Integration of Toxicokinetics in Repeated Dose Toxicity Studies

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Conflict of Interest Statement

The author declares no conflict of interest.
# Abbreviations

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>TK</td>
<td>Toxicokinetics</td>
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<tr>
<td>TD</td>
<td>Toxicodynamics</td>
</tr>
<tr>
<td>ADME</td>
<td>Absorption Distribution, Metabolism Elimination</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under Curve</td>
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<tr>
<td>NOAEL</td>
<td>No Observed Adverse Effect Level</td>
</tr>
<tr>
<td>POD</td>
<td>Point of Departure</td>
</tr>
<tr>
<td>BE</td>
<td>Biomonitoring Equivalent</td>
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<tr>
<td>ILSI</td>
<td>International Life Sciences Institute</td>
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<td>HESI</td>
<td>Health and Environmental Sciences Institute</td>
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<td>MTD</td>
<td>Maximum Tolerated Dose</td>
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<tr>
<td>HDL</td>
<td>High Dose Level</td>
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<tr>
<td>MOA</td>
<td>Mode of Action</td>
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<tr>
<td>HRF</td>
<td>Human Relevance Framework</td>
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Regulatory Testing of AgroChemicals

• An agrochemical product undergoes >100 rigorous studies to support the health, safety and environmental assessments required for registrations.

• From a mammalian toxicology perspective, agrochemicals have the most comprehensive data requirements of any chemical sector, including pharmaceuticals.
Regulatory Testing of AgroChemicals

- Required mammalian toxicology testing for agrochemicals
  - ADME Studies
  - Acute Toxicity Testing
    - oral, dermal, inhalation, skin & eye irritation, sensitization
  - Subchronic Toxicity Testing
  - Chronic Toxicity Testing
  - Carcinogenicity Testing
  - Reproductive Testing
  - Developmental/Teratogenicity
  - Mutagenicity/Genotoxicity Testing (*In vitro* & *In vivo*)
  - Neurotoxicity (Acute & Subchronic)
  - Repeated Dermal
Integration of Toxicokinetics for Agrochemicals

• Integrated Toxicokinetics (TK) utilized by Pharma for many decades

• Gaining recognition for chemicals more recently:
  • Highlighted by ILSI-HESI ACSA publications (2006)
  • Followed by new EU data requirements (EC 1107/2009)

• Implemented by heritage Dow AgroSciences as a default for integrated toxicology testing strategies since 2007
Toxicokinetics

Administered Dose (mg/kg body weight/day) vs. Internal Dose (ug/g blood)

- Molecule A
- Molecule B
Saturation of kinetic processes

KMD = Kinetically-derived Maximum Dose

Administered Dose (mg/kg body weight/day)

Internal Dose (ug/g blood)

Molecule A

Molecule B
Drivers to Integrate Toxicokinetic Data

Drivers

Scientific

Regulatory
Integration of Toxicokinetics for Agrochemicals

✔ Scientific Drivers

✔ Better correlation of biological effects
  • to the systemic concentrations
  • in the context of human exposure

✔ Better interpretation of the safety data
  • in the context of confounding factors resulting from excessive stress from high dose level.

✔ Selection of relevant dose levels in longer term studies

✔ With no additional use of animals, TK data is generated from same set of animals

✔ Species to species differences are better understood

✔ Route to route differences are better understood

✔ Better extrapolation of concentrations and dose levels from in vivo to in vitro and vice versa.
### EU-Regulatory Drivers

<table>
<thead>
<tr>
<th>Document Type</th>
<th>Summary of Requirements/Recommendations</th>
<th>Reference</th>
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</table>
| EC 1107/2009  | TK required in short-term & long-term studies  
Dose level selection should take into account saturation of absorption                                                                                                                                  | EC (2009)   |
<p>| Guidance Chapter R.7c | Use TK to support Dose Setting Decisions for Repeated Dose Studies – “the highest dose-level should not exceed into the range of non-linear kinetics”                                                                                     | ECHA (2008) |
| Guidance Chapter R.7c | “Even though toxicokinetics is not a toxicological endpoint and is not specifically required by REACH, the generation of toxicokinetic information can be encouraged as a means to interpret data, assist testing strategy and study design, as well as category development, thus helping to optimise test designs” | ECHA (2012) |
| EFSA Scientific Report | The first key recommendation is the need for human TK data in hazard assessment to better understand interspecies differences, human variability. Such TK data will ultimately link exposure, internal dose and toxicity using physiologically-based models for risk assessment purposes. | EFSA (2014) |</p>
<table>
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<tr>
<th>Source</th>
<th>Description</th>
<th>Reference</th>
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<tr>
<td>OPPTS 870.8500</td>
<td>Determine the bioavailability of the test substance after dermal or oral treatment; ascertain whether the metabolites of the test substance are similar after dermal (assuming significant penetration) and oral administration; examine the effects of a multiple dosing regimen on the metabolism of the test substance after administration.</td>
<td>US EPA (1996)</td>
</tr>
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</table>
| OPP HED                                     | Recommends ‘use of innovative approaches’
“The highest dose tested should not be above a dose that results in saturation of absorption.”                                                                                                   | GD #G2003.02  |
| EPA Guidelines-Risk Assessment Forum        | “The high dose in long-term studies is generally selected to provide the maximum ability to detect treatment-related carcinogenic effects while not compromising the outcome of the study through excessive toxicity or inducing inappropriate toxicokinetics (e.g., overwhelming absorption or detoxification mechanisms).” | EPA (2005)     |
| EPA Framework Document                      | “All available lifestage-specific TK data are included and described in order to determine the relevance and impact of the TK data in evaluating the study and to determine the impact of exposure on response across lifestages. TK data can be used to verify that indirect exposure of the fetus or neonate (e.g., via maternal circulation or breast milk) occurred without relying on observable outcomes. In some situations, internal dose can be measured, providing greater confidence in derivation of the dose metrics (Section 4.2.2.3). If TK data are available across lifestages, this information can aid in highlighting key lifestages for the assessment.” | US EPA (2006)  |
| EPA GD                                      | This guidance describes the process for identifying pertinent data useful for quantifying inter- and intraspecies differences to serve as the basis for empirically determined “Data-Derived Extrapolation Factors” (DDEFs). Toxicokinetics is one factor used to determine inter- and intraspecies extrapolation factors (toxicodynamics is the other factor). | US EPA (2014)  |
| Draft Guidelines-Risk Assessment Forum      | Toxicokinetics ("Exposure-to-dose considerations: What is known about the toxicokinetics? How is this influenced by factors such as lifestage, race, sex and genetics?") should be used as one technical element of developing the conceptual model or planning tool for exposure assessment requirements. | US EPA (2015)  |
### OECD-Regulatory Drivers

<table>
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<tr>
<th>Reference</th>
<th>Text</th>
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<td><strong>TG 417</strong></td>
<td>“Studies examining the toxicokinetics (TK) of a chemical substance are conducted to obtain adequate information on its absorption, distribution, biotransformation (i.e. metabolism) and excretion, to aid in relating concentration or dose to the observed toxicity, and to aid in understanding its mechanism of toxicity. TK may help to understand the toxicology studies by demonstrating that the test animals are systemically exposed to the test substance and by revealing which are the circulating moieties (parent substance/metabolites). Basic TK parameters determined from these studies will also provide information on the potential for accumulation of the test substance in tissues and/or organs and the potential for induction of biotransformation as a result of exposure to the test substance.”</td>
</tr>
<tr>
<td><strong>TG 451</strong></td>
<td>“points to be considered in dose selection include: Known or suspected nonlinearities or inflection points in the dose–response TK, and dose ranges where metabolic induction, saturation, or nonlinearity between external and internal doses does or does not occur”</td>
</tr>
<tr>
<td><strong>GD 116</strong></td>
<td>One parameter to inform dose selection and dose level spacing can be “toxicokinetics, and dose ranges where metabolic induction, saturation, or non-linearity between external and internal doses does or does not occur”</td>
</tr>
<tr>
<td><strong>TG 426</strong></td>
<td>“Dose levels should be selected taking into account all existing toxicity data as well as additional information on metabolism and toxicokinetics of the test substance or related materials. This information may also assist in demonstrating the adequacy of the dosing regimen. Direct dosing of pups should be considered based on exposure and pharmacokinetic information”</td>
</tr>
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<td><strong>TG 443</strong></td>
<td>Although not required, TK data from previously conducted dose range-finding or other studies are extremely useful in the planning of the study design, selection of dose levels and interpretation of results. Of particular utility are data which: 1) verify exposure of developing fetuses and pups to the test compound (or relevant metabolites), 2) provide an estimate of internal dosimetry, and 3) evaluate for potential dose-dependent saturation of kinetic processes. Additional TK data, such as metabolite profiles, concentration-time courses, etc. should also be considered, if they are available. Supplementation TK data may also be collected during the main study, provided that it does not interfere with the collection and interpretation of the main study endpoints. As a general guide, the following TK data set would be useful in planning the Extended One-Generation Reproductive Toxicity Study: 1. Late pregnancy (e.g. Gestation Day 20) - maternal blood and foetal blood, 2. Mid-lactation (PND 10) - maternal blood, pup blood and/or milk, 3. Early post-weaning (e.g. PND 28) - weanling blood samples</td>
</tr>
<tr>
<td><strong>GD 151</strong></td>
<td>Information on the differences in the toxicokinetics (e.g., biliary excretion or various membrane transport proteins) between human and the test species may assist in evaluating potential differences in the occurrence and/or potency of the observed effects. Aid selection of the route of administration, choice of vehicle, selection of animal species, selection of dosages, information on probable offspring exposure (in utero or via breast milk) and for interpretation of data obtained from the conduct of TG 443</td>
</tr>
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</table>
Integration of TK in Agrochemical Toxicity Testing Program: Proposal

Source: Saghir et al., RTP, 2012; 63 (2012) 321-332
Toxicokinetics - Utility

- Dose Level Selection
- Route to Route Differences
- Species to Species Differences
- In vivo to in vitro Extrapolation
- Comparison to Human Biomonitoring Data
### Case Example 1:

**Molecule X (Herbicide)**

**Dose-Level selection**

<table>
<thead>
<tr>
<th>TK Parameter</th>
<th>250 mg/kg</th>
<th>500 mg/kg</th>
<th>1000 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>(AUC_{(0-24)}) ((\mu g) eq.h/g)</td>
<td>309</td>
<td>318</td>
<td>Predicted</td>
</tr>
<tr>
<td></td>
<td>Measured</td>
<td>Measured</td>
<td>Measured</td>
</tr>
</tbody>
</table>

**AUC: Area Under curve**

**Subject to Sublinearity**

**Rat Blood AUC Data**
90-day Rat Blood AUC<sub>24</sub> Plot

Expected Linearity

Dose Levels:
100, 300 or 1000 MKD

>100 mg/kg/day
What should be the High Dose Level (HDL) for Longer Term Studies?
HDL Determination for Chronic Rat Study

MTD: MTD
1000 MKD

Sublinear >100 mg/kg/day
No Toxic Effects up to the Limit dose
NOAEL=1000 mg/kg/day
OECD GD116: “Although top dose selection based on identification of inflection points in toxicokinetic nonlinearity may result in study designs that fail to identify traditional target organ or body weight effects, it must be appreciated that metabolic saturation in fact represents an equivalent indicator of biological stress. In this case, the stress is evidenced by appearance of non-linear toxicokinetics rather than appearance of histological damage, adverse changes in clinical chemistry, haematology parameters or decrease in body weight gain.”
Weight of Evidence in HDL Selection

SubLinear Kinetics @>100

NOAEL=1000

Dose-Response Relationship

OECD 116

10, 50, 300 mg/kg/day

Relevance to Human Risk Assessment
HDL Determination for Chronic Rat Study

MTD 1000 MKD

KMD 300 MKD

Lower Point of Departure (PoD)

More Relevant
Case Example 2: Route to Route Differences

• Consumer exposure occurs **orally** from consumption of pesticide residues in food.

• **Dietary administration** represents the most appropriate route of test material administration as it mimics human exposure *via* low levels of residues in food commodities.

• However, rabbit developmental studies are traditionally conducted by **gavage** because of their variable feeding habits and palatability issues when administered *via* diet.
Dietary vs. Gavage Administration

<table>
<thead>
<tr>
<th></th>
<th>Dietary</th>
<th>Gavage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systemic exposure</strong></td>
<td>More consistent exposure during the entire course of study due to natural feeding habits.</td>
<td>Oscillating TK profile with a transient high Cmax followed by low Cmin (depending on the half-life) leading to either acute toxicity when there is rapid increase in systemic exposure and or prolonged periods of little to no systemic exposure</td>
</tr>
<tr>
<td></td>
<td>Consistent exposure is critical in developmental toxicity studies as embryonic and fetal developments occurs continuously during gestation.</td>
<td></td>
</tr>
</tbody>
</table>

**Hannas et al., 2016** demonstrated that with several feeding regimen modifications such as, flavor enhancer and set amount feed allotments- dietary administration could be successfully implemented reducing the variability and palatability

**Source:** B. Hannas et al. *Toxicological Sciences, 154 (1), 2016, 90-100*
Kinetic Differences of Dietary vs. Gavage Administration

- TK data demonstrates more consistent exposure via dietary route
- TK data are valuable to indicate internal dose to the dam and also determine exposure to the fetus

Source: B. Hannas et al. Toxicological Sciences, 154 (1), 2016, 90-100
Molecule Y
Species to Species Differences

- Specific target organ was seen only in dogs and not in rodents

Case Example 3:
- Unique metabolite in dogs
- TK Data
- In vitro comparative metabolism
- Not relevant to humans
Molecule Z (Insecticide)

*In Vivo* to *In Vitro* Extrapolation

- Developmental toxicity was seen only in rats and not rabbits

**In Vivo to In Vitro Extrapolation**

- Cross-species comparison of TK data:

<table>
<thead>
<tr>
<th>Study Type:</th>
<th>Rat Developmental</th>
<th>Rat Reproductive Probe</th>
<th>Rabbit Developmental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal Plasma Concentration (µg/g)</td>
<td>24.0-35.5</td>
<td>14.3-41.9</td>
<td>16.8-25.8</td>
</tr>
</tbody>
</table>

- Species differences are **due to dynamics**, not kinetics
- Integrated TK narrowed hypotheses for MoA studies
In Vivo to In Vitro Extrapolation

Developmental Toxicity

Rat

In Vivo Studies

Molecule Y (Insecticide)

TK Data

Extrapolation

MOA/HRF

In Vitro/Ex-Vivo MoA

Humans

Not relevant to humans

MOA: Mode-Of-Action
HRF: Human Relevance Framework

Source:
2. C. terry et al. RTP, 75 (2016) 89-104
Case Example 5: Comparison to Human Biomonitoring Data

Current Risk Assessment Paradigm

- Exposure Reference Guidance Values (ADI or RfD)
- External Administered Dose levels (NOAEL)

External

- mg/kg

Internal

- mg/L

Biomonitoring Equivalent (BE)

- mg/L

Human Biomonitoring Data

Levels of chemical in Biological samples (blood, urine or tissues)

Gold Standard
Calculation of Internal Exposures- BE

• Facilitation of Biomonitoring Equivalents (BE) approach

**Figure 1.** Parallelogram illustrating the BE concept. Pathways 1 and 2 illustrate the potential uses of human and animal pharmacokinetic data to identify BE values. Figure from Hays et al. (2008b).
Urinary 2,4-D biomonitoring data in the context of the BE values corresponding to the updated US EPA RfD. Symbols indicate the median concentration in the study; bars extend to the 95th percentile.

Source: Aylward and Hays. RTP, 2015; 73 (2015) 765e769
Integration of Toxicokinetics for Agrochemicals

Factors for consideration

• Global regulatory acceptance?

• **Extent of metabolism** (could be challenging for molecules that have complex and extensive metabolism)

• Technical challenges
  • Analytical method validation
    • Consistent methods across the sectors
    • Timing of availability of methods within regulatory timelines
  • Sampling methods & techniques- advanced methods to reduce sampling amounts and stress to animals that could confound the safety data collection

• Use of PBPK and *in vitro* methods could further reduce animal use and generate data that is relevant for human health risk assessments
There are important scientific and regulatory drivers for obtaining kinetic data in conjunction with standard toxicity studies. Can be integrated with no additional animals. Integration of TK can provide valuable insight:
- Instrumental in dose level selection—KMD approaches can be more relevant for human health risk assessments than MTD
- *In vivo* to *in vitro* extrapolation
- Cross species/routes/lifestages Comparison
- Better interpretation of the data in the context of human exposure

Moves us closer to exposure-based dose setting ≡ relevance to humans
Acknowledgements

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Thank you for your attention!