Opportunities and Challenges Related to Saturation of Toxicokinetic (TK) Processes: Implications for Risk Assessment

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• Initiated in 2017
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THE TOPIC “FORMERLY KNOWN AS KMD”

• KMD: Kinetically-derived maximum dose
  • “Toxicity studies should ideally be conducted at kinetically linear doses or slightly above the point of departure from linearity or kinetically-derived maximum dose (KMD)” (Saghir 2015)
  • “Limiting the highest test dose to the inflection point of the onset of non-linear behavior” (Saghir 2013)

• Related concepts:
  • “The highest dose level should not exceed into the range of non-linear kinetics” (REACH Chapter R.7c)
  • “There is little value in increasing the administered dose if it does not result in increased plasma concentration of parent or metabolites” (ICH S5(R3))

OPTIMIZE THE ABILITY OF TOXICITY TESTS PERFORMED IN A SMALL # OF ANIMALS TO BE USED / APPLIED IN RISK ASSESSMENT
PROBLEM FORMULATION

• Toxicology studies that utilize KMD are often submitted for the purpose of interpreting dose-response data from repeated dose animal studies to assess human health risks of occupational and environmental chemical exposures. However, there is no agreed upon scientific guidance that clearly specifies what data are necessary and sufficient, in a fit-for-purpose context, to evaluate such studies.

• There are no specific criteria on how to incorporate/integrate all available data streams, including, but not limited to toxicokinetic and exposure information, to inform study design in repeated dose animal studies for environmental chemical exposures.
FALL 2020 VIRTUAL SYMPOSIUM

• Co-sponsored by U.S. Environmental Protection Agency Office of Pesticide Programs (EPA-OPP), NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and HESI

• Plenary held 30 September 2020: 450 multi-sector participants from 22 countries
  • First public forum for scientific discussions related to the concept and applications of the KMD
  • Recording, slides, and Q&A available at: https://ntp.niehs.nih.gov/whatwestudy/niceatm/3rs-meetings/past-meetings/kmd-2020/kmd-2020.html

• Two-day breakout sessions were held 7/8 October 2020 for invited participants to further discuss key topics related to KMD
SYMPOSIUM OBJECTIVES

• MOTIVATION: There are no scientific guidance on how to use a KMD approach to interpret or design repeated dose animal toxicity studies for environmental chemicals

• Highlight lessons learned on the following:
  • Determining dose proportionality
  • Conducting statistical analysis to determine a KMD

• Discuss the situations where the KMD concept can be applied

• Discuss when the use of the KMD approach might be limited or not possible
FRAMING THE DISCUSSION

• Internal concentration is better predictive of the initiation and degree of toxicological responses than administered dose

• Dose-response relationships are the result of toxicodynamic (TD) AND toxicokinetic (TK) processes

• Understanding dose-response is a critical tenet of toxicology – both in designing and interpreting studies

• Various international guidance documents stress:
  • TK should be considered to inform dose level selection
  • The highest dose should not be above a level that results in saturation of absorption

HOW CAN WE BEST UTILIZE TK (AND TD) INFORMATION WHEN DESIGNING AND INTERPRETING ANIMAL TOXICITY STUDIES FOR VARIOUS REGULATORY PURPOSES?
KEY CONCEPTS

• Dose-response:
  • Toxicokinetics (TK)
    • ADME → target tissue exposure from a given administered dose
  • Toxicodynamics (TD)
    • Interactions of the chemical with target molecules / cells / target tissues / organs & subsequent responses

WHAT DO WE NEED TO KNOW TO LINK EXTERNAL EXPOSURE TO EFFECTS?

HOW CAN WE USE THAT INFORMATION TO DESIGN BETTER ANIMAL STUDIES?
Opportunities and challenges related to saturation of toxicokinetic processes: Implications for risk assessment

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Incorporating human exposure information in a weight of evidence approach to inform design of repeated dose animal studies

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Concerns over consideration of TK saturation in study design & interpretation

**INFLECTION POINT:** There is no inflection point in the sigmoid-like relationship between administered dose and internal concentration of a chemical the exhibits nonlinear TK.

**EXPOSURE:** Human exposure levels hard to predict; makes it difficult to ensure that top dose selected using TK information is sufficiently high.

**3Rs:** Obtaining TK data requires additional animal use, violating 3Rs principles.

**TK STUDY LIMITATIONS:** Animal TK not reflective of human exposure scenarios; animal – human TK is different; TK endpoints from animal studies may not be appropriate internal dose metric for toxic moiety.

Heringa et al., 2020
INFLECTION POINT – IS IT APPROPRIATE?

• Deviation from linearity (non-linearity) may be due to various ADME processes (e.g., saturation of absorption, saturation of clearance, saturation of metabolism, capacity-limited transport); in some cases, TD can alter TK

• Non-linearity is important to consider when interpreting or designing studies to avoid over – or under-estimation of risk

It is not about the calculation of a point!
WHAT DOES NON-LINEARITY TELL US?

- Kinetic modeling + in vitro ADME + in vivo ADME data with a pre-defined criterion
- In vitro studies to identify a dose-dependent transition in MOA + kinetic data + information related to which is the active moiety
- Use TK modeling throughout!

Tan et al., 2021
EXPOSURE

• Many robust and reviewed models and methods to estimate human exposure – an integral part of the risk assessment process!
• Various case studies conducted to compare human exposure estimates with animal PODs (e.g., LOAELs & NOAELs)

<table>
<thead>
<tr>
<th>Case Basis</th>
<th>Chemicals</th>
<th>Exposure Scenarios</th>
<th>Exposure routes</th>
<th>Exposure estimation methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monitoring data</td>
<td>106 pesticides</td>
<td>Occupational &amp; residential non-dietary</td>
<td>Inhalation, dermal</td>
<td>Monitoring data + exposure factors</td>
</tr>
<tr>
<td>Weight fractions</td>
<td>Industrials (chemical agnostic)</td>
<td>Occupational</td>
<td>Inhalation, dermal</td>
<td>ChemSTEER (chemical agnostic)</td>
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<tr>
<td>OSHA monitoring</td>
<td>504 industrial chems</td>
<td>Occupational</td>
<td>Inhalation</td>
<td>ChemSTEER (chemical-specific data)</td>
</tr>
<tr>
<td>Chemical properties</td>
<td>448 chemicals w/in vivo tox data</td>
<td>Residential &amp; consumer</td>
<td>Inhalation, dermal, oral</td>
<td>SEEM2 model</td>
</tr>
<tr>
<td>Use / activity</td>
<td>Industrials (chemical agnostic based on exposure bands)</td>
<td>Occupational &amp; consumer</td>
<td>Inhalation, dermal, oral (consumer only)</td>
<td>Worst-case levels based on REACH screening level exposure models</td>
</tr>
</tbody>
</table>

Lowe et al., 2021
EXPOSURE

• The average combined dermal and inhalation exposures to pesticides estimated based on conservative assumptions was 0.74 mg/kg bw/day, which was ~175X lower than the average dermal NOAEL for conventional pesticides (130 mg/kg bw/day)

• The highest average daily dose predicted for occupational worker dermal exposure was 0.0072 mg/kg bw/day (EPA ChemSTEER model)

• Most of the air exposure data in the U.S. Occupational Safety and Health Administration’s Chemical Exposure Health Data (99% out of 78,616 samples) were < 1 mg/kg bw/day, with the median being 9E-5 mg/kg bw/day
Exposure

Establish Exposure Guidance Value
Account for uncertainty factors $UF_A$ and $UF_H$ to ensure protection of human health
E.g., RfD, MCL, OEL

Evaluation of Animal Toxicity Data
Identify Point of Departure in animal toxicity studies
E.g., LOAEL, NOAEL, BMD

Animal Toxicity Studies
Select appropriate doses, including the top dose
e.g., limit dose, MTD, KMD

Lowe et al., 2021
3Rs (REDUCTION, REFINEMENT, REPLACEMENT)

- Unnecessary animal suffering
  - Study may need to be terminated or lose top dose group
  - Unreliable results due to biological stress / metabolic shifts
  - Data not relevant (potential dose-dependent transitions)

- Repeat studies may be required to demonstrate toxicity

Weight of Evidence (WOE)
- Available from early safety testing studies & NAMs
- Can be obtained via microsampling

**Too Hot**
High

**Too Cold**
Low

**Just Right**
TK STUDY LIMITATIONS

• Limitations in animal TK studies and biological species differences that impact extrapolation to human-relevant endpoints is not new!

• Well-developed methods commonly used in risk assessment to address extrapolation:
  • Intra-species
  • Route to route
  • Lifestage
  • Inter-species

• Many *in vitro* approaches using species-relevant biomaterials have been appropriately validated to investigate kinetic differences between animals and humans
WEIGHT OF EVIDENCE

Shorter-term repeat dose animal studies

MOA & Dose-dependent transition

In vitro studies w/animal and human cells + IVIVE

Aggregate Exposure Pathway (AEP)

Internal Exposure
- Amount Absorbed
- Inhaled Amount
- Amount in Blood

Target Exposure
- Protein
- Organ

Macro-Molecular Responses
- Receptor/ligand binding
- DNA/RNA
- Protein oxidation

Cellular Responses
- Gene activation
- Protein production
- Altered signaling

Organ Responses
- Altered physiology
- Disrupted homeostasis
- Altered tissue development/function

Adverse Outcome Pathway (AOP)

- Lethality
- Impaired Development
- Impaired Reproduction
CASE EXAMPLES – WEIGHT OF EVIDENCE

- 90-day rat study
- No toxicity up to limit dose of 1000 mg/kg bw/day
- Saturation of absorption at doses >100 mg/kg bw/day
- 300 mg/kg bw/day chosen for top dose for 2-year study
- In 2-year study, saturation of absorption seen at 300 mg/kg bw/day (6 mo) and 50 mg/kg bw/day (12 mo)
- If limit dose was used for 2-year study, dose spacing would have resulted in all dose levels > saturation of absorption

Tan et al., 2021
CASE EXAMPLES – WEIGHT OF EVIDENCE

- 90-day mouse study; top dose of 1250 ppm for males; 3000 ppm for females (based on 28d study)
- Effects observed at mid and high dose levels – liver effects (males & females); kidney effects (male)
- No effects observed within linear systemic exposure range
- Saturation of elimination seen above 750 ppm in males
- Saturation of absorption seen above 1500 ppm in females
- Top dose for 18-mo study set at 750 ppm for males and 1250 ppm for females using TK + apical effects information
- Liver adenomas and carcinomas observed in 18-mo study; MOA determined not human relevant in follow-up studies
- **Doses selected based on the WoE approach were high enough to result in toxicity**

Tan et al., 2021
CASE EXAMPLES – WEIGHT OF EVIDENCE

Top dose informed by kinetic data

Florpyrauxifen Benzyl

Human Estimated Dietary
Estimated Dietary
0.0005 mkd

ADI
0.5 mkd

NOAEL
Rat 2 y
50 mkd

High Dose
2 y
300 mkd

Fenpicoxamid

Human Estimated Dietary
Estimated Dietary
0.00595 mkd

ADI
0.05 mkd

NOAEL
Mouse 1.5 y
32.1 mkd

High Dose
1.5 y
361 mkd

Sulfoxaflor

Human Estimated Dietary
Estimated Dietary
0.008 mkd

ADI
0.04 mkd

NOAEL
Mouse 1.5 y
10.4 mkd

High Dose
1.5 y
98 mkd

Tan et al., 2021
### CONCLUSIONS

| Characterizing interspecies differences in TK and TD strengthens the biological basis for extrapolations and comparing responses – across species, exposure routes, and dose levels |
| Saturation of ADME processes can impact design an interpretation of animal toxicity tests; dosing above saturation provides no additional useful information |
| A WoE approach should be used to inform dose selection and interpretation of D-R in repeat dose animal studies [TK, TD, exposure, MOA] |
| Clear scientific justification and rationale should be provided re: selection of top dose |
| Top doses are consistently several orders of magnitude higher than anticipated human exposure levels; resources are available to estimate exposure |
| TK data can be collected without additional animal use – in vivo and in vitro |
| The internal dose metric should be used to understand the impact of saturation on the D-R relationship |
| Must be fit for purpose - regulatory requirements need to be considered |

It is not about the calculation of a KMD or an inflection point!
UPCOMING SESSIONS

SOT 2022 Session: Utilizing Multiple Lines of Evidence to Optimize the Design and Interpretation of Long-Term, Repeated-Dose Animal Studies to Inform Human Health Risk Assessment  
*Chairs: Jeanne Domoradzki & Qiang Zhang*

ICT 2022 Session: Putting the Puzzle Together: Multiple Lines of Evidence to Inform Design and Interpretation of Long-Term, Repeated-Dose Animal Studies to Inform Human Health Risk Assessment  
*Chairs: Michelle Embry & Jos Bessem*s
ONGOING & FUTURE WORK

Upcoming paper: Predicting non-linear relationships between external and internal concentrations with physiologically-based pharmacokinetic modeling
• Toxicology and Applied Pharmacology special issue on “Advances in Research Strategies and Approaches for Toxicity Testing of Environmental Exposures”

Ongoing efforts:
• Use of in vitro & in silico information to inform study design of repeat-dose animal studies [TK and TD]
  • Working group formed & paper outline drafted
• Follow-up PBPK modeling work in collaboration with Scitovation – adding Monte Carlo and optimization capabilities to create a generic PK modeling package to analyze non-linear kinetic data
  • Ongoing discussions / work
• Interpreting D-R relationships based on administered dose and systemic exposure using a modeling approach
  • To be started 1Q 2022
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Workshop speakers & participants!
REFERENCES


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