Safety & Environmental Assurance Centre

Identifying and Characterising Stress Pathways of Concern for Consumer Safety in Next Generation Risk Assessments

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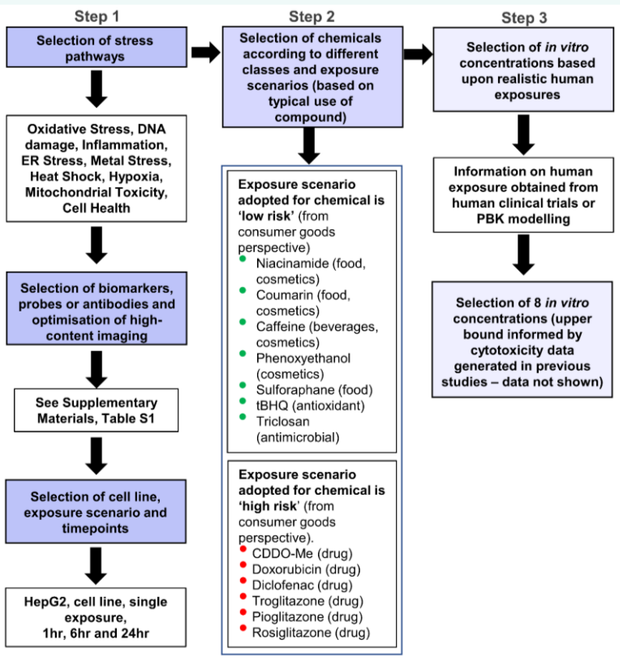
1. Introduction

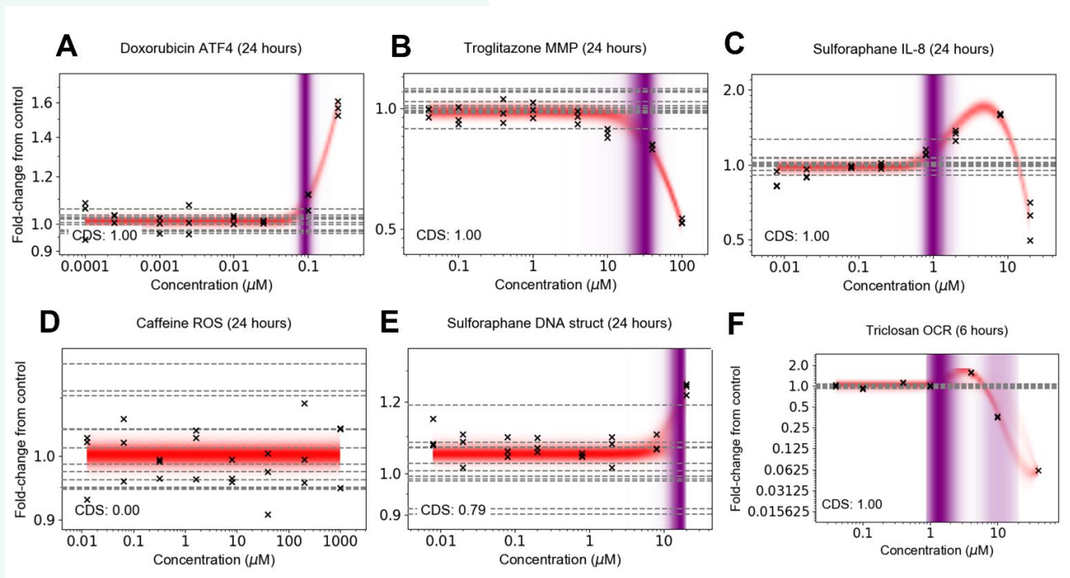
A cell stress panel has been developed to cover the major cellular stress pathways identified in Simmons et al (2009), together with mitochondrial toxicity and other biomarkers reflecting the health and physiology of the cell. To evaluate the relevance of the panel for chemical risk assessment purposes, compounds were selected that are known to either be toxic to humans at defined exposures (and therefore present a 'high risk' from a consumer safety perspective) and have a mode-of-action associated with cellular stress (e.g. doxorubicin, troglitazone, diclofenac), or compounds widely used in consumer products and generally regarded as 'low risk' to humans (e.g. caffeine, niacinamide and phenoxyethanol). A Bayesian model was developed to quantify the evidence for a biological response, and if present, a credibility range for the estimated point of departure (PoD) was determined. PoDs were compared with the plasma Cmax associated with the typical substance exposures and indicated a clear differentiation between 'low' risk and 'high' risk chemical exposure scenarios. The results presented in this work show that the cellular stress panel can be used, together with other new approach methodologies, to identify chemical exposures that are protective of consumer health.

2. Overview of the cellular stress panel



Overview of composition of the stress panel and experimental design for benchmark data generation.

3. Bayesian modelling of concentration-response



We sought to quantify (for each data set) whether a positive response could be detected (a **Concentration Dependency Score,** or **CDS**), and if so at what concentration the **Point of Departure (PoD)** occurs

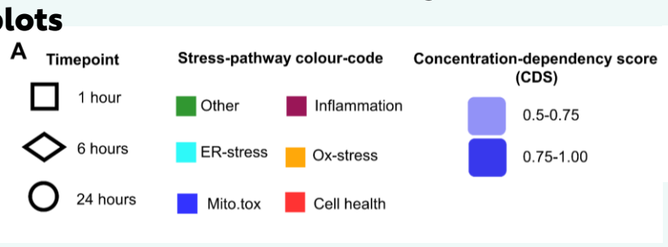
The Bayesian model uses gaussian processes to model the concentration-dependent changes in the mean response

Gaussian processes allow a wide variety of concentration-response curves to be described flexibly without having to adopt any specific shape.

Examples of some of the model outputs are provided on the right, in fig panels A-F.

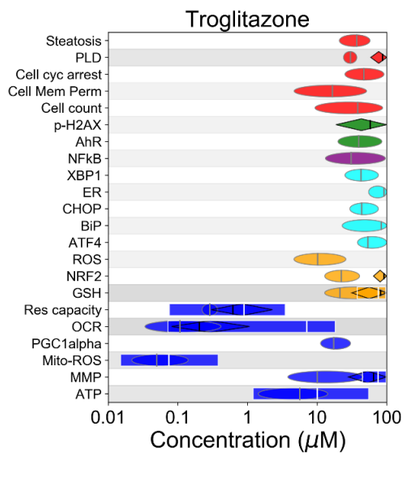
4. Cell stress panel identifies a Specific Mode of Toxicity for a subset of Substances: Mitochondrial Toxicants and Nrf 2 Activators

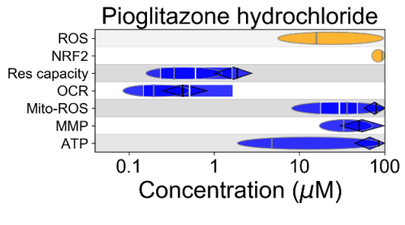
4.1 Overview of PoD summary plots

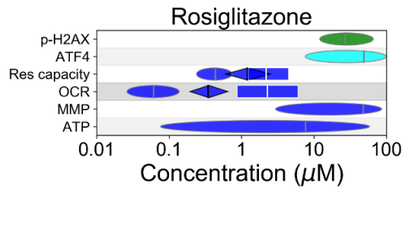


(A) Information on the PoD timepoint, stress pathway and CDS are indicated using shape, color and depth of shading. (B) The credibility range for the representative PoD is indicated using the width of the symbol, the median is given by a vertical grey line

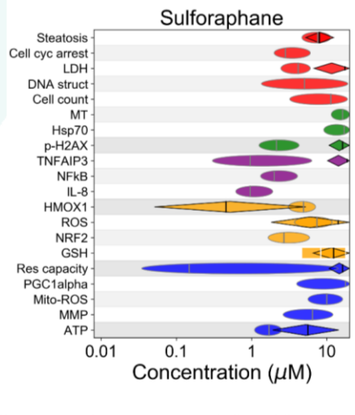
4.2 For the mitochondrial toxicants the lowest PoDs were derived from Mitochondrial toxicity assays

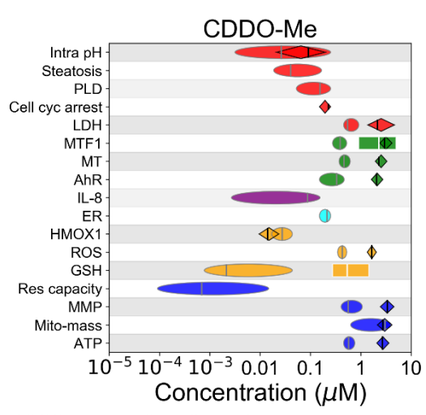




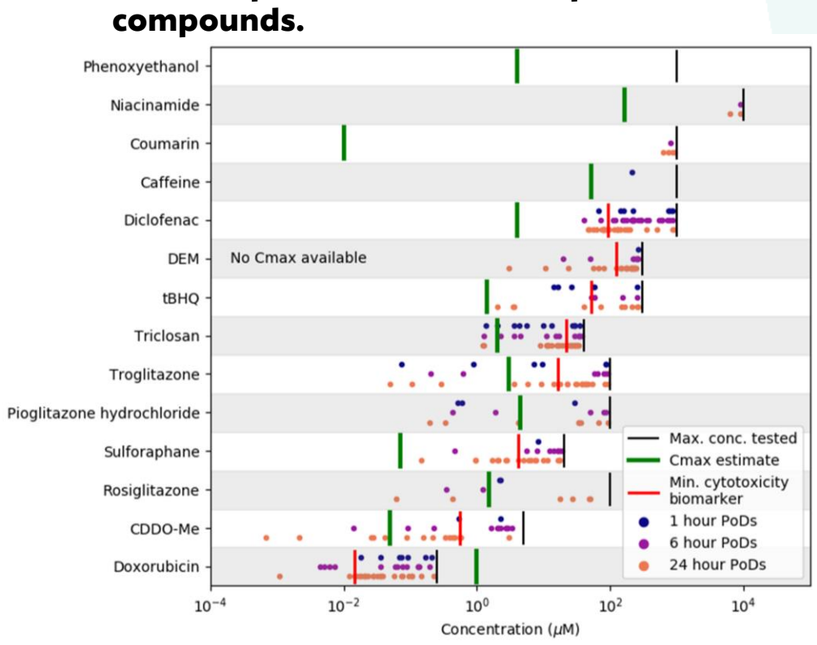


4.2 For the Nrf-2 activators (e.g., Sulforaphane and CDDO-Me) the lowest PoDs were triggered below cytotoxicity were associated with changes in glutathione content, Heme Oxygenase 1 (HMOX1) and reserve capacity via the extracellular flux assay





4.3 Results from the panel indicate a clear differentiation between the 'low-risk' and 'high-risk' compounds at human exposure levels based on typical use-case scenarios for those compounds.



- Overview of PoD modes (corresponding to concentration-response datasets where the CDS is larger 0.5) and associated Cmax estimates for each substance.
- The ordering of the chemicals along the y-axis is determined by ranking chemicals based on the mean of all displayed PoDs.

Conclusions

- Known non-stressor compounds trigger significantly fewer stress pathways and at higher concentrations than the known stress-inducing compounds.
- For compounds known to be bioactive sub-cytotoxic PoDs are observed that can be related to the compounds' known mode of toxicity.
- Overall, the results provide a strong indication that the panel could serve as an assay for identifying and characterising stress pathways of concern, as part of a weight of evidence-based risk assessment