A Representative Approach to Investigate In Vitro Species Differences in Crop Protection Active Ingredient Metabolism

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INTRODUCTION

The scientific opinion of the European Food Safety Authority (EFSA) on the proposed design of in vitro comparative metabolism (IVCM) studies to evaluate pesticide active substances was reviewed by CropLife Europe (CLE) member experts. The study requirement originates from Commission Regulation (EU) No 283/2013. The IVCM study objective 'is to evaluate whether all metabolites formed in the human in vitro test system as a surrogate of the in vivo situation are also present at a comparable level in animal species tested in toxicological studies, and, therefore, if their potential toxicity has been appropriately covered by animal studies'. EFSA proposes a study design which uses hepatocytes for identification of Unique Human Metabolites (UHM) and Disproportionate Human Metabolites (DHM), investigates 5 different species, and proposes a 3×3×3 sampling matrix per each species, with the assumption that the capabilities of in vitro test system sufficiently mimic the in vivo situation. In addition to the detailed description of experimental set-up, the opinion expands to include a description of toxicity testing and risk assessment and extensive discussion on future trends.

METHODOLOGY

The member experts identified seven major areas of concern 1) Legal Requirements – The regulation 283/2013 has no reference to DHM; 2) DHM assessment – the proposed design may not be suitable to address a DHM situation due to the inherent limitations of the in vitro system, and (if required) should consider existing knowledge of in vivo metabolism and apply a weight of evidence approach; 3) Definition of an identification threshold is needed – 5 % of applied radioactive dose; 4) Experimental set-up – The proposed extensive 3 × 3 × 3 approach may not be suitable; 5) Long-term incubations for slow-metabolising compounds – Option to waive based on in vivo metabolism information; 6) Timing of comparative in vitro studies – Needs to allow flexibility in timing of the studies for different registration strategies, and 7) Recommendations for the future – Topics (e.g. reactive metabolites, isoform specific metabolism, stereoisomers, and PBK modelling) should be considered for a separate opinion.

RESULTS

Industry experience exists for IVCM studies in microsomes & hepatocytes to identify UHMs following Whalley et al 2017. A representative approach to investigate in vitro species differences in metabolism is proposed. Stepwise, a pilot experiment using cryopreserved hepatocytes from humans & another appropriate species (e.g. rat) at 1 or 2 incubations (1-20 µM, ~105 dpm) & multiple timepoints (e.g. 5, 30, 60, 120, 240 min) is conducted. Additional endpoints include cell (Trypan) & positive control ECOD for viability. The pilot phase is key to establishing analytical (14C & LC/MS) methods. Based on the pilot results, a definitive experiment with multiple species (human, rat, mouse, rabbit & dog) is designed using a single incubation concentration, 2 time points to cover changes in early & total metabolite formation (e.g. 30 & 240 min). Pilot & definitive experiments always include assessment of non-specific binding, positive & negative controls, extraction efficiency & recovery. Detection limits will be case specific, but the goal is to identify human metabolites formed at $\geq$5 % of the radioactive dose whether or not they are detected in other species, with detection to as low as 1 %.

DISCUSSION

This representative approach will be demonstrated by use of example data. A weight of evidence approach and understanding of the metabolic pathway is important to cross-species comparison since Phase I and Phase II activity is known to differ among species. A flexible experimental approach is needed to account for chemical specific properties and recognise the limitations of relying on in vitro system. Identification of a UHM after consideration of the aforementioned points will require further investigation and potential toxicological evaluation.