



Prototype Pathway Research for Toxicity Testing in the 21st Century (TT21C) – A Case Study using DNA Damage Characterisation

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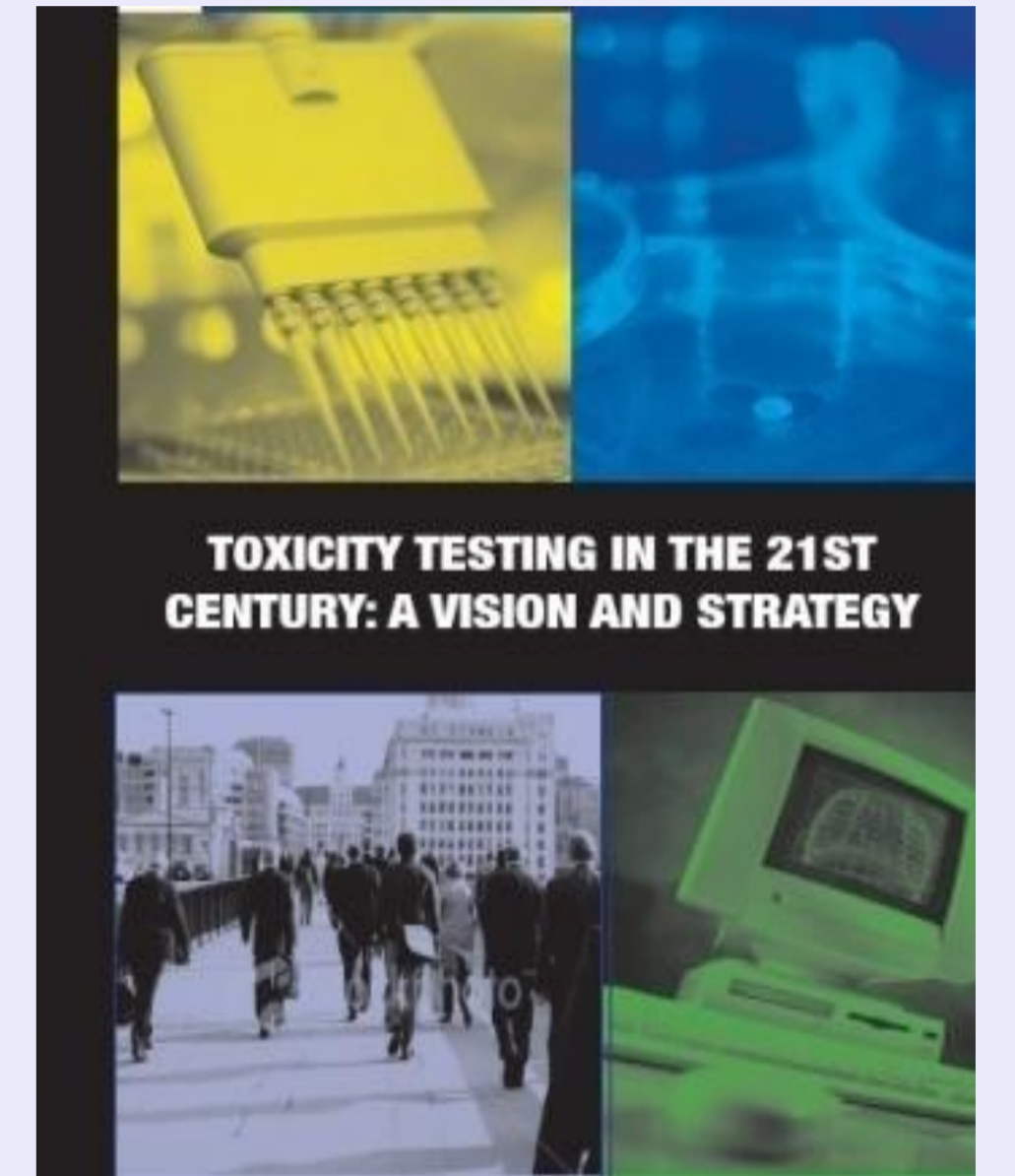
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Background

Assuring the safety of consumer products without the need to conduct animal tests on their ingredients is a considerable challenge. In 2004, Unilever published a novel conceptual framework for making consumer safety decisions that could potentially enable animal tests to be replaced (Fentem *et al.*, ATLA, 2004). This framework focused on the development of new risk assessment approaches that would allow the integration and interpretation of non-animal data. Our on-going research activities aim to explore the feasibility of these new approaches and to integrate new thought-leadership in this space (led by the Hamner Institutes), with the developments in TT21C.

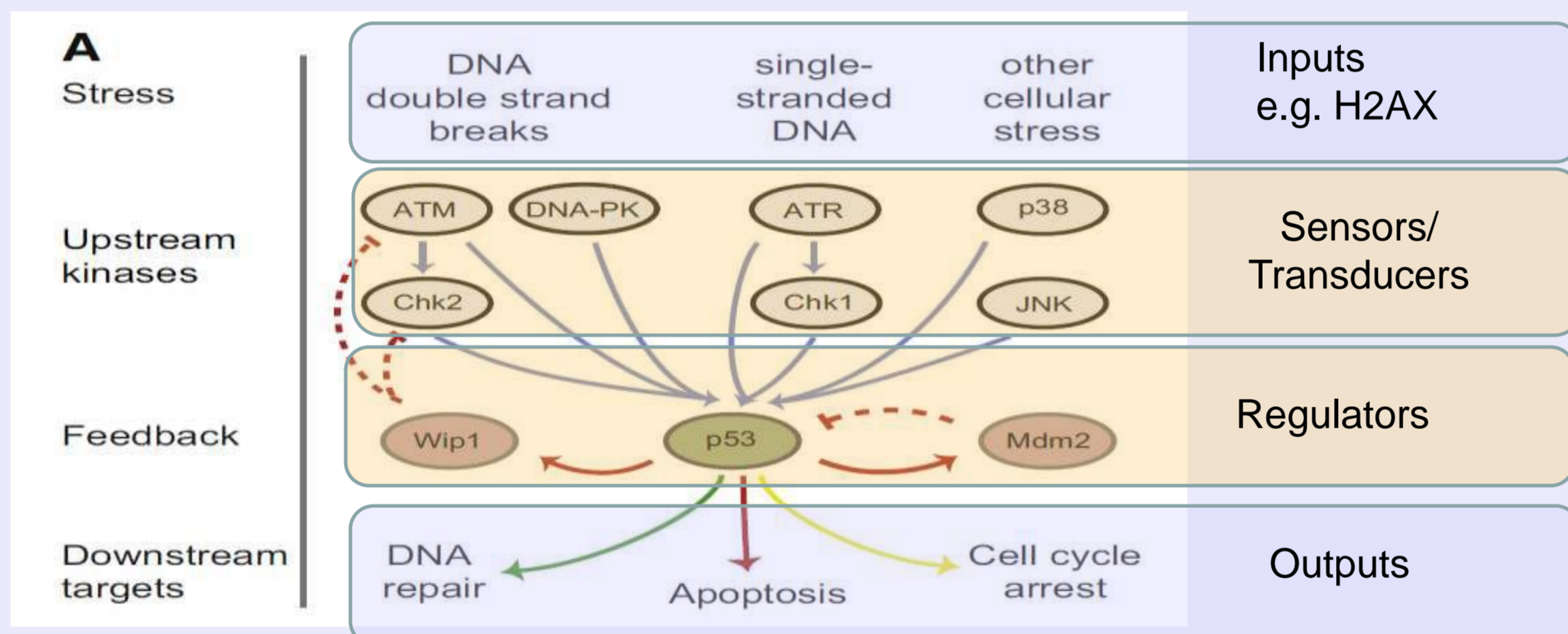
Toxicity Testing in the 21st Century (TT21C)

In 2007 the National Research Council in the US published a report which called for a transformation of 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 of human origin” (Krewski *et al.*, J. Tox. Env. Health Crit. Rev. 2010). Unilever and The Hamner Institutes have forged a **strategic partnership** to pursue the aims of that report. The work presented here illustrates some first steps in this type of ‘pathways-based’ risk assessment, using DNA damage as a prototype ‘tox-pathway’.



Our Prototype ‘Toxicity Pathway’

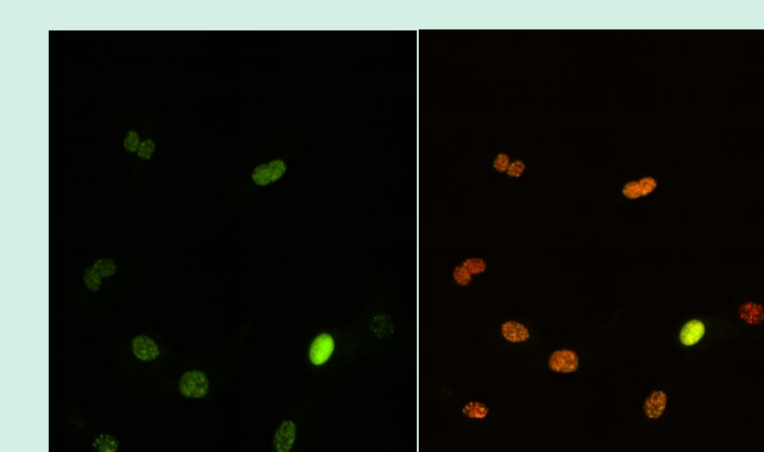
The term ‘toxicity pathway’ refers to key cellular processes which, when disrupted, can lead to adverse effects. In order to prove the concept of a **pathways-based risk assessment** we chose to examine changes in the p53 DNA damage/repair pathway in response to case study chemicals. This pathway is critical as it is involved in regulating the response of cells to DNA damage, protecting cells by regulating DNA repair, cell death, or cell cycle arrest.



Characterisation of consumer use scenario of the product in question (volume applied, frequency, area applied, population characteristics)



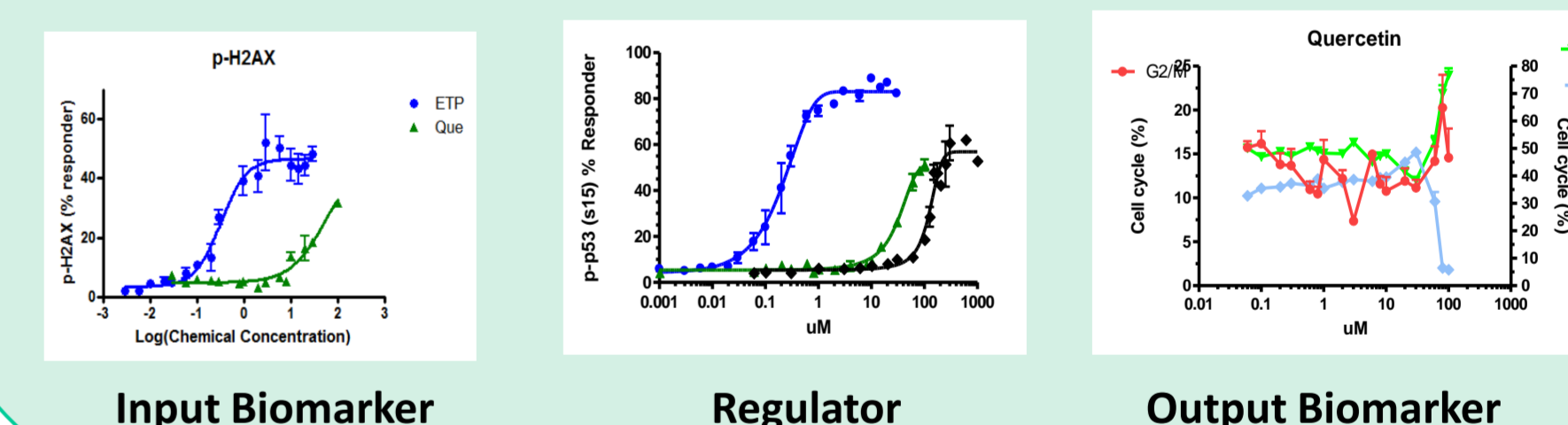
Establishing biomarkers important in the p53 pathway & measuring their fluctuation in relevant systems:



High content assays (HCA) to quantify DNA damage and biomarker response have been established using both high content imaging and flow cytometry systems providing single cell measures on cell populations

The figure above shows phospho-p53 levels (green) and the double strand DNA damage marker gammaH2AX (red) in single cells following treatment with DNA damage inducer. Yellow dots show points of co-localisation of biomarkers within the nuclei

In vitro dose response data shows the concentration threshold for the initiating effect (DNA damage): the figures below show flow cytometry data from 18 point dose response curves for positive control compounds (blue & black) and case study chemical (green)



Input Biomarker, Regulator, Output Biomarker

Exposure & Consumer Use Assessment

High-content information *in vitro* assays in human cells & models

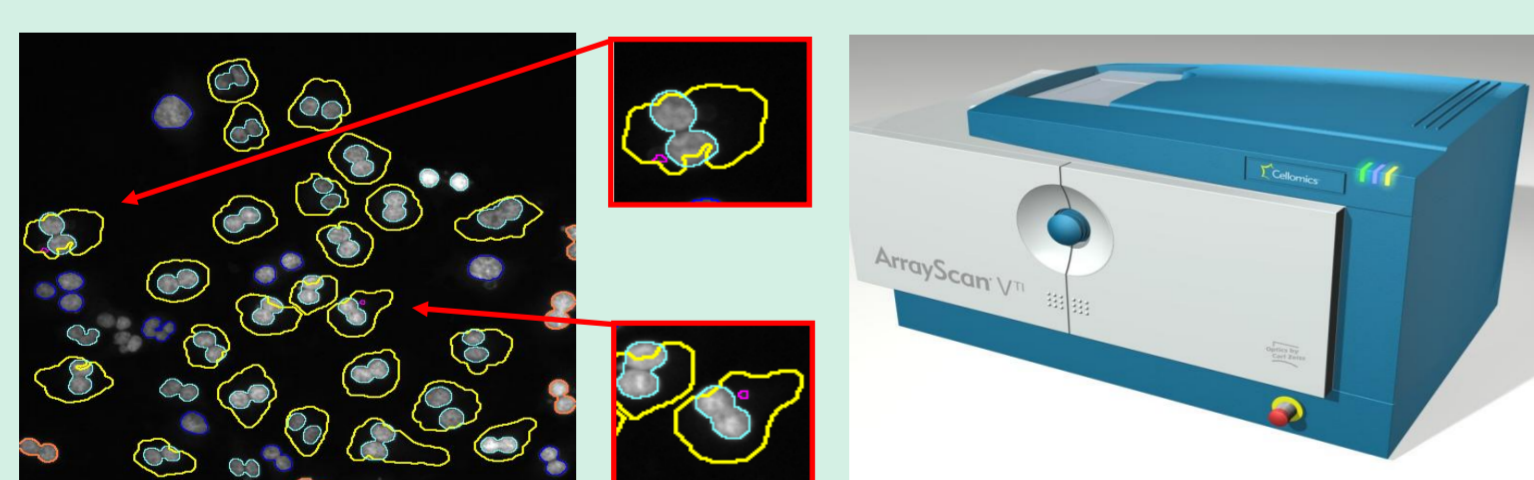
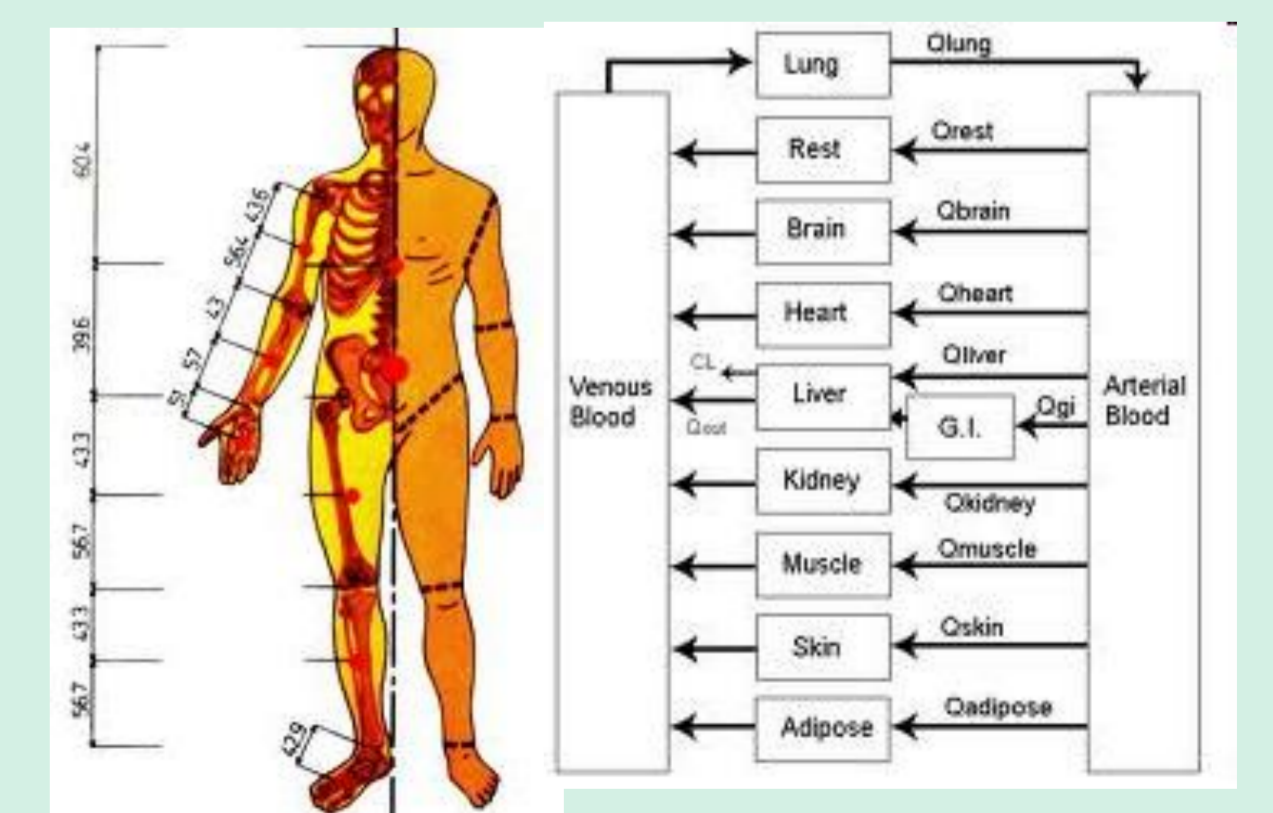
Dose-response assessments

Computational models of the circuitry of the relevant toxicity pathways

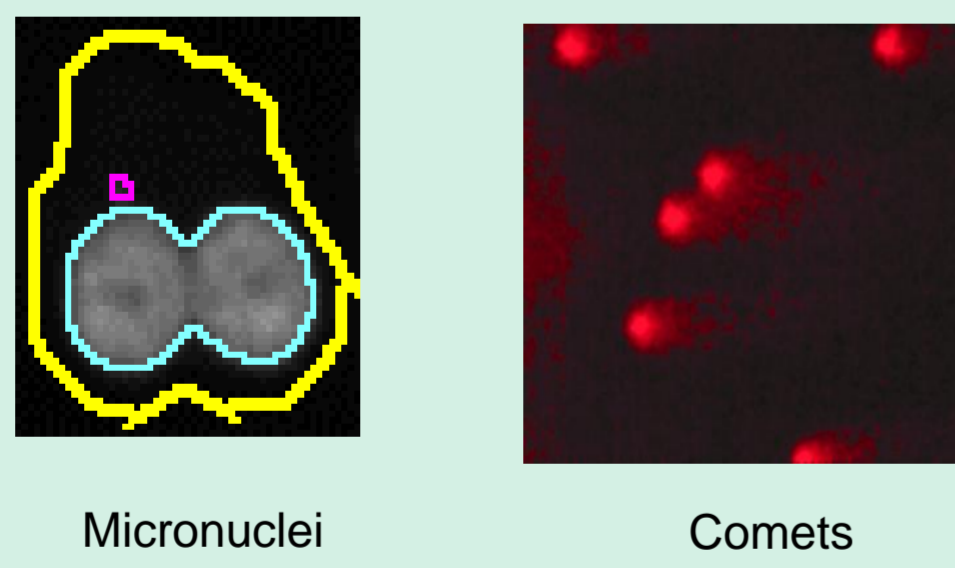
PBPK models supporting *in vitro* to *in vivo* extrapolations

Risk assessment based on exposures below the levels of significant pathway perturbations

PBPK modelling to estimate levels of the ingredient in critical tissues following consumer exposure to the product



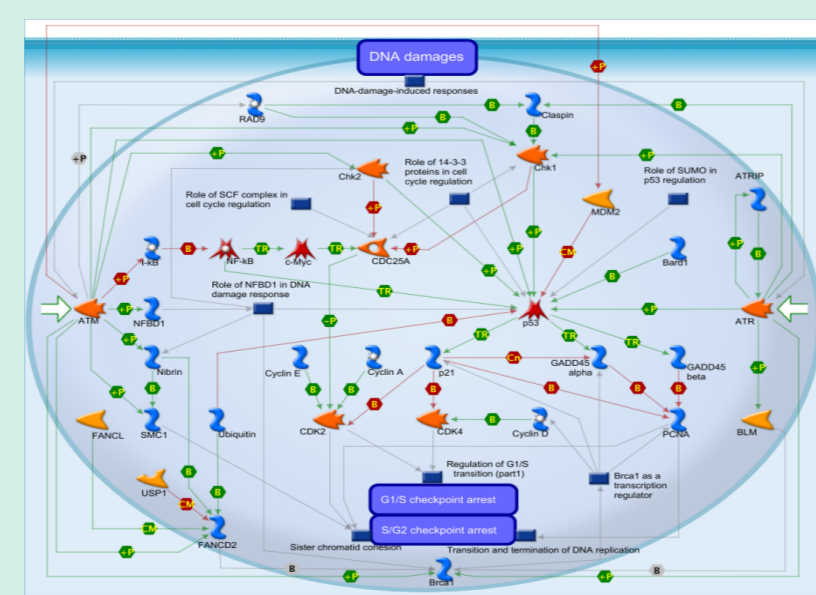
In vitro dose response data from the HCA micronucleus assay (Bioimaging & FACS) and the Comet assay demonstrating a threshold effect



Statistical analysis to determine if the observed dose response fits a linear or threshold model

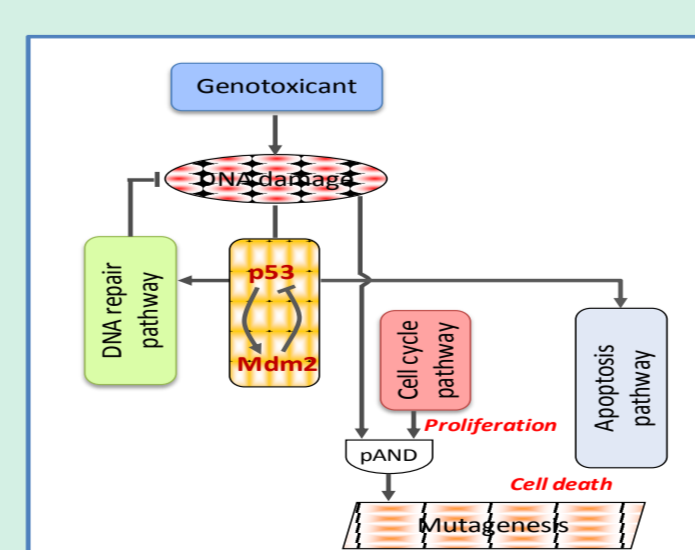


Characterization of the p53 pathway to explain why the threshold exists

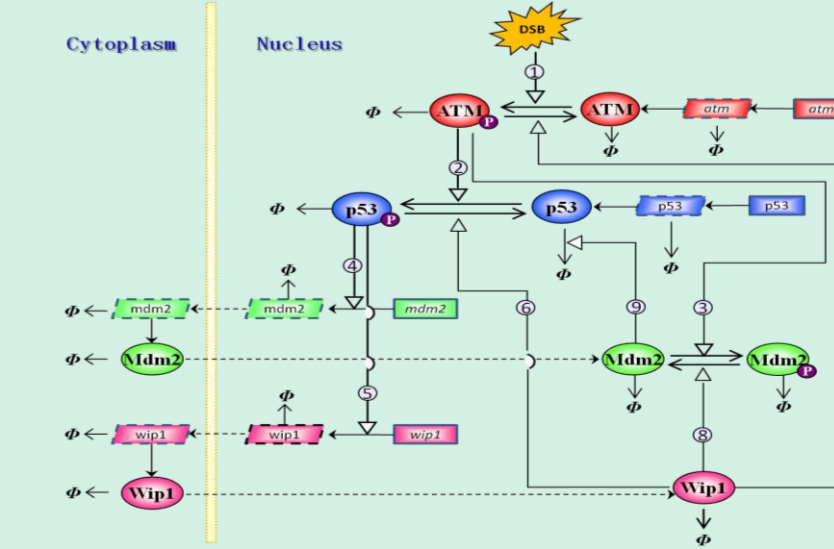


Genego biological pathway model

Constructing computational models of the circuitry of the p53 pathway to model changes caused by exposure to the chemical



Conceptual model



Implementation model using HCA/transcriptomic inputs

