# Activation of Adaptive Cellular Networks and Hormetic Dose Response Relationships

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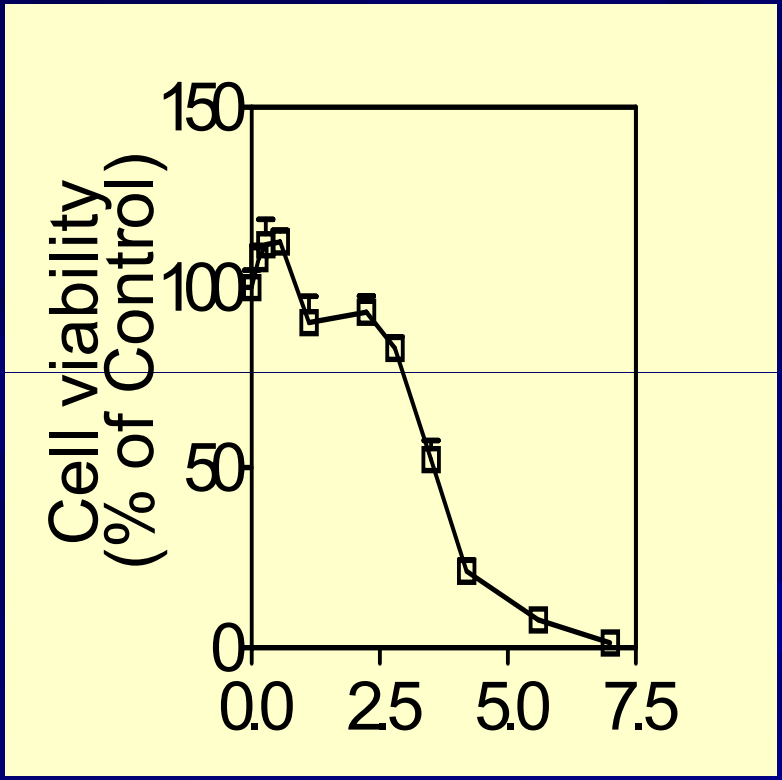
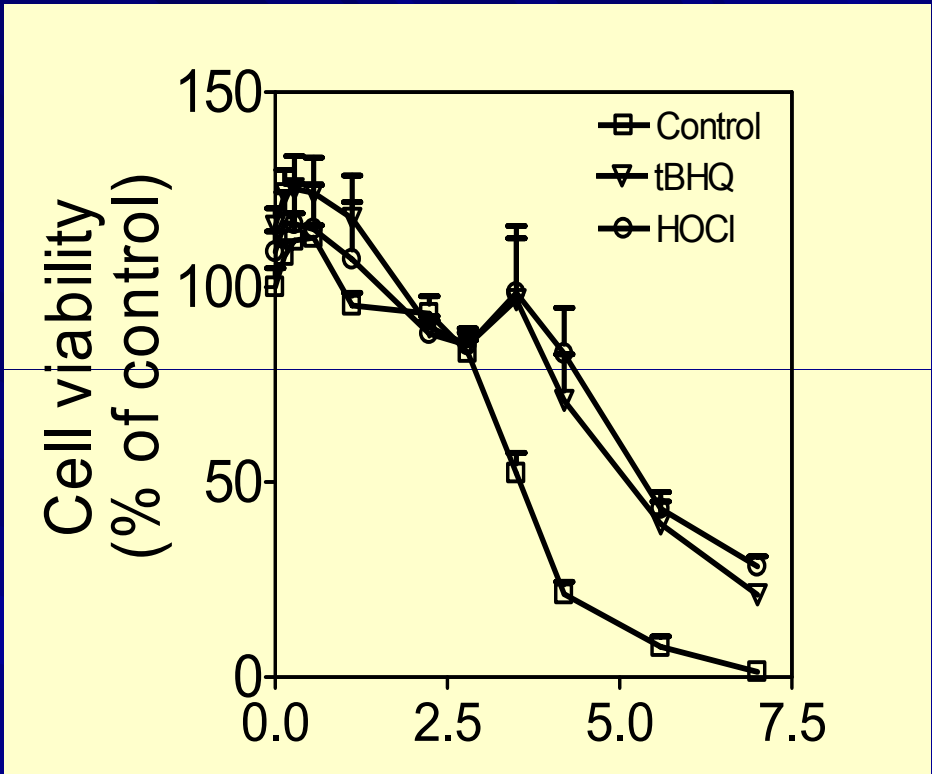
# Toxicity of hypochlorous acid in Mouse RAW cells




In vitro results with various cell types show a region of increased viability before regions of toxicity

Pi et al. (2007)

# Outline

- Risk assessment challenges for irritant gases in developing Reference Concentrations (RfCs)
- Biological models of thresholds, dose-dependent transitions and hormesis versus safety factors
- Choosing prototype irritants for study - e.g., irritant gases, hepatic enzyme inducers
- Moving ideas of hormesis into the risk assessment process

# Reference Concentrations (RfCs) – the Process

$$RfC = \frac{\text{Benchmark Concentration} \times \text{Duration} \times \text{DAF}}{UF1 \times UF2 \times UF3 \ldots\ldots\ldots}$$

# For Chlorine:

- To develop an RfC that takes into account:

  (1) Tissue dosimetry of HCl and HOCl within the respiratory tract

  (2) Dose dependent modes of action of chlorine in these tissues, and

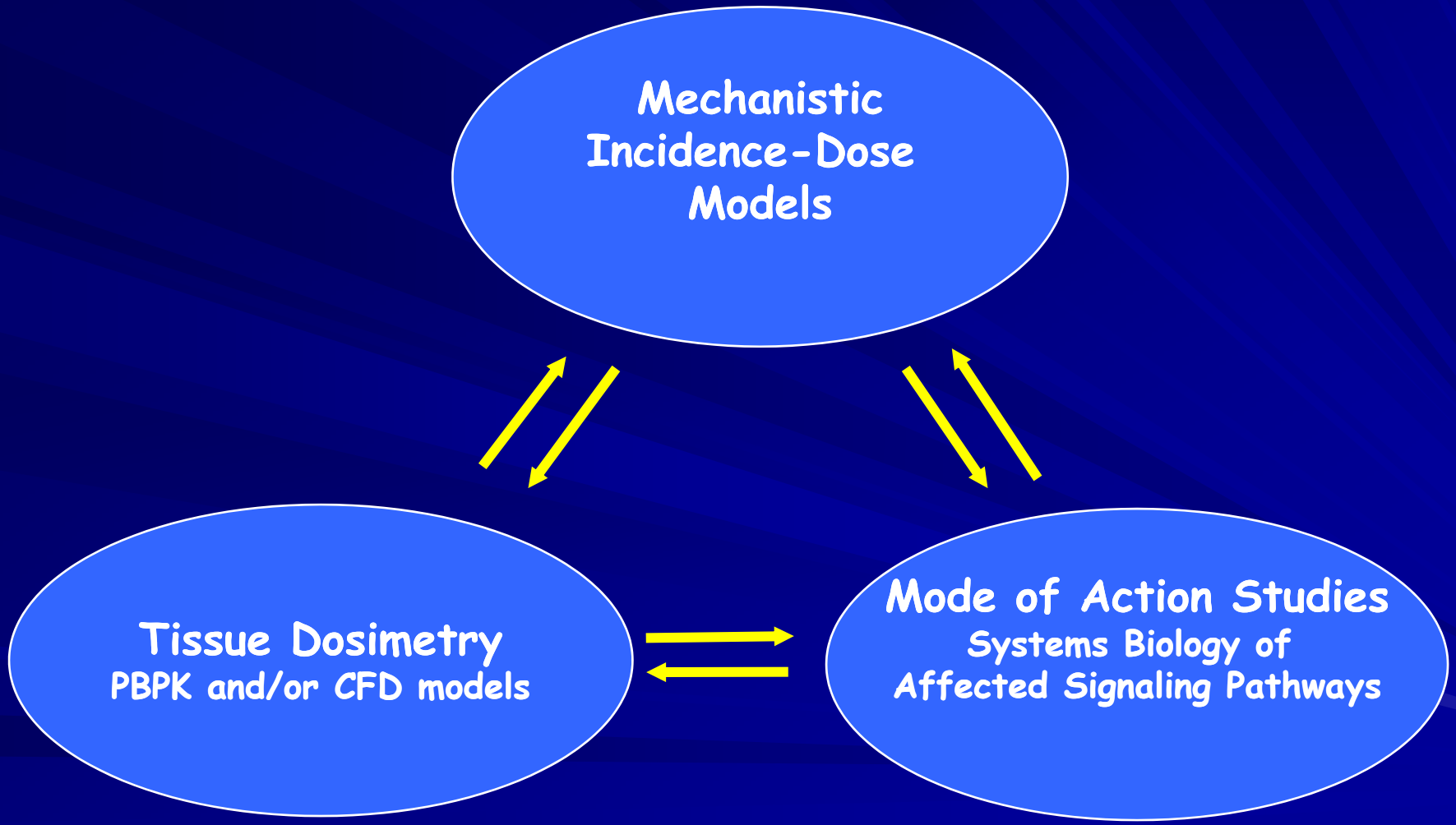
  (3) Cell response modeling of activation of adaptive cell response pathways

  (4) Places U-shaped from in vitro responses in a risk assessment context

# Why chlorine?

- It is a common water disinfectant, a synthetic intermediate for many commodity chemicals, and a possible target for terrorist use as a weapon.
- Good traditional toxicity data base, including an inhalation bioassay and a clear mode of action as a cellular oxidant

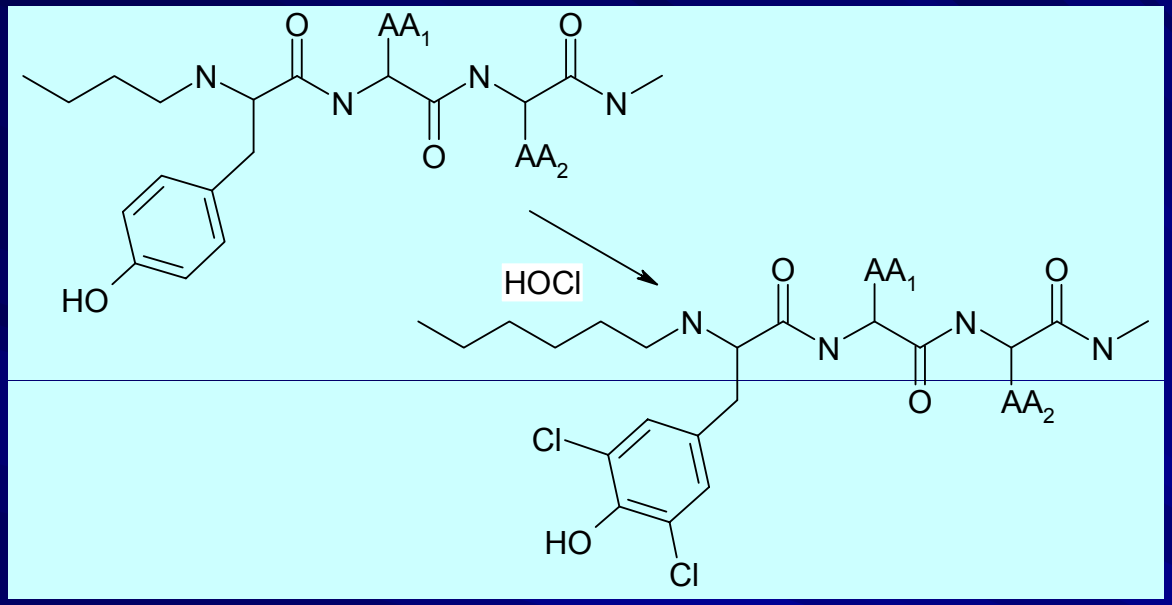
# Mechanistic Approaches to Irritant Gas Risk Assessments



**Hypothesis:** Oxidative stress from HOCl is the predominant mode of action for chlorine irritancy in the respiratory tract

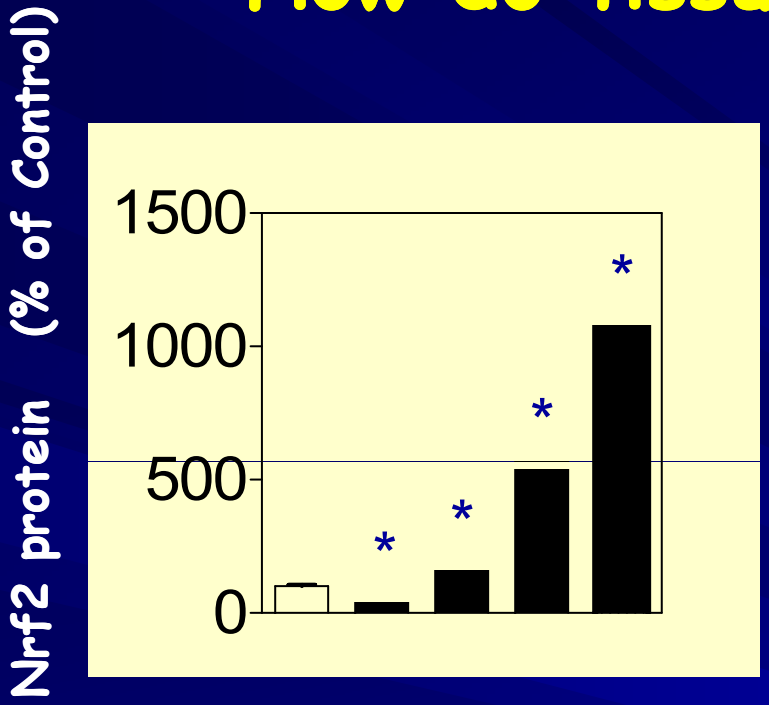
- Chlorine rapidly hydrolyzes in aqueous conditions
- $Cl_2 + H_2O \rightarrow HOCl + HCl$
- Nasal responses observed at several ppm $Cl_2$ compared to 100 ppm for HCl

# What does Chlorine do to tissues?

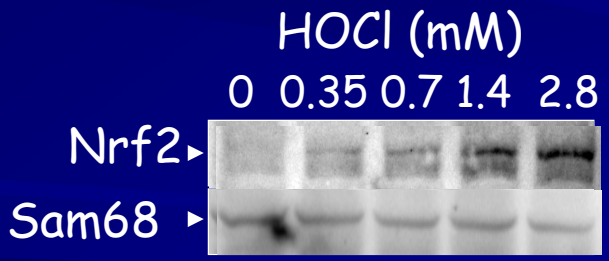



Reactions of HOCl with tissue produces a variety of oxidized and chlorinated products, including chlorinated aromatic amino acids. These products serve as a local biomarker for the presence of HOCl in tissues (Jarabek and Sochaski)

# How do tissues respond?

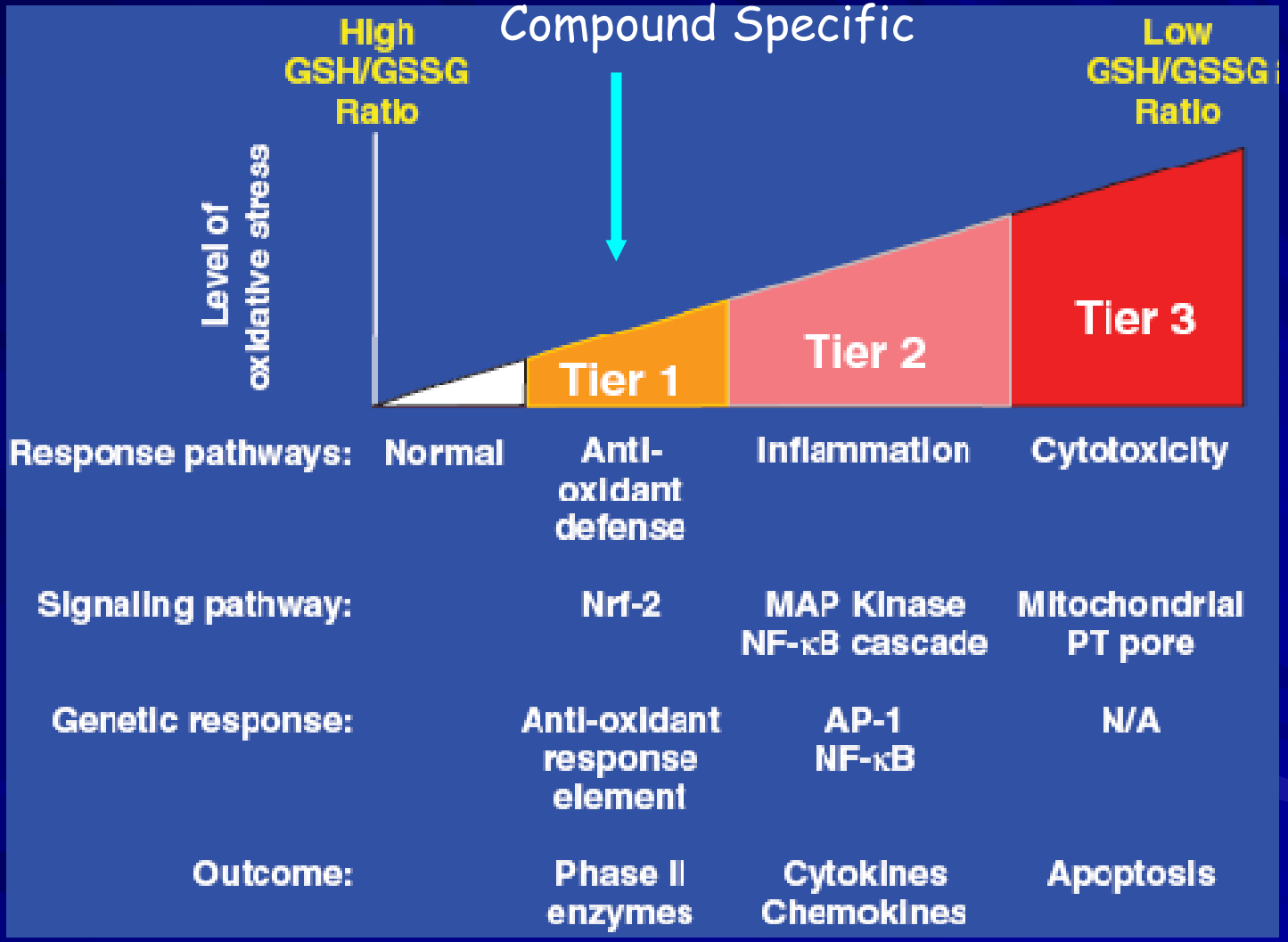



Treatment of RAW cells clearly induces Nrf2, the primary mediator of anti-oxidant stress signaling.



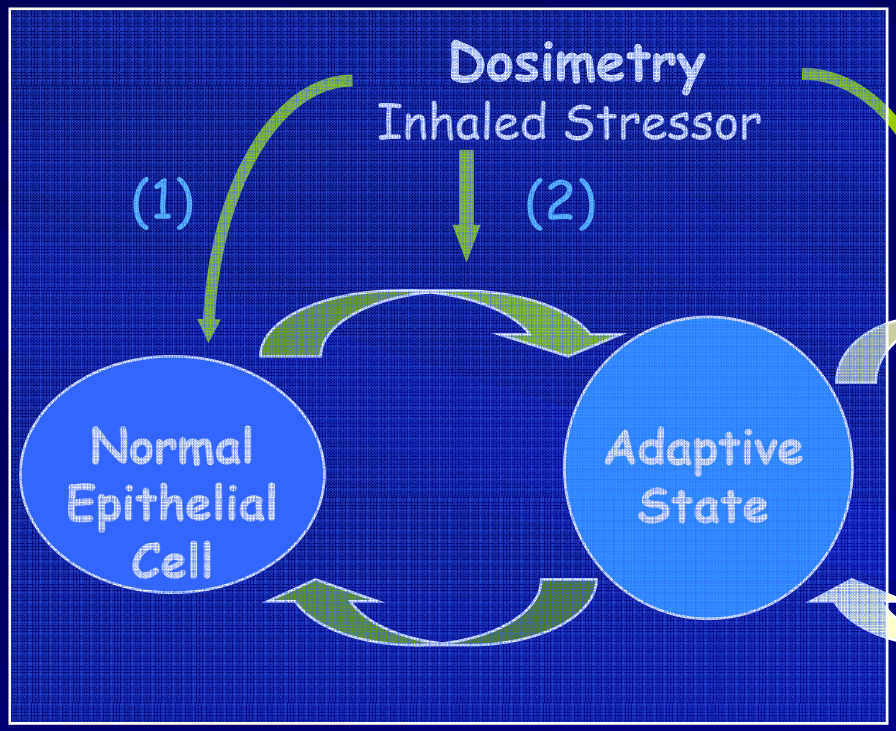

Pi et al.

# Then, as concentrations increase,




Studies based on work of Andre Nel and others (Science, 2006)

# Mechanistic Hierarchical Dose Response Model

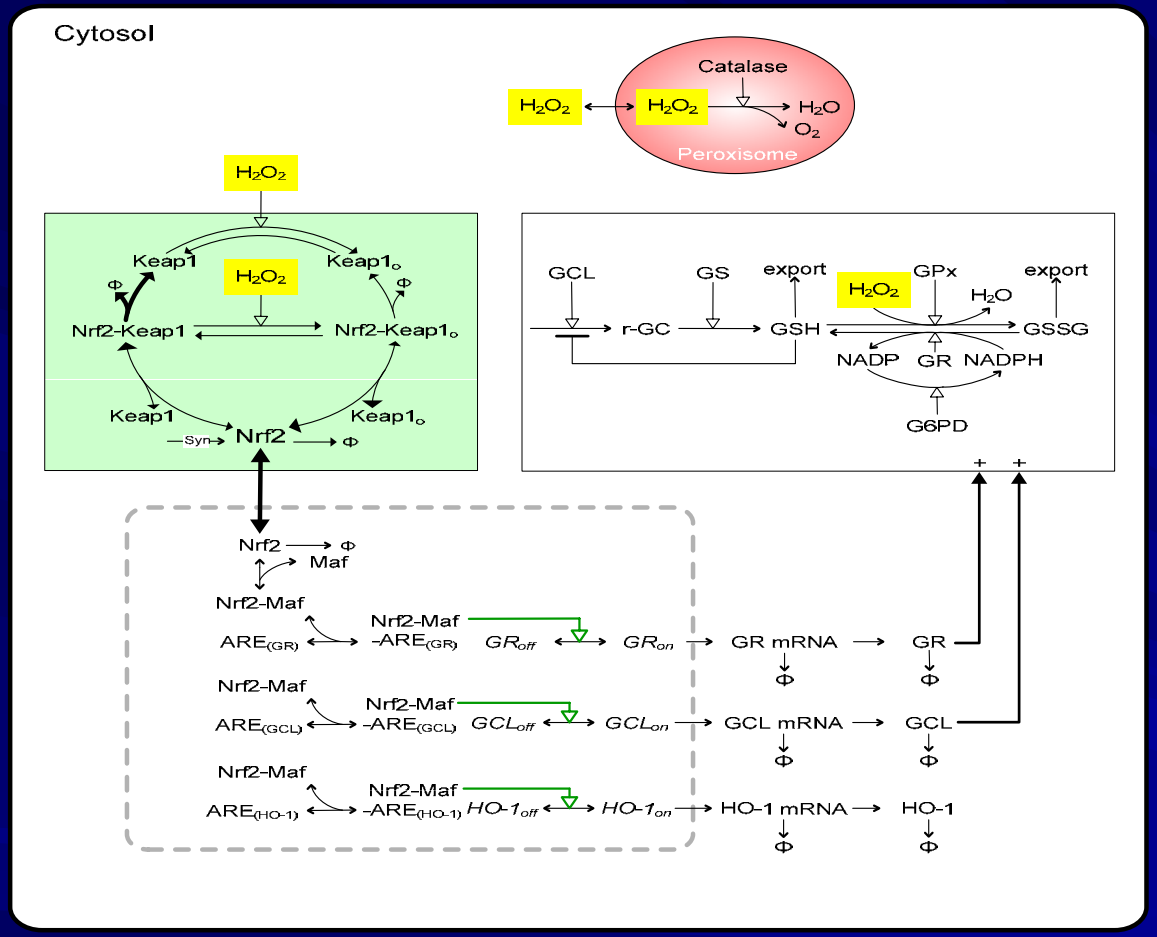



Tissue Phase Reactions

$Cl_2$ → HOCl + HCl

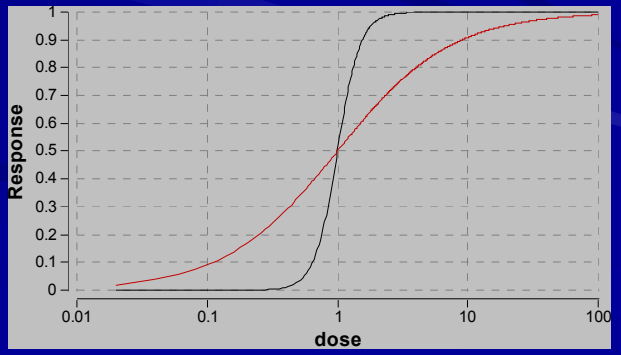
Stressed State → Pathology, Necrosis, Atrophy

# A model for oxidative stress in Pathway Assist- automated model building and dose response

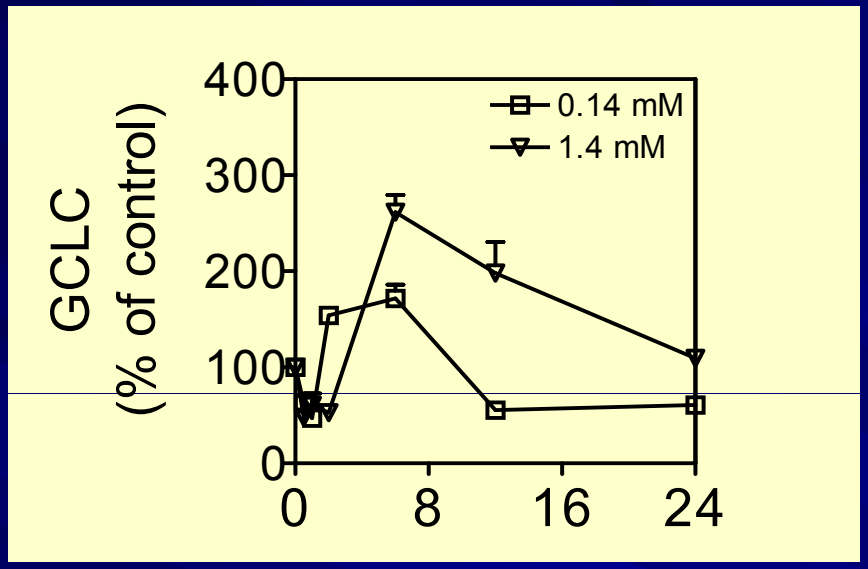


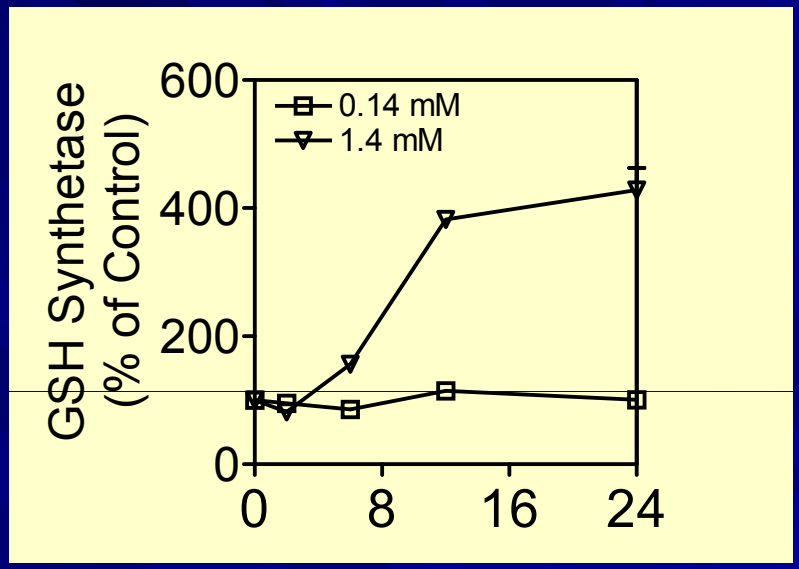

Zhang et al. (in progress)

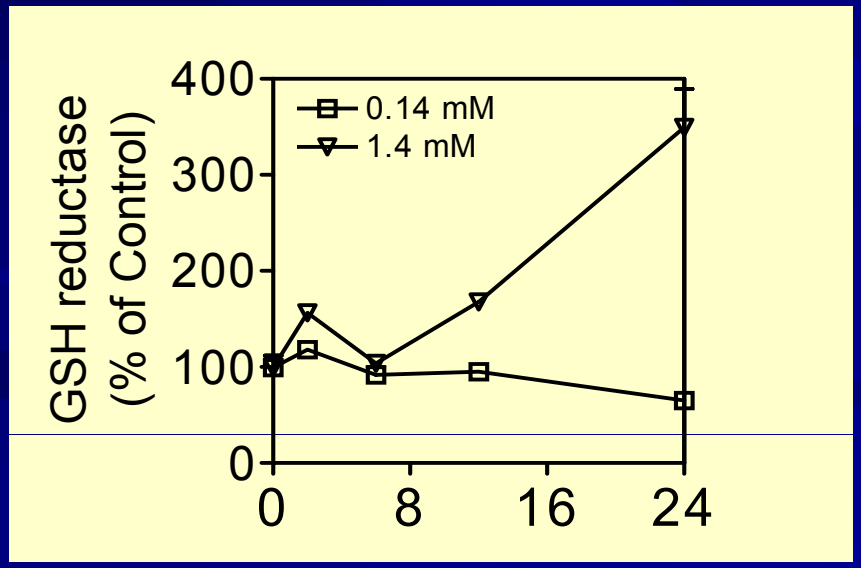
## Dose-Responses

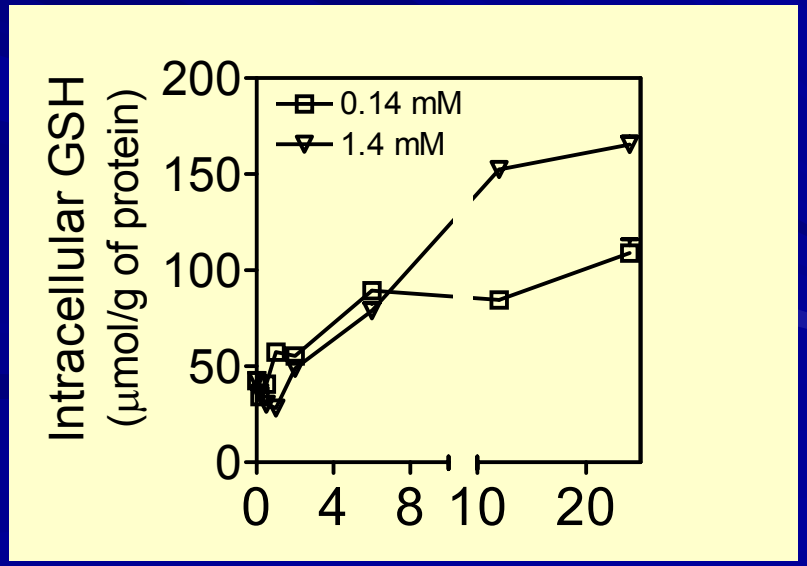


# Controlling Cell Anti-oxidant Synthesis



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# Dose Response Relationship in Anti-Stress Gene Regulatory Networks

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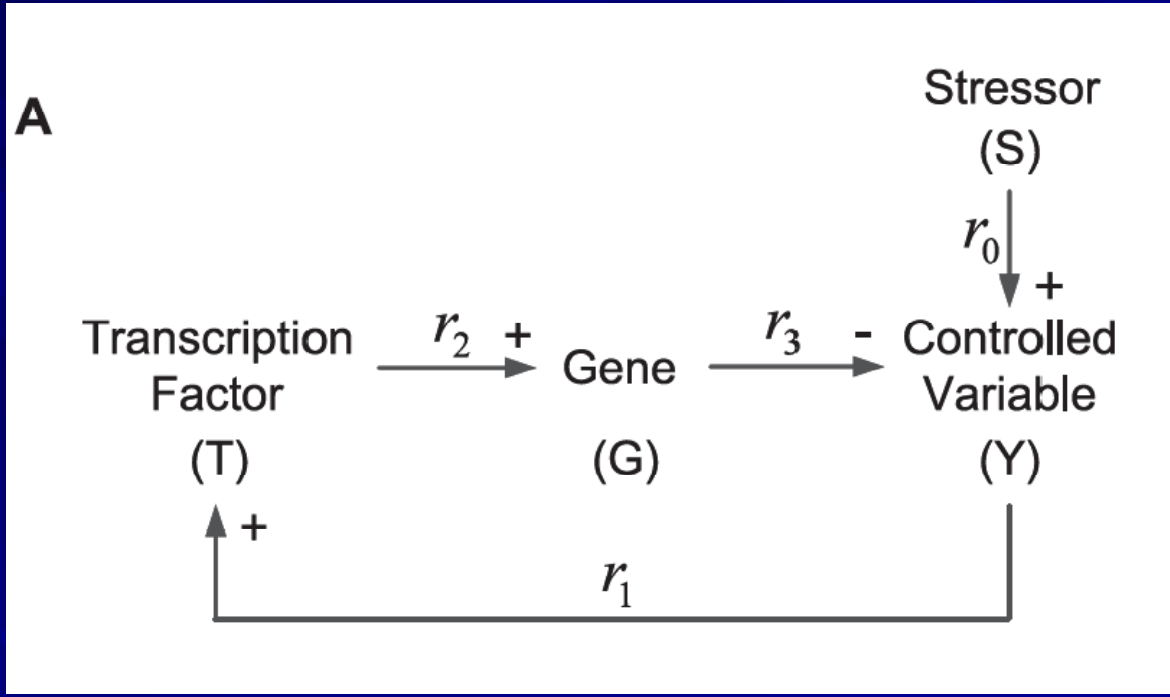
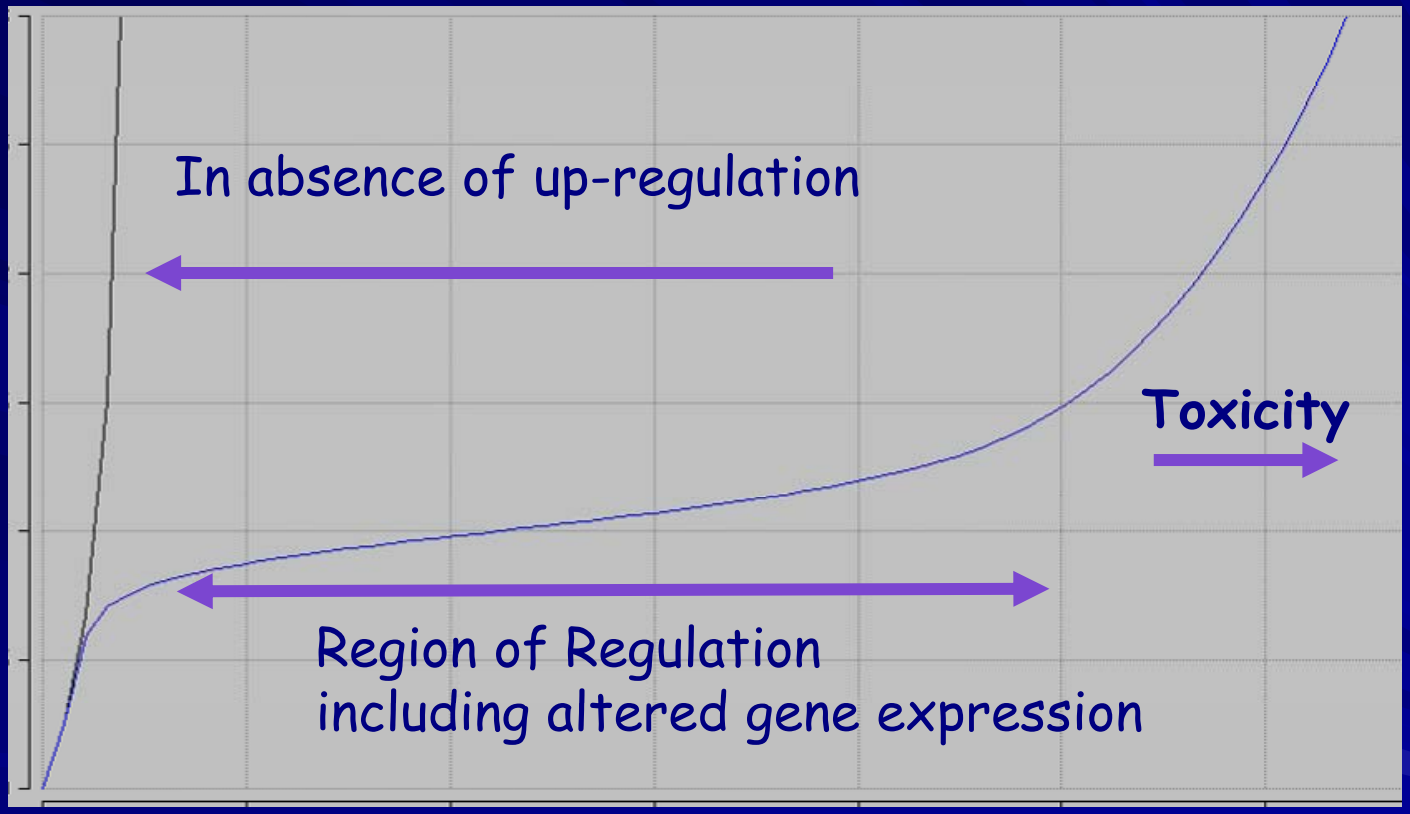
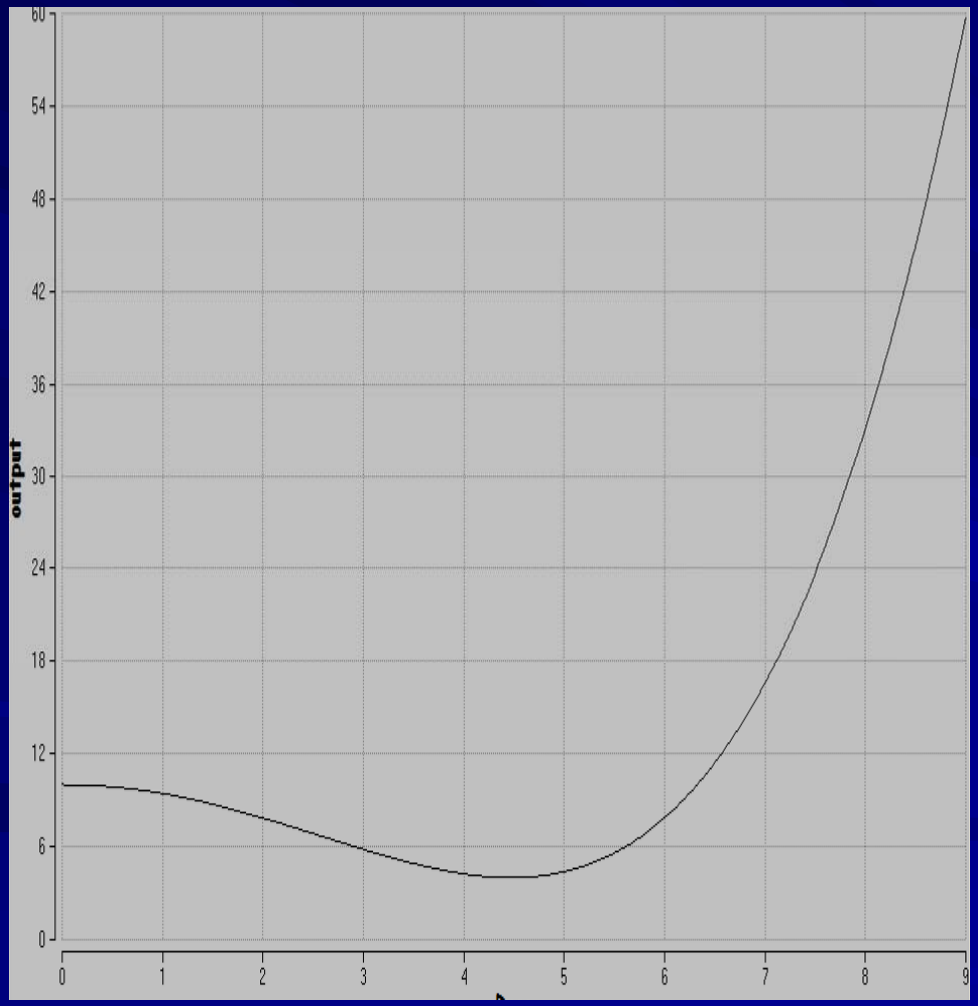



Normal adaptive feedback processes, based on negative feedback with feed forward control. Homeostasis

# Consequences of Feedback Loop on Stressor Levels in Cells

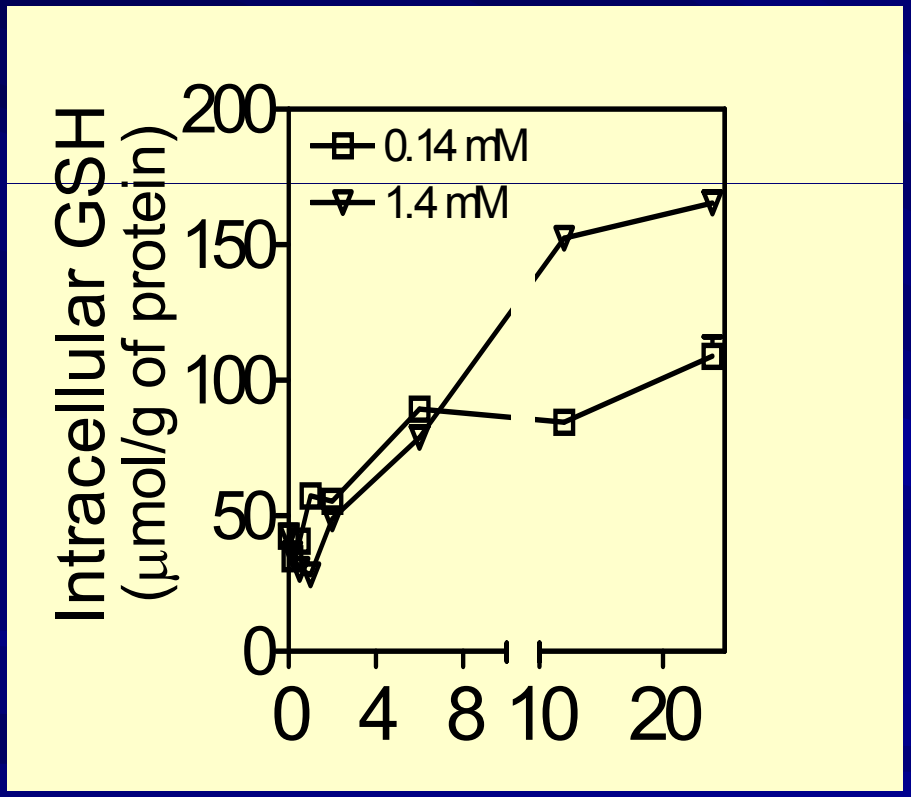


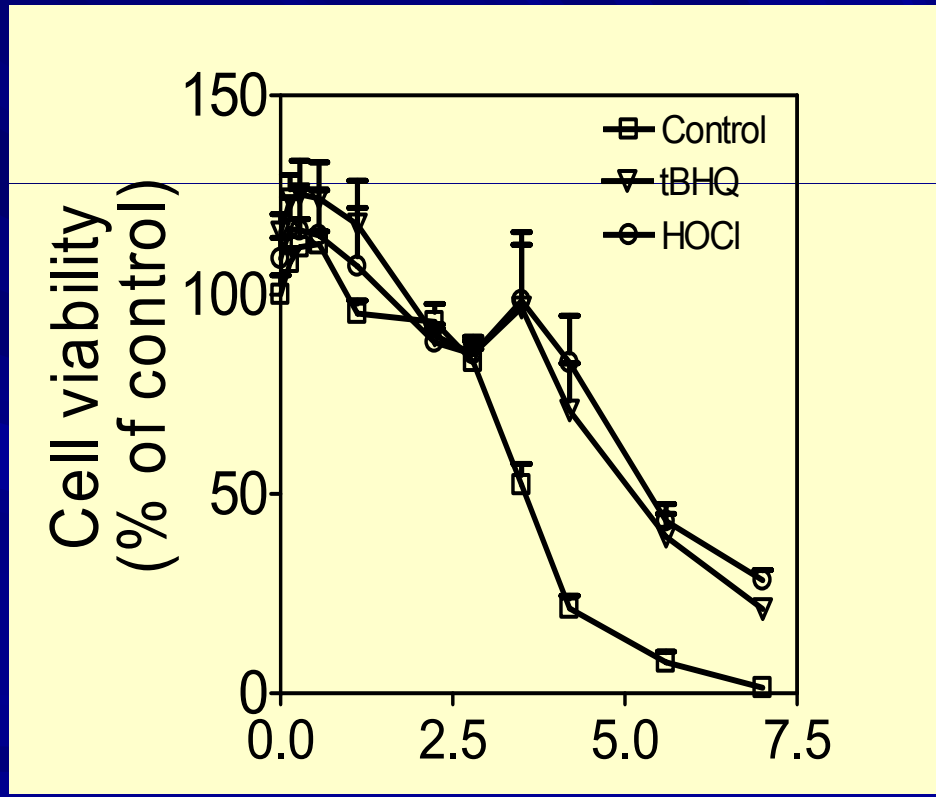
# Incidence – Dose Curves in vivo



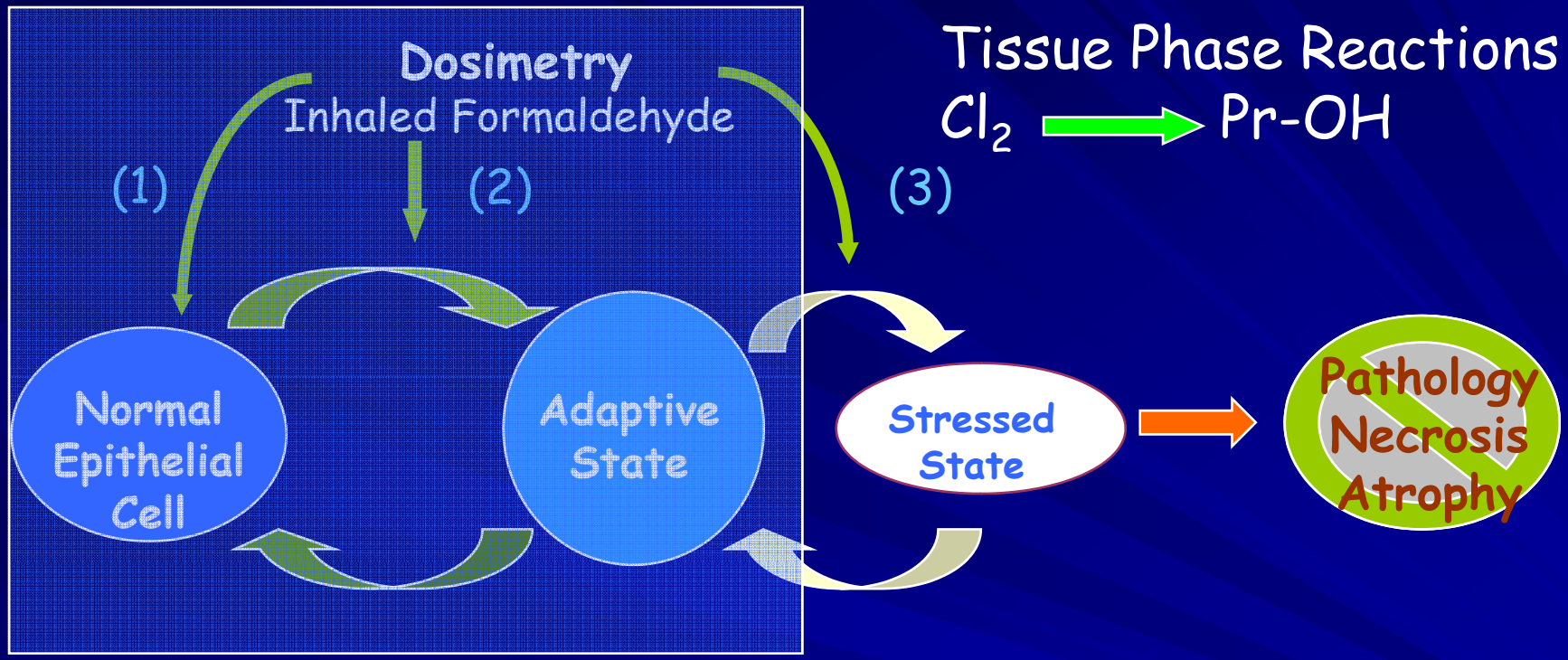

Subsequent dose response curves for toxicity in the intact animal will have an initial threshold for activation of the stress response and then a controlled region before transitioning to overt toxicity. U-shaped responses possible due to altered energy uses with up-regulation of batteries of anti-stress factors in tissues

# Prior exposure protects against HOCl exposures in vitro and enhances U-shaped dose response



# Mechanistic Dose Response Model with Genomic Data




- Use specific in vivo studies to develop a dose response model for activation of **oxidative stress pathways** following formaldehyde exposure and **differentiate dose regions** that activate adaptive responses at low concentrations and inflammatory and necrotic processes as concentrations increase

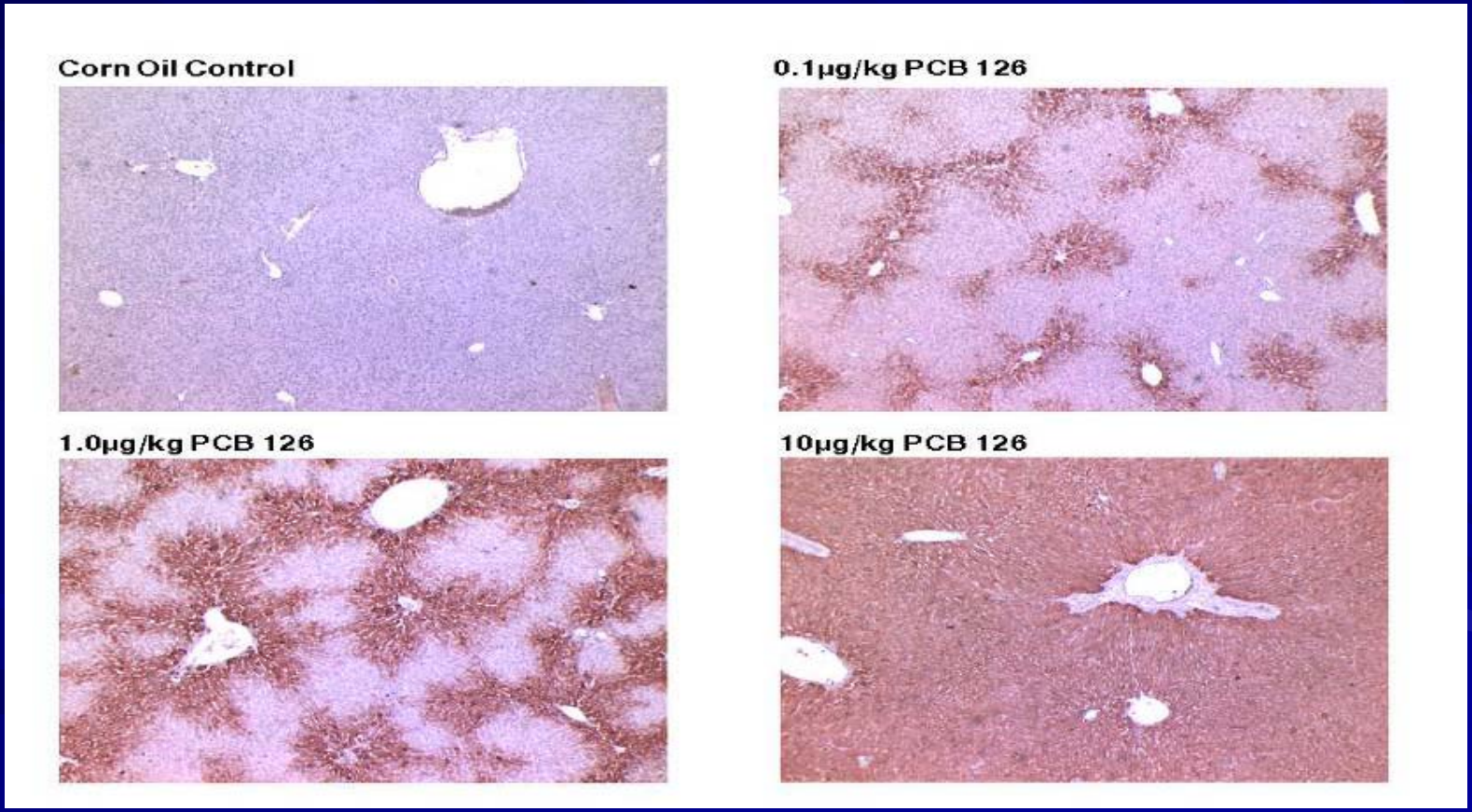
# It has to be interdisciplinary

- In vivo exposures – limited genomic evaluations, oxidative markers, tissue/organ responses (Ms. Jarabek)
- Genomic studies in vitro with epithelial cells in culture (Dr. Yin Chen)
- Confirmation of and mechanistic studies of anti-oxidant response activation (Dr. Jingbo Pi)
- Pathway modeling of activation of Nrf-2 signaling ( Dr. Qiang Zhang)
- Functional Genomic mapping of Nrf2 signaling (Dr. Courtney Woods, Exxon-Mobil Post-Doctoral Fellow)
- ACC-LRI supported programs at The Hamner Institutes

# Conclusions

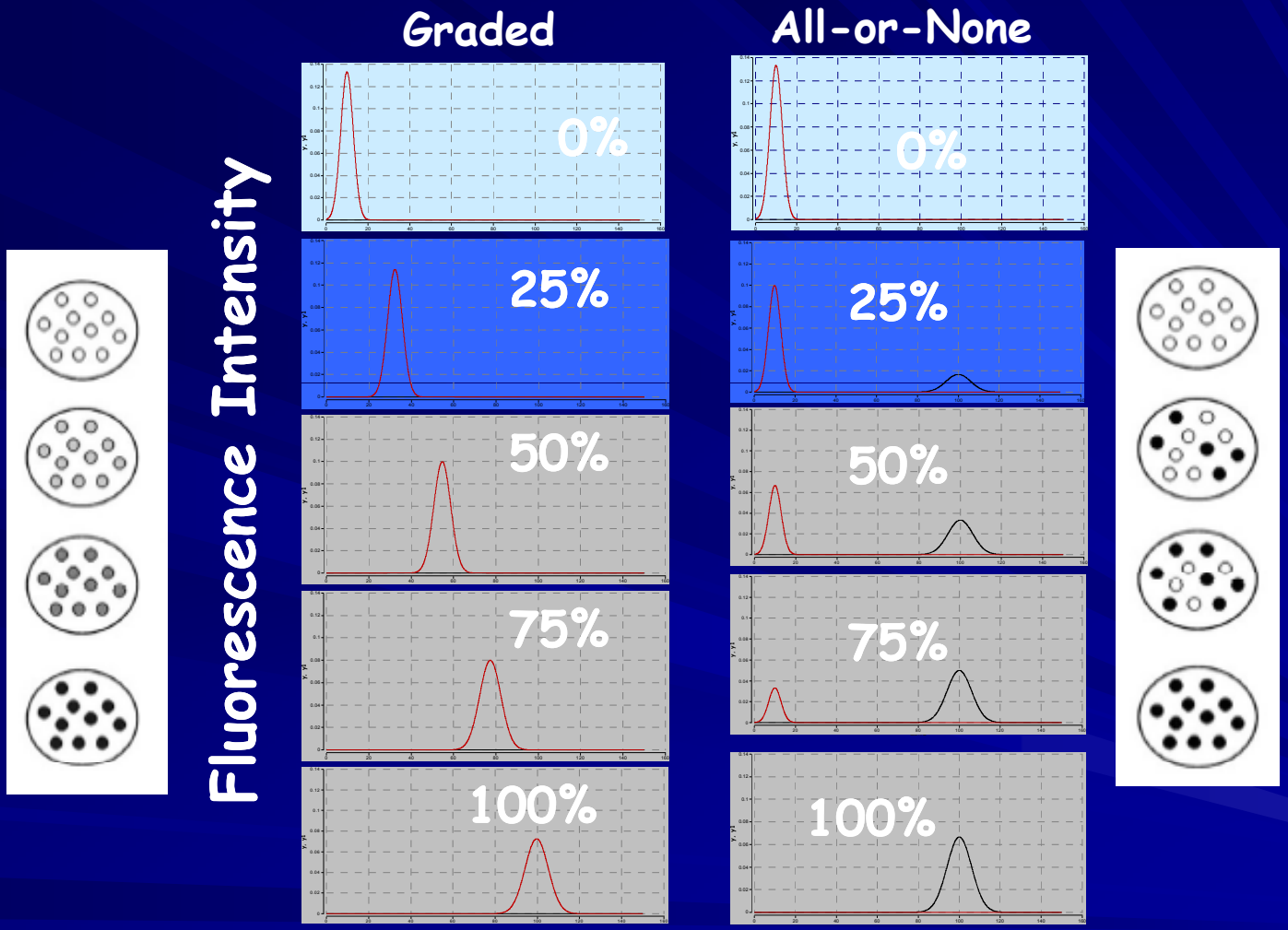
- Hormesis and thresholds are likely related to activation of adaptive pathways, i.e., to regions of homeostasis
- Use in risk assessment requires interdisciplinary development of several compelling, mechanistic prototypes - such as with irritant gases or with some hepatic enzyme inducers
- In the absence of such well-developed examples showing basis for hormesis and non-monotonic responses, risk assessments will hold fast to threshold and low-dose linear methodologies that are now favored

# Cellular responses to stressors are frequently dichotomous



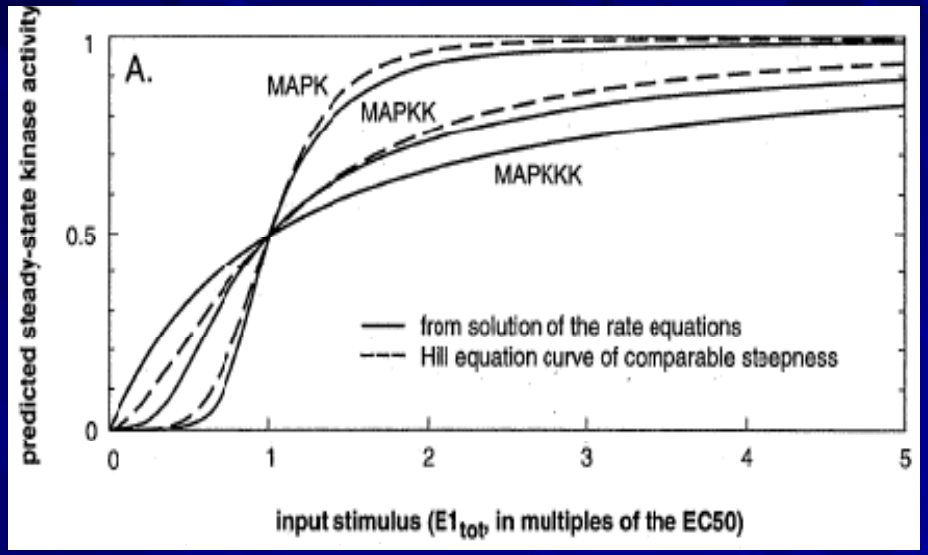

*Chubb et al. (2004)*

# With Binary rather than Graded Responses




Population Distribution – Cells with GFP

# MAP-Kinase modules provide cellular switches




| Stimulus | PG | PDGF | Integrin | Oxidative Stress | IL-1 |
|---|---|---|---|---|---|
| Activator | Activator | RasGTP | Rac1 | Src | TRAF6-TAB1/2 |
| MKKK | MOS | C-Raf1 | MEKK1 | MEKK2 | TAK1 |
| MKK | MEK1 | MEK | MKK4 | MKK5 | MKK6 |
| MAPK | p42 | MAPK | JNK1 | ERK5 | p38 |
| Substrate | Rsk1,2 | TFs | C-Jun | MEF2 | MNK1 |