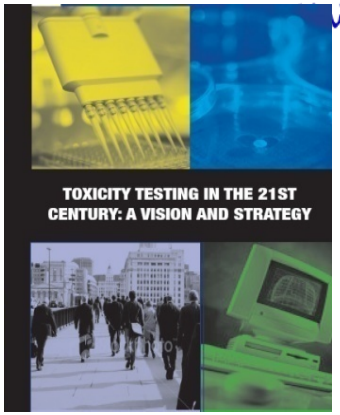
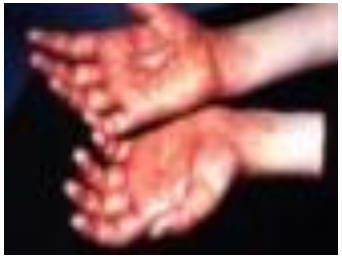
INDUSTRIAL OPPORTUNITIES & APPLICATION OF ALTERNATIVE TESTING METHODS FOR RISK ASSESSMENT

ANDREW WHITE
SAFETY & ENVIRONMENTAL ASSURANCE CENTRE

THE CONSUMER IS KING/QUEEN





Safety remains non negotiable

CAN WE USE A NEW INGREDIENT SAFELY?

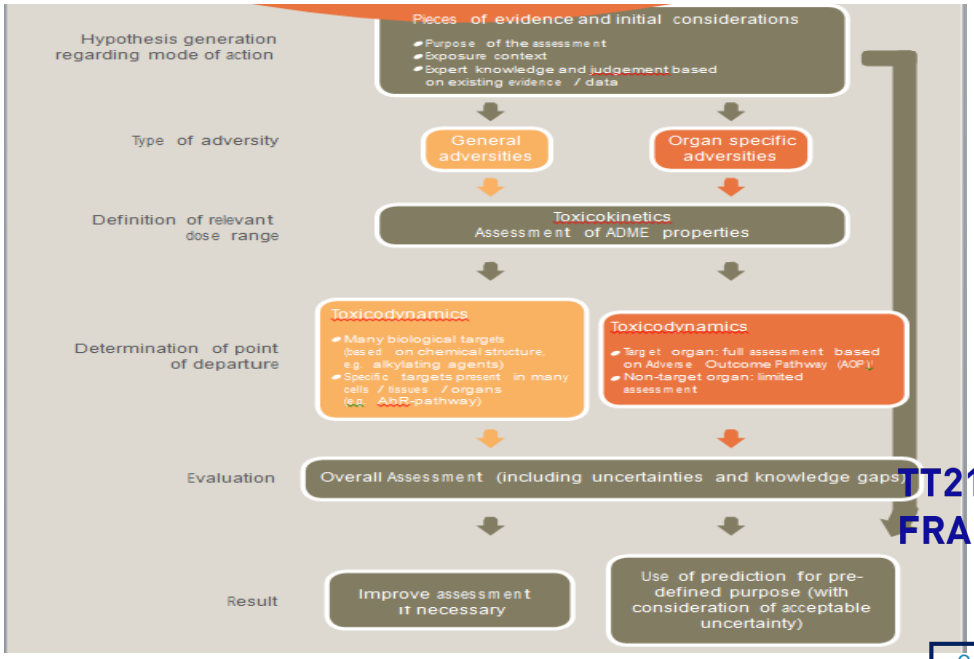
Risk-based approach:
Can we use **x** percent
of ingredient **y**
in product **z**?

PERSONAL CARE CONSUMER PRODUCTS INDUSTRY CAN BE SUCCESSFUL IN THIS

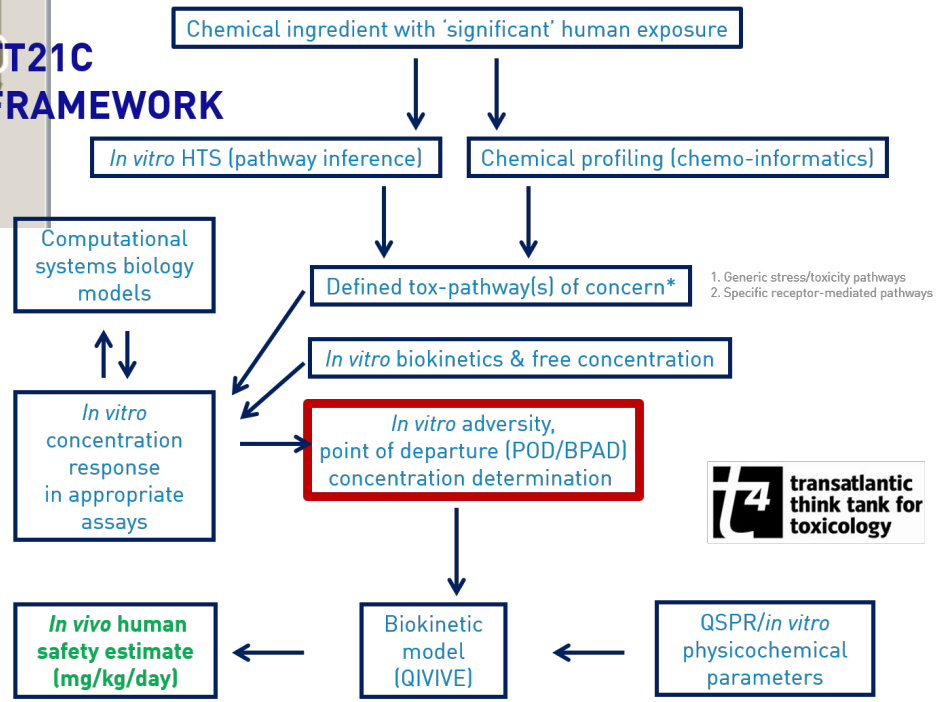
1. Chemical ingredients not generally intended to be pharmacologically active (compare Pharmaceutical Co.)
2. Low bioavailability and often topical exposure
3. Open regulatory environment

Making an exposure-led safety decision based on confidence that the safe level is within or below the adaptive *homeostasis* response, captured by appropriate *in vitro* systems and complemented with network computational models

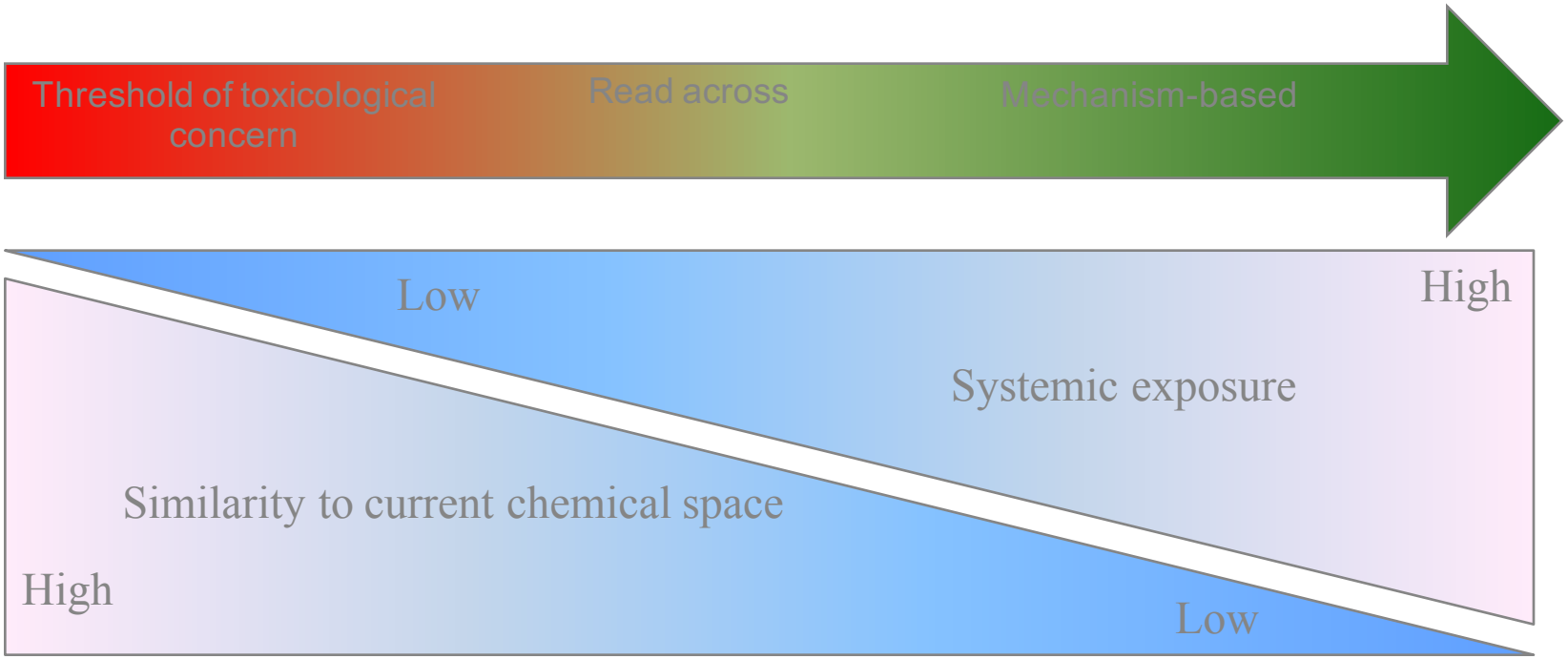
EMERGING DECISION FRAMEWORKS



TT21C FRAMEWORK



FIT FOR PURPOSE DEVELOPMENTS COVER CONTINUUM OF APPLICATIONS DEPENDANT ON CHEMICAL CONTEXT



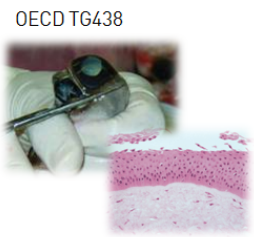
NOW:
Low freedom to operate

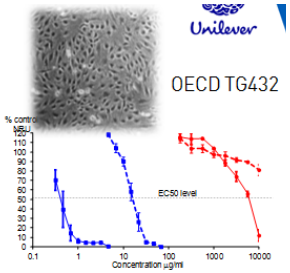
AMBITION:
High freedom to operate

Underpinned by international scientific co-operation and regulatory acceptance

PROGRESS OF IN VITRO TOOLS

- Maximise the use of existing tools risk assessment





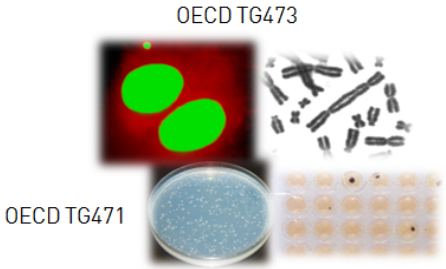
Eye irritation

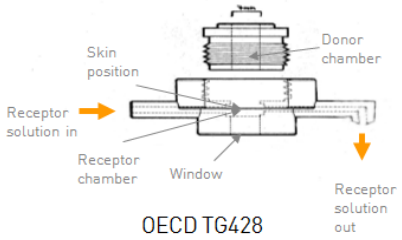
Skin corrosion / irritation

Phototoxicity

Genotoxicity

Skin Penetration





CURRENT SCIENTIFIC REALITY:
NON-ANIMAL APPROACHES FOR SAFETY DECISIONS

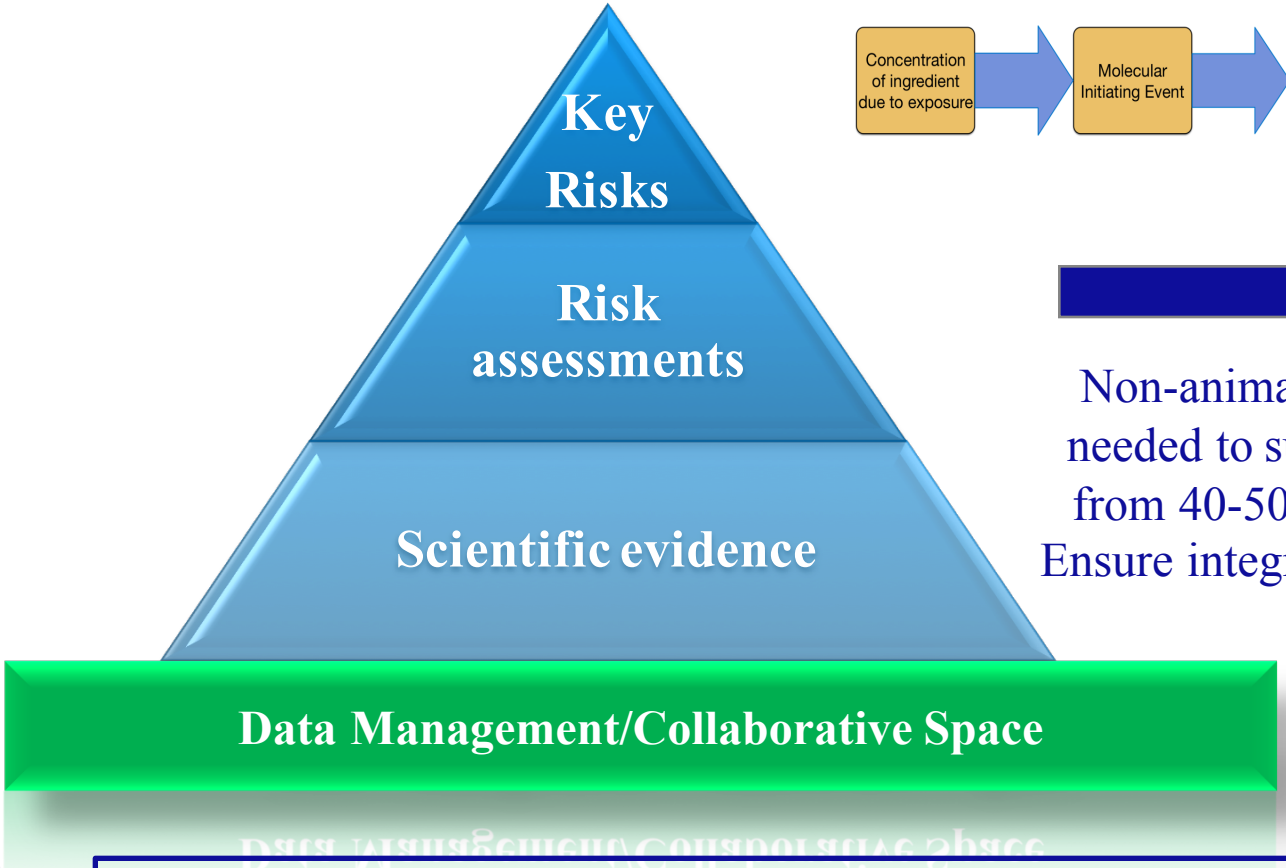
Human Health Toxicology Endpoint	Timeline for Replacement of Animal Testing [Note: Regulatory Acceptance would require an additional 4-8 years]	Comments
Repeated dose toxicity	No timeline for full replacement could be foreseen	Ongoing work still at research stage
Carcinogenicity	No timeline for full replacement could be foreseen	Current *in vitro* test methods are inadequate for generating the dose-response information required for safety assessment
Skin Sensitisation	2017 – 2019 for full replacement	Several non-animal test methods under development & evaluation; data integration approaches for safety assessment required
Reproductive Toxicity	No timeline for full replacement could be foreseen	Ongoing work still at research stage >2020 to identify key biological pathways
Toxicokinetics	No timeline for full replacement could be foreseen	Ongoing work still at research stage 2015 – 2017: prediction of renal & biliary excretion and lung absorption

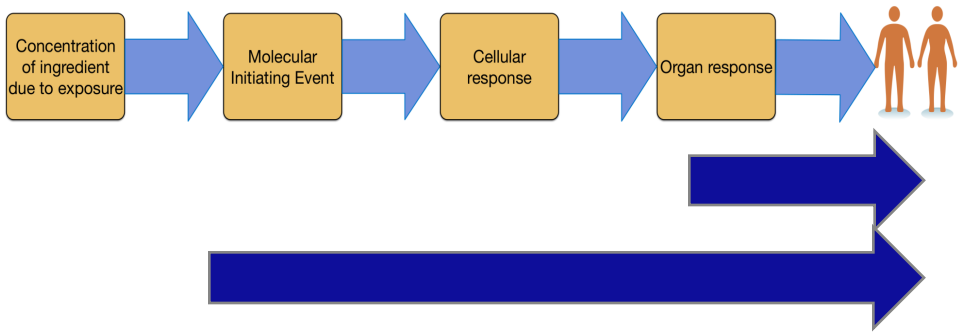
Adler et al (2011), Archives in Toxicology, **85** 367-485

Compiled from Adler et al (2011)

(1) KEY NEEDS / CHALLENGES

Need the underpinning scientific data that enables key risks to be identified and assessments to be conducted.

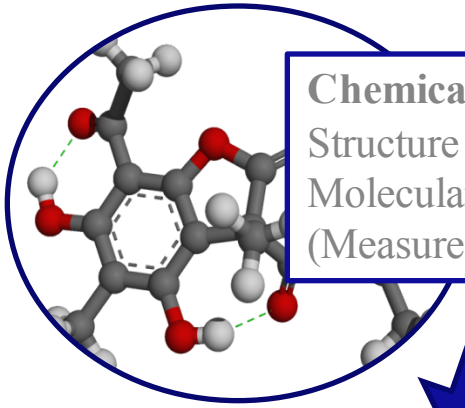




Non-animal approach means that data needed to support a decision has grown from 40-50 pieces up to several 1000s. Ensure integration, provenance & storage.

Need: a knowledge platform that supports common tasks through integration of biological, chemical & toxicological data

DATA INTEGRATION

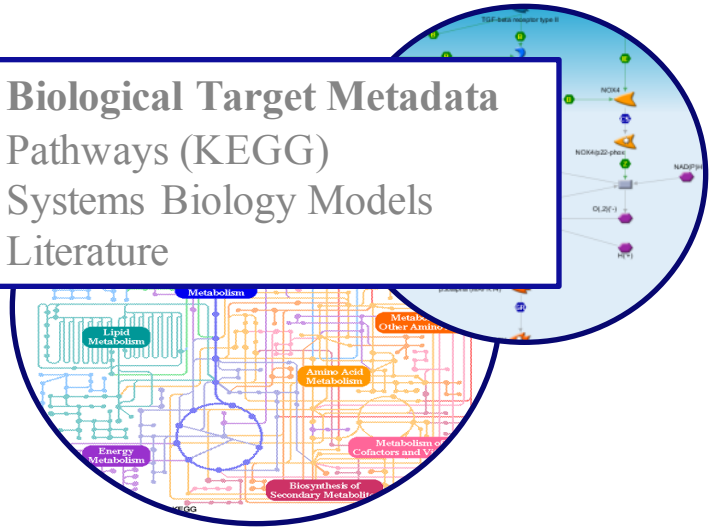


Chemical
Structure
Molecular Properties (chEMBL)
(Measured/Predicted)

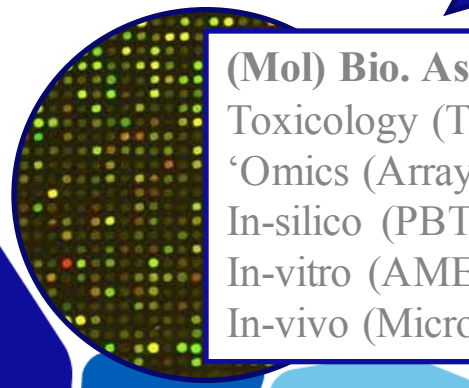
Medical and Pharma
Diseases (OMIM)
Adverse events and Clinical trials (ClinicalTrials.gov)

Computational Toxicology
Integration of data sources
Assessment of veracity/relevance
Presentation of findings

Weight of Evidence risk assessment



Biological Target Metadata
Pathways (KEGG)
Systems Biology Models
Literature



(Mol) Bio. Assays/Predictions
Toxicology (ToxCast, AcTOR, DEREK)
'Omics (ArrayExpress/GEO)
In-silico (PBTK, Toxtree, models)
In-vitro (AMES, Micronucleus)
In-vivo (Micronucleus, TD50s, CPDB)

ADVERSE OUTCOME PATHWAY (AOP) FRAMEWORK

Chemical Research in Toxicology
Perspective

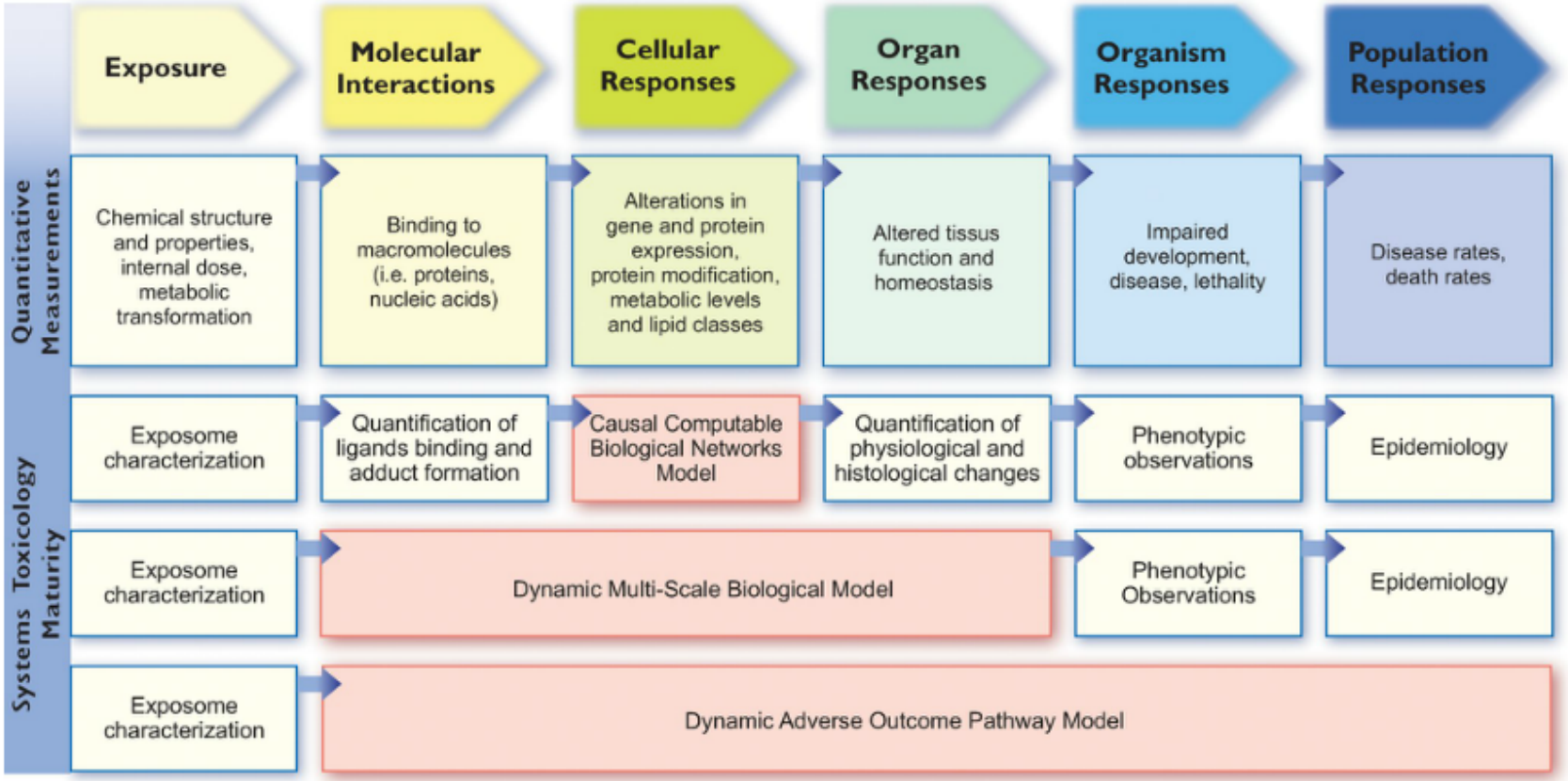
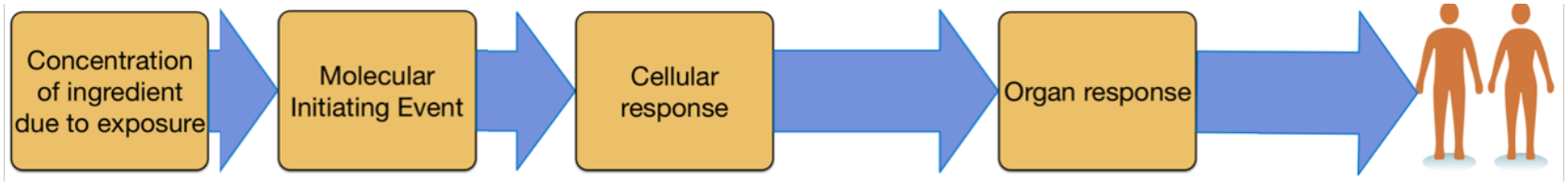


Figure 2. Steps that define the Systems Toxicology paradigm, from biological network models to dynamic adverse outcome pathway (AOP) models.

Sturla et al. Chem Res Toxicol 2014 27(3):314-29

Non-animal test methods for Skin Sensitisation



In vitro skin penetration OECD TG 428	structure-activity relationship (SAR) models	Nrf2 pathway activation assays (e.g. KeratinoSens - OECD TG 442D, LuSens)	Human T cell proliferation assays (e.g. hTCPA)
toxicokinetic skin models	peptide reactivity assays (e.g. DPRA – OECD TG 442C)	Reconstructed Human epidermis activation assays (e.g. SENS-IS, SenCeeTox)	Artificial lymph node tissue models
		Dendritic cell activation assays (e.g. h-CLAT, U-Sens ™, VITOSens, GARD, IL-8 Luc Assay)	

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

Unilever

1. Skin Penetration

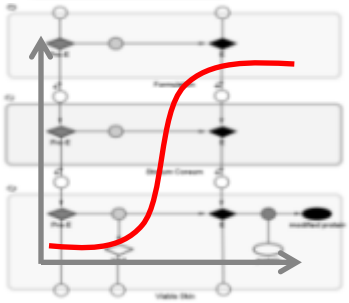
2.Electrophilic substance: directly or via auto-oxidation or metabolism

3-4. Haptenation: covalent modification of epidermal proteins

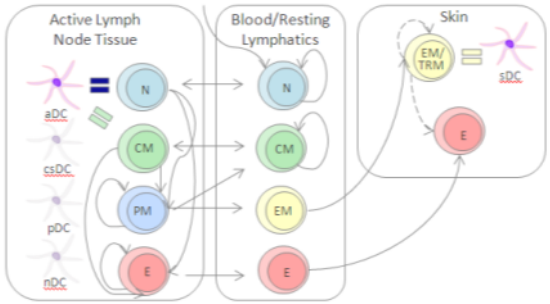
5-6. Activation of epidermal keratinocytes & Dendritic cells

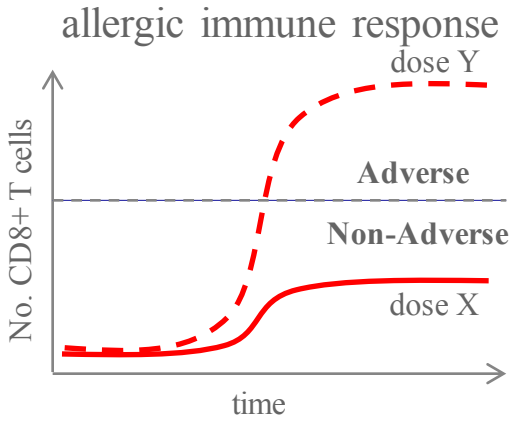
7. Presentation of haptenated protein by Dendritic cell resulting in activation & proliferation of specific T cells

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



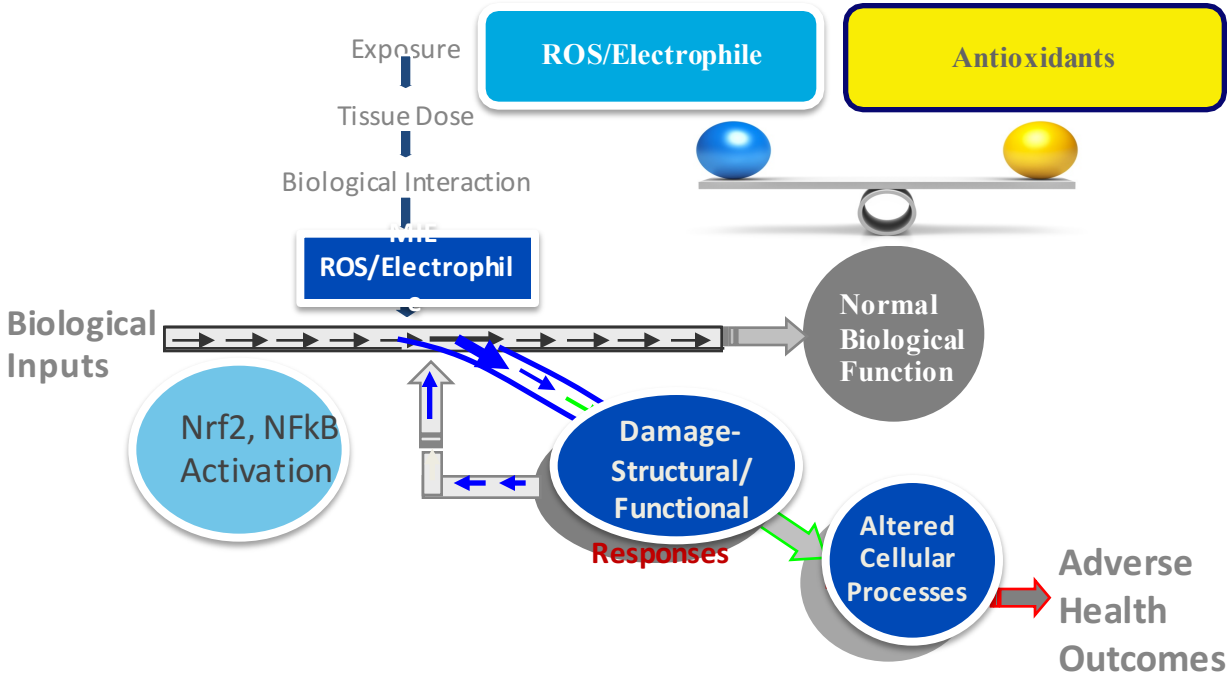
haptenated skin protein prediction





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

Case Study 2. Biological Pathway Perturbation -OXIDATIVE STRESS

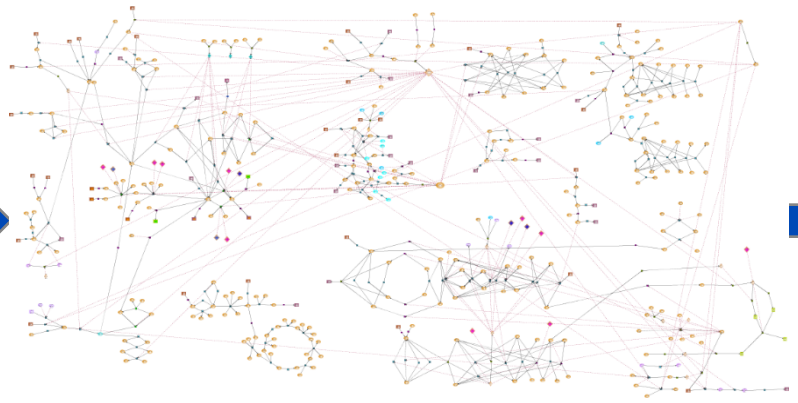


Determining the tipping point between adaptive and adverse effect is critical for chemical risk assessment

Systems Biology Model

Model Input

ROS
-In cytosol
-In mitochondria
Enzyme activities
-γGCS
-GPx
-GR
Oxidative phosphorylation
-Complex 1
-Complex 2
-F0F1 ATPase



Model Output
Cellular defenses
- GSH, GSSG
- Nrf2, NFkB activation
Cellular damage signals
- 4HNE, MDA
- Protein oxidation
- Mitochondrial pore opening
- DNA Damage
Cell death
- Apoptosis
- Necrosis

Chemical → Assay Input Parameter → Model Network → Model output

SUMMARY

Key issue for Risk Assessment – translatability and acceptance

- Significant progress has been made to date
- Confidence in these new approaches will grow through providing examples
- Pragmatic and fit for purpose needs to drive approach
  Exposure based waiving and read across approaches
- Biological knowledge is rate limiting & evolving
  How many AOPs are there
- Uncertainty prevails (both parameter & model)
- How close is my model prediction to reality?
  How do we assess functionality/relevance for integrated testing approaches combining in silico and in vitro outputs.

THANK YOU

www.TT21C.org

1990 – 2015

SEAC 25 YEARS OF SCIENCE

Unilever's Safety & Environmental Assurance Centre (SEAC): helping to shape innovations that are safe for our consumers and workers, and better for the environment.

SEAC was created 25 years ago by bringing together all relevant scientific expertise across Unilever in a single group.