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Letter to the editor:

WHICH CONCENTRATIONS ARE OPTIMAL FOR *IN VITRO* TESTING?

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Dear Editor,

Currently, many research activities in the field of *in vitro* models focus at predicting human organ toxicity, e.g. of the liver (Gomez-Lechon et al., 2014; Grinberg et al., 2014, 2018; Frey et al., 2014), kidney (Li et al., 2013, 2014; Sjögren et al., 2018; Sjögren and Hornberg, 2019), heart (Nemade et al., 2018; Chaudhari et al., 2018) or developmental toxicity (Krug et al., 2013; Waldmann et al., 2014; Shinde et al., 2017). All studies face a similar challenge, which is the choice of concentrations for *in vitro* testing (Leist et al., 2017). This question has recently been discussed in an editorial of the Archives of Toxicology, where the authors pointed out that *in vitro* tests are usually performed at a concentration range around and above the plasma peak concentrations (C_{max}) of a drug in humans (Hengstler et al., 2020). A typical strategy is to test relatively high concentrations, often 20- or even 200-fold higher than the human plasma C_{max}. The choice of high concentrations was justified by the observation that usually higher concentrations are required in the culture medium to induce cell damage compared to the C_{max} that is known to cause adverse effects *in vivo*. We made a similar observation in a recent study using human hepatocytes (Albrecht et al., 2019; Gu et al., 2018). The factor by which *in vitro* concentrations have to be higher than the corresponding *in vivo* plasma concentration in order to cause similar biological effects in the target cells is the *in vitro–in vivo* scaling factor.

However, it should be considered that it is not yet clear if all compounds require identical *in vitro–in vivo* scaling factors. It cannot be excluded that e.g. compounds, whose toxicity depends on specific metabolic pathways may require higher scaling factors than compounds that do not require bio-activation. Moreover, scaling may also depend on the mechanism of toxicity (Hengstler et al., 2020). To gain more insight into the requirements of optimal scaling it is not helpful to test only one or two concentrations, e.g. 20- and/or 200-fold the C_{max} and on this basis decide if a compound is positive or negative in the *in vitro* assay. Rather a concentration-dependent test with not too high dilution factors, such as 2- or at most 3.16-fold is helpful to be able to precisely determine the concentration when toxic effects occur, expressed e.g. as EC_{10} or EC_{50}. This is a precondition to be able to elucidate if groups of compounds categorized e.g. by metabolic activation or mechanism of action require different scaling factors. A limitation to be overcome in the future is that too few studies established high quality, reproducible concentration response relationships for sufficiently high numbers of test compounds that allow a systematic comparison to the human *in vivo* situation.

Conflict of interest

The author declares no conflict of interest.

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