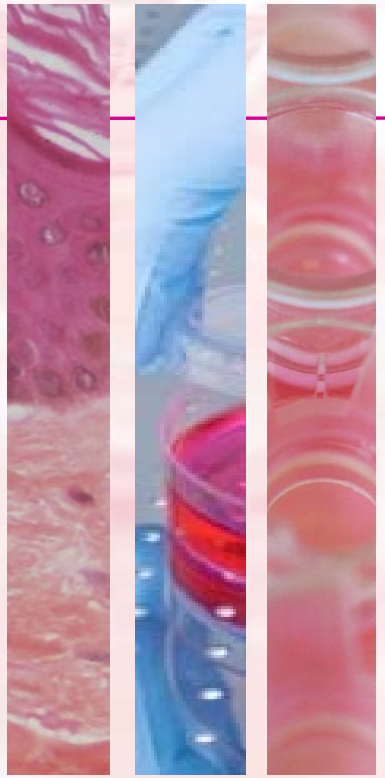
Cutaneous Metabolism

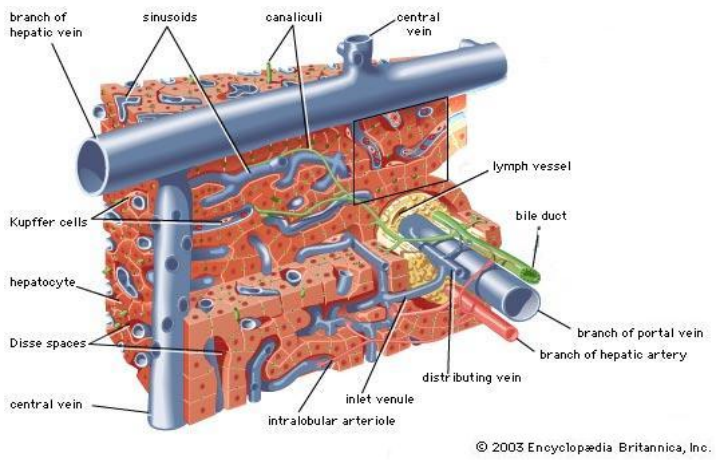
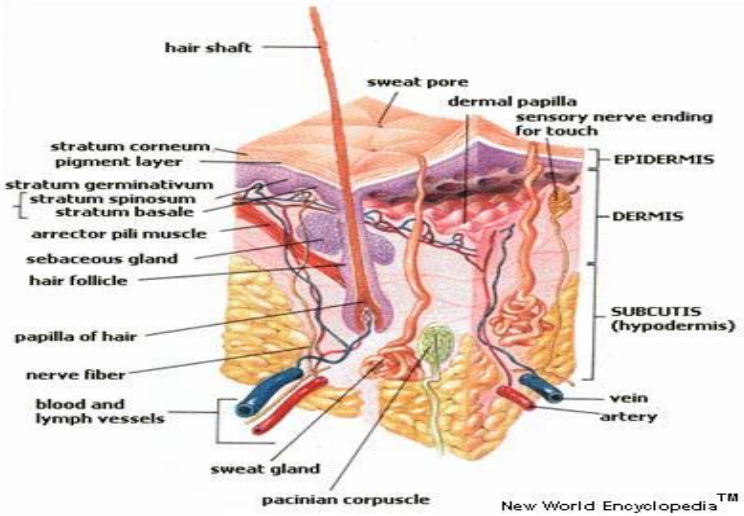
Joan EILSTEIN

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



SKIN METABOLISM MEETING

EDINBURGH 20 May 2011

	LIVER	SKIN
		
Main cells type	**Hepatocytes**	**Keratinocytes**
Main functions	**Endogenous & Drug metabolisms**	**Barrier function, thermoregulation…**
Weight	**2% body weight**	**15% body weight, 2 m²**

VS

CHARACTERIZATION OF THE SKIN METABOLIC CAPABILITIES

REACh
Chemicals characterization

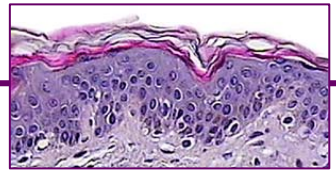
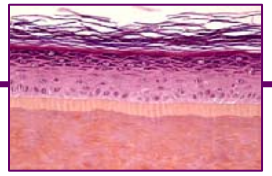
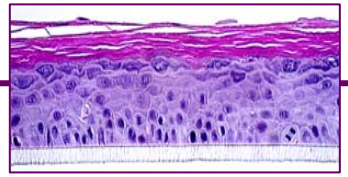
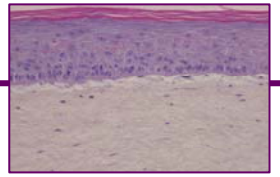
&

7th Amendment to the Cosmetic Directive
Non animal approaches

IN VITRO ASSAYS DEVELOPMENT

USE OF RECONSTRUCTED HUMAN SKIN MODELS

Reconstructed Human Skin Models

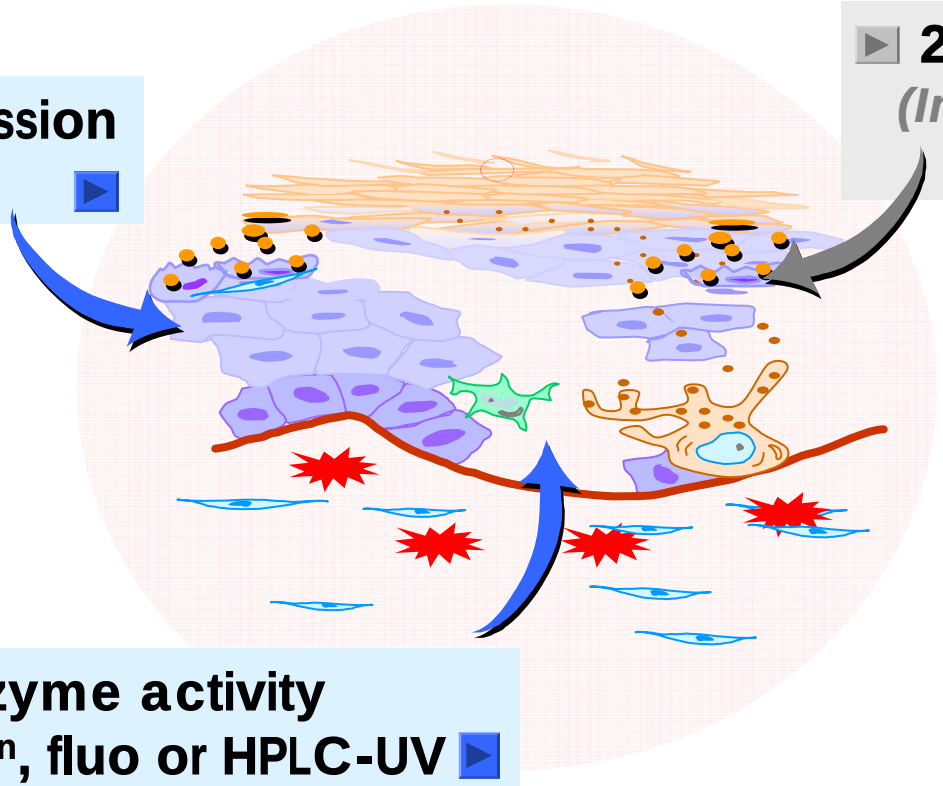
SkinEthic laboratories

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

1. Gene expression (RT-PCR)

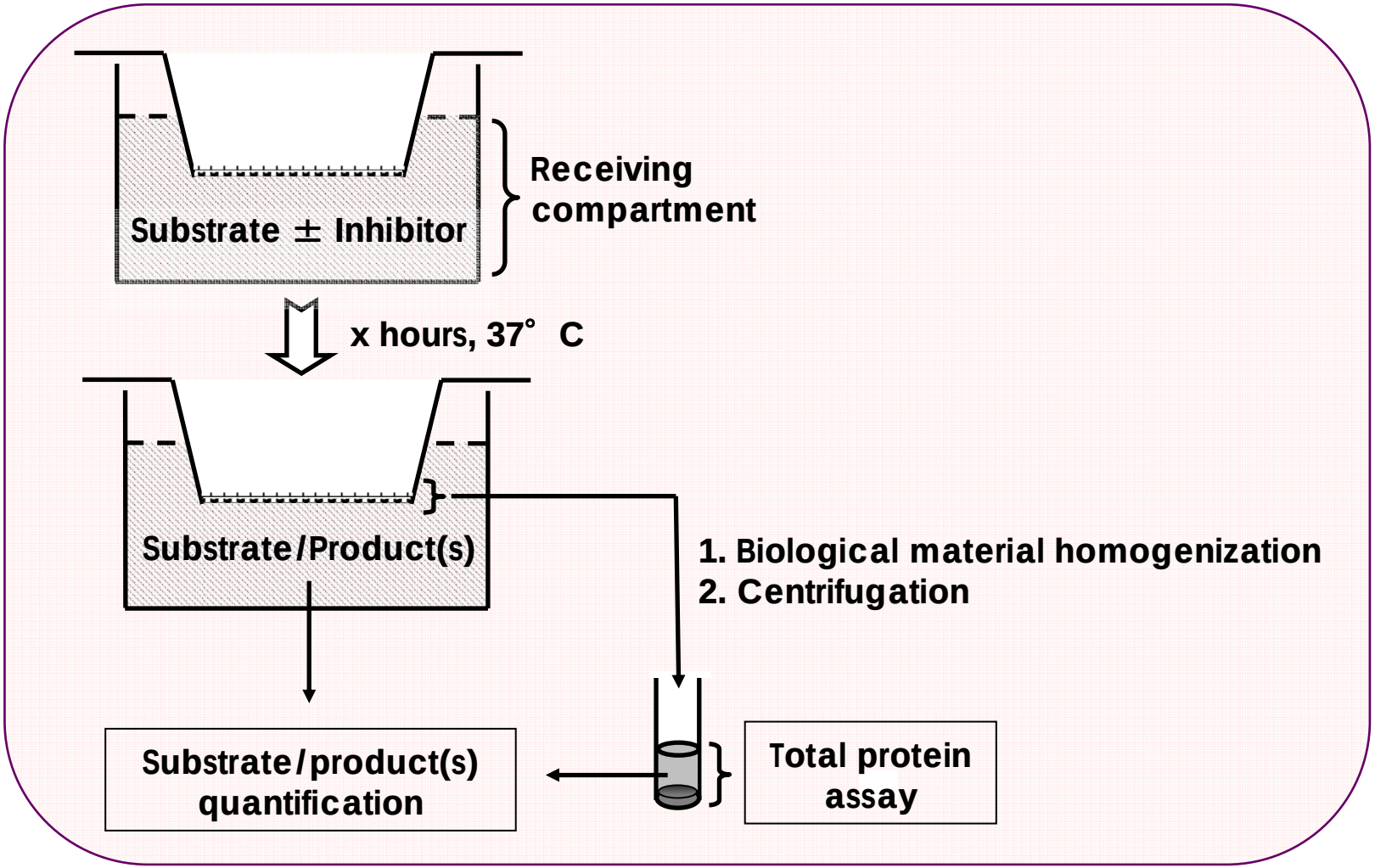
2. Protein expression
(Immuno histochemistry)
(Western blots)



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

: *Fluorogenic radiolabelled or UV visible absorbing substrates*

Activity Studies - General Procedure



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

Expression Profiles of Phase 1and 2 Metabolizing Enzyme

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 1

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	(+/−)	(+/−)	(+/−)	(+/−)	(+/−)	

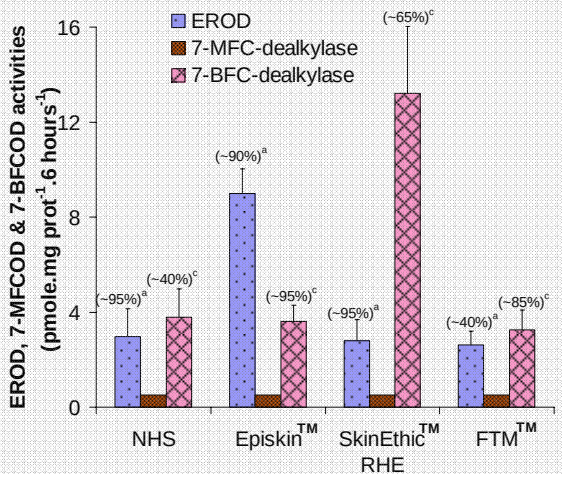
Gene codes	Total human skin	Human dermis	Human epidermis	Episkin™	FTM	Physiological functions
ADH1B	++++	++++	–	–	–	Alcohol oxidation into aldehyde Retinol oxidation into retinal
DRHS8 or 17b-HSD 11	+	++	(+)	(+)	(+)	5a-androstane-3a, 17b-diol oxidation into Androsterone
EPHX1	++	+++	+	+	+	Arene & aliphatic 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 2

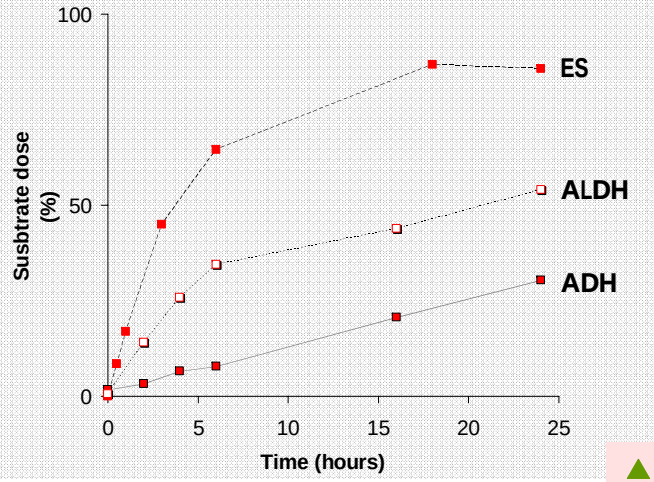
Gene codes	Total human skin	Human dermis	Human epidermis	Episkin™	FTM	Physiological functions
GSTP1	++++	++++	++++	++++	++++	Reduced glutathione conjugation to hydropobic electrophiles
GSTT1	++	++	++	+	++	
GSTM5	+	++	(+)	–	–	
SULT2B1b	+++	+	++++	++++	++++	Cholesterol & DHEA sulfation
SULT1A1	++	++	(+)	(+)	+	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 — Phase I Metabolizing Enzyme Activities

CYP450 (n=4)

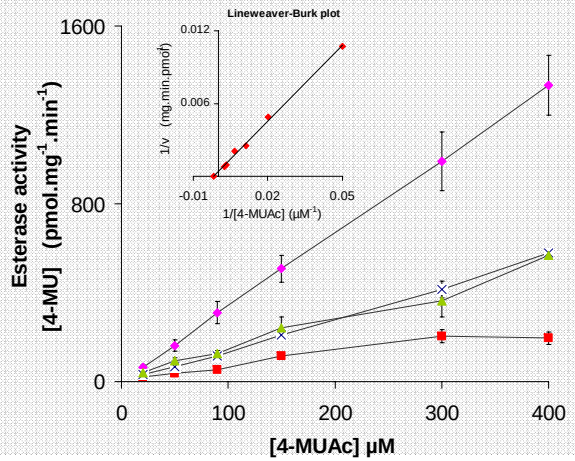


Time-course study (n=2)

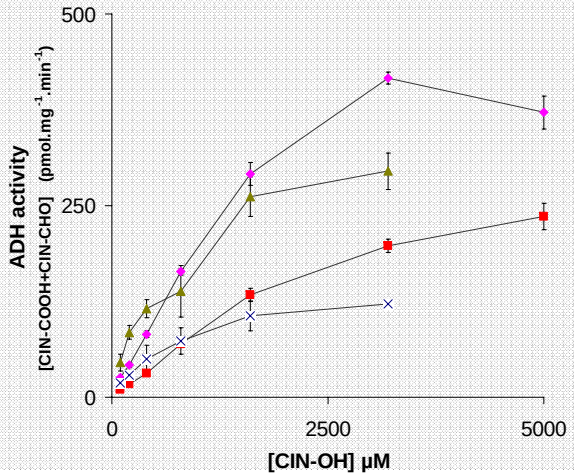


▲ : NHS,
■ : Episkin™,
X : SkinEthic™ RHE,
◆ : FTM

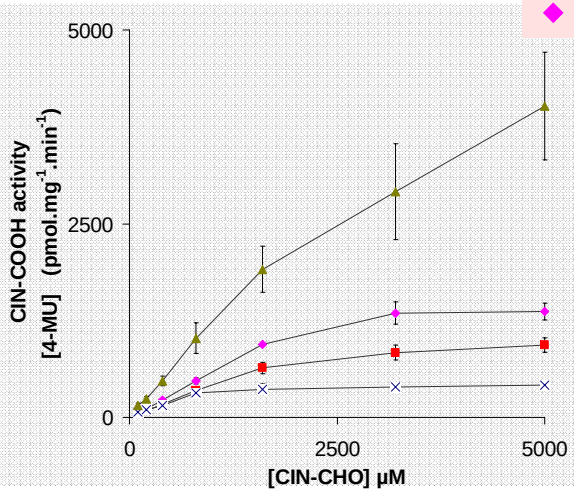
Esterases (n=4)



ADH (n=4)



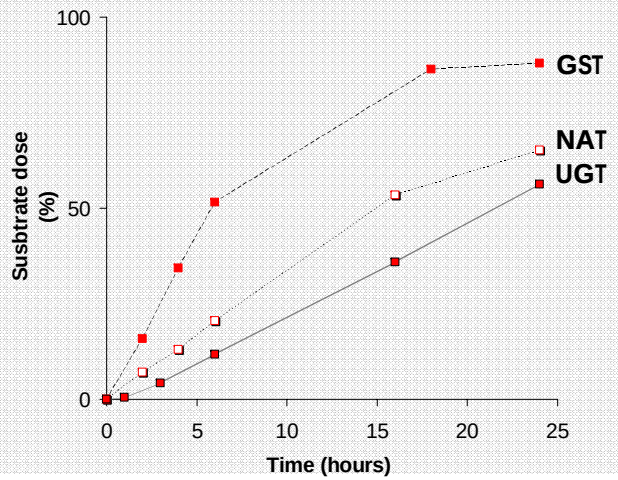
ALDH (n=4)



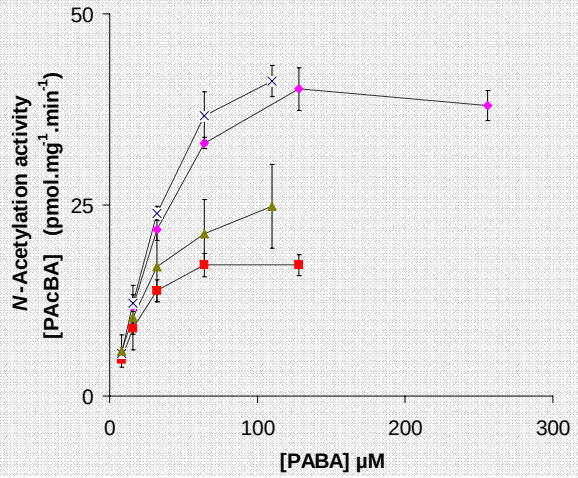
Normal Human Skin vs Models: Km_{app} and $Vmax_{app}$ different but V_{max}/K_m ratio equivalent

CUTANEOUS METABOLISM — Phase II Metabolizing Enzyme Activities

Time-course study (n=2)

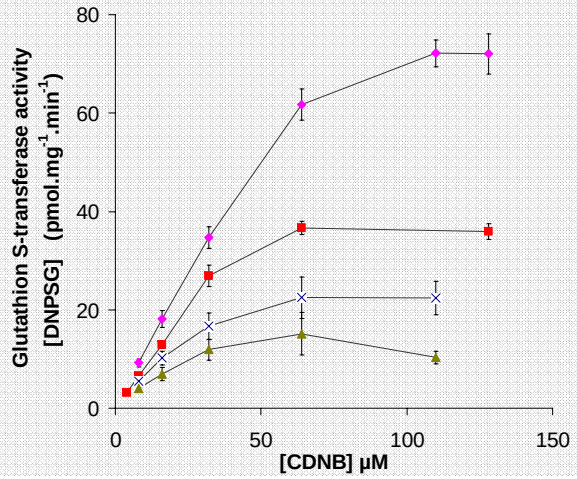


NAT (n=4)

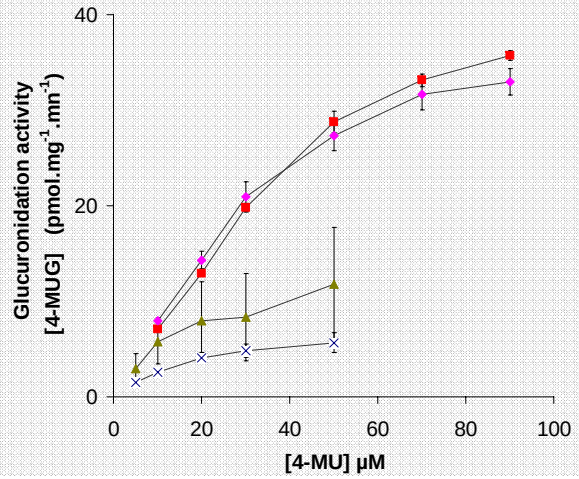


▲ : NHS,
■ : Episkin™,
X : SkinEthic™ RHE,
◆ : FTM

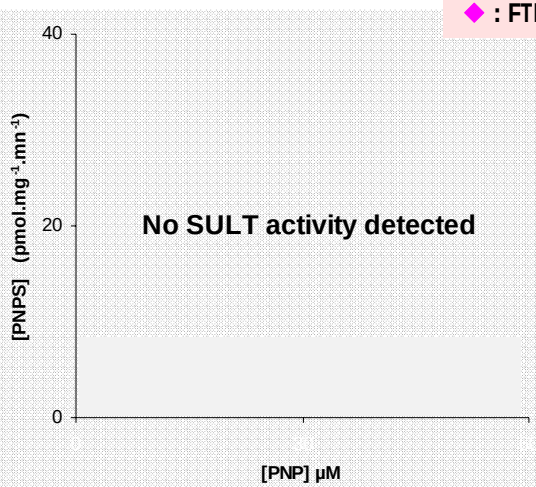
GST (n=4)



UGT (n=4)



SULT (n=4)



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	V_{max}/K_m ratio	2.1 ± 0.1	1.1 ± 0.2	1.6 ± 0.1	3.8 ± 1.0
ALDH		1.6 ± 0.3	0.6 ± 0.1	0.8 ± 0.1	0.9 ± 0.04
UGT		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
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}, NAT_{FTM})

GENERALY MODELS ARE SIMILAR TO NHS IN TERM OF METABOLIC CAPABILITIES

ACTIVITIES

- **Low basal expression and activity of CYP450 involved in «Drug metabolism» (!! induction !!)**
- 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

- Other enzymes to be quickly tested:
  - Phase I: Peroxidase
  - Phase II: COMT

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

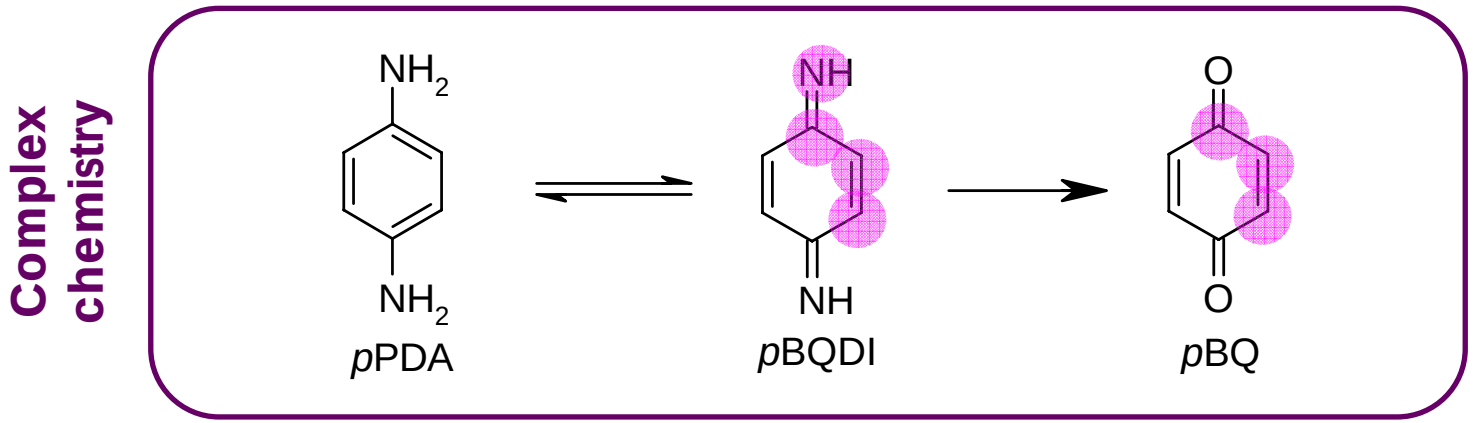
HAPTEN
+
PROTEIN

Mechanism Nu/E

Mechanism R•

ANTIGENIC COMPLEX

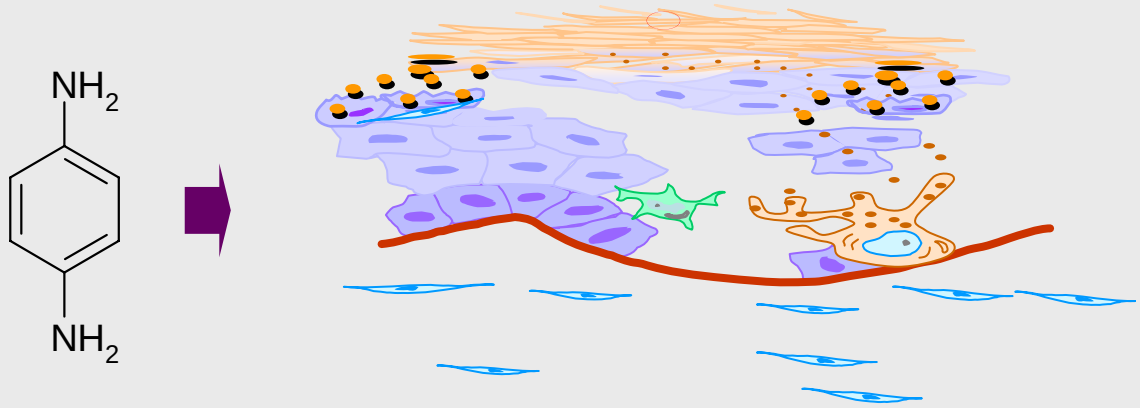
FIRST CASE STUDY:



Roberts, D.W., Lepoittevin, J.-P. Hapten-protein interaction. In allergic Contact Dermatitis: The Molecular Basis (Lepoittevin, J.-P., Basketter, D.A., Goossens, A, Karlberg, A.-T., Eds.) pp 81-111, Springer-Verlag Berlin, Heildelberg, New York (1998)

CUTANEOUS METABOLISM

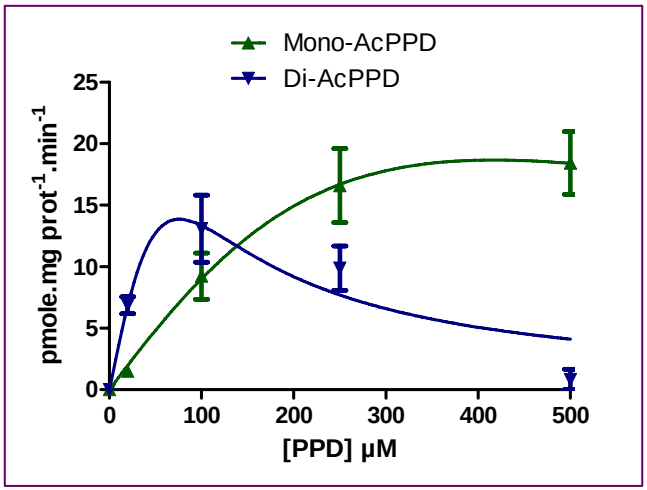
*p*PDA



SKIN IS A DETOXIFICATION ORGAN

NAT1 affinity: *p*PDA << Mono-Ac*p*PD

SATURATION OF DETOXIFICATION SYSTEM → TOXICITY



Nohynek, G.J., Duché, D., Garrigues, A., Meunier, P.A., Toutain, H. and Leclaire, J. Toxicol. Letters 158, 196-212 (2005)

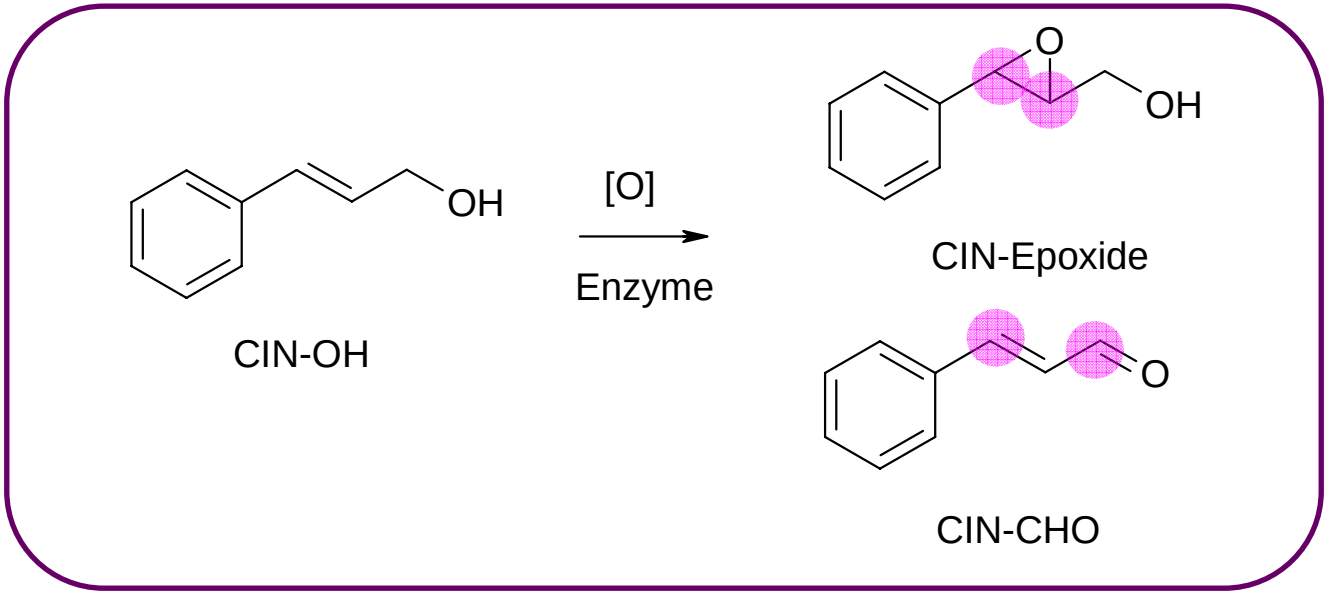
HAPTEN
+
PROTEIN

Mechanism Nu/E

Mechanism R•

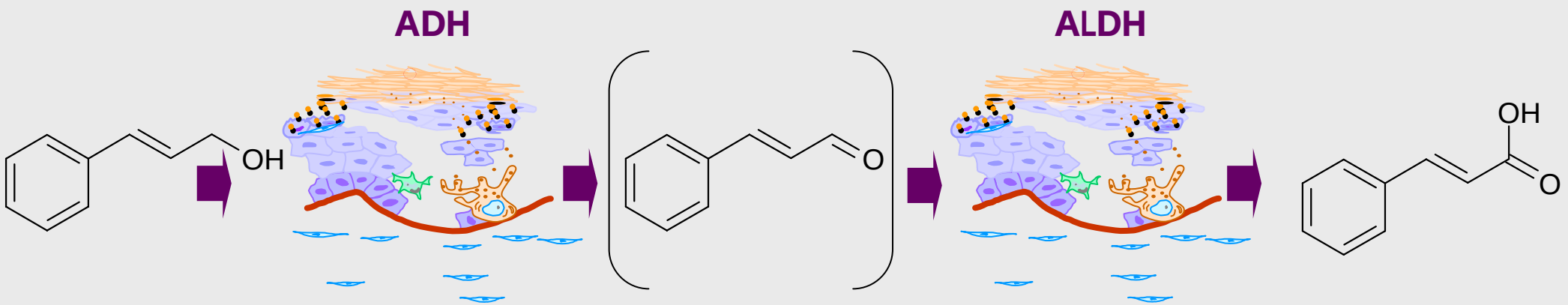
ANTIGENIC
COMPLEX

SECOND CASE STUDY:



CUTANEOUS METABOLISM

CIN-OH



SKIN IS A DETOXIFICATION ORGAN

Activity	NHS (V_{max}/K_m)
ADH	0.3 ± 0.03
ALDH	1.6 ± 0.3

SATURATION OF DETOXIFICATION SYSTEM → TOXICITY

HAPTEN
+
PROTEIN

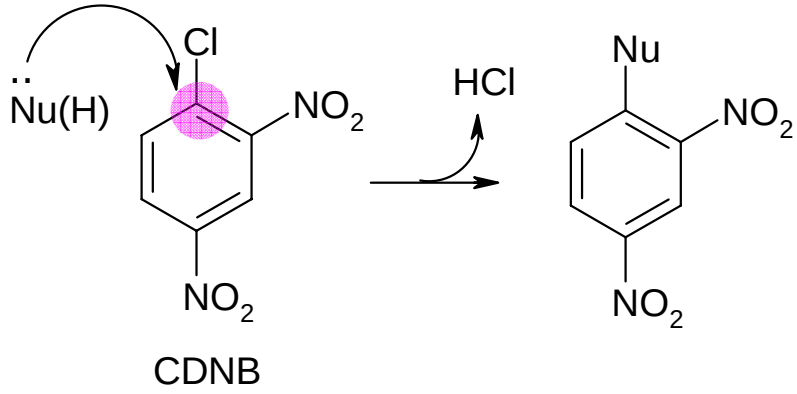
Mechanism Nu/E

Mechanism R•

ANTIGENIC COMPLEX

THIRD CASE STUDY:

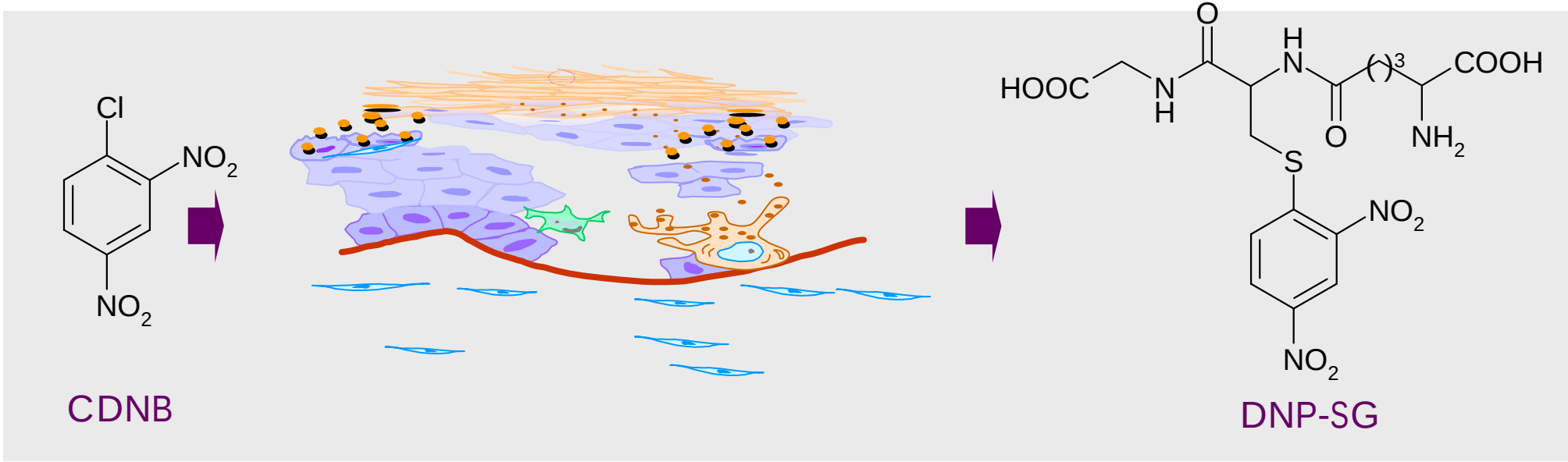
S_NAr



Roberts, D.W., Lepoittevin, J.-P. Hapten-protein interaction. In allergic Contact Dermatitis: The Molecular Basis (Lepoittevin, J.-P., Basketter, D.A., Goossens, A, Karlberg, A.-T., Eds.) pp 81-111, Springer-Verlag Berlin, Heildelberg, New York (1998)

CUTANEOUS METABOLISM

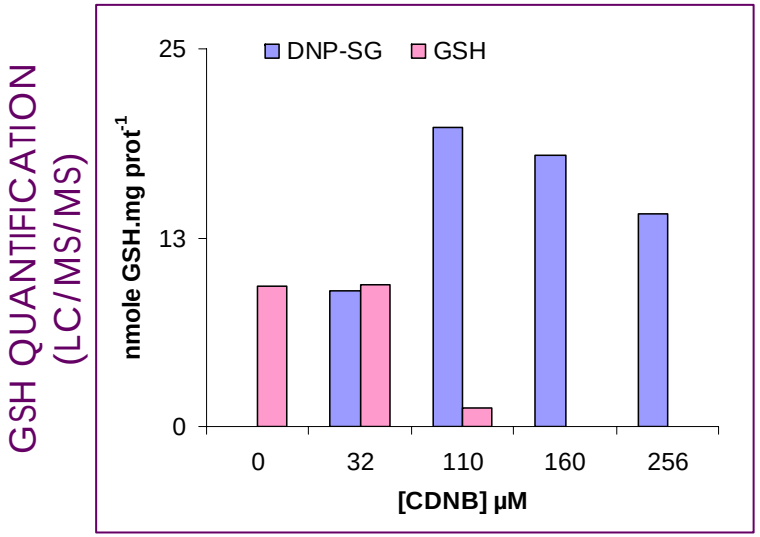
CDNB



SKIN IS A DETOXIFICATION ORGAN

GSH HOMEOSTASIS UNTIL $[CDNB]_{max}$

SATURATION OF DETOXIFICATION SYSTEM
DEFAULT OF COFACTORS
→ TOXICITY



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**

Thanks to…

L'OREAL

Guillaume Léreaux
Daniel Duché
Jean-Roch Meunier

CYP450 experiments:
Oroxcell SAS