Cutaneous metabolism – progress in the new Century

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Overview

- Importance in 2011
- Historical perspective (pre 2000)
- Cytochromes P450
- Esterases
- Other oxidoreductases
- Phase II enzymes
- Concerns and the future

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

What we knew up to ca 2000

- Cytochromes P450
  - mRNA: 1A1, 1B1, 2B6, 2E1, 3A4, 3A5
  - Protein: as above in humans, 1A1/2 in mice, 1A1, 1B1, 2E1, 3A1 in SD rats. 3A "constitutive" in HEKs, aromatase and 3A in ex vivo human skin/sebaceous glands
  - AhR, EROD, PROD activities detected
  - 1A1 in epidermis, others localised to basal layer, sebaceous glands, hair follicle cells

What we knew up to ca 2000

- Esterases
  - Histochemical and functional detection of esterase activity in numerous skin species. Easily released during homogensation
  - Cytosolic generally higher than microsomal
  - detected in all layers of skin (but less in dermis)
  - Importance during percutaneous absorption identified e.g. Fluazifop butyl

What we knew up to ca 2000

- Alcohol and aldehyde dehydrogenase
  - Catalytic activities (alcohol to carboxylic acid) reported in intact skin and subcellular fractions
  - Protein expression (ADH1, 2 and 3; ALDH 1 and 3) detected in skin by Western blotting
  - Histochemical localisation to epidermis and appendages. Little ADH2 detected.

What we knew up to ca. 2000

- Flavin-containing monooxygenases
  - Very few reports in skin
- NAD(P)H Quinone oxidoreductases
  - Detected in rodent epidermal cytosol at higher levels than in liver
  - Inducible by substrates and 3-MC
  - Easily detectable and inducible in keratinocytes in culture

What we knew up to ca. 2000

- Phase 2 enzymes
  - Glutathione transferases (mainly pi isoform)
  - Sulphotransferases (isoforms?)
  - Glucuronyl transferases – range of substrates reportedly conjugated
  - N-acetyl transferases (NAT-1)
    - Rapid N-acetylation of aromatic amines

CYP activity 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
- Yengi et al 2003
  - Main isoforms expressed were 1B1, 2B6, 2D6, 3A4 (2C18, 2C19, 3A5)

CYP expression and activity in human in vitro "skin equivalent" models

- In EpiDerm, 1B1, 2C19, 2D6, 3A4 (weak) and 3A5 constitutively expressed.
- Both 1A1 and 1B1 expression and EROD activity enhanced with 3-methylcholanthrene Hayden et al 2008 The Toxicologist S895
- 1A1, 1B1, 2E1, 2C and 3A5 in four types of organotypic skin model (1A1, 1B1 inducible) Neis et al. 2010 Skin Pharm Physiol 23, 29-39

CYP expression and activity in human in vitro “skin equivalent” models

- Expression of 87% of genes consistent between EpiDerm and ex vivo human skin.
- Basal expression of CYP in Epiderm low but highly inducible with 3-MC

  Hu et al. 2010 Toxicol In Vitro 24, 1450-1463
- Episkin and Full Thickness Episkin showed lower CYP (and FMO) expression that ex vivo epidermis/dermis

  Luu-The et al. 2009 J Steroid Biochem Mol Biol 116, 178-186

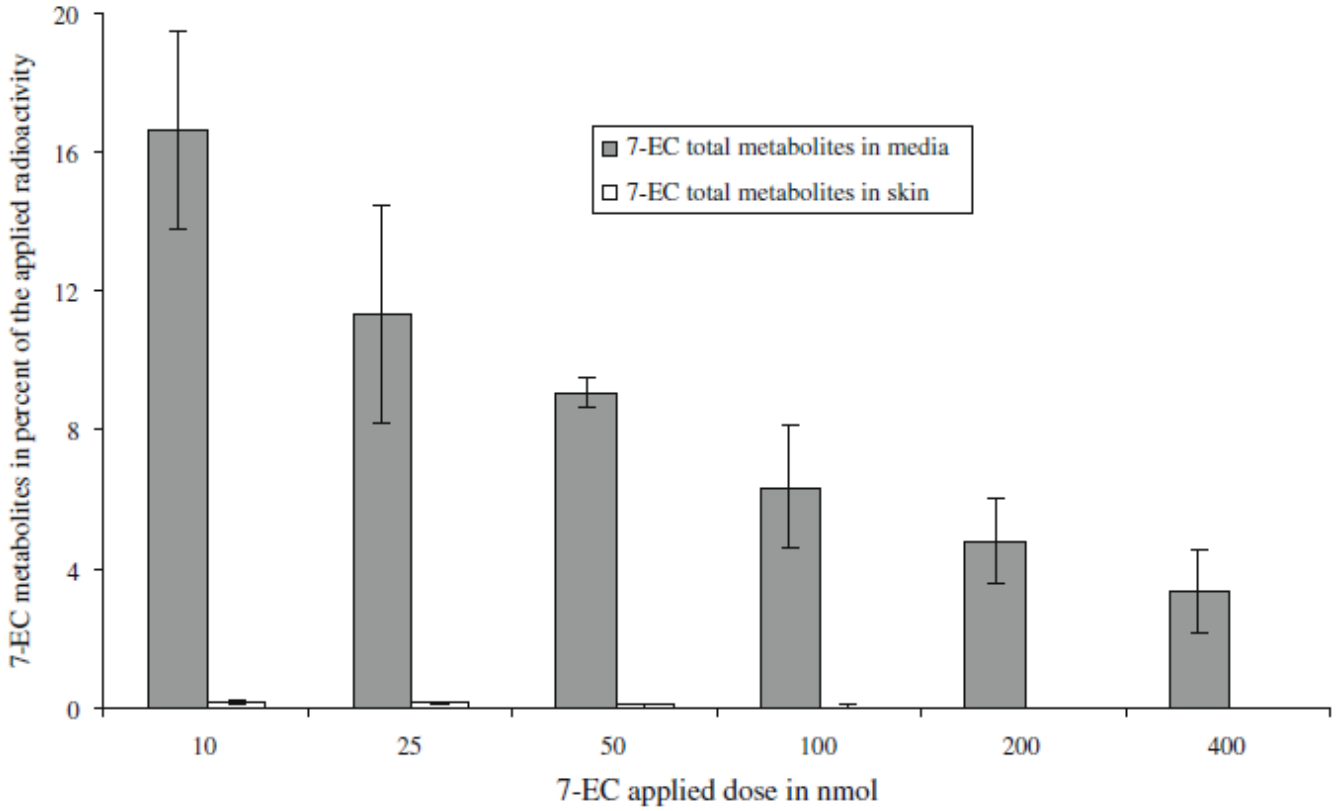
CYP expression and activity in human in vitro “skin equivalent” models

- CYP1A, 1B and 3A family activities detected in EpiSkin using probe substrates.
- 2B6, 2E1, 2C18 detected but too low to quantify. No correlation between mRNA expression and catalytic activity

  Eilstein et al. 2010 Toxicol In Vitro 24, 1450-1463
- Activity “good correlation with human skin”

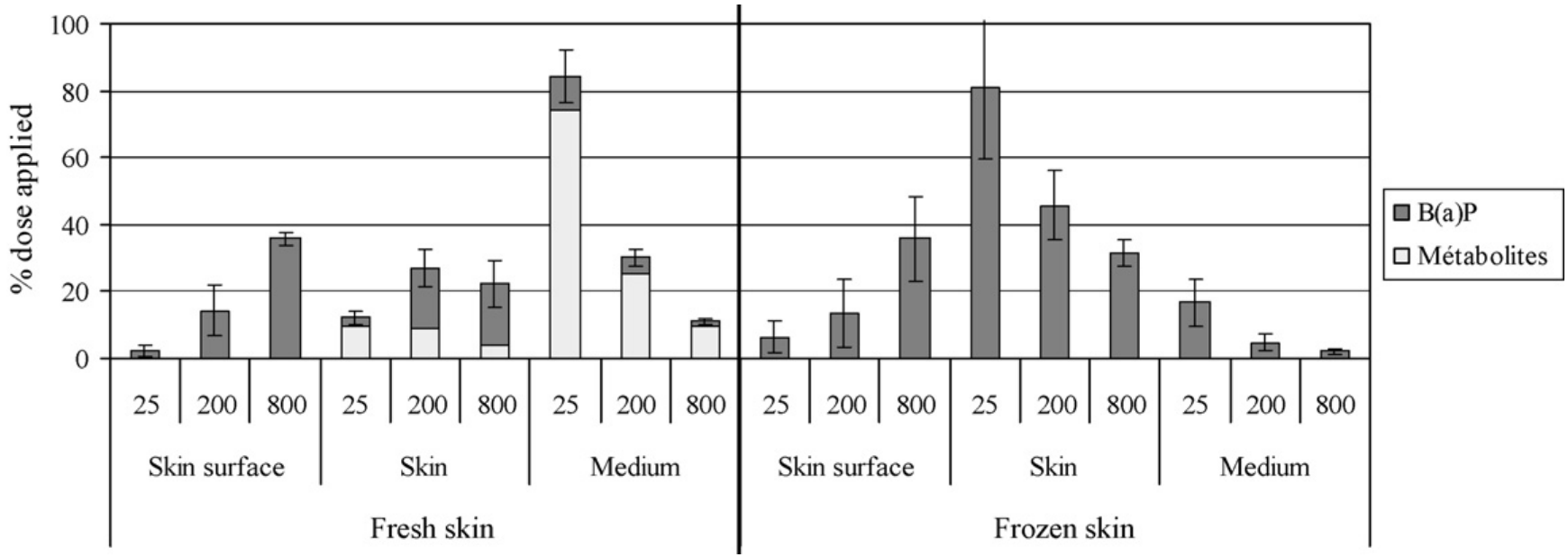
  Gotz et al 2010 Toxicol Lett 196S S145

Consequences of CYP activity in ex vivo pig skin – 7-ethoxycoumarin



Jacques et al. 2010 Toxicol in Vitro 24, 1426

Benzo[a]pyrene in pig skin



Parent and metabolites after 72 h in organotypic culture
Jacques et al. (2010) Toxicology Letters *199 22–33*

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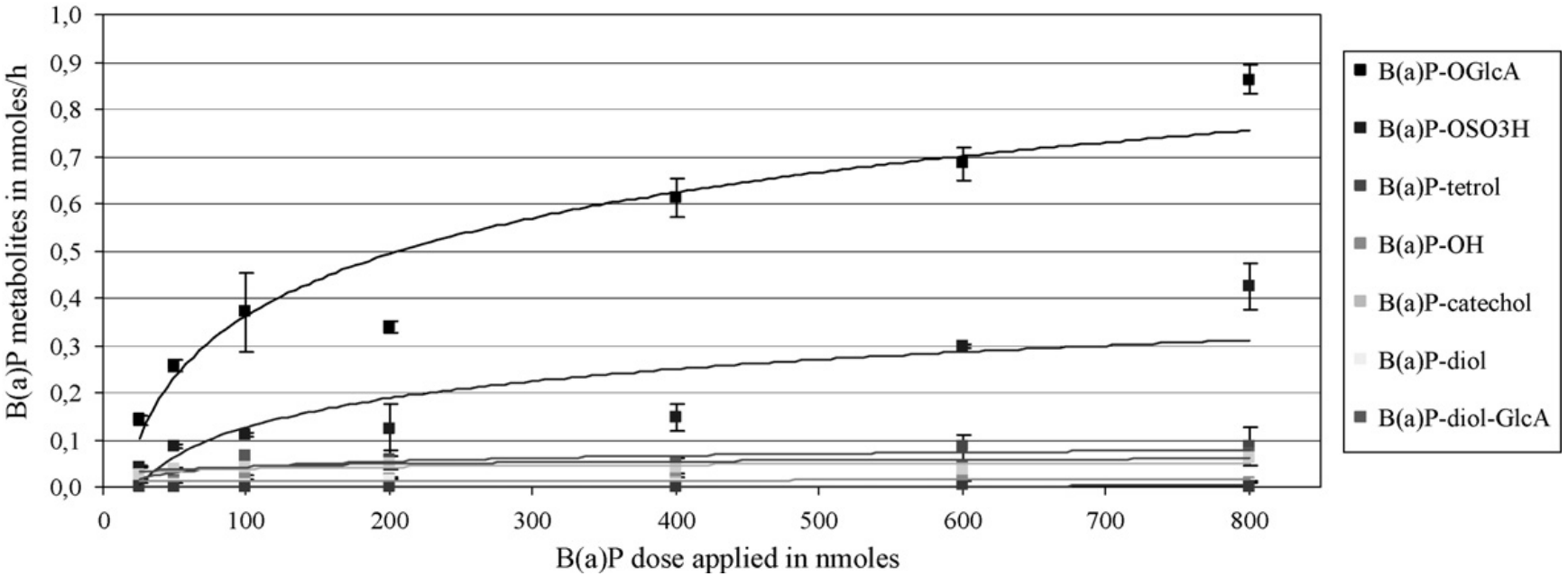
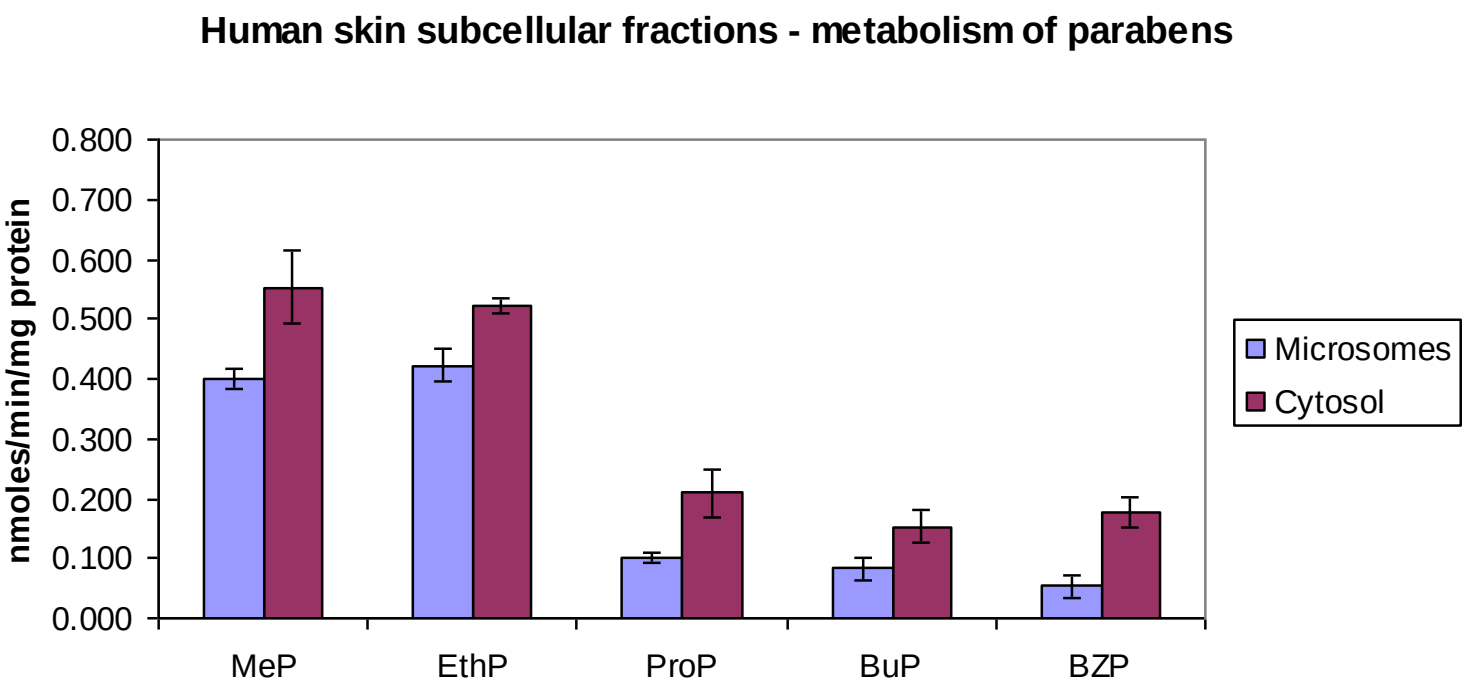


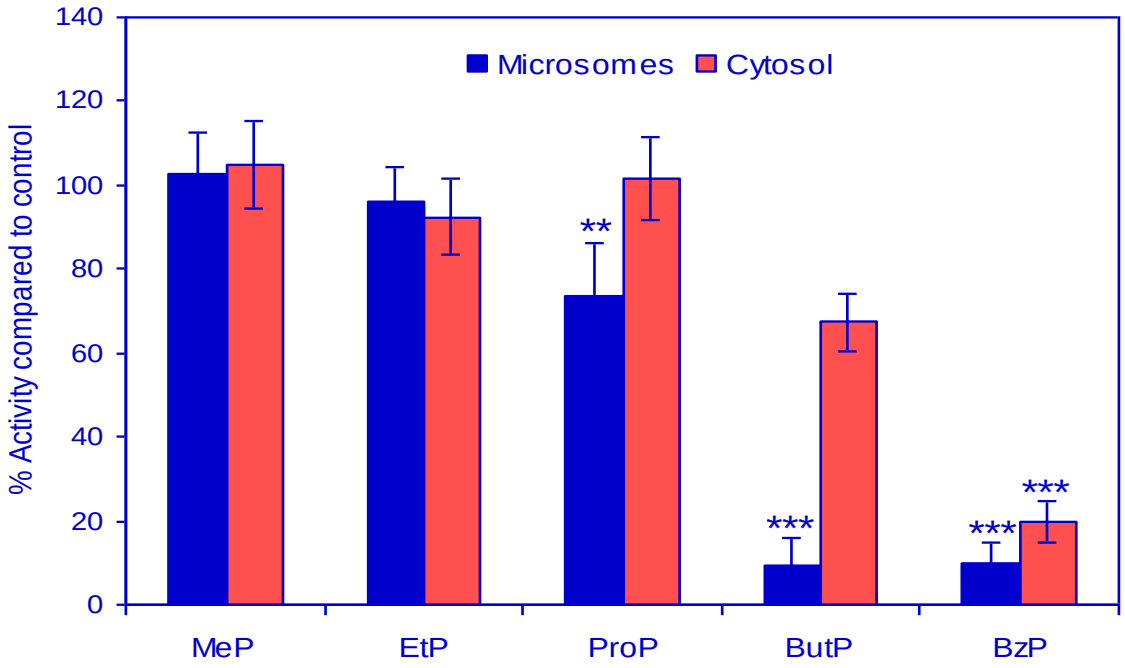
Metabolites in culture medium after 72 h in organotypic culture
Jacques et al. (2010) Toxicology Letters *199 22–33*

Esterase expression and activity



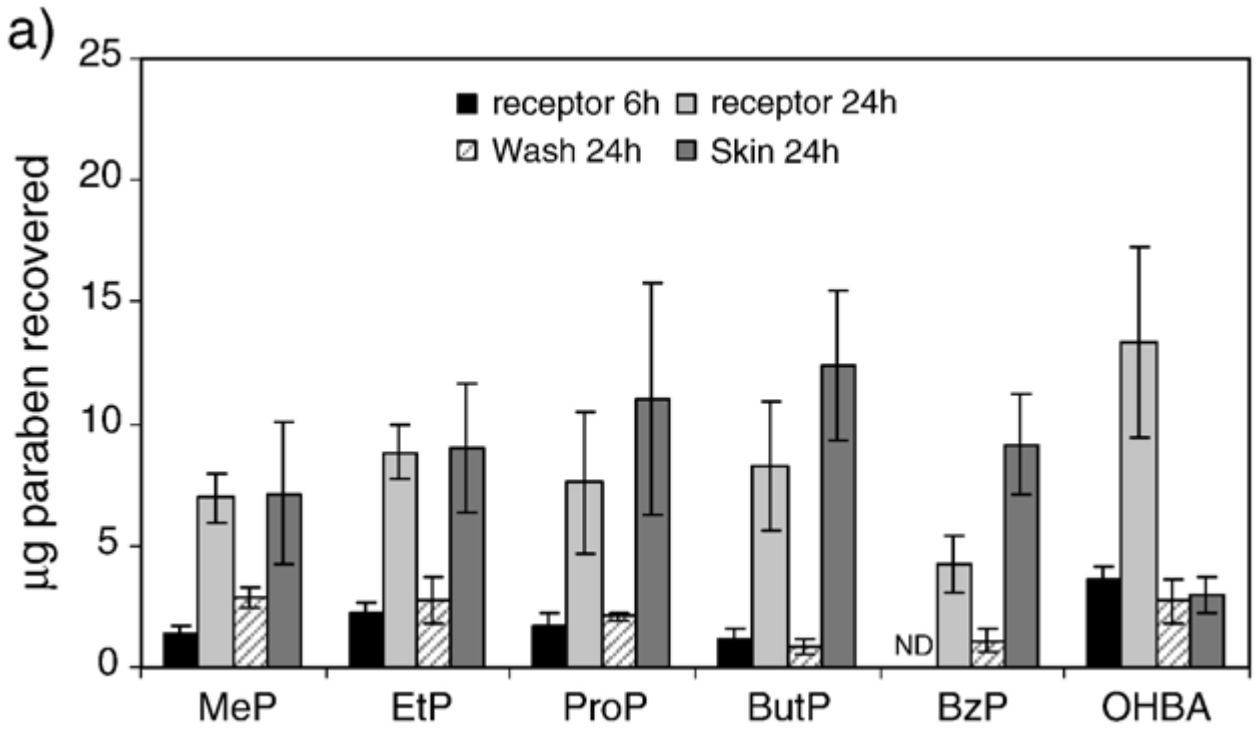
Jewell et al 2007 Toxicol Appl Pharmacol 225, 1-22

Inhibition of paraben hydrolysis by loperamide (hCE2 inhibitor)

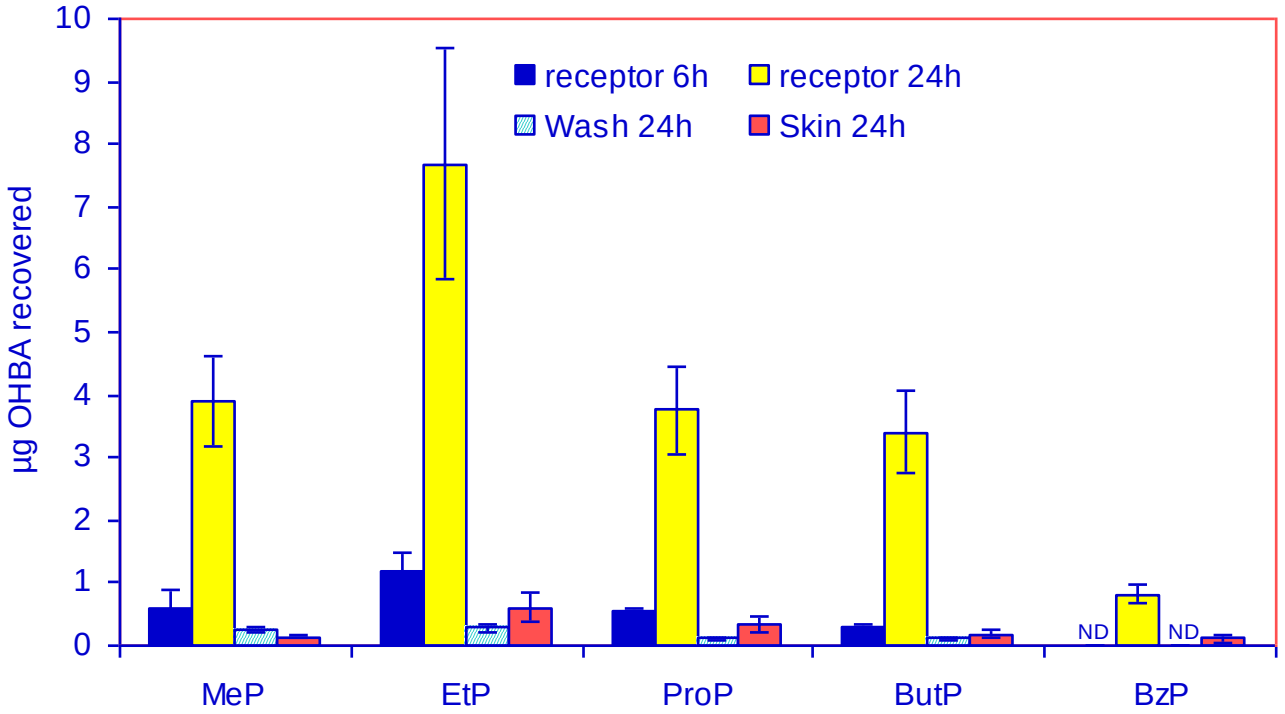


Jewell et al 2007 Toxicol Appl Pharmacol 225, 1-22

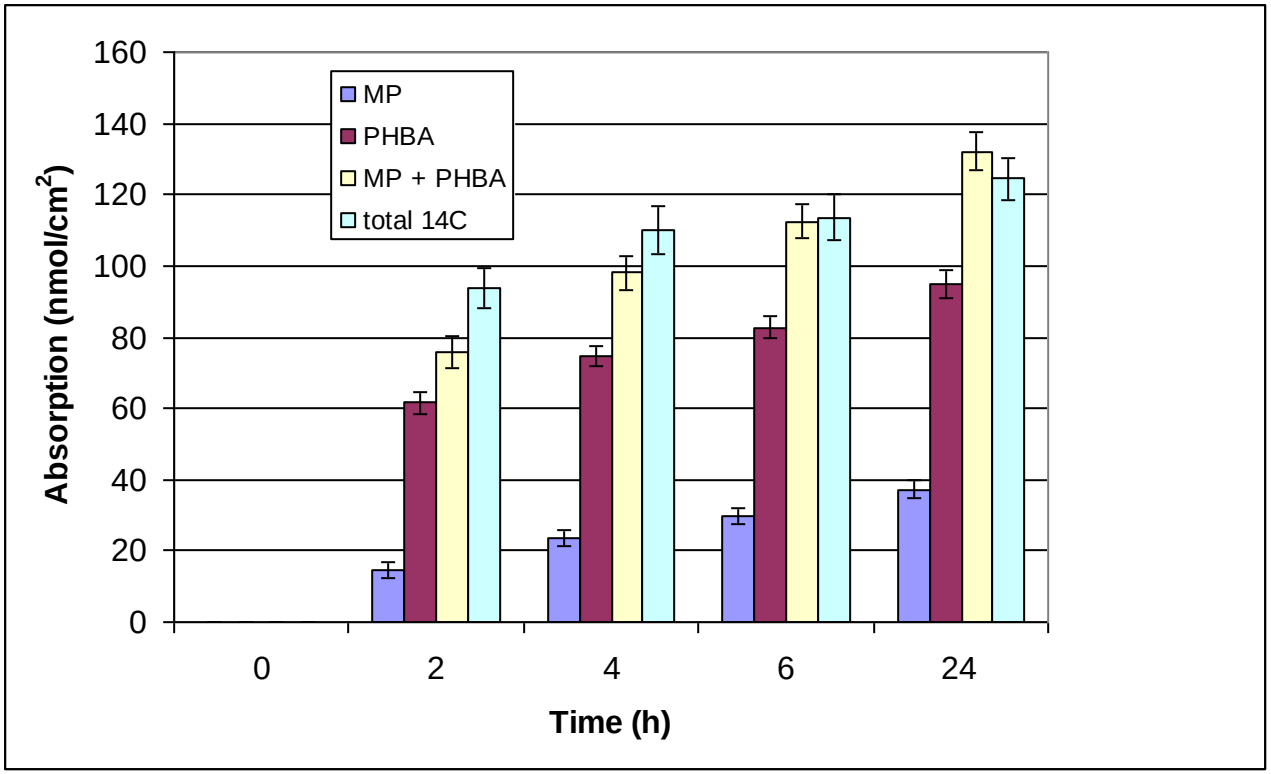
Distribution of parabens in human skin



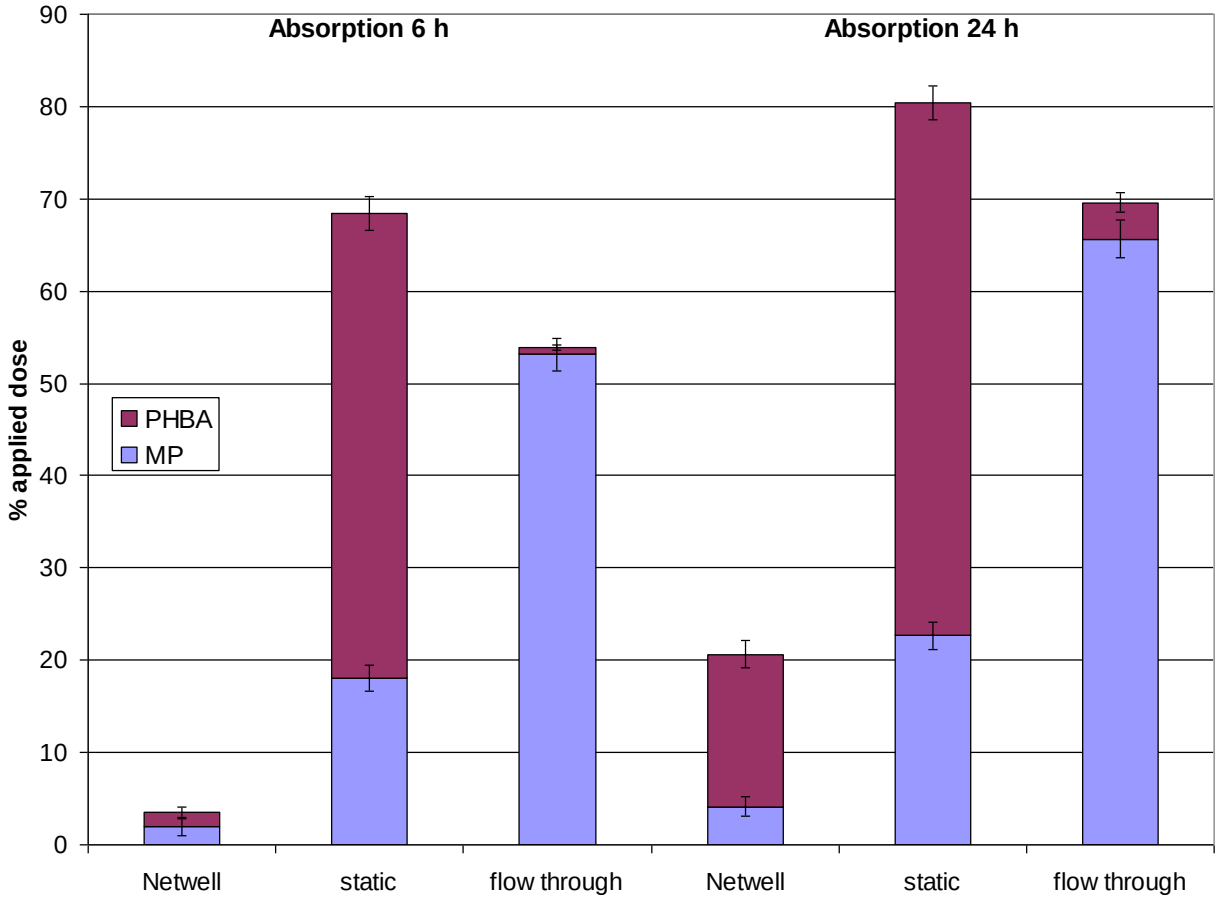
Formation of pOHBA from parabens in human skin



Absorption and metabolism of MP in static cells



Static cells and short term culture versus flow through cells



Esterase activity in skin equivalents

- Glyceryl arachidonate metabolised to arachidonic acid in Epiderm (3% of applied dose), but penetration rate much higher than in viable human skin
- Esterase expresion (mRNA) and activities (4-methylumbelliferyl acetate) measured at comparable levels in human skin, Episkin and Skin Ethic RHE
- hCE-1 not expressed in HaCaTs, hCE-2 extensively expressed

Esterase activity in skin equivalents

- Comparison of models (epidermis full thickness) with ex vivo human skin
- Activity towards prednicarbate and FDA
- Prednisolone the main metabolite after 24 h, epidermis≈FT>ex vivo skin
- Similar pattern in V_{max} towards FDA, K_m not different between models

Klipper et al (2010) J Invest Dermatol 130 S31

Ester Prodrugs

- Esmolol proprionate – higher flux and greater in vivo effect
- N-monoalkyl carabamate prodrugs of NTX more hydrolysed that N,N' dialkyl prodrugs
- Similar conversion of NTX prodrugs on EpiDerm and human skin – valerate extensively hydrolysed to NTX in both systems

Other oxidoreductases

- ADH isoforms in skin differ from liver (methyl pyrazole inhibition, substrate selectivity); species differences
- ALDH 1A3 involved in retinoic acid metabolism, upregulated by RA in keratinocytes, skin equivalent cultures and ex vivo skin but not in fibroblasts
- Under control of AhR
- ALDH1A3 overexpressed in Epiderm cf buttock skin

Other oxidoreductases

- FMO 1 mRNA detected all human skin biopsies tested, FMO 5 in 7/8, FMO 3 and 4 in 50%
- In cultured keratinocytes, FMO 1 not detected, FMO 3, 4, 5 markedly reduced
- HaCaT cells: FMO 1 absent, FMO 4 threefold higher than human skin, FMO 3 and 5 similar to human skin. Role in adduction of dapsone and sulfomethoxazole?
- FMO 1 underexpressed, FMO 5 overexpressed in Epiderm

Phase 2 enzymes

- Gene expression of SULT2B1 (and 1E1), UGT1A8, 1A10 overexpressed in Epiderm
- GSTM 5, SULT1A1, 1A4 underexpressed
- UGT2A1, SULT1A2 absent in Epiderm, but expressed at mod. to low levels in ex vivo skin
- Considerable UGT activity detected in EpiDerm with good reproducibility

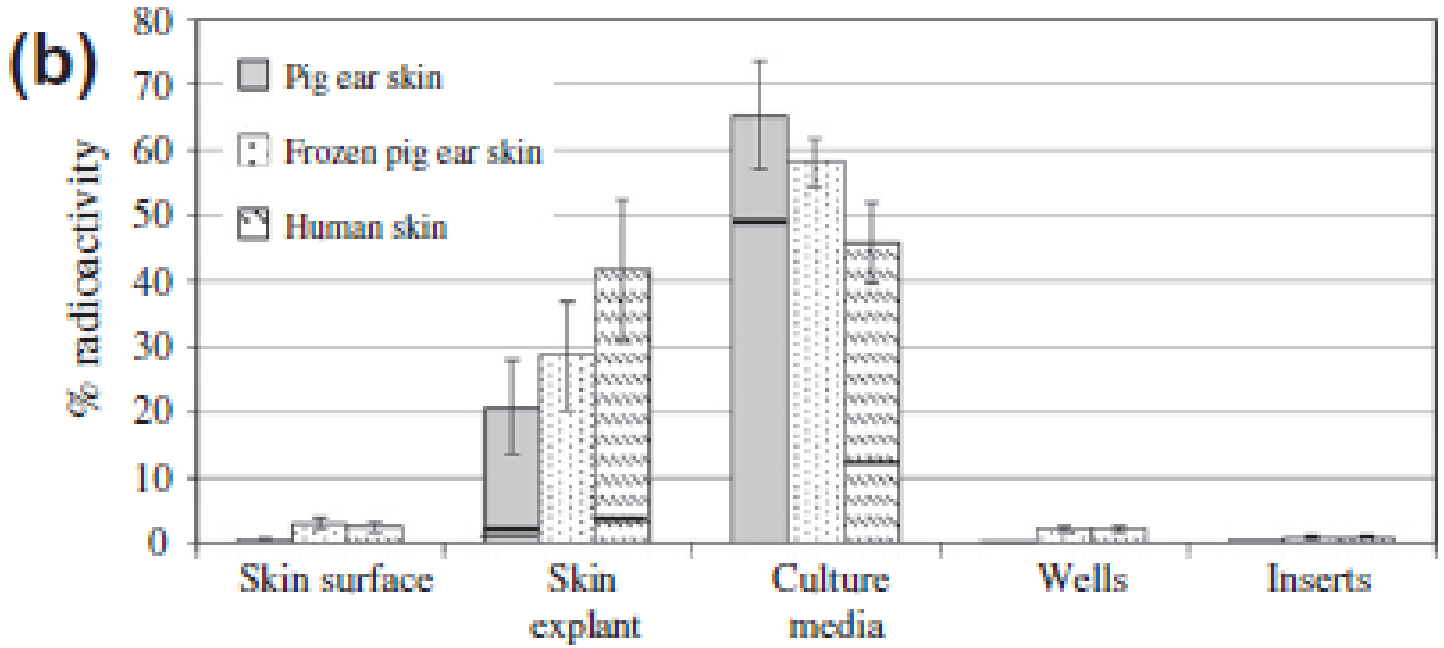
Phase 2 enzymes

- EpiSkin, Skin Ethic and normal human skin showed much lower UGT activity than esterase activity. SULT activity was undetectable with phenols but more strongly detected with steroids
- Several NAT (NAT1 and 5) and GST isoforms expressed in all three skin types; clearances between skins were “equivalent”, although kinetic parameters K_m and V_{max} differed. High variability between samples/donors.

Phase 2 enzymes

- High baseline catalytic activity for GST and UDP-GT detected in EpiDerm, not enhanced by 3-MC.
- Reconstructed epidermis quantitatively transformed p-aminophenol into its N-acetyl derivative, whilst p-phenylenediamine was transformed to mono- and di N,N' acetylated derivatives.
- NAT-1 activity in HaCaTs towards PABA 3.4 fold higher than NHEKs

Ex vivo conjugation of bisphenol A (50 nmol) in pig and human skin



Zalko et al. (2011) Chemosphere 82, 424-430

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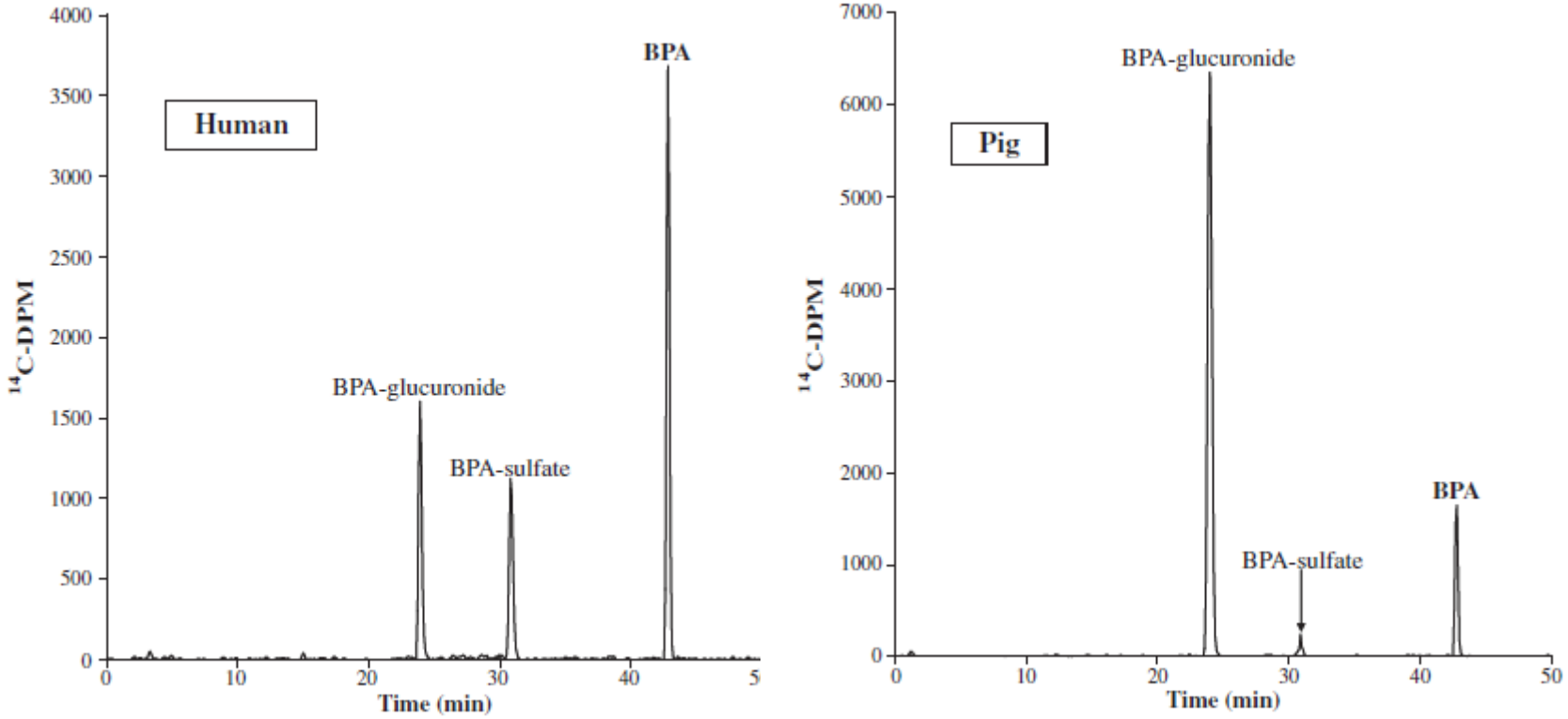
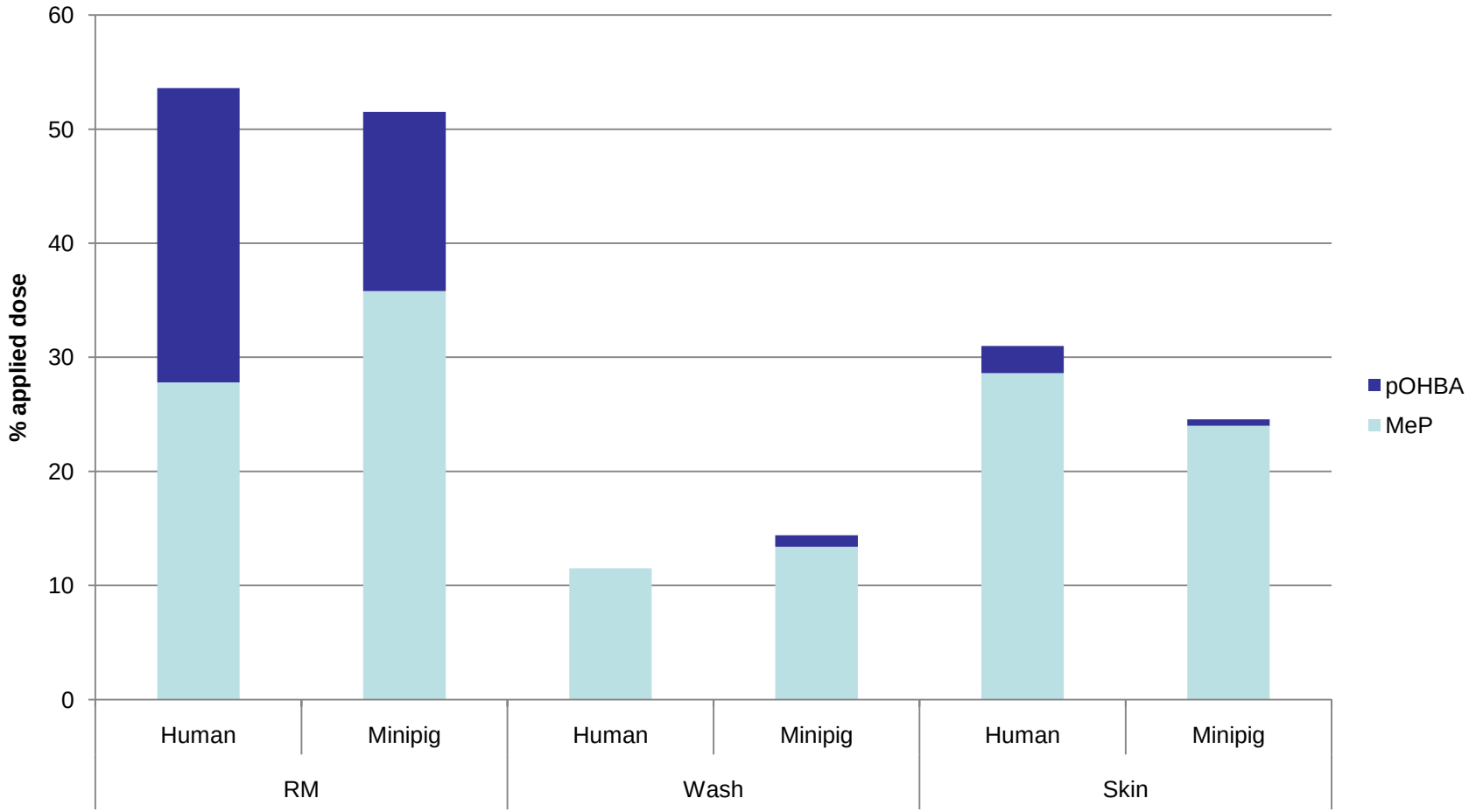


Zalko et al. (2011) Chemosphere 82, 424-430

Species differences

- GST activities in human skin subcellular fractions five fold higher than rat or minipig skin
- Esterase activity towards ethyl nicotinate high in rodent spp, much lower in human skin

Metabolism of parabens



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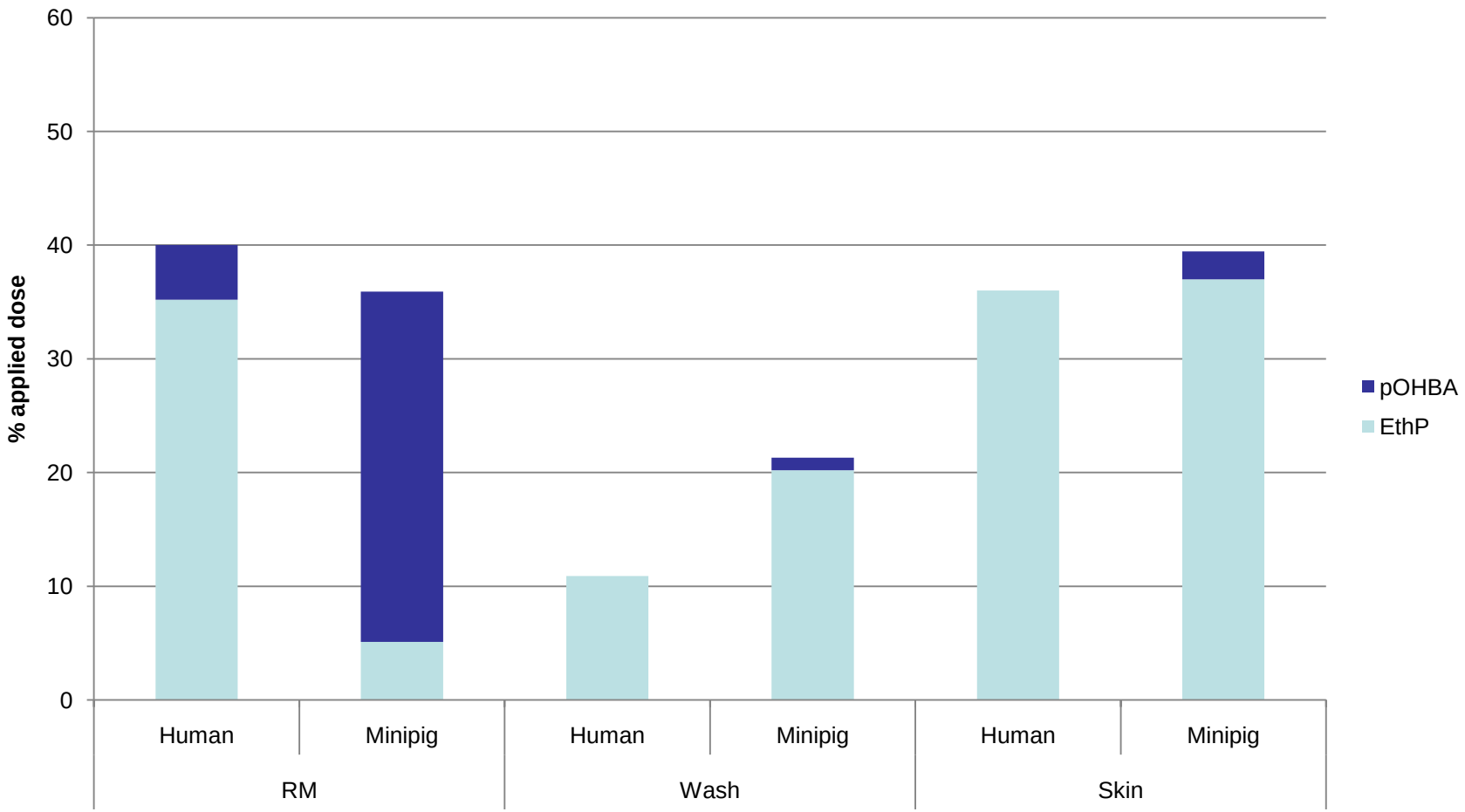

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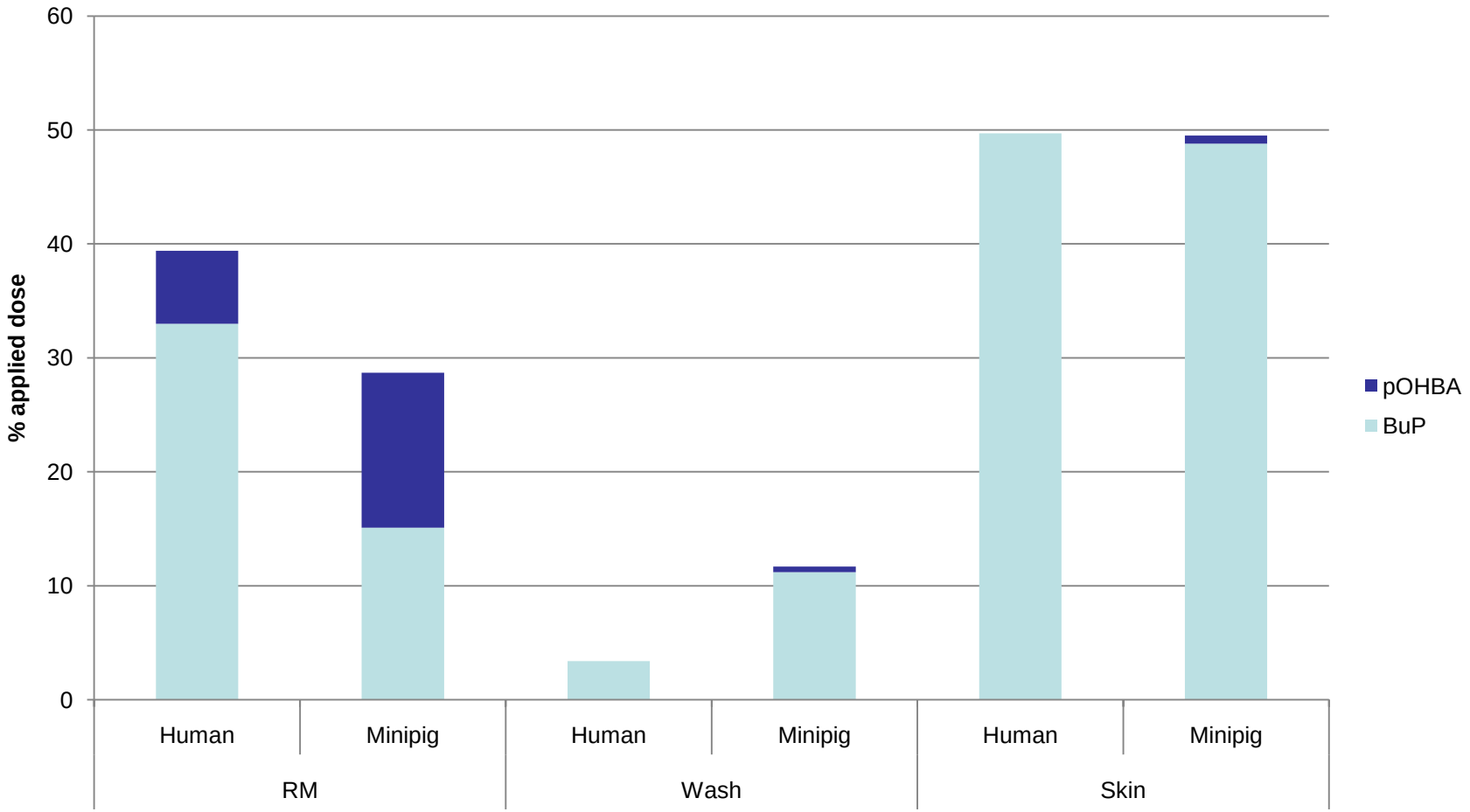
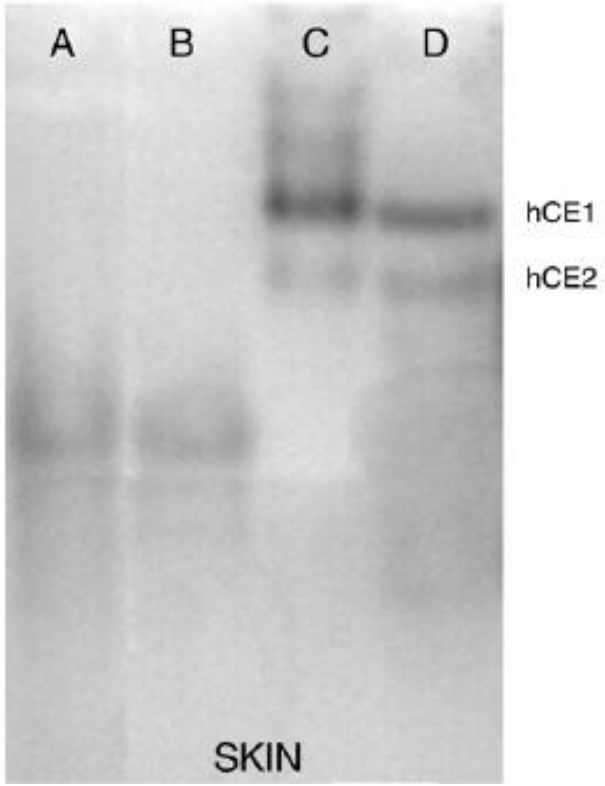
Metabolism of parabens



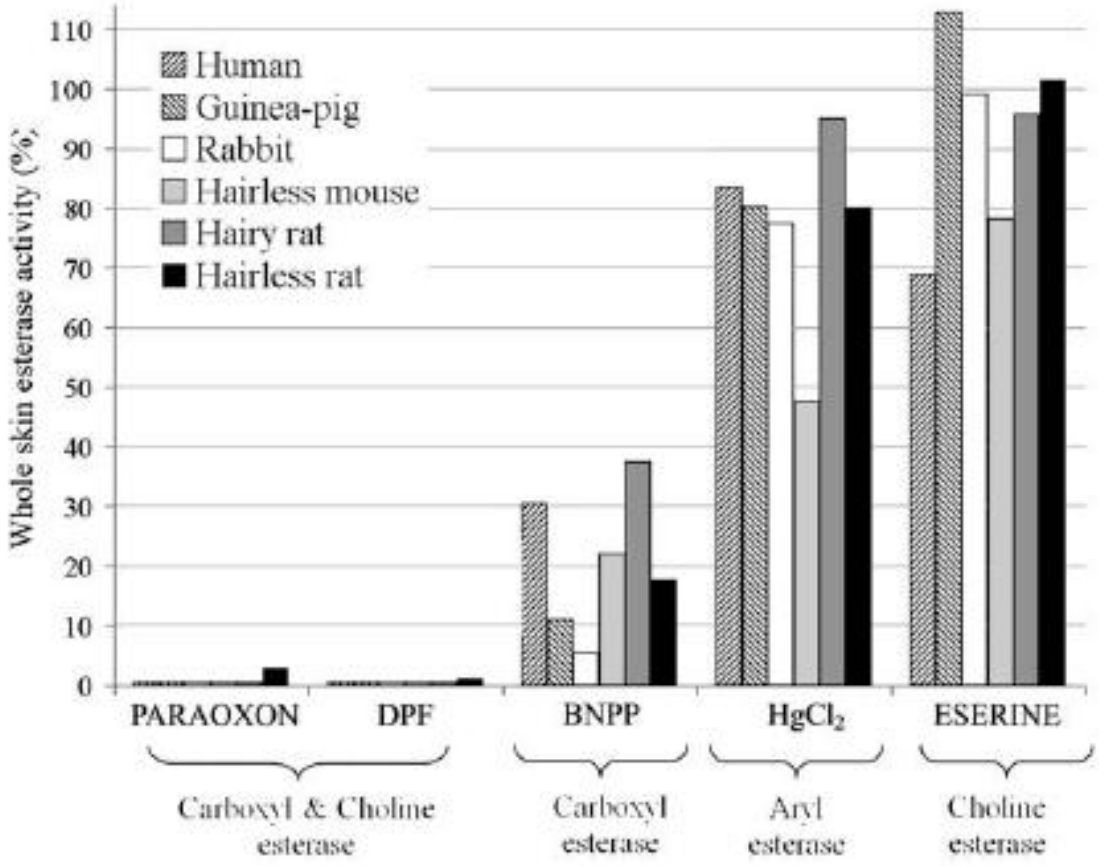
Native gels stained for esterase activity



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

Concerns

- Absorption versus metabolism in “Short term culture” – conditions do not reflect those in vivo; “reabsorption” from receptor medium into skin
- How can we assess “metabolic competence” of skin ex vivo and skin equivalents (i.e. what should we use as our positive control)

Short term culture

Short term culture

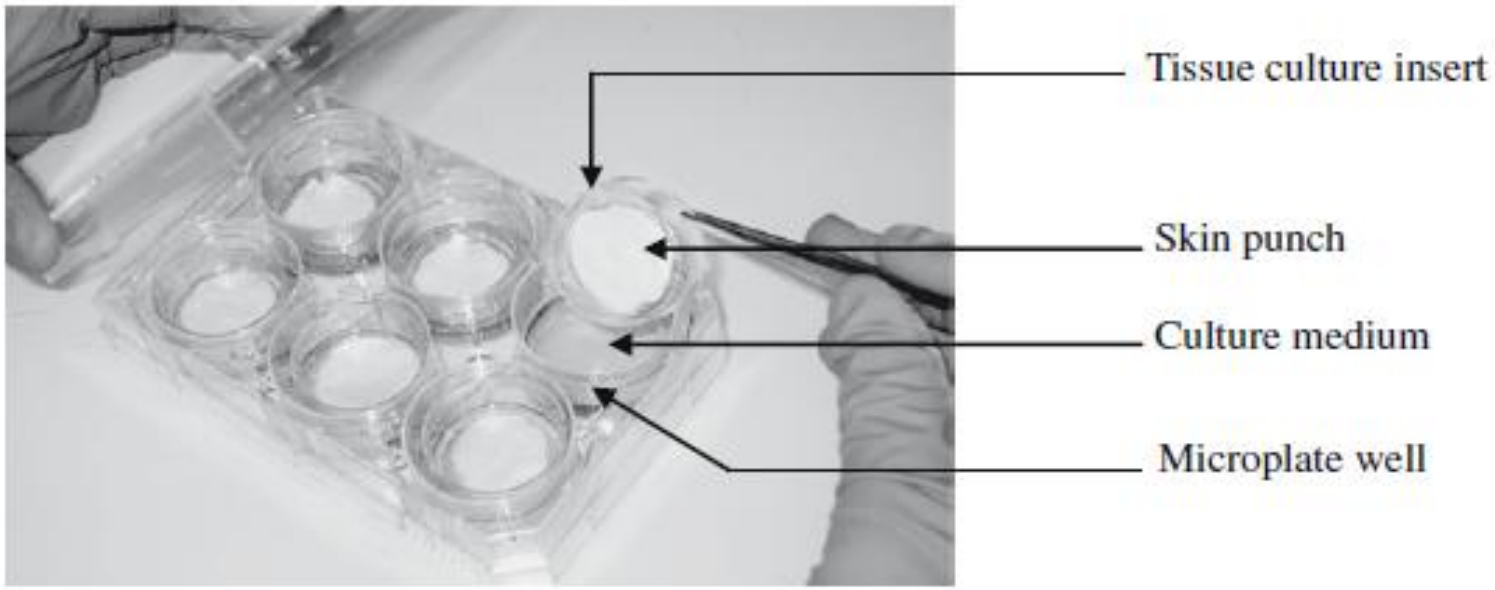


Fig. 1. Photography of pig ear skin short-term culture, in a 6-well plate.

Jacques et al. Toxicology in Vitro 24 (2010) 1426–1434

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Summary

- There has been an increased appreciation of the importance of skin metabolism and much important new research has been done since 2000
- Skin equivalents show considerable promise as experimental models for skin metabolism but some concerns remain

The future

- Development of standard protocols for “qualitative” and quantitative predictions of metabolic activity in skin to replace the local lymph node assay
- A better understanding of the relationship between metabolism of endogenous and xenobiotic substrates
- Novel in vivo/in vitro approaches to skin absorption and metabolism

Acknowledgements

- Faith Williams
- Clive Roper
- Chris Jewell
- All contributors to the field of cutaneous metabolism research past and present

Skin Metabolism one day meeting

- Charles River Europe, Edinburgh
- 20th May 2011
- Details to be available on Skin Forum website