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The Use of Transcriptomics in Product Safety Evaluation; an Agrochemical Perspective

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September 16, 2014

workshop toxicogenomics
the emergency of a new research and regulatory paradigm
September 15th and 16th 2014
Universidade Federal do Paraná

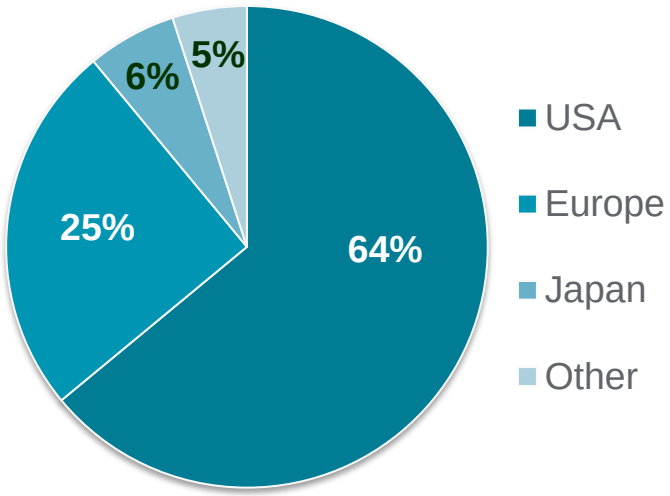
The Use of Toxicogenomics in Chemical Safety Testing

- Current safety testing is geared to produce and accept descriptive data from high-dose animal studies.
  - Interpretation of this information has effectively protected our health and safety for decades
  - Extrapolations across species, from high-dose to low exposures, from descriptive endpoints in mammals to human correlates → all expertly performed using well-established criteria
  - However, hindered by the lack of underlying mechanistic information.
- Concern over chemical safety for humans and the environment has led to the goal of **better understanding the modes of action** underlying toxic effects and safety issues.
  - Regulatory requirements are becoming more concerned about the modes of action (MOA) related to the potential safety issues
  - MOA can identify biomarkers and make prediction of effects easier
- The future of safety testing lies in utilization of emerging technologies that **refine our understanding of biological processes**
  - Identifying effects of chemicals on critical biological pathways

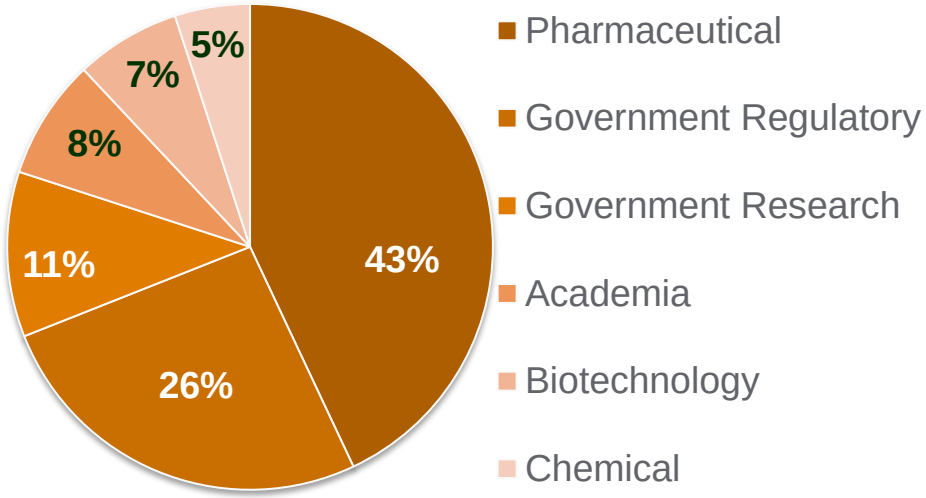
Current Use of Toxicogenomics in Toxicology

Toxicogenomics (TGx) – providing valuable information on the effects of drugs and chemicals at a molecular level; providing a more complete understanding of their potential systemic effects.

Geographical Distribution of Survey Respondants*



Sector Distribution of Survey Respondants



* 112 respondants out of ~300 requests.
Pettit et al., 2010 EHP Vol.118 No.7

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Current Use of Toxicogenomics in Toxicology (continued)

In Vitro Applications of TGx

Formats used: cell lines [86%], primary cultures [84%], organ cultures [30%]
Purpose: identify MOA [86%], compare with in vivo [66%], identify biomarkers [61%], compare cross-species [52%]

In Vivo Applications of TGx

Study designs: short-term [1-2 weeks] Using rat, mouse, or dog with liver as primary tissue evaluated.
Purpose: systems biology approach to analyzing and interpreting database [38%]

50% use

We are here

qRT-PCR measurements vs. Microarray measurements

Target characterization

23%

60%

Primarily to confirm chip results

45%

25%

18%

Confirm safety biomarkers

Confirm efficacy biomarkers

Repeated measures on a small number of transcripts

Pettit et al., 2010 EHP Vol.118 No.7

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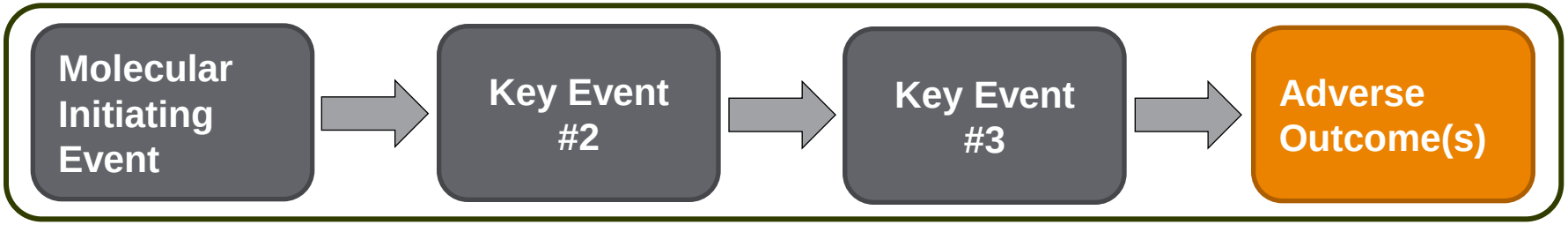
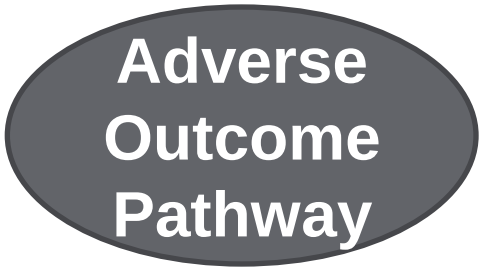
Today's Talk: Discussion on the development and use of MOA and AOPs for Agrichemicals' Risk Assessments

- The goals of predicting and preventing adverse effects for crop protection active ingredients
- Types of toxicity and some examples:
  1) **Mode of Action** (MOA) as the starting point for Adverse Outcome Pathway building, e.g. Cancer MOAs
  2) **Adverse Outcome Pathway** (AOP) knowledge utilized to predict safety, e.g. herbicides (HPPD inhibitors)

QUESTION: What is the difference between a MOA and AOP?



Modes of Action (MOA) vs. Adverse Outcome Pathways (AOPs)



Determine whether animal MOA is plausible and relevant to humans.

WHY

Improve predictions of toxicity via decreased uncertainty.

A regulatory assessment framework based on the concept of pathways.

WHAT

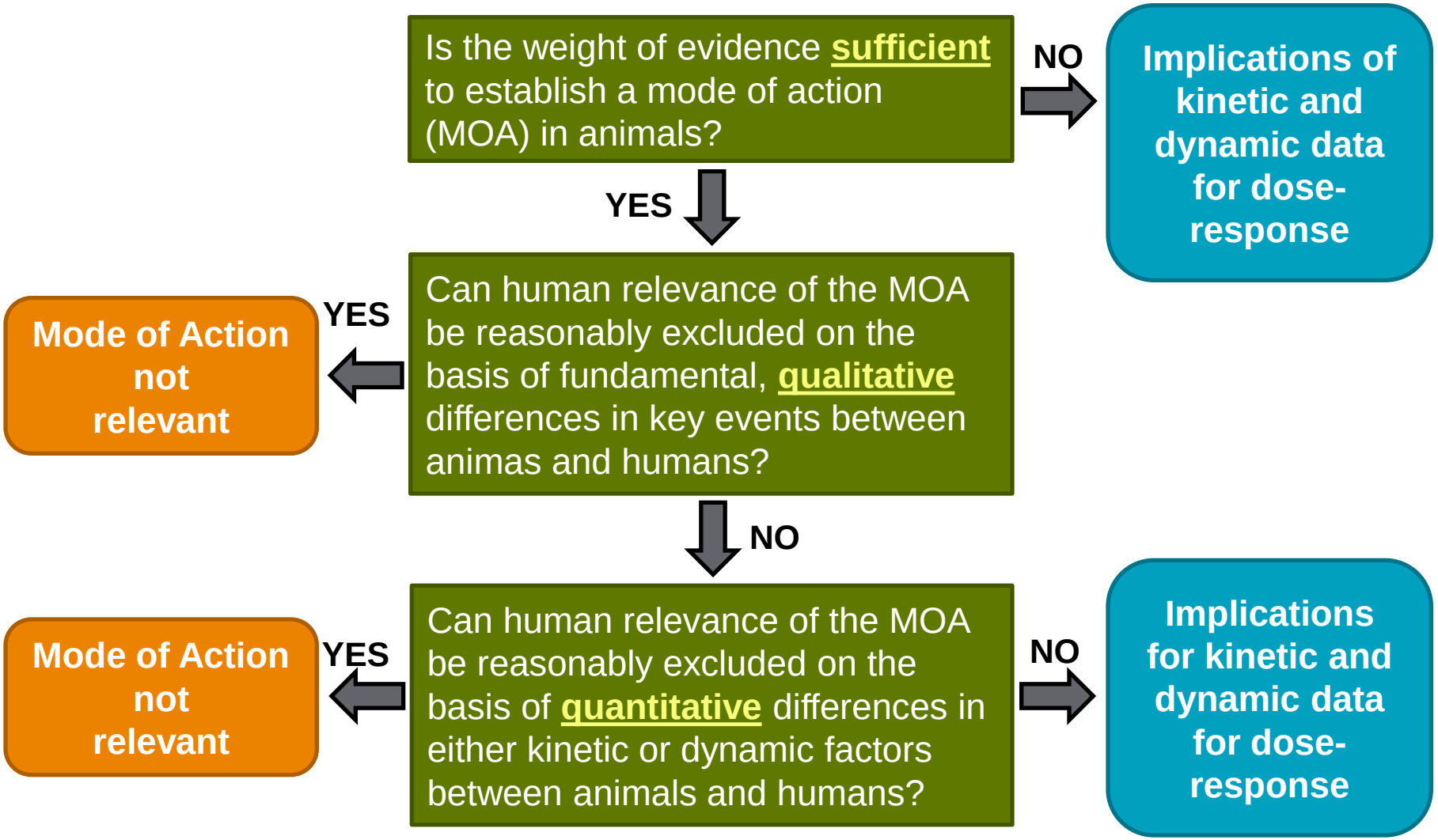
A conceptual and practical tool to capture descriptions of toxicological processes.

Describes key, rate-limiting, and quantifiable events that lead to adverse outcomes.

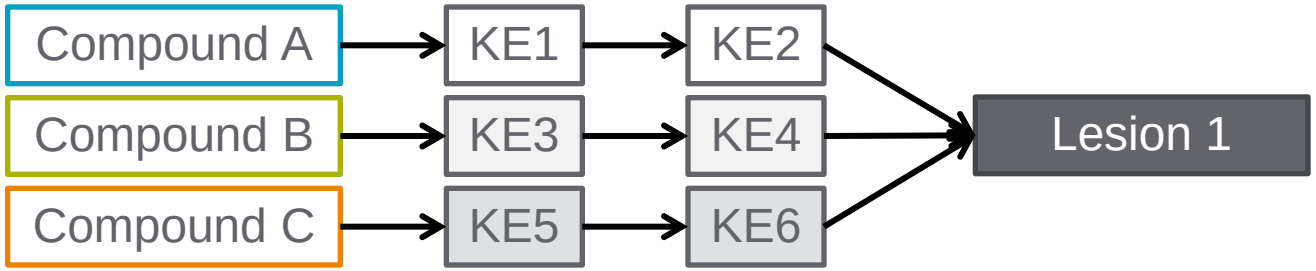
HOW

Based on empirically established links between MIE and adverse health outcome.

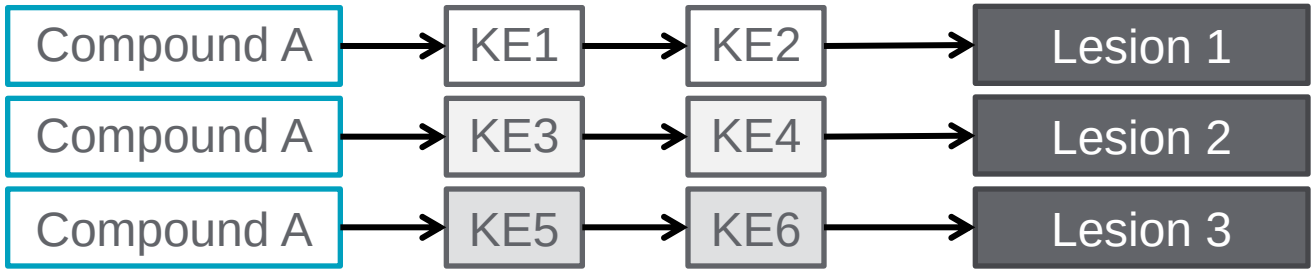
Mode of Action/Human Relevance Framework



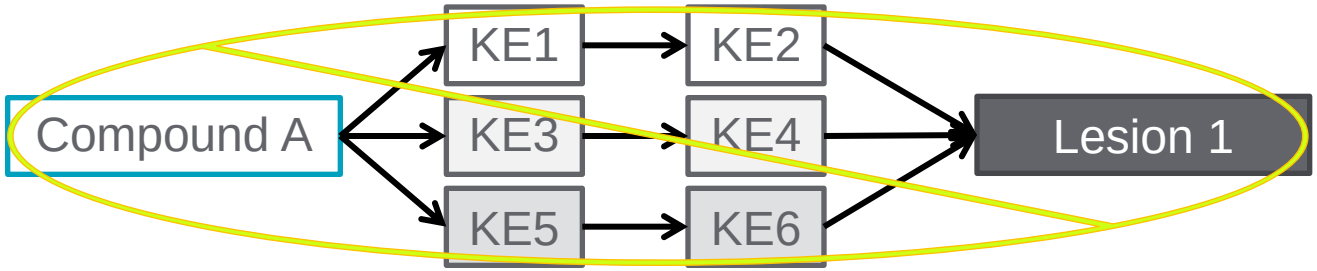
Multiple Modes of Action, but only one MOA/chemical/lesion



Different chemicals may produce the same lesion through different MOAs



A chemical may produce different lesions through different MOAs and thus the chemical can have multiple MOAs



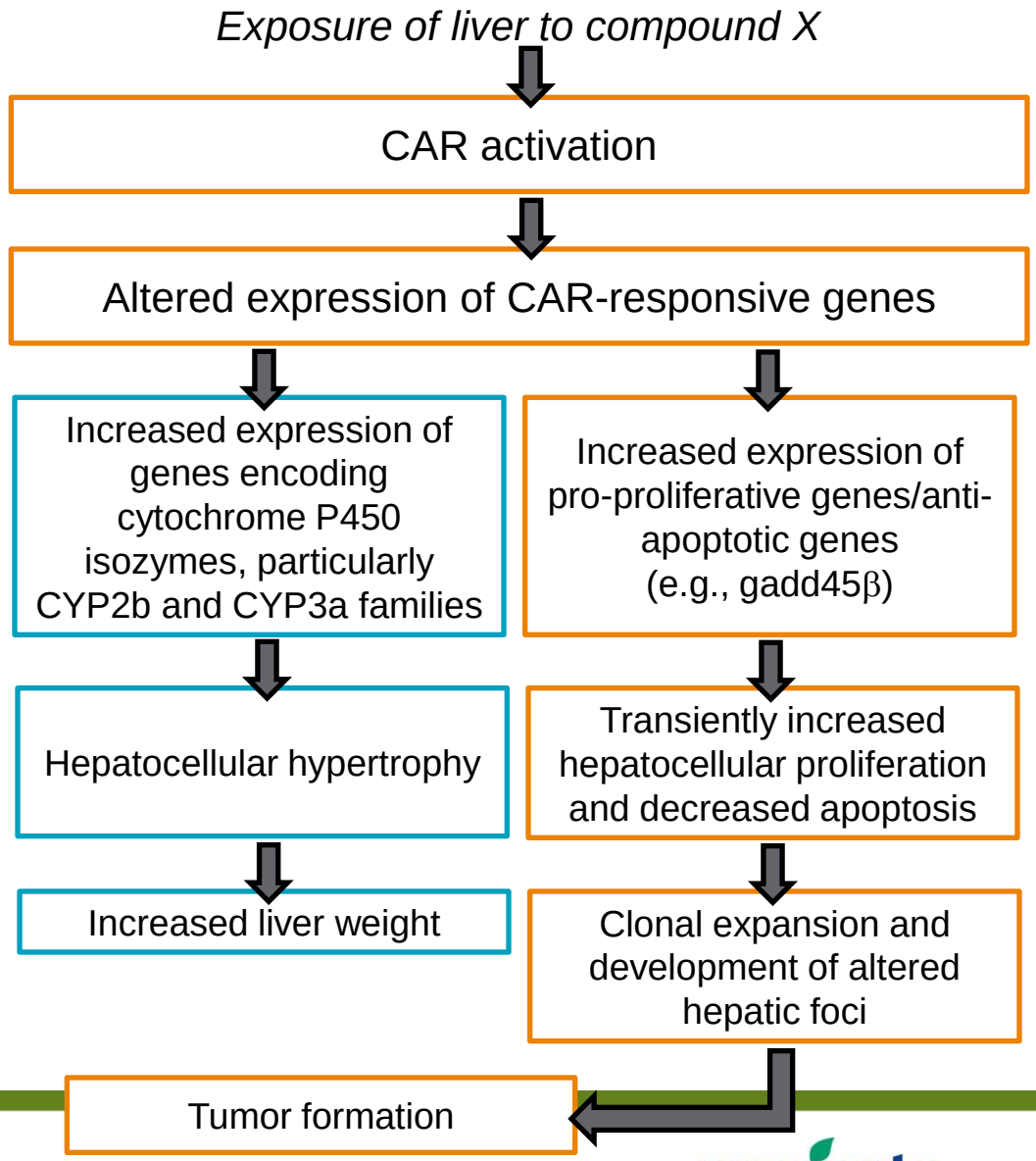
A chemical does not produce a lesion through multiple *separate* MOAs, the key events are organized into a single MOA

Mode of Action: a cascade of measureable events ending at an adverse outcome.

Key Event: an empirically observable precursor step to the adverse outcome; typically not sufficient to induce the adverse outcome without other key events.

Associative Event: biological processes that are reliable indicators or markers for key events.

Modulating Factors: biological responses that could modulate the dose-response or key events or adverse outcomes (e.g. oxidative stress).



Prediction of adverse outcomes begins with an understanding of the causes of toxicity

Toxicity Type	Definition: Effects that are ...	
1	**... caused by the pesticide-MOA being exhibited in a mammal**	**Pest species and mammals have similar targets, e.g. shared receptors or enzymes**
2	**... caused by the chemical properties of the molecule or another biological activity, that are <u>not</u> related to the pesticide-MOA.**	**Chemical types that are reactive leading to tissue damage, mutagenesis, "non-target" effects on other target proteins**
3	**... caused by "high-dose" effects during *in vivo* studies on essentially non-toxic compounds – as a consequence of no dose limiting toxicity**	**Paradoxical non-specific effects in model systems dosed to achieve unfeasibly high exposure levels**

Molecular Initiating Events?

(MIEs)

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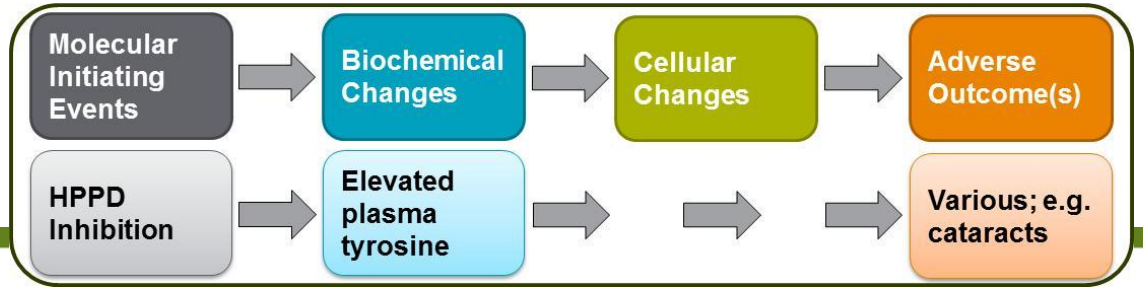
Type 1: Toxicity due to Pesticidal MOA
Developing an AOP for use in Risk Assessment

- **Case Study: predicting human safety at realistic dose levels**
  - AIM: a proof-of-concept human health risk assessment
    - Exposure Scenario: children exposed hand-to-mouth activity whilst playing on treated turf
    - Extrapolation modeling performed using a PB-PK/PD model built on the AOP
  - Pesticidal MOA: 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibition
- **Can the AOP be used for quantitative risk assessment?**
  - What degree of pathway perturbation leads to an adverse effect?
    - Rodent studies suggest tyrosine concentration >1000nM results in adverse effects.
    - FDA guidance based on Human genetic tyrosinemias inform us what level of pathway perturbation (i.e. plasma tyrosine) is needed for disease to occur in humans (a 500nM threshold is protective)



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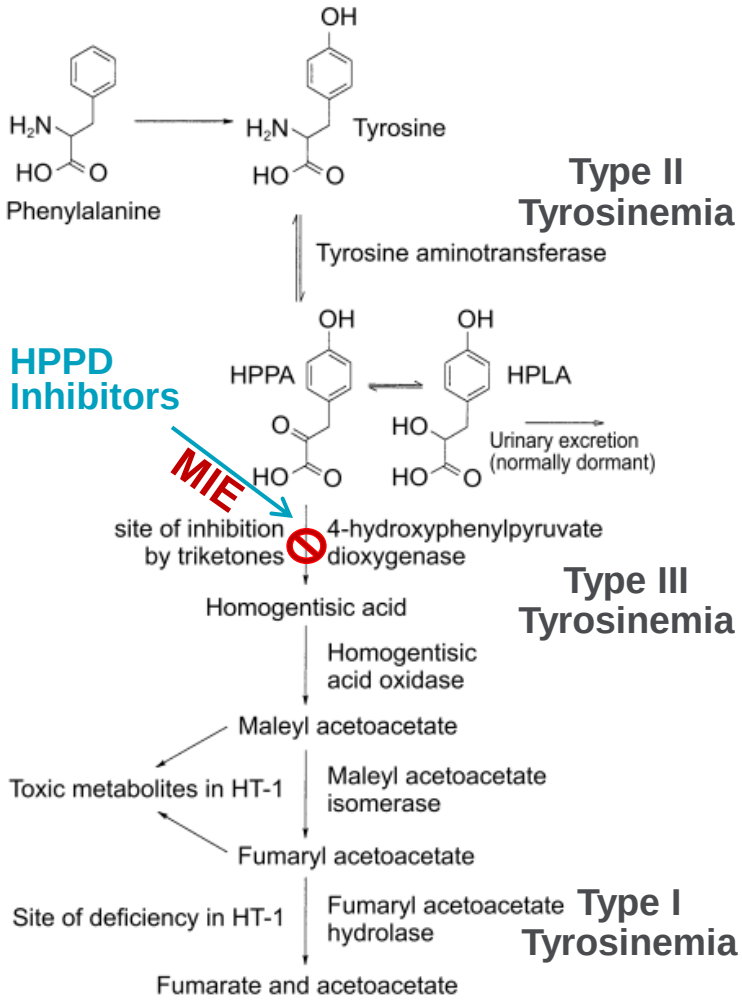
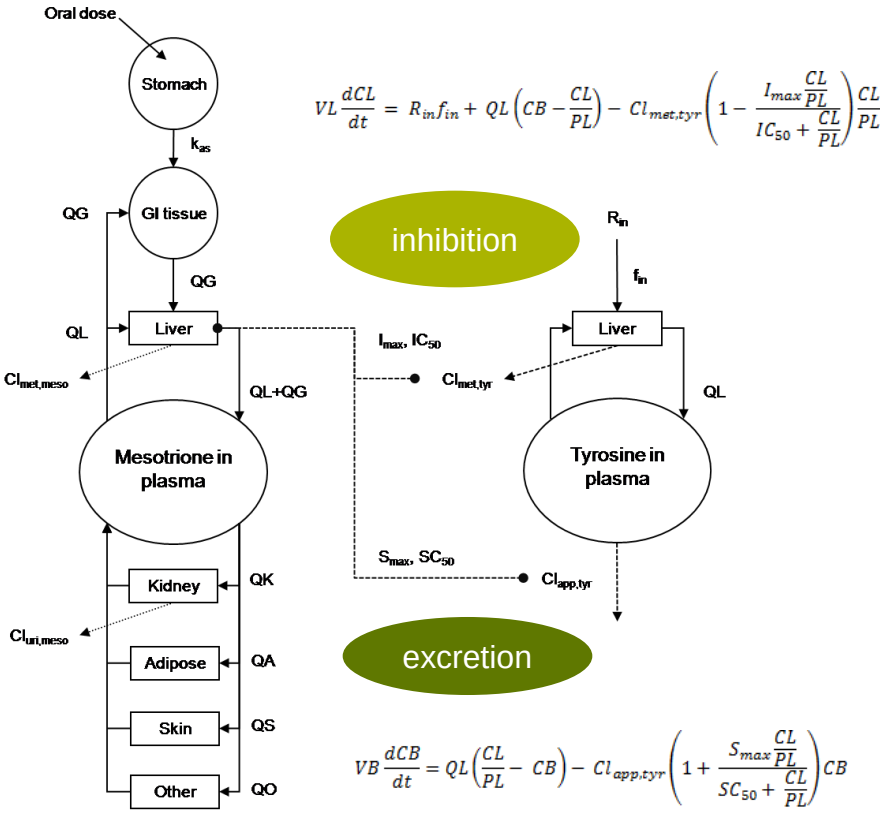
Information Used in the Risk Assessment

The tyrosine catabolism pathway



British Journal of Clinical Pharmacology
Volume 52, Issue 2, pages 169-177, 20 DEC 2001 DOI: 10.1046/j.0306-5251.2001.01421.x
http://onlinelibrary.wiley.com/doi/10.1046/j.0306-5251.2001.01421.x/full#f1

Generating a PB-PK/PD model based on AOP



Data sources:

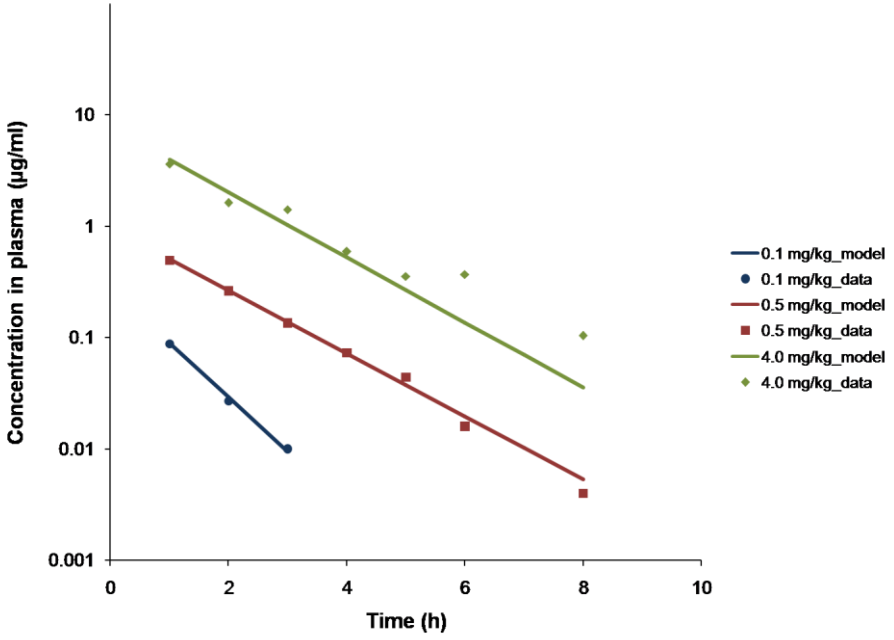
Values we measured - tissue partition coefficients, HPPD IC_{50} was measured *in vitro*

Values obtained from the literature - model structure & physiological parameters

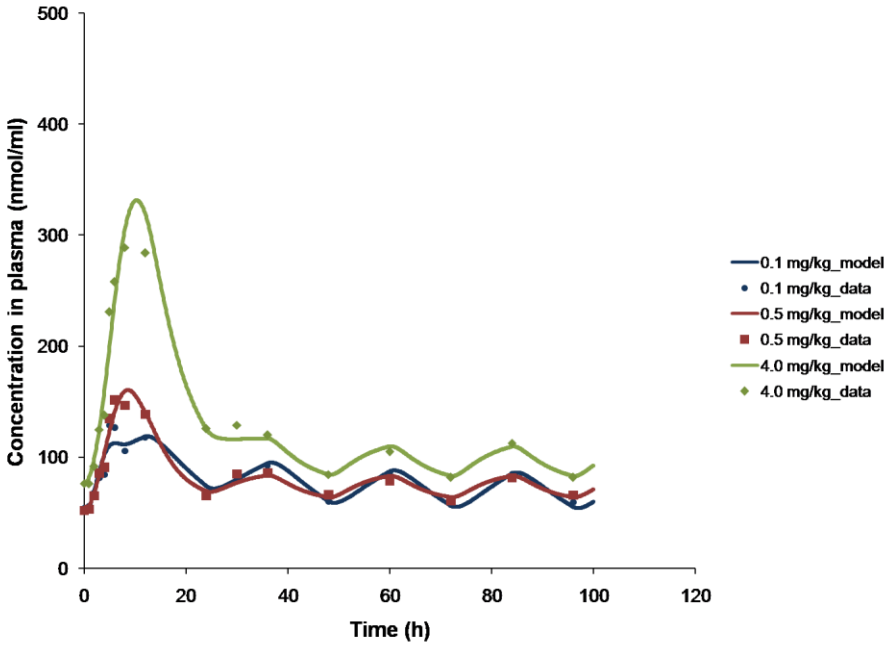
Information Used in the Risk Assessment (continued)

Evaluation of the PBPK/PD model against public time-course data (Hall et al., 2001) shows a good fit of the data

Mesotrione plasma time-course profile in humans



Tyrosine plasma time-course profile in humans



Human PK data generated using volunteers under guidance of the Declaration of Helsinki

British Journal of Clinical Pharmacology
Volume 52, Issue 2, pages 169-177, 20 DEC 2001 DOI: 10.1046/j.0306-5251.2001.01421.x
http://onlinelibrary.wiley.com/doi/10.1046/j.0306-5251.2001.01421.x/full#f1

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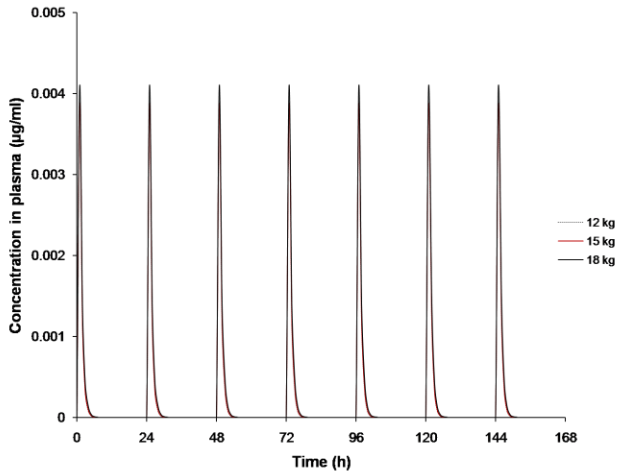
Type 1: Toxicity due to Pesticidal MOA
Extrapolation to the Risk Assessment Requirement

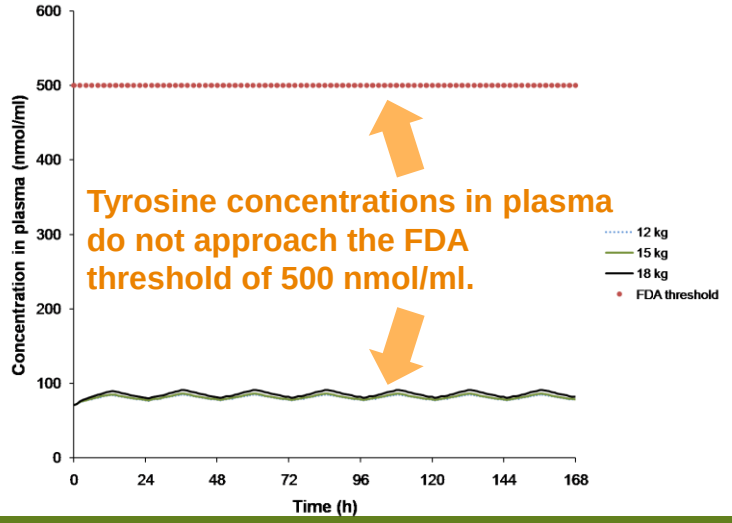
- **Risk context:** 1 h/day exposure to mesotrione on treated turf for 1 week among 3 year old children.
- **Exposure assessment:** estimated from EPA Standard Operating Procedure for Residential Risk Assessment
- **Toxicity pathway:** HPPD inhibition
- **Dose-response & extrapolation model:** The PBPK/PD model was scaled to children by adjusting body weight. We used the CDC growth chart for 3 year old children: 12 kg (5th percentile) to 18 kg (95th percentile).
- **Simulation output included mesotrione and tyrosine concentrations in plasma.**

Simulation of plasma mesotrione and tyrosine in exposed 3-year old children:
this scenario is unlikely to be a concern

Simulated plasma mesotrione



Simulated plasma tyrosine



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Type 1: Toxicity due to Pesticidal MOA
Case Study Key Learnings

1. This approach can give useful information to inform risk assessment.
2. We needed to understand that the MIE was a key event interacting with a disease pathway.
3. We needed to understand the extent of pathway perturbation needed for disease measured using a relevant biomarker.
    a) This was informed by human disease knowledge and rodent studies.
    b) It was not required to have a full understanding of all the intermediate events in the AOP to inform the risk assessment.
4. We needed to measure the internal dose.
    a) Human data was useful to parameterize the PK and PD elements of the model.

Type 2: Toxicity due to other effects
Developing an AOP from Mode-Of-Action knowledge

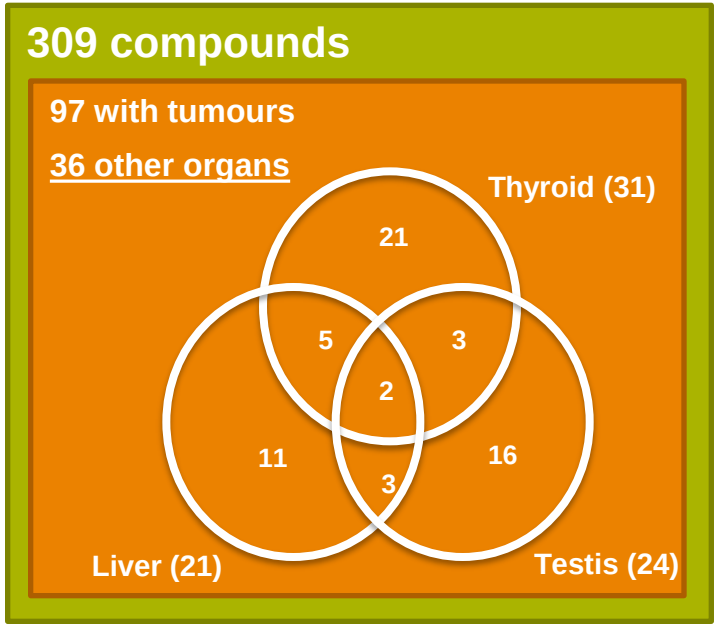
- Case Study: predicting carcinogenicity
  - AIM: a proof-of-concept use of MOA to demonstrate reasonable certainty of no harm

Carcinogenesis as a case study

Rat

Analysis of the ToxRef DB from EPA

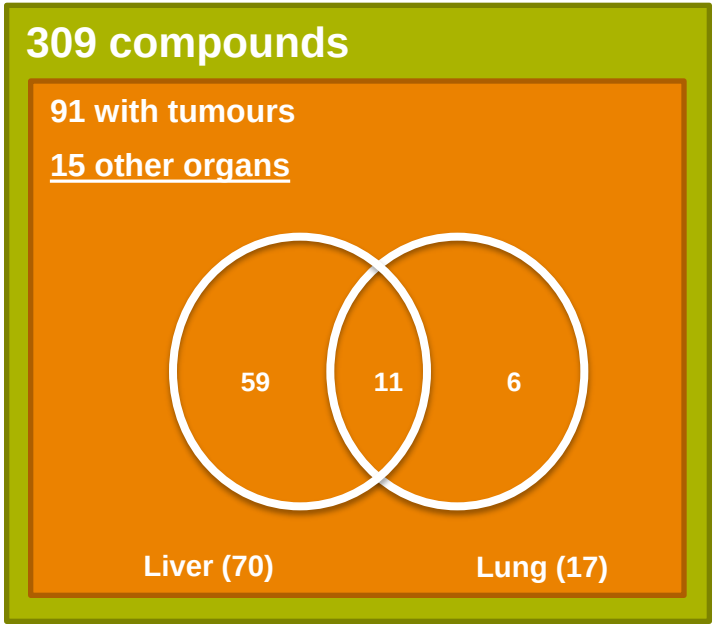
- 31% get rat tumours
- 63% of tumours are in liver, thyroid or testis



Mouse

Analysis of the ToxRef DB from EPA

- 29% get mouse tumours
- 83% of tumours are in liver or lung (77% liver)



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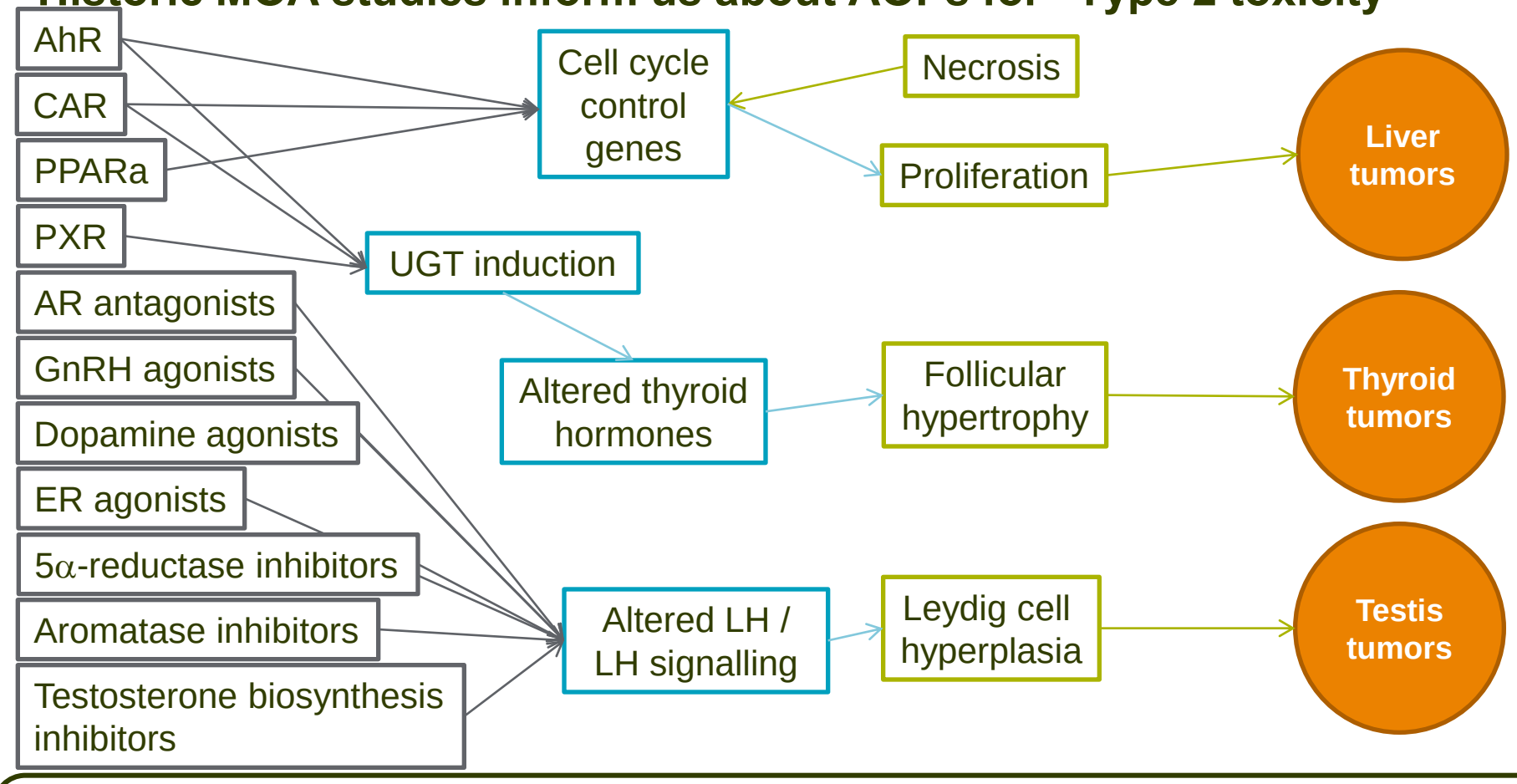
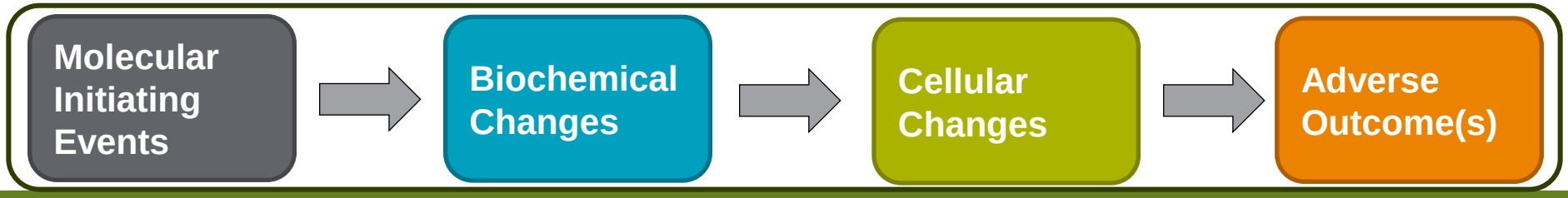
Type 2: Toxicity due to other effects

Historic MOA studies inform us about AOPs for "Type 2 toxicity"





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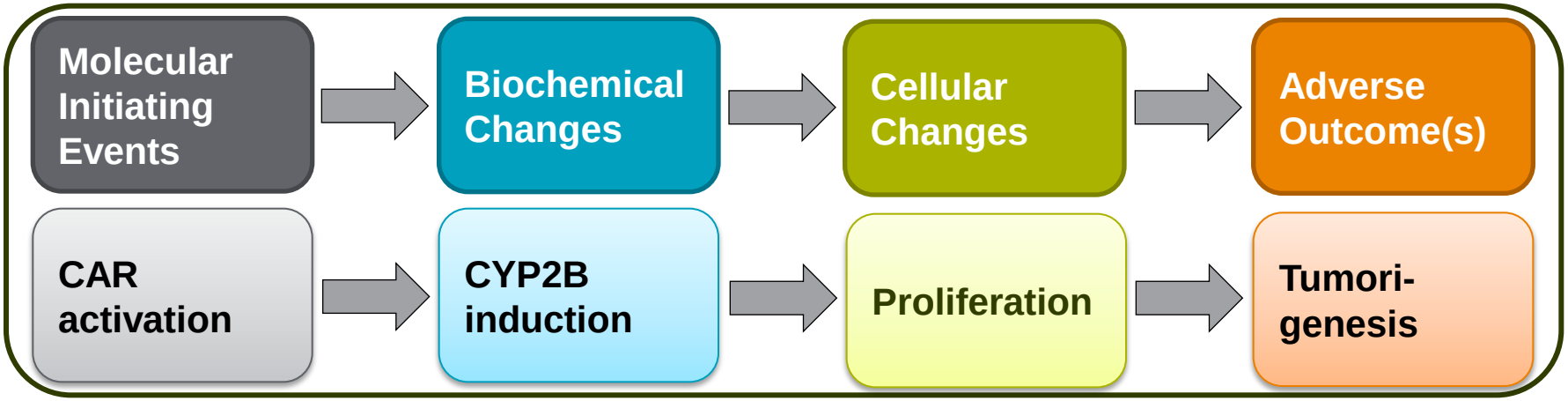
Type 2: Toxicity due to other effects

Single dose toxicity study can detect P450s and hepatocyte proliferative genes

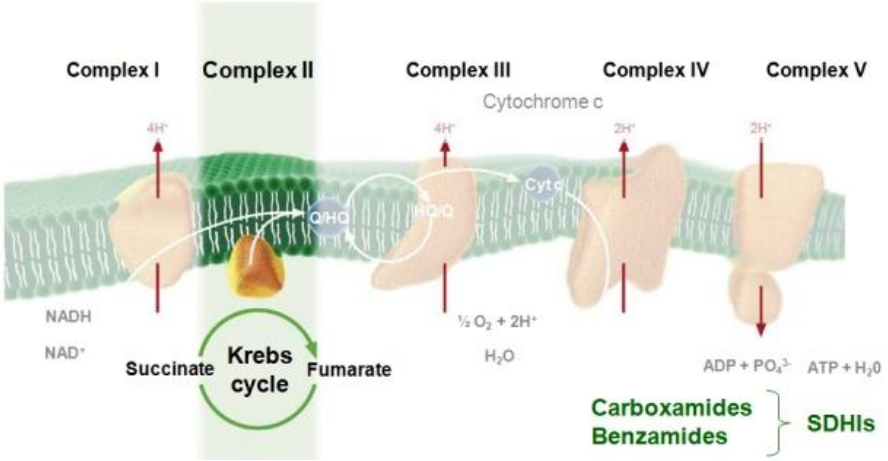
- All CAR-activators induce CYP2B inducers, and proliferation genes at doses that have carcinogenic effects.
  - These genes can be measured in focused single dose studies to help select compounds with reduced risk of developing tumours.



Prediction of carcinogenesis potential – Case Study

Characteristics of Compound X

- Chemically similar to fungicidal succinate dehydrogenase inhibitors
- Liver and thyroid tumours via P450 CYP2b enzyme induction mechanism



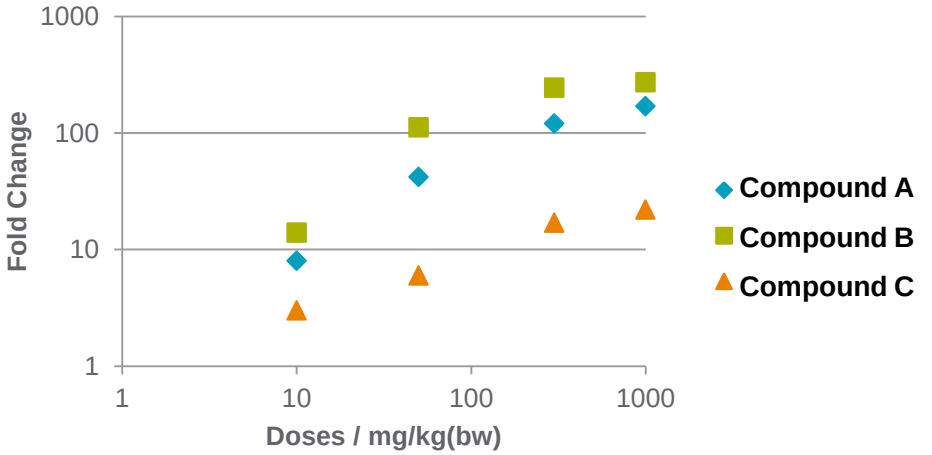
Study Design

- Single dose toxicity study with toxicokinetics and toxicogenomics to identify options for mitigation of liver cancer risk.
- Dose 0, 10, 50, 300, and 1000 mg/kg (bw) to assess acute toxicity.
- Dose second set of rats with same doses and measure gene expression changes at 24 hours.

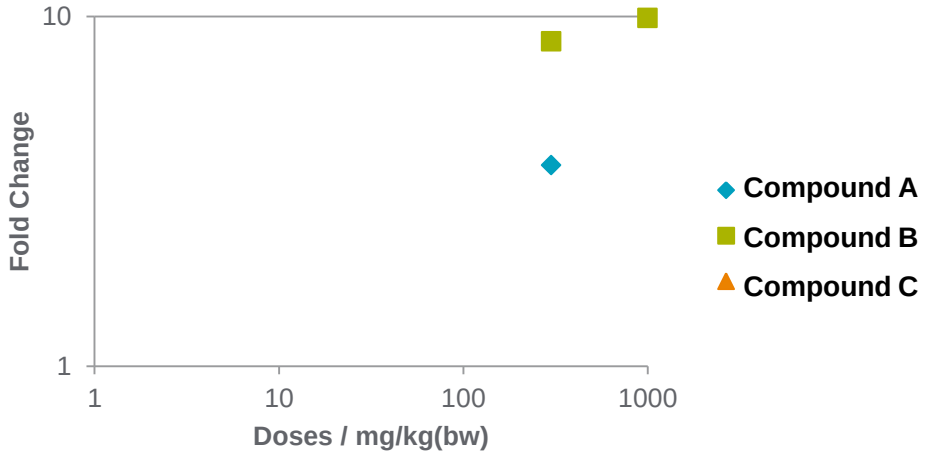
Single dose toxicity study:
Subclasses of this compound can be discriminated in their ability to induce P450s and hepatocyte proliferative genes

- Tested a representative of each class: Compound A, B, and C
  - No acute toxicity
  - All show exposure of parent
  - All are CAR-activators: CYP2B inducers, phase II, GST & ox stress biomarkers plus proliferation markers at high doses ≥300 mg/kg (bw)

Cyp2B mRNA fold change



GADD45b fold change



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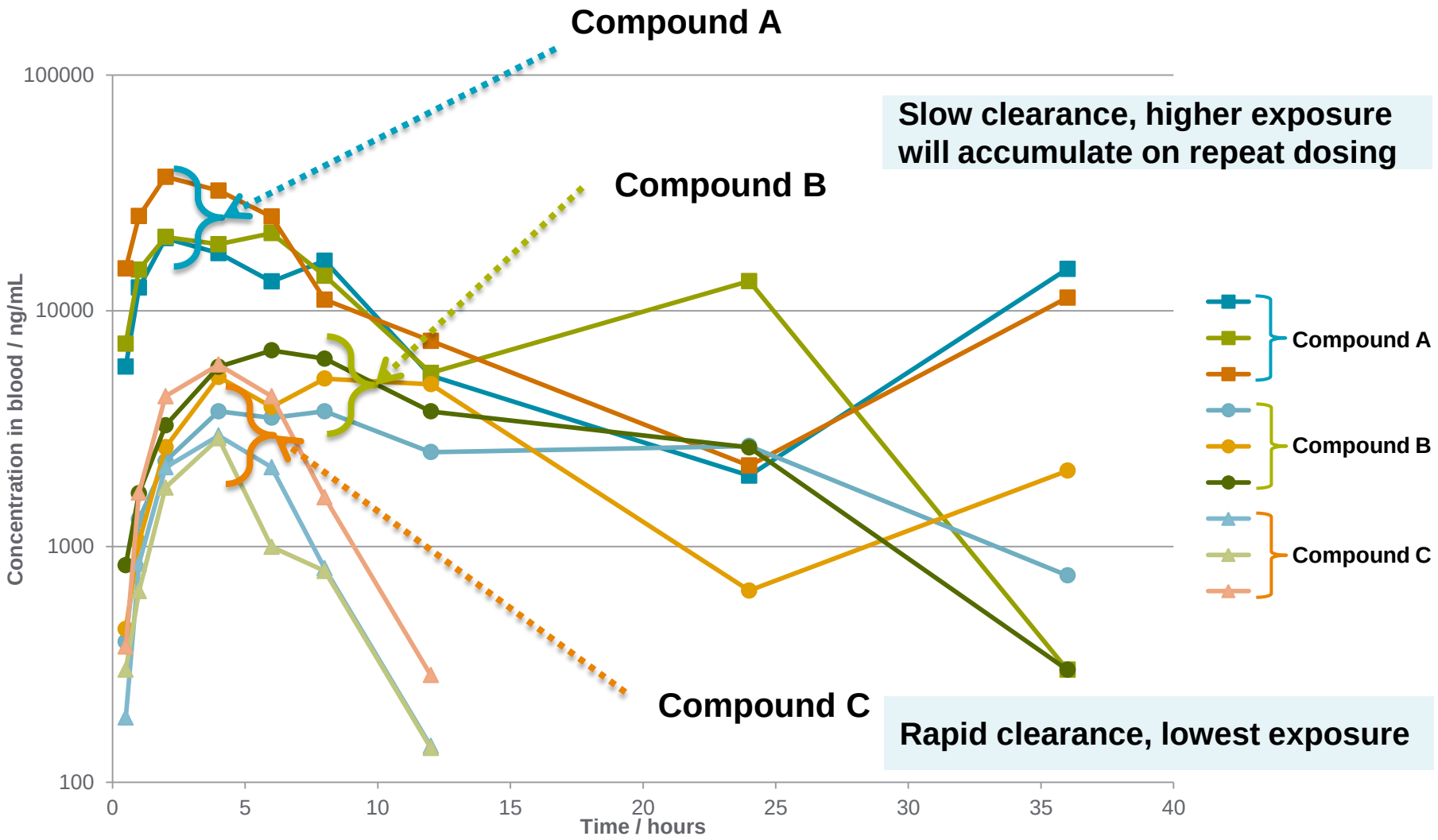
Compound X subclasses have different pharmacokinetic profiles



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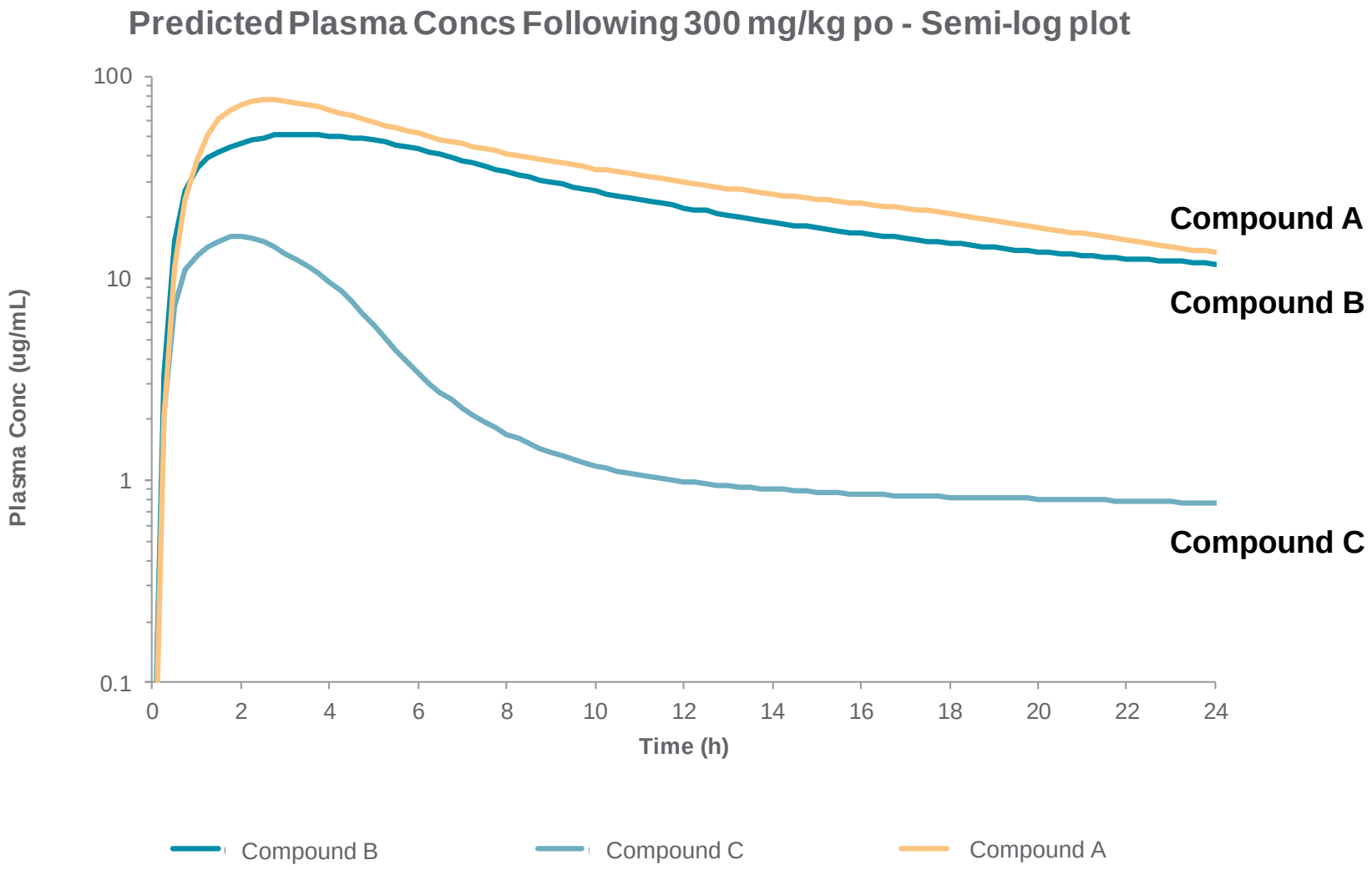
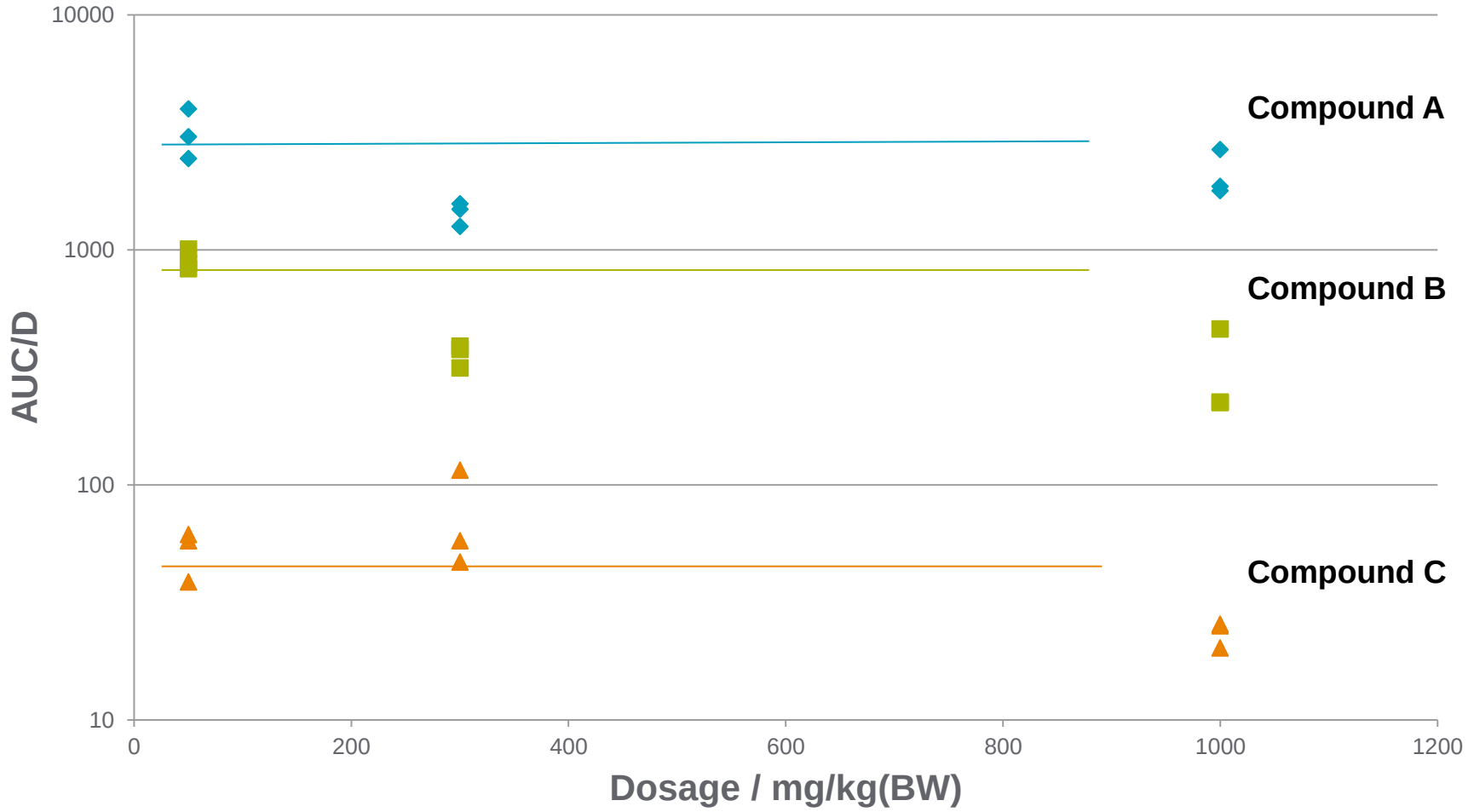
Acute study tested the hypothesis of Compound X simulated pharmacokinetic profile: 300 mg/kg is very similar to actual



Identified options for dose limitation due to non-linearity of AUC with dose: saturation of absorption



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Dose-response models can link perturbations to more integrated responses

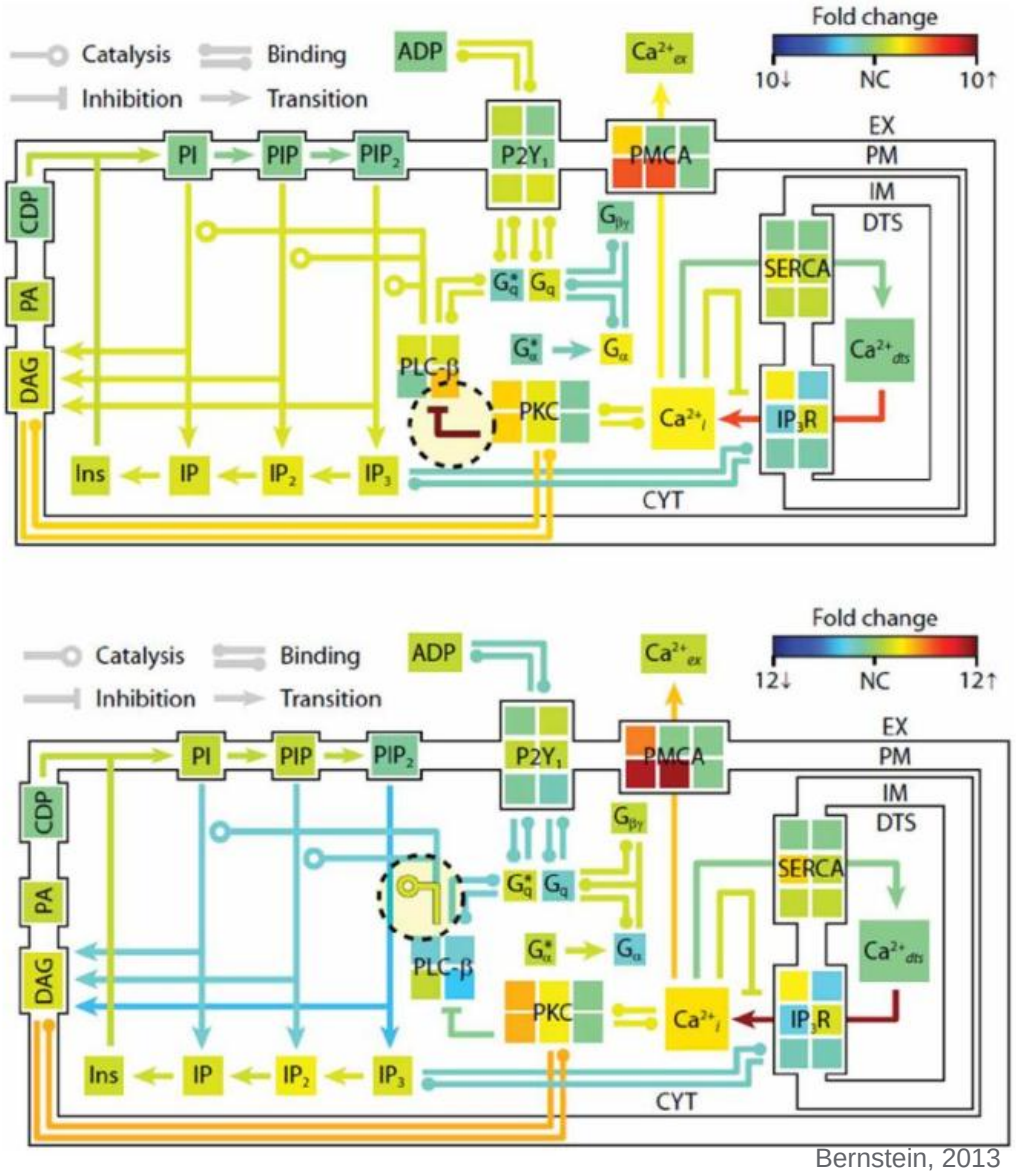
Systems biology description of a pathway is used to generate biologically realistic dose-response models.

Dose-dependent transition studies for pathway activation are used to understand the linkage of cell and tissue level responses

Perturbations

↳ **Adaptations**

↳ **Adversity**



Bernstein, 2013

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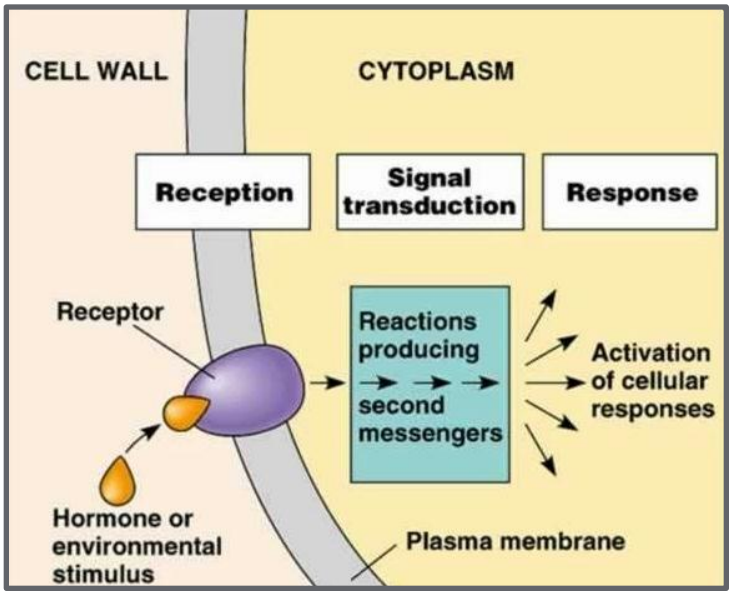
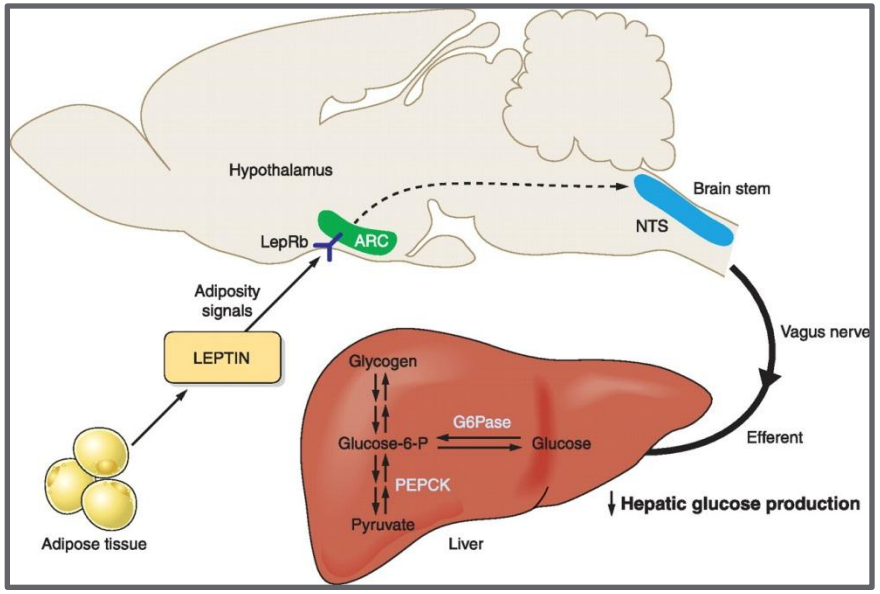
Interpretation of molecular changes requires context

- The MOA behind a specific type of toxicity cannot be explained simply by studying the genetics of the cells themselves.
- As an example, the liver generates and sustains many different cell types and biological functions *because* of patterns that recur on the scale of cellular, tissue, organism, and the wider ecosystem.



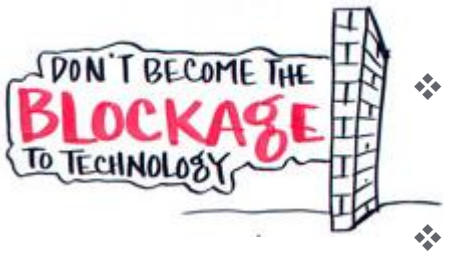


Assess pathways, integrate tissue responses, and in some cases evaluate metabolites.

Achievements with Toxicogenomic Technologies

Greatest impacts of toxicogenomics data:

1. Understanding biological mechanisms
2. Identification of biomarker candidates
3. Identification of species differences



- Analytical capabilities
- Understanding the data in its context
- Interpretation of the data

Greatest benefits of sharing data:

1. Identification and consensus-building around novel biomarkers
2. Standardization &harmonization of approaches & interpretation
3. A method to inform the regulatory community about the application of the data

The future of toxicogenomics is dependent on:

1. Data quality (**ACCURACY** of data)
2. Quality of interpretation (**PERCEPTION** of data)
3. Use of data standards for reporting the data
4. Open dialogue between stakeholders = expand the progress of the technology
5. Train toxicologists and regulators about need for new approach and the tools and methods that will be involved in the transition

Pettit et al., 2010 EHP Vol.118 No.7

Applications of Toxicogenomics in R&D and Risk Assessment

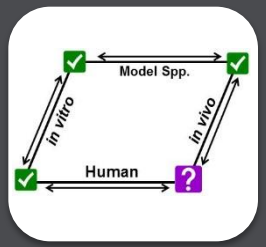
- **Safety testing** will become more focused on human biology and less dependent on animal studies, dialing-in on low dose exposures
- **Prioritization** of studies for focused short term screening studies *in vivo*
- **Dose-response models** will be more diverse for target and integrated cellular responses for links to possible outcomes
- **Pathway targets** will have refined understanding of initiation events
- **AOP databases** for established toxicological MOAs are being developed
  - Focus is on cancer, developmental and reproductive toxicity
  - Identify data gaps
- Developed to give **quantitative understanding** and for use in risk assessment
- There are societal/harmonization/legal/regulatory barriers to use in Registration, but there are actions to lead the change:
  - AOPs utilized as agreed knowledge
  - AOPs utilized to demonstrate reasonable certainty of no harm

The Science of Toxicogenomics is Improving Risk Assessment

MIXTURES

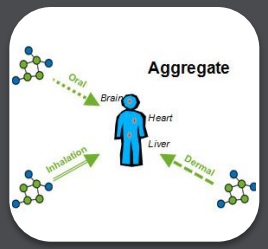
Use MOA data to design toxicogenomic experiments to better inform this area of study

CROSS-SPECIES EXTRAPOLATION



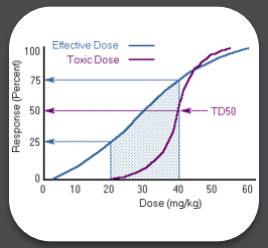
Enhance the confidence in extrapolation through the use of human cell lines (in vitro)

EXPOSURE ASSESSMENT



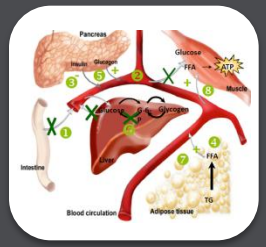
Identify genetic patterns associated with exposure to individual chemicals and perhaps chemical mixtures

DOSE-RESPONSE RELATIONSHIPS



Enhance understanding of how a given exposure level affects a toxic response

MECHANISTIC INFORMATION

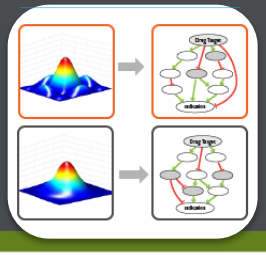


Improve insight on the mechanisms by which chemical exposures cause diseases

HAZARD SCREENING

Enabling rapid screening of potential toxicity of chemicals in development or the environment

VARIABILITY IN SUSCEPTIBILITY



Use of genetic information to identify susceptible subpopulations and overall susceptibility within a population

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Obrigada pela sua atenção.

Eu ficaria feliz em responder suas preguntas.

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IDEAS
Perspective
Social
Unique
Motivation
Creativity
Knowledge
Facts
Research
People
Intuition
Results

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