21ST CENTURY SAFETY SCIENCE AND NON-ANIMAL APPROACHES AT UNILEVER

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Slides available at www.TT21C.org

CAN WE USE A NEW INGREDIENT SAFELY?

Will it be safe
- For our consumers?
- For our workers?
- For the environment?



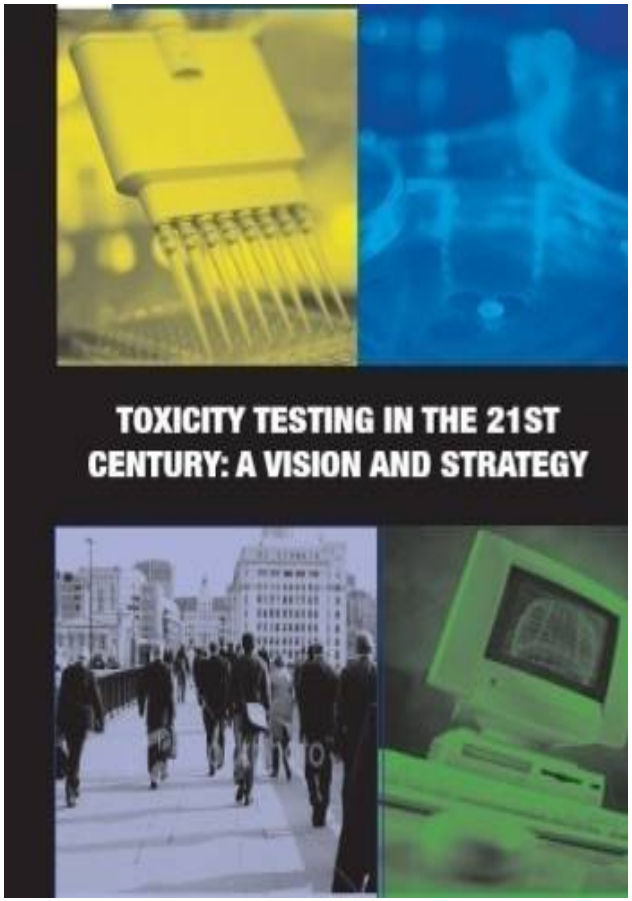


Can we use x% of ingredient y in product z?





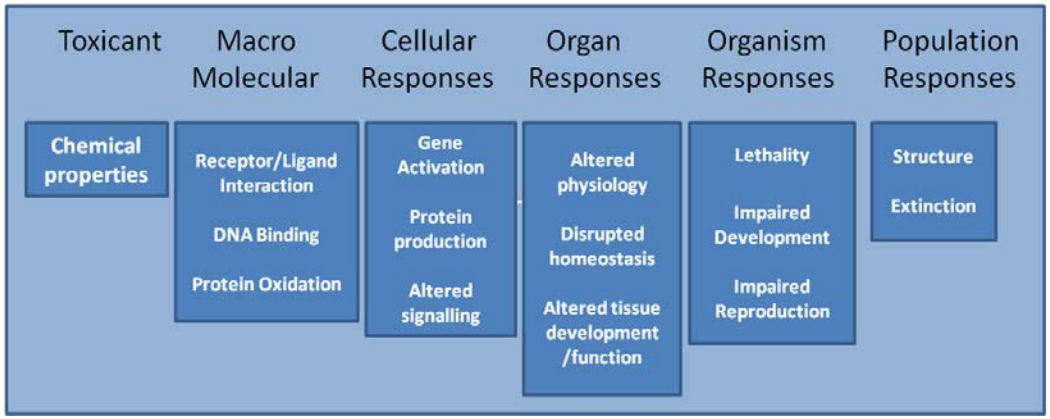

US NRC REPORT JUNE 2007



"Advances in toxicogenomics, bioinformatics, systems biology, epigenetics, and computational toxicology could transform toxicity testing from a system based on whole-animal testing to one founded primarily on *in vitro* methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin."

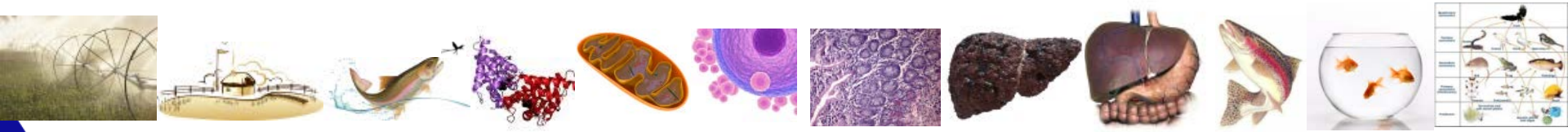
ADVERSE OUTCOME PATHWAYS (AOP) SOURCE TO OUTCOME PATHWAYS (S2OP)

- Proposal for a template and guidance on developing and assessing the Completeness of Adverse Outcome Pathways



Adapted from OECD (2012)

- Source to Outcome Pathways (Crofton et al, 2011)



EXAMPLES OF CASE STUDIES TO EXPLORE PATHWAYS-BASED RISK ASSESSMENT AT UNILEVER

- Skin Allergy Risk Assessment

- Systemic Toxicology Risk Assessment
  - DNA damage

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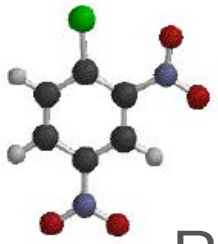
OUR CHALLENGE: HUMAN HEALTH RISK ASSESSMENT FOR SKIN SENSITISATION WITHOUT ANIMAL TESTING

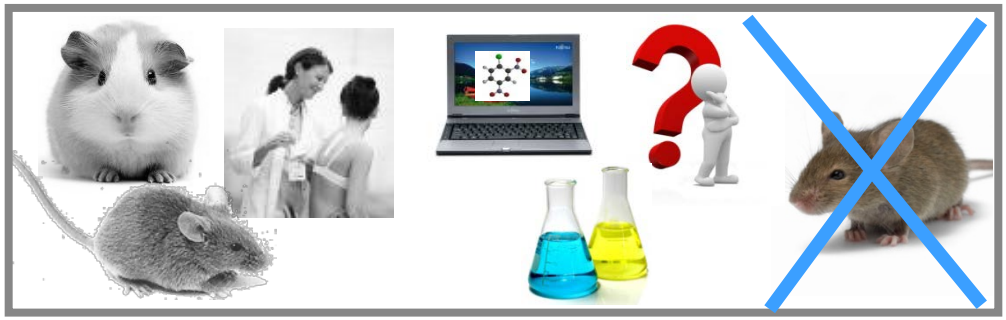


Risk ?

Exposure

x

Hazard



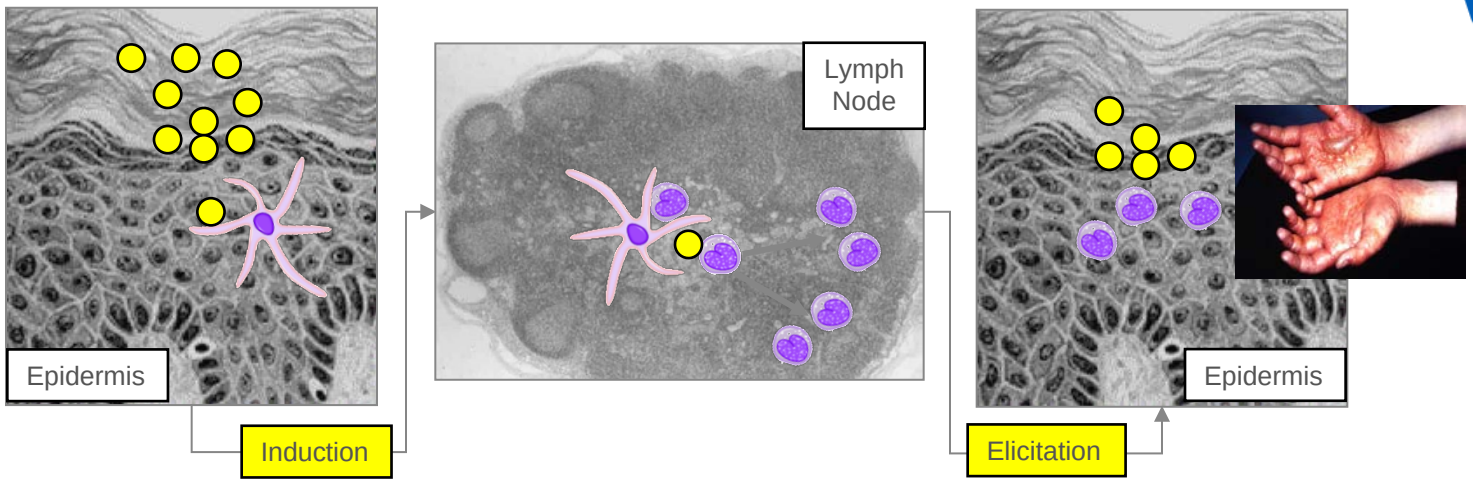
Historical Non-animal *In Vivo*

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

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

» Developing a mathematical model of the mechanism of skin sensitisation in humans

ADVERSE OUTCOME PATHWAY FOR SKIN SENSITISATION: CAPTURING OUR CURRENT MECHANISTIC UNDERSTANDING



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

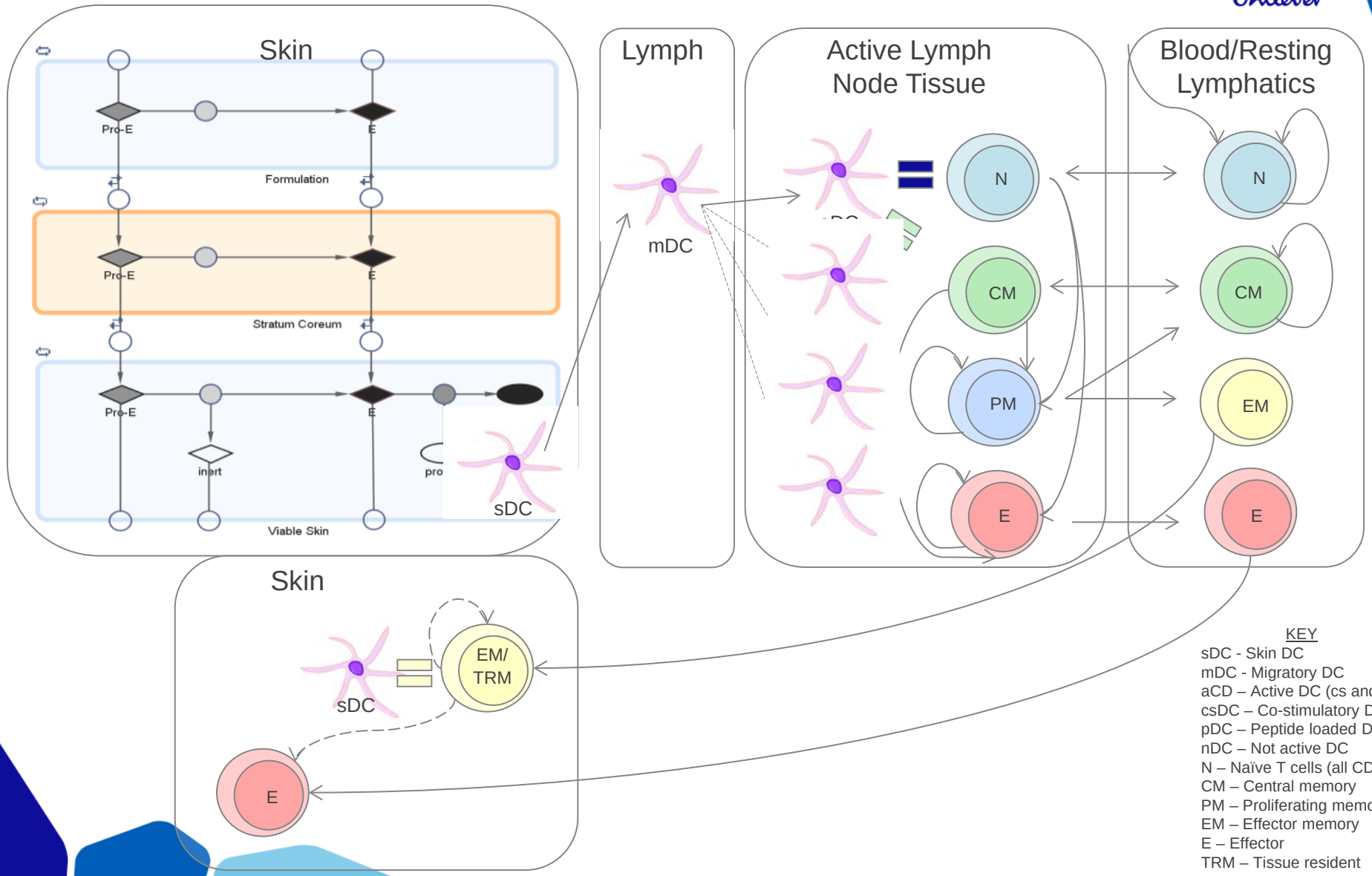
7. Presentation of haptenated protein by Dendritic cell resulting in activation & proliferation of specific T cells

8-10. 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**

Modified version of flow diagram from 'The Adverse Outcome Pathway for Skin Sensitisation initiated by Covalent Binding to Proteins', OECD report

SKIN SENSITISATION CD8+ T CELL MATHEMATICAL MODEL SCOPE

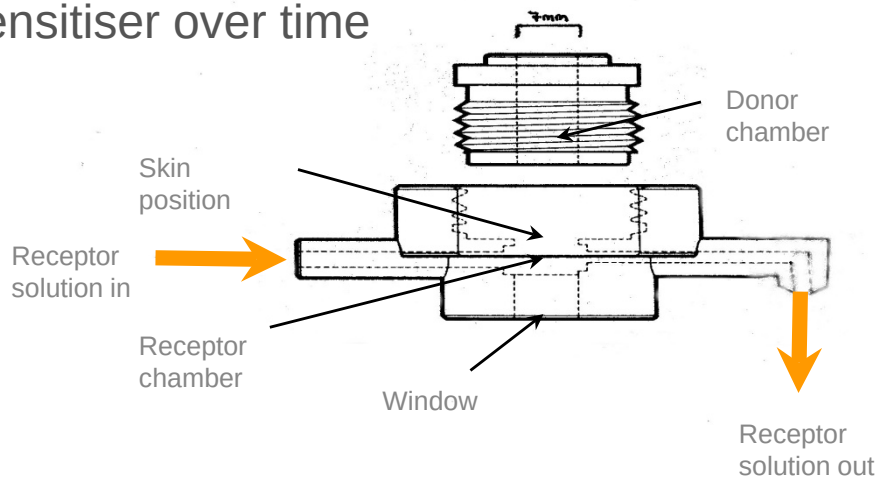


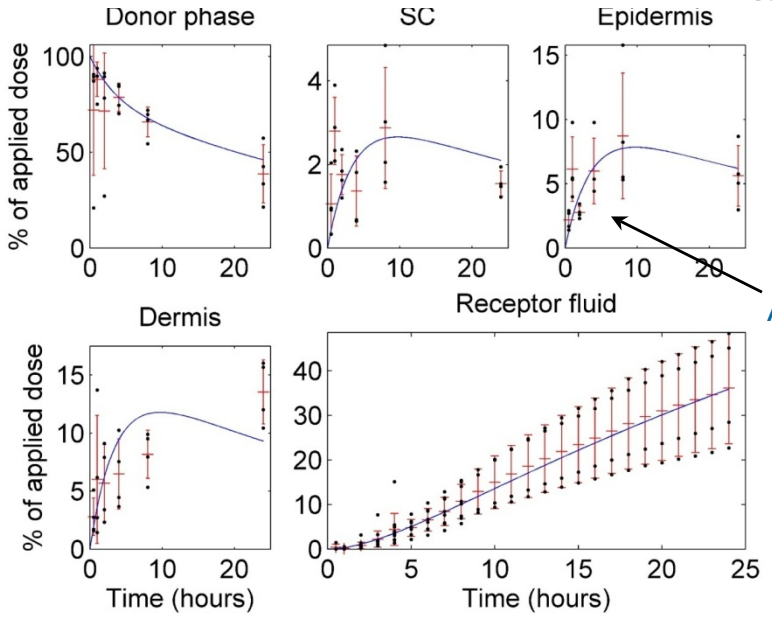
KEY

sDC - Skin DC
mDC - Migratory DC
aCD – Active DC (cs and p)
csDC – Co-stimulatory DC
pDC – Peptide loaded DC
nDC – Not active DC
N – Naïve T cells (all CD8+)
CM – Central memory
PM – Proliferating memory
EM – Effector memory
E – Effector
TRM – Tissue resident memory

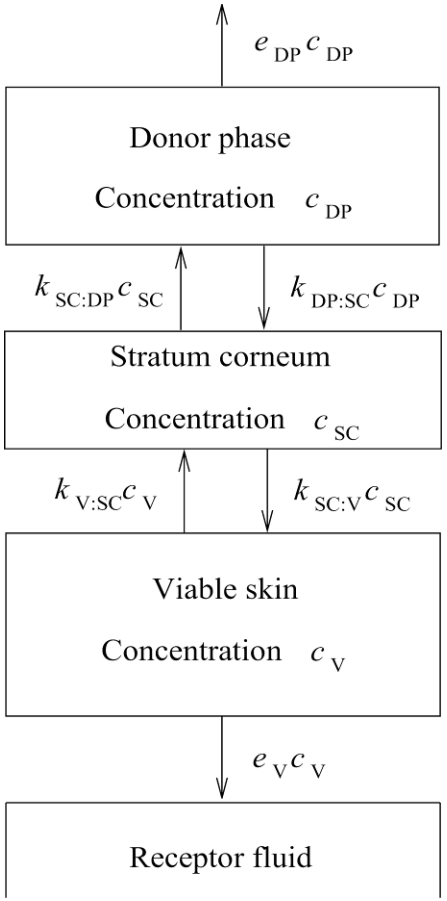
MATHEMATICAL MODELLING OF NON-ANIMAL SKIN PENETRATION DATA

Apply pharmacokinetic modelling to determine how skin bioavailability parameters (e.g. Cmax, tmax, Area Under Curve (AUC)) vary for skin sensitiser over time





AUC/Dose = 12.2hr



Davies *et al*. 2011. *Toxicol Sci.* **119**. 308-18

MORE COMPLEX T CELL MODELS…..

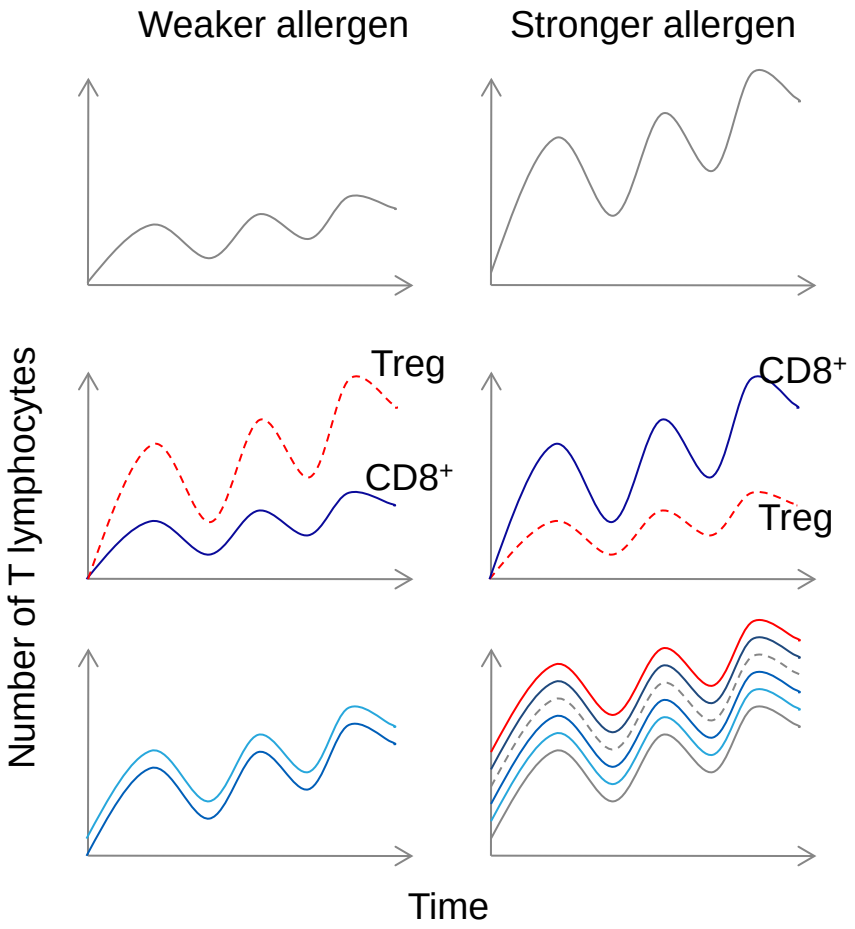
Our current model tests Hypothesis (a)

» magnitude of antigen-specific CD8 response drives severity of response

Hypotheses (b) & (c) will be explored via ‘next generation’ mathematical models:

» Quality of the T cell response (balance of Tregs, CD8, CD4..) drives severity

» Breadth of T cell response drives severity of response

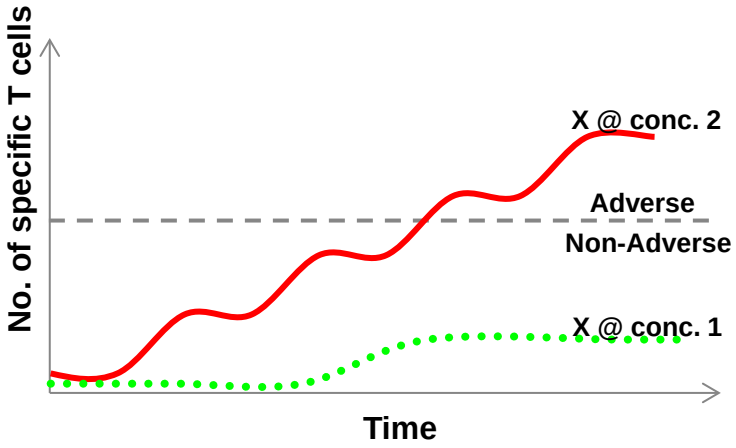


Kimber *et al*, 2012, *Toxicology* **291**18-24

http://www.altex.ch/resources/Mackay_fc.pdf

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 for clinical benefit
- patients already sensitised to chemicals, correlating the degree of sensitisation with the number of antigen-specific T cells

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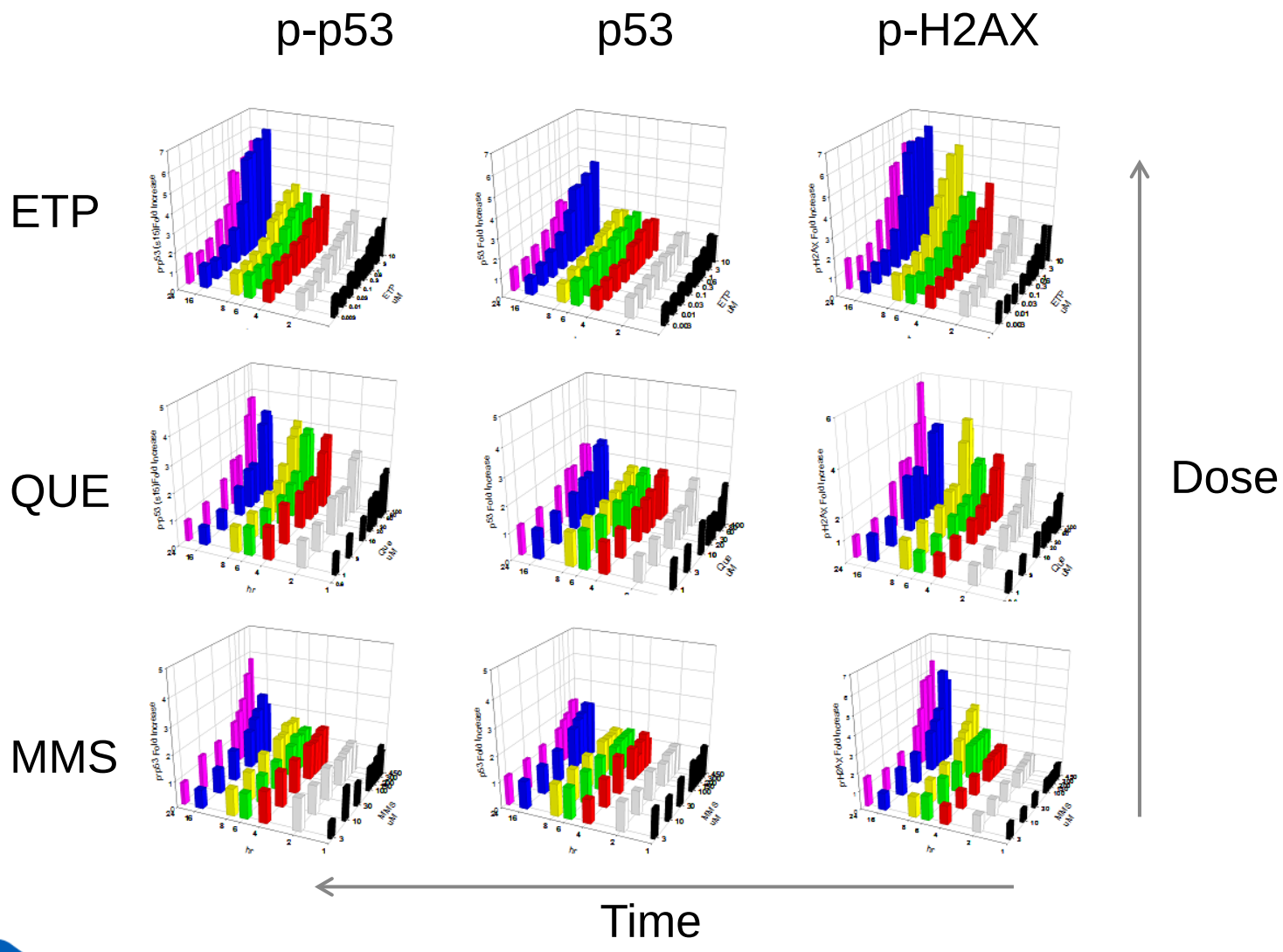
A TT21C PROTOTYPE TOX PATHWAY (AOP): GENOTOXICITY/DNA DAMAGE

- Joint research program with Hamner Institutes
- Develop tools to assess DNA-damage stress pathways
- Examine dose-dependent transitions for case-study mutagenic compounds
- Apply data to develop a computational systems biology model of the p53-mdm2 network
- Q: Can we use genotoxicity tox-pathway in TT21C paradigm to:
  - Provide Genetic Toxicology risk assessment and
  - Provide a prototype proof of principle for TT21C/AOP

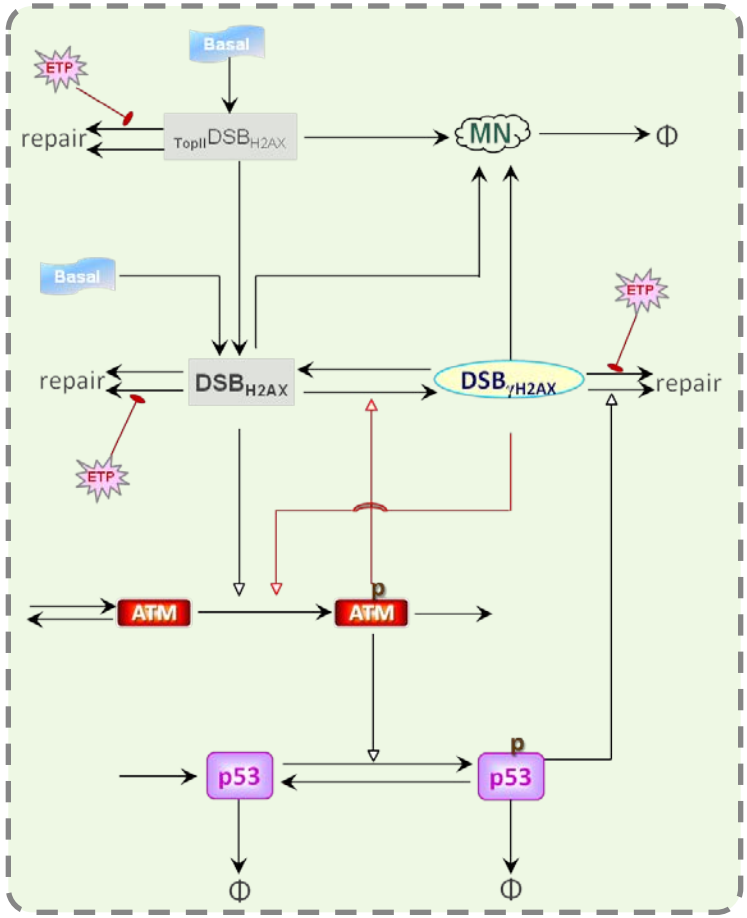
Unilever

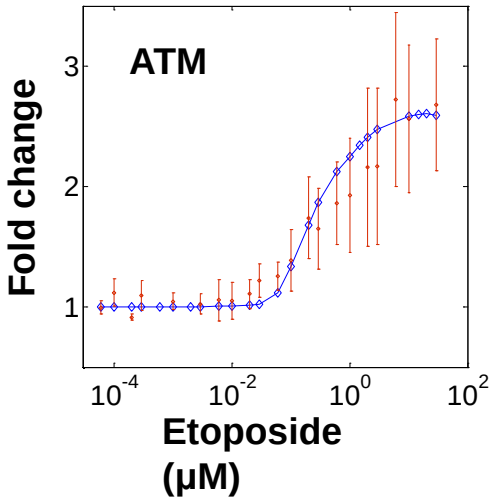
The Hamner Institutes for Health Sciences
Where Great Minds & Medicine Meet

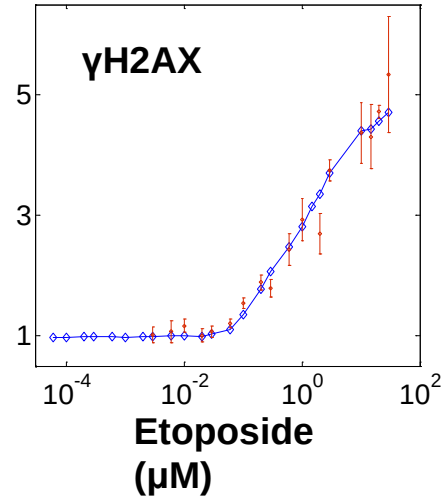
TIME/DOSE: DNA DAMAGE & P53 ACTIVATION

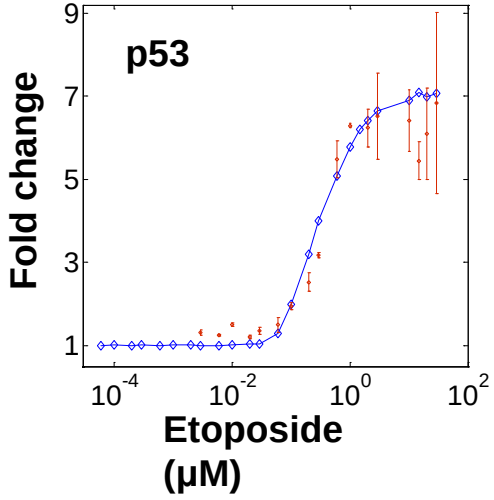


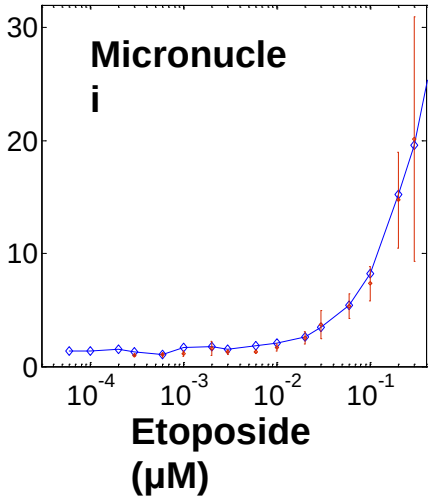
MODELLING ULTRASENSITIVITY IN P53 ACTIVATION



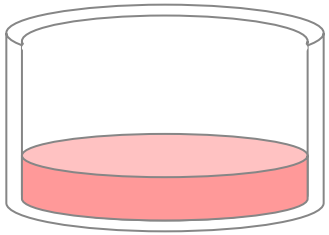






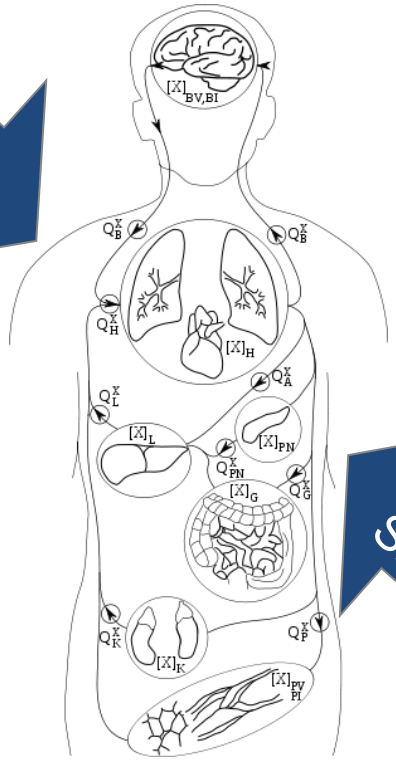


IN VITRO TO *IN VIVO* (HUMAN EXPOSURE) EXTRAPOLATION



In vitro adaptive/adverse threshold concentration (µM) – measuring & modelling **FREE CONCENTRATIONS**

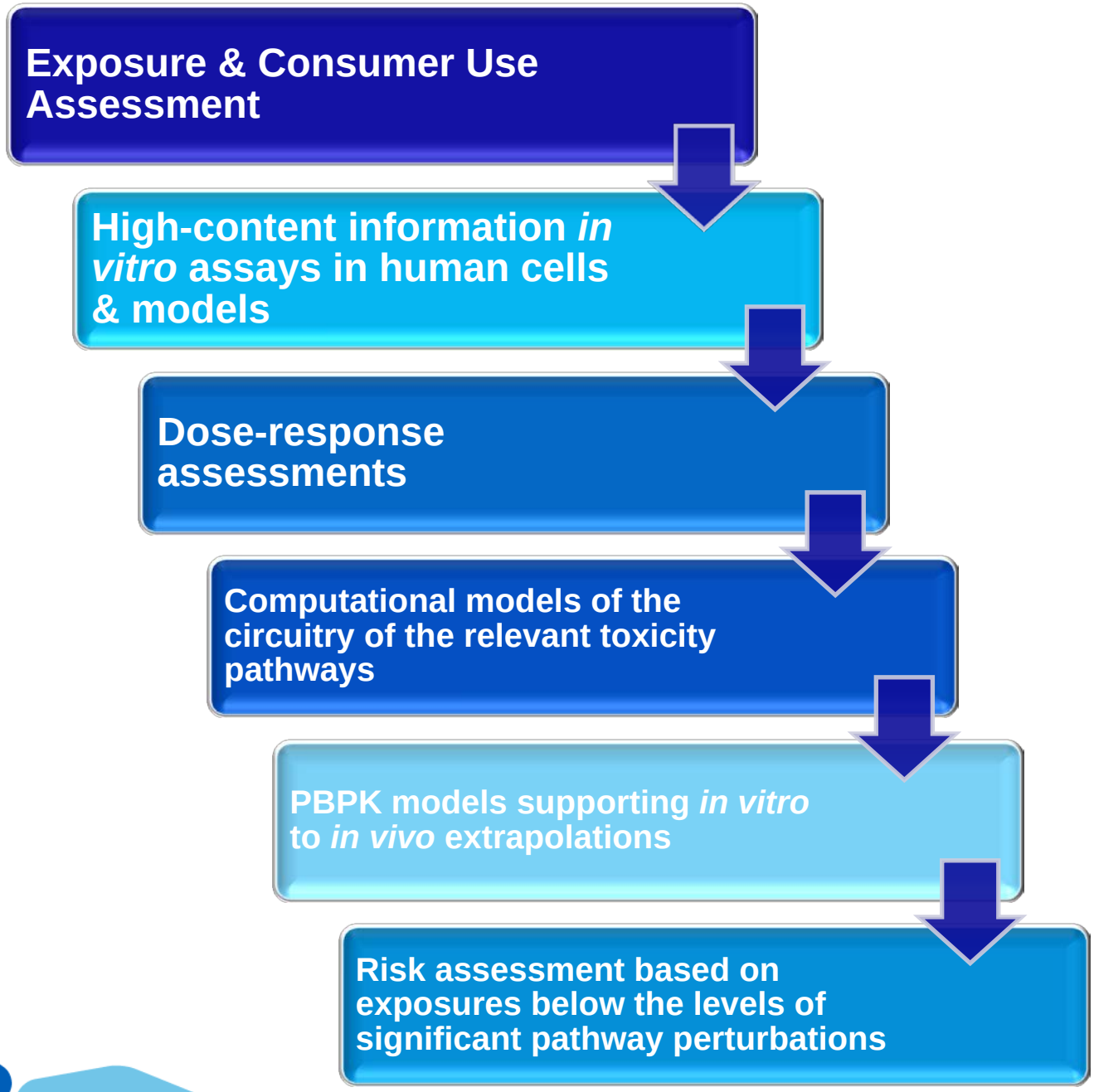
In vitro to in vivo extrapolation



Target site concentration (µM)

PBPK Skin penetration

Exposure mg/kg/day


Exposure & Consumer Use Assessment
High-content information *in vitro* assays in human cells & models
Dose-response assessments
Computational models of the circuitry of the relevant toxicity pathways
PBPK models supporting *in vitro* to *in vivo* extrapolations
Risk assessment based on exposures below the levels of significant pathway perturbations

A LONG-TERM VISION: SOURCE TO OUTCOME PATHWAY-BASED SAFETY RISK ASSESSMENT

To **reduce uncertainty** within our risk assessments…

…and **replace** our current reliance on apical endpoint studies…

...we will focus on characterising the **key impacts**…

...of marketing any new ingredient via:

- **fully integrated** exposure and hazard assessment at **different levels** of biological organisation
- greater **mechanistic understanding** of ingredient properties to allow extrapolation from **Molecular Initiating Events (MIE**s) to an **adverse outcome**
- better communication of **acceptable risk** using defined **protection goals** (**consumer, environmental**)

ACKNOWLEDGEMENTS

Yeyejide Adeleye, Maja Aleksic, Nora Aptula, Mahesh Batakurki, Emma Butler, Paul Carmichael, Stella Cochrane, Sarah Cooper, Carol Courage, René Crevel, Richard Cubberley, Tom Cull, Claire Davies, Michael Davies, Eliot Deag, Matthew Dent, Sue Edwards, Julia Fentem, Chris Finnegan, Paul Fowler, Antonio Franco, Nichola Gellatly, Nicola Gilmour, Stephen Glavin, Dave Gore, Todd Gouin, Steve Gutsell, Colin Hastie, Juliette Hodges, Geoff Hodges, Sandrine Jacquoilleot, Gaurav Jain, Penny Jones, Sarah Kang-King-Yu, Anja Lalljie, Yvan Le Marc, Moira Ledbetter, Jin Li, Cameron MacKay, Ian Malcomber, Sophie Malcomber, Stuart Marshall, Gavin Maxwell, Helen Minter, Craig Moore, Beate Nicol, Sean O'Connor, Deborah Parkin, Ruth Pendlington, Juliette Pickles, Mike Pleasants, Oliver Price, Fiona Reynolds, Jayne Roberts, Nicola Roche, Paul Russell, Ouarda Saib, David Sanders, Paul Sanderson, Gary Sassano, Andrew Scott, Sharon Scott, David Sheffield, Nikol Simecek, Wendy Simpson, Ilias Soumpasis, Chris Sparham, Richard Stark, Vicki Summerfield, Diana Suárez-Rodriguez, Dawei Tang, Jeff Temblay, Sivaram TK, Roger van Egmond, Carl Westmoreland, Andrew White & Sam Windebank

WORKING WITH SCIENTIFIC PARTNERS GLOBALLY