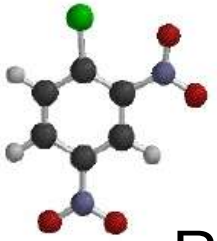
Skin Sensitization MoA/AOP pathway elucidation:
Applying the Skin Sensitization AOP to Risk Assessment

Gavin Maxwell, Unilever (gavin.maxwell@unilever.com)

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

Human Health Risk Assessment for Skin Sensitization



Risk ?

Exposure

X

Hazard

Historical Non-animal *In Vivo*

- 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 risk assess without new animal test data?
  1. Identify pathways driving human adverse response = qualitative AOP
  2. Develop test methods to predict key toxicity pathways
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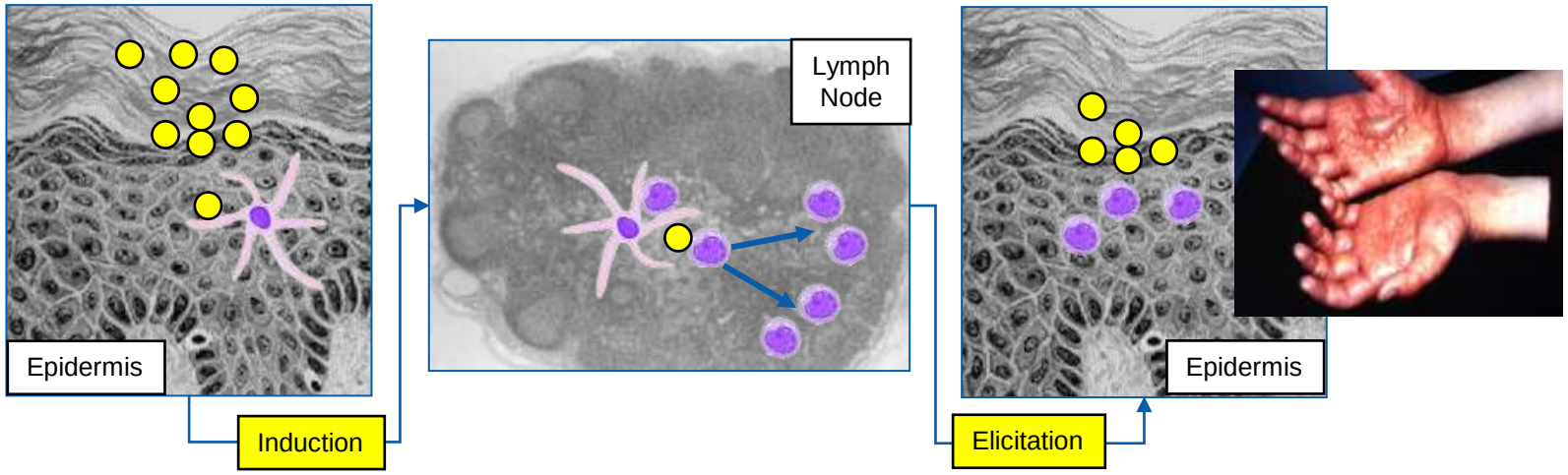
Identifying the toxicity pathways driving the human adverse response



A future approach to measuring relative skin sensitising potency: a proposal

Alternative (non-animal) methods for cosmetics testing: current status and future prospects—2010

Jowsey et al. **2006**. *J. App. Toxicol*. **26**. 341-350.

Adler et al. **2011**. *Arch. Toxicol*. **85**. 367-485.

From Toxicity Pathways to Adverse Outcome Pathways (AOPs)…

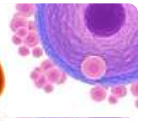

Source

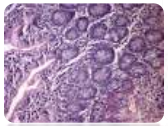

Environmental Containment


Exposure


Molecular Initiating Event


Organelle and Molecular Assemblies Effects


Cellular Effects


Tissue Effects


Organ Effects


Organ Systems Effects


Individual Effects


Population Effects


Community Effects

Toxicity Pathway

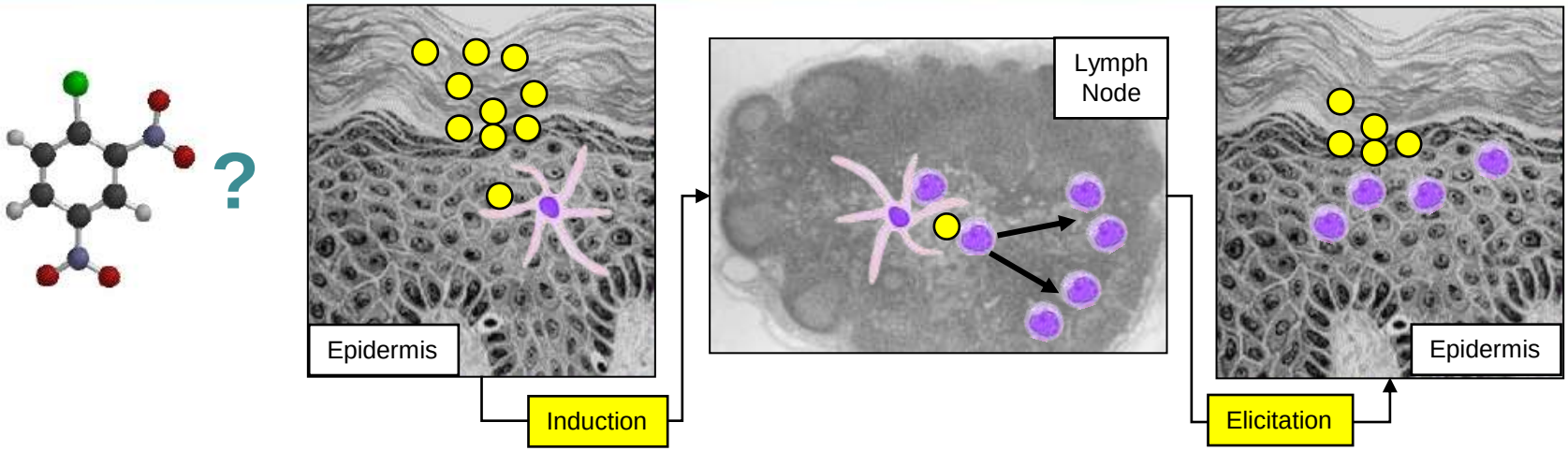
Mode of Action

Adverse Outcome Pathway

Source to Outcome Pathway

Adapted from Kevin Crofton 2010, OECD AOP Meeting Definitions

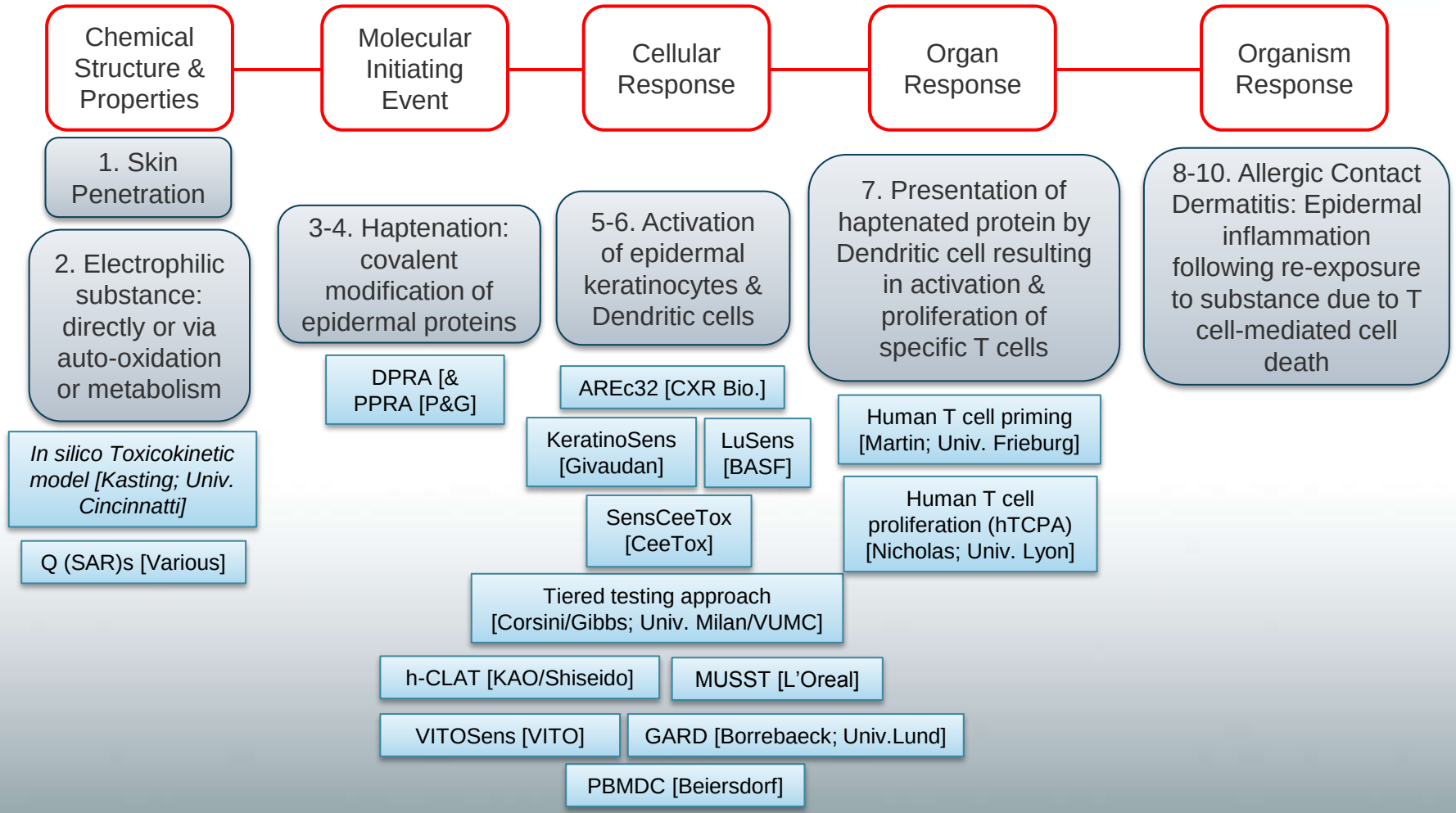
Identify the toxicity pathways driving the human adverse response



Chemical Structure & Properties	Molecular Initiating Event	Cellular Response	Organ Response	Organism Response
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**

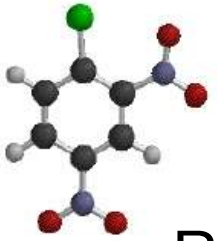
Modified version of flow diagram from 'The Adverse Outcome Pathway for Skin Sensitisation initiated by Covalent Binding to Proteins', OECD report (Draft: 14th Dec 2011)

Develop non-animal test methods to predict key toxicity pathways



Ref: Skin Tolerance TF non-animal toolbox for skin sensitisation

Human Health Risk Assessment for Skin Sensitization



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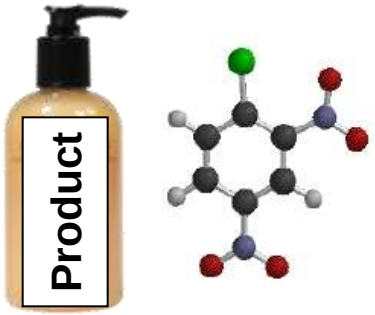
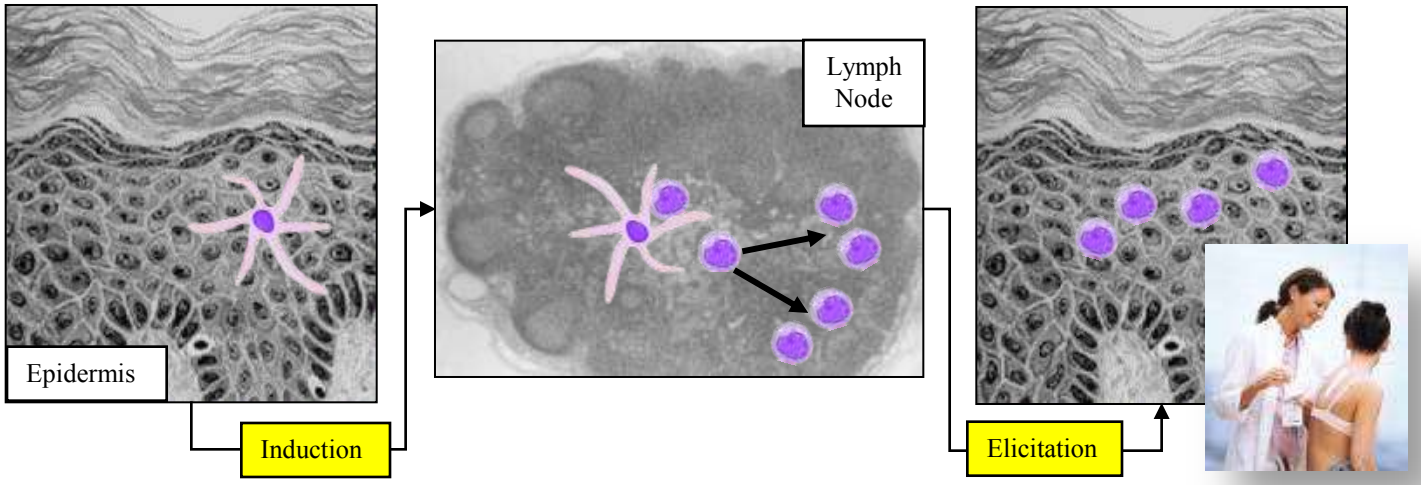
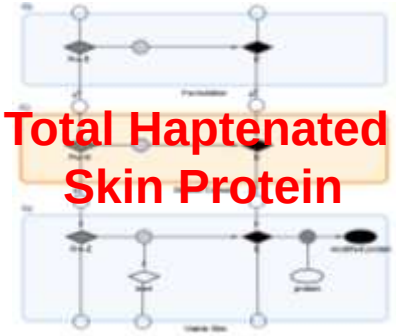
Quantitative AOP for Skin Sensitisation: mechanistic integration of non-animal data to predict whether a given human exposure is adverse

Chemical Structure & Properties → Molecular Initiating Event → Cellular Response → Organ Response → Organism Response



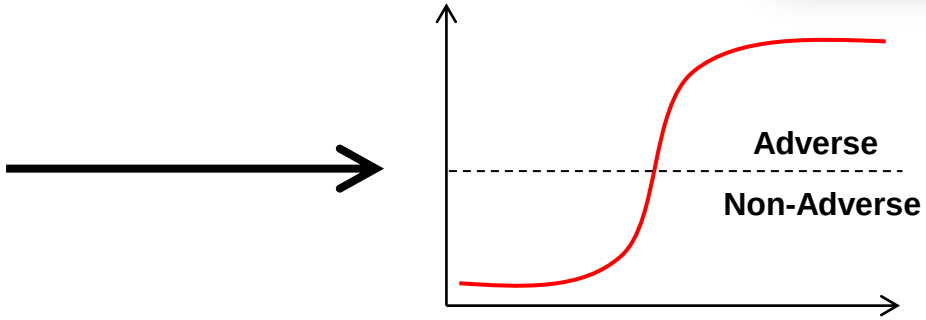
applied dose





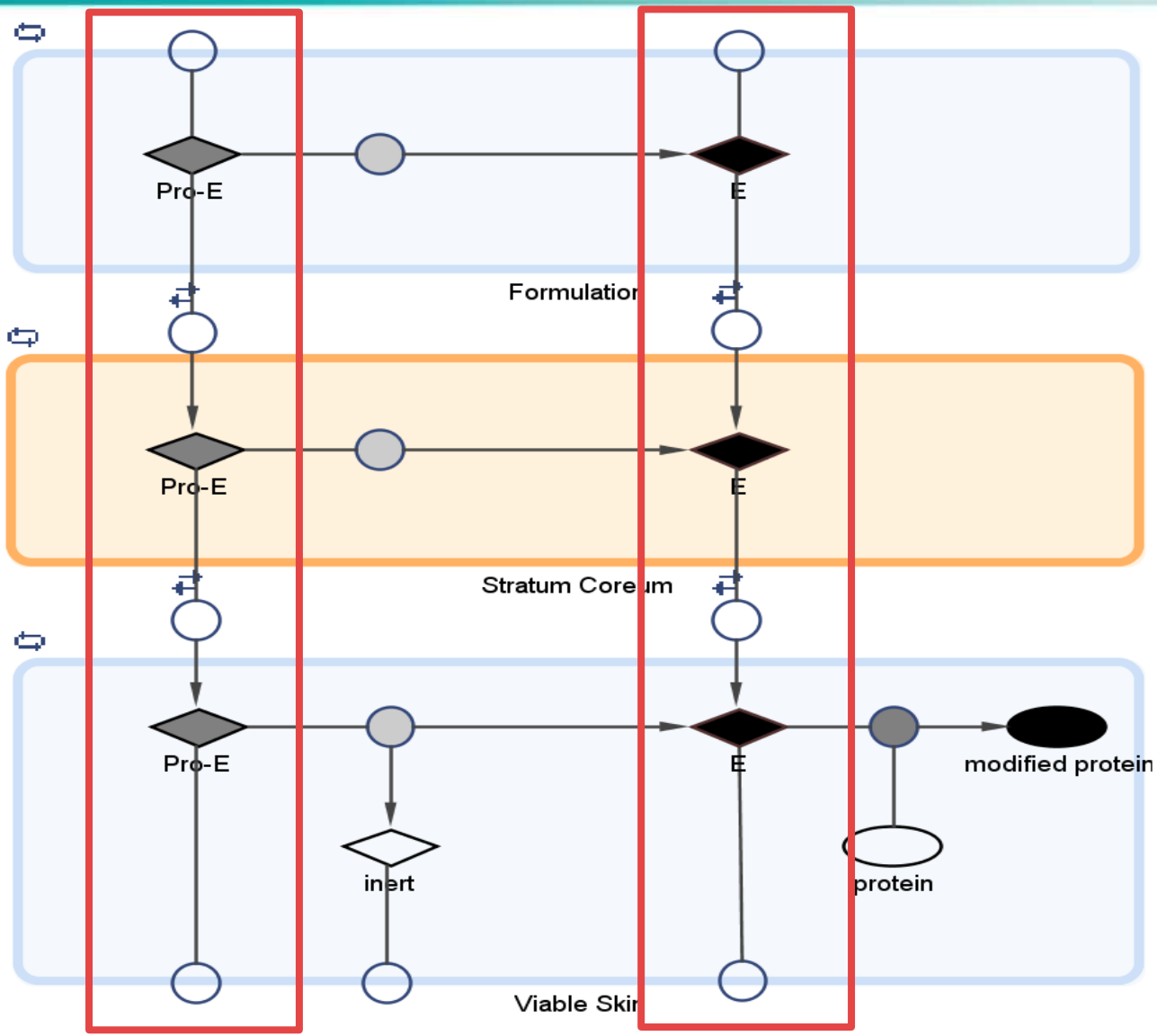
?

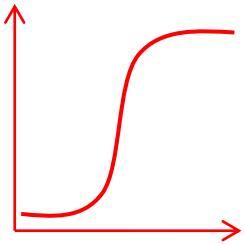
in vitro



in vivo

Applied Dose → Total haptenated skin protein

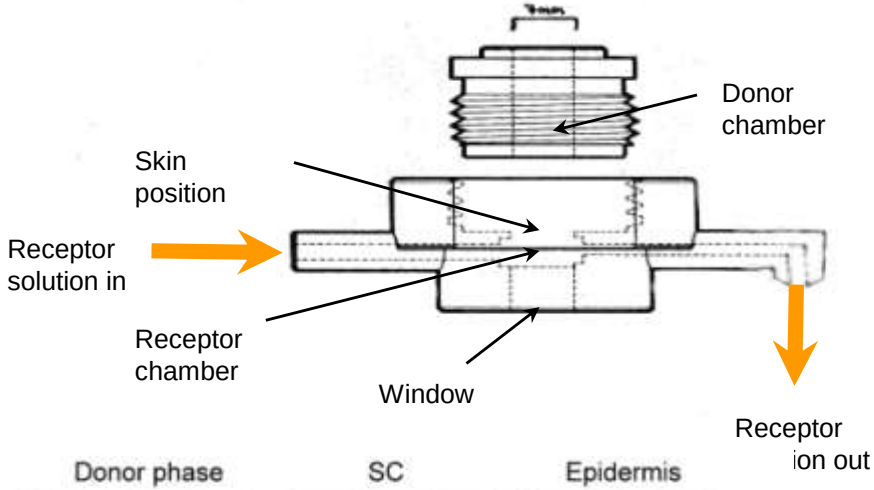


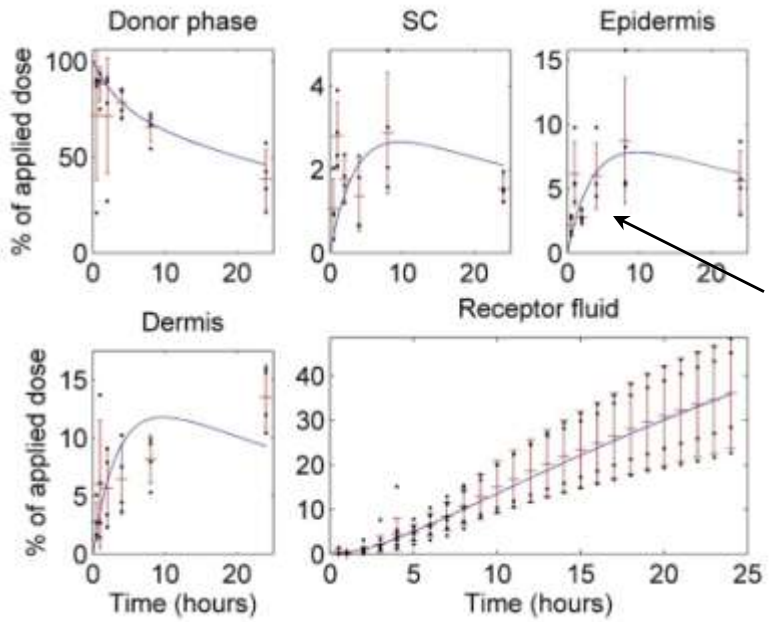


Skin Disposition

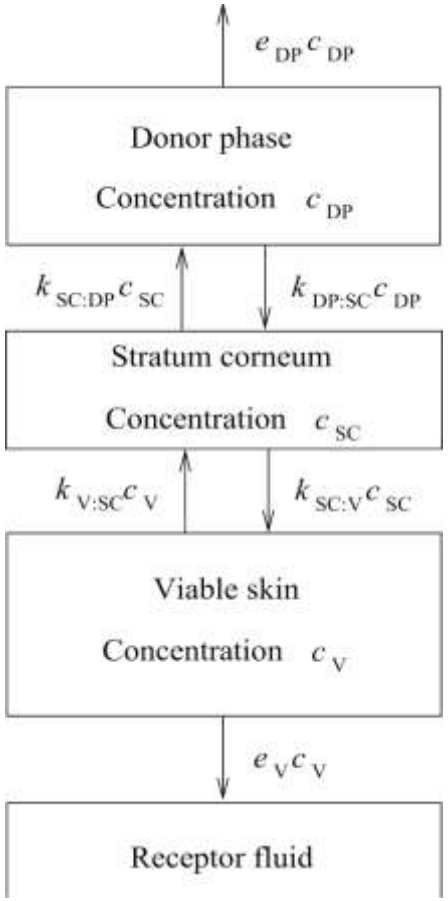
Model input: skin disposition

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



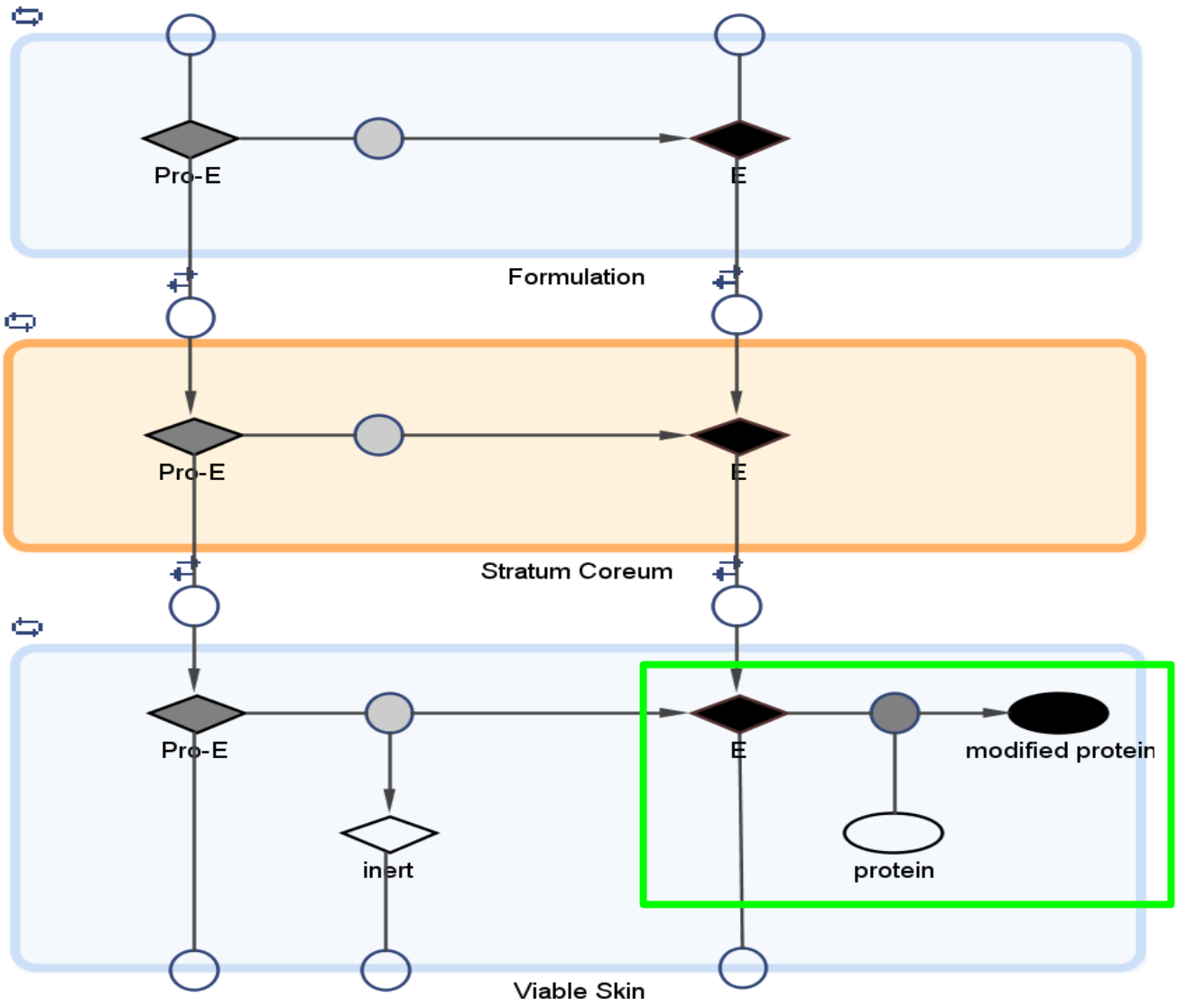


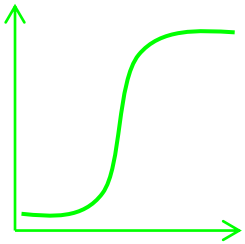
AUC/Dose = 12.2hr



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

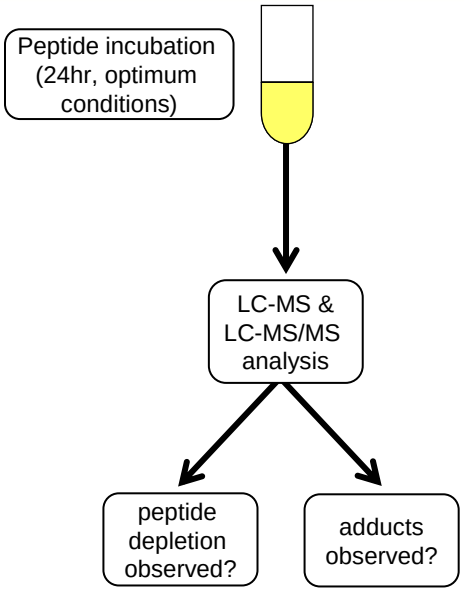
Applied Dose → Total haptenated skin protein





Haptenation

Model input: haptenation



- Peptide depletion and adduct formation measured by LC/MS/MS
- Six different target amino acids each within a different model peptide
- If no adducts are observed chemical is assumed to be non-reactive and therefore non-sensitising (without transformation)

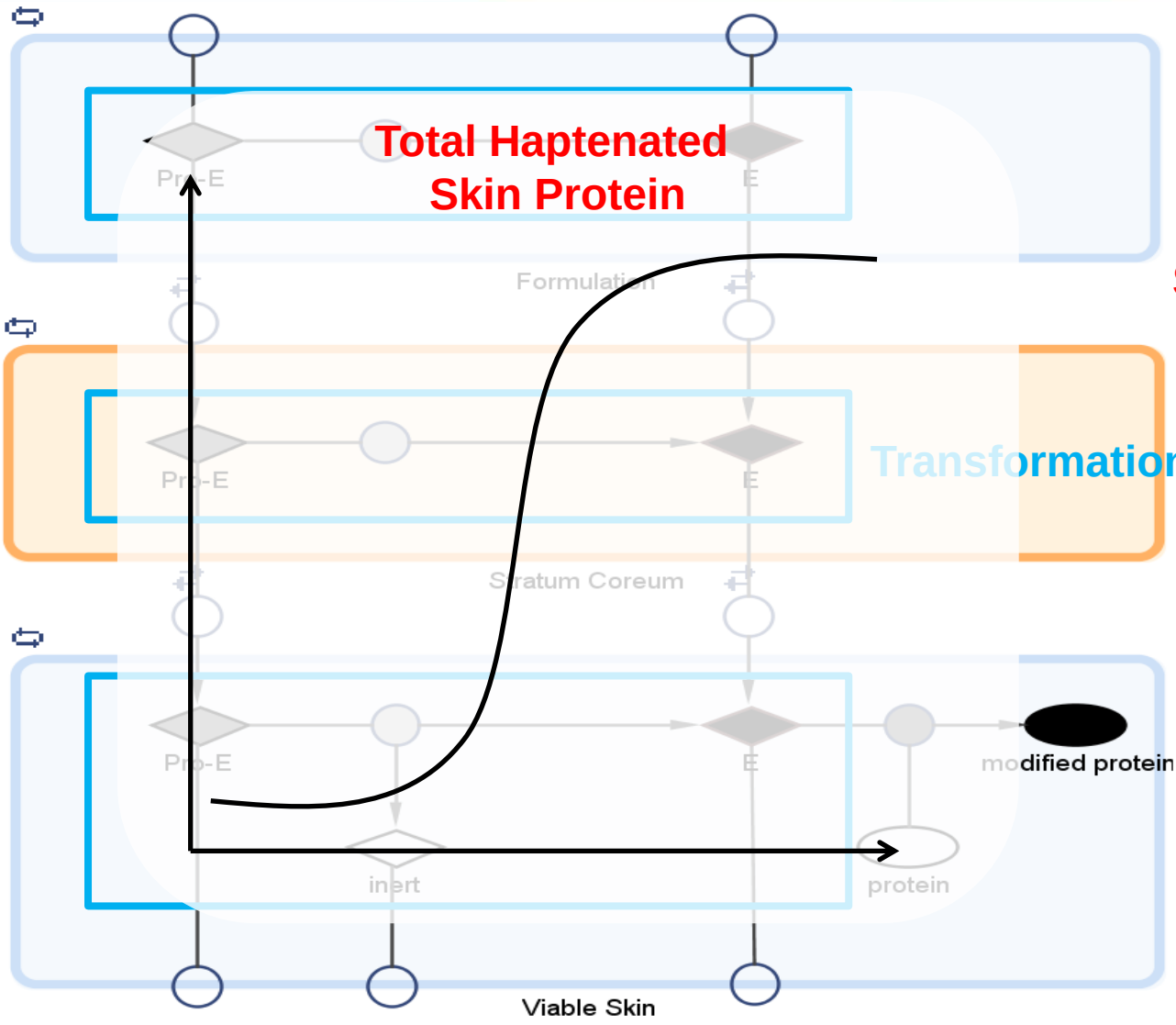
Ac F A A C A A	cysteine
Ac F A A K A A	lysine*
Ac F A A H A A	histidine
Ac F A A R A A	arginine*
Ac F A A Y A A	tyrosine*
NH_2 F A A A A A	N-terminus
Ac F A A A A A	negative control
Ac F A G A G A	internal standard

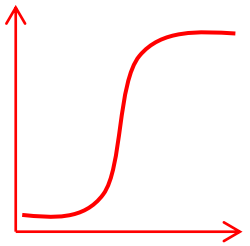
* @pH10

- To improve quantification of reactivity we are currently developing approaches for measuring the kinetics of haptenation

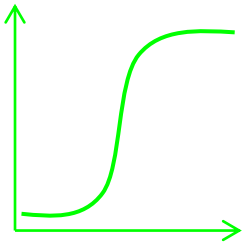
Aleksic et al (2009) Toxicol Sci, **108,** 401-11

Applied Dose → Total haptenated skin protein

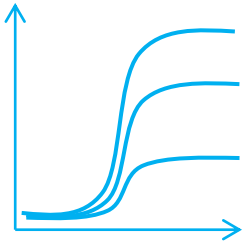




Skin Disposition

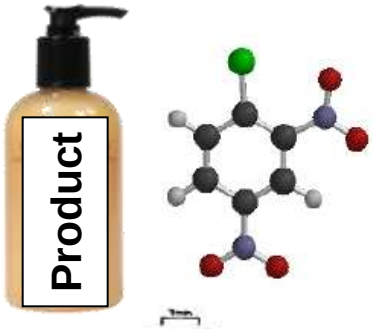


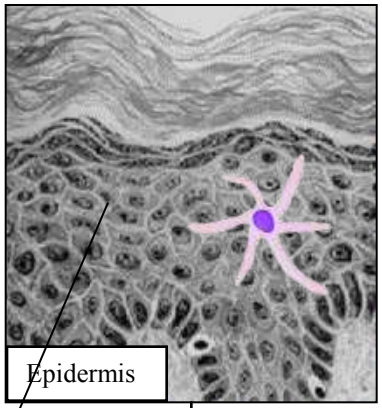
Haptenation



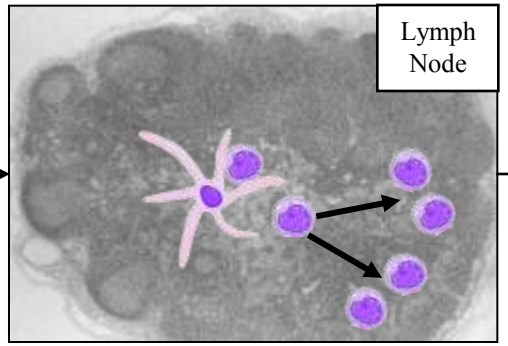
Quantitative AOP for Skin Sensitisation: Mechanistic integration of non-animal data to predict whether a given human exposure is adverse

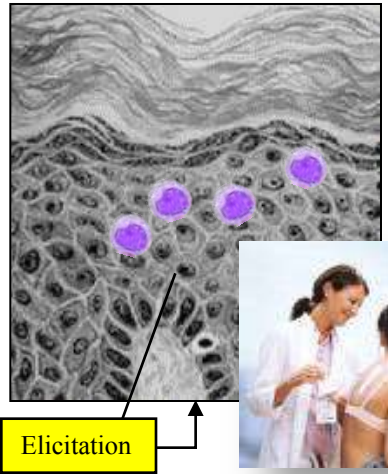
Chemical Structure & Properties → Molecular Initiating Event → Cellular Response → Organ Response → Organism Response



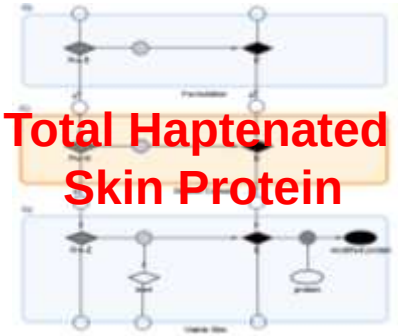


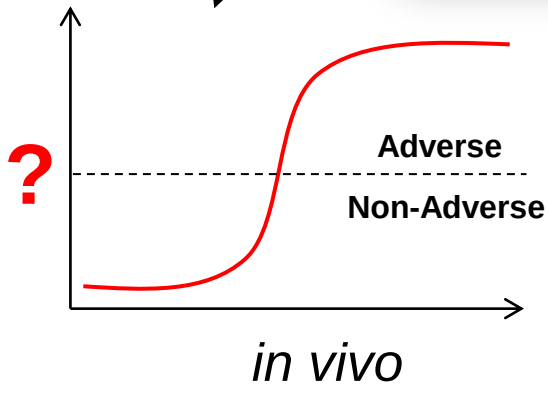
Induction





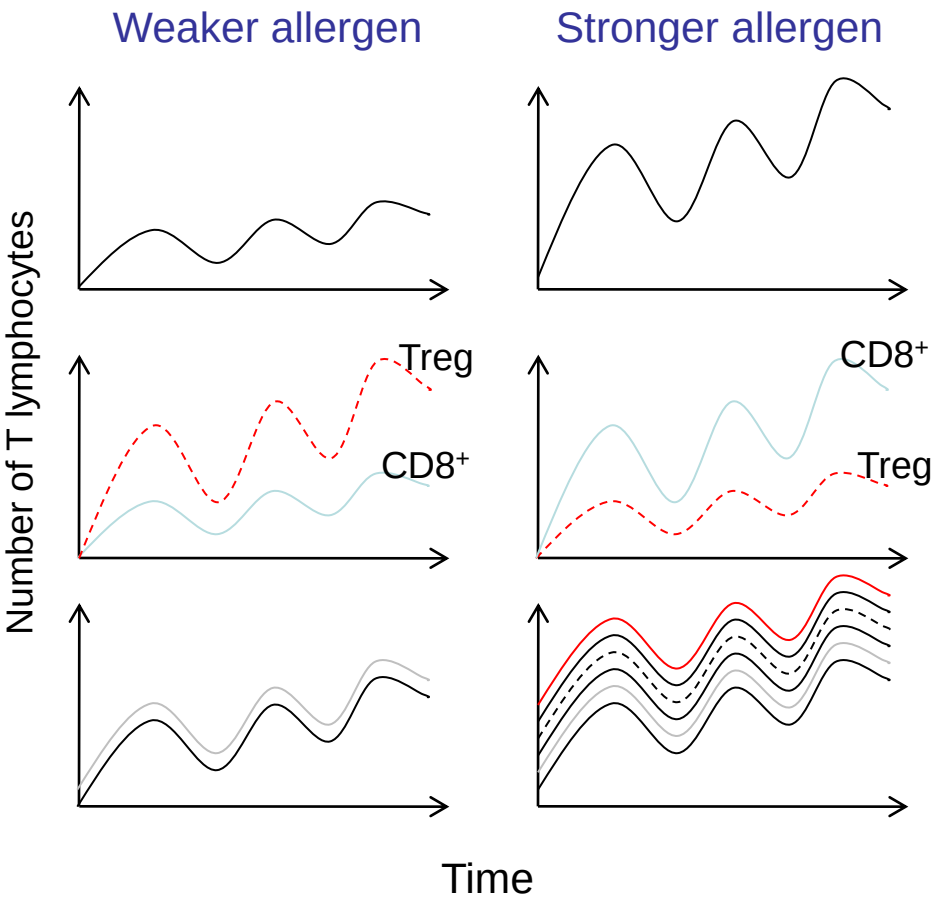
Elicitation





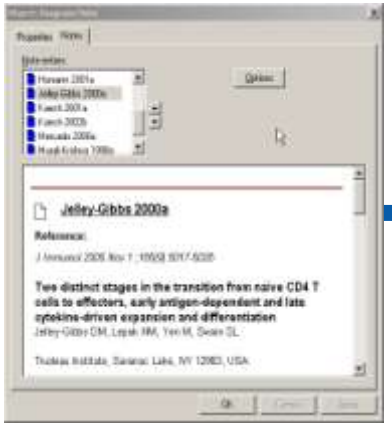
‘T lymphocytes: Orchestrators of Skin Sensitisation’ workshop

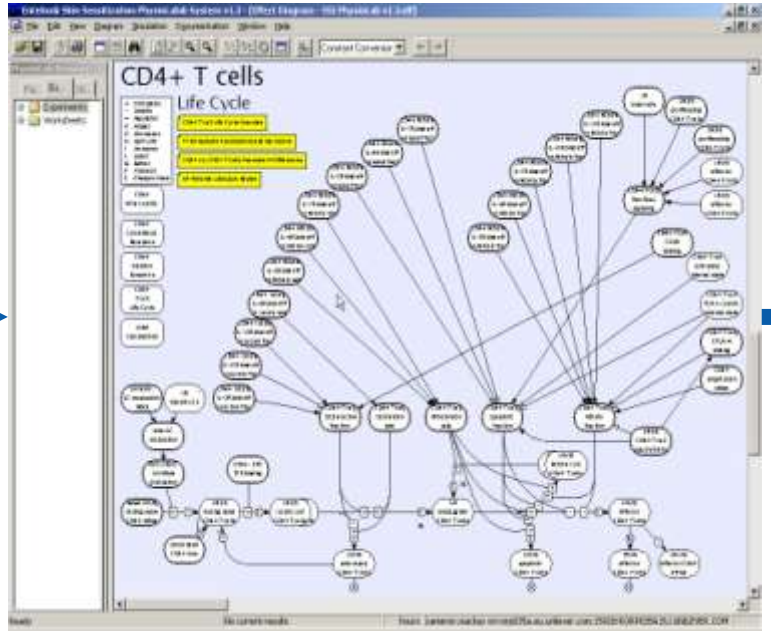
- Immunologists, toxicologists & mathematical modellers – 2 day workshop in May 2010, London
- What are the characteristics of the T cell response that could reflect human skin sensitiser potency?
  - **Magnitude**: What is the extent of sensitiser-induced T cell response (volume, kinetics & duration)?
  - **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?

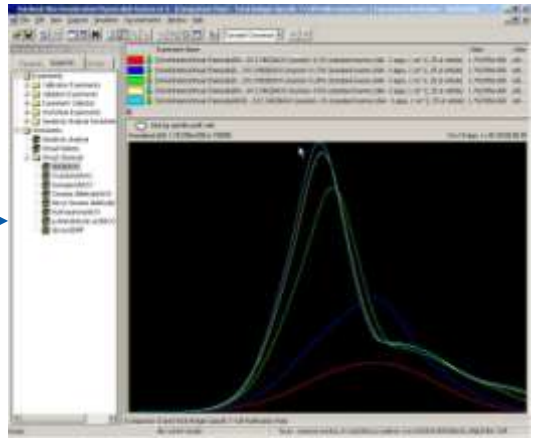


Kimber *et al*. 2012. *Toxicology*. **291**. 18-24

Mathematical model of the induction of Skin Sensitisation: 2006-2008







Mathematical model informed by published literature

Qualitative model schematic panel for key T cell pathways

Simulating experiments using **quantitative** ODE mathematical model

Maxwell G. & MacKay C. 2008. *ATLA*. **36**. 521-556

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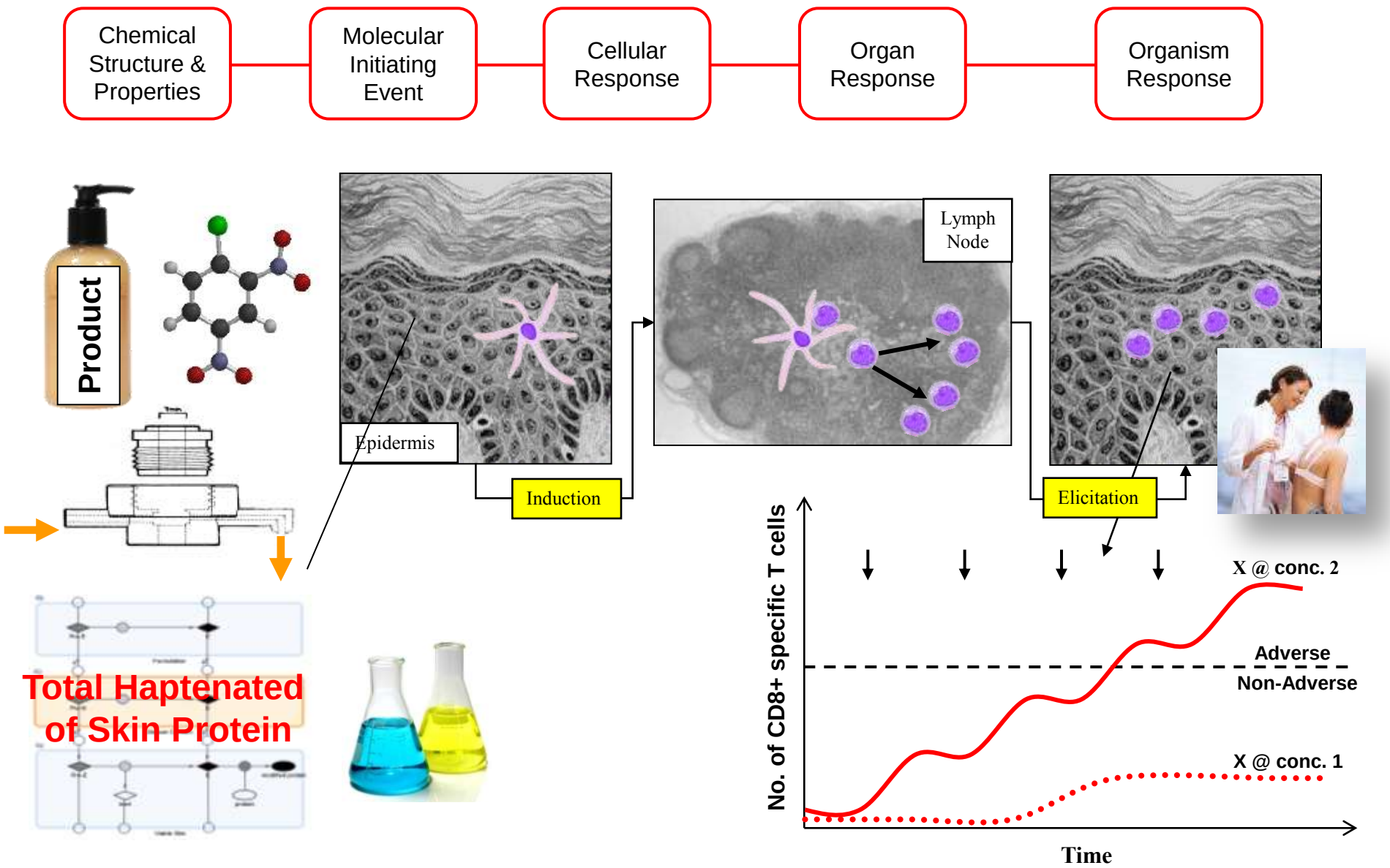
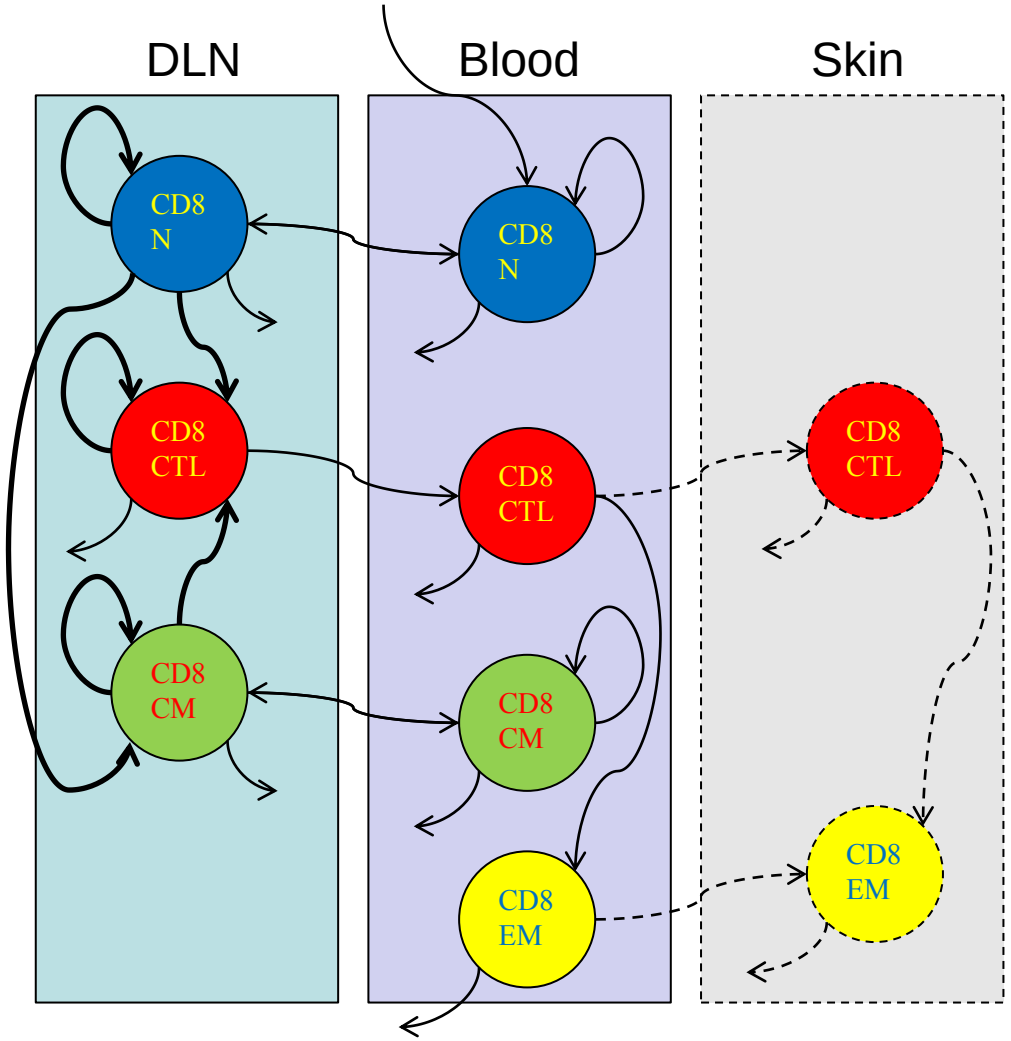
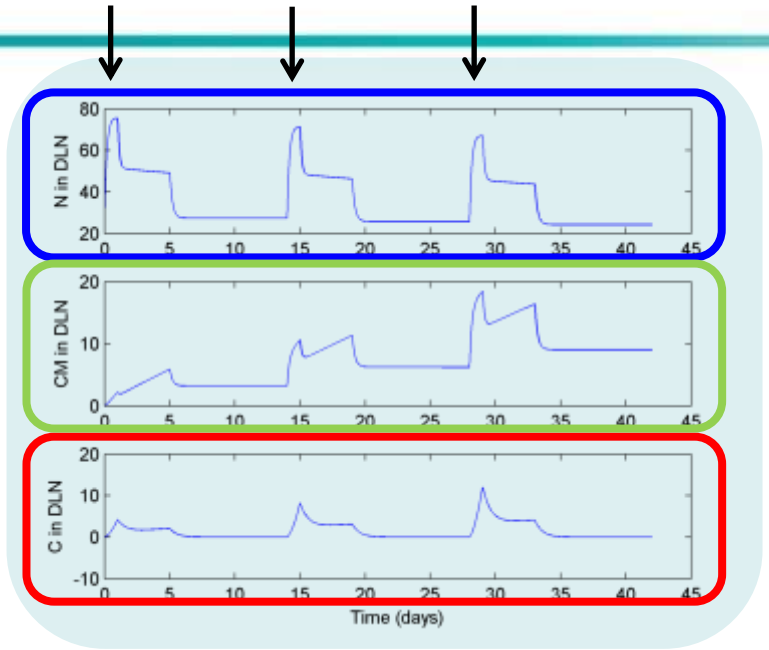


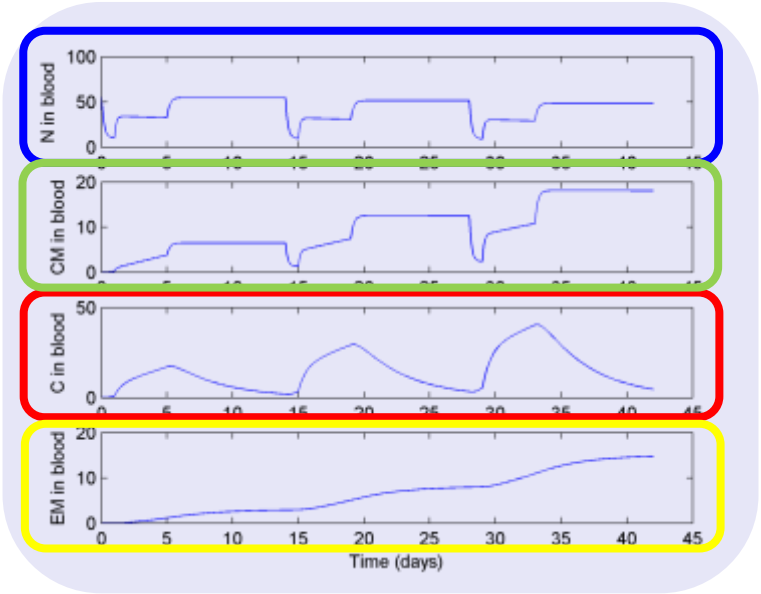
Current ‘pragmatic’ model scope

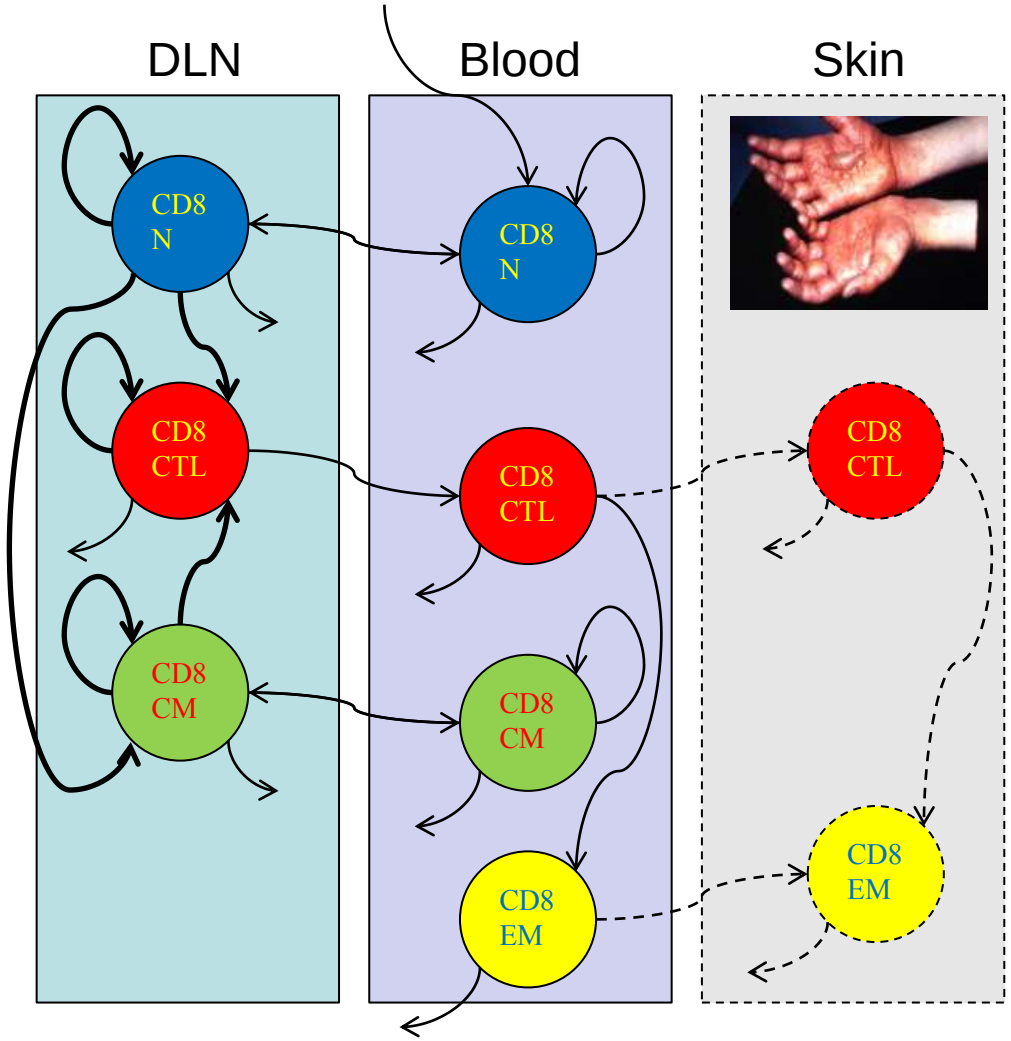
- Current model scope is focussed upon modelling the magnitude of CD8+ (effector, CTL) T cell response
  - Include subsets of central memory, effector memory, naïve and cytotoxic T cells ($CD8^+$ T cell populations only)
  - Only model T cell clones that are specific to antigen
- Human sensitiser-specific T cell data is not available:
  - Make use of relevant literature data
  - Initiate new research to generate sensitiser-specific data to test and improve model



Current model predictions:
3 exposures at 2 week intervals







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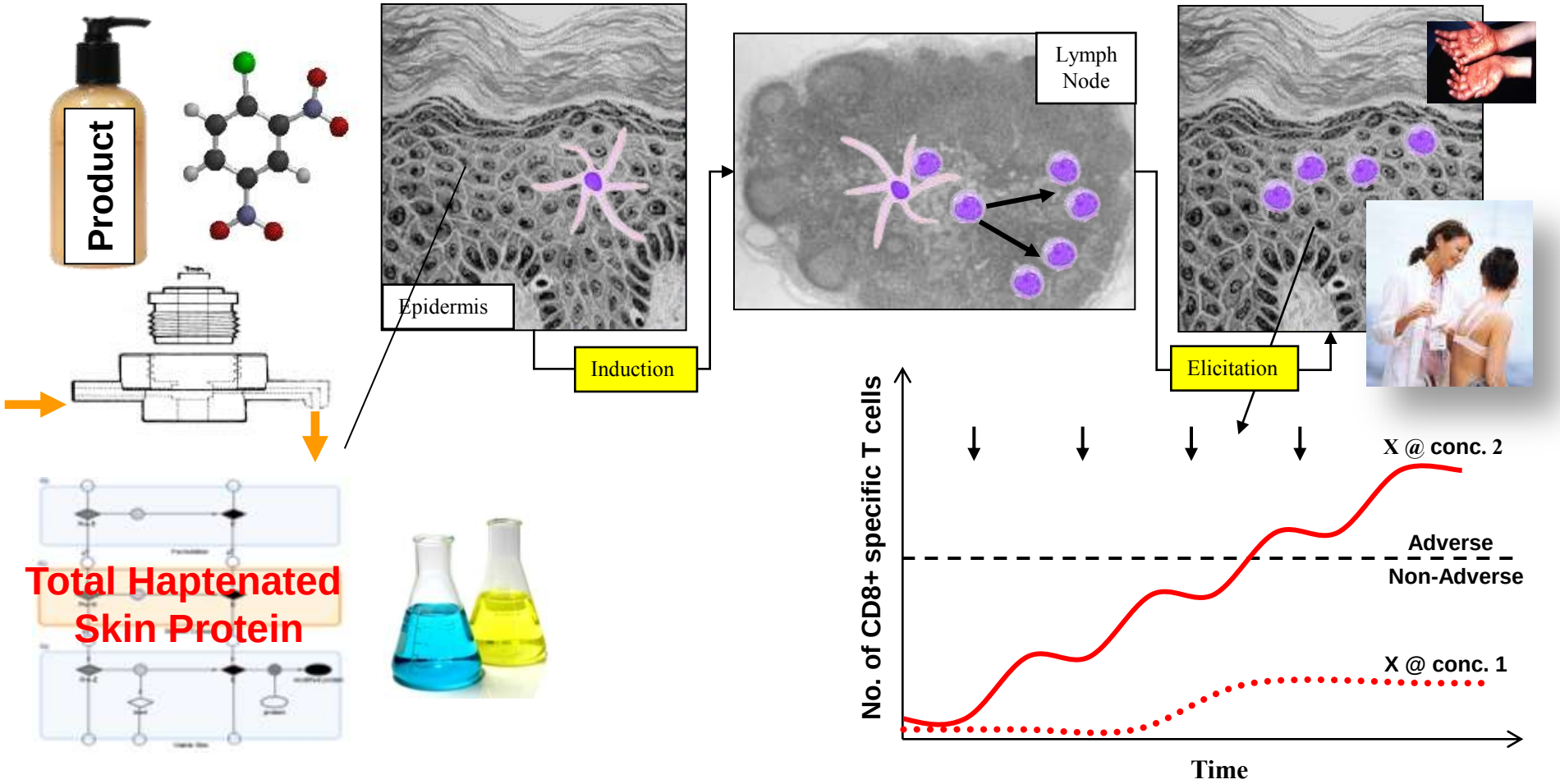
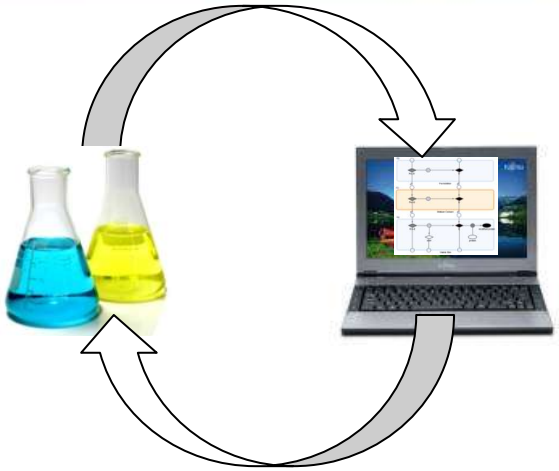
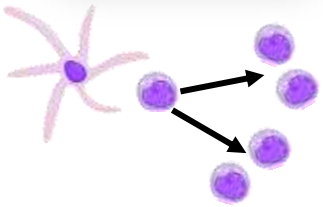


Next Steps

- Iterative refinement of model scope using relevant literature & experimental data
  - e.g what is the optimum T cell response parameter(s) to measure/predict?
  - wet-dry cycle approach



- Generate sensitiser-specific datasets to inform or benchmark model predictions
  - e.g. benchmarking the T cell response:
    - characterising induction of hapten-specific T cell responses in patients undergoing sensitiser treatment for defined clinical benefit
    - characterising ‘mature’ T cell response in individuals attending dermatology patch test clinics for diagnosis of existing allergic contact dermatitis



Personal thoughts/insights

1. Identify pathways driving human adverse response
   - wet-dry cycle approach accelerates pathway characterisation
   - multi-disciplinary teams are difficult to steer but necessary

2. Develop test methods to predict toxicity pathways
   - AOP invaluable for focussing method development
   - methods need to be designed to inform model predictions

3. Will response be adverse for given exposure scenario?
   - need to consider up-front how adversity will manifest
   - modelling required for *in vitro* to *in vivo* extrapolation
   - risk assessment case studies encourage pragmatism

Thank You

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