Building scientific confidence in metabolic similarity in read-across through the use of *in vitro, in silico* and analytical data

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The underlying principle of read-across is that the biological activity of a chemical is inherent in its molecular structure. Analogues are typically identified by structural similarity then evaluated on the basis of their bioavailability, reactivity and metabolic similarity. While structural similarity is the major tenet in read-across, a critical consideration is whether structural differences impact biological activity. This source of uncertainty can potentially be addressed with toxicokinetic (TK) information. Here we report progress on a case study to investigate the feasibility of using *in vitro* high-throughput metabolism experiments in concert with *in silico* metabolism predictions to substantiate biological similarity to enable quantitative read-across. Parent chemicals were incubated with a suspension of primary rat hepatocytes. Possible metabolites were predicted i*n silico* using expert systems (Meteor Nexus and the simulators within TIMES). Suspect screening analysis was performed using liquid chromatography mass spectroscopy (LC/MS) and the *in silico* predicted metabolites. Four target chemicals representing different read-across scenarios were identified: 2 proof-of-concept examples and 2 test cases. Candidate source analogues were identified based on structural similarity and information availability for the two proof-of-concept chemicals (similar metabolism (methyl eugenol & estragole) and different metabolism (2-nitrotoluene & 4-nitrotoluene)), whereas for the two test cases, the experimental and *in silico* results will be integrated to substantiate the validity of the source analogues to inform selection of the most appropriate analogue. To date, *in silico* metabolism predictions have been generated for the proof-of-concept chemicals and compared to the *in vivo* metabolic profiles reported in the literature to assess their agreement. The metabolism predictions from the different tools complemented each other in capturing the primary pathways, and in particular the pathway that has been associated with the genotoxicity (nitrotoluenes) but no tool correctly captured all the metabolites observed. *This abstract does not necessarily represent US EPA policy.*