Opportunities and Challenges in Using the Kinetically Derived Maximum Dose Concept to Refine Risk Assessment

Summary

On September 30, 2020, the Health and Environmental Sciences Institute (HESI), the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and the U.S. Environmental Protection Agency (USEPA) Office of Pesticide Programs (OPP) co-sponsored a symposium webinar titled “Opportunities and Challenges in using the Kinetically Derived Maximum Dose Concept to Refine Risk Assessment”. The symposium was the first public forum on scientific discussions related to the concept and applications of the kinetically derived maximum dose (KMD). Over 450 multi-sector participants from 22 different countries attended this symposium.

The current definition of a KMD refers to the dose above which the systemic exposures depart from being proportional to external doses. This non-linear external-internal dose relationship arises from saturation or limitation of pharmacokinetic (PK) process(es), such as absorption or metabolism. The importance of PK information is widely acknowledged when assessing human health risks arising from exposures to environmental chemicals, as PK determines the amount of chemical at potential sites of toxicological responses. Regulatory agencies often receive submissions of studies that utilize the KMD or non-linear kinetic concepts to design repeated dose animal studies, or to interpret results from these chronic studies. However, there is no agreed-upon scientific guidance that clearly specifies what types of data and analyses are necessary and sufficient, in a fit-for-purpose context, to evaluate the impact of non-linear PK on dose-response studies. In addition, there are no specific criteria on how to incorporate/integrate all available data packages, including, but not limited to, PK and exposure information to use the KMD approach as an option for top-dose selection in repeated dose animal studies.

This KMD symposium was a first step to engage the stakeholders in activities related to the use of the KMD approach. Presentations within the symposium focused on these issues, with illustrative case studies used to convey approaches and challenges. A recording of the symposium and presenters’ slides are available at https://ntp.niehs.nih.gov/go/kmd-2020. A HESI working group has also been formed to develop more consistent, transparent practices in analyzing dose proportionality, defining a KMD, and using the KMD approach to refine risk assessment.

Presentation Abstracts

Use and evaluation of KMD data at USEPA *(Anna Lowit, USEPA)*

PK refers to the study of chemical movement into, through, and out of a living organism, and it determines the amount of chemical available to elicit a toxicological response in tissues from a given external exposure. PK data can provide a stronger biological basis to reduce some uncertainties associated with interspecies, intraspecies and route-to-route extrapolations in chemical risk assessment. The USEPA OPP routinely uses PK data and information to help interpret results from laboratory toxicity studies and evaluate the relevance of these results for human health risk assessment. One such use is to adopt the KMD approach in lieu of the traditional maximum tolerated dose (MTD) in the design of chronic mammalian toxicity studies. Toxicity findings at doses above a KMD, especially when internal concentration of the toxic moiety increases at a disproportionately higher rate than increased external dose, are likely due to altered physiology and overwhelmed adaptive/repair mechanisms in animals. Thus, these toxicity findings may not be relevant to humans exposed at much lower levels. In recent years, the USEPA OPP has received multiple submissions that involve a KMD analysis, but the quality of these submissions has varied, suggesting a need to standardize the KMD approach for broad regulatory use and facilitate global harmonization.

Overview of OECD discussions on dose selection in chronic toxicity studies *(Anne Gourmelon, OECD)*

Test Guidelines issued by the Organisation for Economic Co-operation and Development (OECD) are harmonized test methods that can be used to generate data that are accepted across countries adhering to the Mutual Acceptance of Data system. Discussions on harmonization of approaches for dose selection in chronic toxicity and carcinogenicity studies have a long history at the OECD. Toxicity data have several purposes. For example, use of toxicity data for hazard classification typically requires the demonstration of adverse effects at the upper part of the dose-response curve and the detection of a No Observed Adverse Effect Level (NOAEL) via statistical analysis. On the other hand, use of toxicity data for risk assessment requires dose-response data that are relevant to human exposures by considering other pieces of evidence, such as exposure and toxicokinetics. While guidance is available on the conduct and design of chronic toxicity and carcinogenicity studies and flexibility is allowed, the design of these studies remains of matter of debate. The tension persists between regulatory authorities that would like to place the top dose at or slightly above the saturation of some kinetic process, and those that require the measurement of clear/significant adverse

effects for hazard classification. With the large number of data generated over the last decades and knowledge gathered on toxicokinetics and toxicodynamics, it should be possible to propose an optimal and standardised study design that can meet specific purposes. Options on the table include using statistics to better allocate animal numbers across four dose groups, or as a last resort, to add a dose group to avoid repeating these costly studies. A series of OECD webinars to exchange information on various regulatory needs was started in 2019. The next steps will be determined in light of the current dialogue. At a minimum, basic principles and considerations should be agreed upon to move towards better design of chronic toxicity studies.

Maximum tolerated dose (MTD): Concepts and background *(Chad Blystone, NTP)*

The MTD is used to select the highest exposure in longer term studies, such as chronic and carcinogenicity studies. These long-term exposure studies provide critical data to assess human risk. Endpoints evaluated to determine an MTD often include histopathology, absorption, distribution, metabolism, and excretion (ADME), clinical observations, and clinical pathology observed in shorter term studies. The primary focus of the MTD is on apical toxicity endpoints that provide the greatest confidence in predicting survival outcomes from long term exposure. Exposures that are too high would result in animal loss, impeding the conduct of the study; and exposures that are too low would decrease the ability to detect an effect. The power to detect an effect in animal studies is limited by group sizes and sensitivity of the animal model, and the background incidence rate may or may not be known. Maximizing exposure, under the MTD approach, increases the probability of detecting an effect under these conditions. Furthermore, the MTD provides a degree of certainty in dose response interpretation, in which marginal responses at lower exposures may be discounted without confirmation at the highest exposure. The MTD approach is a pragmatic way to evaluate potential chemical toxicity under conditions of limited numbers of animal groups, unknown animal model sensitivity, and uncertainty in dose response interpretation, where undetected effects could have larger repercussions in the wider human population.

Concepts of non-linear pharmacokinetics and KMD *(Alan Boobis, Imperial College London)*

In toxicity studies in experimental animals, external dose is typically used to extrapolate to acceptable human exposure, and hence, the interpretation of such studies is critically dependent on dose selection. The fate of administered chemicals in the body depends on ADME processes. In many cases, these involve transporters or enzymes, with saturable capacity and specificity for their substrates. In addition, only chemical that is in free solution is

available for absorption and hence the physicochemical properties of a substance, *e.g.* its water solubility, can affect the extent of absorption. All of these can lead to very marked non-linearity between administered dose and systemic exposure. Evidence from the use of human medicines has shown that such nonlinearities can be observed not only *in vitro*, but also *in vivo* in human subjects. Considerations of nonlinear kinetics apply to the active moiety responsible for the toxicity of the compound. In the case of the parent, systemic kinetics are the determinant of response. In the case of minor metabolites, the kinetics of the individual metabolite will determine the response. While saturation of its formation or detoxication may have negligible effect on the systemic kinetics of the parent, this could markedly affect the relationship between dose and response. With the move towards adverse outcome pathway-based testing, the quantitative relationship between dose, key event response and outcome is paramount to appropriate data interpretation. The maximum dose used in toxicity testing is often many orders of magnitude greater than worst-case human exposure. Limited solubility and/or saturation of ADME processes can lead to marked non-linearity between dose and plasma/active-site concentration, and hence response. This can confound interpretation of dose-effect relationships and extrapolation to human-relevant exposures; there may be substantial over- or underestimation of risk to exposed populations. Kinetic considerations are therefore essential in both study design and data interpretation.

Implications of non-linear PK in toxicity testing and interpretation of dose-response data *(Salil Pendse, Nuventra Pharma Sciences)*

Many PK processes are mediated by enzymes or transporters, and these processes may become saturated at high doses. To better understand how the saturation of a PK process influences the internal vs. external dose relationship, a case study was conducted using physiologically based PK (PBPK) modeling. A three-compartment PBPK model for rats was constructed using a customized version of the open-source modeling tool PLETHEM. Eight hypothetical chemicals were used in the simulations to represent realistic chemicals with a wide range of physical-chemical properties and metabolic constants. Quantitative structure-activity relationship (QSAR) models built into PLETHEM were used to predict partition coefficient values for each of the chemicals. Three saturable kinetic processes, described as Michaelis-Menten kinetics, were investigated one at a time: saturation of parent metabolism, saturation of metabolite clearance, and saturation of oral absorption. In all cases, the simulation results showed that the area under the curve (AUC) of parent or metabolite was proportional to external doses at very low doses. In cases where the metabolism of the parent was saturated, the parent AUC showed transition to a non-proportional domain. However, the parent AUC remains

proportional to external doses when the clearance of metabolite was saturated. Both AUCs of parent and metabolite reached a plateau when oral absorption was saturated. Given the same saturable PK process, the AUC vs. external dose relationships were different for the parent compound, the metabolite, or the sum of both, suggesting that knowing the toxic moiety is critical when determining a KMD.

Determining an inflection point from external-internal dose data *(Philip Villanueva, USEPA)*

Currently, there is no guidance on how to analyze data on external-internal dose levels to determine at which measured or statistically determined external dose levels the internal doses are significantly nonproportional to external doses (*i.e.*, KMD). Various methods exist (such as comparing fold differences used to estimate KMDs), but these do not necessarily use the full dataset (such a relationship is fit based on the average internal concentration for dose groups), quantitatively consider potential limitations of the data (such as observed variability within dose groups), nor provide robust estimates and confidence bounds for KMDs. Using a case study, we explored piecewise regression as a statistical tool for determining when the relationship between internal doses and external doses significantly depart from proportional based on data submitted to the USEPA. Piecewise regression can incorporate individual observations and the observed variability, model a regression relationship using all dose groups, and provide statistical tests to determine if there is significant departure from an assumed proportional relationship between external and internal dose. In collaboration with subject matter experts in toxicology and pharmacokinetics, who provide critical insight into biological relevance and plausibility of any models being fit, statisticians can translate questions about data characteristics into mathematical and testable statements. Any statistical analysis should appropriately transform the data to meet any underlying assumptions of the statistical analysis and attempt to incorporate all dose groups and individual observations to appropriately characterize the variability and modeled relationship. For piecewise regression, it is also necessary to ensure the modeled relationship is approximately linear and that the variances are normal and heterogeneous across dose groups. Future work could include additional case studies and statistical simulations to determine the optimal number of dose groups, number of animals per dose group, and to evaluate other potential regression models or statistical tools for estimating KMDs.

Estimating human exposures and comparison to doses used in testing *(Jeff Dawson, USEPA)*

Monitoring and modeling approaches used to assess potential human exposure levels for different areas of chemical space were presented. The intent of this effort was to provide a basis for understanding how exposure information can inform the use of KMD. Exposure assessments are based on fit-for-purpose, scientific approaches, as well as how they can be tailored to meet specific requirements of a risk assessment while accounting for resource availability. To augment confidence in using KMD, it is critical to understand exposure patterns, levels, and the uncertainty and variability associated with them. A more illustrative example using USEPA pesticide information was developed, with exposures predicted using publicly available calculators for regulatory scenarios associated with occupational and consumer uses of pesticides in the U.S. (a total of 923 predictions). Several other examples were also developed, based on monitoring and modeling approaches to highlight different types of exposure scenarios and exposure assessment purposes, including the U.S. Occupational Safety and Health Administration monitoring data (~2.3 million monitoring events), the USEPA ChemSTEER model for predicting exposures for industrial chemicals, and screening-level European Union modeling approaches to predict upper bound exposure levels. In all examples, predictions were found to be generally much lower than the summarized hazard data (such as NOAELs and lowest adverse effect levels). Thus, KMD values are anticipated to be orders of magnitude higher than human exposures in a chemical lifecycle. In summary, exposure assessments are varied, and many rigorous tools are globally available. Exposures are unlikely to approach KMD levels based on the analyses completed to date.

Dose-setting and considerations for the 3Rs *(Fiona Sewell, NC3Rs)*

For scientific, ethical, and business reasons, it is critically important that appropriate doses are selected for repeated-dose regulatory toxicology studies that are conducted in animals. However, there is a lack of clear agreement on how to evaluate data and approaches to determine the top dose for these studies. Selection of top doses that are too high or too low have a negative impact on the 3Rs (reduction, replacement and refinement of animal uses). Dosing that is too high may lead to unnecessary animal suffering, unreliable results, and/or data that may not be relevant to realistic human exposures but may still trigger additional *in vivo* studies to explore observed toxicities. Conversely, doses that are too low can mean that repeat studies may be requested from some regulatory authorities in order to demonstrate toxicity, using additional animals. It is critical to get the balance right and ensure the most scientifically

appropriate doses are selected to add the most value, while considering the 3Rs. Toxicokinetic (TK) data can be used to help inform dose selection, so that toxicity studies are conducted with the considerations of human relevance and 3Rs. However, there is limited guidance on how TK data should be incorporated in the design of these studies. Incorporating TK data does not necessarily require the use of additional animals. For example, the use of microsampling has gained acceptance as the standard blood sampling method for rodent TK data without the need for additional TK satellite groups. With the increased interest in exposures to determine the hazard/risk assessment for chemicals, the adoption of microsampling would facilitate provision of more TK data while applying the 3Rs.

Integration of TK into toxicity studies and dose level setting in repeat dose studies *(Jeanne Domoradzki, Corteva)*

TK integration into toxicity studies starts with determining kinetic parameters early in the safety testing program. Probe ADME studies are conducted, and the time-course blood, plasma, and urine samples are collected to determine appropriate biomarkers (parent and major metabolites) to be monitored in future repeated-dose toxicity studies. In repeated-dose studies, additional TK data are generated at steady state to understand the systemic exposure to the test material. The integrated TK data obtained across toxicity studies follow 3Rs principles (without the use of additional/satellite animals) and provide critical information to understanding differences in response across doses, species, strains, sexes, and life stages. In cases where nonlinearity of the dose–response curve can be identified, the high doses for the subsequent longer-term studies can be selected based on the KMD and apical endpoints. In other words, both TK and toxicodynamics are considered to better inform dose selection in longer-term studies so that results from these studies are relevant to human exposures. Determining the KMD has been the subject of international debate, and several examples of determining dose proportionality (AUC vs. dose) and selection of dose levels for chronic studies are presented to demonstrate how both TK and toxicodynamics data are incorporated in the process for more informed selection of doses.

Integrating KMD/TK Data with MoA and Other Information in a Weight of Evidence Approach *(Harvey Clewell, Ramboll US Consulting, Inc.)*

Typically, the basis for a KMD is evidence demonstrating a nonlinearity in the relationship between administered dose and blood concentrations in animal studies. However, a nonlinearity in kinetics, by itself, may not be sufficient to support a conclusion that effects observed in animal studies at doses above the KMD would be irrelevant to human exposures at lower doses. The

presumption that there is a dose-dependent transition in the mode of action (MoA) for toxicity associated with exceeding the kinetic nonlinearity needs to be supported by additional mechanistic evidence. Some examples of evidence that could support the application of a KMD to limit dosing in animal studies include saturation of absorption that limits the potential for systemic toxicity; saturation of metabolic activation that limits the production of a toxic metabolite; and saturation of a protective detoxification pathway that enhances production of a reactive metabolite. *In vitro* metabolism studies are particularly useful for supporting the use of a KMD by identifying concentrations associated with a nonlinearity in metabolism in both the experimental animal and human. Quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) using the metabolism parameters identified in these studies can then be used to determine human equivalent KMDs. In the future, KMDs can be identified and supported using only *in vitro* studies: *in vitro* metabolism studies coupled with QIVIVE to identify a KMD and *in vitro* assays (e.g., glutathione depletion) to provide MoA information in support of applying the KMD to limit dosing. Moreover, *in vitro* studies could be used to identify a dose-dependent transition in the MoA for a chemical that could serve as a basis for determining a maximum relevant dose to limit animal studies even in the absence of a KMD.