SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety

In Vitro to *In Vivo* Concordance for Toxicity Prediction and Use in Safety Assessments

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Analysis of *In Vitro* to *In Vivo* Concordance Studies for Food Safety Assessment in Humans

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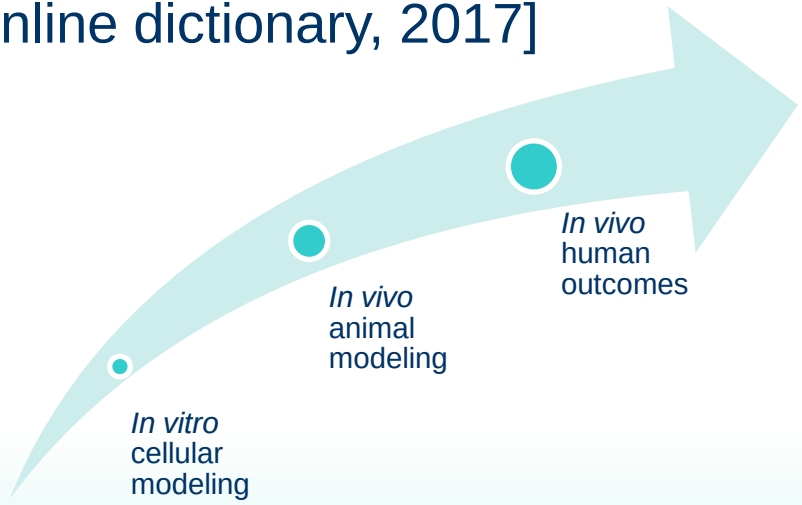
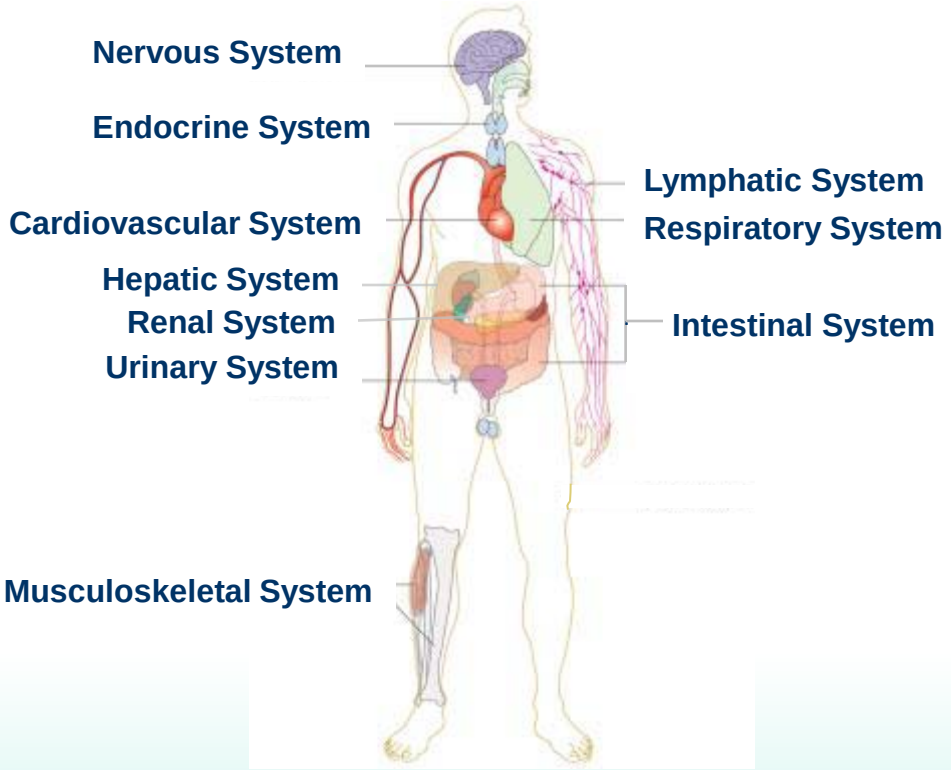
Conflict of Interest Statement

- No conflicts of interest to declare

Rationale and Overview

Concordance is “a state in which things agree and do not conflict with each other.” [Merriam-Webster online dictionary, 2017]



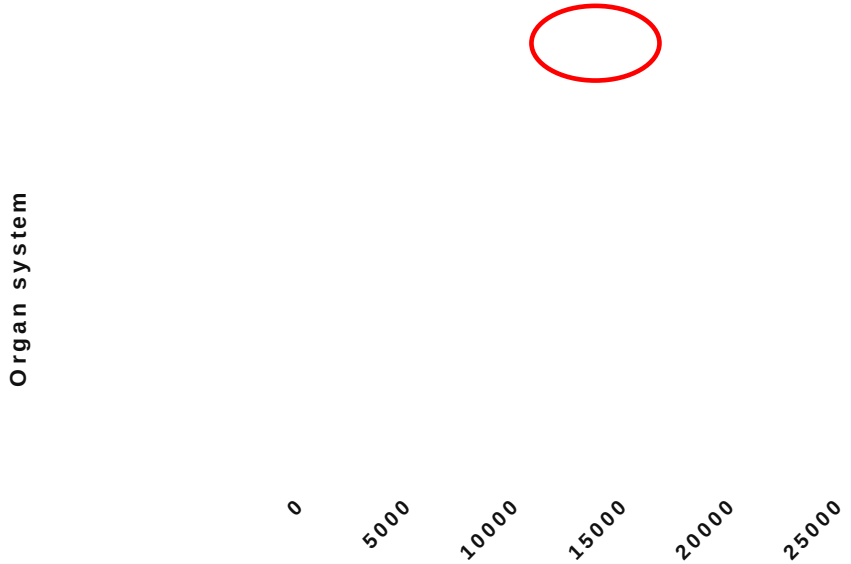


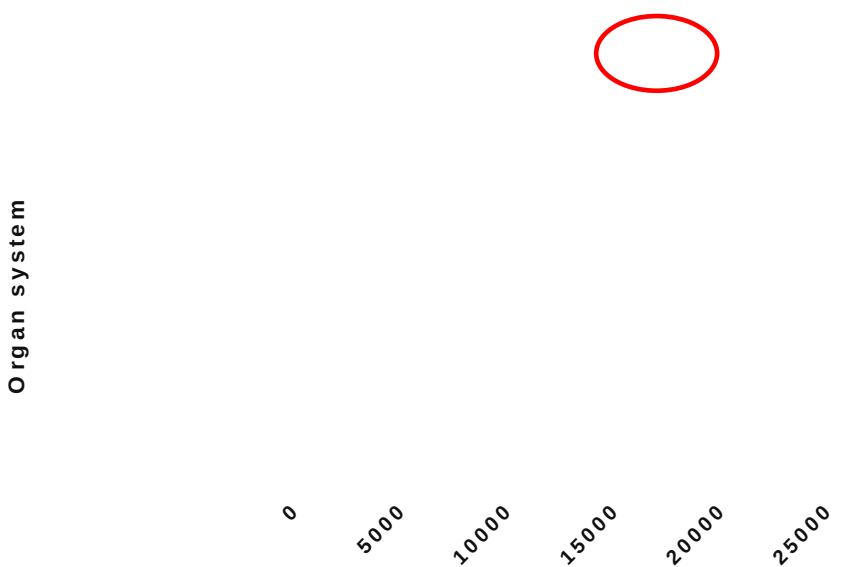
Rationale and Overview

Understanding the level of concordance between *in vitro* and *in vivo* outcomes will help establish the value of *in vitro* models for many purposes:

- Understanding modes of action
- Pharmacokinetic features
- Prediction modeling of ADME and potential toxicity
- Risk assessment

Parallel Trends in Research Using *In Vitro* and *In Vivo* Cellular Modeling





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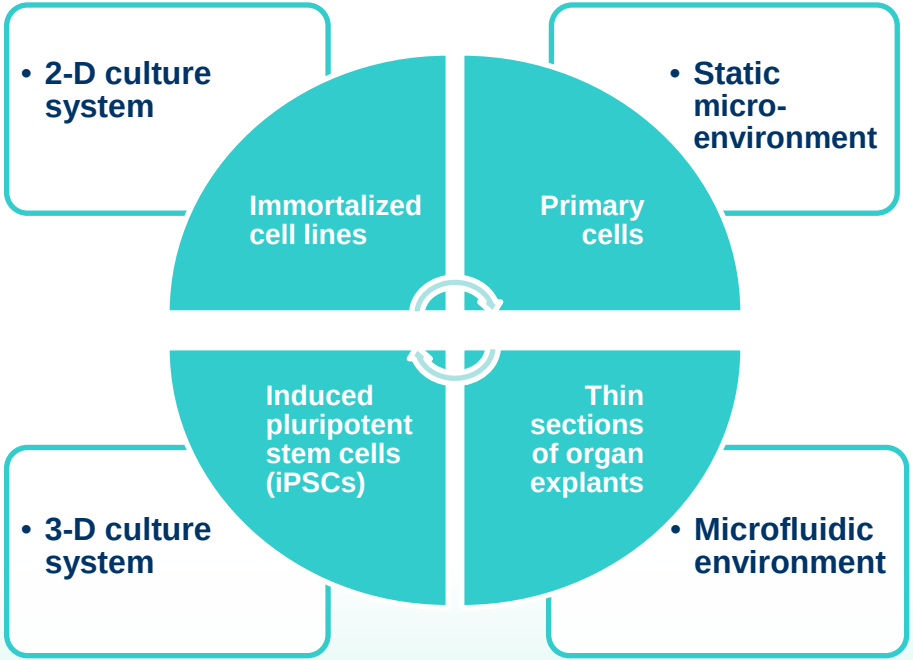
Considerations About Concordance Evaluation

What are some reasonable expectations relating to assessing the concordance between *in vitro* and *in vivo* outcomes?

- What types of models are being used?
- What aspect of an *in vivo* system is being modeled *in vitro*?
- Is the concordance between them being assessed qualitatively or quantitative?
- Can concordance assessment be extrapolated to both healthy and disease models?

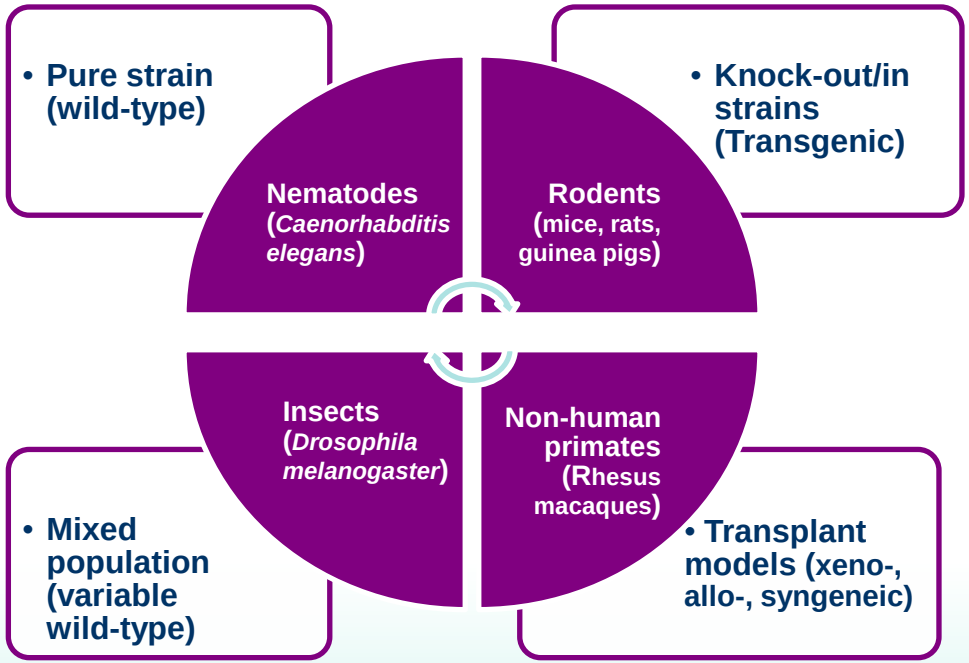
In Vitro Cellular Modeling

There are several categories of *in vitro* models to study toxicity including:



In Vivo Modeling

There are several categories of *in vivo* models to study toxicity including:



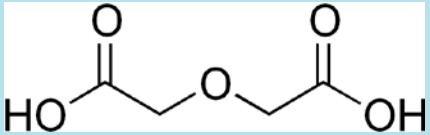
Establishing Concordance

The process of comparing outcomes from *in vitro* to *in vivo* systems is diverse among the research community.

- Guidance on the use of *in vitro* methods:
  - OECD official testing methods [www.OECD.org]
  - Tox21 *in vitro* assays [https://ncats.nih.gov/tox21/projects/assays]

- Published approaches to establishing concordance:
  - Due to the large variety of *in vitro* and *in vivo* models available, there is a wide variety of rubrics and statistical tests that can be reasonably used to decide on the extent of agreement between systems. (e.g., Olaharski et al. 2009; Wang and Gray 2015; Mossoba et al. 2017; Sprando et al. 2017)

Concordance Case Study: Diglycolic Acid



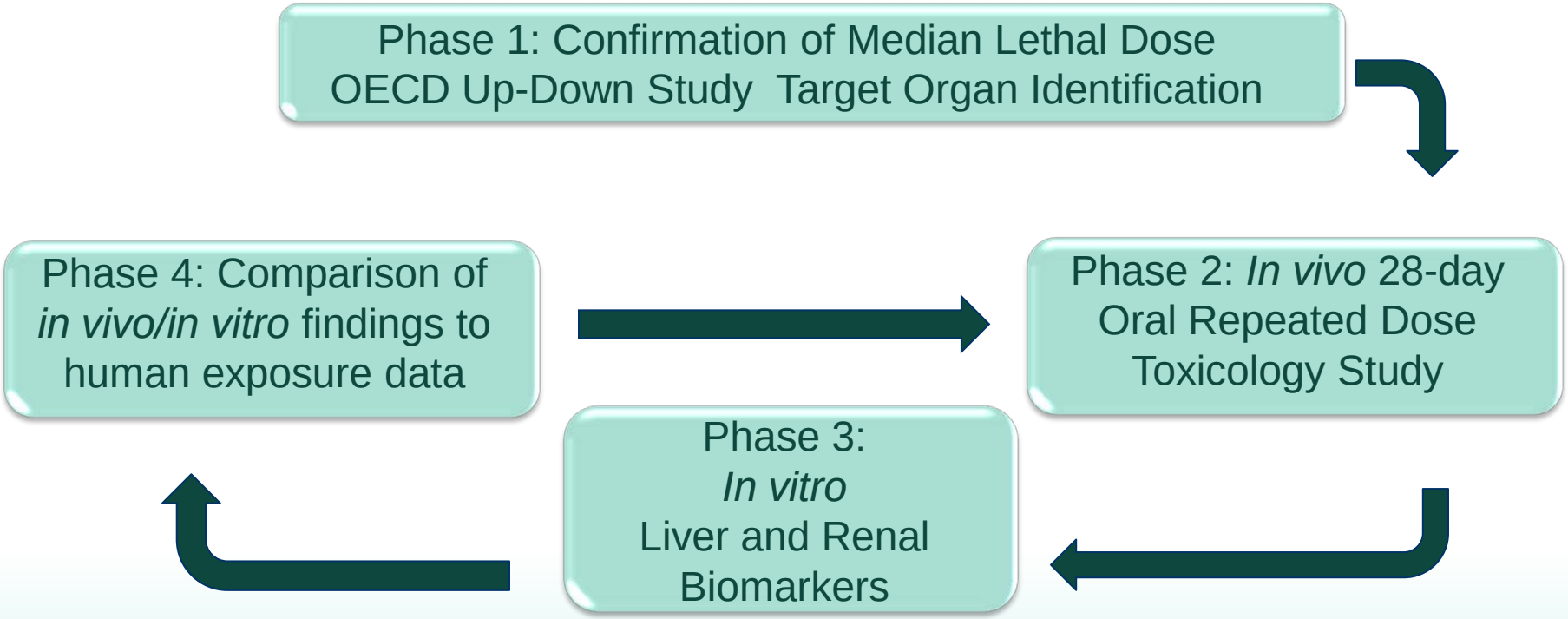
Diglycolic acid (DGA) is an impurity produced during the synthesis of several carboxymethyl carbohydrate preparations (e.g., CMC, CMS) used in food products:

- Frozen dairy products, cake baking mixes and syrups.

Studies conducted between 2009 and 2011 identified DGA as a minor metabolite (4% max) of diethylene glycol (DEG) and one of two active agents responsible for the human renal toxicity.

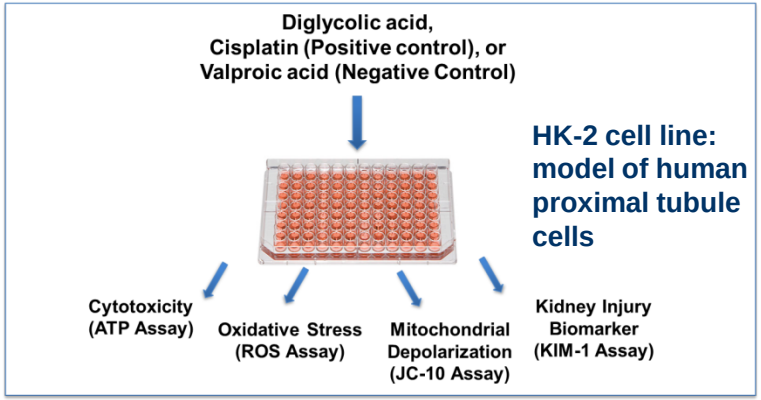
- Mass poisonings with products adulterated with diethylene glycol have resulted in renal toxicity and DGA is a metabolic by-product of DEG.
- A case study documenting accidental ingestion of DGA showed direct evidence of renal and other toxicities.

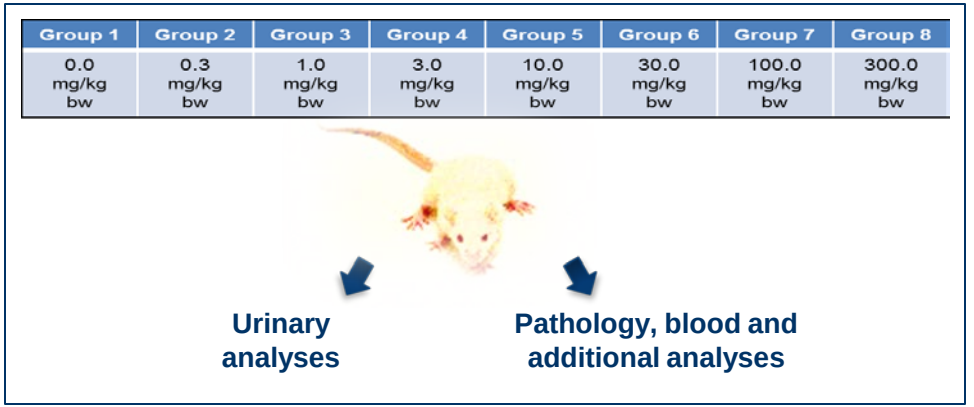
Concordance Case Study: Diglycolic Acid



Concordance Case Study: Diglycolic Acid

In the absence of comprehensive safety data on DGA, an *in vivo* safety study in rats was performed and complemented with *in vitro* cellular testing to understand the concordance between these systems.



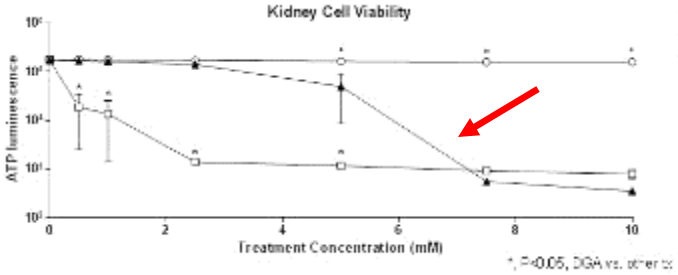


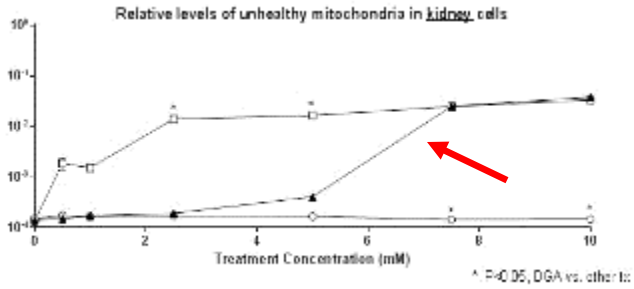
- Data from the *in vitro* human cell study were compared to data from the *in vivo* rat study as well as human case report outcome.

Case Report on DGA: *In Vitro* Findings

Directly exposing HK-2 cells to DGA *in vitro* for 24 hours resulted in:

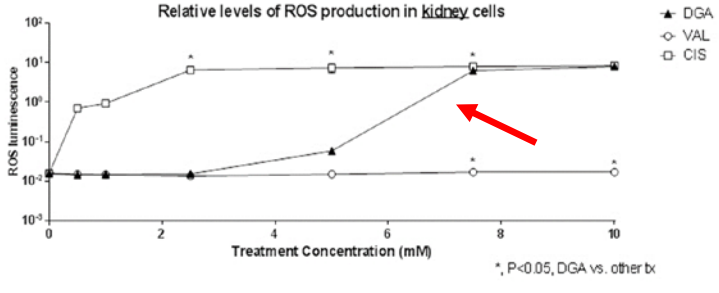
HK-2 cells exhibited reduced ATP production significantly.

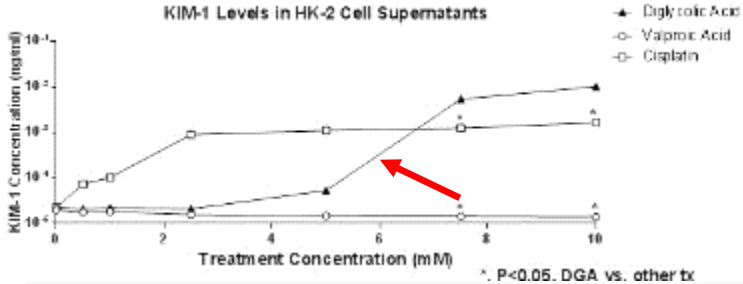




Decreased MMP; membrane integrity is affected.

Reactive oxygen species production was greatly increased.



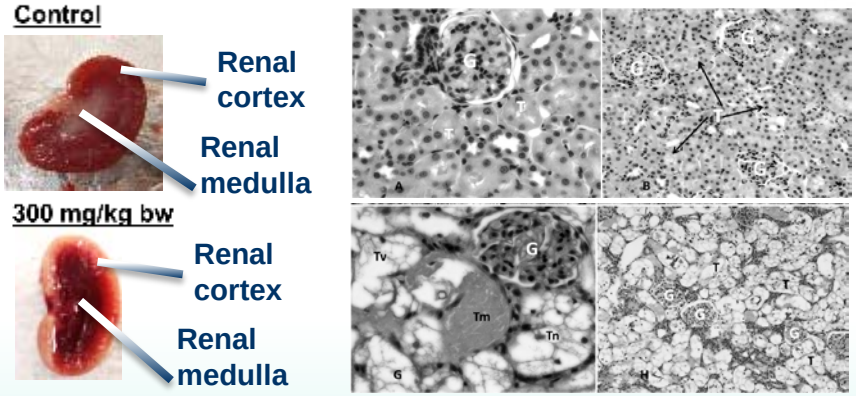


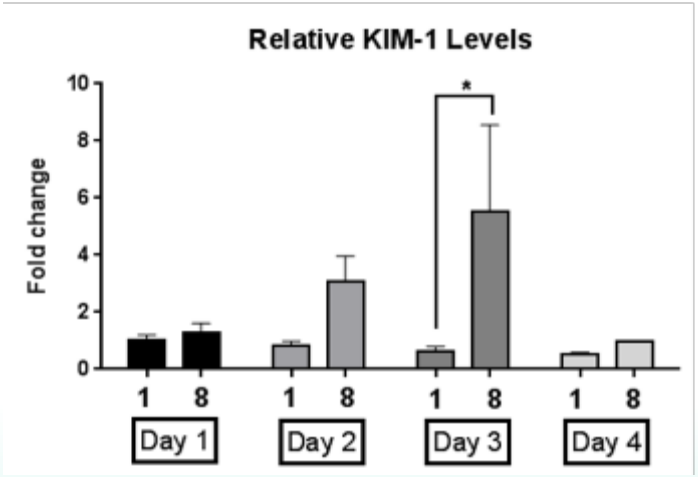
Relative KIM-1 expression superseded +ve control cisplatin levels.

Case Report on DGA: *In Vitro* Findings

All animals were dosed with daily oral doses for 28 days, except for the high dose group (300 mg/kg body weight), which could not survive past 5 days. (Sprando et al., 2017)

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
0.0 mg/kg bw	0.3 mg/kg bw	1.0 mg/kg bw	3.0 mg/kg bw	10.0 mg/kg bw	30.0 mg/kg bw	100.0 mg/kg bw	300.0 mg/kg bw





Case Report on DGA: *In Vitro* to *In Vivo* Concordance

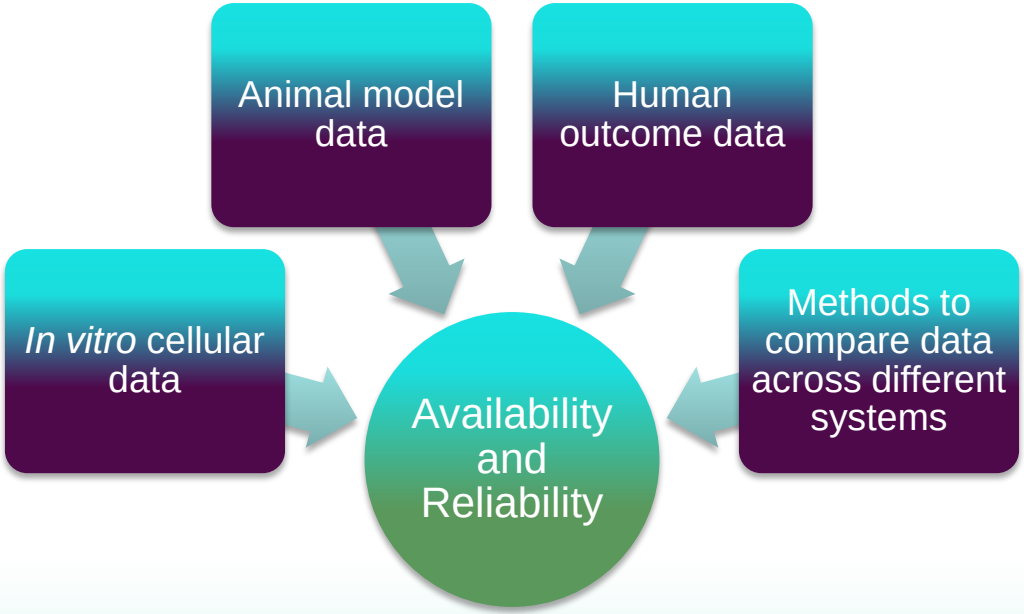
HK-2 *in vitro* data were concordant with both the *in vivo* rat model data as well as the reported human exposure data:

- *In vitro* findings: HK-2 cells that were directly exposed to DGA for 24 hours *in vitro* showed clear evidence of decreased cellular and mitochondrial health and increased oxidative stress. Treated cells also upregulated the biomarker KIM-1 in a dose-dependent manner.

- *In vivo* findings: Treated rats demonstrated injurious high-dose renal effects: elevated BUN, CREA, and KIM-1 levels, tubular necrosis, renal failure.

- Human Exposure Data: Above observations are consistent with effects reported for an unintentional human exposure case report (Roscher et al. 1975).

Variables to Consider When Establishing Concordance



Variables to Consider When Establishing Concordance

Some cases of concordance are clear-cut, others are not.

- Disparities exist among approaches by researchers in trying to compare *in vitro* to *in vivo* data.
  - These variables are present for both food and drug toxicity studies.
  - The availability and reliability of data from *in vitro*, *in vivo*, human are important along with the different approaches to compare data across systems.

In Vitro Outcome Considerations

Availability/reliability of toxicity data from *in vitro* cellular systems depends on:

- Accurate dosing in a direct exposure model.
  - *In vitro* dosing is dependent on solubility of the food or food ingredient. For example, lipids are often modified with albumin or first dissolved in appropriate solvents (e.g., DMSO, ethanol, ethyl acetate) prior to use in cell culture.
  - The range of doses used *in vitro* may not reflect an *in vivo* situation. Even when *in vivo* blood levels are used as a guide, organ-specific concentrations may differ greatly (e.g., higher local concentration in the proximal tubules of the kidneys or lower local concentrations in the brain due to the BBB).
  - Definitions of acute vs. chronic dosing are often arbitrary.

In vitro Outcome Considerations

- Some variations in general cell culture methods, growth conditions exist.
  - Primary cells as well as cell lines used by different researchers are subjected to different media (esp. FBS), and occasional contamination by *Mycoplasma* or even other cell types.
- Cell lines that are over-passaged undergo genetic drift.
  - Over time, polymorphic and telomeric changes take place.
  - Culture conditions may alter the differentiation state and epigenetic status.
  - Source of cell lines may not be taken into consideration when modeling *in vivo* outcomes (e.g., gender, age, species of cell line source).
- Co-culturing cell types introduces new variables.
  - Bacteria added to intestinal cell models may improve the *in vitro* model.

In Vitro Outcome Considerations

- Converting 2-D cell models to 3-D representations of solid organs.
  - Culturing multiple monolayers may better mimic specific organs than others.
  - 3-D printing of multiple cell types may better represent organs.
  - Compartmentalized cell culture systems (e.g., Transwell) may improve cell functionality in part by inducing cell polarization.
  - Newer technologies that impose stretch forces on cells are also emerging.
  - The necrotic core that can form inside a cellular spheroid may confound data.

In Vitro Outcome Considerations

- Microfluidic systems vs. static *in vitro* cellular culture.
  - Current microfluidic systems still rely on 1 or 2 cell types to represent entire organs, but has potential to accurately mimic organ function.
  - Organ-on-a-chip may permit multiple organs to be connected together to mimic the effects of toxic outcomes affecting downstream organs.
  - High-throughput design is not readily available, decreasing the statistical power of typical *in vitro* experiments.

In Vivo Outcome Considerations

The availability/reliability of toxicity data from *in vivo* models depends on:

- The fidelity of the selected model to represent human outcomes.
  - Inbred strains of rodents are popular *in vivo* models and using more than one strain could improve its concordance human outcomes.
- Accounting for potential discrepancies in vulnerability to toxicity between genders.
  - e.g., female rats are more likely to show toxicity than male rats.
- The ability of disease models to display disease phenotypes.
  - The increased vulnerability of humans to toxins is often related to a disease state.

In Vivo Outcome Considerations

- Conserved cellular signaling pathways among animals offers a mechanistic window into understanding toxicity.
  - Despite lacking the same anatomy as humans (e.g., tear ducts, liver, perspiration glands), non-mammalian *in vivo* models often have conserved cell signaling pathways.
- *In vivo* models with humanized organs can yield a unique way to study human toxicity in an experimental model.
  - When *in vivo* models are transplanted with human blood or solid organs, exposure to toxins can be assayed in a physiological environment.

Human Outcome Considerations

The availability/reliability of toxicity data in human subjects depends on:

- Clinical trial data collected by industry/academia/government are:
  - Not always published into the public domain.
  - Not as commonly available for food and ingredients as for pharmaceutical drugs.
- Accidental ingestion or food poisoning events are available through online databases.
  - Scale of event(s) vary widely (sparse occurrences vs. mass poisonings).
  - Sometimes events are population-specific.
- Toxicity data may be due to unusual circumstances.
  - Mistaken identities of different species of foods (e.g., fish).

Establishing Concordance Between Model Systems

When deciding on whether data generated from an *in vitro* model are concordant with *in vivo* outcomes, it is important to note that:

- Portions of *in vitro* models can yield data that are concordant with *in vivo* outcomes.
  - Transporter protein expression on proximal tubules may yield data on toxin entry into cells that is concordant with *in vivo* situations, but brush border enzyme expression may be absent.
  - Mechanistic information may only be necessary to establish mechanistic concordance, as opposed to full toxicological data.

Establishing Concordance Between Model Systems

- Relying on *in vivo* outcomes from nematode, insect, or animal models to establish concordance with *in vitro* systems could be undermined by the concordance of the *in vivo* model with human outcomes.
  - Toxicity observed in humans can sometimes be measured using *in vitro* cellular models, but not necessarily *in vivo* systems.
- The ability of potential toxins to be processed *in vivo* via ADME pathways could undermine the relevance of any *in vitro* data.
  - Ingested foods may bypass certain organs, thereby reducing their actual exposure *in vivo*. Lack of toxicity *in vivo*, therefore, may not be correctly captured in an *in vitro* cellular model of direct exposure.

Summary

When discussing concordance, defining what functional or mechanical aspect of an *in vitro* model is being used to assess its *in vivo* counterpart is critical.

- Functional characteristics should help guide model selection.
  - Multiple endpoint analyses would help avoid artefactual data and better establish levels of concordance (e.g., as shown in our DGA study with elevated KIM-1 levels *in vivo* correlating to upregulating of KIM-1 *in vitro*).
- *In Vitro* to *In Vivo* Concordance for Toxicity Prediction and Use in Safety Assessments is still in development.
  - As toxicologists continue to collect concordance data, how to improve *in vitro* modeling for better concordance will become more clear.

Concluding Remarks on Establishing Concordance

In vitro modeling and methods continue to improve over time, as official guidance on how to determine the equality of their endpoints increases.

- As the quality of *in vitro* modeling improves, concordance with *in vivo* outcomes will also improve.
- Collecting data from multiple *in vitro* or *in vivo* models will likely offer more leverage to establish concordance with greater confidence.
- The diversity in endpoint and kinetic assays (*in vitro* and *in vivo*) will create new opportunities to generate more complete comparisons of selected systems.
- Efforts from researchers to scrutinize *in vitro* and *in vivo* data will also lead to a better understanding of sources of discordance.

How to Overcome Discordance with New Tools

Advanced technologies are emerging to help improve the quality of *in vitro* cellular models and methods

- Increasing number of organs are being developed as organ-on-a-chip systems, which can be interconnected.
- Stem cell differentiation protocols are being developed to increase the number of cell types of various organs to have a more comprehensive collection of *in vitro* models that represent both male and female organs.
- Genetic tools that can help model diseases of interest are no longer prohibitively expensive.

Outlook on the Future of Food Safety Assessment

In vitro cellular methods can yield rapid results on mechanistic aspects of toxicology testing, especially as better models are being developed.

In vivo approaches allow for a systemic understanding of toxicity.

- Running experiments in parallel and comparing the results will provide information on how to improve *in vitro* cellular models.
- Integrating the two approaches will therefore create better *in vitro* models and improve concordance for food safety assessments.

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Acknowledgements

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