

Skin equivalents – how do they contribute to skin metabolism and differ to ex vivo skin?

Skin Forum Skin Metabolism 20th May 2011, Edinburgh (UK)
Christine Jäckh

Keeping in mind the proposed breakout topics: Are we doing it right?

How do we answer qualitative questions?

How do we answer quantitative questions?

How do we assess metabolic "competence" and positive controls?

3D models and their utility ?

How useful are in vitro animal models in predicting human metabolism?

What is the future? What do we need to increase our confidence in the data?

How useful are in vitro animal models compared to human skin?

Native human skin



cornified cell layer

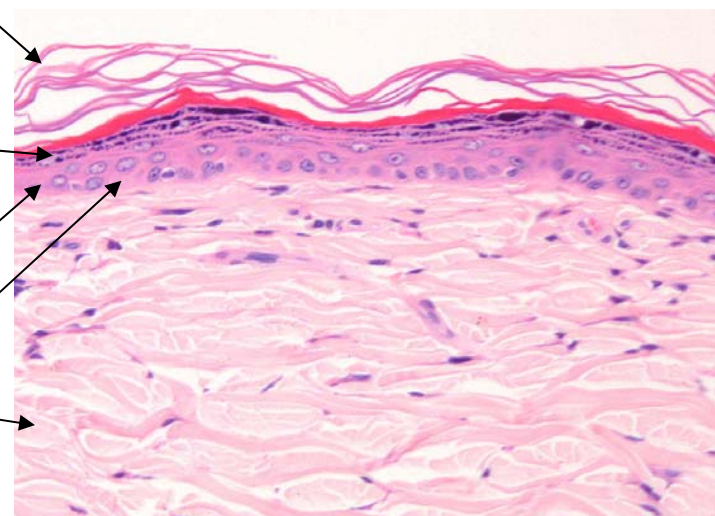
granular cell layer

spinous cell layer

basal cell layer

dermis

Native rat skin



Gröters (BASF)

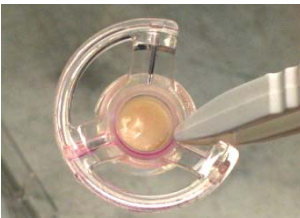
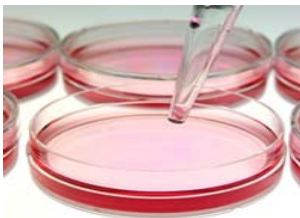
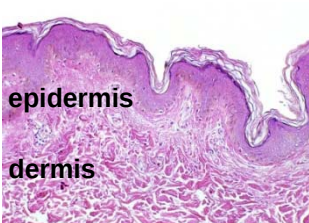
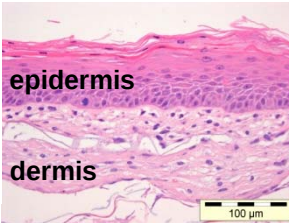
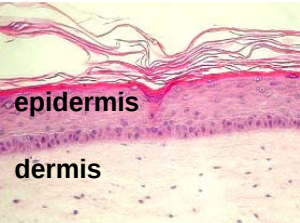
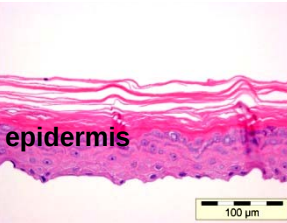
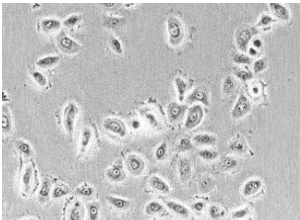
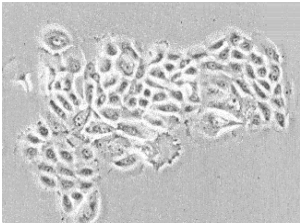
In vitro systems

- + human like tissue
- + human gene expression
- + standardized
- + human like metabolism
- Regulatory acceptance

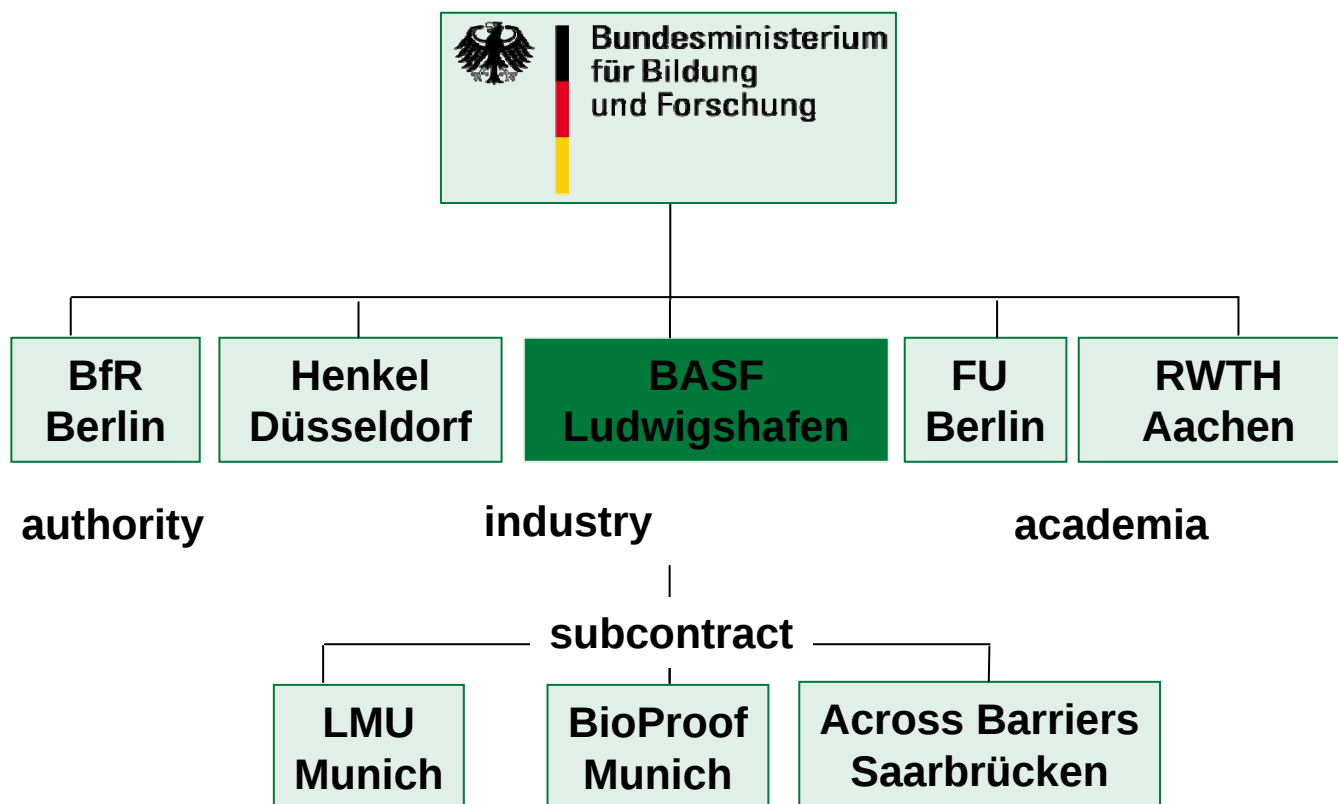
Species difference

- Barrier difference
- morphological differences
- metabolism
- + large database on metabolic profiles of test compounds from in vivo studies

Human 3D models and their utility ?

native human skin	Phenion® FT (Henkel)	EpiDermFT™ (MatTek)	EpiDerm™ (MatTek)	Monolayer cell cultures
				
				
				
D: ~ 0.5 mm A: 1.7 cm ² Ø: 0.7 cm adult abdominal	D: ~ 2.0 mm A: 1.3 cm ² Ø: 1.3 cm neon. foreskin	D: ~ 1.0 mm A: 1.0 cm ² Ø: 1.2 cm neon. foreskin	D: ~ 0.5 mm A: 0.6 cm ² Ø: 0.9 cm neon. foreskin	Keratinocytes Fibroblasts

BMBF- funded project:
„Characterization of the metabolic capacity of *in vitro* skin models in order to identify an optimal model for skin toxicology assays as well as for higher tier assessment of exposition of substances with dermal biotransformation.“



BASF:
 Robert Landsiedel
 Eric Fabian
 Christine Jäckh
 Veronika Blatz
 Katharina Guth

BfR:
 Andreas Luch
 Frank Henkler
 Joseph Brinkmann
 Susanne Trappe
 Manfred Liebsch

FUB:
 Monika Schäfer-Korting
 Günter Weindl
 Wiebke Klipper
 Franziska Bätzer

Henkel:
 Kerstin Reisinger
 Selin Kanamkudam

RWTH Aachen:
 Hans F. Merk

Characterization of enzyme activities of Cytochrome P450 enzymes, Flavin-dependent monooxygenases, N-Acetyltransferases and UDP-Glucuronyltransferases in human reconstructed epidermis and full-thickness skin models.
 Jäckh C, Blatz V, Fabian E, Guth K, van Ravenzwaay B, Reisinger K, Landsiedel R.; *Toxicol In Vitro*. 2011

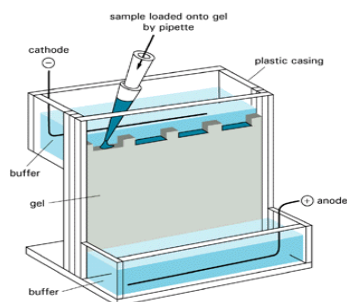
How do we assess metabolic "competence" and positive controls?

Gene expression

RNA

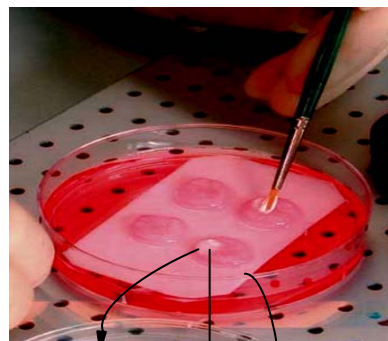


Protein

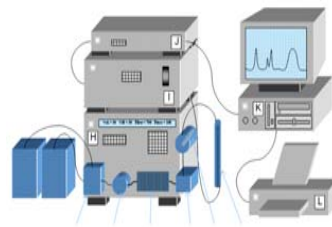


Biotransformation of test substances

topic

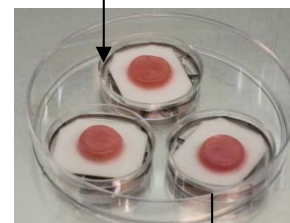


wash
skin receptor

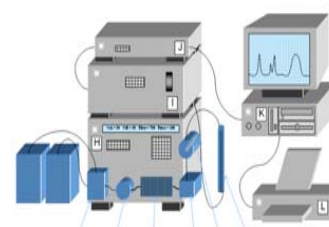


systemic

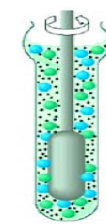
model substrate
into medium



medium for
metabolite detection



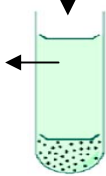
fractions



homogenization
• potter
• ultrasound

S9

9.000 x g
15 min

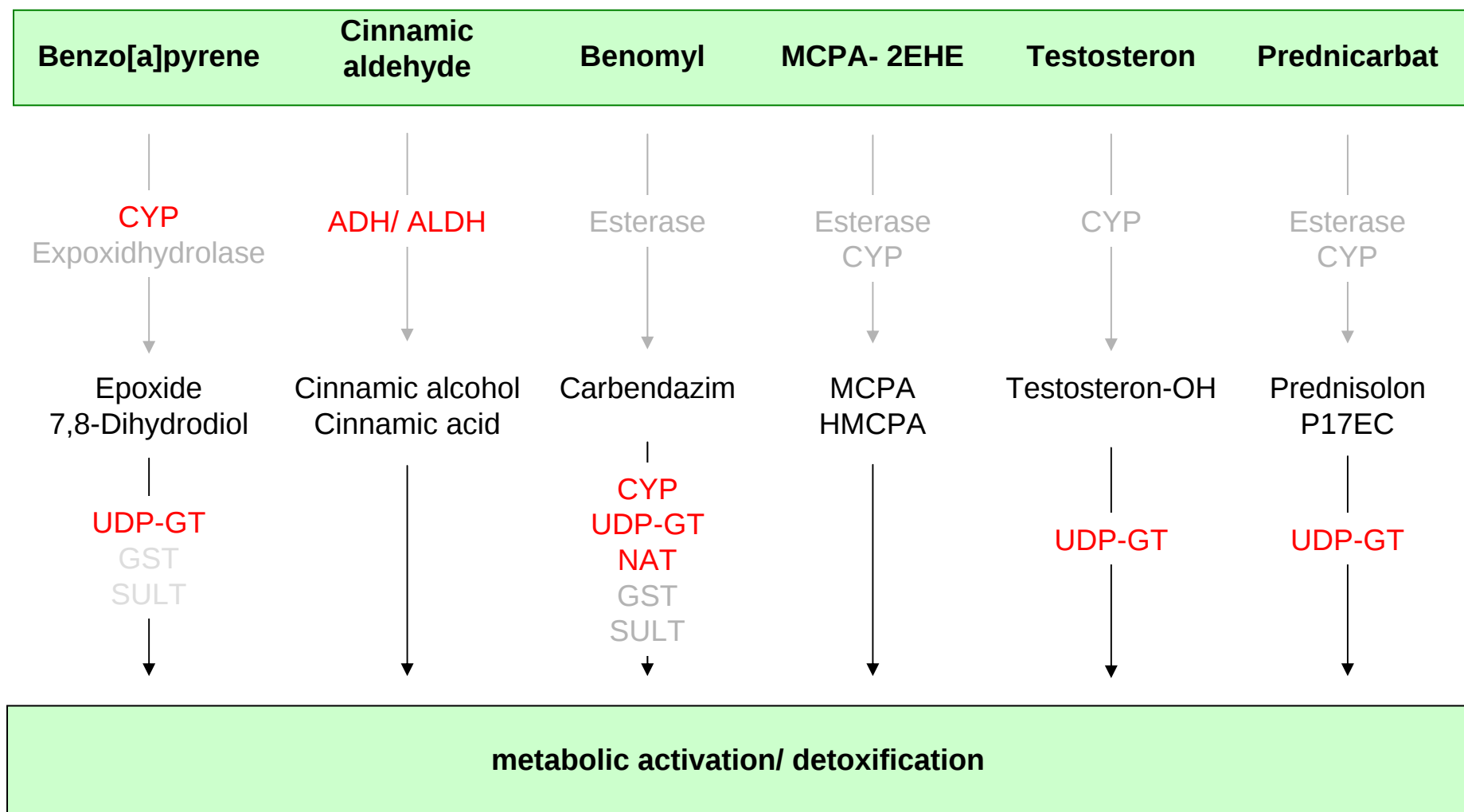


cytosol

100.000 x g
2 x 60 min

microsomes

Enzyme selection according to biotransformation of selected test substances



Enzyme expression is compartment specific and is decreasing with the complexity of the system

transcription										
	enzymes	native skin		EpiDerm	PhenionFT		EpidermFT		Keratinocytes	Fibroblasts
		Epidermis	Dermis		Epidermis	Dermis	Epidermis	Dermis		
RWTH Aachen*	CYP1A1	+		-	+		-		+	+
	CYP1B1	++		+	+		+		++	++
	CYP3A4	++		+	+		++		-	-
	CYP2A6	+		+	+		n.a.		-	-
	CYP2B6	-		-	-		-		-	-
	CYP2C8	+		+	+		+		n.a.	n.a.
	CYP2C9	+		+	+		+		n.a.	n.a.
	CYP2C19	+		+	+		+		n.a.	n.a.
	CYP2D6	-		+	+		-		n.a.	n.a.
	CYP2E1	++		+	+		n.a.		+	+
	CYP3A4	++		+	+		++		-	-
Henkel*	FMO1	++/+++	++/++	+/+	+/+	++/++	+/-	+/+	-/-	+/+
	FMO3	-/-	++/++	-/-	-/-	+/++	-/-	+/+	-/-	+/+
	NAT 1	++/+++	++/++	++/++	++/++	++/++	+/+	+/+	+/++	+/++
	UGT	++/++	++/+++	++/+++	++/+++	++/++	n.a.	n.a.	++/++	-/-
	ADH1A	-/-	-/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	ADH1B	-/-	++/++	-/-	-/-	++/++	-/-	++/++	-/+	+/+
	ADH1C	-/-	++/+	-/-	-/-	+/+	-/-	+/+	-/-	+/+
	ALDH1A1	++/+++	++/+++	+/+	++/++	++/++	+/+	++/++	+/+	++/+++
	ALDH2	++/+++	++/++	++/+++	++/++	+/+	+/+	++/++	+/+	+/+

*K. Reisinger (Henkel); H.F. Merk (RWTH Aachen)

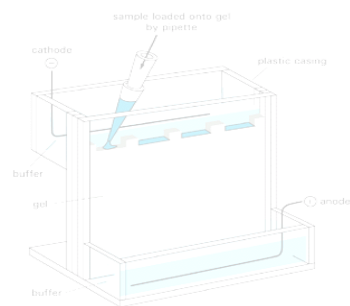
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Gene expression

RNA

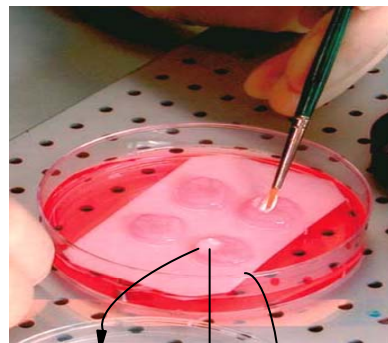


Protein

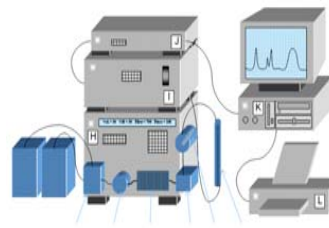


Biotransformation of test substances

topic

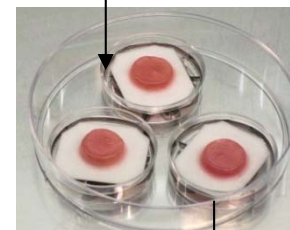


wash
skin receptor

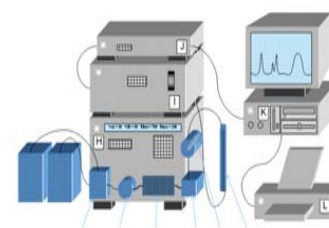


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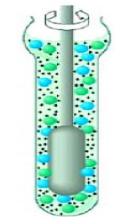
model substrate
into medium



medium for
metabolite detection



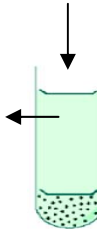
fractions



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S9

9.000 x g
15 min

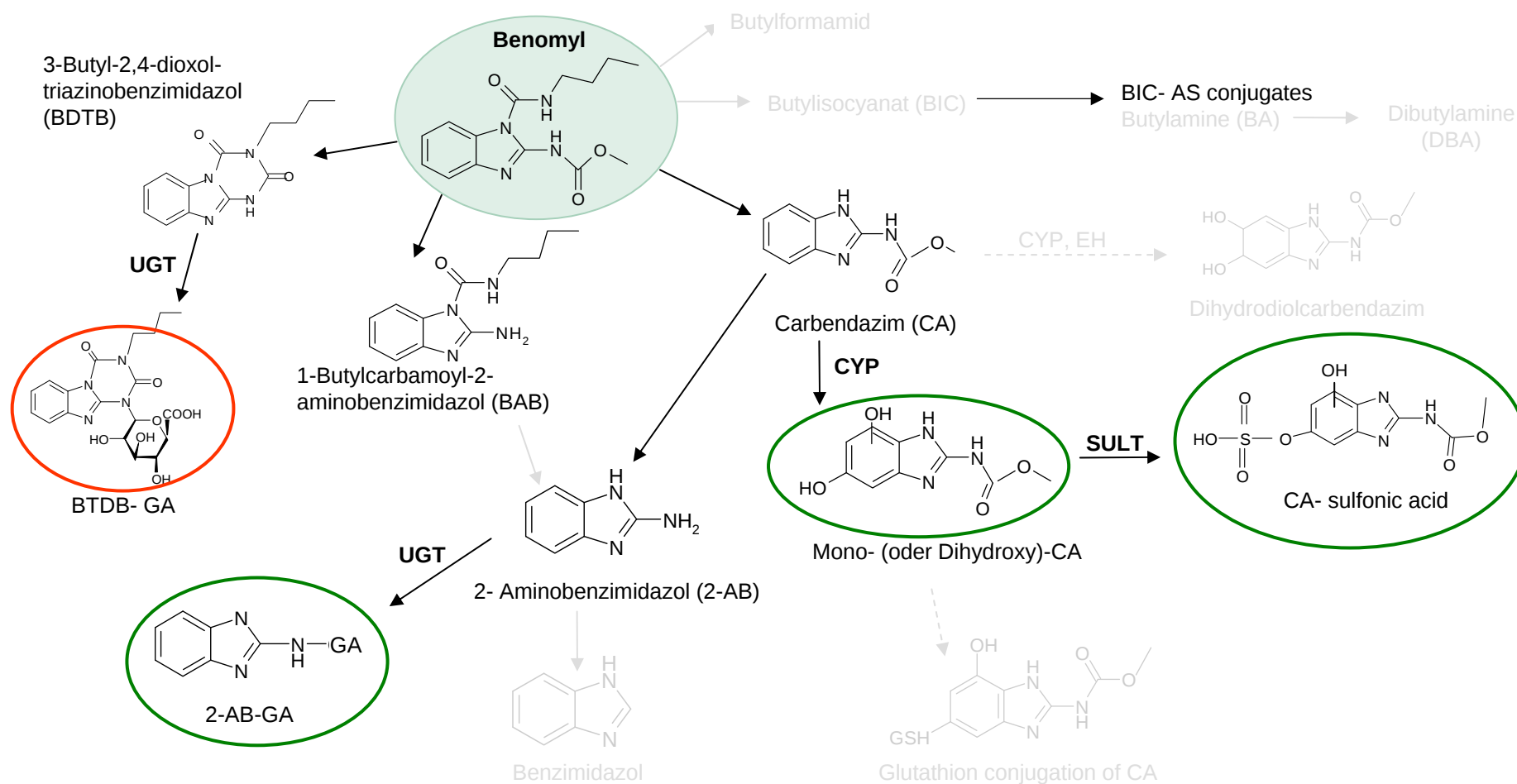


cytosol

100.000 x g
2 x 60 min

microsomes

Biotransformation of Benomyl reveals low hydroxylating potency in skin



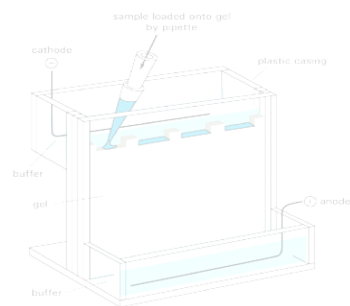
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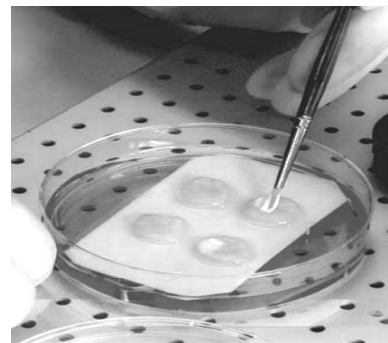


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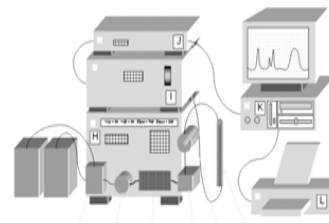
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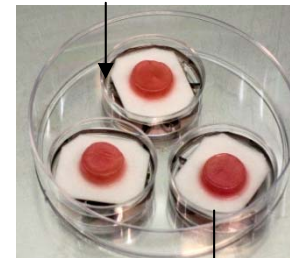
wash

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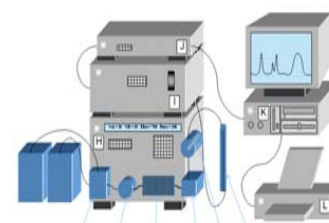


systemic

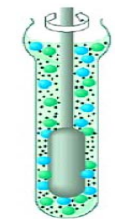
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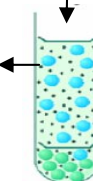


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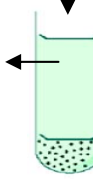
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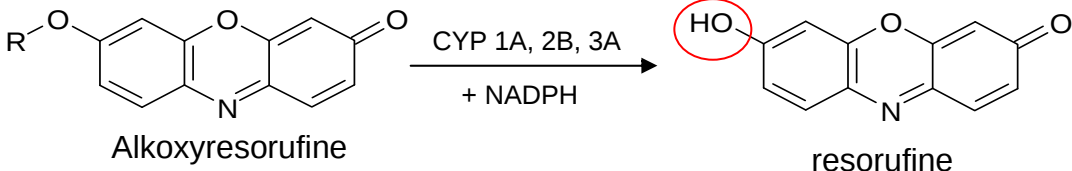
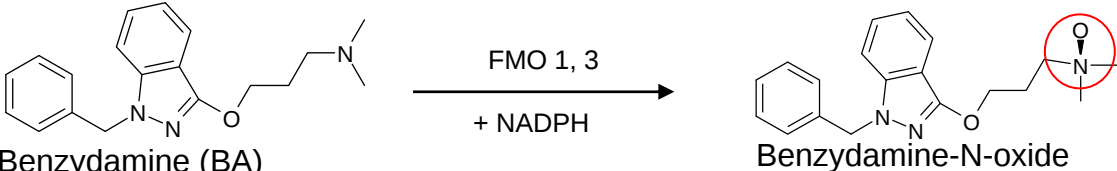
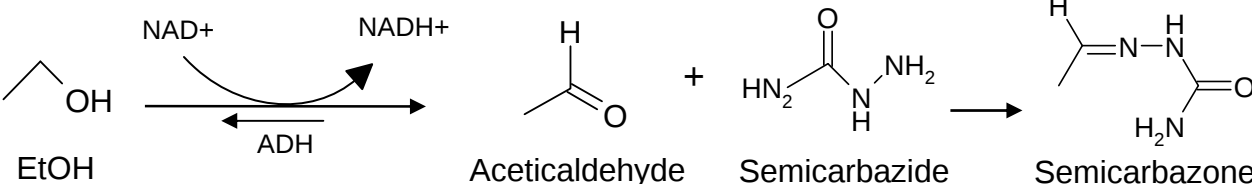
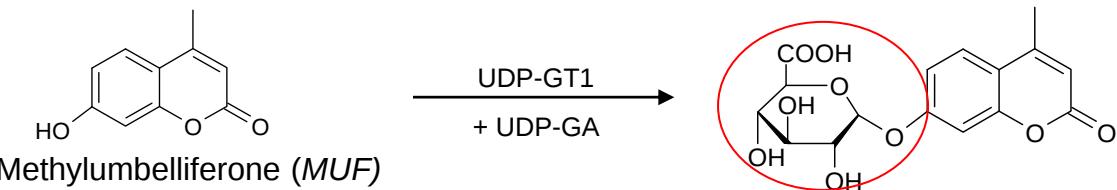
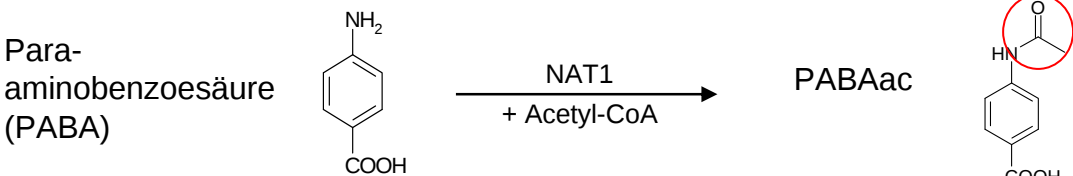


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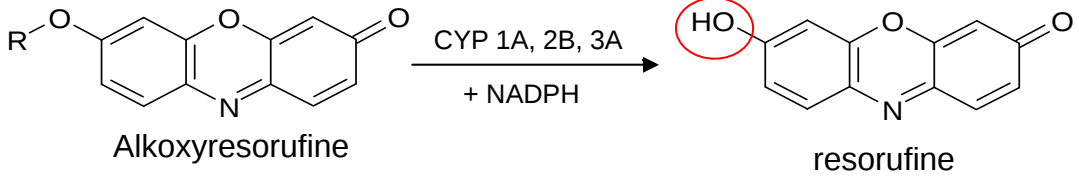
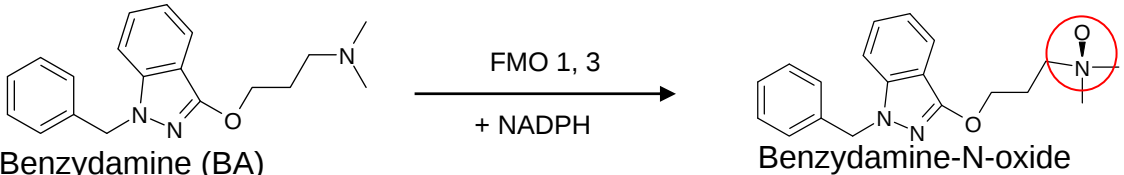
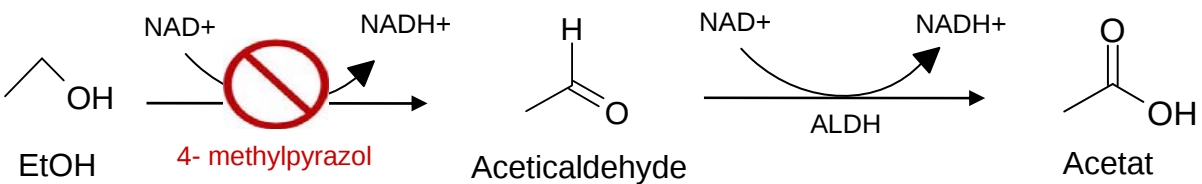
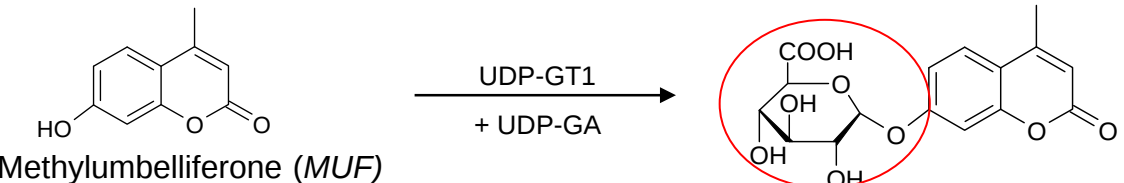
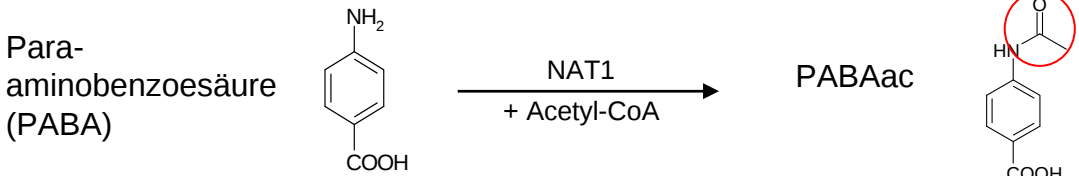
How do we assess metabolic "competence"?

Incubation of model substrates in subcellular fractions

CYPs:	 Alkoxyresorufine resorufine	Fluorescence ($\lambda_{\text{ex}} = 550$; $\lambda_{\text{em}} = 585$)
FMO:	 Benzydamine (BA) Benzydamine-N-oxide	HPLC- Fluorescence ($\lambda_{\text{ex}}:305$; $\lambda_{\text{em}}: 375$)
ADH ALDH:	 EtOH Acetaldehyde Semicarbazide Semicarbazone	NADH ⁺ Absorption (340 nm)
UDP- GT:	 Methylumbelliferone (MUF)	Fluorescence ($\lambda_{\text{ex}}:315$; $\lambda_{\text{em}}: 365$)
NAT:	 Para-aminobenzoic acid (PABA) PABAac	HPLC- UV (263 nm)

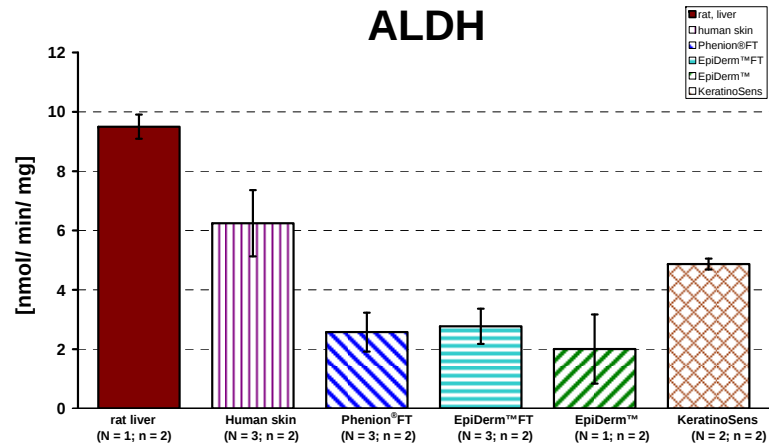
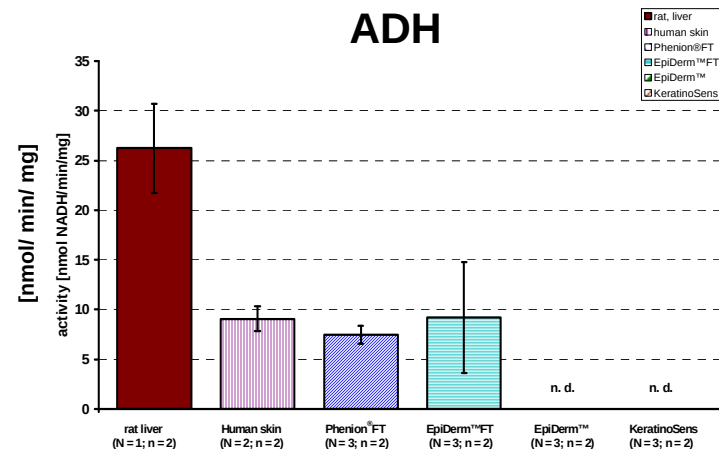
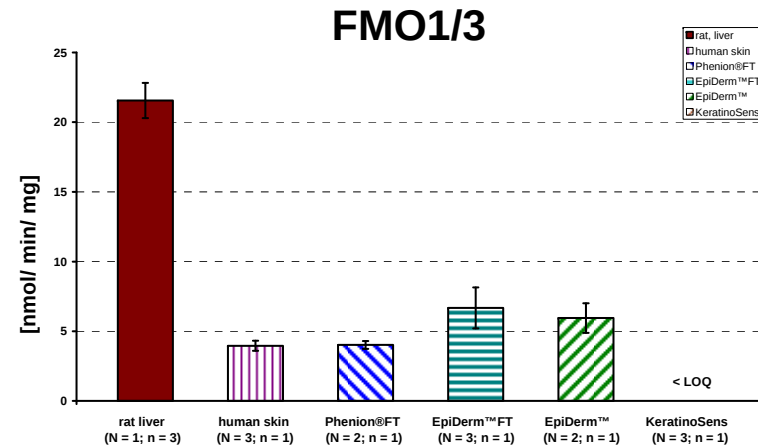
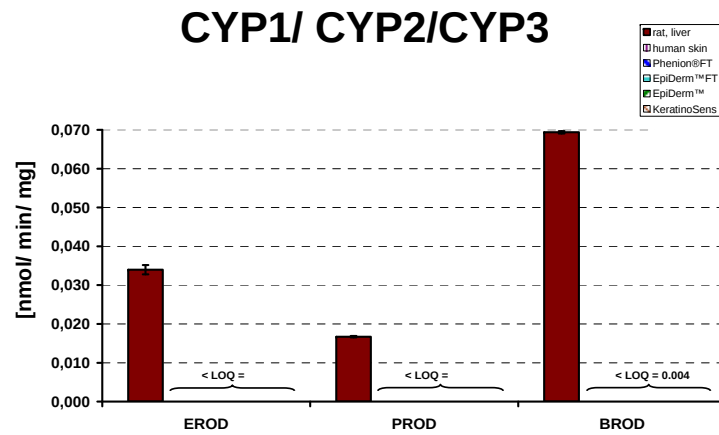
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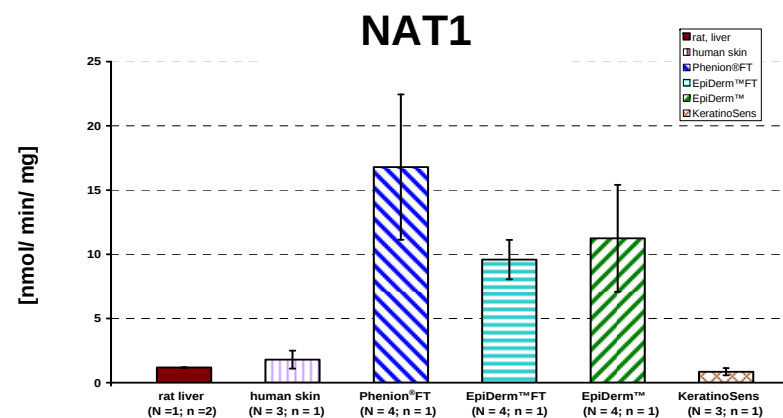
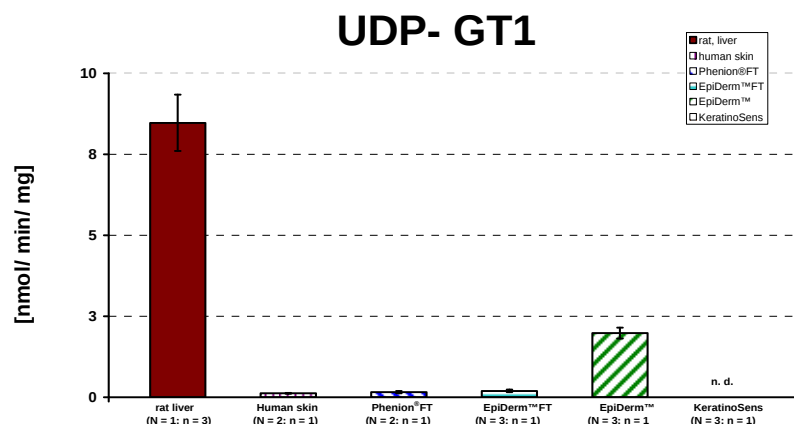
How do we assess metabolic "competence" ?

Results subcellular fractions



How do we assess metabolic "competence" ?

Results subcellular fractions



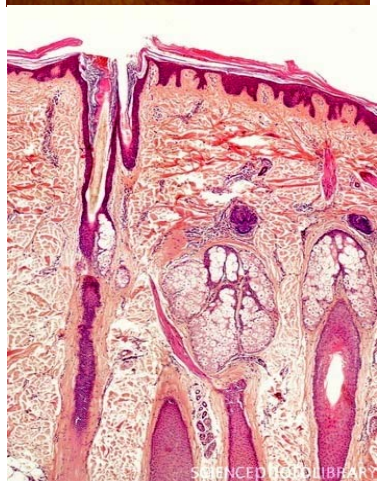
Summary and Conclusion

- Metabolic competence of the skin differs from the hepatic system
- Enzyme expression differs within epidermis and dermis
- Enzyme expression is decreasing with the complexity of the in vitro system
- Lack of hydroxylated metabolites and undetectable EROD/ PROD/ BROD activity indicates low basal CYP enzyme activity in the skin
- Full-thickness skin models demonstrate ADH, ALDH, FMO, UDP-GT1 and NAT1 enzyme activity which is comparable to native human skin

Full-thickness skin models represent an interesting alternative to native human skin for toxicological testing

What is the future?

What do we need to increase our confidence in the data?



Native human skin is more complex than 3D models

- Sebaceous glands and follicle cells possess higher biotransforming capacities than keratinocytes (Coomes et al., 1984; Finnen et al., 1984; Baron et al., 1988)
- Improvement of enzyme assay sensitivity
- Measurement of biotransformation of more test compounds in comparison to native human skin
- Analysis of inductive effects of test compounds

Final read out

Prove of biotransformation by testing toxicological endpoints