3-D human skin models – appropriate test systems for toxicology testing?

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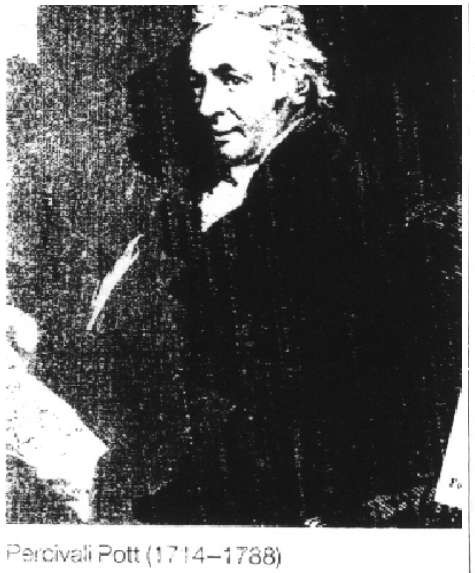
The Chemical Company

Cutaneous metabolism

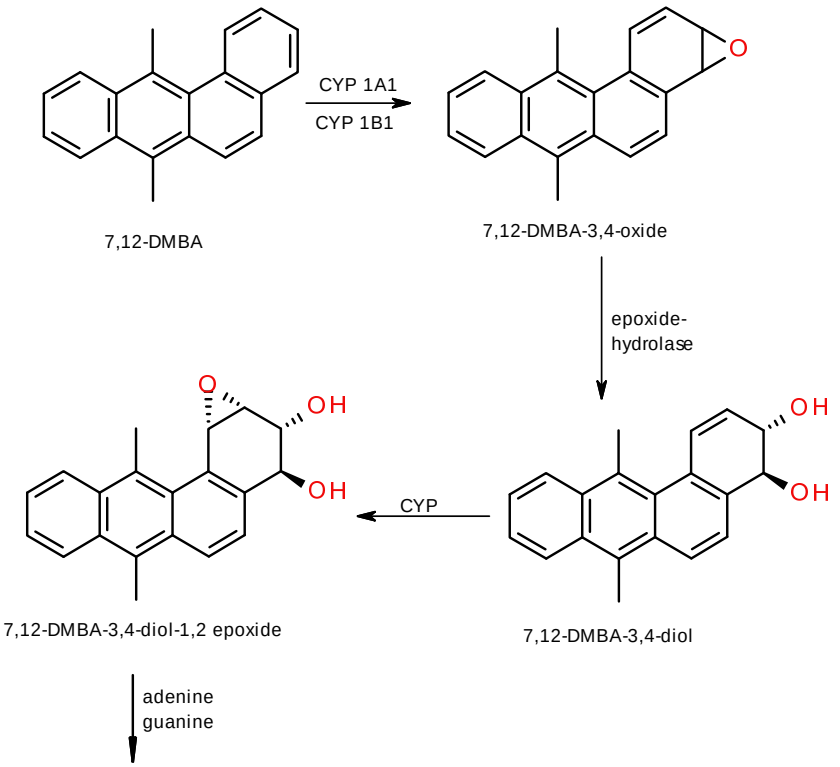


Sir Percivall Pott (1714–1788)

The London Sweep

- 18th century: Sir Persivall Pott first linked the exposure of London chimney sweeps to soot and resulting scrotum cancer
- 20th century: polyaromatic hydrocarbons (PAH) were identified as cancerogenic agent of the soot
- and: association of skin cancer with CYP1A and CYP1B

- PAH: e. g. 7,12-Dimethylbenz[a]anthracene

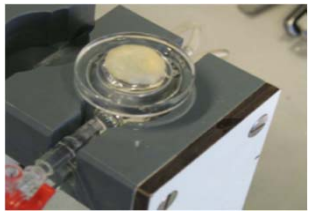
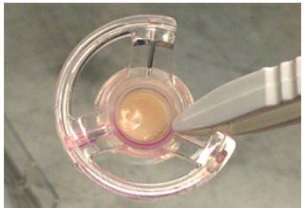
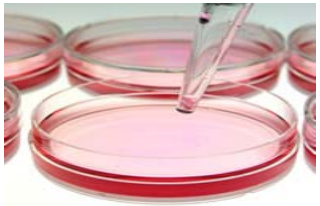
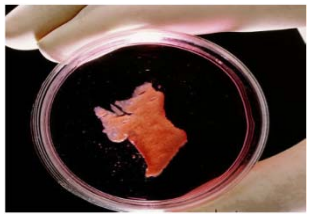
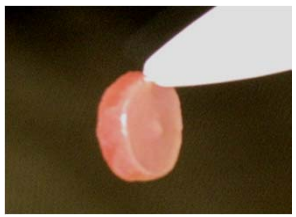
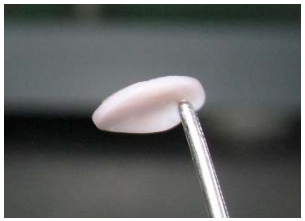
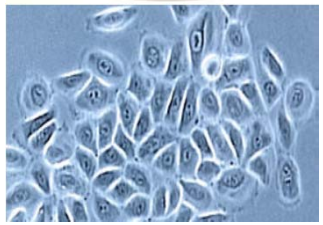
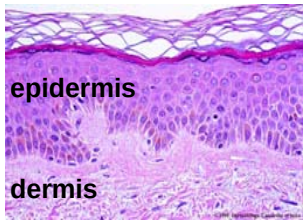
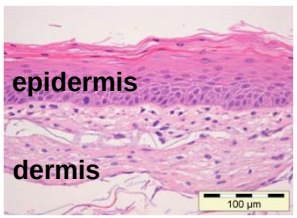
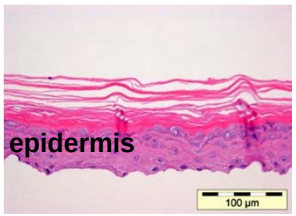
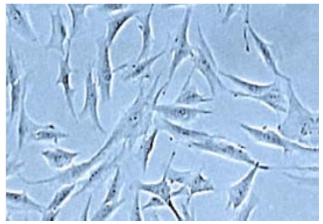


Usage of 3-D skin models in toxicology

- 7th Amendment of EC Cosmetics Directive 76/768/EWG
  - Ban on animal testing for cosmetics and its ingredients in March 2013

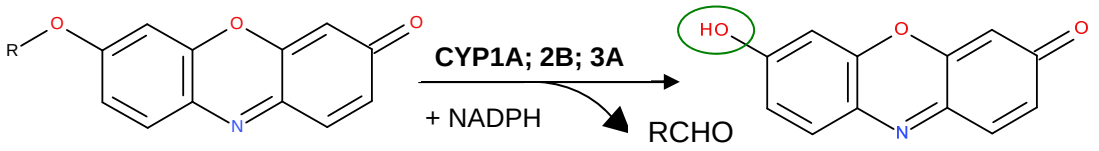
- Advantages of 3-D skin models
  - Human origin
  - Commercially available
  - Morphology

Dermal systems

ex vivo human skin	Phenion®FT (Henkel)	EpiDerm™FT (MatTek)	EpiDerm™ (MatTek)	Monolayer cell cultures
				
				
				
Dermatomed or full thickness	D: ~ 2.0 mm A: 1.54 cm^2 Ø: 1.4 cm neonat. foreskin	D: ~ 1.0 mm A: 1 cm^2 Ø: 1.13 cm neonat. foreskin	D: ~ 0.5 mm A: 0.64 cm^2 Ø: 0.9 cm neonat. foreskin	keratinocytes fibroblasts

Enzyme activity assays

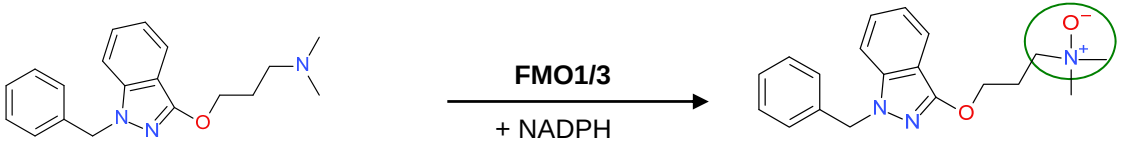
CYP:



alkoxyresorufine — resorufine

(λ_{ex}= 550 ; λ_{em}= 585)

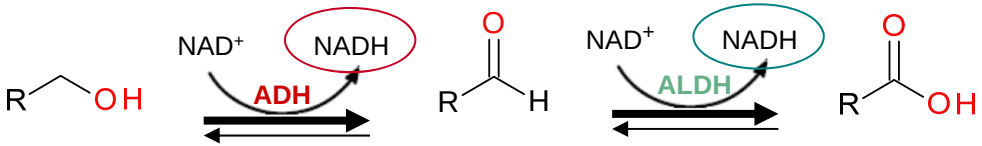
FMO:



benzydamine (BA) — benzydamine-N-oxide

(λ_{ex}= 305 ; λ_{em}= 375)

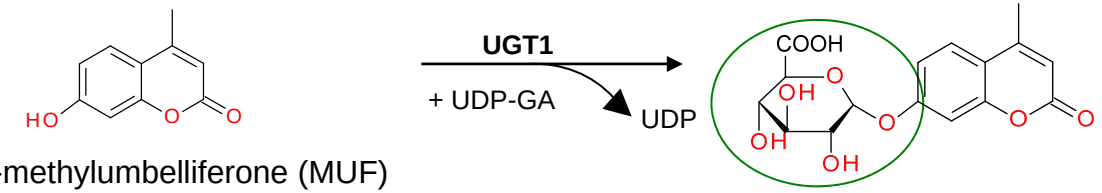
ADH/ALDH:



alcohol — aldehyde — acid

(λ = 340 nm)

UGT:

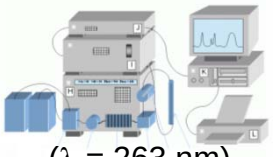


4-methylumbelliferone (MUF)

(λ_{ex}= 315 ; λ_{em}= 365)

NAT:

para-aminobenzoic acid (PABA)



(λ = 263 nm)

Enzyme activities of dermal systems

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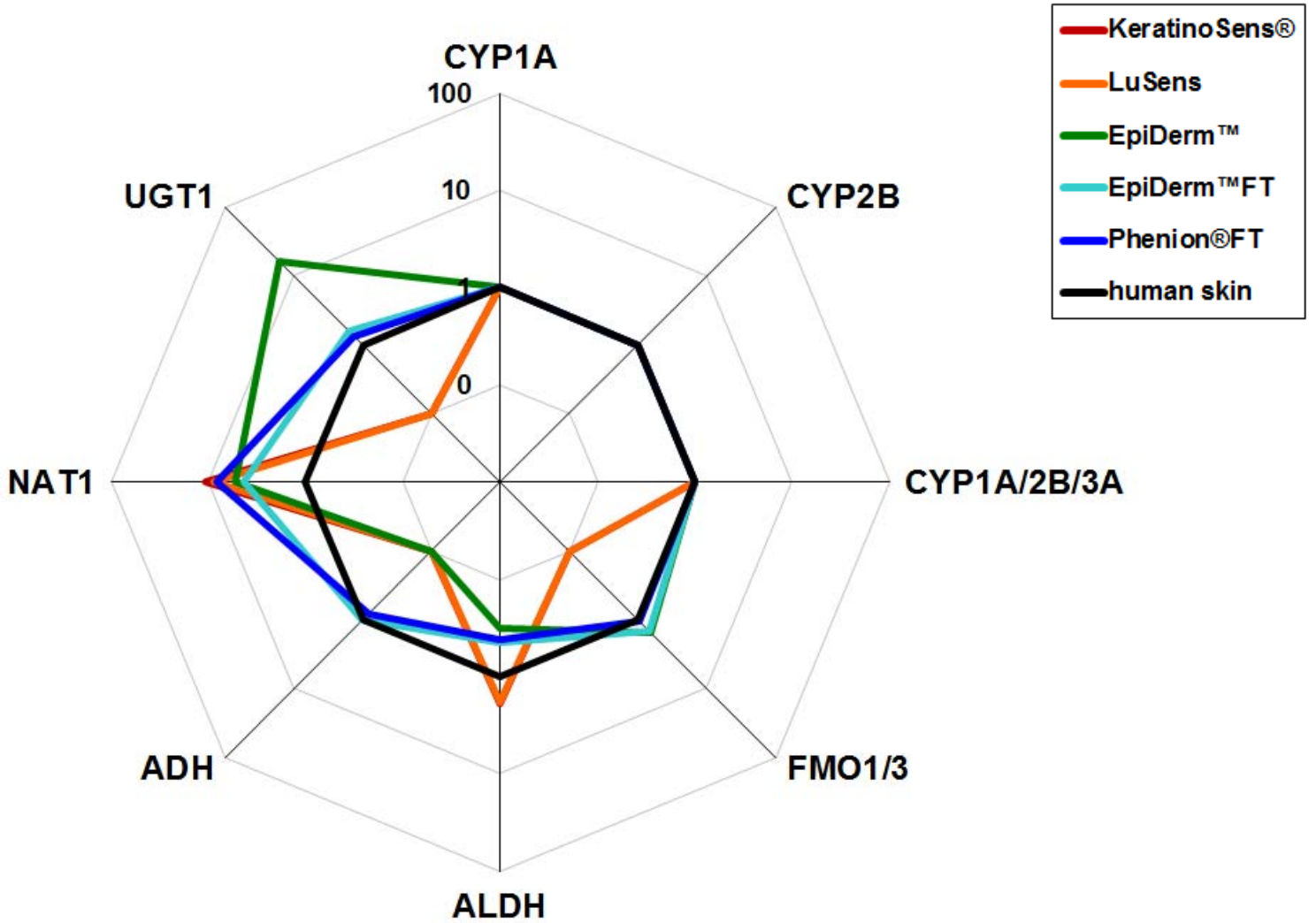
enzyme activities [nmol/min/mg protein]									
enzyme	subcellular fraction	LOD	Human Skin	Phenion®FT	EpiDerm™FT	EpiDerm™	Keratino Sens®	LuSens	rat liver
CYP1A	microsomes	0.001	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.03 +/- 0.012
CYP2B	microsomes	0.0025	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.02 +/- 0.002
CYP1A/CYP2B/CYP3A	microsomes	0.002	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.07 +/- 0.003
FMO1/ 3	microsomes	0.25	3.9 +/- 0.5	4.0 +/- 0.2	5.8 +/- 0.3	6.0 +/- 1.0	< LOD	< LOD	28.6 +/- 2.3
ALDH	cytosol	0.79	6.2 +/- 1.1	2.6 +/- 0.7	2.8 +/- 0.6	2.0 +/- 1.2	12.0 +/- 6.3	11.4 +/- 5.1	9.5 +/- 0.4
ADH	cytosol	1.57	9.2 +/- 1.2	7.5 +/- 0.9	9.1 +/- 5.6	< LOD	< LOD	< LOD	26.2 +/- 4.5
UGT1	microsomes	0.025	0.1 +/- 0.01	0.2 +/- 0.1	0.2 +/- 0.1	2.0 +/- 0.2	< LOD	< LOD	8.4 +/- 0.9
NAT1	S9	0.033	1.8 +/- 0.7	14.8 +/- 4.6	7.8 +/- 1.6	9.5 +/- 3.8	19.1 +/- 8.4	15.6 +/- 6.2	1.1 +/- 0.04

LOD = Limit Of Detection

Data published:
Jäckh et al., 2011
Henkler et al., 2011
Götz et al., 2012
Fabian et al., 2013

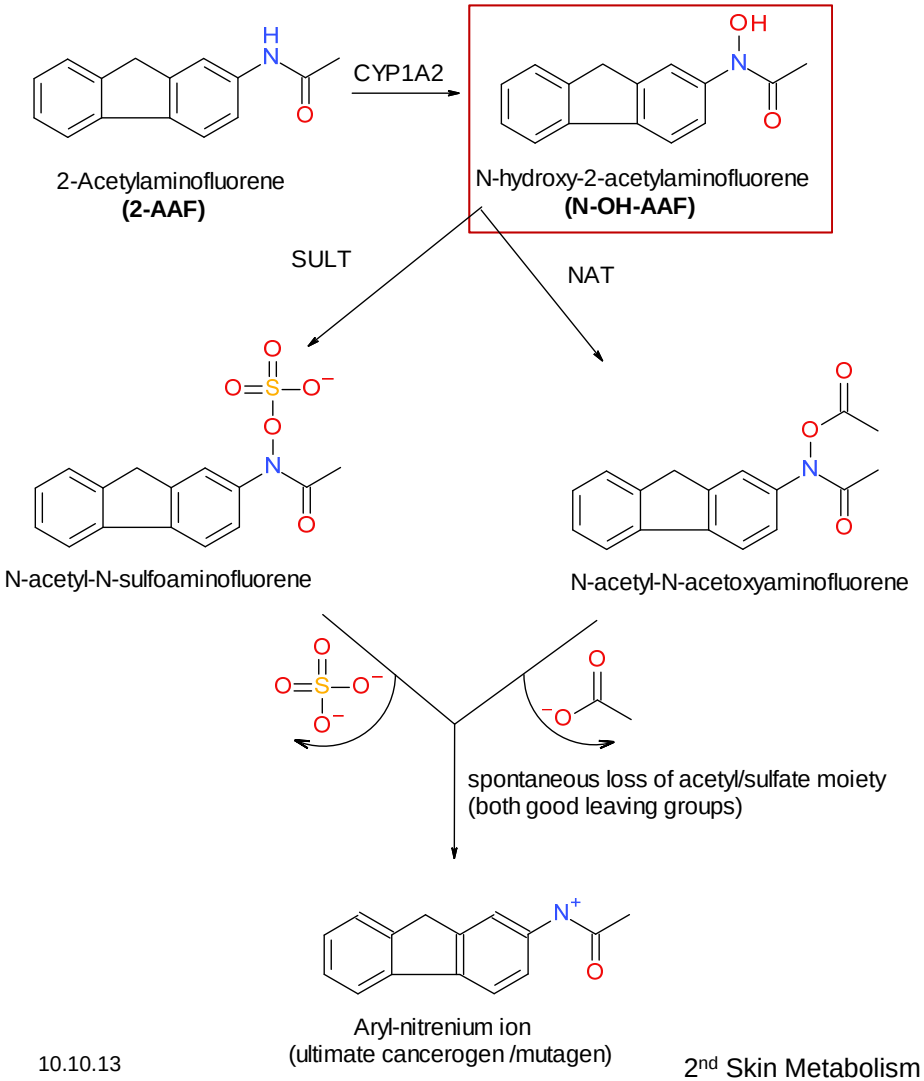
Enzymatic profile of dermal systems

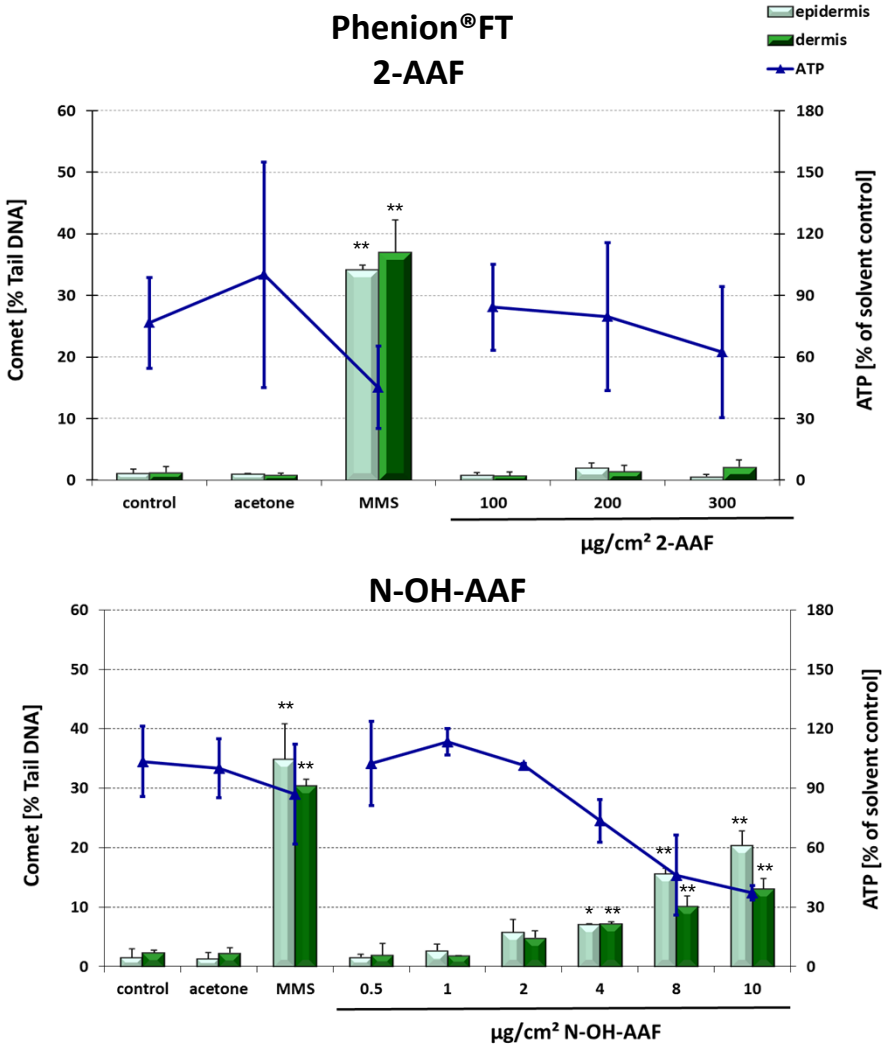


Question:

Can we use 3-D skin models for genotoxicity testing?

Comet Assay using 3-D skin models
Pro-mutagen: 2-AAF





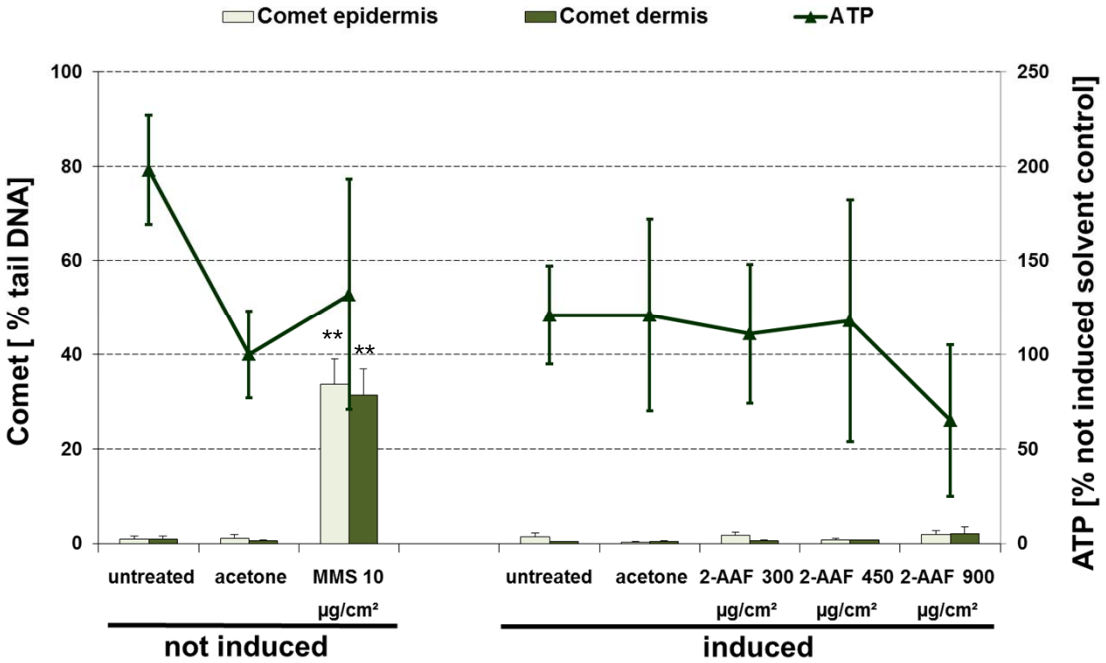
Answer:

Yes,

but the knowledge about the profiles of xenobiotic metabolizing enzymes (XME's) should be taken into account!

CYP induction

CYP induction cocktail					
substance	**conc. [µM]**	**induction pathway**	**CYP isoform**	**detection method**	**result**
ß-Naphthoflavone	10	AhR	CYP 1A	EROD	< LOD
Phenobarbitone	2000	CAR	CYP 2B	PROD	< LOD
Dexamethasone	0.1	PXR	CYP 3A	BROD	< LOD



Further inducers used:

- ✓ Aroclor 1254
  [10 – 100 µM]
  24 – 72 h
- ✓ ß-Naphthoflavone
  [25 – 200 µM]
  48 h
- ✓ 3-Methylcholanthrene
  [25 – 200 µM]
  48 h

➡ AROD activities below LOD

Summary and conclusions

- 3-D skin models mimic the metabolic profile of native human skin better than keratinocyte cell lines
- Full-thickness skin models are closer to human skin than epidermis skin model
- Comet Assay is an appropriate test method for detection of DNA damage using 3-D skin models

The profiles of XME's should be considered for data assessment of pro-mutagens

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Thank you for your attention!