

L'ORÉAL

# Cutaneous Metabolism

**Joan EILSTEIN**

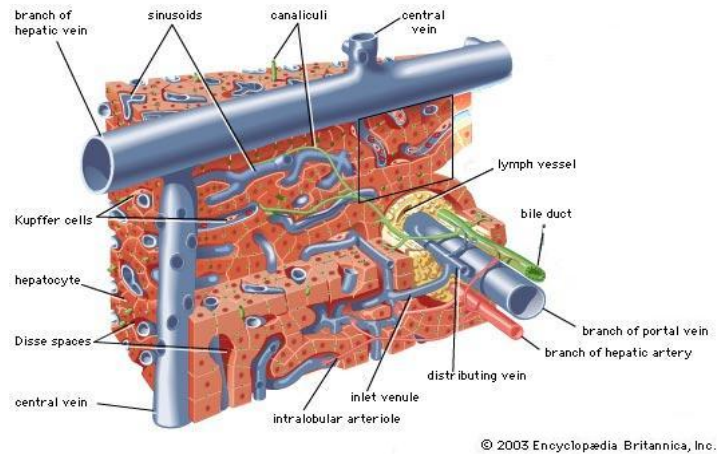
*ADMET - Metabolism  
Predictive Methods and Models Development Department  
L'OREAL Advanced Research*

SKIN METABOLISM MEETING

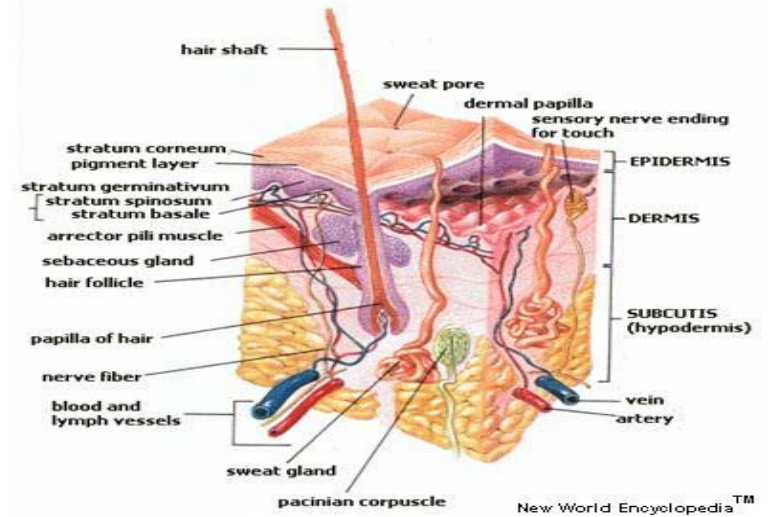
EDINBURGH 20 May 2011



## LIVER



## SKIN



VS

Main cells type

Hepatocytes

Keratinocytes

Main functions

Endogenous & Drug metabolisms

Barrier function, thermoregulation...

Weight

2% body weight

15% body weight, 2 m<sup>2</sup>

## CHARACTERIZATION OF THE SKIN METABOLIC CAPABILITIES

REACH  
Chemicals characterization

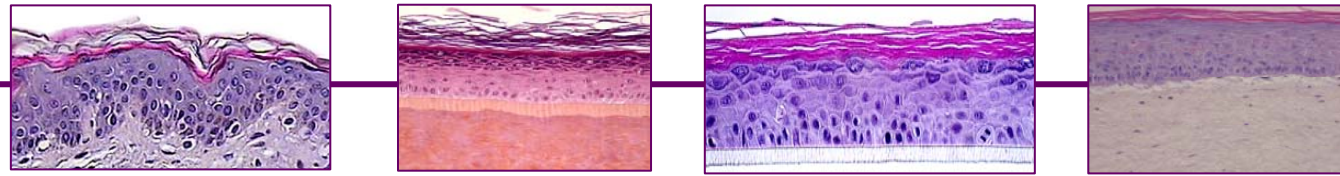
&

7<sup>th</sup> Amendment to the Cosmetic Directive  
Non animal approaches



*IN VITRO ASSAYS DEVELOPMENT*

USE OF RECONSTRUCTED HUMAN SKIN MODELS



**SkinEthic**  
laboratories



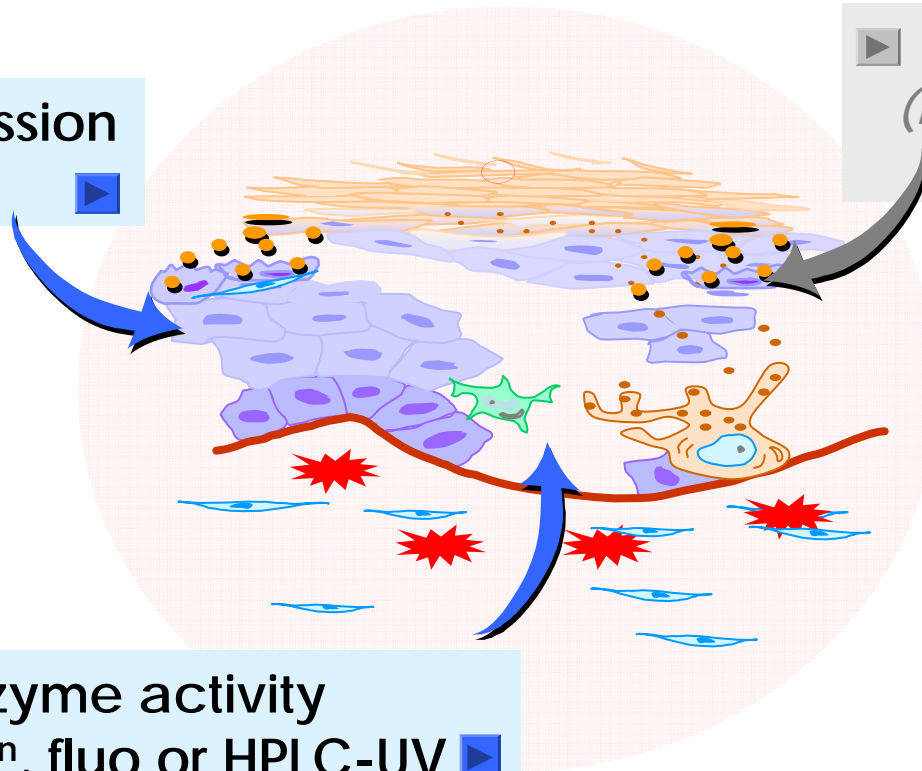
TYPE	ORIGIN
<b>Normal Human Skin (NHS):</b> <i>Epidermis/Dermis (BIOPREDIC)</i>	<ul style="list-style-type: none"> <li>• Mammoplasties</li> </ul>
<b>Episkin™:</b> <i>Reconstructed human epidermis</i>	<ul style="list-style-type: none"> <li>• NHK (mammoplasties)</li> <li>• Pool of 4 – 5 donors</li> <li>Support: BPER</li> </ul>
<b>SkinEthic™ RHE:</b> <i>Reconstructed human epidermis</i>	<ul style="list-style-type: none"> <li>• NHK (foreskin/abdo)</li> <li>• 1 donor/Pool of 2 donors</li> <li>Support: Polycarbonate</li> </ul>
<b>Full thickness of Episkin™:</b> <i>Reconstructed human epidermis / equivalent dermis</i>	<ul style="list-style-type: none"> <li>• NHK (mammoplasties)</li> <li>• Pool of 4 – 5 donors</li> <li>Support: Polycarbonate</li> </ul>

## COMPARISON OF METABOLIC CAPABILITIES BETWEEN NORMAL HUMAN SKIN AND MODELS

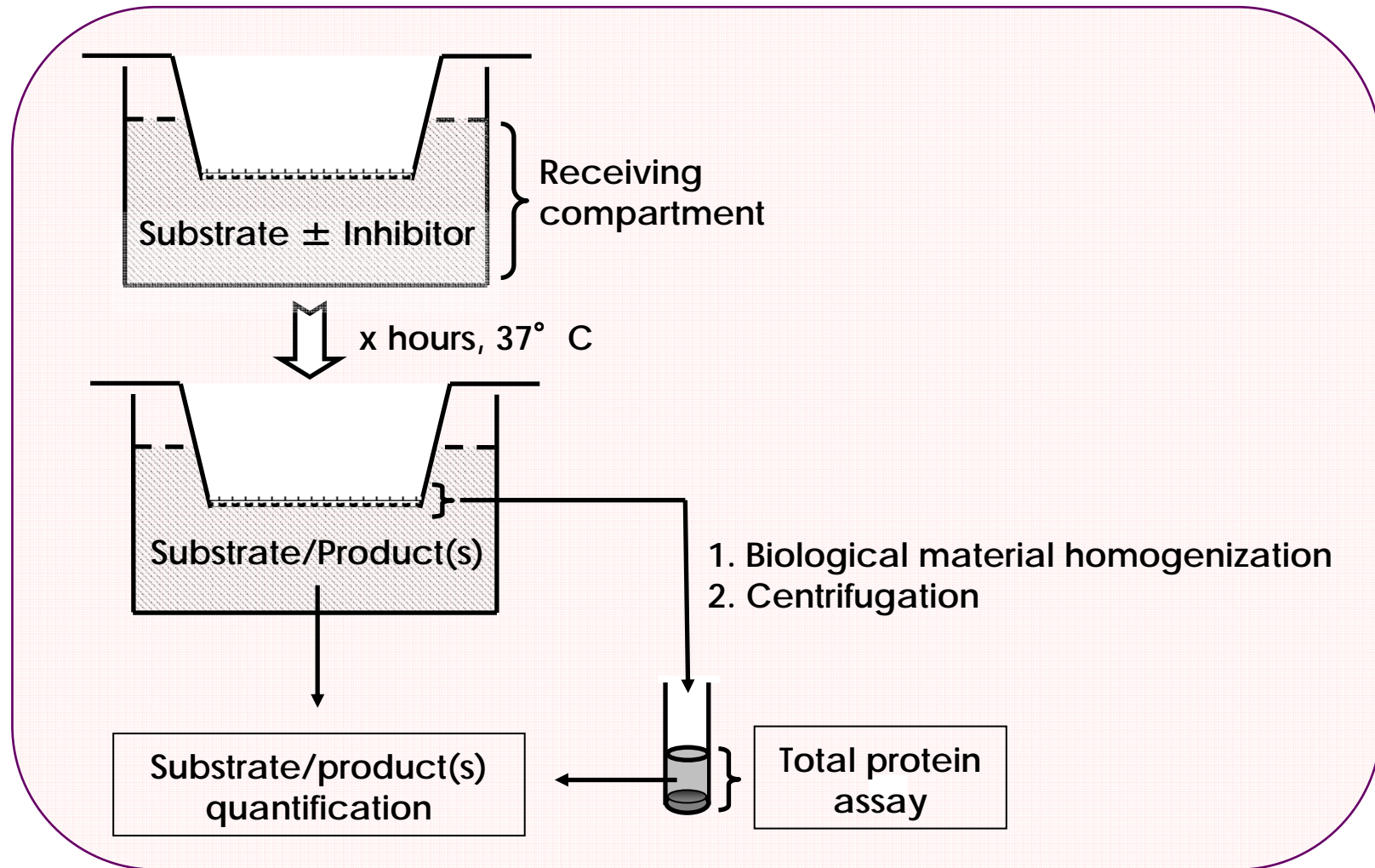
1. Gene expression  
(RT-PCR) ▶

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

3. Enzyme activity  
Radio, MS<sup>n</sup>, fluo or HPLC-UV ▶



 : Fluorogenic radiolabelled or UV visible absorbing substrates



1. Time-course study: Incubation time determination

2. Dose-effect study: apparent enzymatic parameters  $\rightarrow K_m, V_{max}$  and  $V_{max}/K_m$  ratio

# CUTANEOUS METABOLISM

## Expression Profiles of Phase 1 and 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<sup>a,\*</sup>, Daniel Duche<sup>b</sup>, Corinne Ferraris<sup>b</sup>, Jean-Roch Meunier<sup>b</sup>, Jacques Leclaire<sup>b</sup>, Fernand Labrie<sup>a</sup>

++++> 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

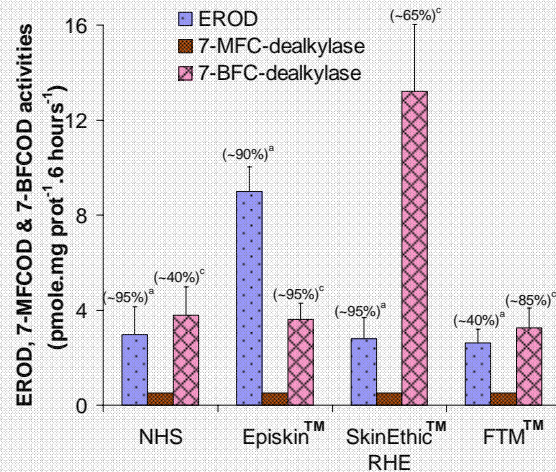
Gene codes	Total human skin	Human dermis	Human epidermis	Episkin™	FTM	Physiological functions
CYP4B1	+	(+)	+	+	(+)	Arylamine N-hydroxylation, Lauric acid ω-hydroxylation
CYP26B1	+	+	+	(+/-)	(+)	Retinoic acid metabolism
CYP39A1	+	(+)	+	(+)	(+)	24-Hydroxy cholesterol
CYP2J2	+	(+)	+	(+)	+	7 α-hydroxylation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

Gene codes	Total human skin	Human dermis	Human epidermis	Episkin™	FTM	Physiological functions
ADH1B	++++	++++	-	-	-	Alcohol oxidation into aldehyde
DRHS8 or 17b-HSD 11	+	++	(+)	(+)	(+)	Retinol oxidation into retinal
EPHX1	++	+++	+	+	+	5α-androstane-3α, 17β-diol oxidation into Androsterone
EPHX2	++	++	++	(+)	+	Arene & aliphatic epoxide hydrolysis
HADH2	++	++	++	+++	+++	3-Hydroxybutyryl-CoA mitochondrial β-oxidation
AKR1C2 or 3α-HSD	+	+	(+)	+++	++	DHT reduction into 3-diol
AKR1C1 or 20α-HSD	+	+	(+)	+++	++	Progesterone reduction into 20α-hydroxyprogesterone
FMO1	(+)	(+/-)	(+)	-	(+/-)	N-oxidation of secondary & tertiary amines
FMO2	(+)	(+)	(+/-)	(+)	+	
FMO3	(+)	(+)	(+/-)	-	-	
FMO4	(+)	(+)	(+)	(+)	(+)	
FMO5	(+)	(+)	(+)	(+/-)	(+)	
STS	(+)	(+)	(+)	(+)	(+)	Cholesterol, DHEA & estrone sulfates hydrolysis
NOS1	(+/-)	(+/-)	(+)	(+/-)	(+/-)	
NOS2A/ADH7/AKR1C4	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)	

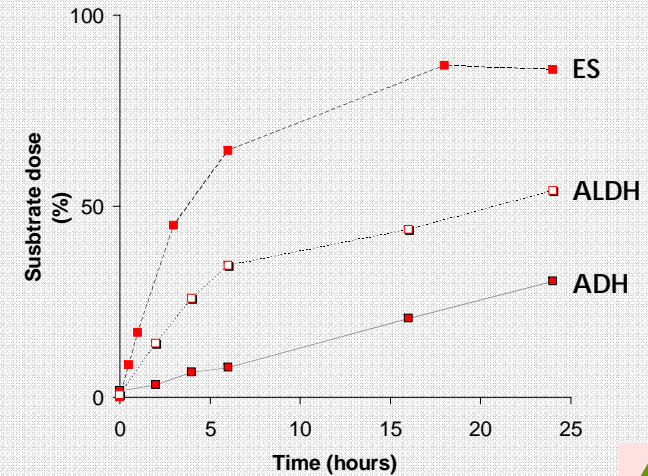
### PHASE 2

Gene codes	Total human skin	Human dermis	Human epidermis	Episkin™	FTM	Physiological functions
GSTP1	++++	+++	+++	+++	+++	Reduced glutathione conjugation to hydrophobic 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

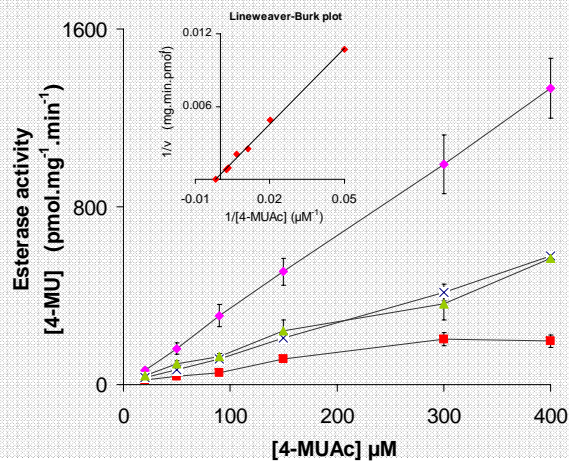
### CYP450 (n=4)



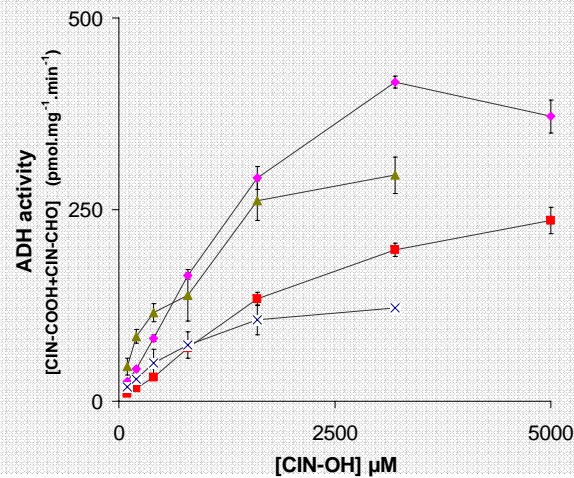
### Time-course study (n=2)



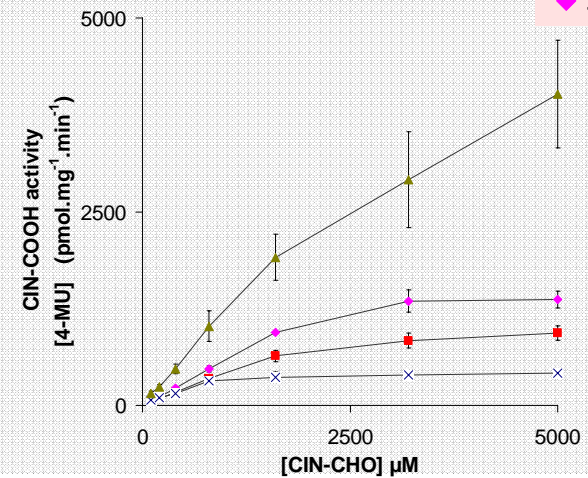
### Esterases (n=4)



### ADH (n=4)



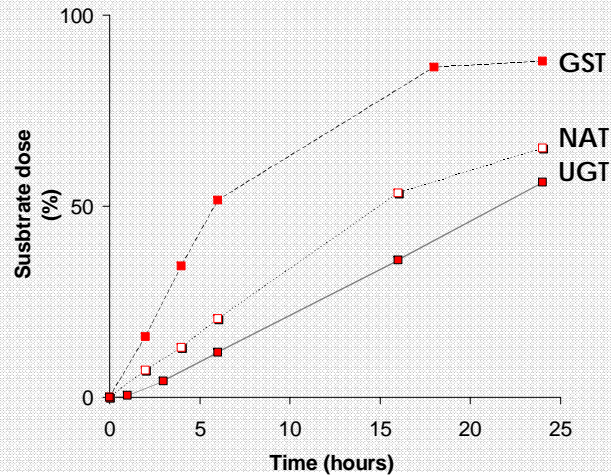
### ALDH (n=4)



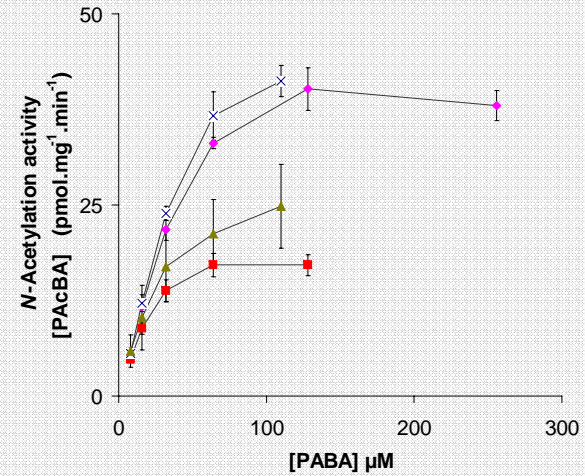
▲ : NHS,  
■ : Episkin<sup>TM</sup>,  
× : SkinEthic<sup>TM</sup> RHE,  
◆ : FTM

Normal Human Skin vs Models:  $K_{m_{app}}$  and  $V_{max_{app}}$  different but  $V_{max}/K_m$  ratio equivalent

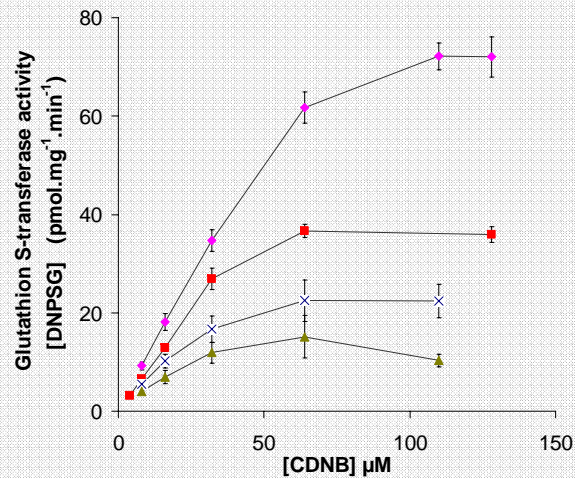
Time-course study (n=2)



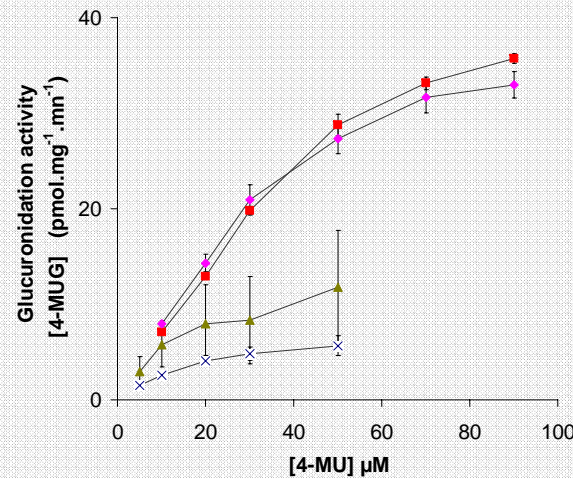
NAT (n=4)



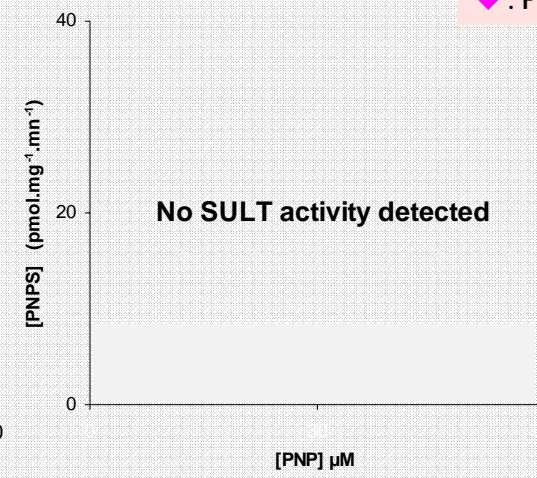
GST (n=4)



UGT (n=4)



SULT (n=4)



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

Normal Human Skin vs Models:  $K_{m_{app}}$  and  $V_{max_{app}}$  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  $V_{max}/K_m$  ( $\mu\text{L.mg protein}^{-1}.\text{min}^{-1}$ )

## MODEL COMPARISON PER ACTIVITY:

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

GENERALLY 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

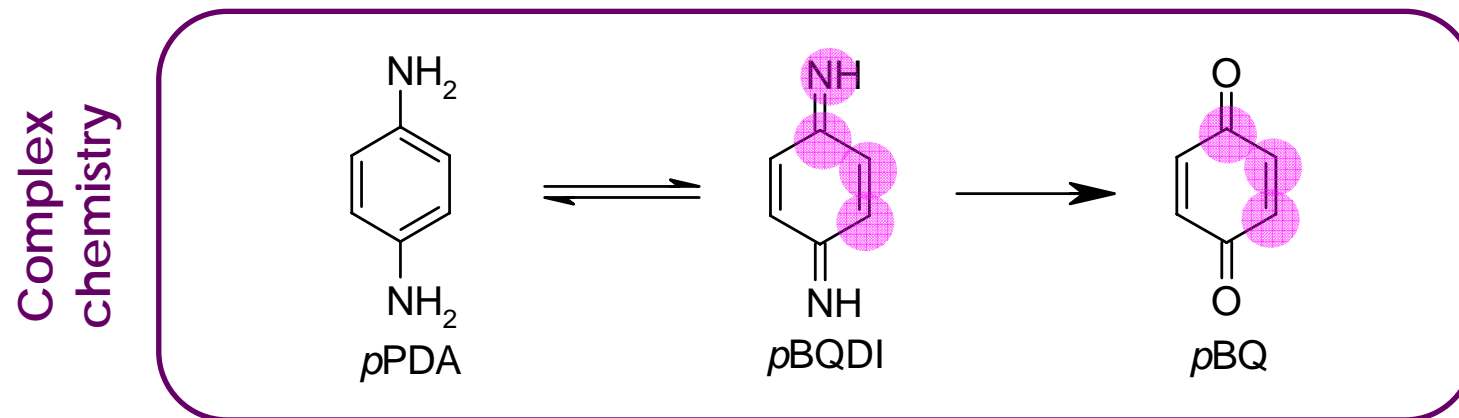
Apparent enzymatic parameters were calculated and compared between reconstructed skin models and with normal human skin:

- Affinities ( $K_m$ ) and Maximal velocities ( $V_{max}$ ) are different
- Clearances ( $V_{max}/K_m$  ratio) are often similar

### CUTANEOUS METABOLISM & TOXICITY



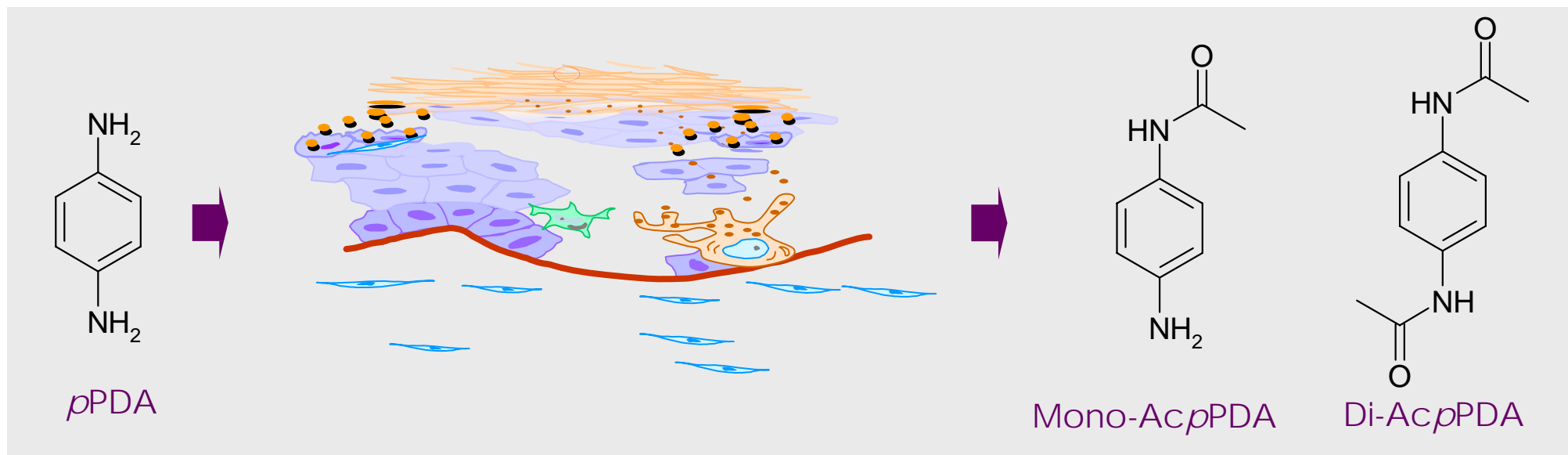
## FIRST CASE STUDY:



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, Heidelberg, New York (1998)

# CUTANEOUS METABOLISM

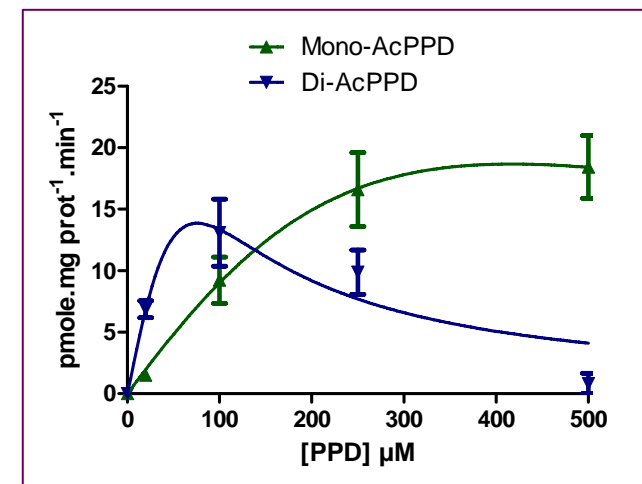
pPPDA



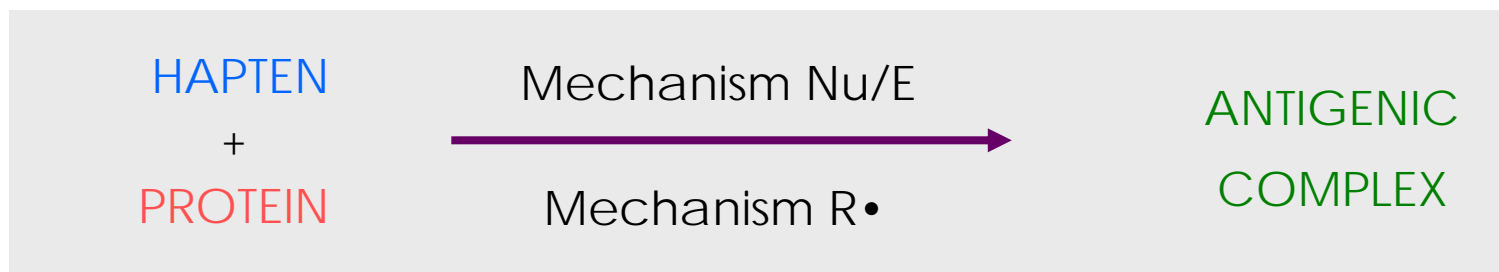
SKIN IS A DETOXIFICATION ORGAN

NAT1 affinity: pPPDA  $\ll$  Mono-AcPPD

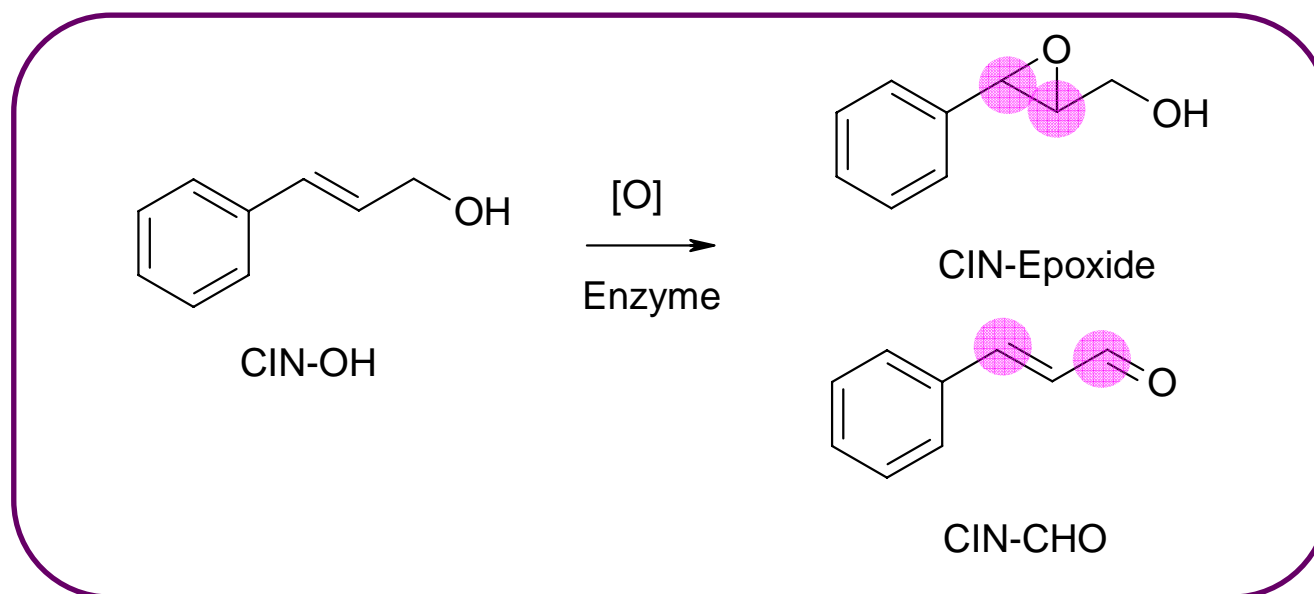
SATURATION OF DETOXIFICATION SYSTEM  $\rightarrow$  TOXICITY



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

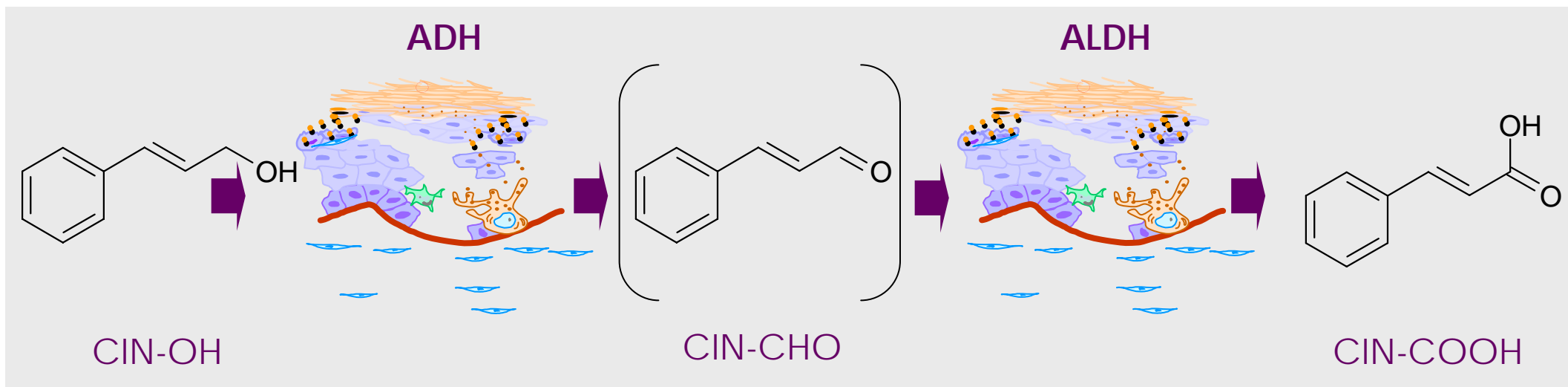


## SECOND CASE STUDY:



# CUTANEOUS METABOLISM

# CIN-OH

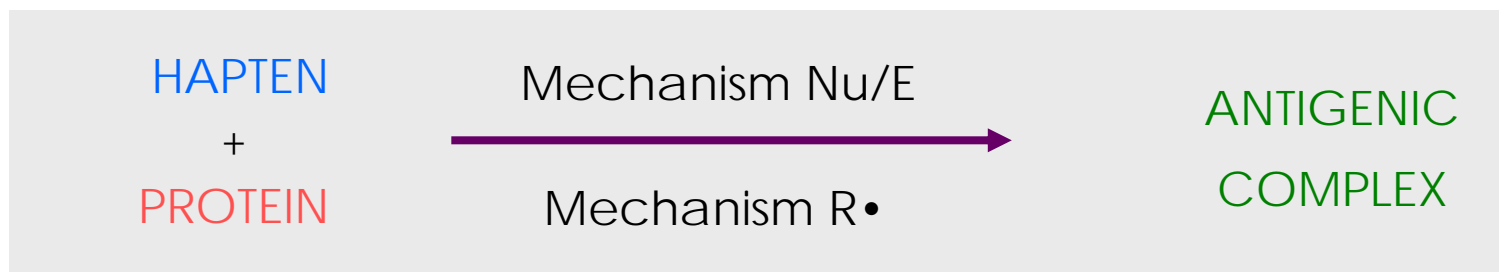


**SKIN IS A DETOXIFICATION ORGAN**

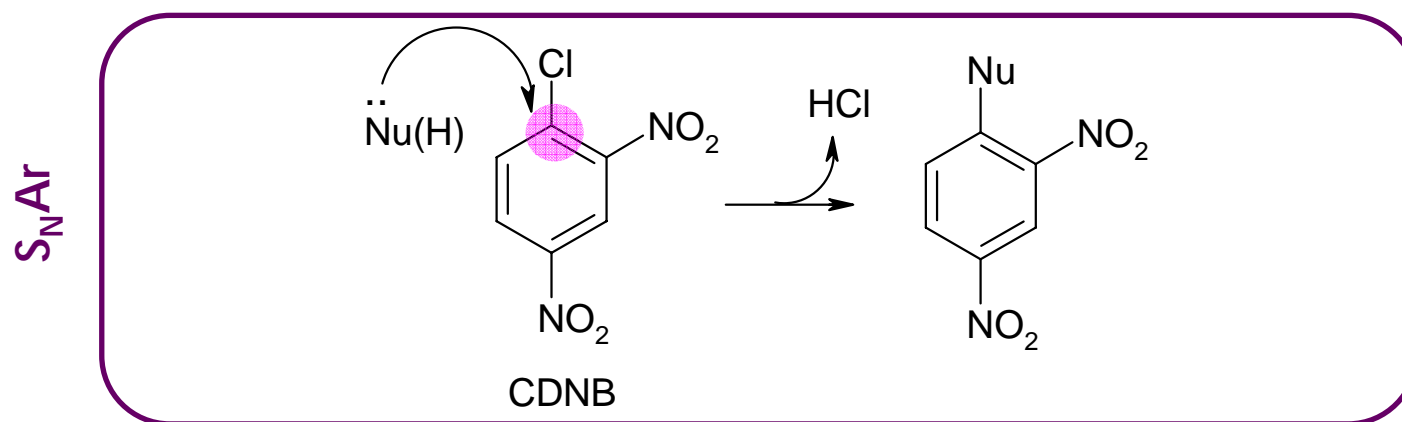


**SATURATION OF DETOXIFICATION SYSTEM → TOXICITY**

Activity	NHS ( $V_{max}/K_m$ )
ADH	$0.3 \pm 0.03$
ALDH	$1.6 \pm 0.3$



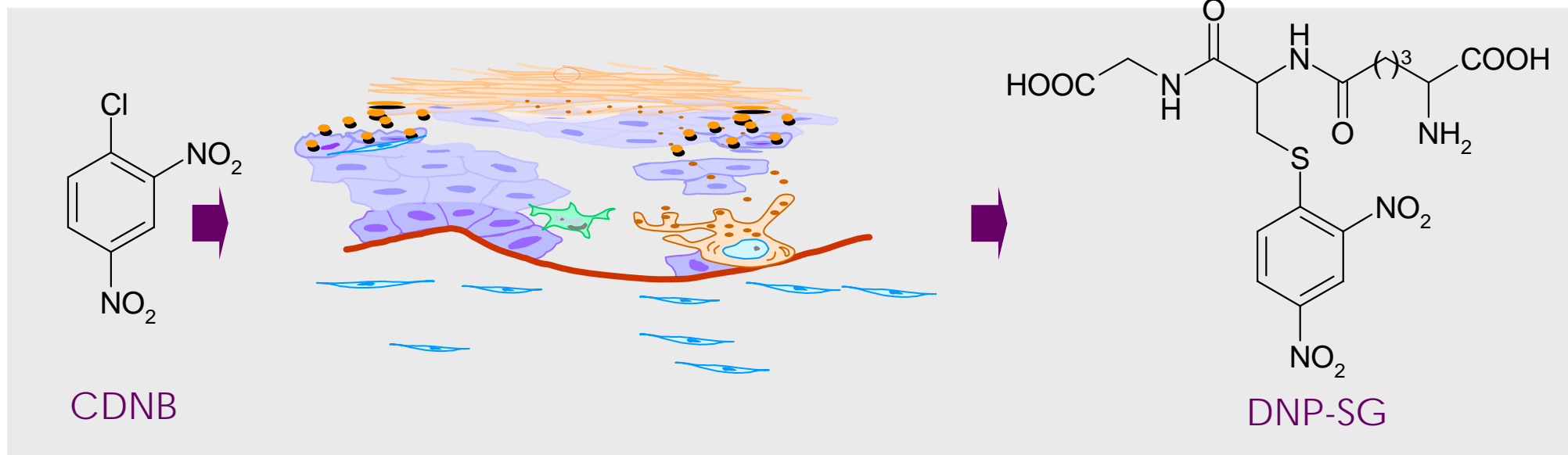
## THIRD CASE STUDY:



*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, Heidelberg, New York (1998)*

# CUTANEOUS METABOLISM

# CDNB



**SKIN IS A DETOXIFICATION ORGAN**

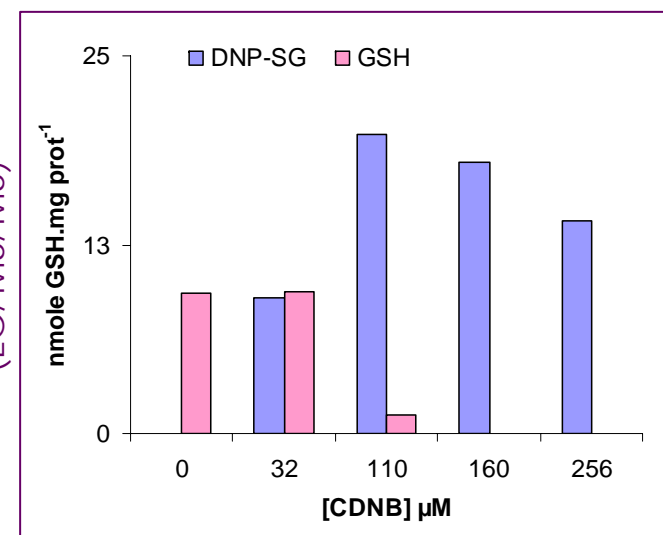


GSH HOMEOSTASIS UNTIL [CDNB]<sub>max</sub>

**SATURATION OF DETOXIFICATION SYSTEM  
DEFAULT OF COFACTORS**

**→ TOXICITY**

GSH QUANTIFICATION  
(LC/MS/MS)



- **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**

**L'OREAL**

Guillaume Léreaux  
Daniel Duché  
Jean-Roch Meunier

CYP450 experiments:  
Oroxcell SAS

