Cutaneous Metabolism and Its Importance for Skin Permeation and Toxicity

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Conflict of Interest Statement

- The presenter (or immediate family members) has/have a significant financial interest[1] in an entity that manufactures and/or distributes a material that is the subject of this session.

Outline/Objectives

- Importance of skin metabolism
- Expression of different xenobiotic metabolism enzymes in skin tissue
- Functional measures of metabolic enzyme activity
- Contribution to absorption and absorption and toxicity–“real and imagined?”
- Effectiveness of *in vitro* model systems for identification of pro haptens and pro genotoxins

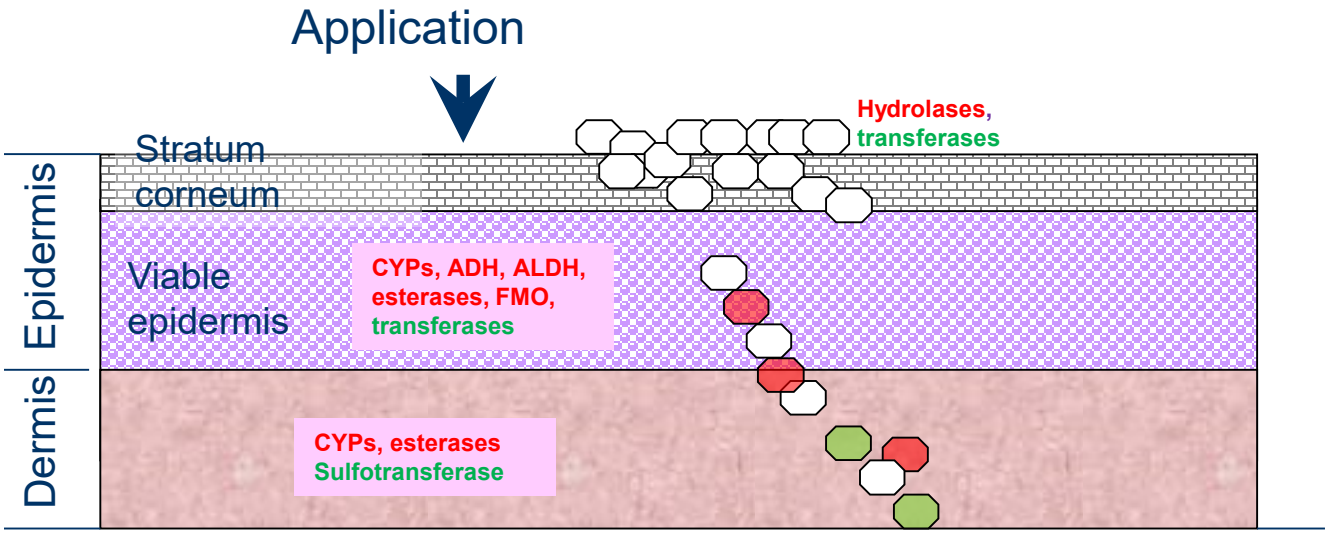
Importance of Cutaneous Metabolism

- Influence on toxicity
  - Activation of chemical agents to toxic metabolites
  - Skin sensitisation – metabolic involvement in replacement for LLNA
  - Detoxification through the dermal route
  - Enhancement of absorption by ester hydrolysis

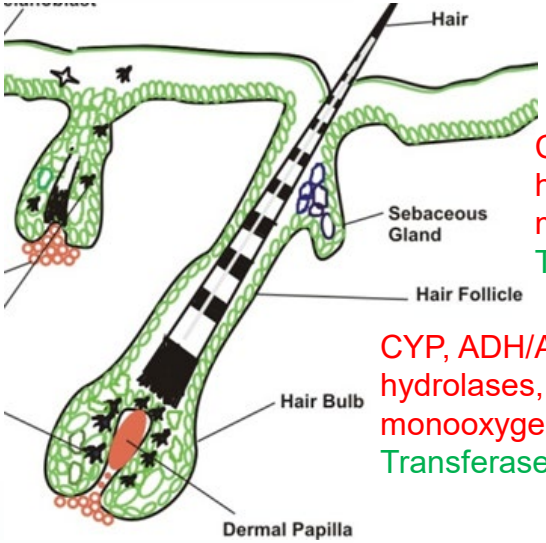
Importance of Cutaneous Metabolism

- Influence on drug delivery
  - Ester (and other) pro drugs
  - Deactivation of therapeutic agents
  - Drug-drug interactions
- Importance for skin physiology
  - Desquamation
  - Filaggrin processing to NMF
  - Other endogenous substrates

Skin Permeation/Metabolism

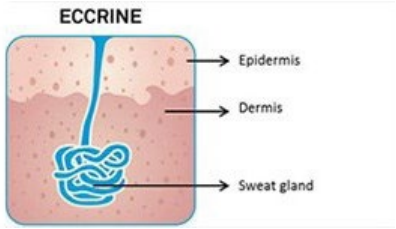


Appendages



CYP, ADH/ALDH, hydrolases, monooxygenases
Transferases

CYP, ADH/ALDH, hydrolases, monooxygenases, Transferases



CYP, ADH/ALDH

CYP Expression *In Vivo*

- Human skin biopsies (healthy volunteers and psoriasis patients) Smith et al. 2003
  - 1B1, 1A1, 2S1 consistently expressed
  - 2E1 in some individuals, higher in lesional skin (as was 2S1)
  - 2S1 highly induced with coal tar treatment
  - 2S1 constitutive and induced expression was highly individual
- Yengi et al 2003
  - Main isoforms expressed were 1B1, 2B6, 2D6, 3A4 (2C18, 2C19, 3A5)

CYP Expression

CYP 2S1

- Extra hepatically expressed isoform
- Strongly expressed in skin “throughout (viable) epidermis” and epithelial cells of appendages
- Under control of AhR, not regulated by differentiation *in vitro*.
- Endogenous substrate – retinoic acid

Saarikoski et al. Toxicology and Applied Pharmacology 207 (2005) S62–S69

Expression Changes During Keratinocyte Differentiation

- 4B1, 2W1, 2C18, 3A4, 2C19, 2C9 all increased expression
- 2S1, 2J2, 1B1, 1A1, 2E1 no change
- 2U1 decreased differentiation

Du et al (2006) Toxicol Appl Pharmacol 213: 135-144

Enzyme Expression *Ex Vivo*/*In Vitro*

mRNA expression Phase I enzymes (Eilstein et al., 2014)

Enzymes	Isoformes	NHS		RECONSTRUCTED HUMAN SKIN MODELS		
		Epidermis	Dermis	Episkin™	FTM	SkinEthic™ RHE
CYP450	1A1	+	(+)	-	-	-
	1A2	(+)	-	-	-	-
	1B1	(+)	+	(+)	(+)	+
	2B6	(+)	+	-	(+)	-
	2C18	(+)	-	-	+	+
	2C19	nd	nd	-	nd	+
	2D6	(+)	(+)	(+)	(+)	(+)
	2E1	(+)	(+)	(+)	+	-
	3A5	(+)	-	(+)	(+)	++
	3A7	(+)	-	-	-	-
Esterases	AADAC	nd	nd	++	nd	++
	CEL	nd	nd	(+)	nd	(+)
	ESD	nd	nd	++	nd	++
	CES1	nd	nd	++	nd	++
	CES2	nd	nd	++	nd	++
	ACHE	nd	nd	-	nd	-
	PLA2G4B	nd	nd	++	nd	++

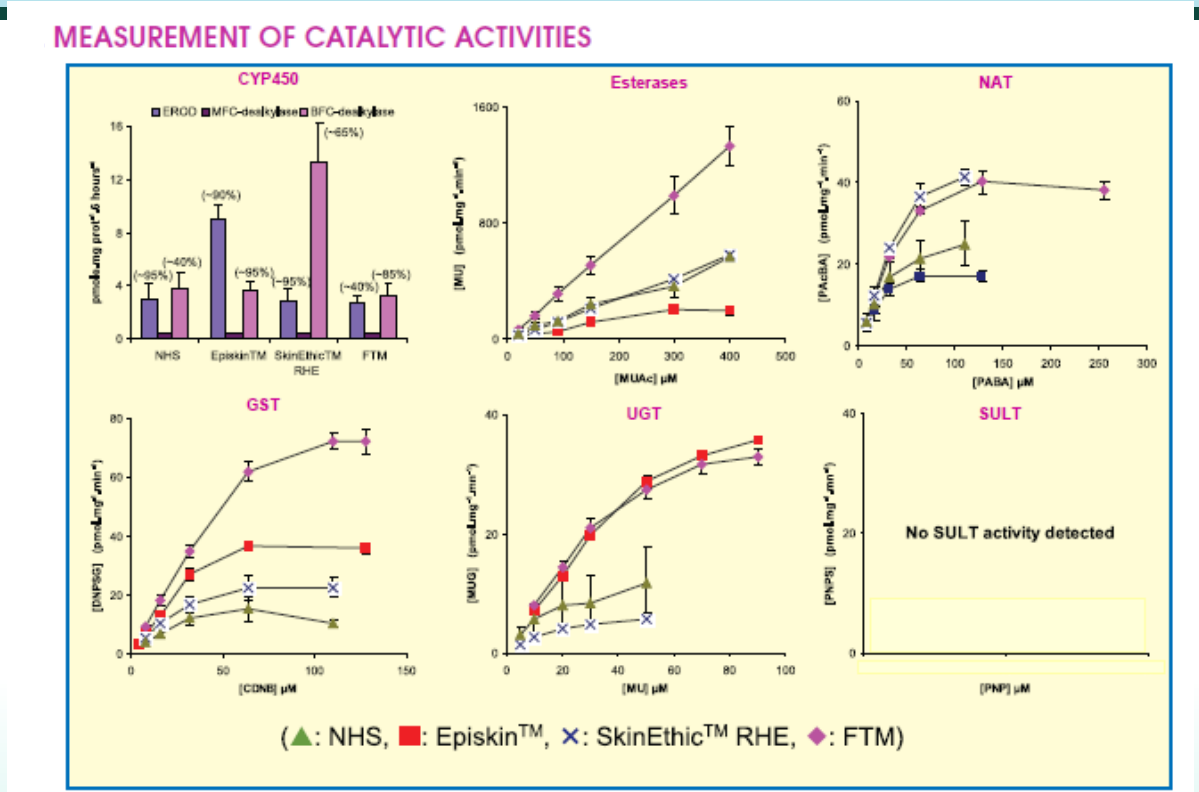
Enzymes	Isoformes	NHS		RECONSTRUCTED HUMAN SKIN MODELS		
		Epidermis	Dermis	Episkin™	FTM	SkinEthic™ RHE
NAT	NAT1	(+)	(+)	++	(+)	++
	NAT2	-	-	-	-	-
	NAT5	++	++	++	++	++
GST	GSTA3	++	(+)	-	nd	(+)
	GSTA4	nd	nd	++	nd	++
	GSTM2	++	++	(+)	nd	++
	GSTM3	++	++	(+)	nd	(+)
	GSTM5	(+)	++	nd	-	(+)
	GSTP1	+++	+++	++++	++++	+++
	GSTT1	++	++	++	++	++
	GSTZ1	nd	nd	++	nd	++

Enzyme Expression *Ex Vivo*/*In Vitro*

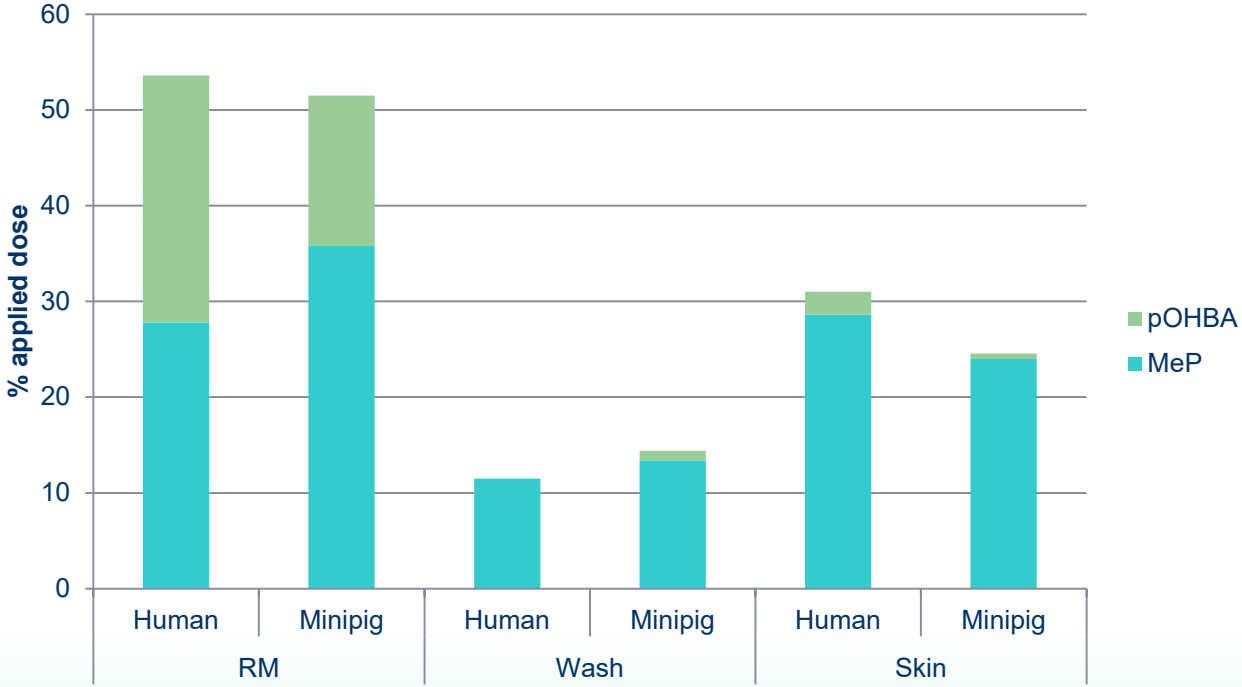
Enzymes	Isoformes	NHS		RECONSTRUCTED HUMAN SKIN MODELS		
		Epidermis	Dermis	Episkin™	FTM	SkinEthic™ RHE
UGT	UGT1A1	nd	nd	-	nd	-
	UGT1A3	nd	nd	++	nd	++
	UGT1A4	nd	nd	(+)	nd	(+)
	UGT1A5	nd	nd	++	nd	++
	UGT1A6	++	(+)	++	nd	++
	UGT1A7	nd	nd	++	nd	++
	UGT1A8	nd	nd	++	nd	++
	UGT1A9	nd	nd	-	nd	-
	UGT1A10	(+)	(+)	++	(+)	++
	UGT2B17	-	(+)	-	-	-
	UGT2B28	-	(+)	-	-	-
SULT	SULT1A1	++	++	(+)	++	(+)
	SULT1E1	(+)	(+)	++	++	++
	SULT2A1	-	-	-	-	-
	SULT4A1	nd	nd	-	nd	(+)
	SULT2B1	+++	++	+++	+++	+++

(Phase II enzymes Eilstein et al., 2014)

Enzyme Activities *Ex Vivo*/*In Vitro*



Metabolism of Parabens in *Ex Vivo* Skin (Jewell et al, 2007)



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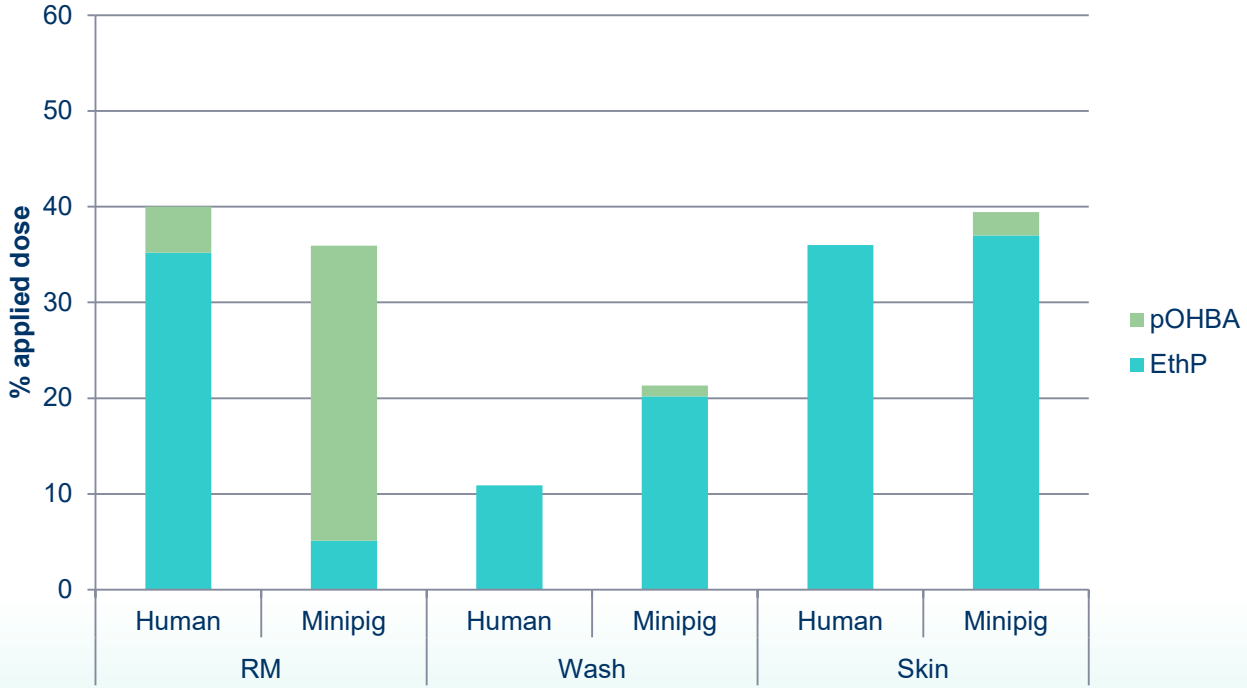

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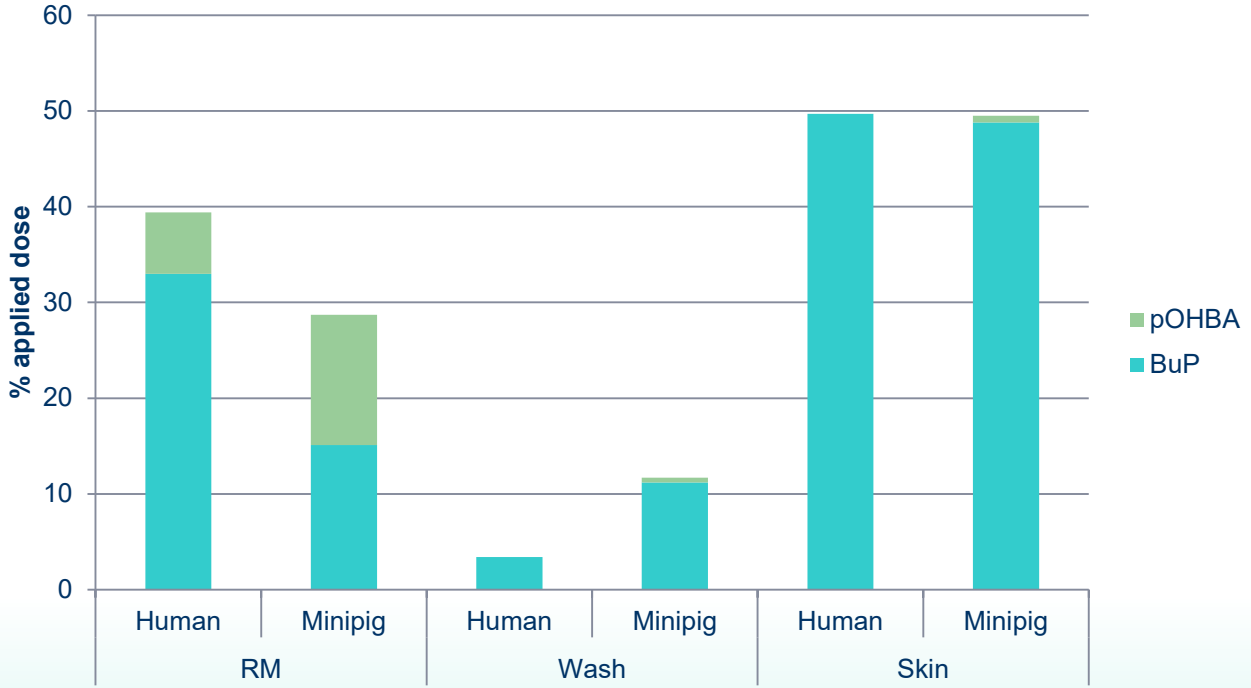
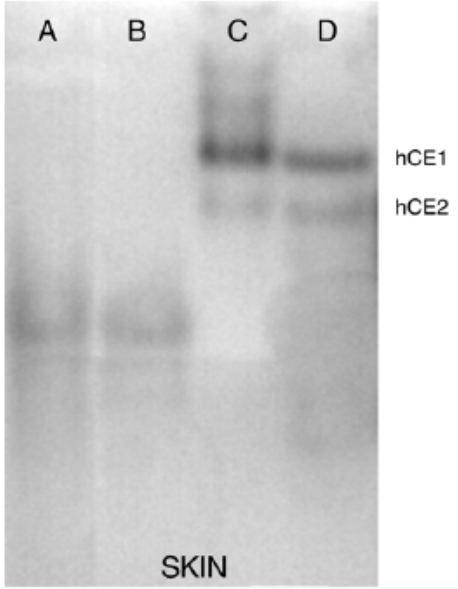


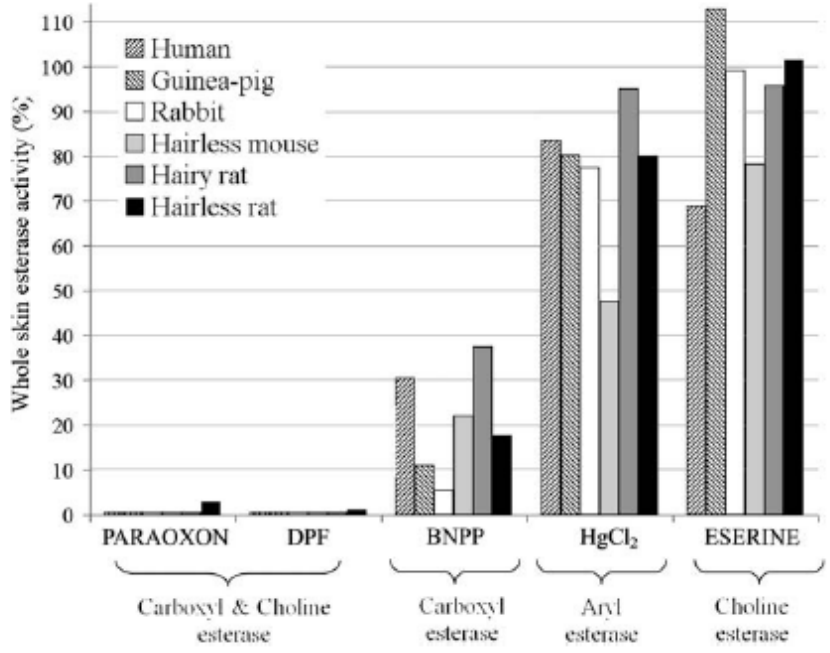
Native Gels Stained for Esterase Activity (Jewell et al 2007)



A Minipig microsomes
B Minipig cytosol

C Human microsomes
D Human cytosol

Metabolism of Dibutyl Phthalate by Filtered Skin Homogenates



Beydon et al.
Toxicology in Vitro
24 (2010) 71–78

DBP 100 μM
Inhibitor 10 μM

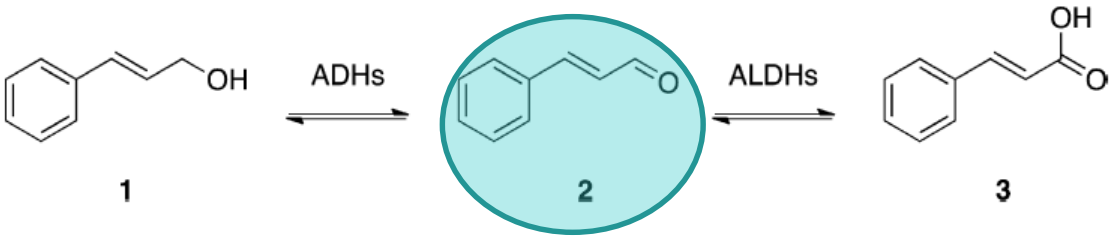
How Can Cutaneous Metabolism Potentially Contribute to "Local" Toxicity?

- Generation of haptens from pro-haptens
  - Skin sensitisation
- Generation of genotoxins from pro-genotoxins
  - Carcinogenesis
- Others
  - Generation of ROS
  - Perturbation of steroidogenesis

Key Steps in the Sensitisation Pathway

- Skin penetration
- Electrophilic substance
  - Direct, autooxidation or metabolic activation
- Haptenation
  - Covalent modification of epidermal proteins
- Activation of keratinocytes and dendritic cells
- Presentation of haptenated protein by dendritic cell
  - Activation and proliferation of T cells
- ACD: inflammation resulting from T cell-mediated cell death

Cinnamyl Alcohol – a Prohapten?



Cinnamyl alcohol

Cinnamaldehyde

Cinnamic acid

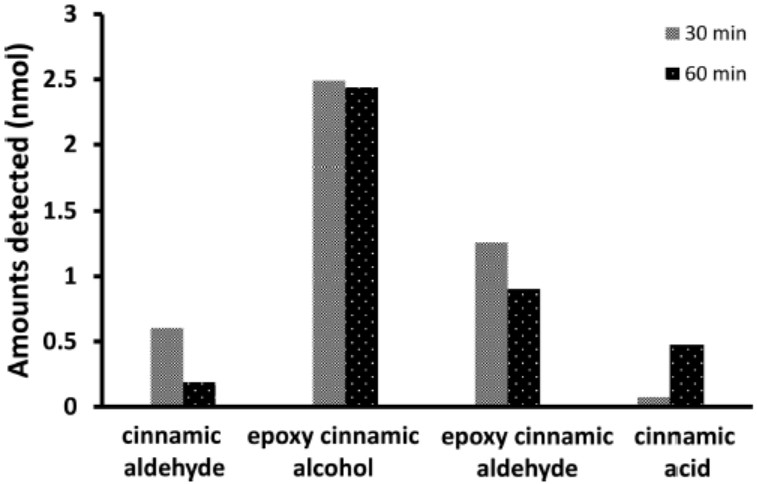
Cinnamyl Alcohol – a Prohapten? Maybe…But…

- More frequent positive patch tests to the alcohol than to the aldehyde when at the same concentration
- Conversion of cinnamyl alcohol to the acid AND reduction of cinnamaldehyde to cinnamyl alcohol have been observed in *ex vivo* skin models (dermatomed and full thickness) but formation of cinnamaldehyde from cinnanyl alcohol has not been demonstrated definitively.
- How could it? Traditional methods rely on diffusion of metabolites from skin tissue. Reactive metabolites will almost certainly not behave in that way.

Cinnamyl Alcohol – Maybe But Not Via Cinnamaldehyde?

- Moss et al 2016 Chem Res Toxicol. 29; 1172-1178
- Combined the use of reconstructed human epidermis (SkinEthic) and high resolution magic angle spinning (HRMAS) nuclear magnetic resonance (NMR) to observe *in situ* and non-invasively chemical interactions between reactive skin sensitizers and nucleophilic residues on amino acids
- No conversion of Cinnamyl alcohol to cinnamaldehyde was detected, though ADH and ALDH activities were confirmed

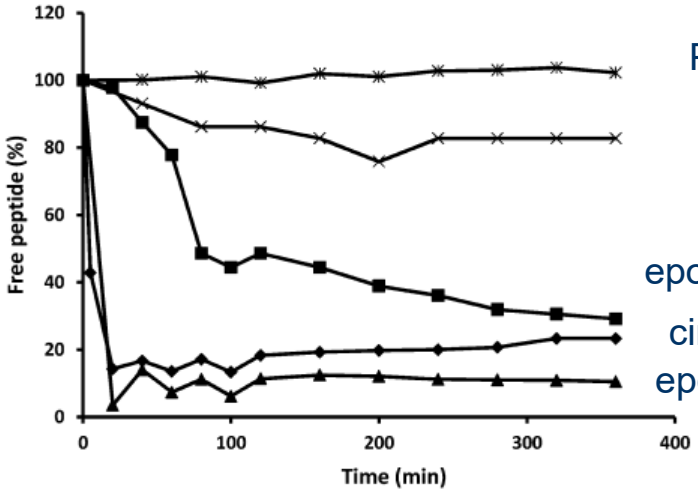
Cinnamyl Alcohol – a Prohapten?



Niklasson et al 2014 Chem Res Toxicol. 27; 568-575
10 μM cinnamyl alcohol incubated with liver microsomes (0.5 mg protein)

Cinnamyl Alcohol – a Prohapten?

Niklasson et al 2014 Chem Res Toxicol. 27; 568-575
Reactivity of compounds: depletion of a model peptide



Purified Cinnamyl Alcohol

Technical grade Cinnamyl Alcohol

epoxy Cinnamyl Alcohol

cinnamaldehyde

epoxycinnamaldehyde

Peptide Reactivity: DPRA

Direct peptide reactivity Assay

- Relies on measurement of depletion of model peptides containing either cysteine (10:1 ratio) or lysine (50:1 ratio) by LC UV or LCMS
- Single 24 h exposure
- % depletion relative to vehicle control
- Prediction of reactivity class:

Mean of cysteine and lysine depletion	Reactivity class	Prediction
0% - 6.38%	Minimal reactivity	Non-sensitiser
6.38% - 22.62%	Low reactivity	Sensitiser
22.62% - 42.47%	Moderate reactivity	Sensitiser
42.47% - 100%	High reactivity	Sensitiser

PPRA – Peroxide Peptide Reactivity Assay

- Designed to better identify pre- and pro-haptens

A range of concentrations of chemical measured (only one used for conventional DPRA)

Cysteine peptide +/- horse radish peroxidase/H_2O_2
Max chemical concn 5 mM

Lysine peptide no HRP/H_2O_2

Max chemical concn 25 mM

EC25 calculated using 3 parameter logistic model; EC25=>0.1 mM are “reactive;” <0.1 mM are “highly reactive.”

Cell-Based Detection of Peptide Reactivity

- KeratinosensTM Assay
  - HaCat cell with Nrf2-Keap1-ARE reporter gene
  - “Metabolically competent”
  - Combination of fold induction and viability used to classify compounds
  - OECD TG 442d adopted Feb 2015
- How metabolically competent is this model?

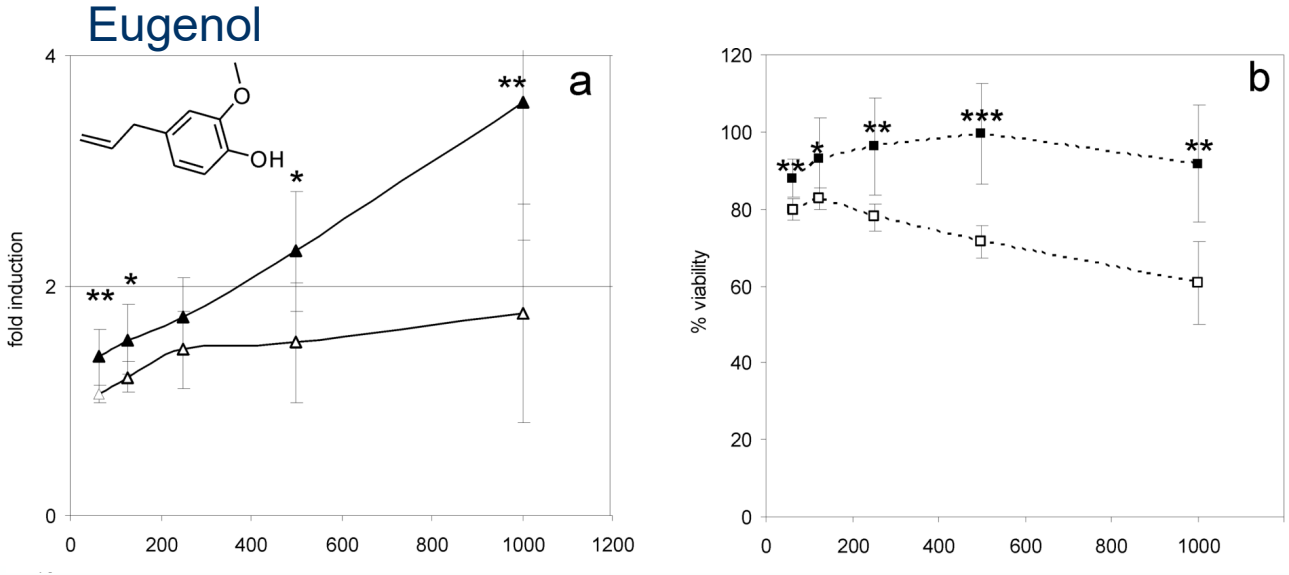
Fabian et al. Arch Toxicol (2013) 87:1683–1696

- Assessed expression and activity of XMEs in Keratinosens™ cell line (inter alia)
  - CYP, FMO, ADH and UGT activities “below LOD”
  - Measurable ALDH activity and esterase activity in Keratinosens cell line
  - Also measurable levels of NAT-1 activity
- mRNA detected for CYP1B1 (CYP1A1 and 2E1)
- Also GSTp1, UGT1A10, ALDH1A1, and 2

Keratinosens S9 Assay
(Natsch and Haupt, Toxicol Sci 135, 356–368 2013)

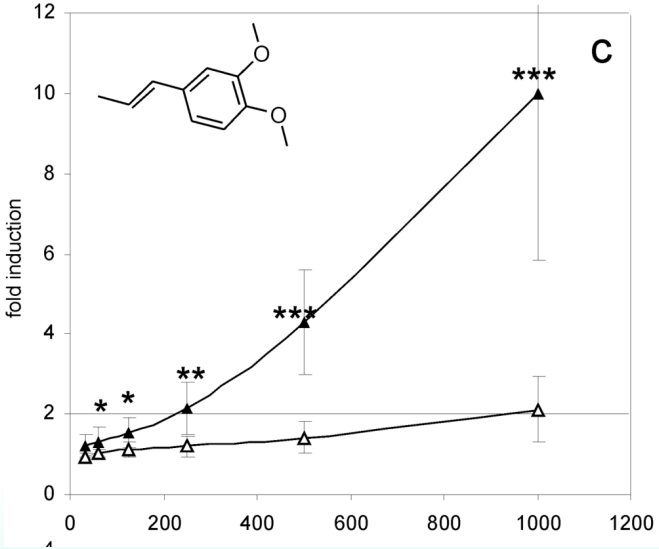
- Used a combination of Keratinosens Assay and Aroclor induced S9
- Ten compounds identified as putative pro-haptens showed an enhanced signal in presence of S9
- Only four non-pro-haptens initially classified as non-sensitisers in absence of S9 were re-classified in new test
- Co-incubation with CYP inhibitors had mixed results.

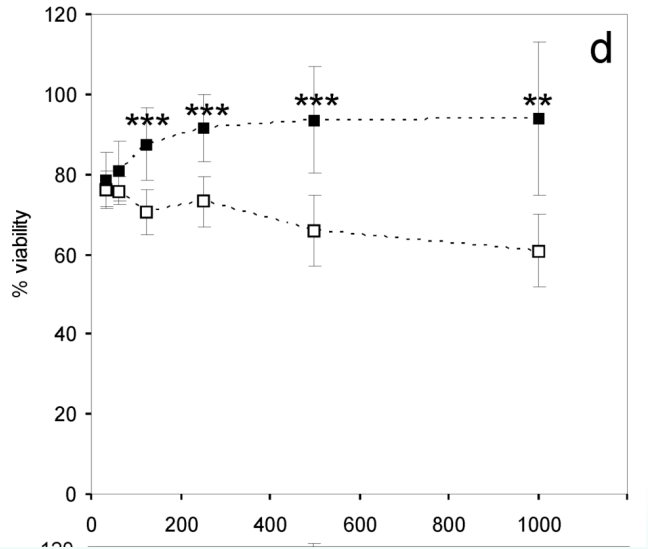
Keratinosens S9 Assay
(Natsch and Haupt, Toxicol Sci 135, 356–368 2013)



Keratinosens S9 Assay
(Natsch and Haupt, Toxicol Sci 135, 356–368 2013)

Methyl iso-eugenol





Keratinosens S9 Assay
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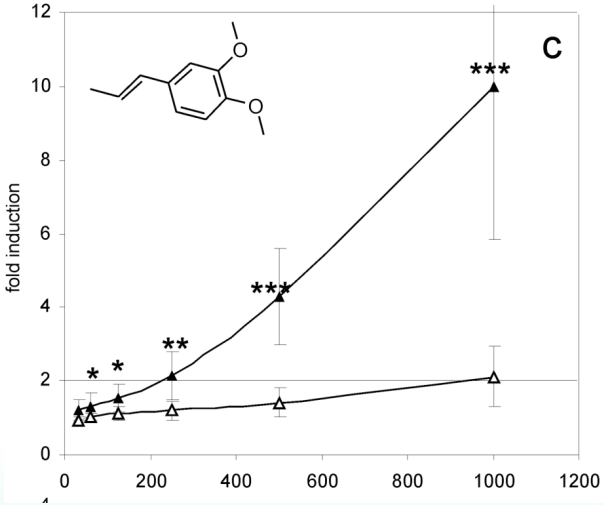
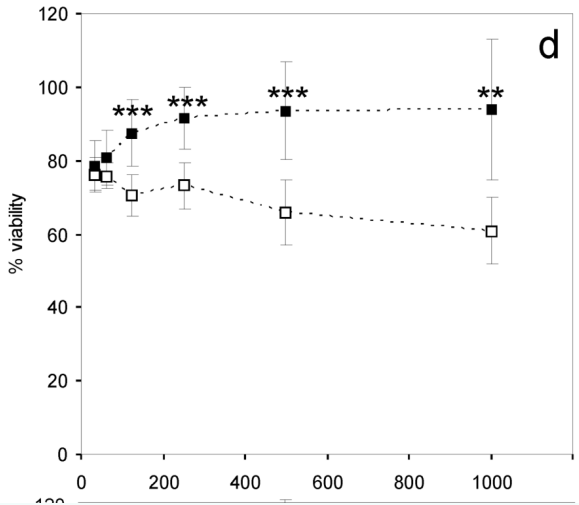
Iso-eugenol (pre-hapten)





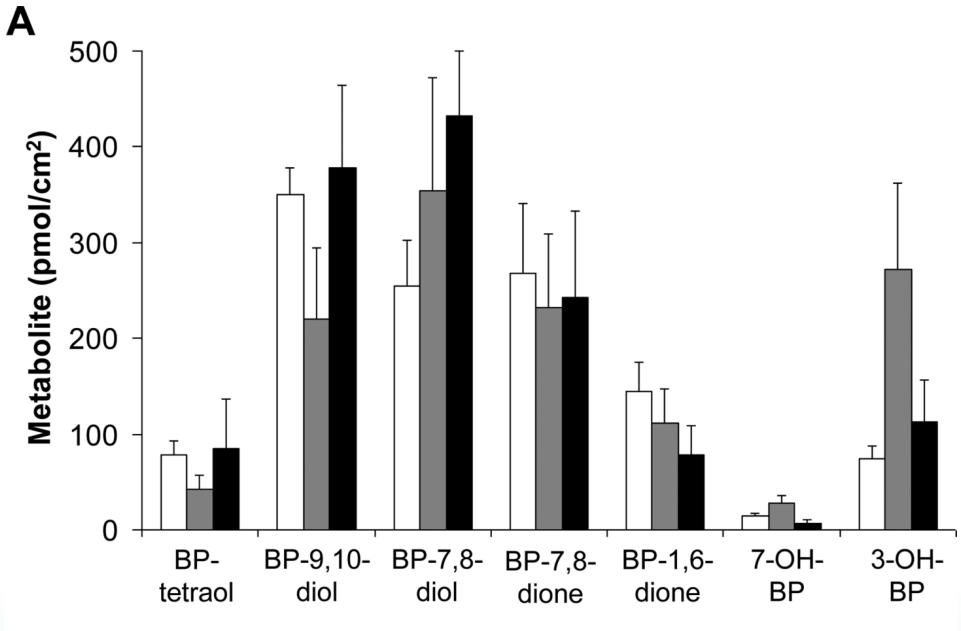
Metabolism of Pro-Genotoxins

Brinkmann et al. Toxicol Sci 131(2), 351–359 2013

- Compared metabolic activity towards Benzo[a]pyrene in *ex vivo* skin, 2D and 3D models
- Analysed metabolite formation by LCMSMS
  - All models were competent to metabolise BaP
  - Seven metabolites were generated, profiles remarkably similar
  - Genotoxicity of these metabolites in skin models was assessed using Comet assay

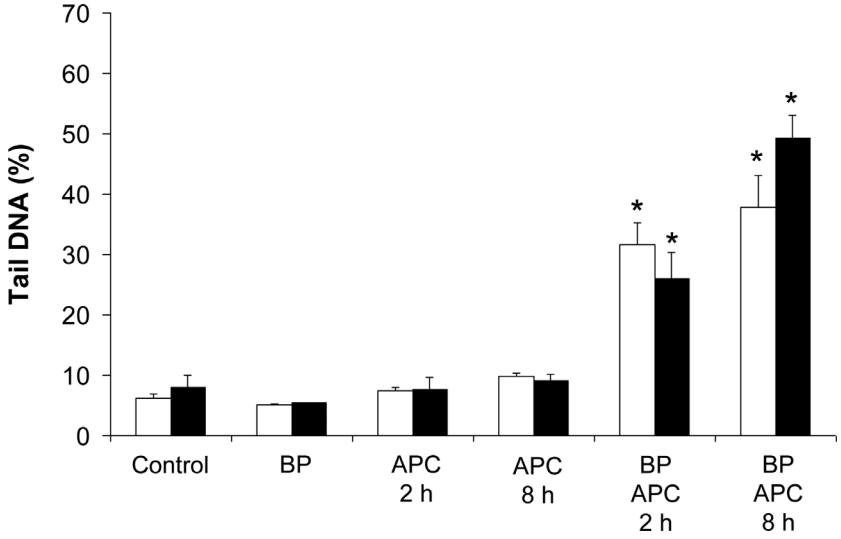
Metabolism of B[a]P (50 nmol/cm^2 in Acetone) by EpiDerm, EpiDerm FT and *Ex Vivo*

Brinkmann et al. Toxicol Sci 131(2), 351–359 2013



Genotoxicity in Keratinocytes (□) and Fibroblasts (■) of EpiDerm FT (50 nmol/cm^2 B[a]P for 48 h)

Brinkmann et al. Toxicol Sci 131(2), 351–359 2013



Aphidicolin (APC), a DNA repair inhibitor, was added to culture medium 2 or 8 h before completion

Summary

- A considerable amount of research into cutaneous enzyme systems has been carried out in recent years, especially in response to legislation regarding *in vivo* testing of cosmetics.
- The metabolic competence of a number of *ex vivo* and *in vitro* skin models has been evaluated over the last few years, and we have some measures of inter-individual variability of enzyme expression and activity
- This will enable us to identify the ability of these models to detect pro-haptens and pro-genotoxins, and identify where gaps remain
- There is still much basic science to be done

References

- Eilstein J, et al (2014) Comparison of xenobiotic metabolizing enzyme activities in *ex vivo* human skin and reconstructed human skin models from SkinEthic. Archives of Toxicology 88, 1681-1694
- Jewell C, et al (2007) Hydrolysis of a series of parabens by skin microsomes and cytosol from human and minipigs and in whole skin in short-term culture. Toxicol Appl Pharmacol 225, 221-228
- Smith et al (2003) Cytochrome P450 Quantitative real-time reverse Transcription…J Invest Dermatol 121, 390-398
- Yengi et al (2003) Quantitation of cytochrome P450 mRNA levels in human skin. Anal Biochem 316, 103-110

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