**Objective:** Oxidative stress and the role of the Nrf2/Keap1 pathway in cellular defenses provides one of the case studies in our development and understanding of approaches to drive the TT21C vision forward with practical application.

- Oxidative stress is caused by excess levels of reactive oxygen species (ROS) damaging cellular components.
- This is one of the key drivers through which many chemicals exert an adverse effect.
- Paradoxically, low levels of ROS are essential for normal cell function. Thus, maintaining a balance between ROS production and elimination is key to ensuring cells remain healthy.
- The transcription factor Nrf2 (NF-E2-related factor 2) acts as a key cellular rheostat by altering levels of key cellular antioxidants and cytoprotective genes.

The key goal is to:

1. Understand the molecular events that lead to adverse effects in order to predict when the cellular adaptive capacity becomes overwhelmed;
2. Using a systems model, correctly interpret effects measured in vitro systems in the context of risk for human health over repeat dosing scenarios.

### 1. Developing the Analytical tools

A range of different assay types have been developed to provide the time- and dose-response data in both hepatocyte and keratinocyte cell lines for a range of biomarkers These relate to either determination of the MIE through to biomarkers of adaptive / adverse cellular damage as a consequence of oxidative stress.

The current focus is now on generating the necessary data that will corroborate and complete the underpinning homeostatic model and build understanding of the level of variation across differing cell lines.

### 2. Building a Predictive Systems Model

The model aims to recapitulate the known core feedback mechanisms to predict the effect of ROS production and/or Nrf2 activation on glutathione, protein oxidation, lipid peroxidation, mitochondrial function, and cell death via apoptosis or necrosis at different doses and times points.

An overview of the model components aligned to a causal pathway is shown below:

![Model components](image)

To date various individual modules of the overall model have been fitted to recapture published data. Examples of GSH depletion, 4-HNE increase and Nrf2 alterations are shown.

Work is now ongoing using the experimental data generated to tune the overall model and to verify the predictions.

### 3. Decision flow

The workflow shows the initial build of how the components of the project can be integrated to build a decision based on experimental data, exposure modelling and the predicted outputs from the systems model.

The aim is to provide a useful tool box to map regions of safety therefore enabling risk assessments within an AOP framework.

### 4. Determining the Biological Pathway Altering Dose (BPAD) Metric for Oxidative Stress

No specific endpoint for oxidative stress is currently defined to benchmark in vitro data against. Evaluation of the available literature indicates a combination of both temporal and magnitude changes in biomarkers including Nrf2 accumulation, GSH, and lipid peroxidation (MDA) levels may provide a decision point to distinguish between adaptation and adverse effects. Distributions in the levels for relevant biomarkers in human plasma under both normal physiological conditions and adversity have been modelled.

Additional further work is required to assess the relative value of the decision criteria in terms of the risk framework and physiological adaptive capacity. Case study assessments are planned/underway alongside further work to examine the uncertainties and feasibility in extrapolating the in vitro data to cover different in vivo tissues.

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**References:**


- Additional work is ongoing with collaborations from De Water, Jones, Li, Middleton, Narasimha M K, A. Raghavan, B.

- Literature on ROS, MDA levels no greater than 2.8 and recovery in Nrf2 or GSH, MDA levels.