

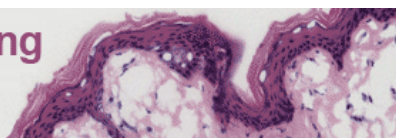


SKIN BIOAVAILABILITY MODELLING FOR RISK ASSESSMENT OF SKIN SENSITIZING INGREDIENTS

TODD GOUIN

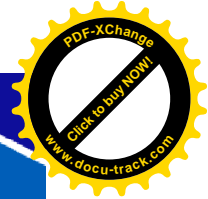
Skin Forum 13th Annual Meeting

London, UK | 24-26 June 2013



MODELLING SKIN PERMEATION SYMPOSIUM
24 JUNE 2013





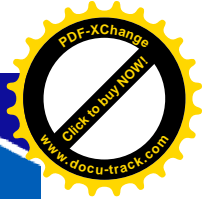
ACKNOWLEDGEMENTS



The work presented here, stems from the discussions at an expert workshop, funded and organised by Unilever and held in Bedford, UK on 31st May & 1 June 2012.

Cameron Mackay, Ann-Therese Karlberg, Gerald Kasting, Jean-Pierre Lepoitttevin, Dean J. Naisbitt, Camilla Pease, Nora Aptula, Alan Boobis, Anna Borje, Paola Carbone, Rob Chilcott, Ed Clarke, Richard Cubberley, Michael Davies, Elena-Gimenez Arnau, Gaurav Jain, Sandrine Jacquilleot, Jonathan Goodman, Michael Hughes, Gavin Maxwell, Xioli Meng, Craig Moore, Arne Naegel, Ruth Pendlington, Juliet Pickles, Ouarda Saib, David Sheffield, Anna Shvedova, Vicki Summerfield, Carl Westmoreland, Simon Wilkinson, Sarah Wootton, Zheyong Zhu.



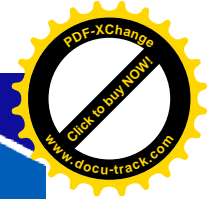


INTRODUCTION



- Risk assessment for skin sensitization and consumer safety
- Reduced reliance on animal testing and increased reliance on *in vitro* and *in silico* tools
- Building mechanistic understanding into the risk assessment paradigm





OECD REPORT:

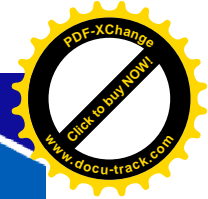
“THE ADVERSE OUTCOME PATHWAY FOR SKIN SENSITIZATION INITIATED BY COVALENT BINDING TO PROTEINS”



Key events of the AOP:

- Penetration of the sensitizing ingredient into the viable layers of the skin
- Modification of skin protein by the sensitizing ingredient (either directly or via metabolic or abiotic transformation of a precursor)
- Production of danger signals and inflammatory mediators by keratinocytes, fibroblasts, and skin-resident dendritic cells
- Maturation and migration of dendritic cells to the local lymph node
- Antigen presentation to specific naïve T-cells, and subsequent T-cell proliferation and differentiation
- The generation of a sufficient population of antigen-specific memory T-cells required to mediate an elicitation response at the site of re-exposure





OECD REPORT:

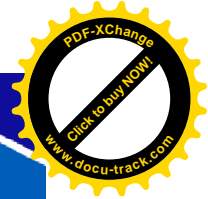
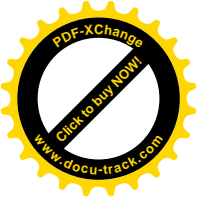
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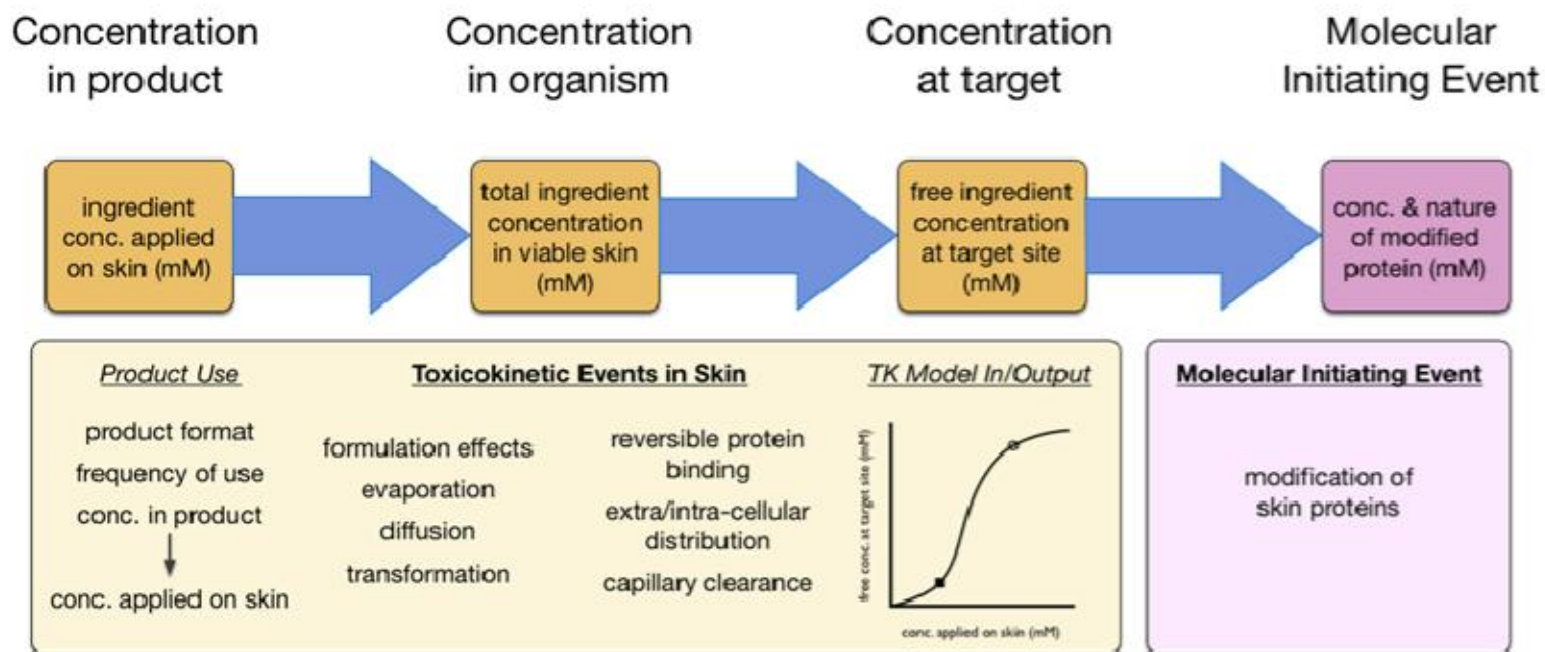
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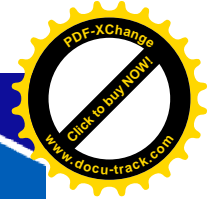
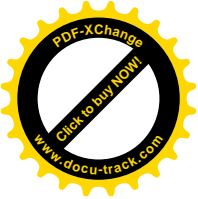
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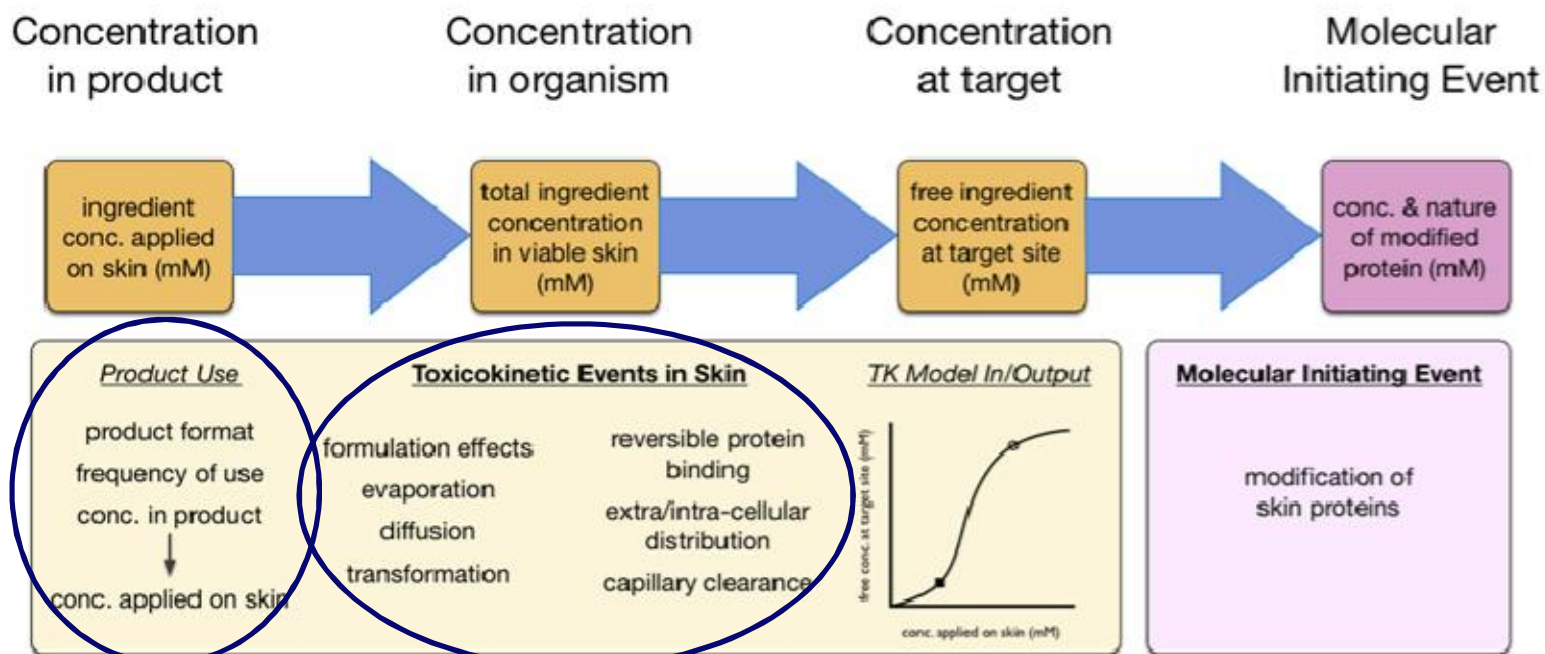


PENETRATION OF THE SENSITIZING INGREDIENT INTO THE VIABLE LAYERS OF THE SKIN





PENETRATION OF THE SENSITIZING INGREDIENT INTO THE VIABLE LAYERS OF THE SKIN



Extensive properties

Intensive properties





INTENSIVE VERSUS EXTENSIVE CHEMICAL PROPERTIES



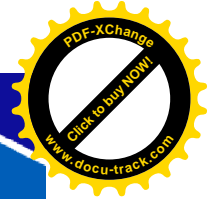
Intensive

- Is a physical property of a system that does not depend on the system size or the amount of material in the system, it is scale invariant.
- Examples include: Chemical potential, solubility in water, K_{OW} , etc.

Extensive

- Quantity of chemical applied to the skin / system dependent





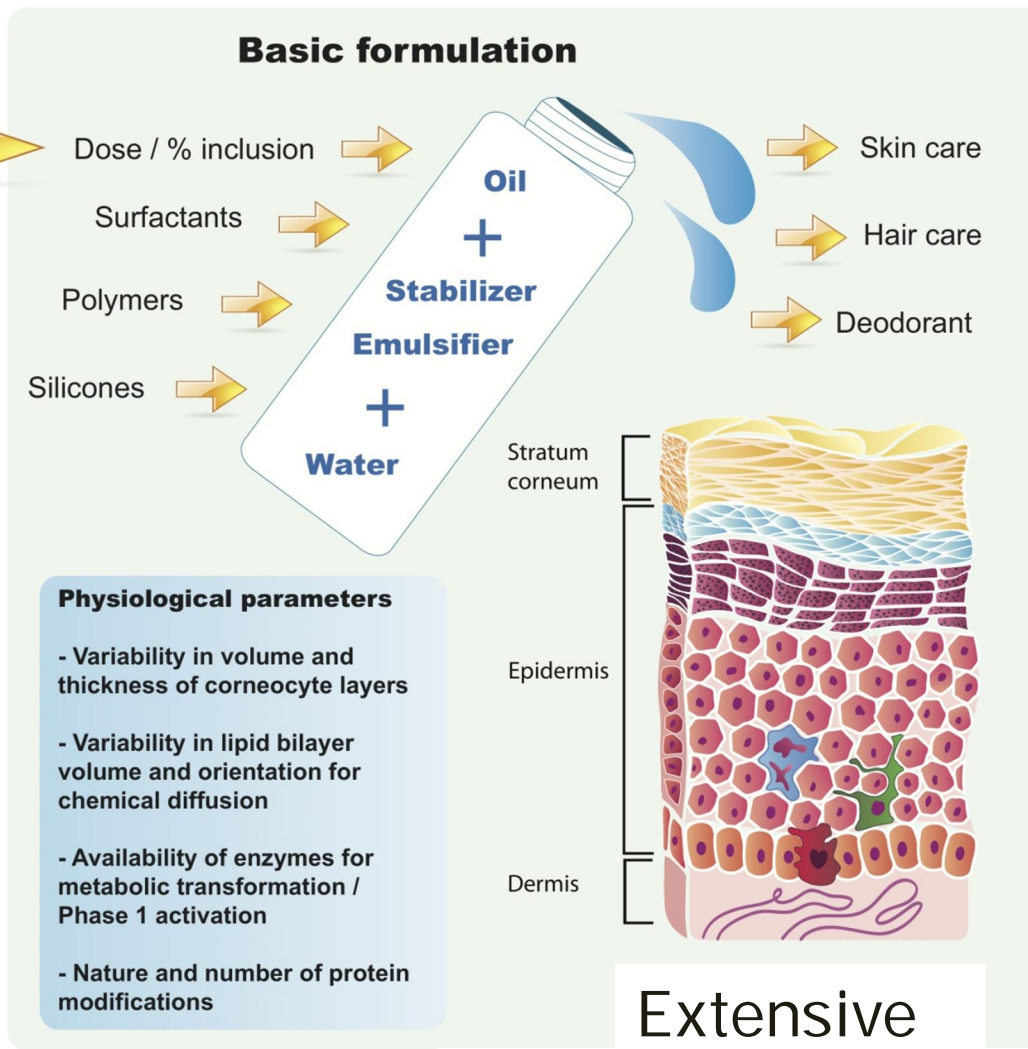
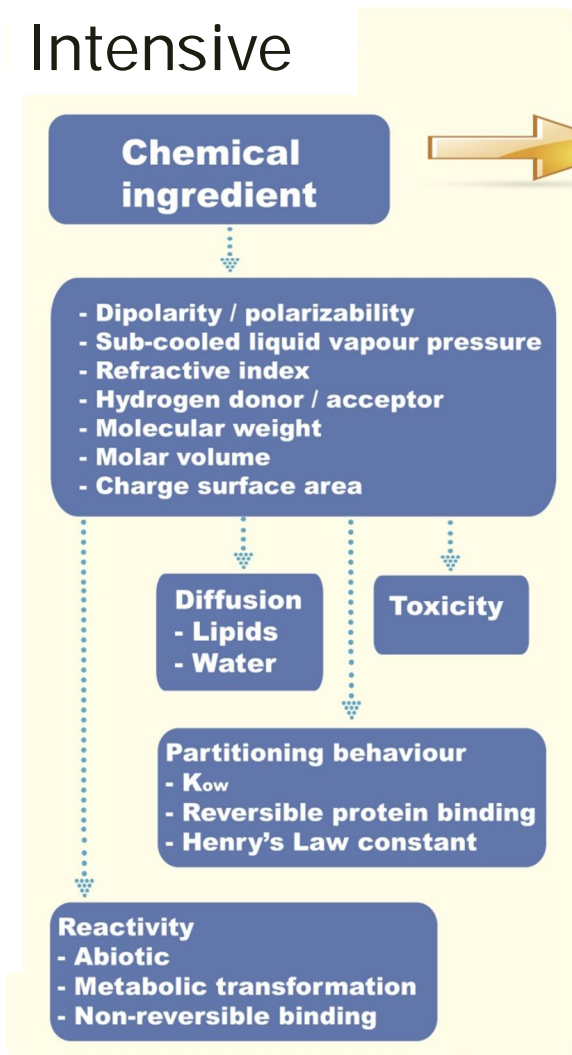
INTENSIVE VERSUS EXTENSIVE

System independent



Intensive

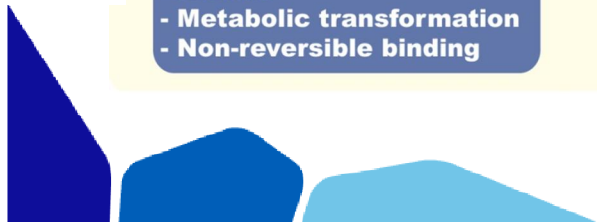
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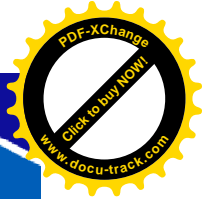
Extensive

System dependent





SKIN DISPOSITION



Despite the competing processes that interact to influence C_{free} a metric regarding skin dosimetry for skin sensitization is required:

“The concentration in skin of the parent chemical and transformed species that is freely available to modify protein and induce an inflammatory response”

This definition expands on previous suggestions that focused on a specific skin layer and also includes transformation products





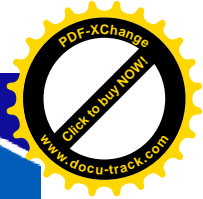
MODELLING CHALLENGE



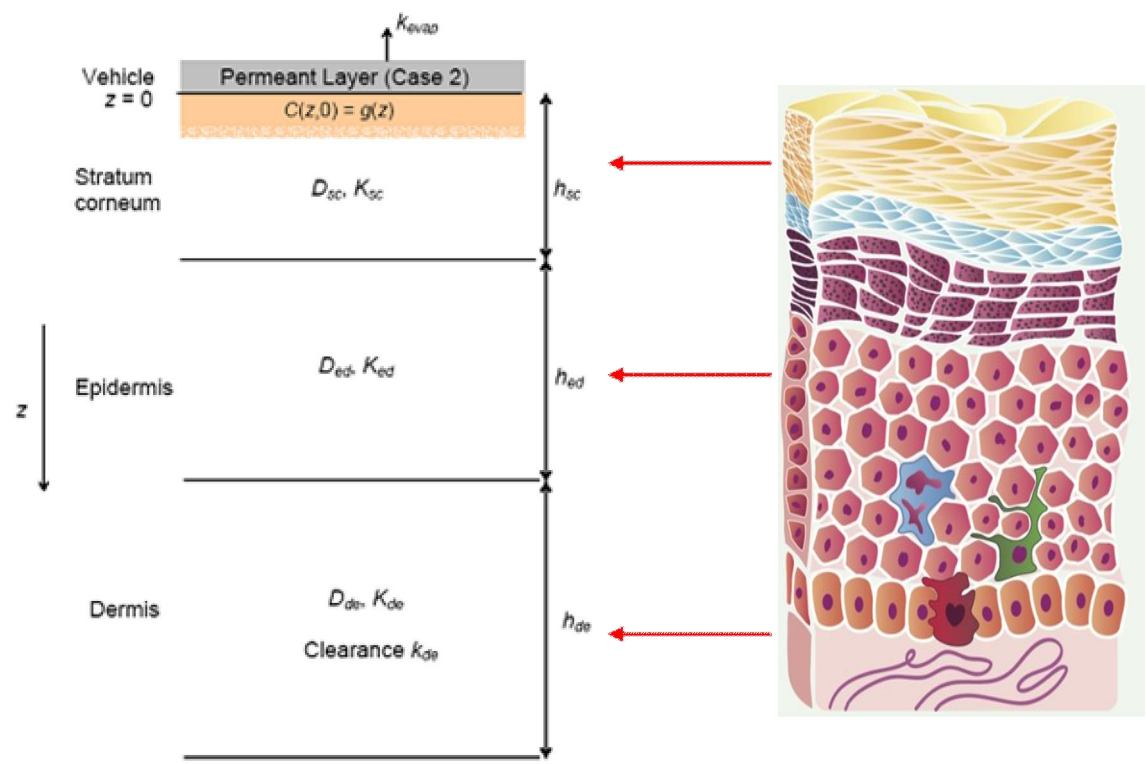
Skin penetration models thus need to include:

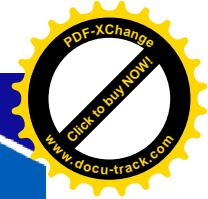
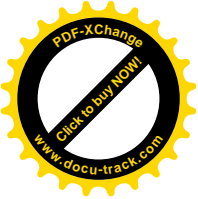
- Multilayer/compartmental
- Formulation effects
- Biotic and abiotic transformation processes





STATE-OF-THE-ART MULTILAYER MODEL

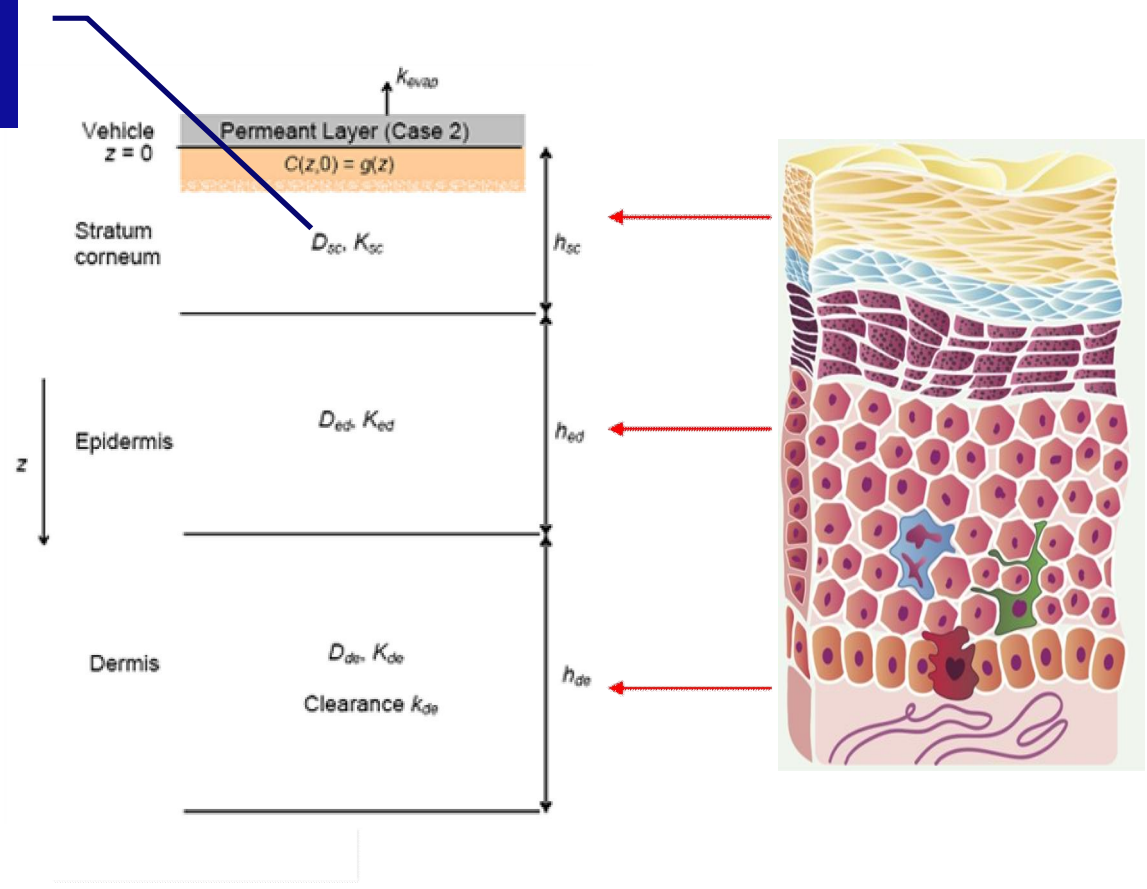


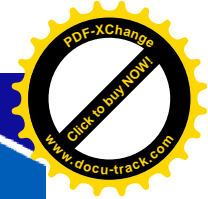
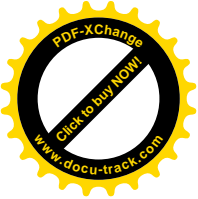


STATE-OF-THE-ART MULTILAYER MODEL



$$D_{SC} = (P_{SC/W} h_{SC}) / K_{SC}$$

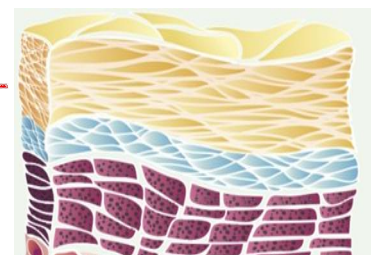
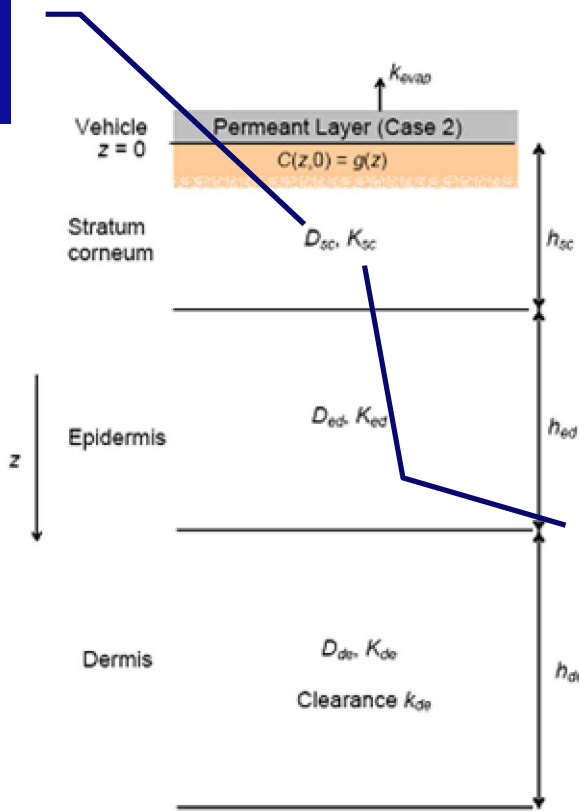




STATE-OF-THE-ART MULTILAYER MODEL



$$D_{SC} = (P_{SC/W} h_{SC}) / K_{SC}$$

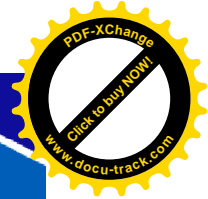


$$K_{SC} = K_{lip/W} \phi_{lip} + K_{Cor/W} \phi_{Cor}$$

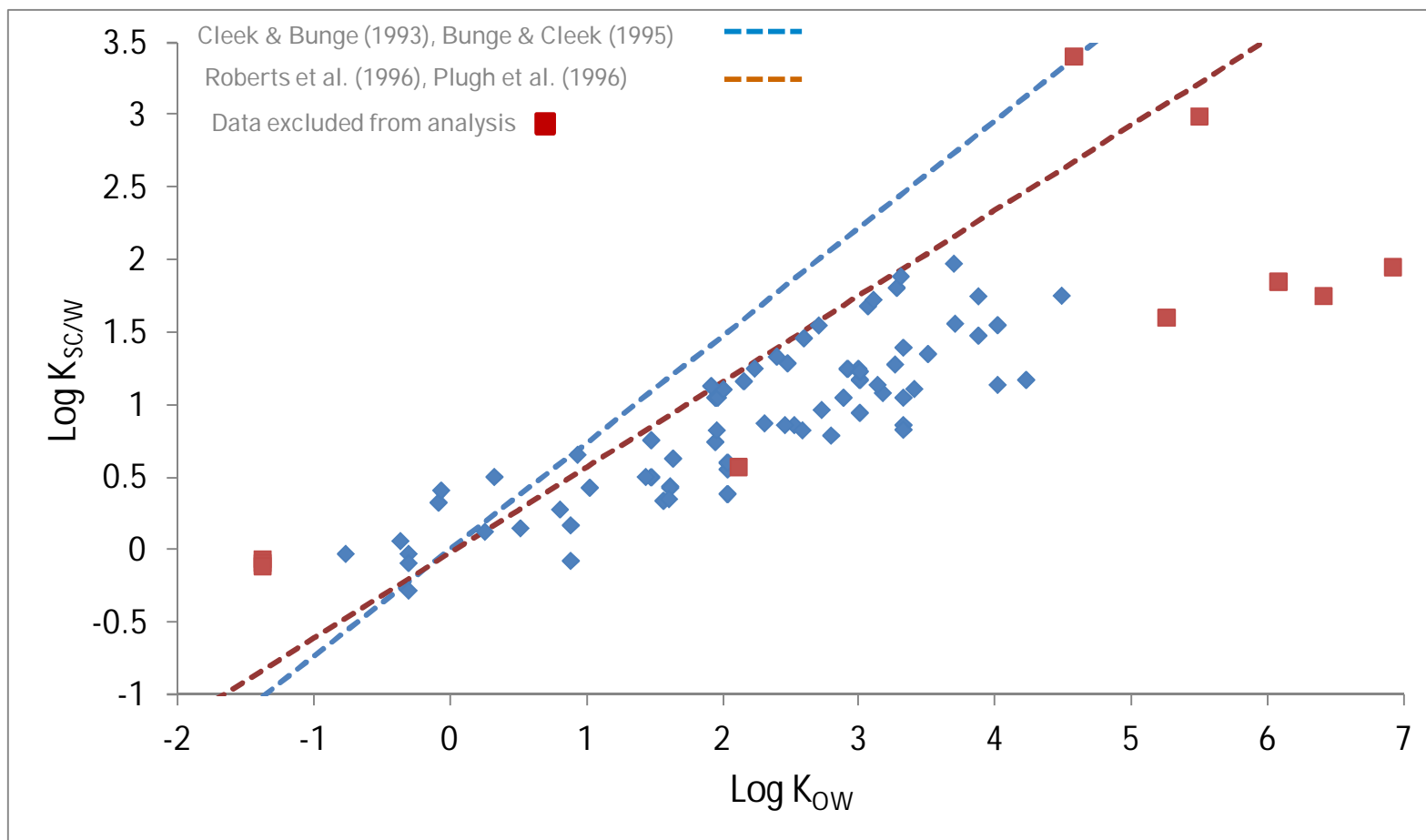
Or

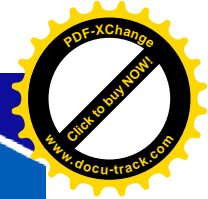
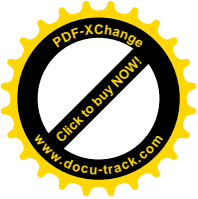
$$K_{SC} = 0.43(K_{OW})^{0.81} \phi_{lip} + (5.4(K_{OW})^{0.27} \omega_{pro} + \nu) / ((w_{pro} \rho_{water} / \rho_{pro}) + \nu)$$

Note: K_{OW} is used in both equations and is a sensitive parameter within the model

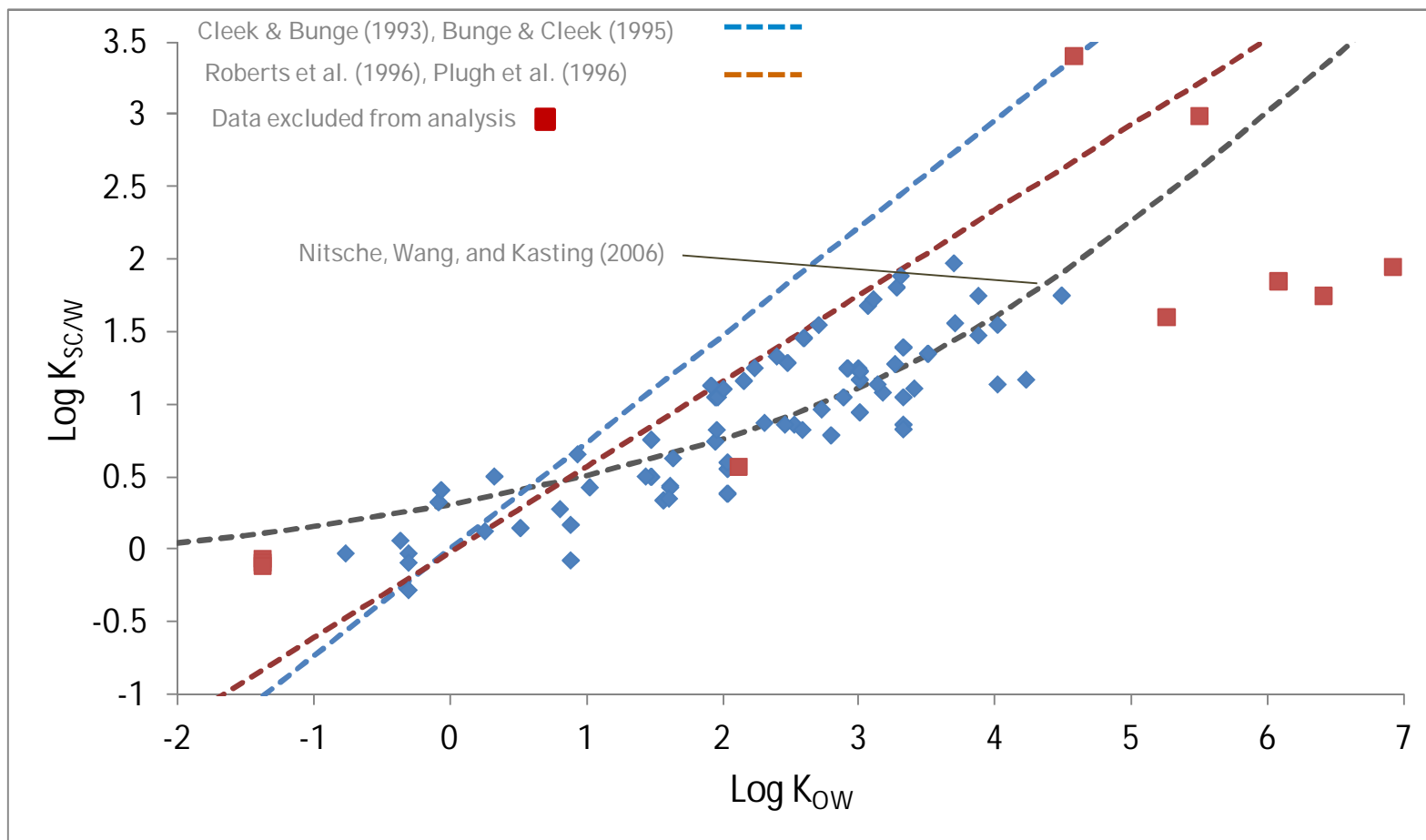


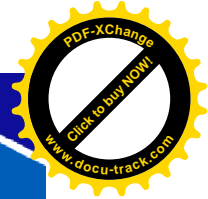
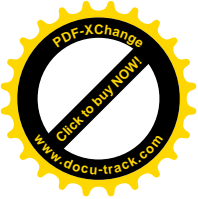
A TWO PHASE ANALYSIS OF SOLUTE PARTITIONING INTO THE STRATUM CORNEUM (NITSCHKE, WANG, AND KASTING (2006))



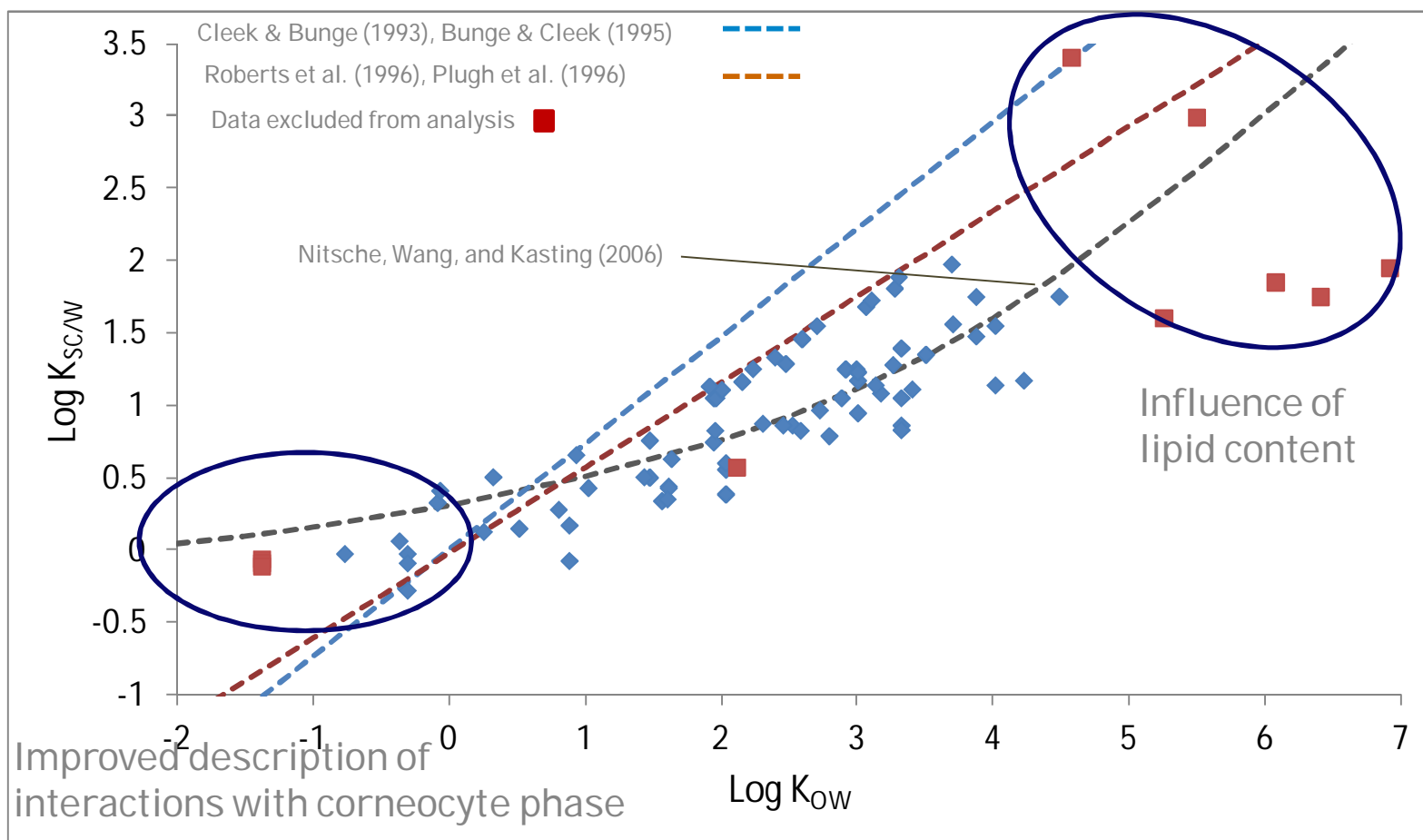


A TWO PHASE ANALYSIS OF SOLUTE PARTITIONING INTO THE STRATUM CORNEUM (NITSCHKE, WANG, AND KASTING (2006))





A TWO PHASE ANALYSIS OF SOLUTE PARTITIONING INTO THE STRATUM CORNEUM (NITSCHKE, WANG, AND KASTING (2006))





LIMITATIONS



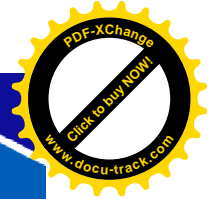
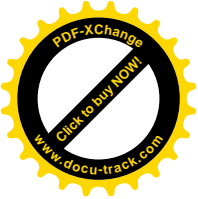
The model represents a reasonable fit for chemicals with $\log K_{ow} \leq 5$

Model is not well characterized for highly hydrophobic chemicals

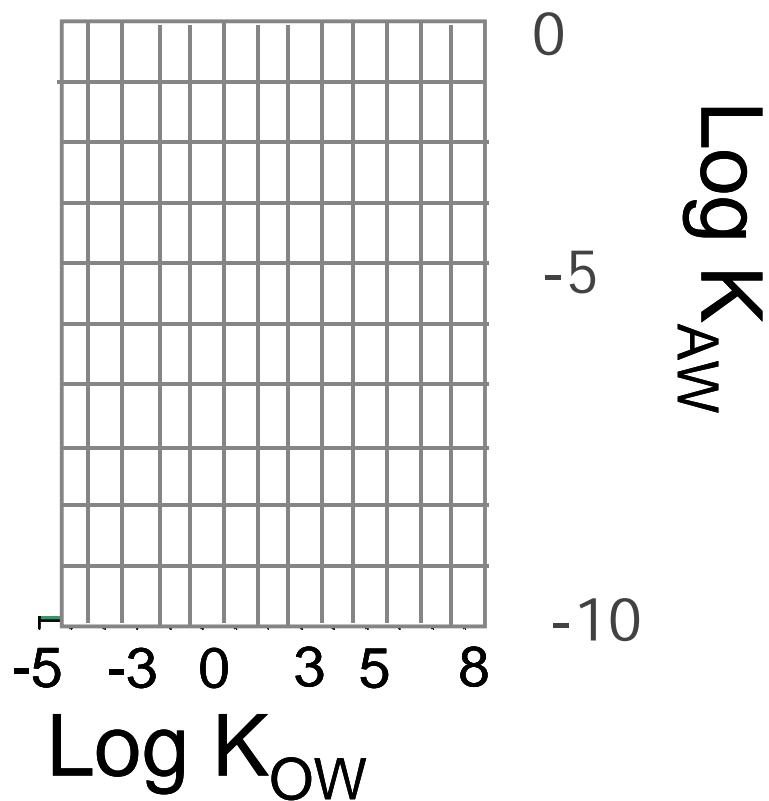
$K_{sc/w}$ measurements are sensitive to the lipid content and composition of stratum corneum samples, which vary significantly.

Need for further study!!





CHEMICAL SPACE DIAGRAM



 Fraction in SC > 80%

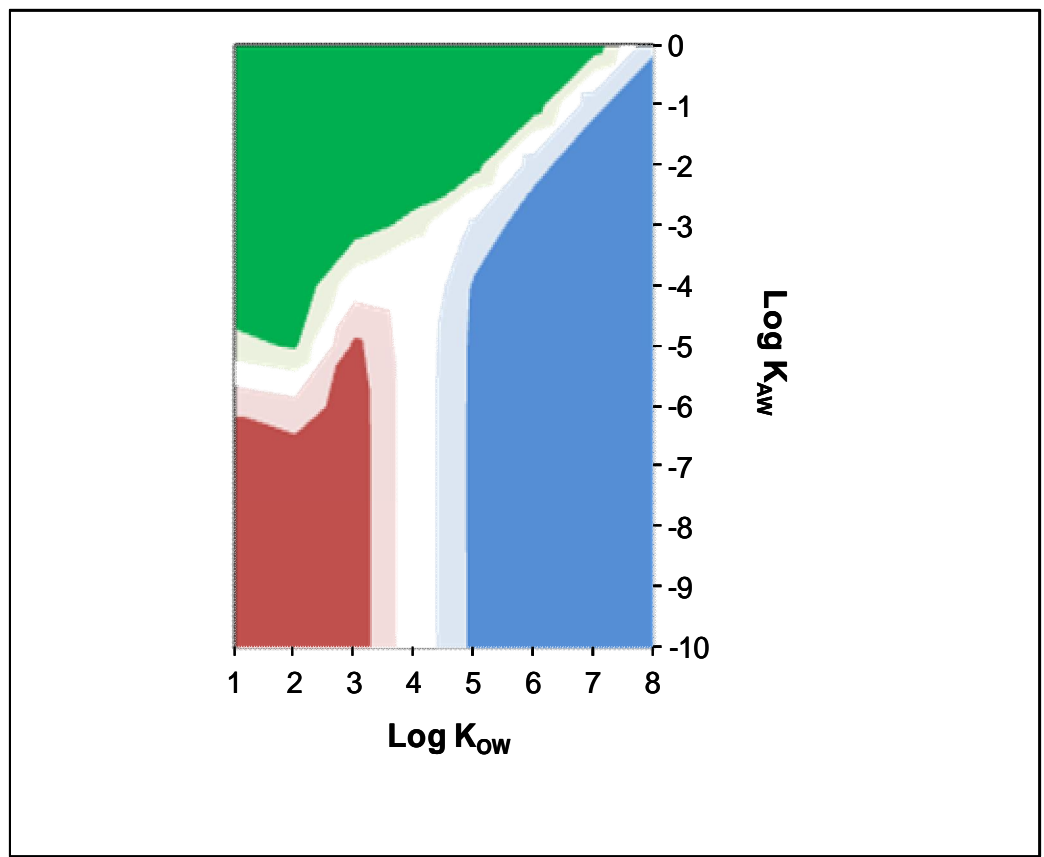
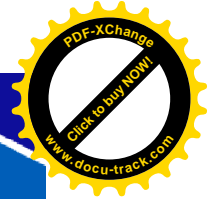
 Fraction in systemically absorbed > 80%

 Fraction volatilises > 80%





NEUTRAL HYDROCARBON MW = 100 CHEMICAL SPACE



Fraction in SC > 80%

Fraction in systemically absorbed > 80%

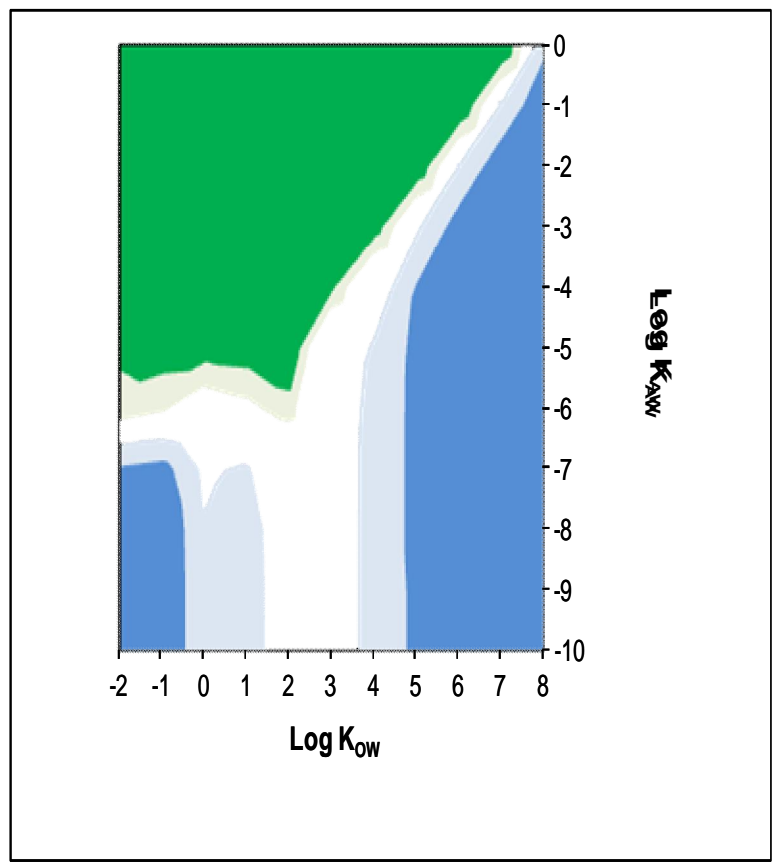
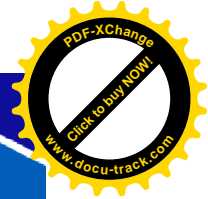
Fraction volatilises > 80%

Ethanol vehicle / partially hydrated





NEUTRAL HYDROCARBON MW = 200 CHEMICAL SPACE



■ Fraction in SC > 80%

■ Fraction in systemically absorbed > 80%

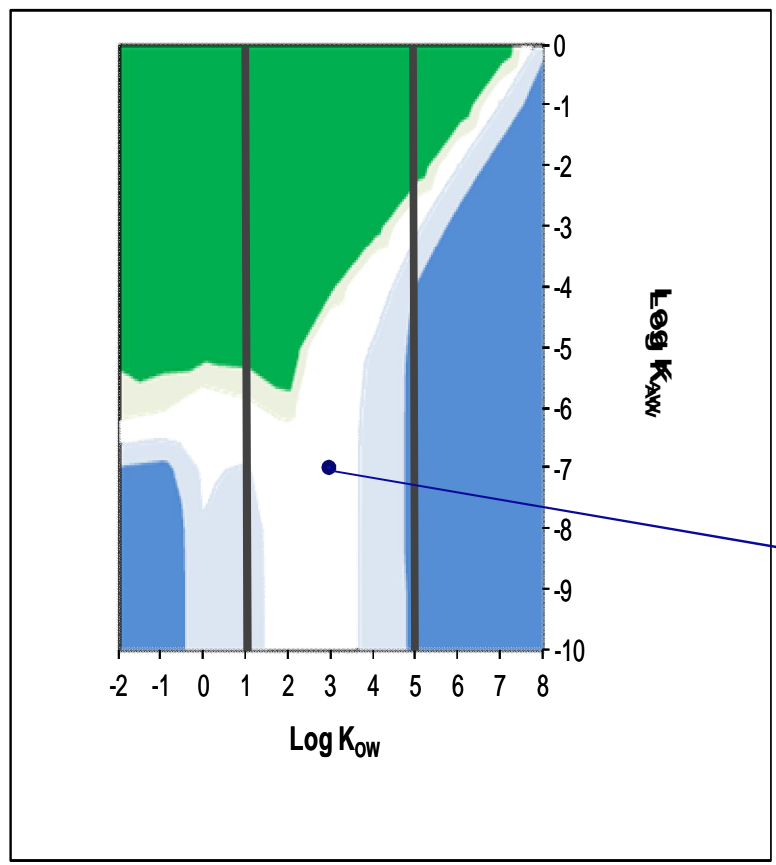
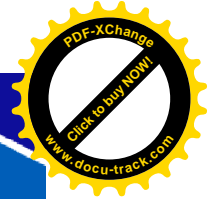
■ Fraction volatilises > 80%



Ethanol vehicle / partially hydrated



NEUTRAL HYDROCARBON MW = 200 CHEMICAL SPACE



49% SC
49% systemically absorbed

- Fraction in SC > 80%
- Fraction in systemically absorbed > 80%
- Fraction volatilises > 80%

Ethanol vehicle / partially hydrated





STRATUM CORNEUM



Stratum corneum consists of:

Lipids

(lipid volume fraction between 3-9%)

protein

(protein volume fraction between 19-55%)

Water

(water volume fraction between 36-78%)





THREE SOLUBILITIES

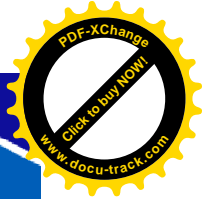


Behaviour of chemical will largely be defined based on interactions with the components of the stratum corneum, which are biologically intensive properties.

Ideally:

- Solubility in water
- Solubility in lipids
- Solubility in protein

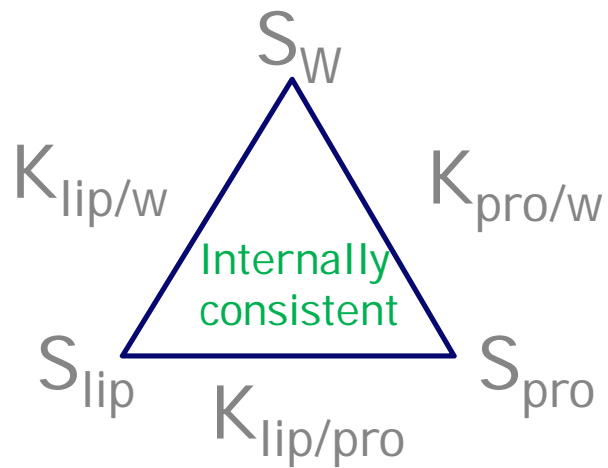




THREE SOLUBILITIES

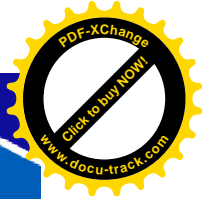


Assuming a closed system



Properties for which data are not readily available, and which are not easily obtained...

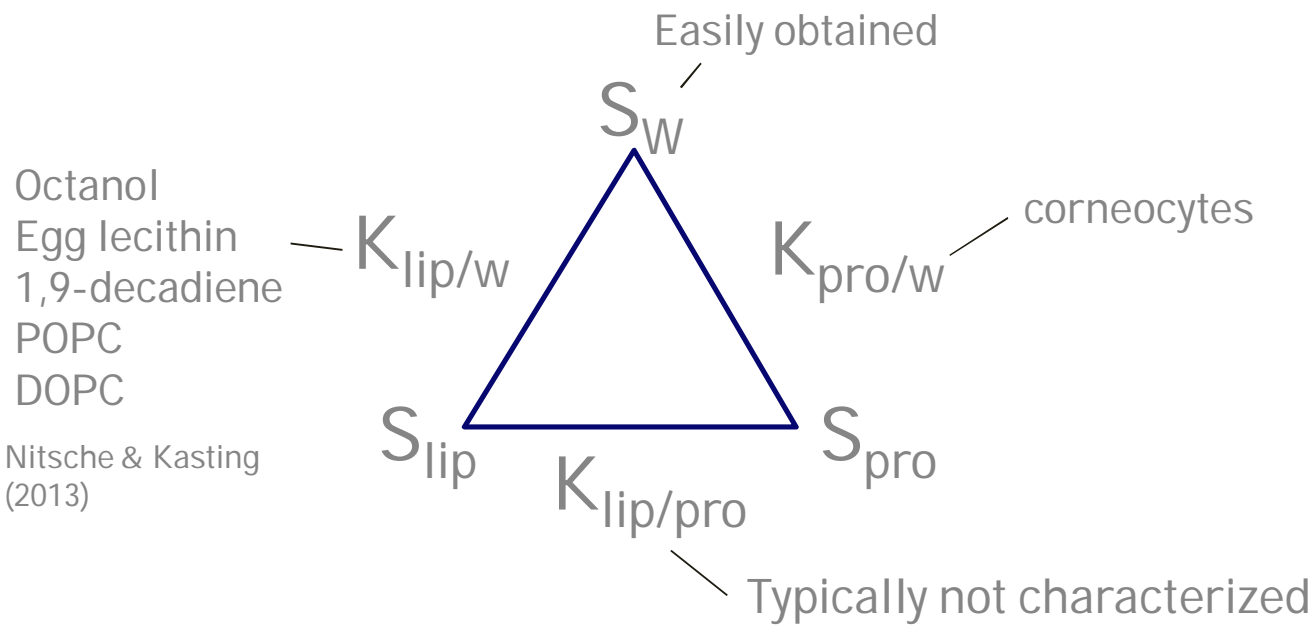




THREE SOLUBILITIES

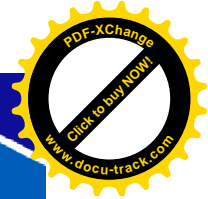
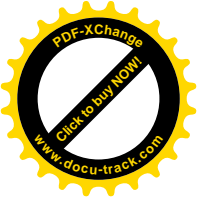


Adopting surrogates



Relationships between surrogates, however, may not be internally consistent





INTENSIVE VERSUS EXTENSIVE



Intensive

Chemical ingredient

- Dipolarity / polarizability
- Sub-cooled liquid vapour pressure
- Refractive index
- Hydrogen donor / acceptor
- Molecular weight
- Molar volume
- Charge surface area

Diffusion
- Lipids
- Water

Toxicity

Partitioning behaviour
- K_{ow}
- Reversible protein binding
- Henry's Law constant

Reactivity

- Abiotic
- Metabolic transformation
- Non-reversible binding

Basic formulation

Dose / % inclusion

Surfactants

Polymers

Silicones

Oil

+

Stabilizer

Emulsifier

+

Water

Skin care

Hair care

Deodorant

Physiological parameters

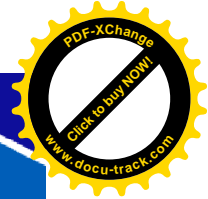
- Variability in volume and thickness of corneocyte layers
- Variability in lipid bilayer volume and orientation for chemical diffusion
- Availability of enzymes for metabolic transformation / Phase 1 activation
- Nature and number of protein modifications

Stratum corneum

Epidermis

Dermis

Extensive / system dependent



INTENSIVE VERSUS EXTENSIVE

System independent



Intensive

Chemical ingredient

- Dipolarity / polarizability
- Sub-cooled liquid vapour pressure
- Refractive index
- Hydrogen donor / acceptor
- Molecular weight
- Molar volume
- Charge surface area

Diffusion
- Lipids
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Toxicity

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Reactivity

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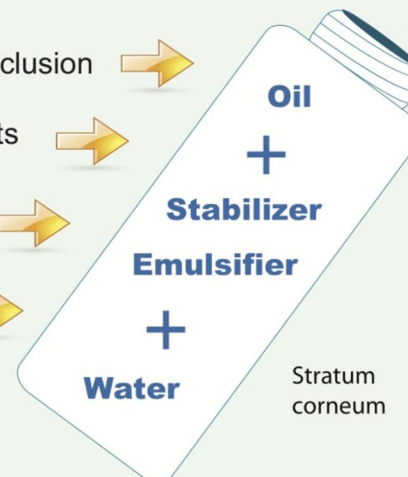
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Dose / % inclusion

Surfactants

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Skin care

Hair care

Deodorant

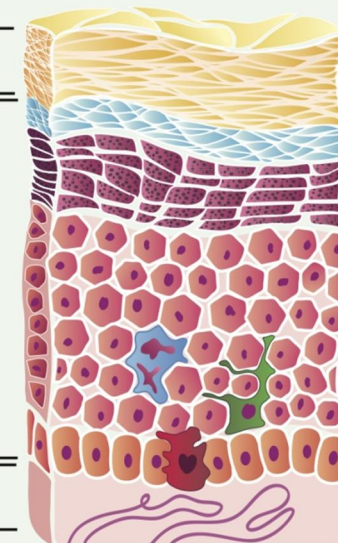
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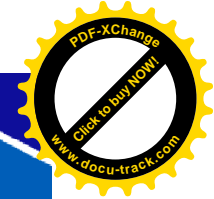
Stratum corneum

Epidermis

Dermis



Extensive / system dependent



ABIOTIC AND METABOLIC CHEMICAL TRANSFORMATION



Sensitizing chemicals can be divided into three categories based on their intensive reactivity, and whether they undergo chemical or enzymatic transformations:

1. *Haptens*

Low molecular weight chemicals that can react directly with a carrier protein to form immunogenic peptide-hapten complexes.

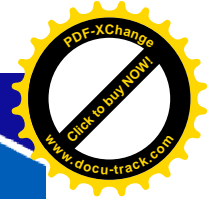
2. *Prohaptens*

Chemical that itself is non- or low-sensitizing but could be transformed into a hapten in the skin through bioactivation, usually via enzyme catalysis/Phase I activation.

3. *Prehaptens*

Chemical that itself is non- or low-sensitizing that is transformed into a hapten outside the skin by simple chemical transformation (air oxidation, photoactivation) and without the requirement of specific enzymatic systems.





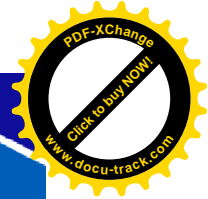
BIOAVAILABILITY RESEARCH NEEDS



Fundamental

- Estimating the kinetics of skin disposition
- Improved understanding of Phase 1 activation mechanisms
- Improved understanding of Phase 2 de-activating mechanisms
- Assessing the impact of formulation on skin disposition





BIOAVAILABILITY RESEARCH NEEDS



Fundamental

- Estimating the kinetics of skin disposition
- Improved understanding of Phase 1 activation mechanisms
- Improved understanding of Phase 2 de-activating mechanisms
- Assessing the impact of formulation on skin disposition

System independent

Associated with intensive properties

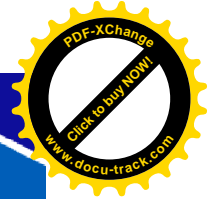
- Assessing the impact of non-covalent binding on the free concentration of a chemical
- Assessing the impact of air oxidation prior to transformation
- Assessing the role of photooxidation

System dependent

Associated with extensive properties

- Investigate whether 3D skin models and cell systems contain relevant metabolism
- Assessing differences between individuals with respect to key metabolising enzymes
- Investigating the role of microbiological transformations on the skin surface





BIOAVAILABILITY RESEARCH NEEDS



Fundamental

- Estimating the kinetics of skin disposition
- Improved understanding of Phase 1 activation mechanisms
- Improved understanding of Phase 2 de-activating mechanisms
- Assessing the impact of formulation on skin disposition

Prioritize reducing uncertainty in key model input parameters

Associated with intensive properties

- Assessing the impact of non-covalent binding on the free concentration of a chemical
- Assessing the impact of air oxidation prior to transformation
- Assessing the role of photooxidation

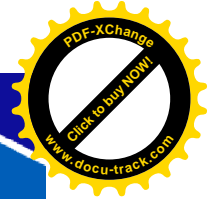
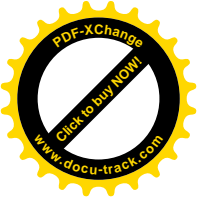
System dependent

Associated with extensive properties

- Investigate whether 3D skin models and cell systems contain relevant metabolism
- Assessing differences between individuals with respect to key metabolising enzymes
- Investigating the role of microbiological transformations on the skin surface

Perform uncertainty/sensitivity analysis to identify key system dependent properties influencing model performance





COMPLEMENTARY APPROACH THE ROLE OF MODELS IN DIRECTING EMPIRICAL STUDIES

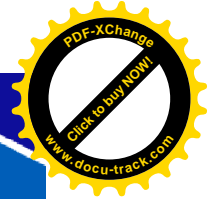


Where possible priority should be given towards improving fundamental understanding and towards accurate quantification of intensive properties, which are used as model input parameters

Assumption is that model estimates will have an inherently high degree of uncertainty associated with highly variable physiological/system dependent properties.

By reducing uncertainty in intensive properties and incorporating new insight gained from improved fundamental understanding it should be possible to utilize the model towards directing research at reducing uncertainty associated with the most sensitive physiological properties

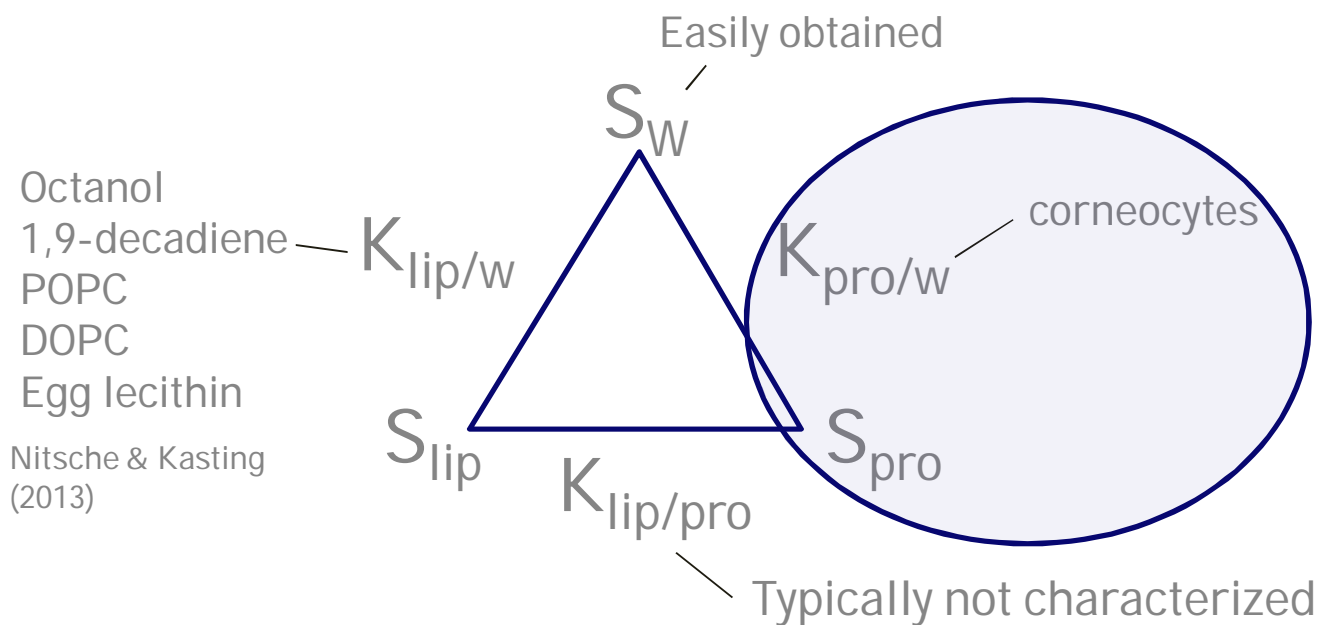




PROTEIN BINDING AND PHYSIOLOGICAL PARAMETERS



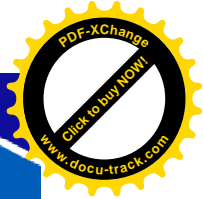
Nature and number of protein modifications



Nature of protein modifications –
fundamental / System independent
 Problematic to assess mechanistically, however, due to significant diversity and complexity

Number of protein modifications –
System dependent





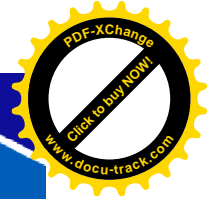
RESEARCH NEEDS – PROTEIN BINDING



Fundamental

- Assessing Immunogenic relevance:
 - » critical sites in skin
 - » critical adduct characteristics
 - » critical reaction mechanisms
- Measurement of protein adducts in human skin
- Identification of new reaction mechanisms and specific sites of binding using model peptides
- Assessing the utility of 3D modelling tools





CONCLUSIONS



- Articulation of our understanding of skin bioavailability in terms of intensive and extensive properties can be used to better prioritize research needs aimed at the development of improved mechanistic models
- Modelling tools should be used to help direct experimental efforts – emphasis on:
 - » complementary approach
 - » reducing model uncertainty
 - » improved mechanistic insight

