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Challenge 20: Metaboderm

Launch Meeting
10 September 2015

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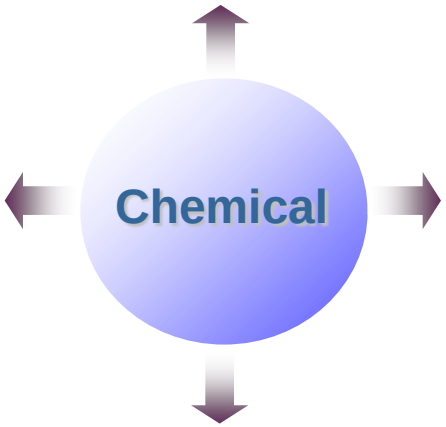
Why are we interested in skin metabolism?

Xenobiotic metabolising enzymes and transport proteins function as a biochemical barrier of the skin

- • Biotransformation process for endogenous compounds and xenobiotics
- • Divided into Phase I (functionalisation reactions, oxidation reduction and hydrolysis) and Phase II (conjugation reactions)
- • Increase hydrophilicity = increased excretion

Inactive Metabolites (typically phase II reactions - transferases)

Detox. mechanism



Active Metabolites

- Increased affinity for the desired target
- Undesirable off-target activity

Active Metabolites

Pro-drug concept

- Betamethasone 17-valerate penetrates the stratum corneum more readily than betamethasone – rapidly cleaved by esterases to yield betamethasone
- Ester prodrugs of naltrexone
- Minoxidil – minoxidil sulphate to stimulate hair follicles

Reactive Metabolites (typically phase I reactions- cytochrome P450)

Irreversible binding to macromolecules, immune mediated toxicity

•Dapsone (skin explants), sulfamethoxazole (KCs), PAHs (benzo(a)pyrene by CYP1A1-arylhydrocarbon hydroxylase)

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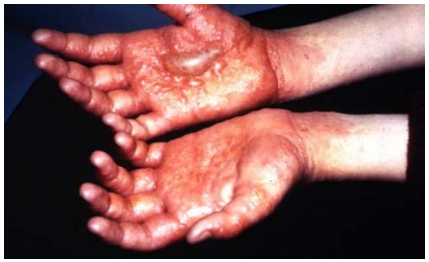
Significance of skin metabolism

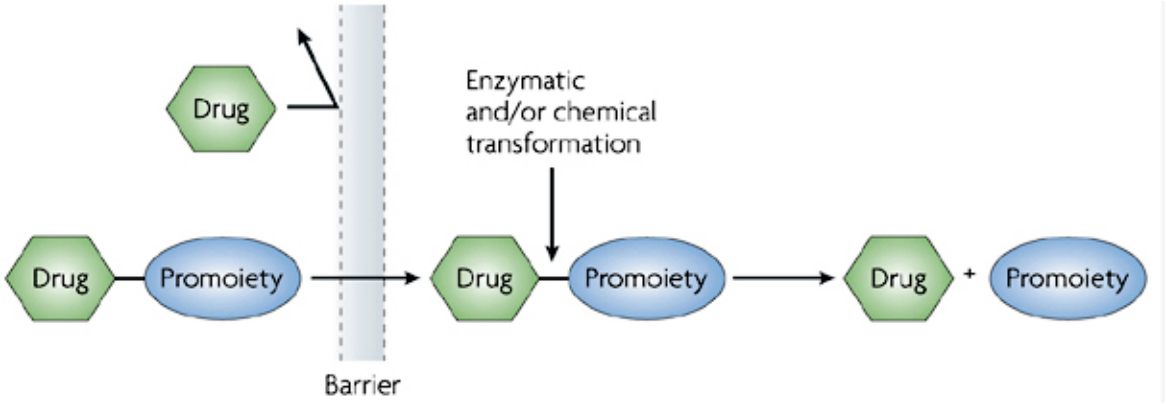


Allergic Contact Dermatitis

20% of known skin allergens would not react with proteins without previous metabolic activation

Dermatologically applied prodrugs rely on metabolism to deliver actives

It is hypothesised that detoxification enzymes increase tolerability to potentially harmful chemicals entering in contact with skin



Prodrugs: design and clinical applications *Nature Reviews Drug Discovery* **7**, 255-270 (Jarkko et al, 2008)

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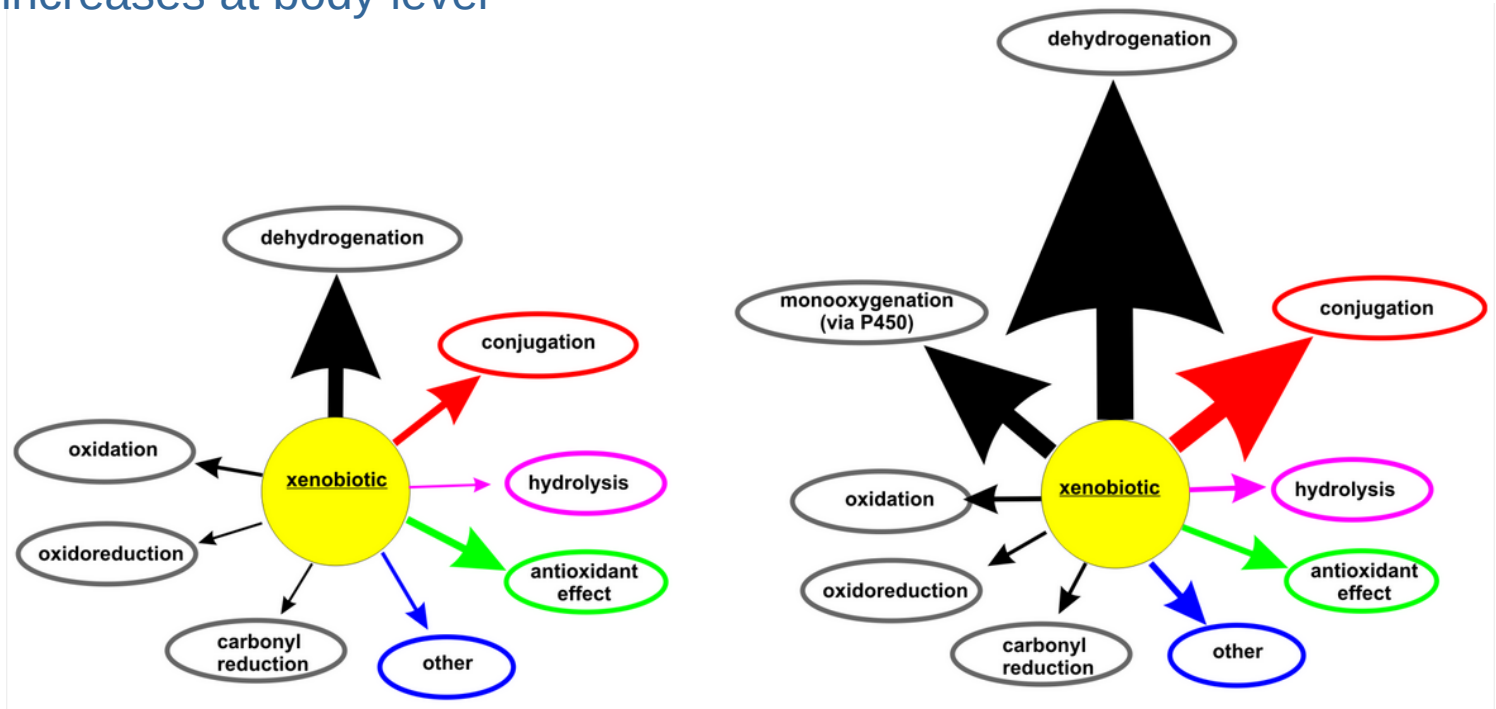
Why are we interested in skin metabolism?

- Skin metabolism is comparable to liver metabolism in terms of the enzymes involved
- Activities reported were significantly lower than in liver w/w
- Skin as an organ covers $2m^2$ of the body so significance increases at body level

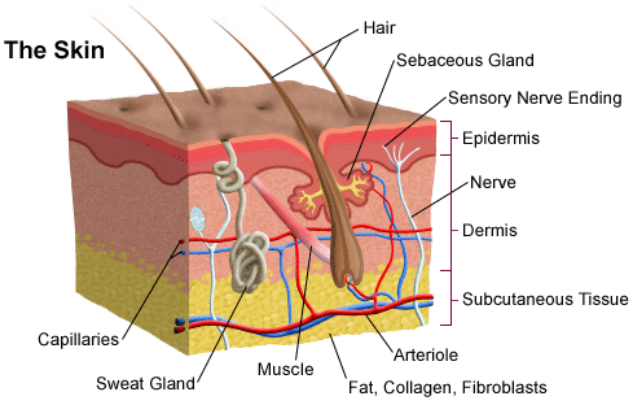


Potential routes of xenobiotic metabolism in skin and liver. Reproduced from van Eijl S et al. (2012) Elucidation of Xenobiotic Metabolism Pathways in Human Skin and Human Skin Models by Proteomic Profiling. PLoS ONE 7(7): e41721. doi:10.1371/journal.pone.0041721

Current knowledge of skin metabolism

Phase I

- CYP (CYP1 family, CYP2C9, CYP2E1, CYP3A)
- Cyclooxygenase
- Alcohol dehydrogenase
- NADH/NADPH quinone reductase
- Esterase
- FMO



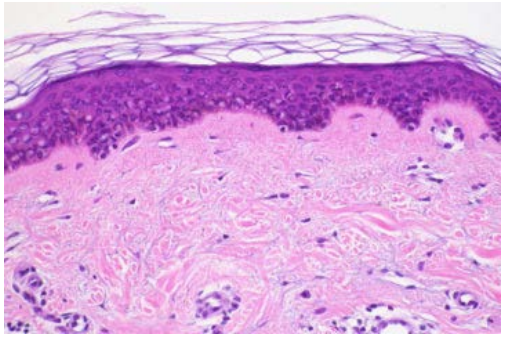
Xenobiotic-metabolizing enzymes in the skin of rat, mouse, pig, guinea pig, man, and in human skin models. Oesch et al. Arch Toxicol. 2014; 88(12): 2135–2190.

Phase II

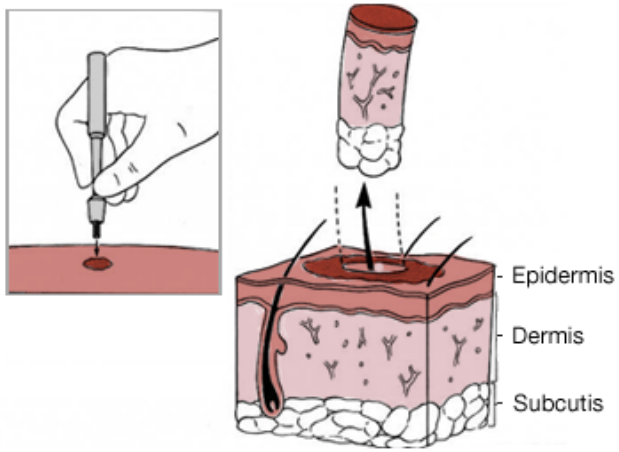
Family:	Individual enzymes detected:	Reference:
UGT	1A6, 1A8, **1A10**, 2A1, 2A3, 2B4, 2B15, 2B17, 2B28	(Hu et al., 2010, Luu-The et al., 2009)
SULT	**1A1**, 1A2, 1A3, 1A4, 1B1, **1E1**, 1E2, 2A1, **2B1**	(Hu et al., 2010, Luu-The et al., 2009, Kushida et al., 2011, Higashi et al., 2004, Dooley et al., 2000)
NAT	**NAT1**, NAT2	(van Eijl et al., 2012, Hu et al., 2010, Luu-The et al., 2009, Bonifas et al., 2010a)
COMT	Single human gene is detected	(van Eijl et al., 2012, Hu et al., 2010, Luu-The et al., 2009)
GST	**Alpha**, **Mu**, **Pi**, Theta, Zeta, Omega	(van Eijl et al., 2012, Hu et al., 2010, Luu-The et al., 2009, Raza et al., 1991, Blacker et al., 1991)

Phase II metabolism in human skin: skin explants show full coverage for glucuronidation, sulfation, N-acetylation, catechol methylation, and glutathione conjugation. Manevski et al. Drug Metab Dispos. 2015 Jan;43(1):126-39.

Challenges



- Low level of Phase I activities
- Analytical challenge to measure reactive metabolites
- Enzymes stability in skin biopsies
- No *in vivo* data exclusively on skin
- Differences between human skin equivalents and *ex vivo* skin



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Current status and gaps

A number of *in vitro* methods exist to:

- Identify metabolites and measure transcutaneous diffusion
- Model the processes of absorption and metabolism mathematically
- Model the pharmacokinetics of xenobiotics mathematically

What is missing?

A method to combine all these methods to:

- Predict absorption and kinetics of xenobiotics *in vivo*
- Validate the method using available *in vivo* data

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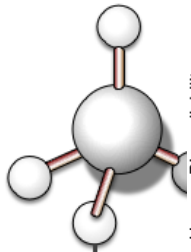
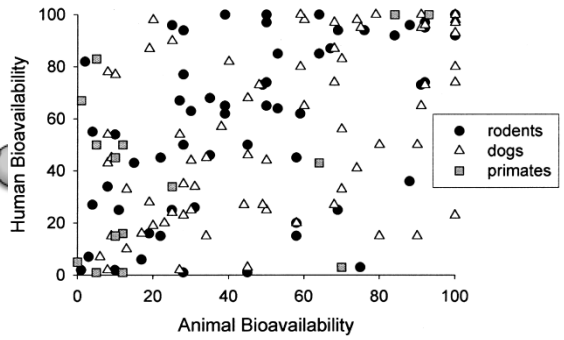
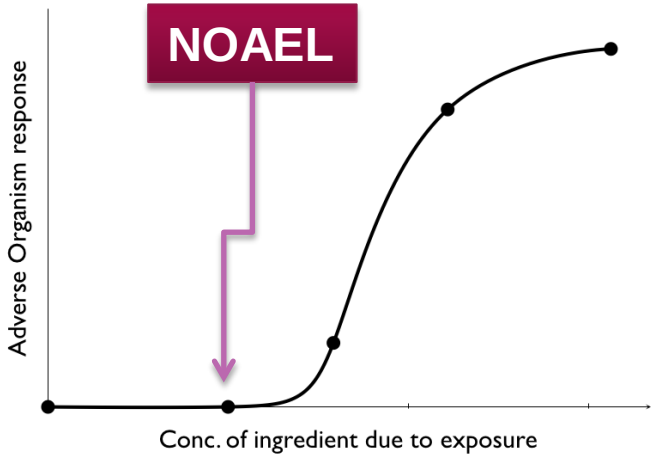
Sponsor Perspectives

Traditional risk assessment strategy







Amount/Conc. of ingredient due to exposure

Hazard Characterisation

Adverse Organism Reponse

Species Extrapolation

Safe Dose in Humans

Model

NOAEL ÷ 10 - 1000

Safety/ Uncertainty factors

Targeted Testing

Uncertainty Factors

Pathways based toxicology

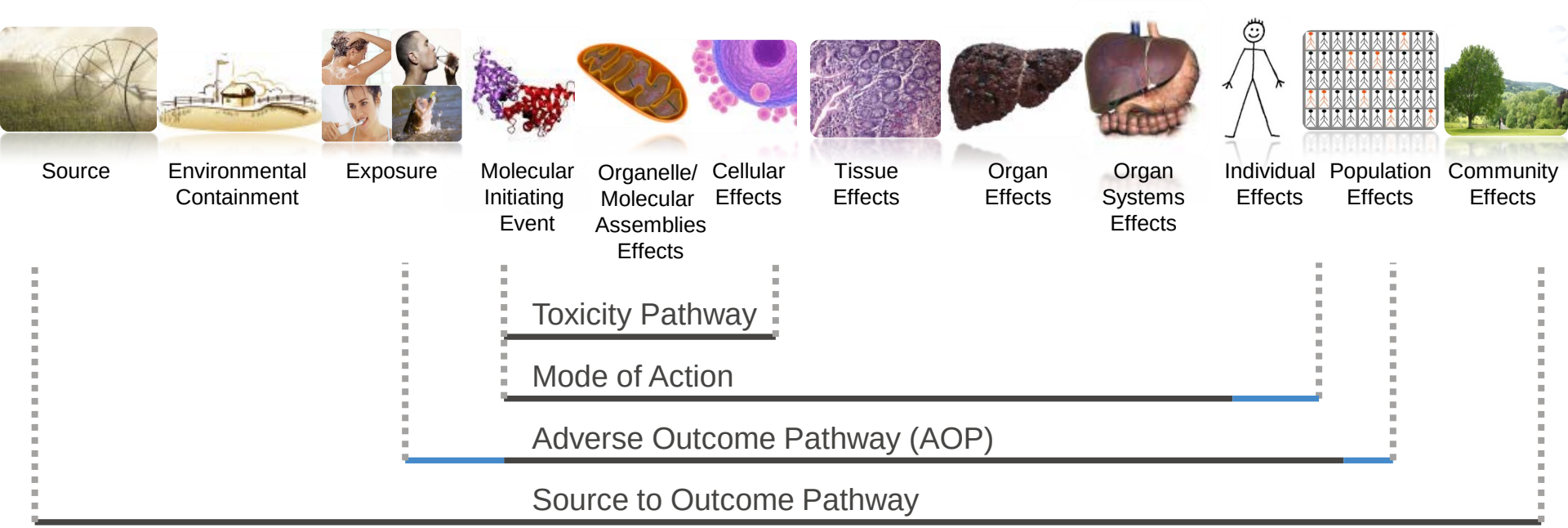
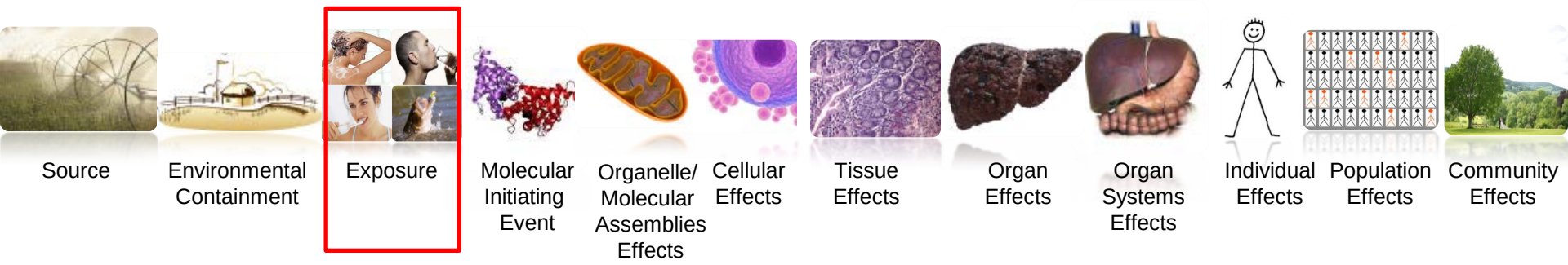


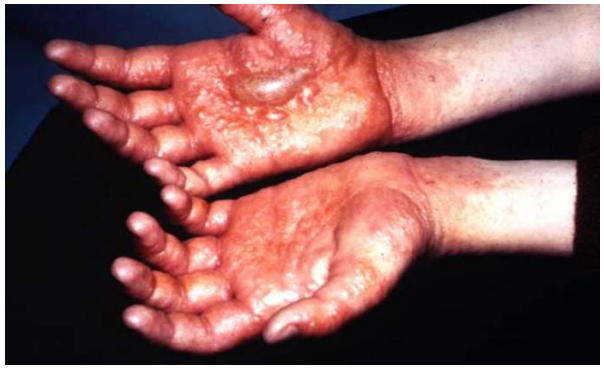
Figure 1. Scope of Pathways Approaches (Adapted from Crofton 2010)

Exposure



- **Metabolism** key component determining local and systemic concentrations of both parent and metabolites
- Metabolite formation may increase or decrease risk

Example: allergic contact dermatitis



Allergic Contact Dermatitis is a delayed-type hypersensitivity response as a result of an external compound reacting with skin proteins

20% of known skin allergens would not react with proteins without previous metabolic activation

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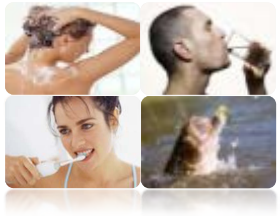
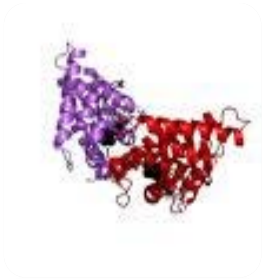
Allergic contact dermatitis pathway

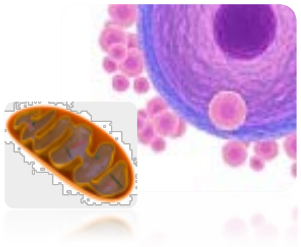


1. Skin Penetration

2. Electrophilic substance: directly or via auto-oxidation or metabolism

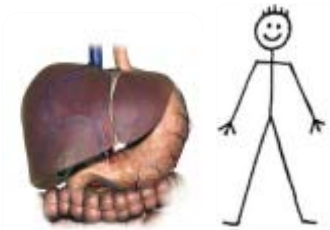


3-4. Haptenation: covalent modification of epidermal proteins



5-6. Activation of epidermal keratinocytes & Dendritic cells

7. Presentation of haptenated protein by Dendritic cell resulting in activation & proliferation of specific T cells



8-11. Allergic Contact Dermatitis: Epidermal inflammation following re-exposure to substance due to T cell-mediated cell death

Key Event 1

Key Event 2 + 3

Key Event 4

Adverse Outcome

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Metabolism and skin sensitisation

Pro-electrophiles

Activation

Pro-haptens

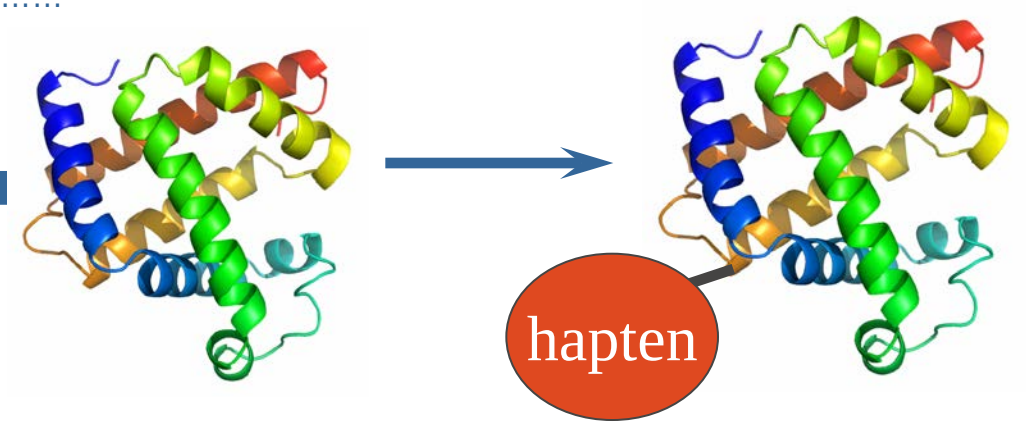
2-aminophenol, eugenol …..

Enzyme mediated activation (reactive electrophilic intermediates - reactive metabolites)

Pre-haptens

PPD, hydroquinone ……

Chemical oxidation to reactive intermediates



electrophilic hapten

Chemical specific T cells recognise a conjugate of the chemical with a peptide - surface of antigen-presenting cells

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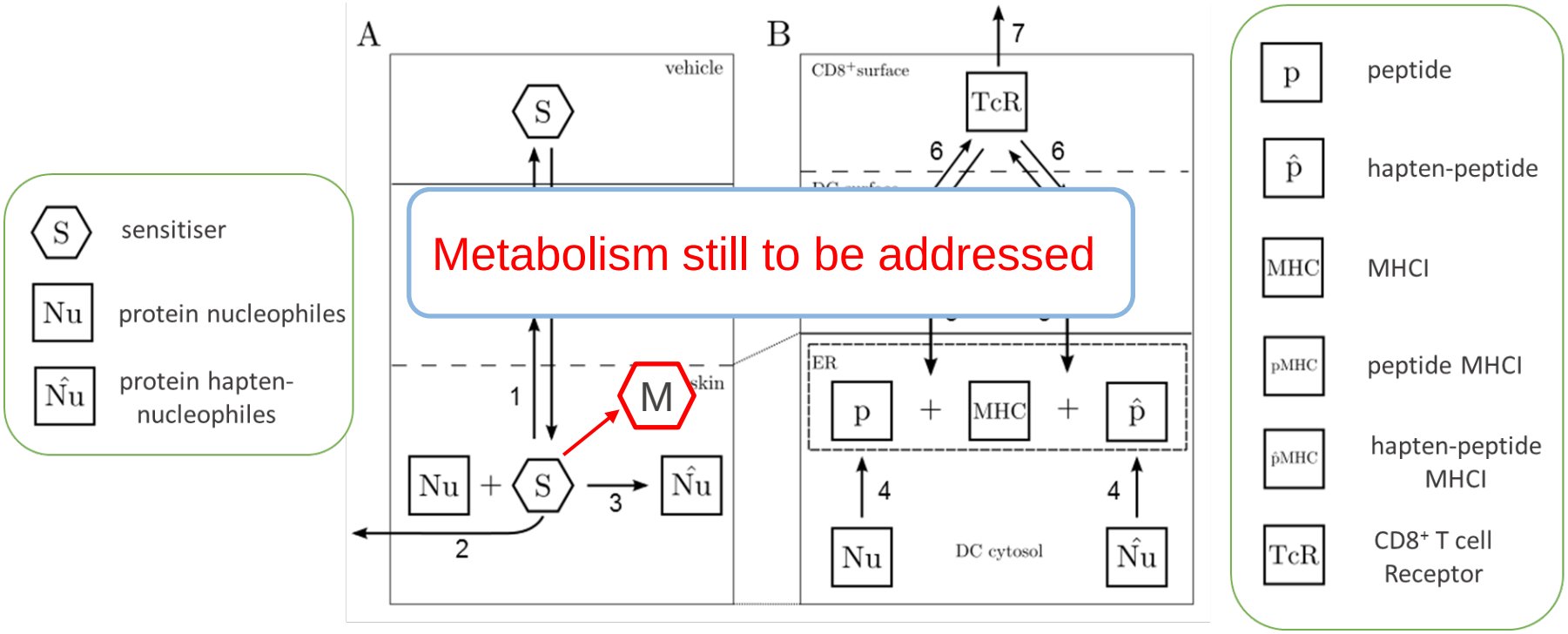
Mechanistic model schematic



GSK Perspective

do more
feel better
live longer

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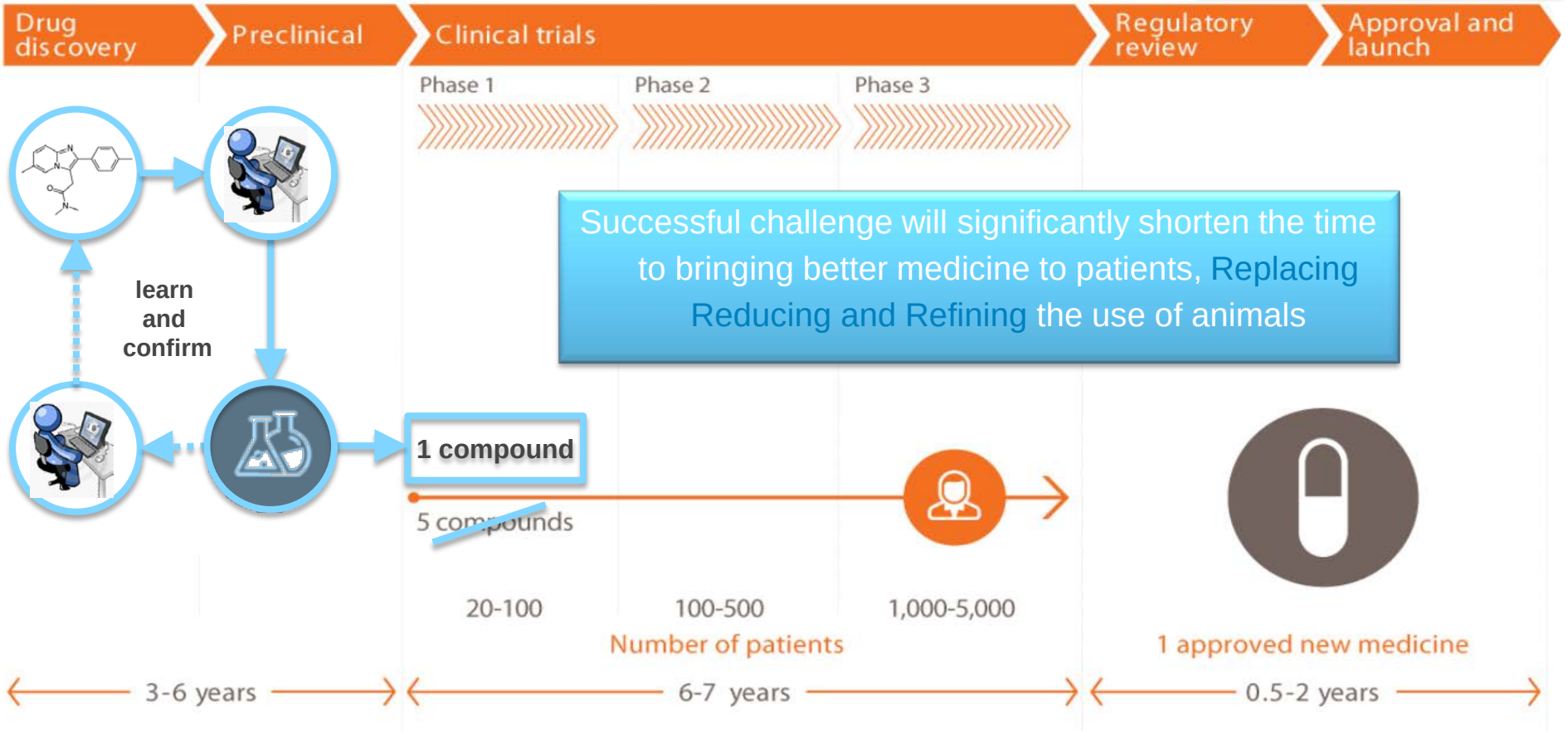
Drug discovery and development



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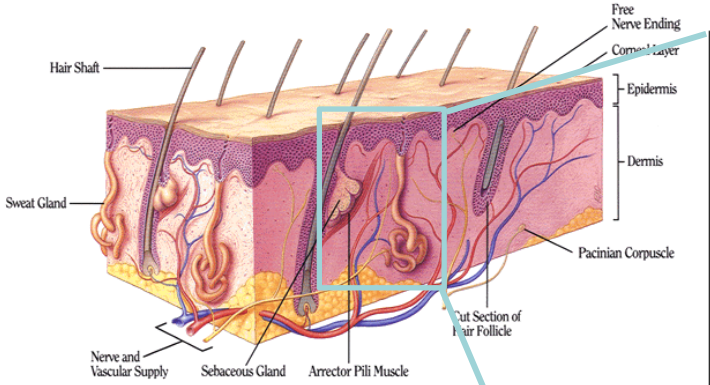
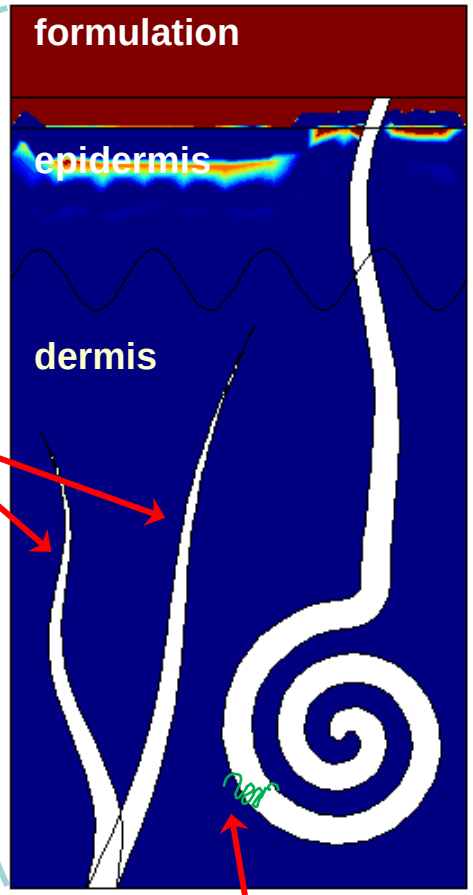
Drug exposure at target is a critical “Pillar of survival”





Metabolism site

Definition of the three Pillars of survival

For a development candidate to have potential to elicit the desired effect over the necessary period of time, three fundamental elements need to be demonstrated:

i. Exposure at the target site of action over a desired period of time
ii. Binding to the pharmacological target as expected for its mode of action
iii. Expression of pharmacological activity commensurate with the demonstrated target exposure and target binding

Understanding drug exposure in the skin both for efficacy and toxicity requires understanding of skin metabolism

Morgan, P., Van Der Graaf, P. H., Arrowsmith, J., Feltner, D. E., Drummond, K. S., Wegner, C. D., & Street, S. D. a. (2012). Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. *Drug Discovery Today*, *17*(9-10), 419–24.

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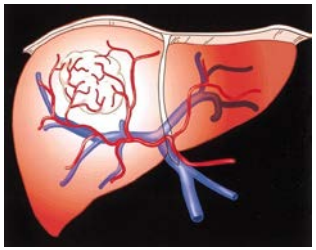
In vitro skin metabolism challenge

LIVER

Scale up to whole liver



Scale up to whole organism

$$CL = \frac{Q_h f_u CL_{\text{int}}}{Q_h + f_u CL_{\text{int}}}$$

SKIN

- Can we do better for metabolism in the skin?
- Can we predict metabolism based only on compound structure

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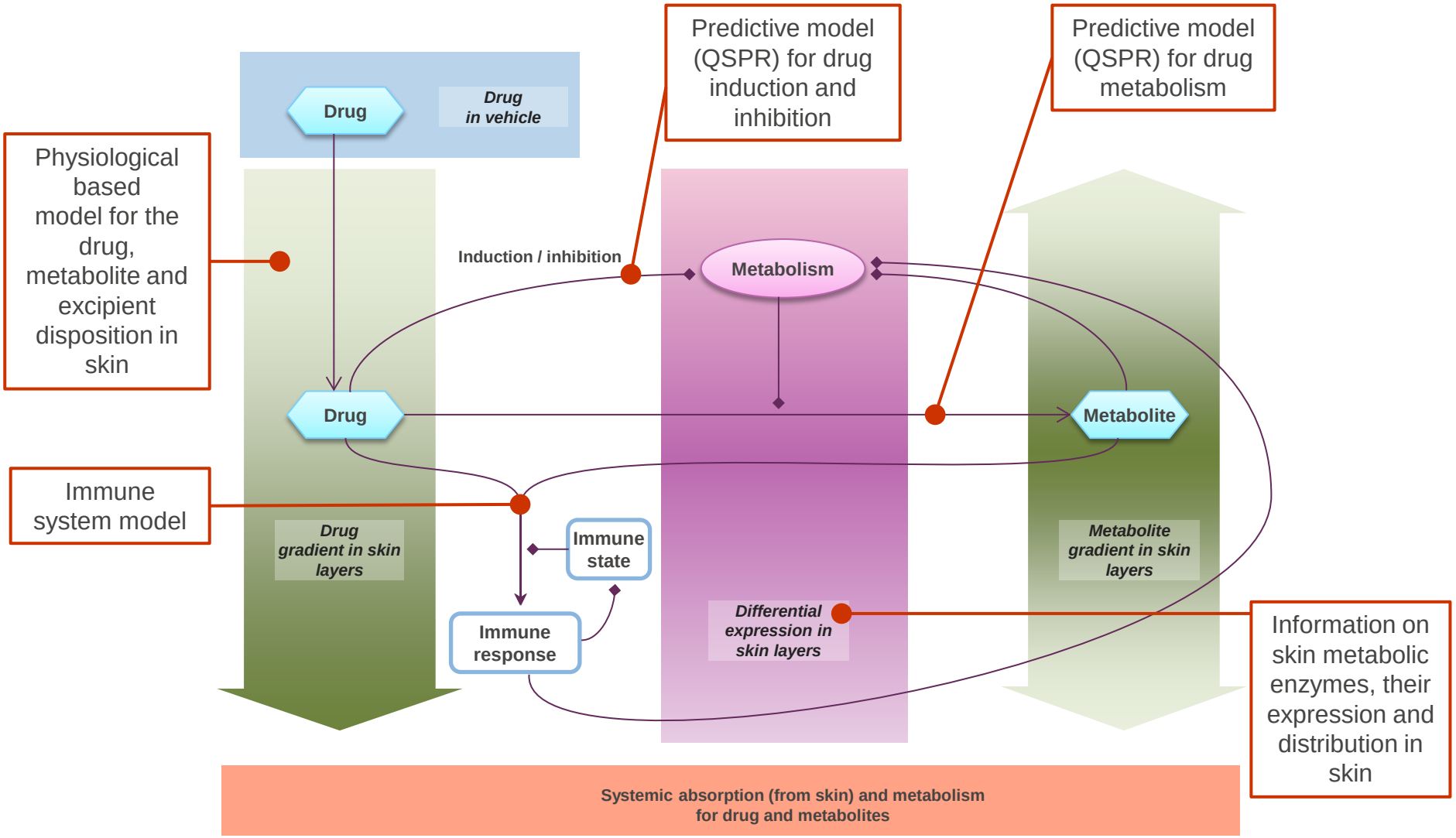
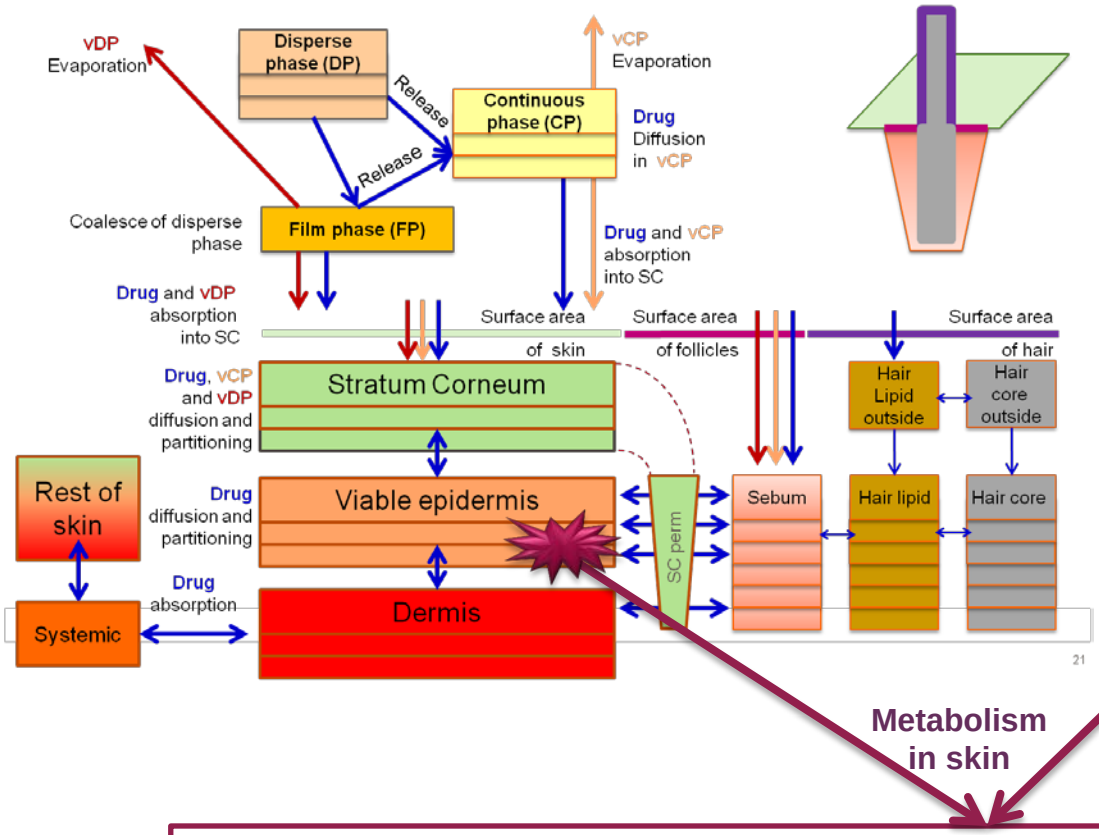
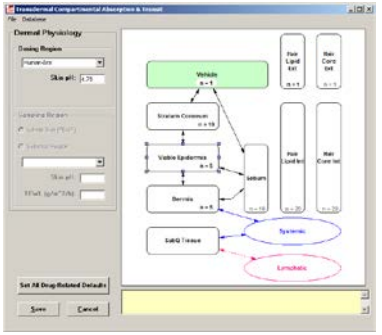
Modeling challenges



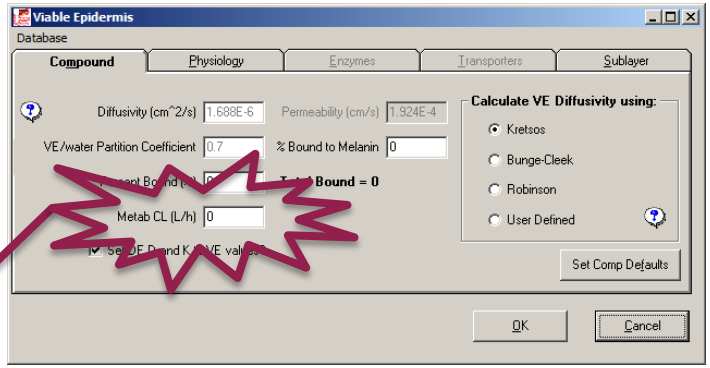
Physiology Based skin absorption model



Transdermal Compartmental Absorption & Transit



TCAT model as implemented in GastroPlus v9.0

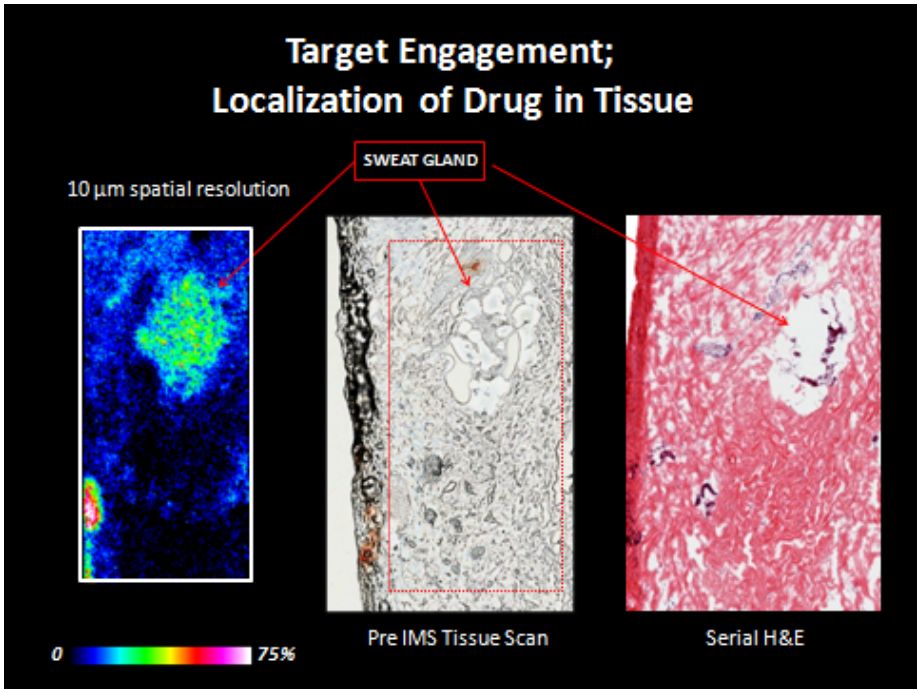


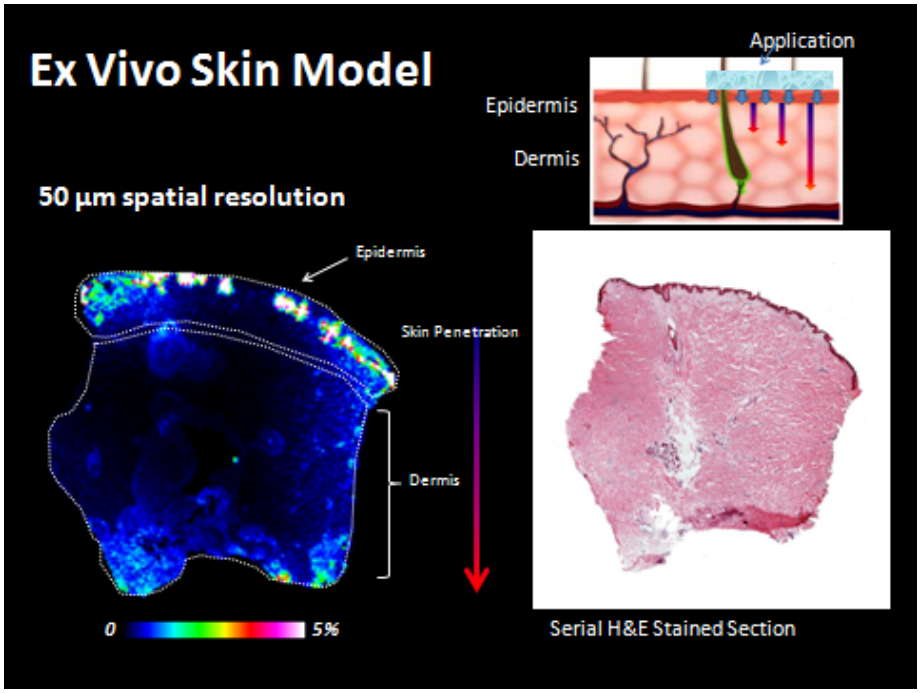
Metabolism in skin

There is little information on how to measure *in vitro* or **predict** skin metabolism rates and routes even though current models are capable of accepting metabolic clearance inputs

GSK can provide modelling expertise

Drug and metabolite distribution in ex-vivo skin





- MALDI is one of the ways to image drug and it's metabolites in tissue
- Better models are needed to explain the observed spatial distribution of drug and metabolites

GSK can provide MALDI imaging expertise

Sponsor Perspectives

Defence Science Interest

Long term Goal: reliable estimates of effects in humans from *in vitro* measures

Dstl's remit is to protect service personnel and first responders.

Interested in both toxic chemicals and therapeutic drugs.

Defence needs for developing new therapeutics are the same as those of pharmaceutical companies.

High emphasis on development of robust methods for extrapolating from *in vitro* measures to *in vivo* and from animal data to human for toxicants and therapeutics. There are/can be no exposed human populations to test therapeutic agents.

Defence Science Interest

Interest in quantitation of measures of cutaneous metabolism to determine:

- effect on pharmacokinetics of xenobiotics
- time course of toxic effects and therapeutic benefits
- use of *in silico* models to predict *in vivo* response from *in vitro* measures

Dstl needs a robust prediction of toxicity or therapeutic effect from more focused animal experiments (refinement and reduction). This Challenge is the first step towards this long term goal.

These goals are similar to those of Occupational Toxicology for protecting health. Similar methods are required for this application of the science.

3Rs Benefits (1)

- For regulatory submissions in the development of drugs for topical administration, a pharmaceutical company will use around 1,000 animals in studies relating to skin toxicity per year- approximately 30% of which involve non-rodent species, in particular, the minipig.

- The development of PBPK models based on dermal exposure is currently an area of active research and these models may be used in addition to *in vivo* approaches to predict pharmacokinetic (PK) and toxicokinetic (TK) properties of candidate drugs. Better understanding of skin metabolism will improve dermal PBPK models, enable better selection of chemicals and reduce the use of *in vivo* toxicokinetic models.

- While a successful Challenge will not completely eliminate the use of these animals in pharmaceutical companies, the novel modelling approaches developed though this Challenge will reduce and replace significant numbers of animals and where animals are still used, minimise the number of required time points/doses. As the market for developing new chemical entities specifically for topical applications is expanding, (Kelly Scientific, 2015), the 3Rs impact of this Challenge will continue to increase.

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3Rs Benefits (2)

- The aim of this Challenge is not to predict animal toxicity data but rather focus on safety risk assessment based on data relevant to human use as outlined in *Toxicity testing in the 21st century: A vision and a strategy* (TT21C) (Krewski *et al,* 2010). Specifically, the tools developed in this Challenge will allow skin metabolism studies to be conducted without the use of animals and also improve approaches to address the impact of xenobiotic metabolism in skin, informing the understanding of dermal and systemic availability of materials applied to the skin in humans.

- A platform which could deliver the Challenge would impact animal use across the *personal care product, pharmaceutical and agrichemical* industries where concerns around skin toxicity exist.

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Challenge 20: Metaboderm

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Deliverables

AIM: To establish, both qualitatively (which metabolites are produced) and quantitatively (concentration of the metabolites produced), the extent to which skin metabolism determines xenobiotic availability in human skin

- Identify studies and test systems to investigate the skin metabolism of topically applied xenobiotics (in vitro/minimally invasive in human)
- Establish suitable analytical techniques for measurement of metabolites
- Use of modelling to provide a kinetic understanding of the extent to which metabolism determines xenobiotic availability in skin

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Deliverables

Phase 1

- Develop an experimental and/or clinical approach to investigate topically applied xenobiotics that is representative of human skin metabolism
- Demonstrate the advantages of this approach compared to existing methods (e.g. liver microsomes or existing 3D skin models)
- Provide data and evidence that the approach can measure both phase I and phase II metabolism
- Present computational approaches which will be developed further in Phase 2 of the Challenge
- Present plans for wider use of the approach in industry (routes-to-market)

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Deliverables

Phase 2

Development and evaluation of the **experimental/clinical** approach to determine:

- Phase I metabolism induction
- Phase I and phase II metabolism pathways, including characterisation of metabolites and their rates of elimination from the skin
- Spatial localization of active metabolic processes in the skin and their relationship to xenobiotic gradients in the skin
- The cellular and subcellular localisation of the metabolic processes

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Deliverables

Phase 2

Development of **computational** approaches with the ability to:

- Predict expected metabolites for a given chemical structure
- Calculate the rates of metabolism that determine bioavailability in skin
- Predict skin exposure for parent chemical and metabolites (PBPK model parent chemical and metabolites in the skin) with consideration given to possible permeation enhancement
- Provide the science and mathematics necessary for the incorporation of skin metabolism kinetics within existing open-source or commercial PBPK software

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Sponsor in-kind contribution

Phase 1

- Provision of known chemicals that have relevance to skin metabolism along with relevant data
- Scientific advice and modelling experience

Phase 2

- In house assessment of the approaches developed through this Challenge as appropriate - to facilitate industry uptake
- Access to relevant findings from ongoing research programmes focussing on toxicity testing in the 21st century (TT21C) approaches to mechanistic-based risk assessment of human relevant toxicity (www.tt21c.org)
- Provision of risk assessment expertise for chemicals used in a personal and home care context, and understanding of their chemistries
- Provide expertise/knowledge gained from in-house *experimental* approaches currently employed for prediction of metabolic fate and PBPK

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Thank you

The Sponsors are happy to discuss the challenge and potential applications with people in the run up to the submission deadline

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