Update in the Characterization of Metabolizing Enzymes in Human Skin and Reconstructed Human Skin Models from SkinEthic™

Joan Eilstein

ADMET - Metabolism
Predictive Methods and Models Development Department
L'OREAL Advanced Research

2nd Skin metabolism Meting 2013
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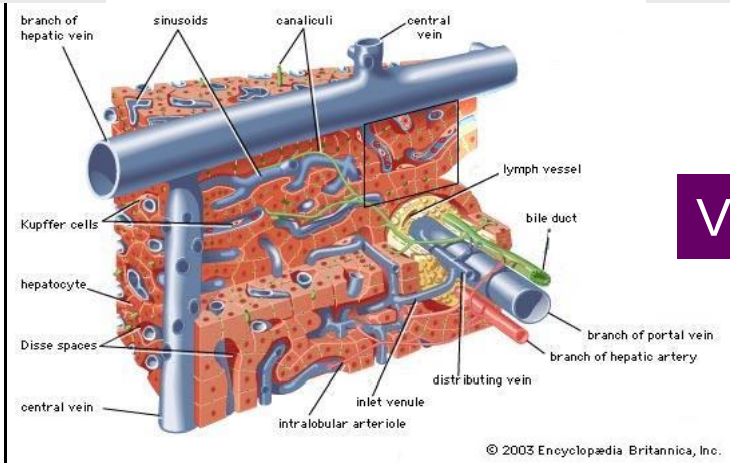
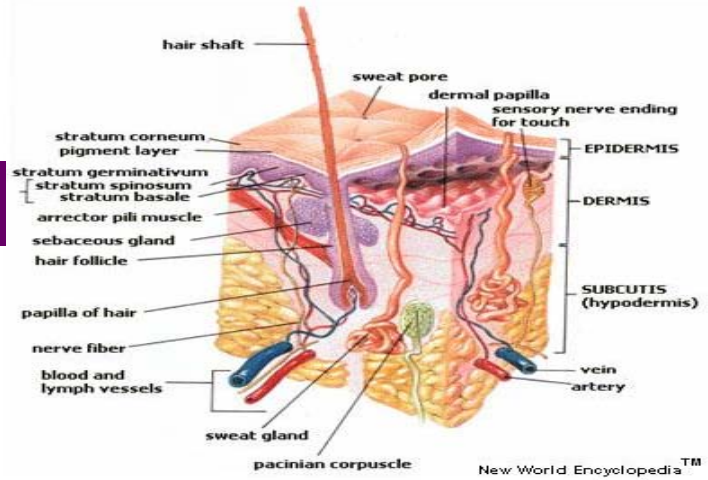
L'ORÉAL
Research & Innovation

Cutaneous metabolism
Generalities

	LIVER	SKIN
		
Main cells type	**Hepatocytes**	**Keratinocytes**
Main functions	**Endogenous & Drug metabolisms**	**Barrier function, thermoregulation…**
Weight	**2% body weight**	**15% body weight, 2 m^2**

VS

Characterization of the skin metabolic capabilities

Cutaneous metabolism
Regulatory context

REACh
Chemicals characterization

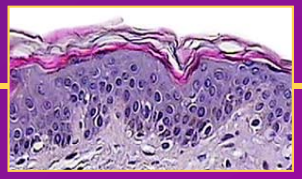
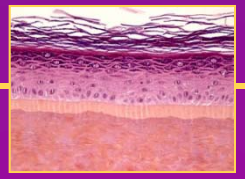
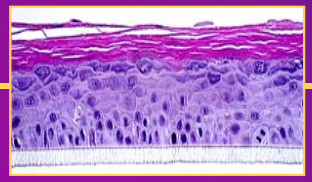
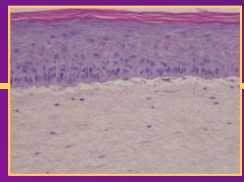
&

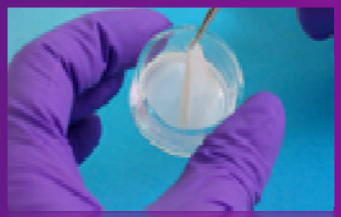
7th Amendment to the Cosmetic Directive
Non animal approaches

***In vitro* assays development**

Use of reconstructed human skin models

Cutaneous metabolism
Reconstructed Human Skin



SkinEthic laboratories

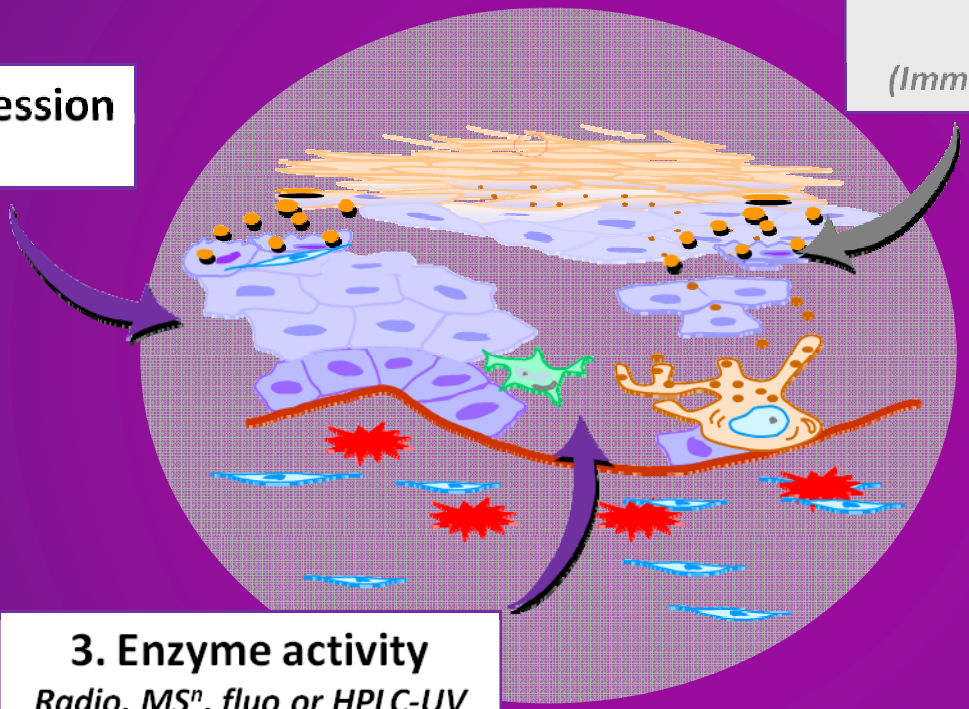
TYPE	ORIGIN
Normal Human Skin (NHS): *Epidermis/Dermis (BIOPREDIC)*	**Mammoplasties**
Episkin™: *Reconstructed human epidermis*	**NHK (mammoplasties)** **Pool of 4 – 5 donors** Support: BPER
SkinEthic™ RHE: *Reconstructed human epidermis*	**NHK (foreskin/abdo)** **1 donor/Pool of 2 donors** Support: Polycarbonate
Full thickness of Episkin™: *Reconstructed human epidermis / equivalent dermis*	**NHK (mammoplasties)** **Pool of 4 – 5 donors** Support: Polycarbonate

Comparison of metabolic capabilities between normal human skin and models

Cutaneous metabolism
Develop Approach

1. Gene expression
(RT-PCR)

2. Protein expression
(Immuno histochemistry) (Western blots)



3. Enzyme activity
Radio, MS^n, fluo or HPLC-UV

: Fluorogenic radiolabelled or UV visible absorbing substrates

Cutaneous metabolism

Expression profiles of phase 1 and 2 metabolizing enzymes

J Steroid Biochem Mol Biol. 2009 Sep;116(3-5):178-86

Expression profiles of phases 1 and 2 metabolizing enzymes in human skin and the reconstructed skin models Episkin™ and full thickness model from Episkin™

Van Luu-The[a,*], Daniel Duche[b], Corinne Ferraris[b], Jean-Roch Meunier[b], Jacques Leclaire[b], Fernand Labrie[a]

++++> 1 million copies/µg total RNA.
+++> 500,000 copies/µg total RNA.
++> 200,000 copies/µg total RNA.
+> 50,000 copies/µg total RNA.
(+)> 10,000 copies/µg total RNA.
(+/−)> 5000 copies/µg total RNA.

Phase I (functionalisation)

Gene codes	Total human skin	Human dermis	Human epidermis	Episkin™	FTM	Physiological functions
CYP4B1	+	(+)	+	+	(+)	Arylamine N-hydroxylation, Lauric acid ω-hydroxylation
CYP26B1	+	+	+	(+/−)	(+)	Retinoic acid metabolism
CYP39A1	+	(+)	+	(+)	(+)	24-Hydroxy cholesterol 7α-hydroxylation
CYP2J2	+	(+)	+	(+)	+	Arachidonic acid metabolism, cis-epoxy-eicosatrienoic acid
CYP4F8	+	+	(+/−)	(+)	(+/−)	Prostaglandin H1&2 19-hydroxylation
CYP4F12	+	(+)	+	(+)	+	Arachidonic acid oxidation to 18-hydroxy arachidonic acid
CYP27A1	+	+	(+)	(+/−)	(+/−)	Cholesterol 27-hydroxylation Vitamin D3 25-hydroxylation
CYP27B1	(+/−)	(+/−)	(+/−)	(+)	+	25-Hydroxy vitamin D3 1α-hydroxylation
CYP7B1	(+)	(+)	(+)	(+)	(+)	27-Hydroxy cholesterol & DHEA 7α-hydroxylation
CYP2B6/2D/2E1/1A1/1B1/2C8/2C18/2F1/3A5	(+)	(+)	(+)	(+)	(+)	7-Ethoxy-4-trifluoromethyl coumarin-O-deethylation
CYP2C9/1A2/3A7	(+/−)	(+/−)	(+/−)	(+/−)	(+/−)	

Gene codes	Total human skin	Human dermis	Human epidermis	Episkin™	FTM	Physiological functions
ADH1B	++++	++++	–	–	–	Alcohol oxidation into aldehyde Retinol oxidation into retinal
DRHS8 or 17b-HSD 11	+	++	(+)	(+)	(+)	5a-androstane-3a, 17b-diol oxidation into Androsterone
EPHX1	++	+++	+	+	+	Arene & aliphatic epoxide hydrolysis
EPHX2	++	+	++	(+)	+	
HADH2	++	++	++	+++	+++	3-Hydroxybutyryl-CoA mitochondrial b-oxidation
AKR1C2 or 3a-HSD	+	+	(+)	+++	++	DHT reduction into 3-diol
AKR1C1or 20a-HSD	+	+	(+)	+++	++	Progesterone reduction into 20a-hydroxyprogesterone
FMO1	(+)	(+/−)	(+)	–	(+/−)	N-oxidation of secondary & tertiary amines
FMO2	(+)	(+)	(+/−)	(+)	+	
FMO3	(+)	(+)	(+/−)	–	–	
FMO4	(+)	(+)	(+)	(+)	(+)	
FMO5	(+)	(+)	(+)	(+/−)	(+)	
STS	(+)	(+)	(+)	(+)	(+)	Cholesterol, DHEA & estrone sulfates hydrolysis
NOS1	(+/−)	(+/−)	(+)	(+/−)	(+/−)	
NOS2A/ADH7/AKR1C4	(+/−)	(+/−)	(+/−)	(+/−)	(+/−)	

Phase II (conjugation)

Gene codes	Total human skin	Human dermis	Human epidermis	Episkin™	FTM	Physiological functions
GSTP1	++++	++++	++++	++++	++++	Reduced glutathione conjugation to hydropobic electrophiles
GSTT1	++	++	++	+	++	
GSTM5	+	++	(+)	–	–	
SULT2B1b	+++	+	++++	++++	++++	Cholesterol & DHEA sulfation
SULT1A1	++	++	(+)	(+)	+	Phenol & catecholamine sulfations
SULT1E1	(+)	(+/−)	(+)	(+)	(+)	Other sulfotransferase isoforms
SULT1B1/2A1	–	–	–	–	–	
COMT	++	++	++	+++	++++	Catechol methylation
NAT5	+	+	+	+	+	Protein N-acetylation
NAT1	(+)	(+)	(+)	(+)	(+)	N-acetylation
NAT2	–	–	–	–	–	
UGT2B28/2B4/1A1/2B17/2B15	(+/−)	(+/−)	(+/−)	(+/−)	(+/−)	Glucuronosyl conjugation

Cutaneous metabolism

Expression profiles of phase 1 and 2 metabolizing enzymes

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Phase I (functionalisation)

Gene codes	Total human skin	Human dermis	Human epidermis	Episkin™	FTM
CYP4B1	+	(+)	+	+	(+)
CYP26B1	+	+	+	(+/−)	(+)
CYP39A1	+	(+)	+	(+)	(+)
CYP2J2	+	(+)	+	(+)	+
CYP4F8	+	+	(+/−)	(+)	(+/−)
CYP4F12	+	(+)	+	(+)	+
CYP27A1	+	+	(+)	(+/−)	(+/−)
CYP27B1	(+/−)	(+/−)	(+/−)	(+)	+
CYP7B1	(+)	(+)	(+)	(+)	(+)
CYP2B6/2D/2E1/1A1/1B1/ 2C8/2C18/2F1/3A5	(+)	(+)	(+)	(+)	(+)
CYP2C9/1A2/3A7	(+/−)	(+/−)	(+/−)	(+/−)	(+/−)

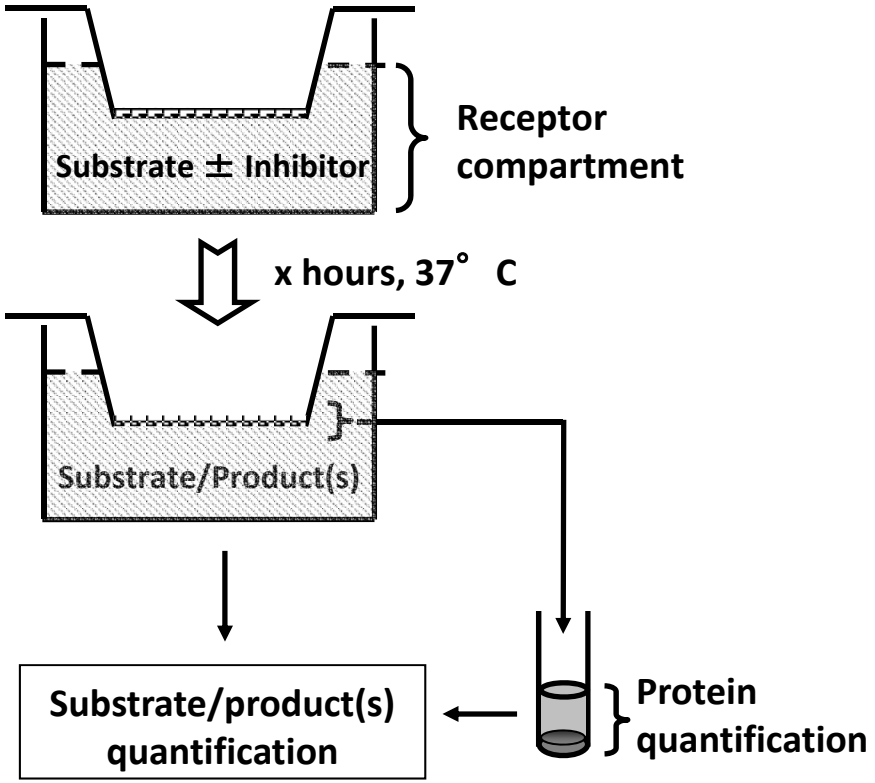
Gene codes	Total human skin	Human dermis	Human epidermis	Episkin™	FTM
ADH1B	++++	++++	−	−	−
DRHS8 or 17b-HSD 11	+	++	(+)	(+)	(+)
EPHX1	++	+++	+	+	+
EPHX2	++	+	++	(+)	+
HADH2	++	++	++	+++	+++
AKR1C2 or 3a-HSD	+	+	(+)	+++	++
AKR1C1or 20a-HSD	+	+	(+)	+++	++
FMO1	(+)	(+/−)	(+)	−	(+/−)
FMO2	(+)	(+)	(+/−)	(+)	+
FMO3	(+)	(+)	(+/−)	−	−
FMO4	(+)	(+)	(+)	(+)	(+)
FMO5	(+)	(+)	(+)	(+/−)	(+)
STS	(+)	(+)	(+)	(+)	(+)
NOS1	(+/−)	(+/−)	(+)	(+/−)	(+/−)
NOS2A/ADH7/AKR1C4	(+/−)	(+/−)	(+/−)	(+/−)	(+/−)

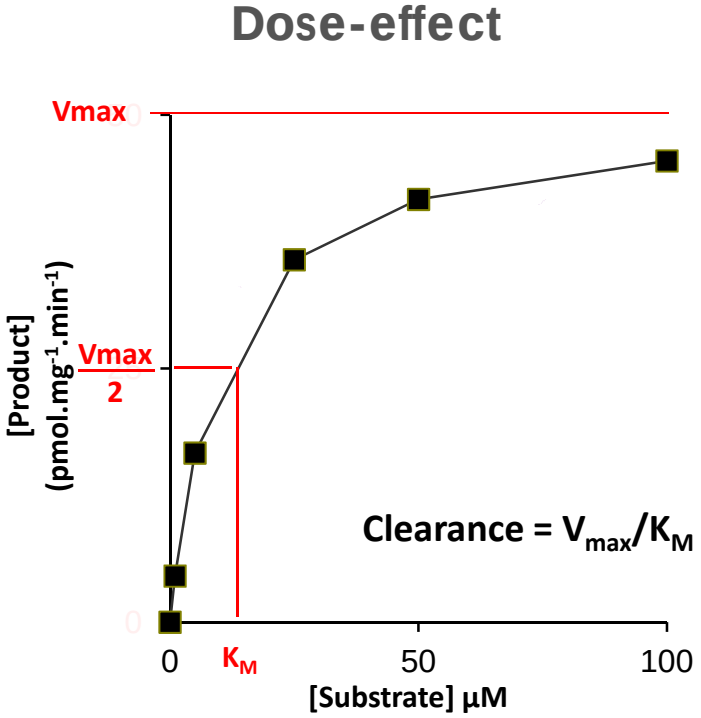
Phase II (conjugation)

Gene codes	Total human skin	Human dermis	Human epidermis	Episkin™	FTM
GSTP1	++++	++++	++++	++++	++++
GSTT1	++	++	++	+	++
GSTM5	+	++	(+)	−	−
SULT2B1b	+++	+	++++	++++	++++
SULT1A1	++	++	(+)	(+)	+
SULT1E1	(+)	(+/−)	(+)	(+)	(+)
SULT1B1/2A1	−	−	−	−	−
COMT	++	++	++	+++	++++
NAT5	+	+	+	+	+
NAT1	(+)	(+)	(+)	(+)	(+)
NAT2	−	−	−	−	−
UGT2B28/2B4/1A1/2B17/2B15	(+/−)	(+/−)	(+/−)	(+/−)	(+/−)

Cutaneous metabolism

Activity studies – General procedure





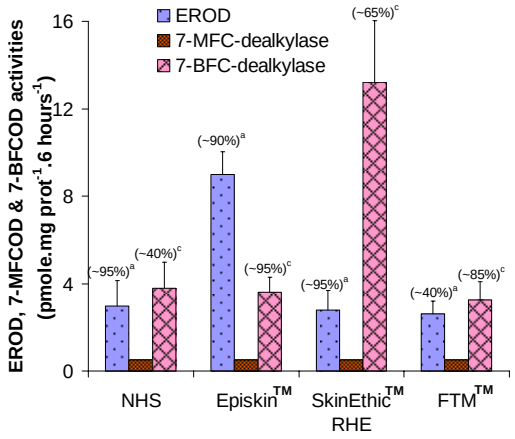
Apparent enzymatic parameters

1. Time-course study: Incubation time determination
2. Dose-effect study: Apparent enzymatic parameters → K_m, V_{max} and V_{max}/K_m ratio

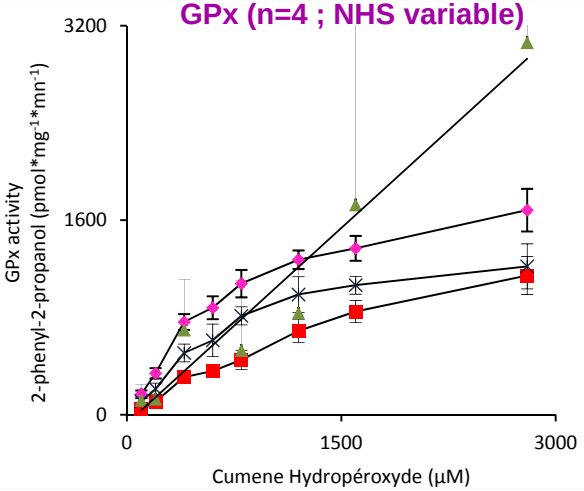
Cutaneous metabolism

Phase I Metabolizing Enzyme Activities

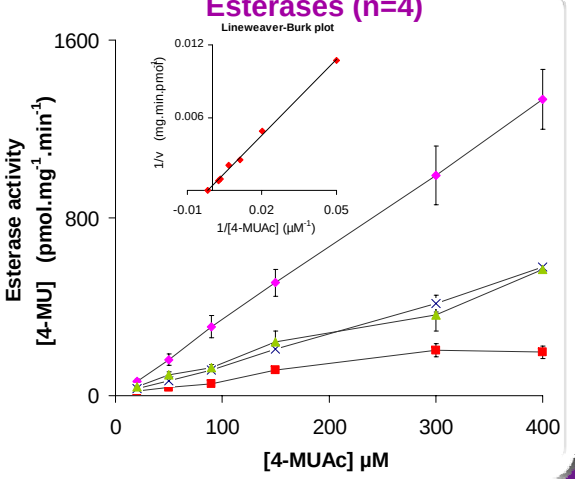
CYP450 (n=4)



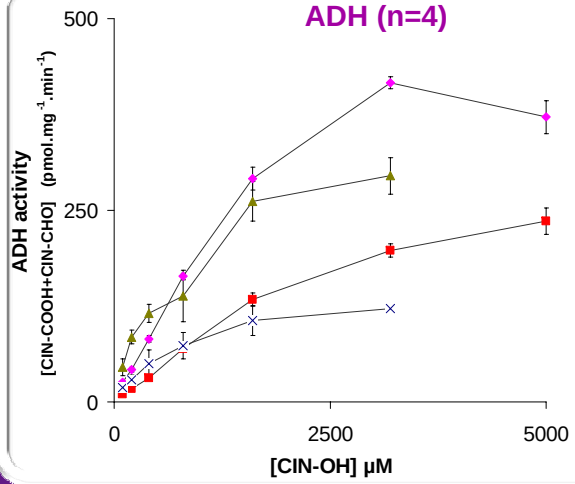
GPx (n=4 ; NHS variable)



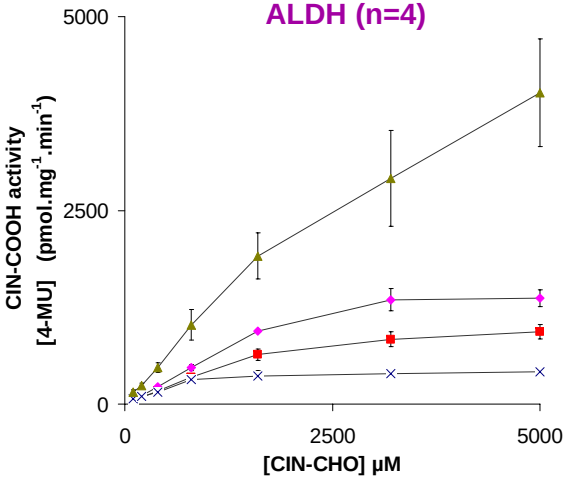
Esterases (n=4)



ADH (n=4)



ALDH (n=4)

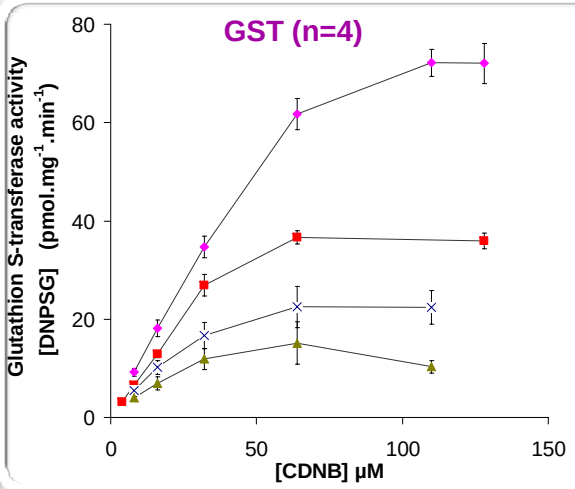


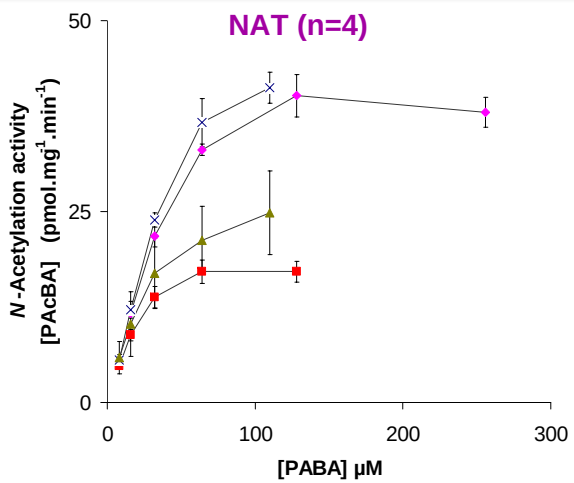
▲ : NHS
■ : Episkin™
X : SkinEthic™ RHE
◆ : FTM

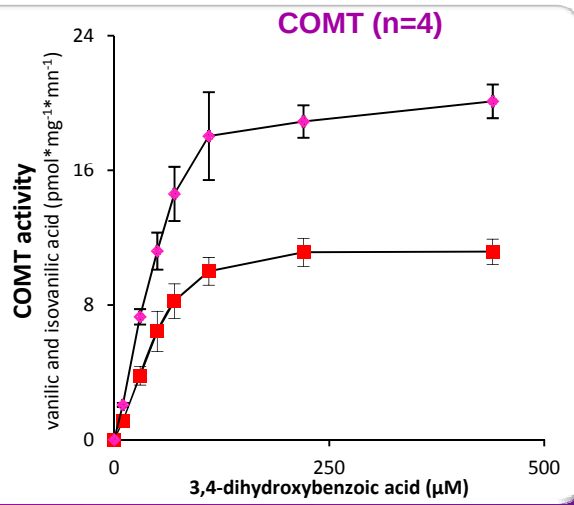
Normal Human Skin vs Models: Km_{app} and $Vmax_{app}$ different but V_{max}/K_m ratio equivalent

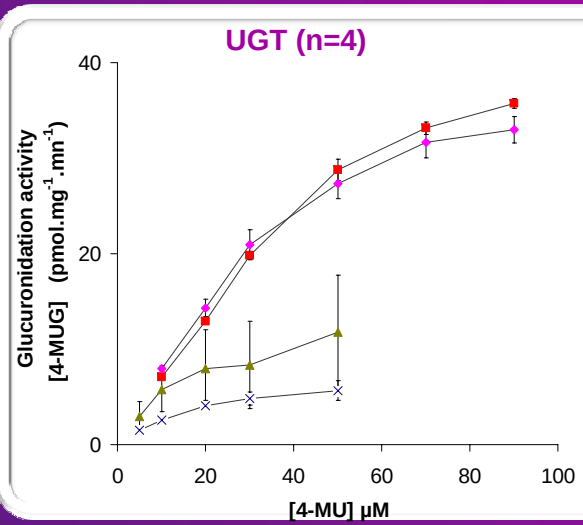
Cutaneous metabolism

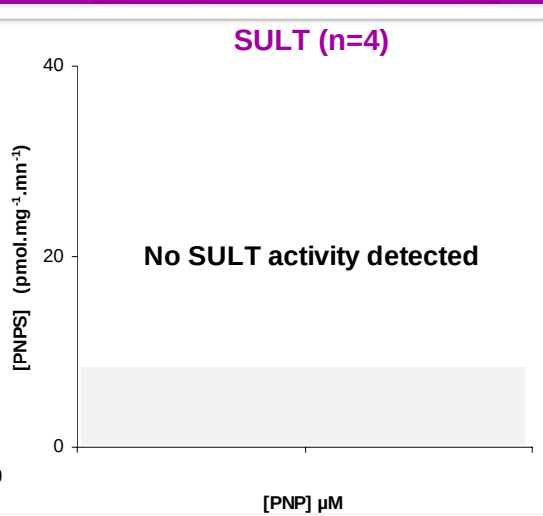
Phase II Metabolizing Enzyme Activities











▲ : NHS
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Normal Human Skin vs Models: Km_{app} and $Vmax_{app}$ different but V_{max}/K_m ratio equivalent

Cutaneous metabolism

Metabolizing enzyme activities comparison

Activity	Apparent enzymatic parameters (Mean +/- SEM)	NHS	Episkin™	SkinEthic™ RHE	FTM
ES		2.1 ± 0.1	1.1 ± 0.2	1.6 ± 0.1	3.8 ± 1.0
GPx		1.5 ± 0.6	0.7 ±0.04	1.7 ± 0.1	2.5 ± 0.1
ALDH		1.6 ± 0.3	0.6 ± 0.1	0.8 ± 0.1	0.9 ± 0.04
UGT	V_{max}/K_m ratio	1.3 ± 0.7	0.9 ± 0.03	0.4 ± 0.1	1.1 ± 0.1
GST		1.0 ± 0.2	1.4 ± 0.1	1.1 ± 0.1	1.6 ± 0.2
NAT1		0.7 ± 0.3	1.0 ± 0.1	1.2 ± 0.1	1.4 ± 0.1
COMT		ND	0.2 ± 0.02	ND	0.4 ± 0.05
ADH		0.3 ± 0.03	0.1 ± 0.01	0.2 ± 0.1	0.3 ± 0.01

Apparent Vmax/Km (µL.mg protein^{-1}.min^{-1})

MODEL COMPARISON PER ACTIVITY:

- NHS clearances are highly variables
- Model clearances are often similar with NHS clearances
  (except for $ES_{EPIS\&RHE}$, ADH_{EPIS}, GPx_{EPIS}, NAT_{FTM})

Generaly models are similar to NHS in term of metabolic capabilities

Cutaneous metabolism
Summary of Activities Studies

ACTIVITIES

- **Low basal expression and activity of CYP450 involved in «Drug metabolism»** ***(!! induction !!)***
- Glutathion peroxydase activity was detected
- High esterase activity (Low affinity with the compound used as substrate…)
- ADH and ALDH were detected

- NAT activity was detected
- GST activity was detected
- UGT activity was detected
- Very low SULT activity except for steroid sulfation
- COMT activity was detected

Other enzymes to be quickly tested:

Phase I: FMO
Phase II: COMT (in progress)
Transporters

COMPARISON

Apparent enzymatic parameters were calculated and compared between reconstructed skin models and with normal human skin:

- Affinities (Km) and Maximal velocities (Vmax) are different
- Clearances (Vmax/Km ratio) are often similar

Cutaneous metabolism & toxicity

Cutaneous metabolism
Conclusion

- **SKIN IS INVOLVED IN METABOLIZING PROCESS** *(potential First Pass Effect)*
- **SKIN IS RATHER A DETOXIFICATION ORGAN THAN A BIO ACTIVATING ONE**
- **TOXICITY APPEARS IN SKIN WHEN:**
  - DETOXIFICATION SYSTEMS ARE OVER EXPOSED TO TOXICANTS
  - REACTIVE MOLECULES ARE RELEASED IN LARGE AMOUNTS
- **RECONSTRUCTED HUMAN SKIN MODELS ARE GOOD ENOUGH PREDICTIVE TOOLS OF SKIN METABOLISM AND TOXICITY**

Cutaneous metabolism

Thanks to ....

L'OREAL

Guillaume Léreaux
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Daniel Duché

CYP450 experiments:
Oroxcell SAS

Normal Human Skin
BIOPREDIC

D. Mansuy
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V. Luu The