Abstract Modelling of Adverse Outcome Pathways:

Problem brought by – Alistair Middleton (Unilever,UK)

Report By

Gerold Baier[1]

Teresa Collins[2]

Joanne Dunster[3]

Ciarán Fisher[4]

Enuo He[5]

Andrzej Kierzek[6]

John King[7]

Florian Klimm[8]

Gary Mirams[8]

Tom Snowden[3]

John Ward[9]

[1]University College London, Faculty of Life Sciences, Gower Street, London, UK, WC1E 6BT

[2]AstraZeneca, Drug Safety Metabolism, Alderley Park, Macclesfield, UK

[3]University of Reading, Department of Mathematics and Statistics, PO Box 220, Reading, Berkshire, RG6 GAX

[4]Simcyp, Blades Enterprise Centre, John Street, Sheffield, UK, S2 4SU

[5]GlaxoSmithKline, R&D, Ware, Hertfordshire, UK

[6]University of Surrey, Faculty of Health and Medical Sciences, Guildford, UK, GU2 7XH

[7]University of Nottingham, School of Mathematical Sciences, University Park, Nottingham, UK, NG7 2RD

[8]University of Oxford, Parks Road, Oxford, UK, OX1 3QD

[9]Loughborough University, Department of Mathematical Sciences, Loughborough, Leicestershire, UK, LE11 3TU

Latest version: 2nd November 2015

Introduction

Cells have a range of gene regulatory and repair mechanisms that allow them to adapt to changes in the extracellular/intracellular environment and thus maintain homeostatic conditions. This ability to adapt is essential to the survival of the organism, maintaining specialised cellular functions within tissues. Dependent on their function cells accumulate 'wear and tear' over the course of their life cycle and there is a natural rate of turnover of cells which varies between tissues. However, under conditions of extraordinary cellular stress, the rate at which this damage accumulates can increase. Cells can sense an 'assault' (e.g. through nuclear receptors) and so adapt the expression of detoxification and repair mechanisms accordingly, thus eliminating the chemical insult and repairing, as far as possible, any damage caused. These chemical insults can take many forms ranging from pharmaceuticals, personal care products, cleaning products, fertilizers or industrial chemicals. In any case it is important to understand the level at which organisms/tissues/cells exposed to such compounds are able to tolerate exposure and how this corresponds to the level of exposure required for these compounds to be effective or useful.

A number of well characterised biological pathways are associated with adverse responses and these can act as markers of cellular stress. Three such pathways were presented to the workgroup: oxidative stress [1], unfolded protein response [2] and DNA damage [3]. Given these examples, the aim was to reduce these three pathways down to a level of abstraction that allowed all three to be modelled through unified adverse outcome pathway (AOP). AOPs aim to structure pathways associated with toxicity into a uniform structure beginning with a molecular initiating event (MIE), the key events (KE) resulting from the MIE and the key event relationships that link these KEs [4]. Such a model would be able to describe the stress responses of all three and given sufficient data do so quantitatively. On studying the pathways the group concluded that there was a unifying behavioural motif between all three pathways and this could be reduced to the level of healthy cells accumulating cellular damage that is repaired through maintenance mechanisms. Given a sufficient stress, the rate at which damage accumulates, becomes more critical and can lead to cell death.

Following discussions, the group decided to tackle the problem posed using three approaches. Firstly, a model describing three distinct states of the cell whereby 'healthy' cells may accumulate 'wear and tear' (non-critical damage such as associated with aging) before tipping over to 'critical' damage requiring the cell to mobilise special adaptive measures to mitigate the damage. The second approach seeks to minimise the problem even further, describing the balance between damage and repair processes. The third approach is complementary to the others, providing insight into how perturbation of a cell system will affect metabolic pathways producing key molecules required by repair mechanism pathways.

These three approaches are presented below.

Model 1 –‘ Crumple-Zone’ model

We devised the model depicted in *Figure 1* which describes a population of cells by dividing them into three groups: *H* the set of ‘healthy’ cells, *W* the set of `worn' cells, and *C* the set of ‘critically damaged’ cells. Additionally, the model explicitly accounts for two variables *R* and *E* that can be abstractly understood to represent the material and energy depleted in repairing the population of cells.

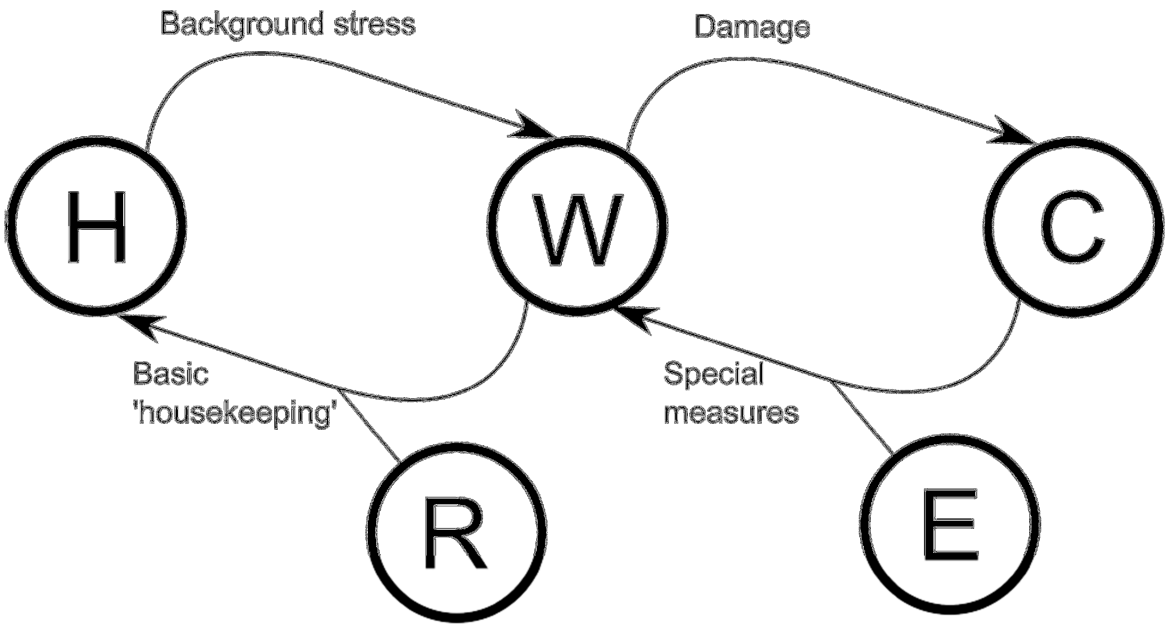


Figure 1: A schematic of an abstract adverse outcome pathway model.

R represents the resources of basal repair and detoxification mechanisms in addressing general wear and tear. Meanwhile *E* accounts for the ‘emergency’ repair mechanisms that can be mobilised to repair critical damage in the cells, but which require activation following the cell sensing damage. Given such a model it is assumed that occurrence of an adverse outcome can be predicted as a function of the proportion of critically damaged cells.

Hence we chose to model the system under mass action kinetics, as defined via the following system of chemical equations:

$$H \xrightarrow{k1} W, \qquad W + R \xrightarrow{k2} H,$$

$$W \xrightarrow{k3} C, \qquad C + E \xrightarrow{k4} W.$$

Now define state-variables *h(t)*, *w(t)* and *c(t)* to represent the time-varying proportion of the cell population that are in healthy, worn, and critical states, such that we have the conservation relation

$$h(t) + w(t) + c(t) = 1$$

Similarly, define time-varying state-variables to describe the amount of the repair resources *R* and *E* available. Such that, overall, we have:

$h(t)$ = [Healthy Cells],

$w(t)$ = [Worn Cells],

$c(t)$ = [Critical Cells],

$r(t)$ = [Basic Repair Resources],

$e(t)$ = [Emergency Repair Resources].

Via the Law of Mass Action the dynamics of these state-variables can then be described by the following system of ODEs:

$$\frac{dh(t)}{dt} = k2r(t)w(t) - k1h(t),$$

$$\frac{dw(t)}{dt} = k1h(t) - k2r(t)w(t) - k3w(t) + k4c(t)e(t),$$

$$\frac{dc(t)}{dt} = k3w(t) - k4c(t)e(t),$$

$$\frac{de(t)}{dt} = k7 - k8r(t) - k4e(t)c(t).$$

Table 1: **An imagined parameterisation associated with the unstressed abstract adverse outcome pathway model.**

Parameter	Value
$k1$	0.1
$k2$	1
$k3$	0.01
$k4$	0.5
$k5$	0.5
$k6$	0.15
$k7$	0.05
$k8$	0.015

Homeostatic regime

Under normal, background stress we would expect to see a population of cells settle into a steady-state where they are predominantly healthy with a small proportion of cells in a worn state, and a negligible proportion in critical condition. Using this ideal, we sought to inform a basic, intuitive parameterisation of the model that yields this behaviour. This led to the parameterisation given in Table 1 which, at steady-state, yields

- approximately 96% of the cell population in a healthy state;
- approximately 4% in the worn state; and,
- roughly 0.02% in the critical state.

Stress

In a pharmacological context, the model depicted in Figure 1 can be used to account for a number of types of stress by allowing different parameters to be altered in response to drug concentration. While any of the 8 model parameters can be altered to potentially simulate a stress situation, we noted that from both the nature of the model and the example adverse outcome pathways provided these stresses can broadly divided into two classes:

1. actively damaging stresses
2. repair inhibiting stresses

We will address each of these classes of stress in turn.

Actively damaging stresses

In this case the stress caused by the administration of a drug actively causes damage to population of cells; increasing the rate with which healthy cells become worn and worn cells become damaged. This is depicted schematically in Figure 2. Within the model this can be accounted for by increasing the rate of background stress (represented by rate parameter $k1$) and the rate of damage

(represented by rate parameter $k3$). Amongst the three example adverse event pathways provided at the workshop this form of stress most readily corresponds to the example of oxidative stress.

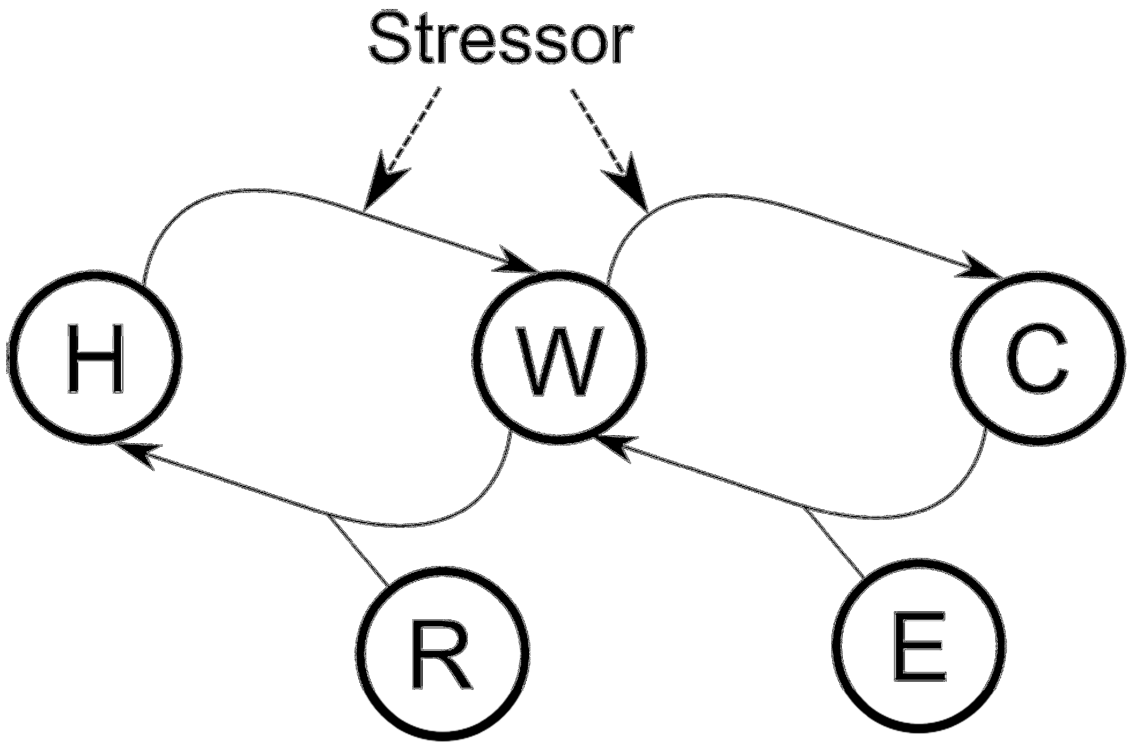


Figure 2: A schematic of actively damaging stress.

Figure 3 depicts an example time-course for actively damaging stressor. Here the system was initially assumed to be in the homeostatic, steady-state regime where at $t = 0$ the administration of an actively damaging stress is simulated via a 10-fold increase in the rates of background stress and damage (such that $k1 = 1$ and $k3 = 0.1$). As can be seen from the simulated results, the system is initially able to compensate for this stress in the first 0.5 units of time by using its emergency repair resources. In the figure, we refer to this period of relative safety as the system's 'crumple-zone'. Once these emergency repair resources have been depleted, however, more critical damage begins to accumulate and is likely to result in an adverse outcome. Under this stressed regime the population of cells eventually reaches a steady-state where

- approximately 13% are in a healthy state
- approximately 5% are in a worn state
- approximately 82% are in a critical state

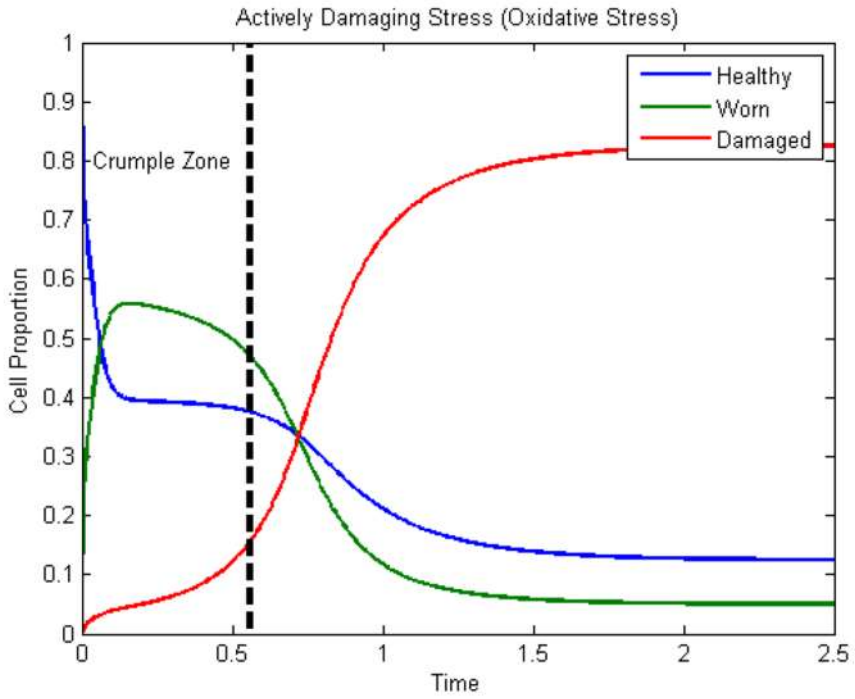


Figure 3: A simulation of the abstract adverse outcome model under an actively damaging stress.

Repair inhibiting stresses

In this case the stress caused by the administration of a drug inhibits the model's repair and detoxification mechanisms; this leads the cell's background wear and tear to steadily accumulate and to eventually tip over into more critical damage. A schematic for this type of stress is depicted in Figure 4. Such a class of inhibitive stress can potentially encompass both the DNA damage and unfolded protein accumulation examples presented at the problem workshop.

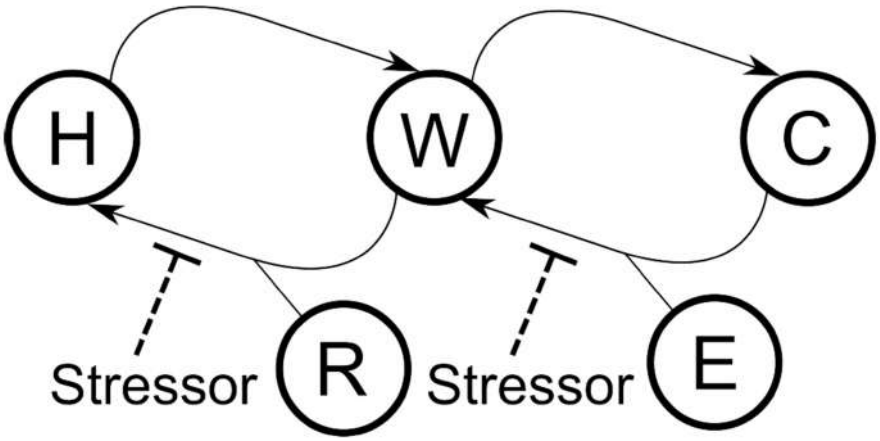


Figure 4: A schematic of repair inhibiting stress.

Given the basic model, there are a number of ways to perturb the parameterisation in order to simulate repair inhibiting stress. Specifically, there are three main possibilities

1. we can reduce the rate at which the repair resources interact with damaged cells by reducing parameters $k2$ and $k4$
2. we can reduce the rate at which the repair resources are synthesised by reducing parameters $k5$ and $k7$
3. we can increase the rate at which the repair resources are degraded by increasing parameters $k6$ and $k8$

Here we chose to explore possibility 2., such that the inhibition of the system's repair resources is caused by limiting their rate of resupply. Simulated results for this type of stress are shown in Figure 5. Specifically, we mimicked this type of repair inhibition by applying a 10-fold reduction to the rates of resupply represented by parameters $k5$ and $k7$. This stress was simulated in two forms:

- Firstly, a permanent step change, where $t \geq 1$ the rates of resupply are reduced to 10% of their original value. Again, the system exhibits the crumple-zone like behaviour until the existing pool of repair resources is exhausted. Once this has been overwhelmed, critical damage rapidly begins to rise.
- In the second case, a pulse of stress was administered where the rates of resupply are reduced to 10% of their original value $1 \leq t \leq 7$. After the stress has been 'switched off' the worn cells are very rapidly repaired as the necessary repair resources are replenished quickly. The critically damaged cells, however, are repaired significantly more slowly.

Under the permanent repair inhibition stress regime the population of cells eventually reaches a similar steady-state to that obtained under the actively damaging stress, such that

- approximately 13% are in a healthy state
- approximately 5% are in a worn state
- approximately 82% are in a critical state

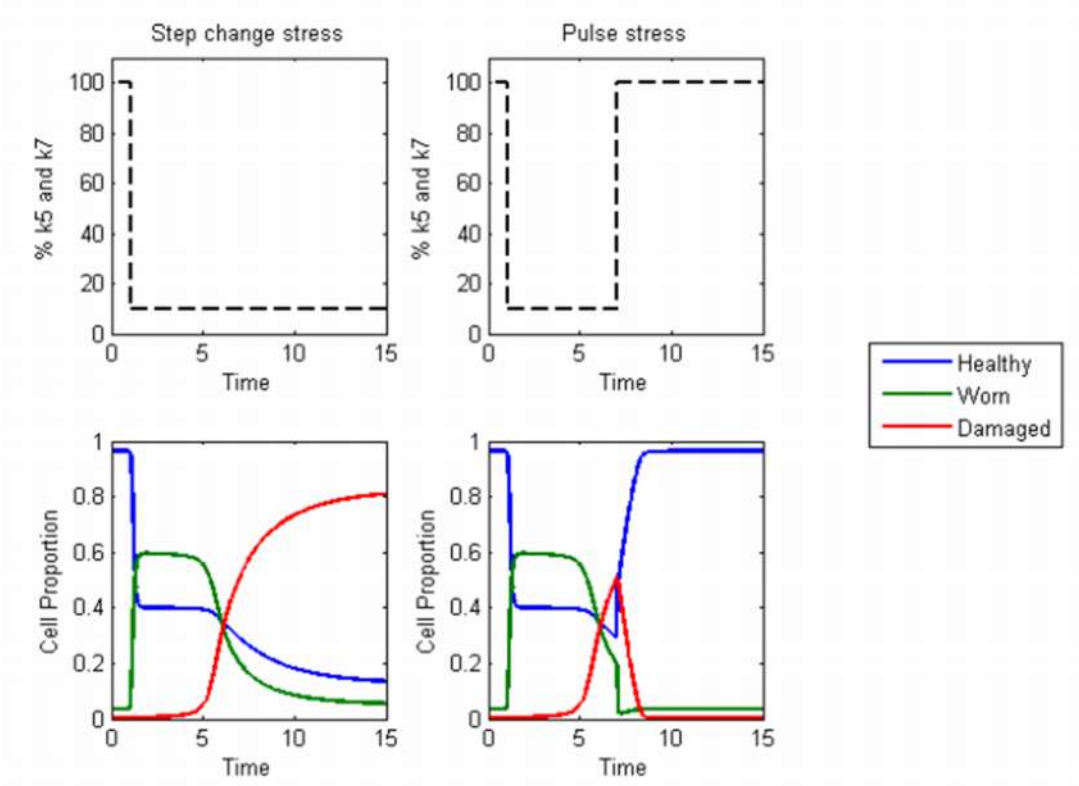


Figure 5: Simulations of the abstract adverse outcome model under repair inhibiting stress.

Repeated dosing and cumulative stress

Finally, we sought to simulate how this system might respond under repeated dosing of a stressor. To look at this we again utilized a repair inhibiting stress that reduces the rate of repair synthesis (represented by parameters $k5$ and $k7$). We assumed that the drug in question was administered intravenously, such that upon administration the stress could be assumed to be instantaneously active before returning via a logistic decay to normal operating conditions. Additionally we assumed that the effect of each individual dose was well within the systems `crumple-zone' such that a single dose was not capable of causing critical damage. Figure 6 shows simulated results for two cases of such repeat dosing.

In the first case, referred to as the long interval repeat dosing case, the model simulates the administration of a repair inhibiting stressor every 6 units of time. In the second, short interval repeat dosing case the same stressor is administered every 3 units of time. In both cases the stress response has fully dissipated by the time of the repeat dose, which can be interpreted as representing the complete pharmacokinetic elimination of the drug by this point. However, in the long interval repeat dosing case the proportion of critically damaged cells reaches a maximal value of 1.6%, which would likely still be considered safe. Meanwhile, in the short interval repeat dosing case the proportion of critically damaged cells can climb to around 48%, potentially leading to an adverse outcome. Crucially, in this short interval case, the system can accommodate the first 2-3 doses, but after this point the repair mechanisms cannot sufficiently replenish themselves before the administration of the next dose.

In essence, then, the system is able to accumulate damage under repeated dosing, even where there is hypothetically no pharmacokinetic accumulation of the drug. This can occur if the dosing interval of a drug is long enough such that it is fully eliminated between doses, but short enough that the `repair resources' are not able to be fully replenished before a repeat dose is administered.

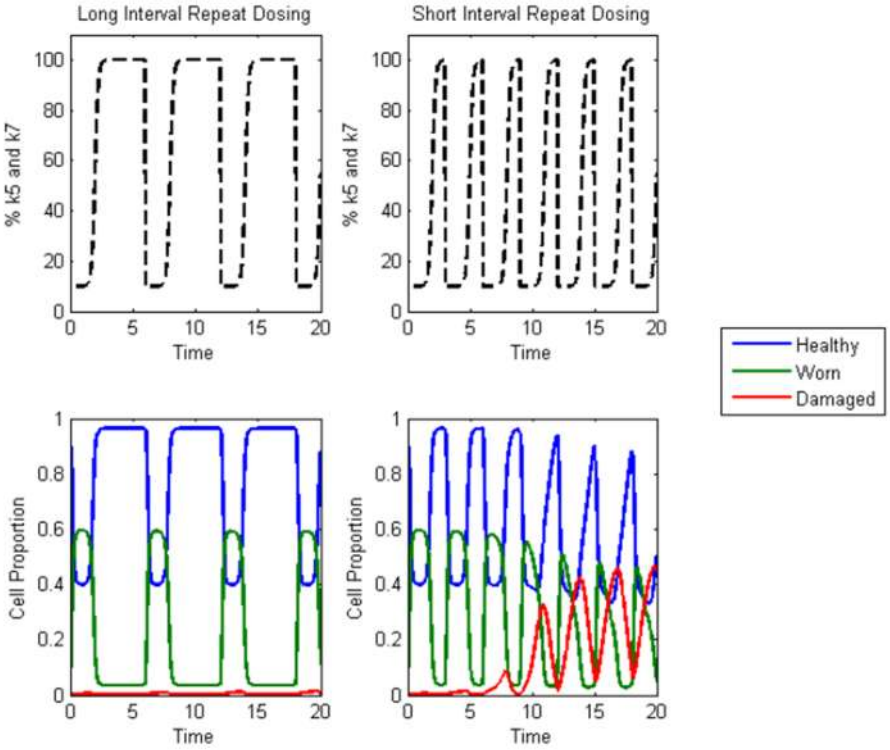


Figure 6: Simulations of the abstract adverse outcome model under repeat dosing of a repair inhibiting stressor.

'Crumple-Zone' Model Summary

As demonstrated above, this model exhibits a phenomenon that we have termed the `crumple-zone'. Specifically, this implies that the population of cells is able to absorb a certain degree of stress without producing any critical damage. It is only if the stress is sufficient to overwhelm the pool of existing repair/detoxification resources, and hence get beyond the crumple-zone, that any more substantial form of damage will begin to accumulate. These crumple-zone dynamics also have interesting implications in the administration of repeat dosing; as was shown, it is possible for the stress effects associated with the administration of a drug to dissipate without having caused any notable damage if the duration of the stress is less than that of the 'crumple-zone'. However, if the dosing is frequent enough and the rate of repair resource replenishment is slow enough, this can still eventually lead to the emergence of more significant damage and the possibility of adverse outcomes.

There were a number of ideas for further work on this model discussed during the workshop that could not be addressed in the time available. These include:

- **More detailed mathematical analysis of the 'crumple-zone' model:** Further exploration of the 'crumple-zone' network should be performed, including a detailed bifurcation analysis. It may be possible that, for certain parameterisations, this model can exhibit bistability (indeed, during the workshop it was possible to show that the steady-state solution of the system was the solution of a cubic which may permit a bifurcation). Additional methods, such as sensitivity analysis or asymptotic analysis, may also provide greater understanding of the dynamical behaviour of this system.
- **Comparative analysis of existing adverse outcome pathway models:** Another approach to the problem of creating a generalised description of adverse out-come pathways that was discussed during the workshop centres on the comparative analysis of existing pathway models. The aim would be to reduce and analyse a range of these networks to obtain highly simplified motifs that still give the same dynamical behaviour. We would then seek to compare and match these simplified motifs in the hopes of finding motifs that can be seen to underlie a wide range of adverse outcome pathways. This would essentially be a graph matching problem, similar ideas are explored in [5].
- **Model verification and parameter fitting:** Finally it may be possible to verify the 'crumple-zone' model for specific drugs with associated adverse out-comes. For example, glutathione levels may represent a good proxy for the pool of repair resources for certain adverse outcomes.

Model 2 - Adverse Outcome Pathway Core Model

The aim of this approach was to create a more minimal framework to describe key features observed in the above mentioned examples of AOPs. These include the healthy state, the hijacking of functional pathways and the accumulation of a reagent that leads to a negative outcome, for example apoptosis.

We decided to start the model as a two state system coupled according to the law of mass action:

$$\frac{dA}{dt} = -D[A] + R[B]\ ;\ \frac{dB}{dt} = -\frac{dA}{dt}$$

The two states *A* and *B* represent two required components in a cellular pathway, for example a folded and unfolded form of protein. We assume that the overexpression of component *B* leads to an adverse outcome (i.e. serves as a biomarker of toxicity). *D* and *R* give the rate constants of the two processes of destruction and repair, respectively. The assumption of conservation of the total population

$$[A] + [B] = Const. = Total$$

allows us to rewrite the system with a single state variable. For *Total* = 1 we get

$$\frac{dB}{dt} = D([B] - 1) - R[B] = D - [B](R + D)$$

This equation has a single stable fixed point at $[B]^* = 1 - R/D$. This fixed point can be interpreted as the *normal* state of the biological system in which it operates. It represents the stable coexistence of the two reagents. The stability guarantees that after small perturbations (for instance due to noise in the environment) the system will return to the normal state and remain near it.

As noted in many of the exemplary AOPs, feedback loops are important in the catastrophic accumulation of the toxic marker *B*. Such feedback loops can be represented as the functional dependency of the rates on the population *B*. Here we want to give two fundamental examples. We include firstly a positive feedback on the destruction process and secondly a negative feedback on the repair mechanism. Note that both may lead to an increase in the negative reagent *B*. We model the positive feedback on the destruction mechanism as a simple linear response $D = d \times [B]$ leading to:

$$\frac{dB}{dt} = d[B] - [B](R + d[B])$$

The negative feedback on the repair mechanism is modelled as a Heaviside step function.

$$R = r \times \theta\,([B] - B_0)$$

$$\frac{dB}{dt} = d[B] - [B](r\,\theta\,([B] - B_0) + d[B])$$

Where *r* is the amplitude of the step and B_0 is the onset threshold. The step function can be seen as a simplified form of a sigmoidal characteristic of the repair mechanism.

Note that both response functions are chosen to be minimalistic; in detailed models more complex functions may be chosen.

In the analysis of this system we are especially interested in the behaviour of the stable state of the system under the change of the destruction parameter *D* which can be interpreted as representing the amount of toxic agent, for example from the AOP of a given drug. Mathematically speaking we are performing a bifurcation analysis. The outcome can be compared to experimentally obtained dose-response curves.

In Figure 7 we illustrate the simulated dose-response curve from a numerical integration of the differential equation and overlay it with the analytical bifurcation diagram of the model. For low toxicity values *D* the stable fixed point$= 0$ (i.e. there is no adverse response). For intermediate toxicity, the stable fixed point is given by the solution $1 - R/D$. This will often coincide with the regime of a stable biological process since a finite amount of *B* is necessary for functionality. The stability of these two solutions interchange at $D = 1$ in a transcritical bifurcation. As a result of the step function form of the feedback this solution abruptly stops to exist when the stability point is above B_0 (dotted vertical line). After this the only stable fixed point is $B = 1$ which represents a state of only the negative reagent being present and thus representing an adverse outcome. This step occurs at $R/(1 - B_0)$.

We note that a supra-linear feedback on the destruction mechanism in the form $D = d \times [B]^n$ (with *n*>1) leads to a bistable system and hysteresis behaviour in the dose-response curve. Such a bistability and hysteresis has been predicted by various detailed models of adverse outcome AOPs.

The two-state system represents fundamental mechanisms of the AOPs in the form of feedback loops and the eventual accumulation of a negative reagent. This negative reagent could stand (e.g. for the unfolded protein molecules in [2]. The simple form of this model allows the analytical derivation of the bifurcation diagram. The system's numerical integration shows that both solutions coincide. The equivalent of the bifurcation diagram can be measured experimentally as a dose-response curve and compared with the analytical prediction of various feedback functions. The two feedback functions must be seen as a gross simplifications of more complex pathways and more realistic functions will need to be included in detailed models.

A point of future research would be the enrichment of such predictions with real world data sets of signalling pathways and the investigation of the specific type of feedback functions and accompanying bifurcations.

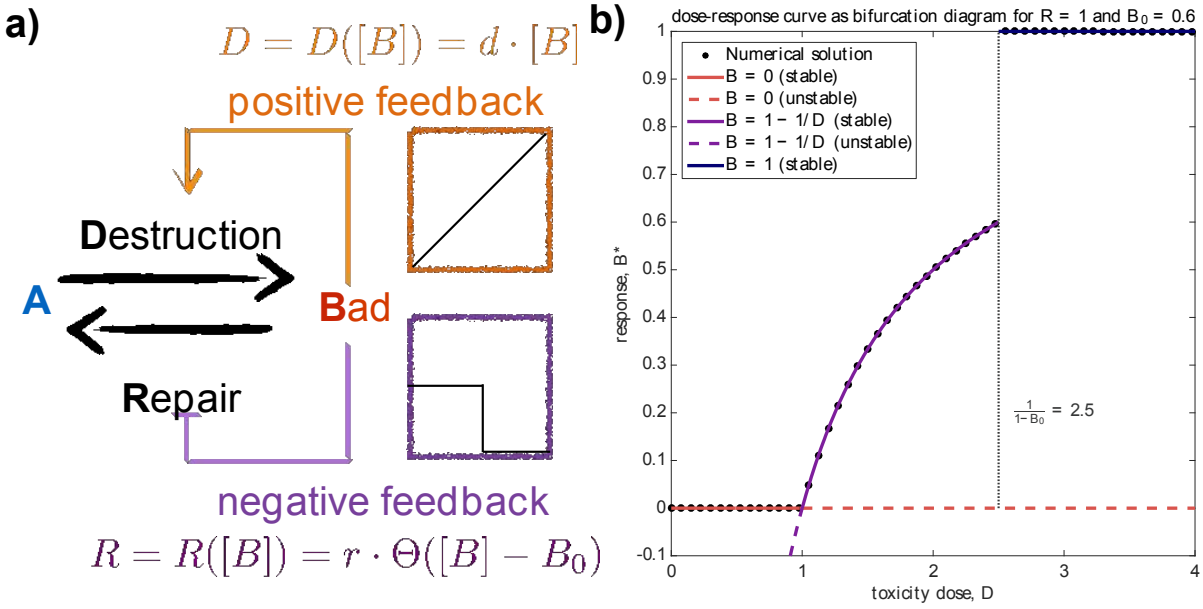
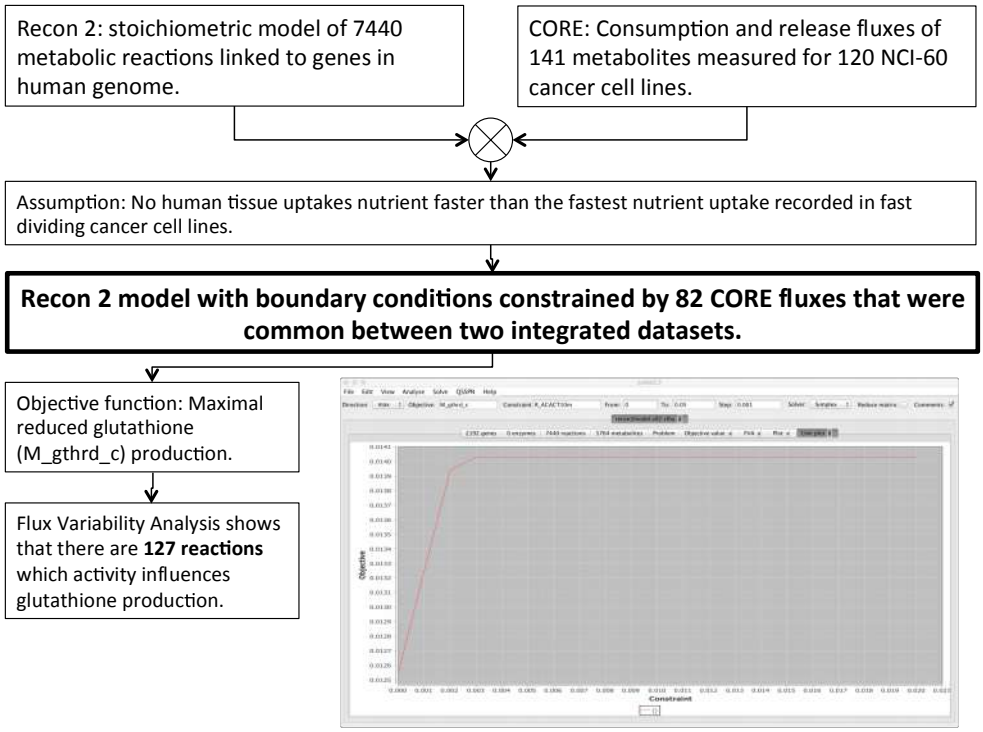


Figure 7: a) Minimal AOP model with two states A and B. Destruction and repair mechanism are influenced via two feedback loops in form of linear response (orange) and a Heaviside step function (purple). b) Numerical integration of the differential equation gives the dose response curve (black dots). Analytical bifurcation diagram matches those results and solid lines representing stable and dashed lines unstable fixed points, respectively. Response values below zero are non-biological and only given to show the exchange of stabilities at the trans-critical bifurcation point $D = 1$.

Model 3 – Complementary Genome Scale Metabolic Network

Mechanistic, bottom up modelling of literature-based molecular network models is an alternative to the phenomenological mathematical modelling approaches presented above. Here we used mechanistic simulation of a human genome scale metabolic network to identify reactions, which influence glutathione availability and so can potentially limit the cells capacity of responding to oxidative and/or xenobiotic exposure. Workflow of the analysis performed with version 2 of SurreyFBA [6] software is shown in Figure 8.



Example reaction identified in analysis: Dependence of maximal glutathione production on Mitochondrial ACAT1 acetyl-CoA acetyltransferase (R_ACACT10m). SurreyFBA2 software.

Figure 8: Constrained based modelling workflow with SurreyFBA2 software. The exchange reactions of Recon 2 model of human metabolism has been constrained with uptake fluxes measured for 120 cancer cell lines from NCI-60 collections. The Flux Variability Analysis revealed that there are 127 reactions, which have to carry non-zero flux if glutathione production is to reach its maximal value. The screenshot of SurreyFBA2 software shows dependence of maximal glutathione production on of the 127 reactions – Mitochondrial ACAT1 acetyl-CoA acetyltransferase. Similar curves would be obtained for each of the reactions identified by FVA. Thus, our analysis identified 127 reactions, which would affect glutathione production if their activity was decreased due to toxic agents or through a genetic polymorphism.

The Recon 2 model of human metabolism, recently developed by an international community [7] contains 7440 biochemical reactions and 5764 metabolites. Recon 2 aims to describe the whole-cell network of biochemical conversions used to generate biosynthetic precursors, energy and reducing power from available nutrients. The variables of the model are reaction fluxes at quasi-steady state. The quasi-steady approximation state is justified by the time separation of metabolic reactions and

metabolic gene expression. Such models can be analysed by a wide range of Constraint Based Methods (CBM) [8], which explore the space of metabolic flux distributions satisfying stoichiometric constraints defined by elementally balanced metabolic reaction formulas and thermodynamic constraints defined by current knowledge on reaction reversibility under physiological conditions. The Recon 2 model is based on the set of genes encoding metabolic enzymes in human genome. However, an additional gap filling step was necessary construction of the model to reproduce basic metabolic capabilities of human cells. Additional reactions were added for which the mediating enzymes are currently not known. This illustrates an advantage of using mechanistic models over metabolic pathway maps; the model needs to provide stoichiometrically and thermodynamically feasible flux distributions for known metabolic functions, whereas pathway maps are not validated. Here, we have imported the Recon 2 model into SurreyFBA and calculated the maximal growth rate of the cell.

The original Recon 2 model has been validated in qualitative simulations, where only the existence of a flux distribution realising the metabolic function of interest was required. This implies that fluxes would not have realistic values in quantitative units, which would limit the application of the model in Quantitative Systems Pharmacology context. However, to provide further parameterisation, we have integrated Recon 2 model with a landmark dataset of metabolic fluxes measured for NCI-60 cancer cell lines [9]. For each of 120 cancer cell lines the consumption/release (CORE) flux for each of 141 extracellular metabolites has been measured by quantitative metabolomics. We assumed that no human tissue would uptake the nutrient faster than the fastest rate observed over 120 fast growing cell lines. This provides upper bounds for uptake and release of metabolites in Recon 2. To be more specific, we have identified 82 extracellular metabolites in Recon 2, which could be unequivocally assigned to metabolites in CORE dataset. For each of these metabolites we constrained the upper/lower bounds of the corresponding exchange reaction to minimal/maximal flux observed across 120 cell lines. Subsequently, we have calculated a maximal growth rate by Flux Balance Analysis [10]. The result of 0.065 1/h was within the range of growth rates observed for NCI-60 cell lines, a more physiological value than 3.198 1/h obtained for the original, unconstrained, Recon 2 model. We concluded that parameterisation of boundary conditions using the CORE dataset constrained the solution space to a physiologically realistic range of quantitative fluxes in mmol/gDW/h units. This CORE constrained model can now be used to draw conclusions about quantitative upper bounds on metabolic capabilities.

To identify reactions across the network linked with glutathione production, we have performed flux variability analysis. The sum of fluxes producing cytoplasmic reduced glutathione was used as an objective function for linear programming optimisation. In FVA, the SurreyFBA software first calculates a maximal objective function value. Subsequently, the objective function is constrained to its maximal value and each reaction in the model becomes objective, which is maximised and minimised. Thus, FVA calculates for each reaction in the model the range of fluxes consistent with maximal value of the original objective, here glutathione production . Reactions, for which flux ranges do not contain zero, must carry flux if objective is maximal. This implies that any decrease in the activity of any of these reactions would impact glutathione production. Our FVA simulations predicted 127 reactions, which influence glutathione availability. Figure 8 shows one example: Mitochondrial ACAT1 acetyl-CoA acetyltransferase. We used SurreyFBA to calculate maximal glutathione production as a function of an upper bound on Mitochondrial ACAT1 acetyl-CoA

acetyltransferase flux. The plot shows that a decrease in this enzyme activity decreases availability of glutathione for downstream processes.

Our analysis shows that availability of glutathione for stress response and drug detoxification can be affected by 127 reactions in global metabolic network. This prediction goes beyond what can be concluded by examining pathway maps; for example Mitochondrial ACAT1 acetyl-CoA acetyltransferase does not belong to the 'glutathione synthesis pathway'. Our results imply that genetic polymorphisms or inhibition of any of these 127 reactions impact on glutathione-mediated stress responses. Thus, these results constitute a proof-of-concept case study of how CBM implemented in SurreyFBA software can be used to identify gene-drug and drug-drug interactions. By facilitating a semi-qualitative interrogation of the producibility of molecules forming part of the repair/elimination pathways modelled above, CBM can inform analysis of AOP by providing insight in to whether these pools of repair resources can be replenished given the availability of nutrients to the cell.

Finally, we note that recently published Quasi-Steady State Petri Net (QSSPN) approach [11] and QSSPN software, now integrated with SurreyFBA, enable integration of Petri Nets (PN) [12] with CBM models. The method has been validated by integration of qualitative PN model of nuclear receptor network with HepetoNet1 [13] genome scale model of liver metabolism. The current version of the QSSPN software has enhanced support for continuous Petri nets representing Ordinary Differential Equation (ODE) models. This opens the avenue for multi-formalism simulation of models containing abstract, qualitative modules, ODE based models describing Physiologically Based Pharmacokinetics and Genome Scale Metabolic Networks.

Conclusion

We have produced two models for simulating AOPs in a quantitative, but abstract form resulting in a framework that reduces all chemical/environmental cell stresses to the level of 'damage' and 'repair'. As noted above, these models would require parameterisation from *in vitro* datasets and further analysis in order to determine their true utility. We also present a complementary genome scale, semi-quantitative approach that can inform toxicity studies by exploring the impact of nutrient status and genotype/phenotype relationships on the cells ability to maintain pools of key molecules for repair/detoxification pathways.

It is worth noting that the AOP models developed here may require increased mechanistic resolution in order to be useful. However, the incorporation of a large-scale mechanistic metabolic model may mitigate this. Also, these models may require some adaption to be able to investigate the impact of chronic stress. The models presented above assume a fixed population of cells and assume that subsequent generations would start from a perfectly healthy background regardless of the stresses on parent cells. These transgenerational effects would be important in studying chronic exposure to cell populations, but this lies beyond the scope of this work.

Here we show that it is possible to reduce the behaviours of the three AOPs (oxidative stress, unfolded protein response and DNA damage) to a single level of abstraction, with varying resolution.

However, we also acknowledge that the incorporation of multiple modelling strategies offers a far more robust approach and that a multi-scale workflow, incorporating both quantitative and qualitative data, is likely to give the greatest benefit in studying cellular stress.

References

[1] Khalil HS, Goltsov A, Langdon SP, Harrison DJ, Bown J, Deeni Y: Quantitative analysis of nrf2 pathway reveals key elements of the regulatory circuits underlying antioxidant response and proliferation of ovarian cancer cells. Journal of Biotechnology 2015 202:12-30.

[2] Erguler K, Pieri M, Deltas C: A mathematical model of the unfolded protein stress response reveals the decision mechanism for recovery, adaptation and apoptosis. BMC Systems Biology 2013 7(1):16.

[3] Li Z, Sun B, Clewell RA, Adeleye Y, Andersen ME, Zhang Q: Dose response modeling of etoposide-induced DNA damage response. Toxicological Sciences 2014 137(2):371-384.

[4] Groh KJ, Carvalho RN, Chipman JK, Denslow ND, Halder M, Murphy CA, Roelofs D, Rolaki A, Schirmer K, Watanabe KH: Development and application of the adverse outcome pathway framework for understanding and predicting chronic toxicity: I. Challenges and research needs in ecotoxicology. Chemosphere 2015 120:764-777.

[5] Gay S, Soliman S, Fages F: A graphical method for reducing and relating models in systems biology. Bioinformatics 2010 26(18): 575-581.

[6] Gevorgyan A, Bushell ME, Avignone-Rossa C, Kierzek AM: SurreyFBA: a command line tool and graphics user interface for constraint-based modeling of genome-scale metabolic reaction networks. Bioinformatics 2011, 27:433–434.

[7] Thiele I, Swainston N, Fleming RMT, Hoppe A, Sahoo S, Aurich MK, Haraldsdottir H, Mo ML, Rolfsson O, Stobbe MD, Thorleifsson SG, Agren R, Bolling C, Bordel S, Chavali AK, Dobson P, Dunn WB, Endler L, Hala D, Hucka M, Hull D, Jameson D, Jamshidi N, Jonsson JJ, Juty N, Keating S, Nookaew I, Le Novere N, Malys N, Mazein A, *et al*.: A community-driven global reconstruction of human metabolism. Nature Biotechnology 2013, 31:419–425.

[8] Lewis NE, Nagarajan H, Palsson BO: Constraining the metabolic genotype–phenotype relationship using a phylogeny of in silico methods. Nature Publishing Group 2012, 10:291–305.

[9] Jain M, Nilsson R, Sharma S, Madhusudhan N, Kitami T, Souza AL, Kafri R, Kirschner MW, Clish CB, Mootha VK: Metabolite Profiling Identifies a Key Role for Glycine in Rapid Cancer Cell Proliferation. Science 2012, 336:1040–1044.

[10] Orth JD, Thiele I, Palsson BO: What is flux balance analysis? Nature Biotechnology 2010, 28:245–248.

[11] Fisher CP, Plant NJ, Moore JB, Kierzek AM: QSSPN: dynamic simulation of molecular interaction networks describing gene regulation, signalling and whole-cell metabolism in human cells. Bioinformatics 2013, 29:3181–3190.

[12] Breitling R, Gilbert D, Heiner M, Orton R: A structured approach for the engineering of biochemical network models, illustrated for signalling pathways. Briefings in Bioinformatics 2008, 9:404–421.

[13] Gille C, lling CBO, Hoppe A, Bulik S, Hoffmann S, bner KHU, dt AKA, Ganeshan R, nig MKO, Rother K, Weidlich M, Behre JOR, tter H-GHU: HepatoNet1: a comprehensive metabolic reconstruction of the human hepatocyte for the analysis of liver physiology. Molecular Systems Biology 2010, 6:1–13.