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Development of non-invasive methods and exposure assessment of flame retardant chemicals

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Non-invasive bio-monitoring and exposure assessment of flame retardant chemicals

» Objective

Development and validation of non-invasive methods for the monitoring of human body burdens; exploration of the utility of non-invasive matrices such as *hair, earwax, nails, saliva and urine* as biomarkers of internal exposure to **flame retardants** (FRs)





Multi-residue method for hair analysis

Hair sample (200 mg)

IS addition: BDE-77, TPhP-d15, TDCPP-d15, TnBP-d27, TCEP-d12, TBEP-d6

Digestion & Extraction

- 3 mL of 10% HNO_3 and 3 mL of Hex:DCM (4:1, *v/v*) over 25 min. at 25 °C followed by 1 min. vortexing and 5 min. centrifugation
- Additional extraction: 2 x 3mL Hex:DCM (4:1, *v/v*)

Fractionation
(Florisil)

Fraction A (PBDEs)
10 mL Hexane + 6mL Hex:DCM (6:1, *v/v*)

Fraction B (PFRs)
10 mL EtAc

Clean-up
Acidified silica gel (44 %, *m/m*)
9 mL Hex + 3 mL DCM

GC-ECNI-MS

LC-MS/MS

Method validation & applicability (1)

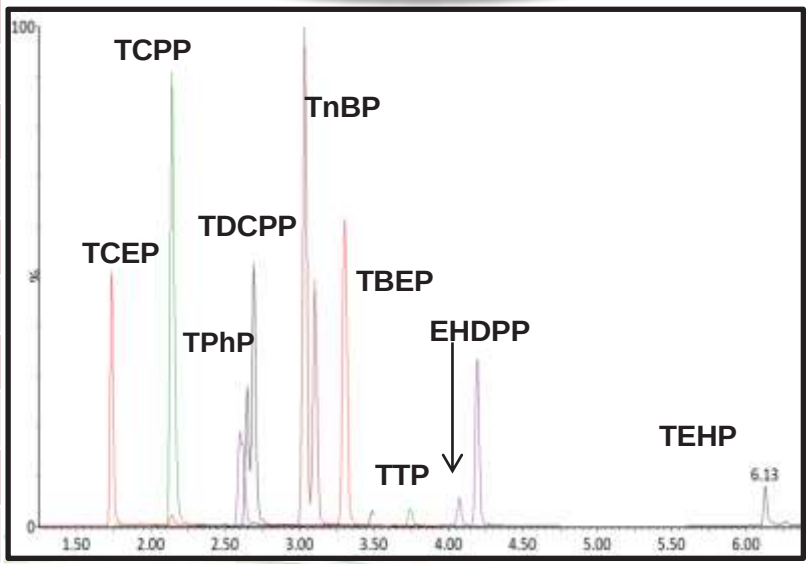
Compound	% Recovery (Qlow)	% Recovery (Q High)	U (%) *	LOQ_m	Levels in hair (ng/g)
BDE-28	82	84	35	0.8	< LOQ
BDE-47	88	91	20	0.8	12
BDE-99	94	98	12	0.8	6
BDE-100	91	94	16	0.8	1
BDE-153	108	114	33	0.8	< LOQ
BDE-154	94	100	13	0.8	< LOQ
BDE-183	112	120	46	0.8	< LOQ
TPhP	103	104	18	4	7 – 237
TDCPP	108	109	23	6	9 – 2968
TCPP	105	103	23	6	17 – 1749
EHDPP	109	107	28	4	9 – 104
TnBP	106	108	19	10	18 – 5032
TCEP	109	113	27	4	11 – 404
TBEP	113	111	24	10	16 – 338
TEHP	106	95	21	3	4 – 322
TTP	101	101	28	8.0	19 – 73

Spiking experiment (HQ & LQ); 3 days, 3 replicates

n= 20; Belgian population

TnBP>TDCPP>TCPP>TCEP>TBEP>TEHP>TPhP>EHDPP>TTP

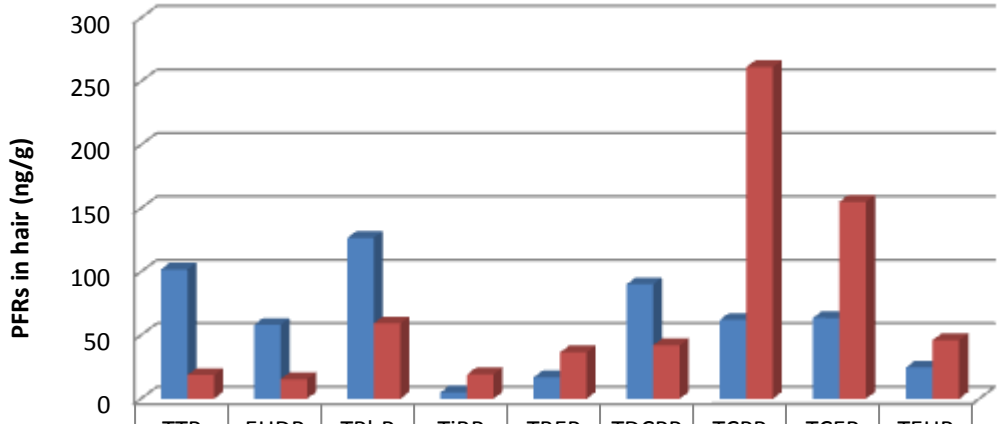
EHDPP, TPhP, TCEP - 100 %
TBEP, TDCPP - 95 %
TCPP, TnBP – 90 %



Method applicability (2)

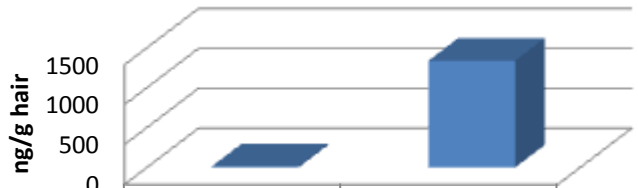
100 % - EHDPP, TPhP, TCPP, TCEP
90 % - TEHP, TDCPP
80 % - TTP
50 % - TBEP
20 % - TnBP

PFRs in Hair from e-waste vs. PFRs in Belgian Hair



	TTP	EHDPP	TPhP	TiBP	TBEP	TDCPP	TCPP	TCEP	TEHP
Median (e-waste samples)	102	58	127	5	17	90	62	64	25
Median (Belgian samples)	19	15	59	19	37	42	261	155	46

TnBP in Hair from e-waste vs. TnBP in Belgian Hair



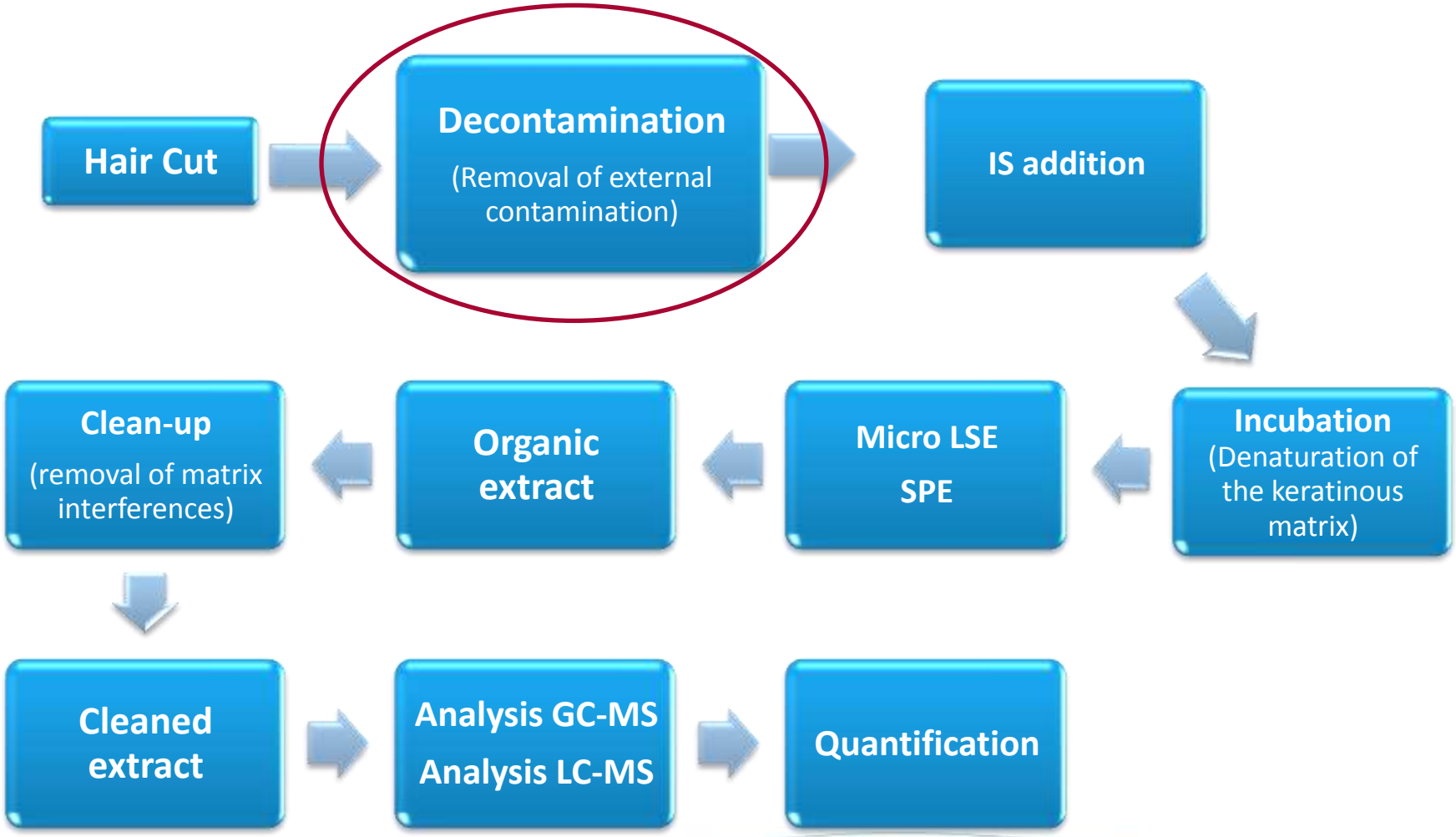
	Median (e-waste samples)	Median (Belgian samples)
TnBP	15	1339

Ear Wax !!!!

Simple extraction in ultrasound
Fractionation on Florisil
Analysis LC-MS/MS

	TTP	EHDPP	TPhP	TiBP	TBEP	TDCPP	TCPP	TCEP	TnBP	TEHP
% Detection EW	40	80	100	100	10	10	70	70	100	40
% Detection Hair	80	100	100	80	50	90	100	100	20	90

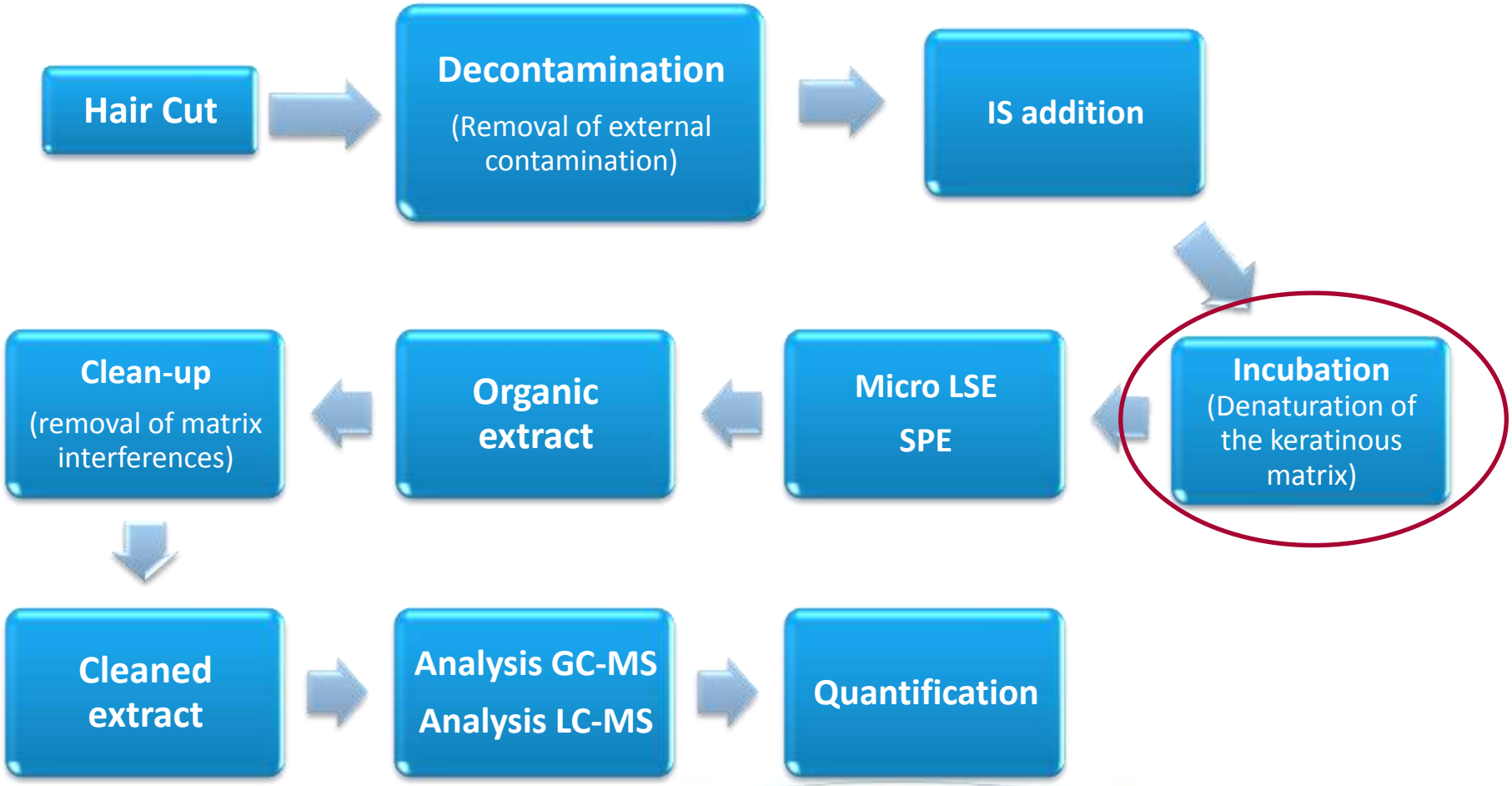
General Scheme of Hair Analysis



Washing Efficiency

- ❑**Hair samples should not be washed before analysis**
- ❑**There is no appropriate medium that can deal with the external decontamination**
- ❑**MeOH as solvent used for direct extraction**
- ❑**Water/Shampoo don't remove external contamination**
- ❑**It's not possible to distinguish external from internal exposure**
- ❑**Hair as biomarker of integrative exposure (passive sampler)**

General Scheme of Hair Analysis



Hair digestion efficiency

SEM – Scanning Electron Microscope

Media:

- 10 % HNO_3
- 20 % HNO_3
- 0.1M HCl
- 0.25M HCl
- 3M HCl
- MeOH
- Hex:DCM (1:1)
- Acetone
- Combined extr. 10 % HNO3 + Hex:DCM (4:1)

} 25 °C; 25 min.

- MeOH
- 3M HCl

} 60 °C overnight

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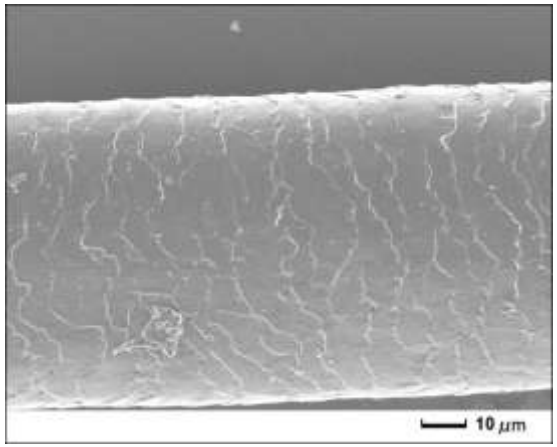
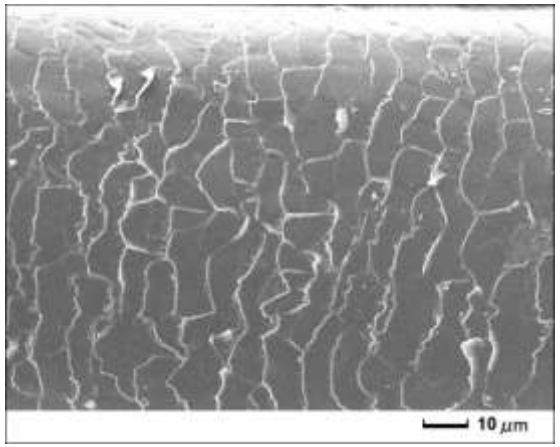
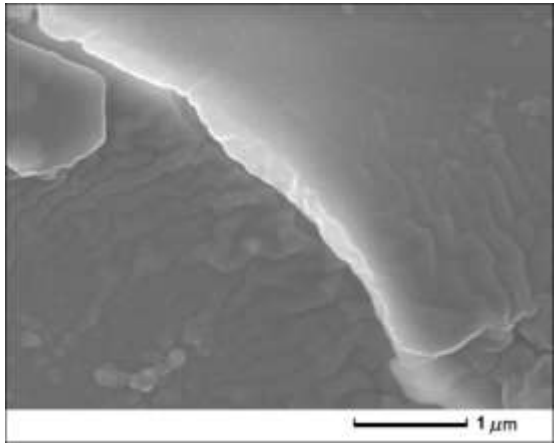
Hair Structure after Treatment in Different Media (1)



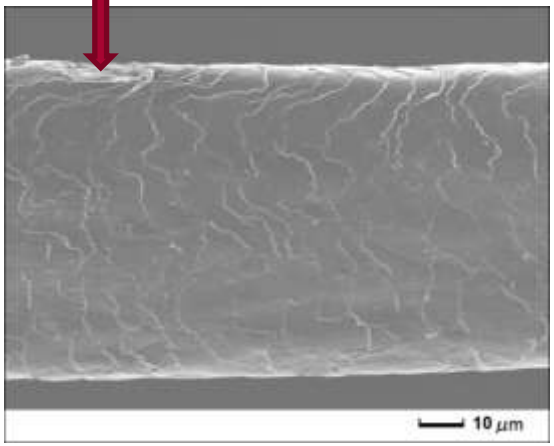
Blank Hair

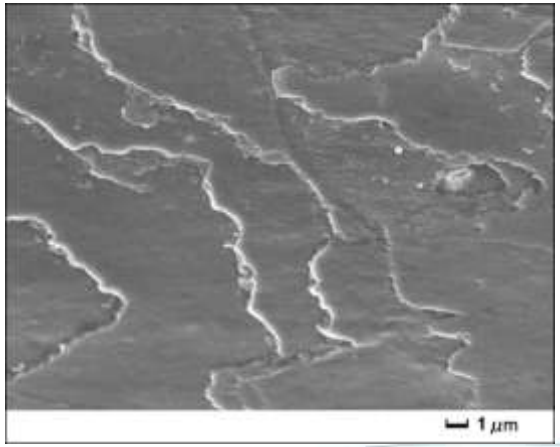


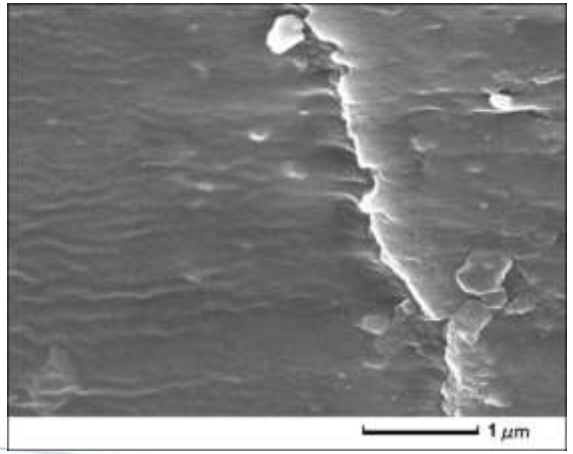
0.1M HCl (more cracks)



3M HCl (additional waves)

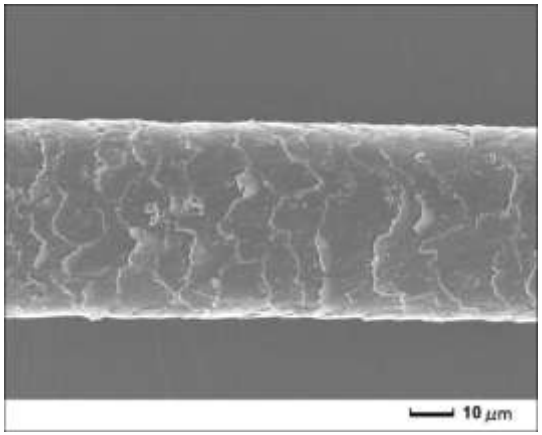




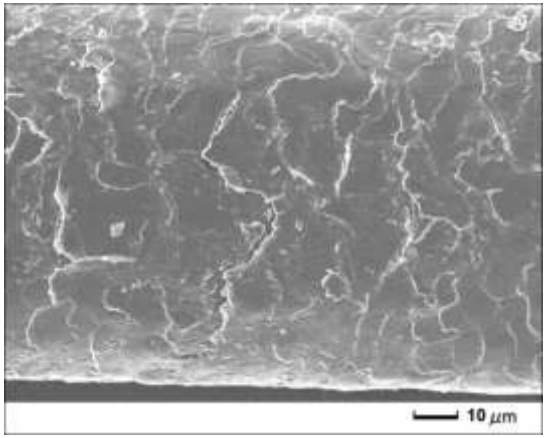


20 % HNO_3 (waves &holes)

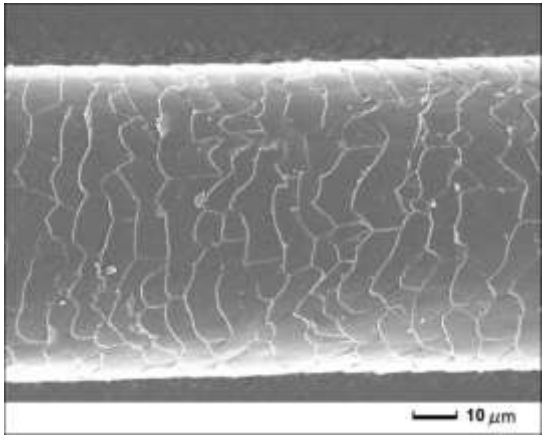
Hair Structure after Treatment in Different Media (2)



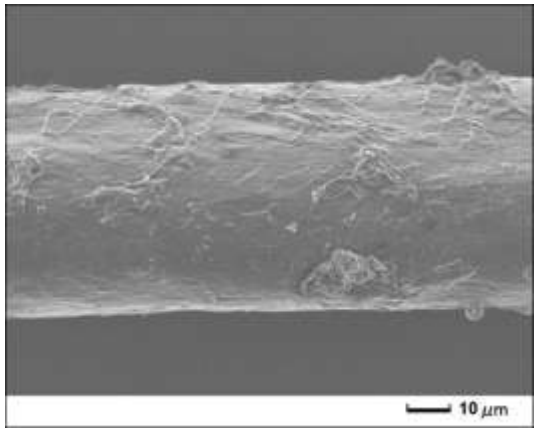
Acetone

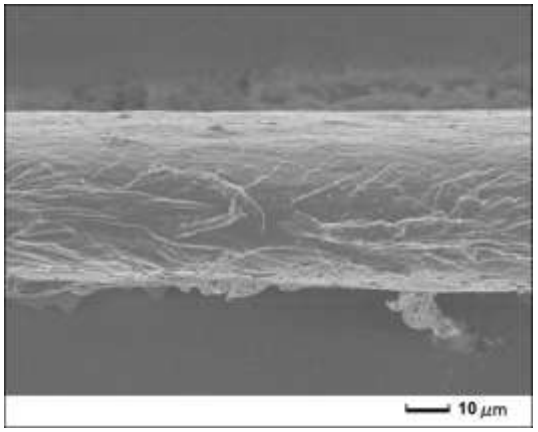


Hex:DCM (1:1)

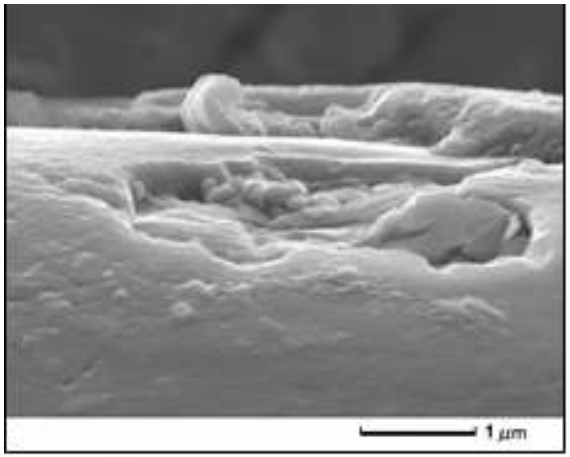


MeOH (60 °C, overnight)





3M HCl, 60 °C; overnight



Combined extraction 10 % HNO_3 + Hex:DCM

Progress up to date

- Poster Presentation and Oral Presentation

at Dioxin 2013 (25-30 August, 2013;Daegu, South Korea)

- Co-author/Participation in review about human biomonitoring
- Course of Interpretation of MS-MS Mass Spectra (at VITO; Hyphen MassSpec; Prof. Dr. Wilfried Niessen) 23 – 25 September 2013
- 2 publications to be submitted by the end of November 2013

1. **Tackling the low sample amounts in biomonitoring studies: broad spectrum flame retardant analysis in hair samples**
2. **Non-invasive biomonitoring for OPEs and PBDEs: new insights in hair analysis**

- Method development for PFR metabolites measurement by direct analysis of urine

THANK YOU FOR YOUR ATTENTION ☺