

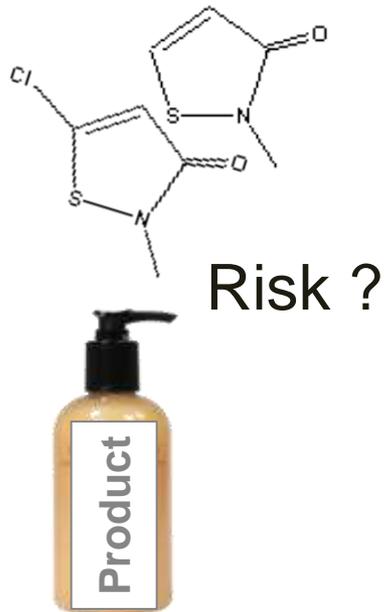
NON-ANIMAL RISK ASSESSMENT FOR SKIN SENSITISATION

DR GAVIN MAXWELL



Unilever

OUR CHALLENGE: HUMAN HEALTH RISK ASSESSMENT FOR SKIN SENSITISATION WITHOUT ANIMAL TESTING



Exposure



X

Hazard



Historical

Non-animal

In Vivo

Expert

We **risk assess** to prevent skin sensitisation in consumers

» What risk does ingredient **X** at conc. **Y** in product **Z** pose to the consumer?

How can we apply our mechanistic understanding of skin sensitisation to human health risk assessment?

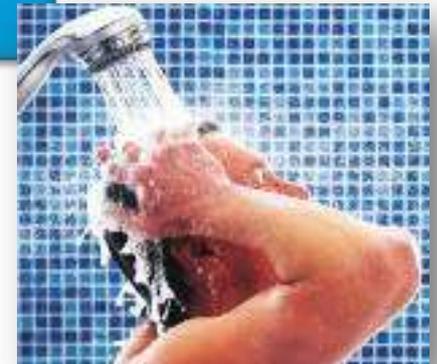
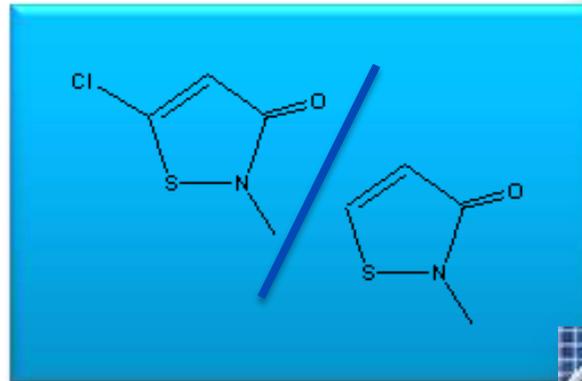
» removing the need for new animal test data...



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QUANTITATIVE RISK ASSESSMENT: CASE STUDY

What risk of skin sensitisation does MCI/MI* at 5ppm (0.0005%) in shampoo pose to the consumer?



*(Methylchloroisoithiazolinone/Methylisothiazolinone)

CONSUMER EXPOSURE TO INGREDIENT IN SHAMPOO



	Amount of product used per day	12.181g	=	Total exposure to product	12181mg *
X	Retention factor	0.01	=	Product remaining on skin	121.81mg **
÷	Skin surface area of application	1430 cm ² ***	=	Product exposure(mg/cm ²)	0.085mg/cm ²
X	Level of ingredient in product (%)	0.0005%	=	Consumer Exposure Level (ug/cm ²)	0.000425 μg/cm ²

* 95th percentile from Industry studies on product type Hall et al 2007

** QRA technical guidance dossier

*** EPA Exposure handbook 1998



DERIVATION OF NO EXPECTED SKIN SENSITISATION INDUCTION LEVEL (NESIL) FOR MCI/MI



Mouse Local Lymph node assay

EC3 = 0.009%

Extreme sensitiser

2.25 $\mu\text{g}/\text{cm}^2$

Human repeat insult patch test

1 $\mu\text{g}/\text{cm}^2$ is established as
No Observed Effect Level (NOEL)

Clear Lowest Observed Effect
Level (LOEL) at 4 $\mu\text{g}/\text{cm}^2$

Weight of Evidence No Expected Skin
Sensitisation Induction Level = 1 $\mu\text{g}/\text{cm}^2$



SENSITISATION ASSESSMENT FACTORS: SAF



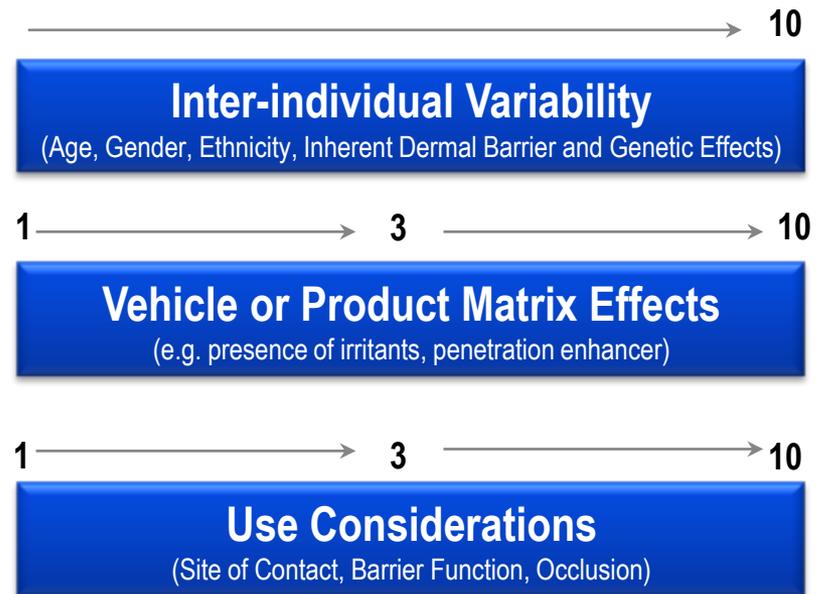
Apply to NESIL to extrapolate from controlled experimental situation to real life exposure scenarios
(Ref: Felter et al 2002)

Three areas of extrapolation/SAF's

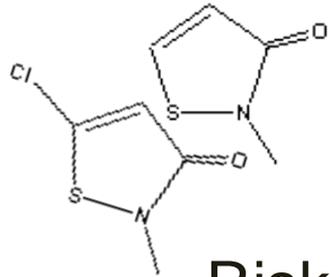
- Inter-individual susceptibility
- Matrix effects
- Use considerations

Product specific

- For a shampoo a SAF of 100 is applied



RISK OF SKIN SENSITISATION FROM INCLUSION OF MCI/MI AT 5PPM (0.0005%) IN SHAMPOO IS ACCEPTABLE



Risk ?



X



0.0004 $\mu\text{g}/\text{cm}^2$
Consumer Exposure
Level (CEL)

0.01 $\mu\text{g}/\text{cm}^2$
Acceptable Exposure
Level (AEL)

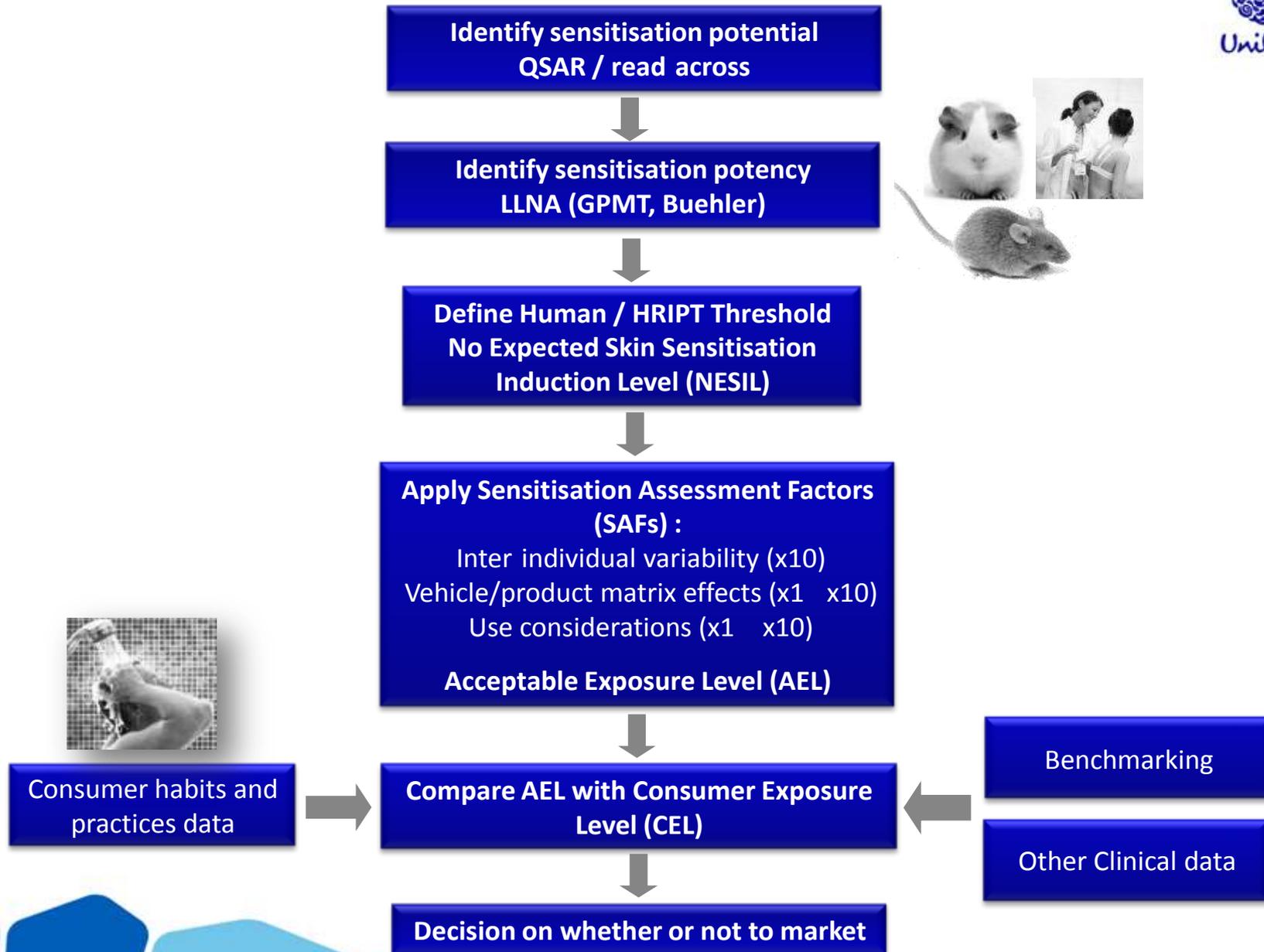
No Expected Skin
Sensitisation
Induction Level
(NESIL) 1 $\mu\text{g}/\text{cm}^2$



0.0001 0.001 0.01 0.1 1 10

MCI/MI Level - log mg/cm²

HOW CAN WE IMPROVE OUR QUANTITATIVE RISK ASSESSMENT APPROACH?



DEVELOPING AOP-BASED APPROACHES TO IMPROVE HUMAN HEALTH RISK ASSESSMENT



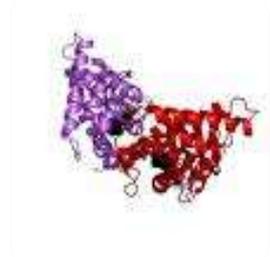
Induction of skin allergy is a multi-stage process driven by toxicity pathways

- mechanistic understanding is captured in Adverse Outcome Pathway (AOP)
- non-animal test methods have been developed; each aims to predict impact of a chemical on one key event
- how can we make risk assessment decisions by integrating this scientific evidence?



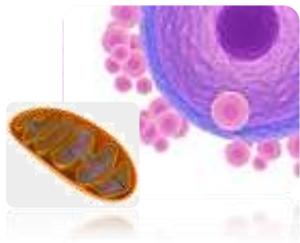
1. Skin Penetration

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



3 4. Haptentation: covalent modification of epidermal proteins

Key Event 1



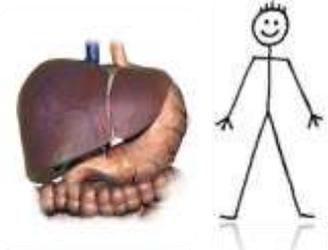
5 6. Activation of epidermal keratinocytes & Dendritic cells

Key Event 2 + 3



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

Key Event 4



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

Adverse Outcome



Modified from 'Adverse Outcome Pathway (AOP) for Skin Sensitisation', OECD report

NON-ANIMAL RISK ASSESSMENT FOR SKIN SENSITISATION: APPLICATION OF MATHEMATICAL MODELLING

1. Skin Penetration

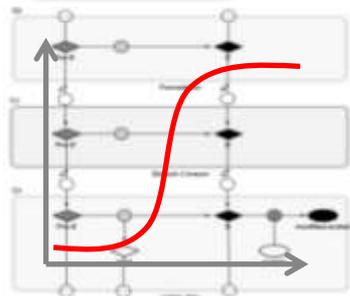
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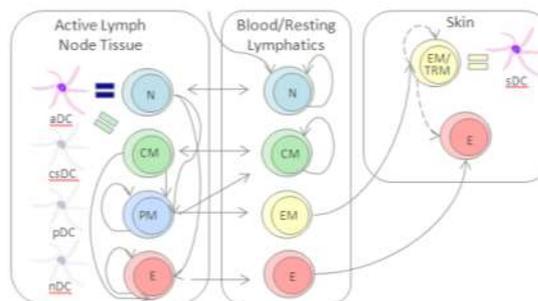
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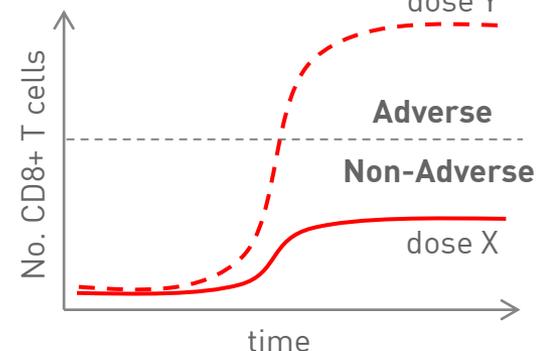
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haptentated skin protein prediction



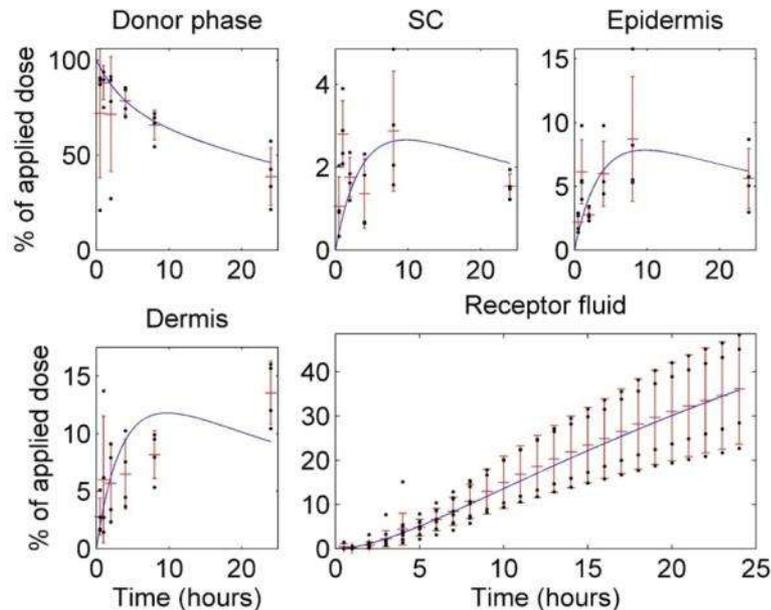
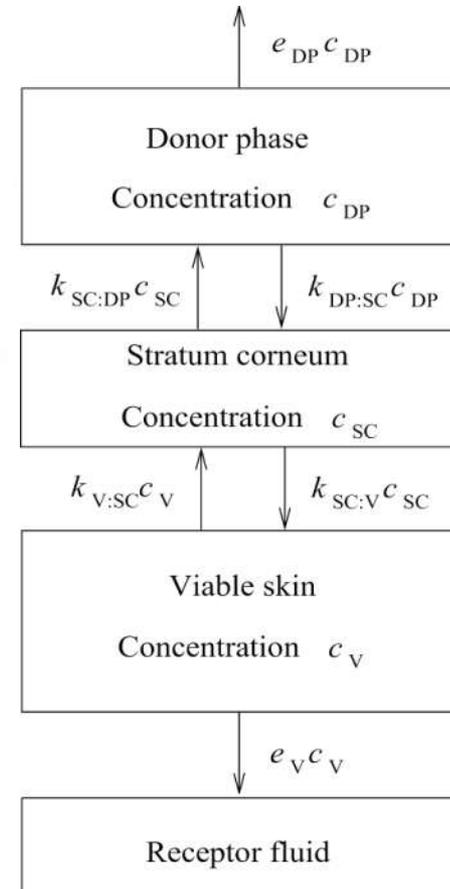
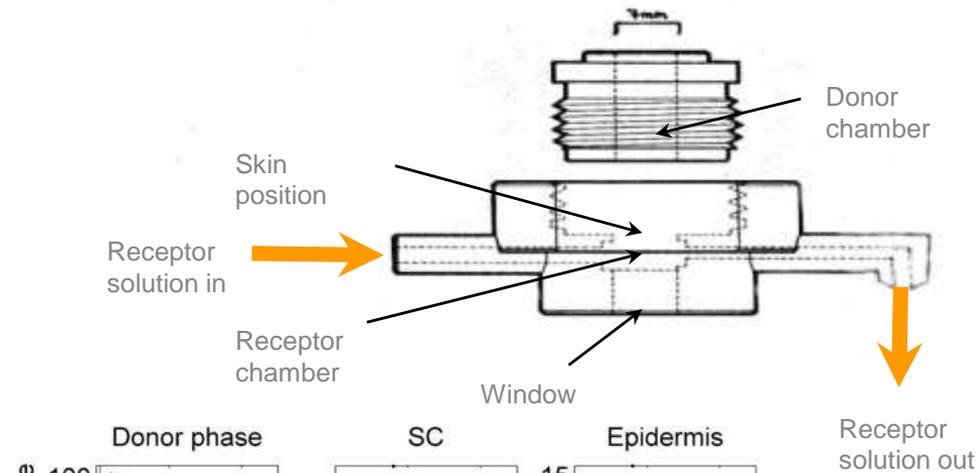
allergic immune response



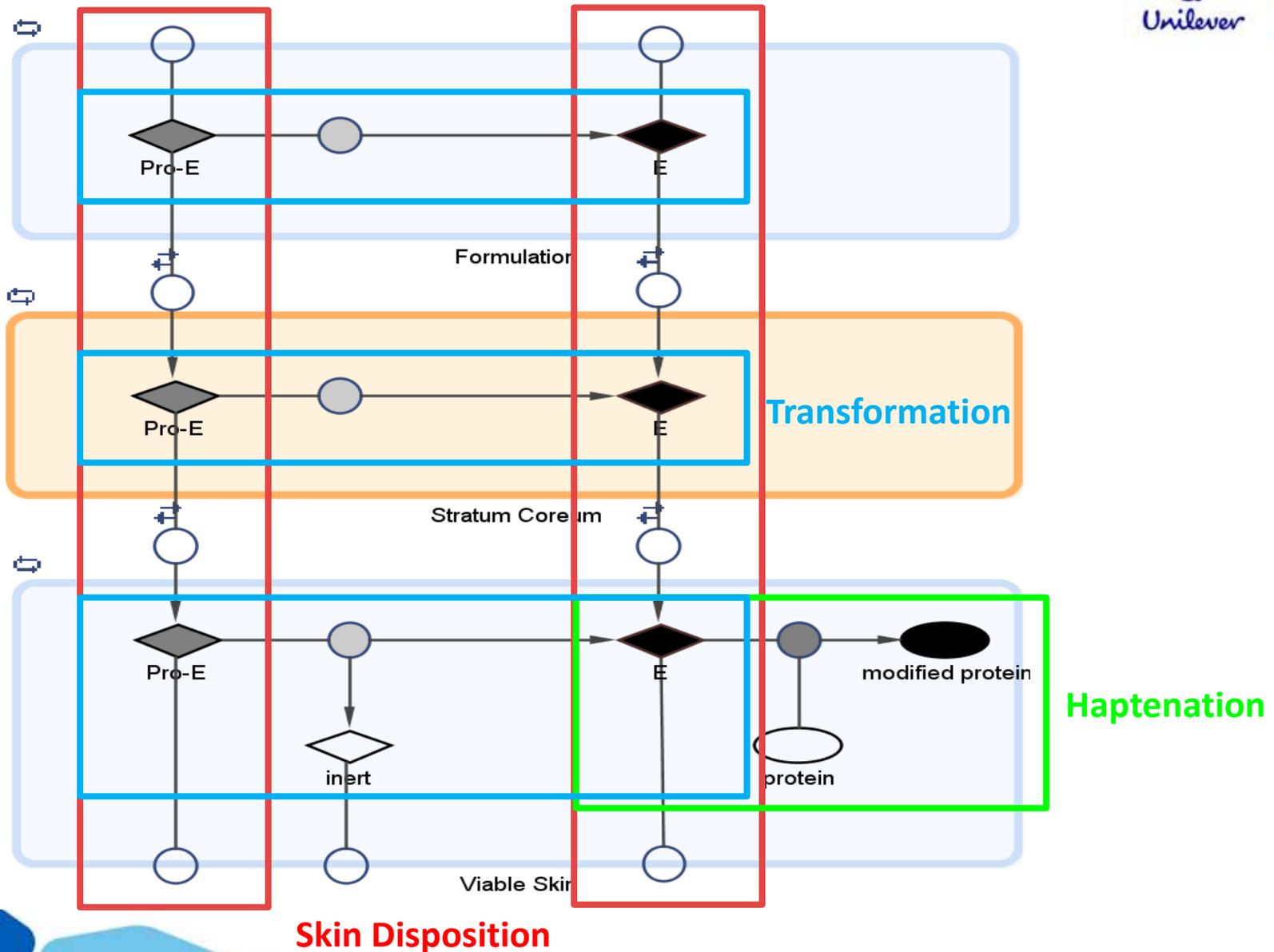
1. Generate relevant non-animal data for both the chemical (hazard) and the exposure scenario
2. Use linked mathematical models to predict human allergic immune response (with non-animal data as model input parameters)
3. Apply human immune response model prediction for risk assessment decision

MATHEMATICAL MODELLING OF NON-ANIMAL SKIN PENETRATION DATA

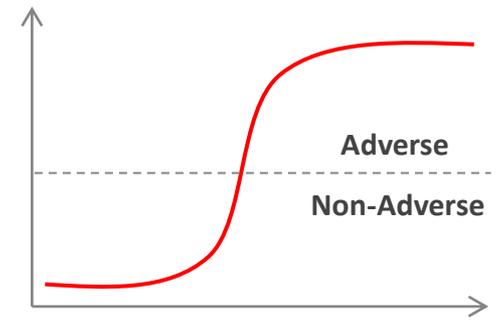
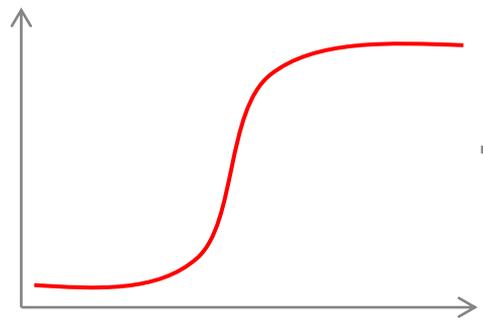
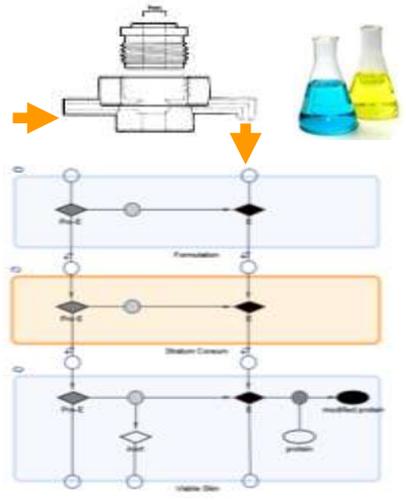
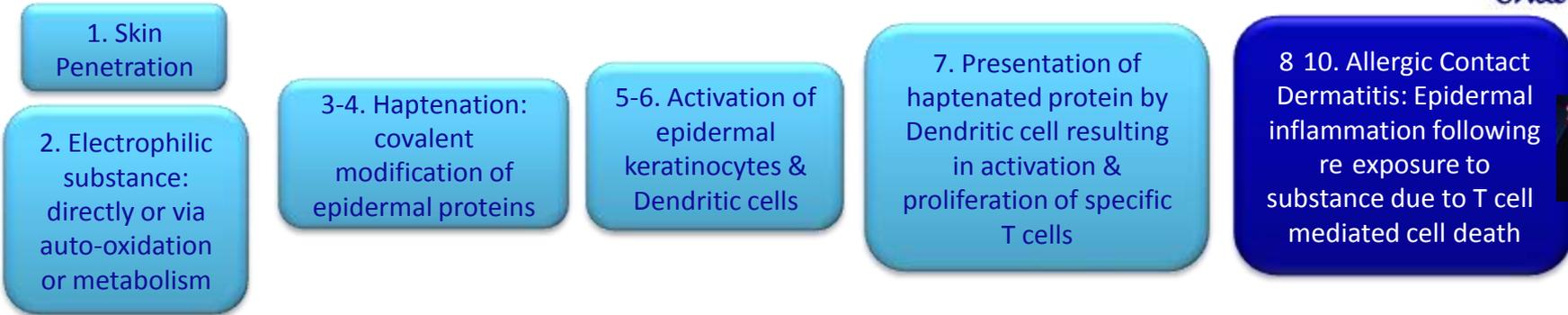
Apply pharmacokinetic modelling to ingredient permeation data and determine the free concentration of ingredient available to cause the molecular initiating event, i.e. modification of proteins in viable skin.



HAPTENATED SKIN PROTEIN MODEL SCOPE (INCLUDING TRANSFORMATION)



Applying non-animal data to predict whether a given human exposure is adverse or not



MODEL PREDICTION = HAPTENATED SKIN PROTEIN

human T cell-mediated immune response

'T LYMPHOCYTES: ORCHESTRATORS OF SKIN SENSITISATION' WORKSHOP

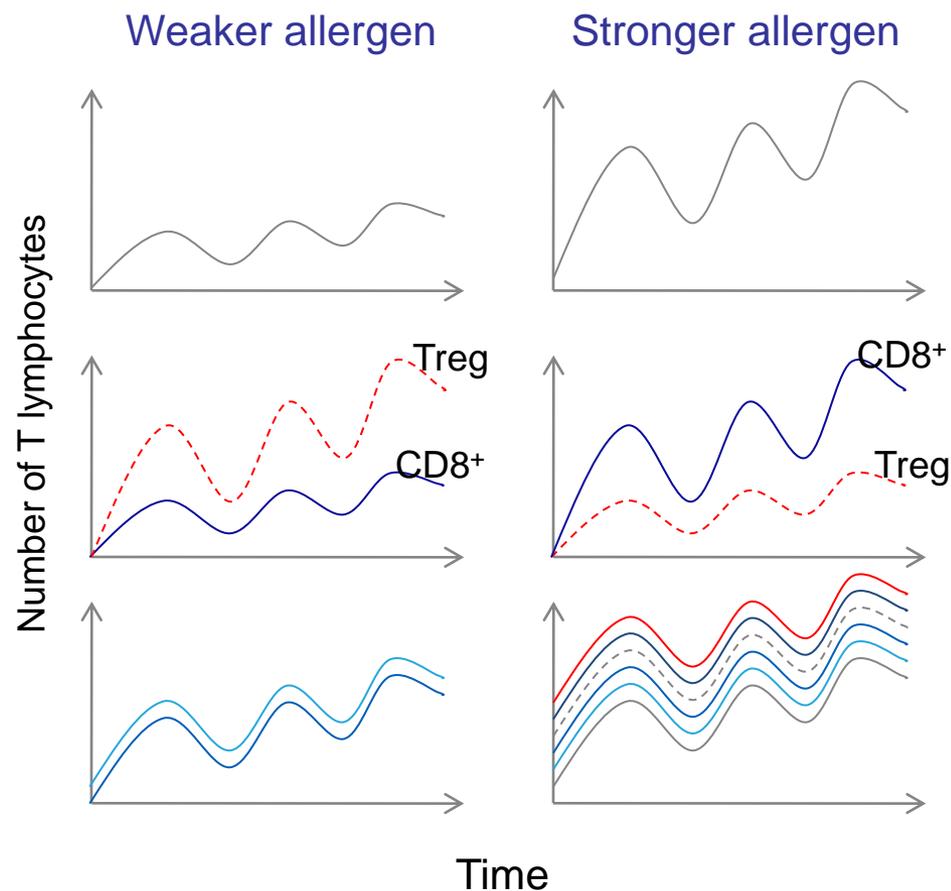


Immunologists, toxicologists & mathematical modellers – 2 day workshop in May 2010, London

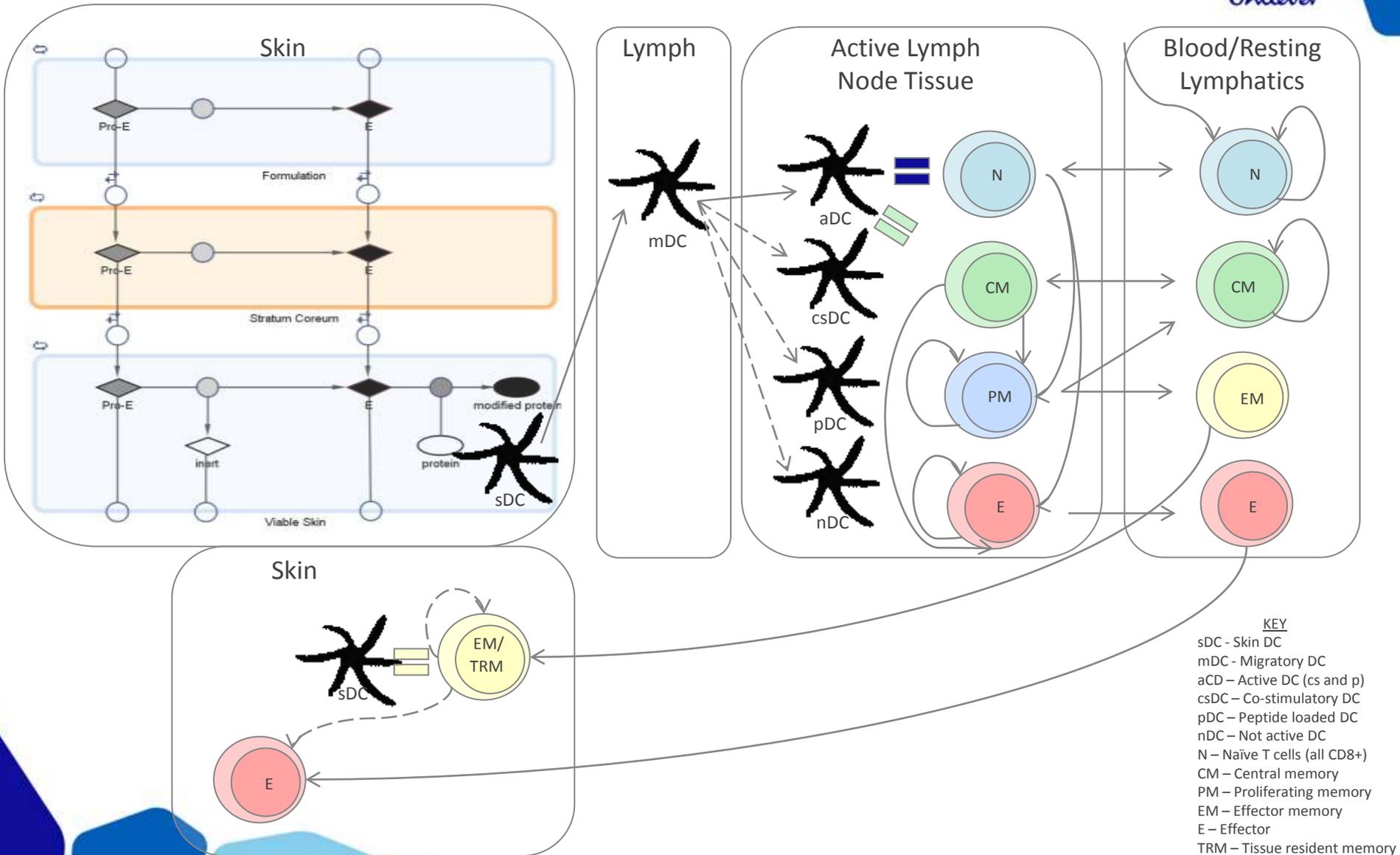
What are the characteristics of the T cell response that could reflect human skin sensitiser potency?

- » **Magnitude:** What is the extent of sensitiser-induced T cell response (volume, kinetics & duration)?
- » **Quality:** Within sensitiser-induced T cell response, what is the balance between the T cell sub-populations?
- » **Breadth:** What proportion of the T cell clonal repertoire has been stimulated by a given sensitiser?

Kimber *et al.* 2012. *Toxicology*. **291**. 18-24

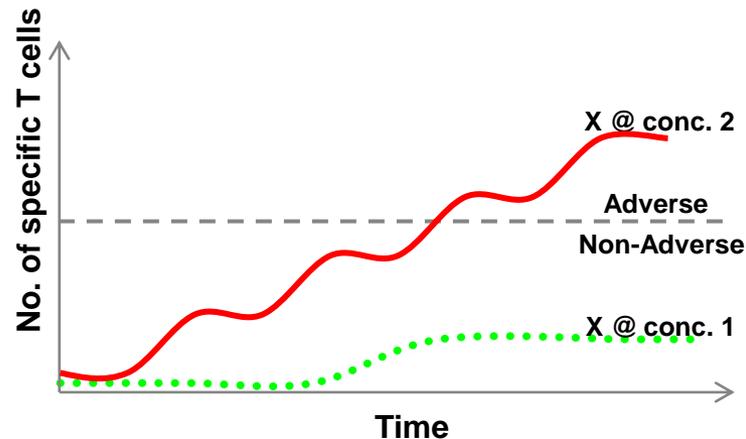


SKIN SENSITISATION MATHEMATICAL MODEL SCOPE



WHAT T CELL POPULATIONS CORRELATE WITH CLINICAL ADVERSITY?

We need human data to benchmark the threshold at which the number of antigen-specific T cells correlates with clinical adversity:



Working with collaborators to inform, test and improve our model:

- » patients undergoing sensitisation (e.g. treatment of viral warts)
- » patients already sensitised to chemicals, correlating the degree of sensitisation with the number of antigen-specific T cells

OUR NON-ANIMAL QUANTITATIVE RISK ASSESSMENT APPROACH FOR SKIN SENSITISATION?



Consumer habits and practices data

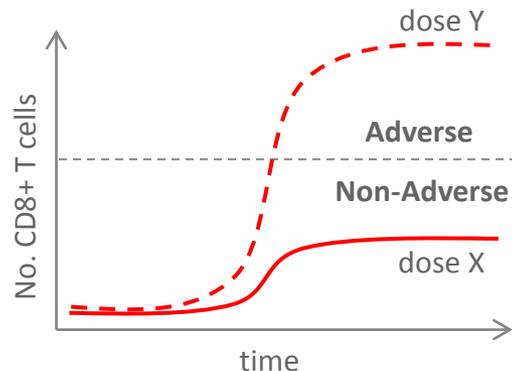
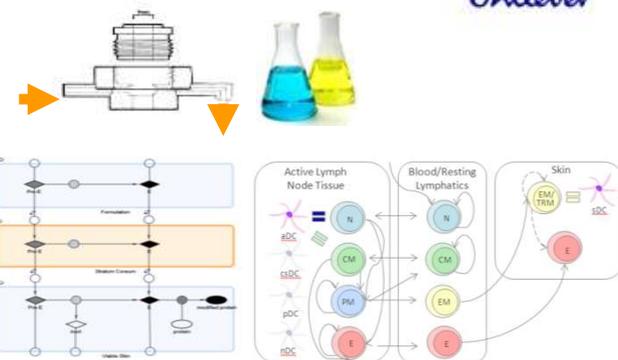
Identify sensitisation potential
QSAR / read across

Generate/apply skin bioavailability & haptentation as model input data for given skin exposure & product type

Generate mathematical model prediction of T cell response for given skin exposure & likely non adverse/adverse threshold (with explicit uncertainties)

Ability to generate supporting clinical biomarker data to demonstrate absence of adverse T cell response at consumer exposure levels

Decision on whether or not to market



Benchmarking

Other Clinical data



CONCLUSIONS



- Improving our quantitative risk assessment approach for skin sensitisation can be achieved through mechanistic interpretation of non-animal data in the context of a defined skin exposure
- Quantitative mathematical modelling of Skin Sensitisation AOP allows us to predict whether human immune response for a given exposure scenario to sensitiser will be adverse (or not)
- To apply our mathematical model to risk assessment decision-making we will also need to generate clinical/human-relevant datasets to confirm/challenge model predictions



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ACKNOWLEDGEMENTS

Maja Aleksic, Richard Cubberley, Michael Davies, Julia Fentem, Nikki Gellatly, Todd Gouin, Gaurav Jain, Sandrine Jacquilleot, Cameron MacKay, Craig Moore, Deborah Parkin, Juliette Pickles, Fiona Reynolds, Ouarda Saib, David Sheffield, Vicki Summerfield, Jeff Temblay, Carl Westmoreland & Sam Windebank

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