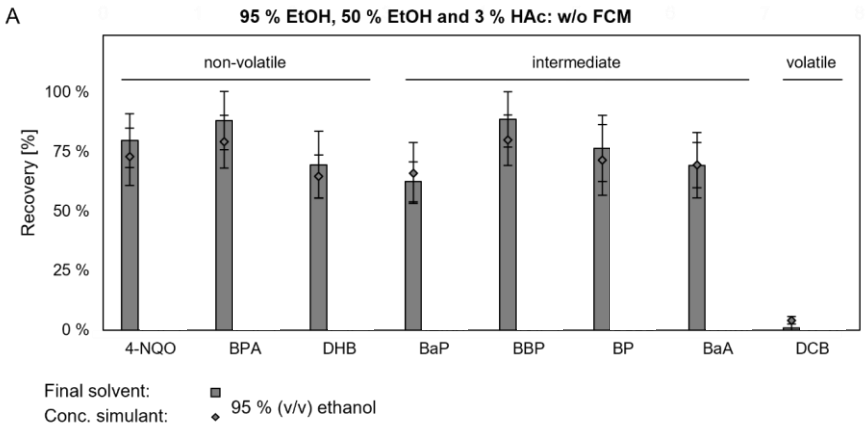
For 95 % ethanol: Pre-concentration by evaporation

Rotary evaporation



Simulants spiked with 100 ppb of model substances, 300-fold concentration

4-NQO: 4-Nitroquinoline 1-oxide, BPA: Bisphenol A, DHB: 1,3-Dihydroxybenzene, BaP: Benzo[a]pyrene, BBP: Benzyl butyl phthalate, BP: Benzophenone, BaA: Benzo[a]anthracene, DCB: 1,4-Dichlorobenzene

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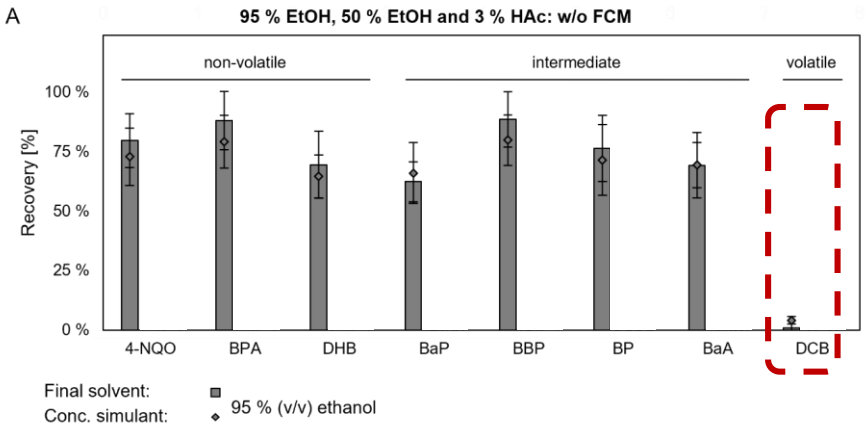

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Evaporation of 95% ethanol: losses of volatiles acceptable

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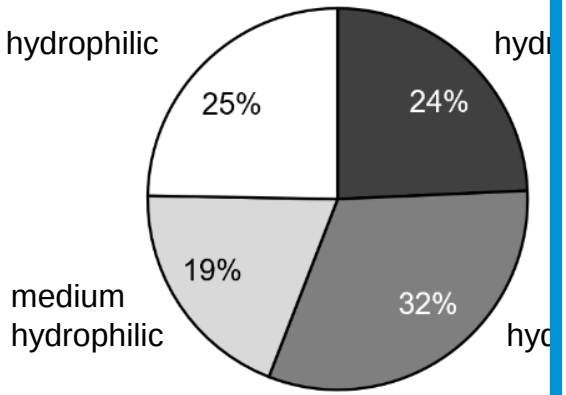

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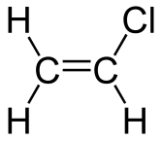
Careful evaporation of **95% ethanol**:

9% most volatile mutagens: not detectable with this method!

Decision: Acceptable



Vinylchloride (Monomer PVC): positive in Ames-Test, but lost during sample preparation

Volatility

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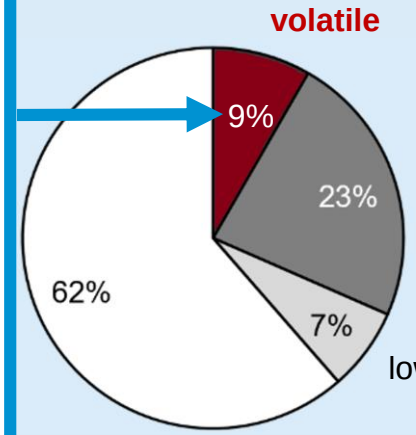

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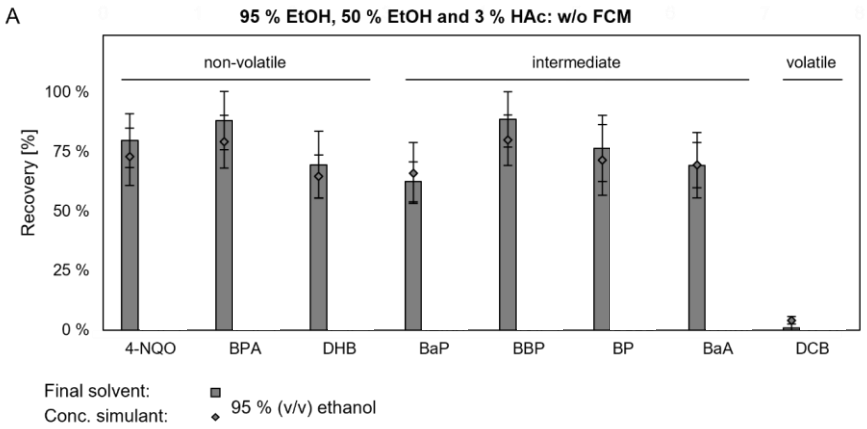

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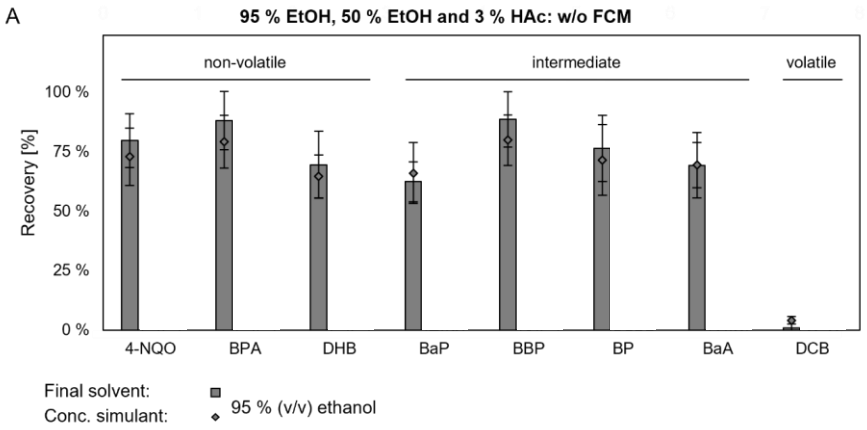
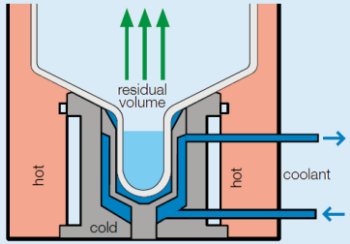
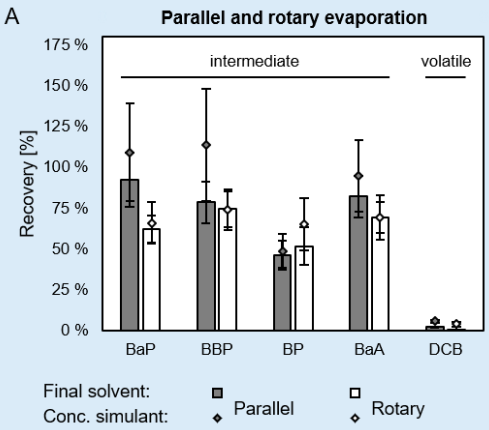


Parallel evaporation





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For aqueous solvents: Evaporation not suitable!

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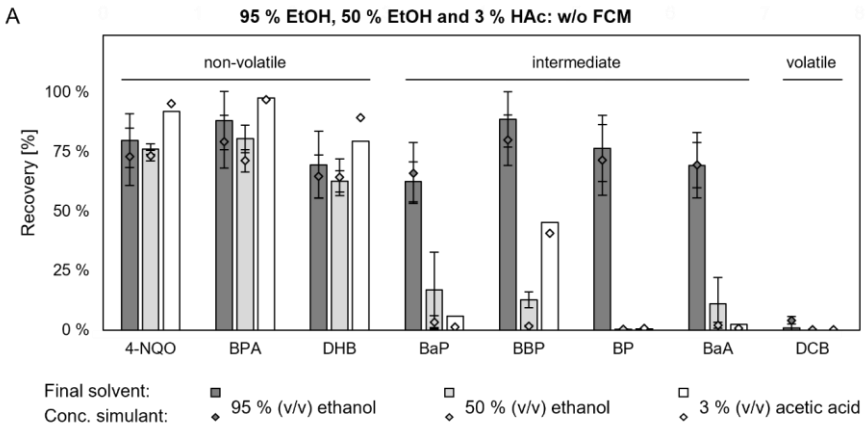

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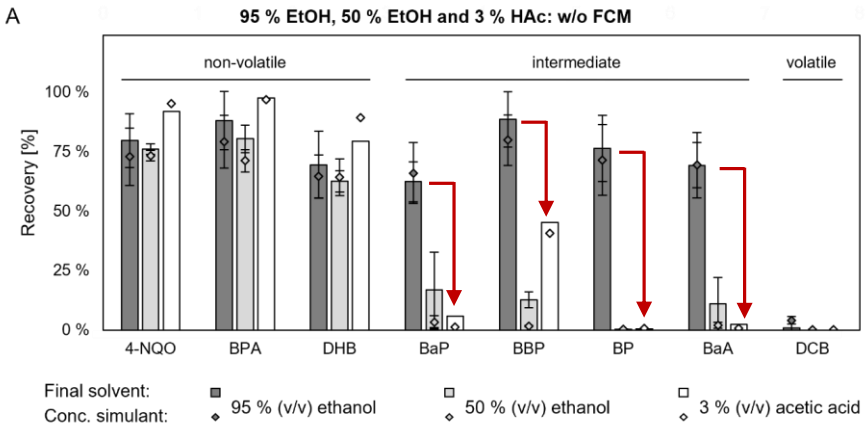

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Evaporation of aqueous solvents: inacceptable losses of volatiles

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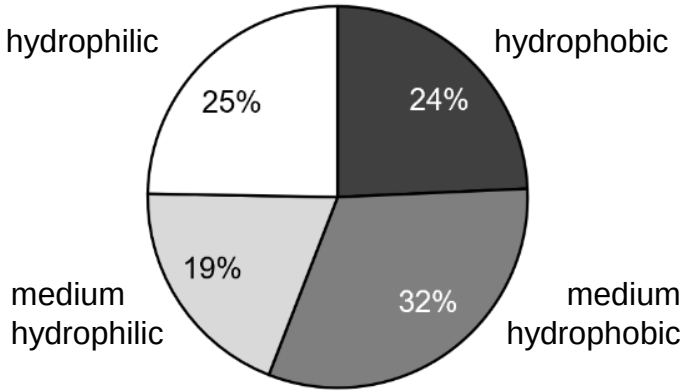

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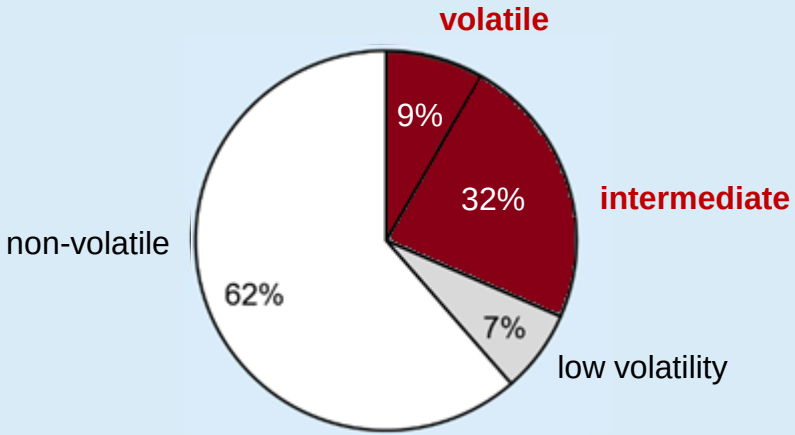

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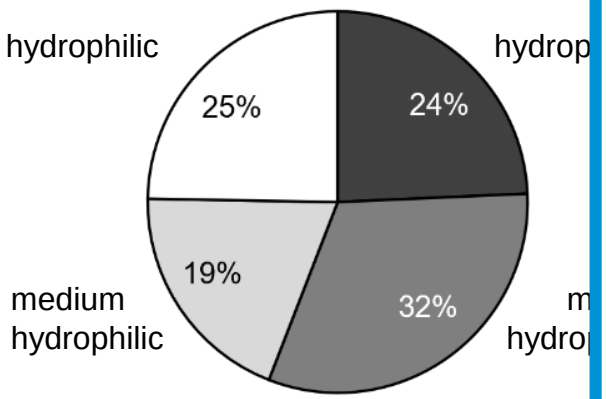

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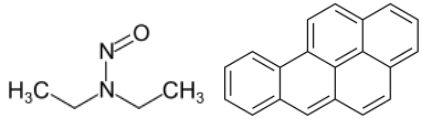
Careful evaporation of aqueous migration solvents **(20% ethanol, 50% ethanol, 3% acetic acid)**

41% most volatile mutagens: not detectable with this method!

Decision: Unacceptable



Diethylnitrosamine or Benz[a]pyrene: positive in Ames-Test, but mostly lost during evaporation of aqueous solvents

Volatility

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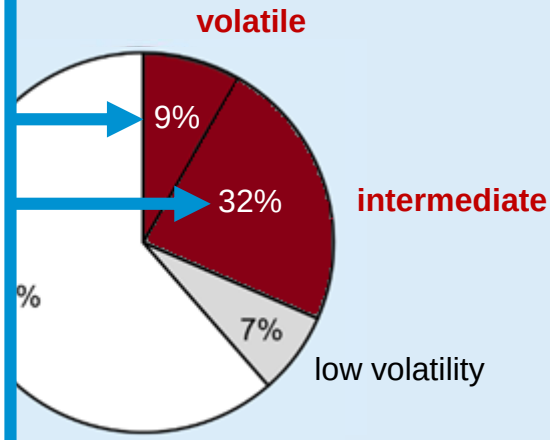

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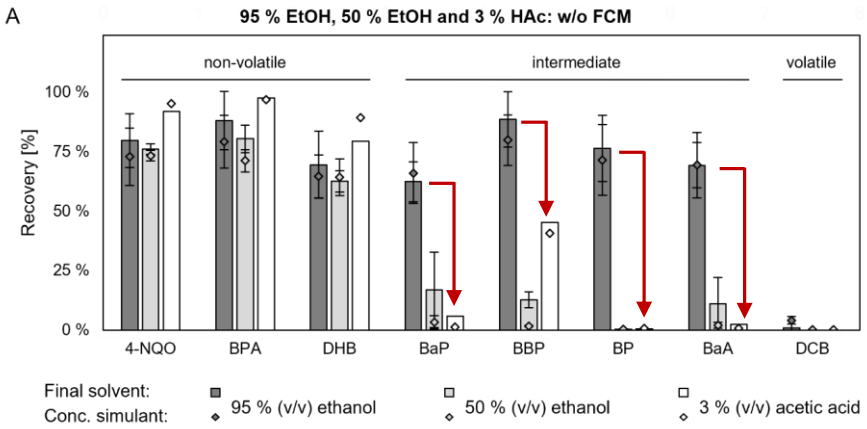

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Alternatives to evaporation

Evaluated in Migratox Project:

- Solvent / Solvent Extraction
- Solid Phase Extraction

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To increase throughput: automated parallel evaporation!

SPE and liquid/liquid extraction: losses of hydrophilic substances

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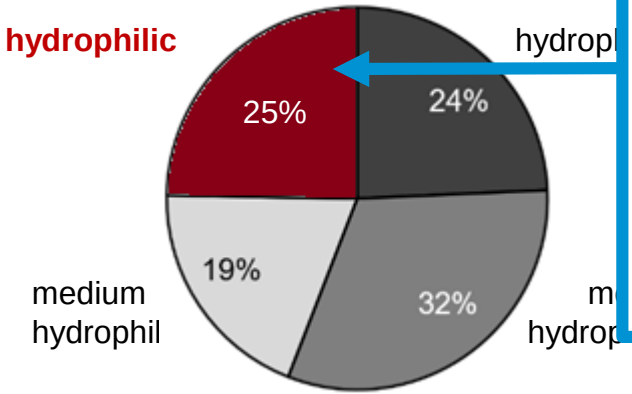

1-Step Solidphase or Liquid/Liquid Extraction loses hydrophilic fraction!

Substances that migrate most easily to aqueous solvents are lost!

e.g. nitrosamines, PAAs

Decision: Unacceptable

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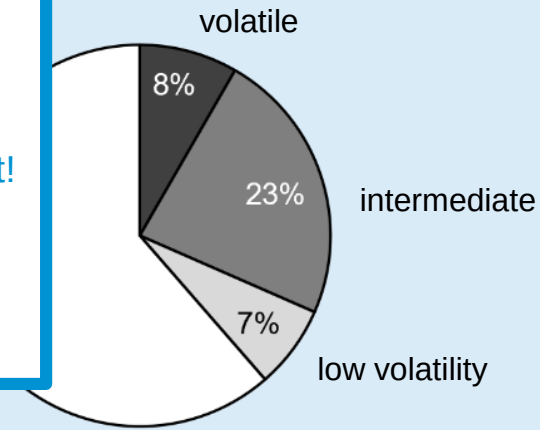

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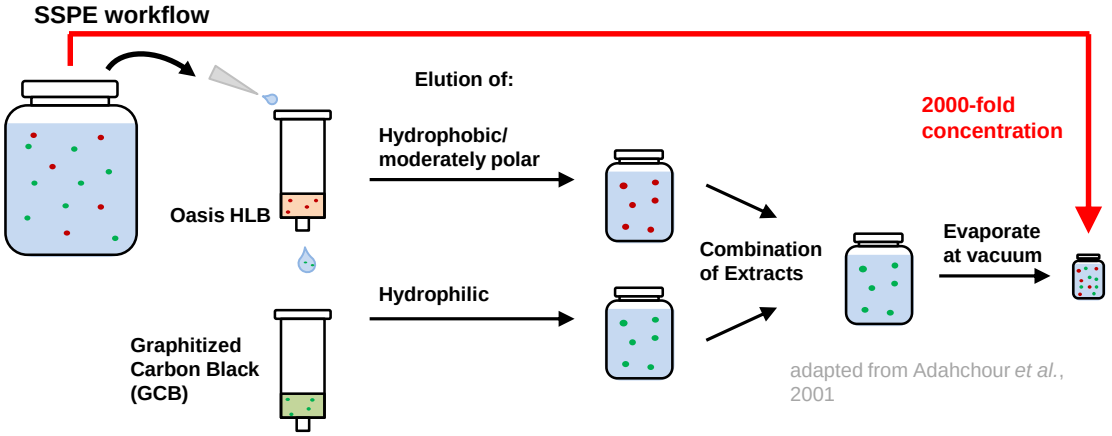
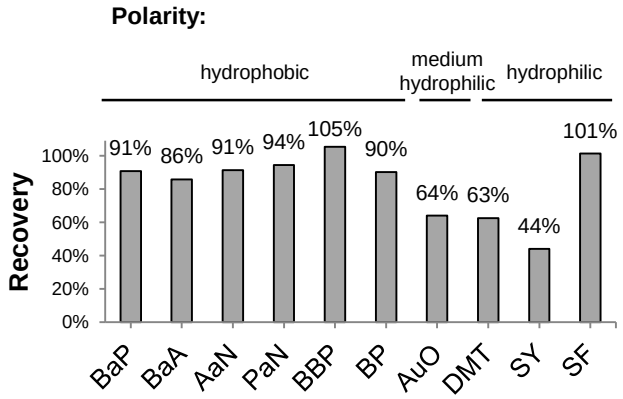
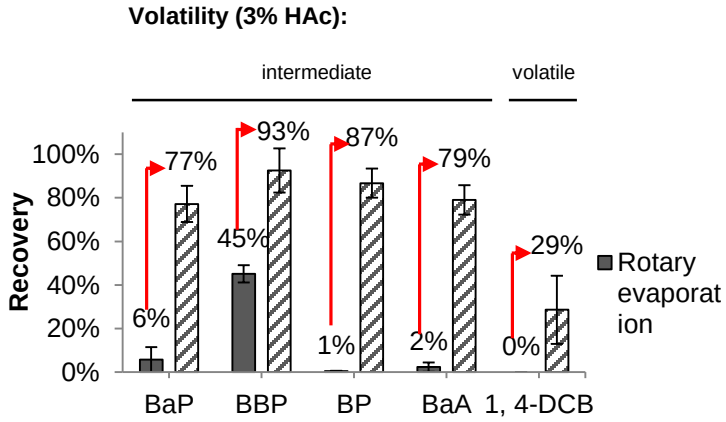
Solution: Complex 2 step Solid Phase Extraction







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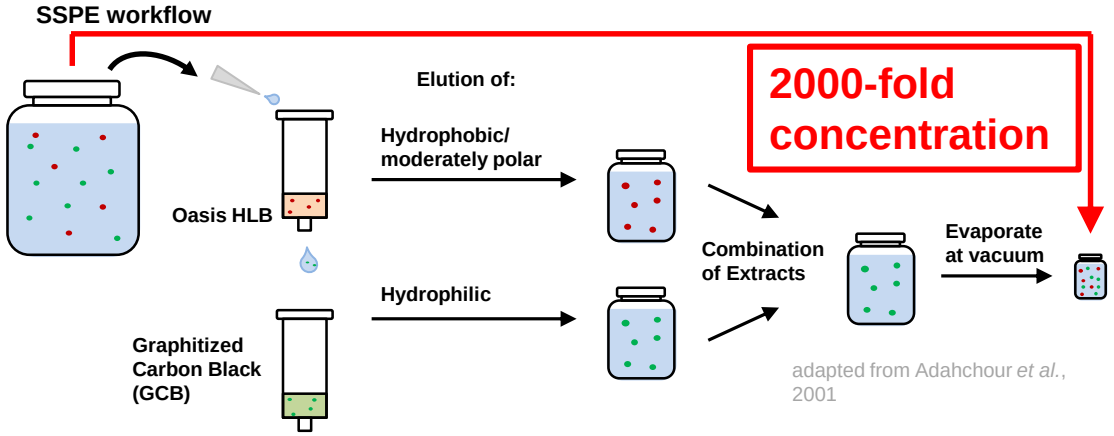

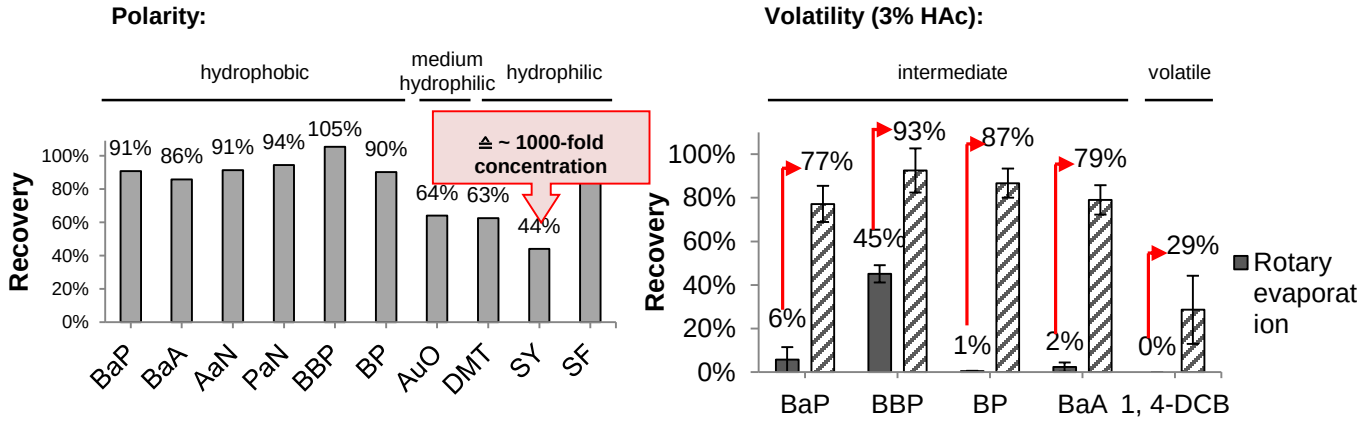

Sample Preparation is key to sensitive and reliable bioassays!

- DNA-reactive substances can have very different physico-chemical properties:
  - from very volatile to completely involatile
  - from very polar and hydrophilic to completely hydrophobic
- No single method can concentrate all of these substances!

- Gentle Evaporation of volatile solvents only loses the 9% most volatile substances
  - Evaporation to dryness has to be prevented
  - Adding DMSO as a keeper prior to the final evaporation step helps to avoid losses

- Evaporation of aqueous solutions (e.g. 20/50% ethanol): inacceptable losses of volatiles

- Two-Step Solid Phase Extraction Method allows to concentrate polar and non-polar mutagens fro aqueous solvents

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