Questions and Comments Received From Webinar Attendees Directed to Specific Speakers: Alan Boobis or Anne Gourmelon

Q: Maximum tolerated dose and kinetic maximum dose (KMD) are arbitrary threshold concepts, both determined following a weight-of-evidence assessment of gradual, experimentally determined dose-related changes in toxicokinetics (TK) and toxicodynamics (TD). We recognize there are differences in development and confidence in use for the two approaches. However, scientifically, considering potential interspecies qualitative and quantitative differences in TK and TD, are the two approaches equivalent in underestimating or overestimating human health protection goals (hazards/safety), or is one intrinsically stronger and why?

A: It could be argued that KMD is intrinsically stronger, as it is more based on mode of action. Maximum tolerated dose is empirical and takes no account of the determinants of the dose, specifically whether they have any relevance to the human exposure scenarios. KMD requires knowledge of the kinetics of the compound, and hence provides for the opportunity to consider relevance to human exposures.

Questions and Comments Received From Webinar Attendees Directed to Specific Speakers: Alan Boobis

Q: Are the terms "bioavailability" and" internal dose" interchangeable?

A: No. Bioavailability is unitless with a value between 0 and 1, with bioavailability for oral and other routes expressed relative to i.v. bioavailability, which is considered to be full bioavailability with a value of 1. Internal dose is an actual dose/amount, usually expressed as the amount entering systemic circulation.

Q: The current practice is maximum tolerated dose (MTD) plus mechanistic studies to show whether the findings are caused by saturation. The kinetic maximum dose (KMD) approach cannot detect low incidence of cancers (or other chronic effects) in lab animals if the default continues to be a group size of 50. Is there a good way to compensate for this, other than using higher doses such as the MTD in studies? As we know, epidemiological studies have even lower powers.

A: Study design optimization approaches, including Bayesian adaptive study designs and other Bayesian study design optimization approaches, can be used to determine how to optimally assign animals to dose groups, and which doses we should use in order to identify optimal doses. There is often a presumption that KMD will result in the use of lower maximum doses. That is not correct. If kinetics is linear at the top dose, it is possible that the KMD will be the same as the MTD. When the KMD (if appropriately selected) is lower than the MTD, this implies information at the MTD is not informative of hazard or risk to humans.

Q: Some deduction from my side elaborating on your key messages. If we are serious about predicting human and environmental health effects with any tool we have now in our hands as a result of advances in scientific knowledge over the last 30 years, we should not ignore in our test batteries methods that give information on polymorphism, induction, and inhibition of Phase I and Phase II biotransformation enzymes to be able to interpret dose-response relationships of new chemical entities.

A: Fully agree, with appropriate toxicokinetic (TK)/toxicodynamic considerations, so that, for example, the *in vivo* toxicological consequences of polymorphism of enzymes of metabolism are interpreted appropriately.

Q: For efficacy considerations of a drug (i.e. half-maximal inhibitory concentration [IC50] of an enzyme), is the free fraction of a drug in plasma predictive of the free fraction in a target organ?

A: The free fraction in plasma will predict the free fraction in a target organ, but only if there is no active transport into or out of the target tissue (which takes place, for example, for paraquat in renal and lung cells).

Q: What experiments do you think should be added to standard testing packages for pesticides or chemicals that would help inform the TK profile/KMD approach?

A: I think there is a role for *in vitro* characterization of enzyme specificity and kinetics (and transporters, if appropriate) in humans and relevant test species.

Q: Can we anticipate enzyme saturation as a key toxic effect and in lieu of overt toxicity?

A: I have never been a fan of treating enzyme saturation as a toxic effect *per se*. It is a biochemical phenomenon and relates to kinetics. The consequences depend upon the concentration-effect relationship, which is a toxicodynamics question.