



In vitro, ex vivo, in vivo toxicology, the terminology issue



The science of toxicology more and more moves away from the use of animal models, now focusing to a great extent on experimentation on a lower level of biological integration than intact organisms, preferably from human origin. This move was strongly intensified after the seminal report of the US National Academy of Sciences, “Toxicity testing in the 21st century: a vision and a strategy”, now ten years ago (NRC, 2007). The advantages of the approaches, with the emphasis on the study of mechanisms of toxicity rather than apical endpoints have been highlighted since then in many reports, thus emphasizing the role of in vitro methodologies in our discipline (Blaauboer et al., 2012; Wilmes et al., 2013). Since these studies can be combined with in silico models for the biokinetic behaviour of compounds (Tsaouin et al., 2016), this also can play an important role in the applied science of toxicity testing, and thus in risk assessment procedures in the near future (DeJongh et al., 1999; Louisse et al., 2010; Blaauboer et al., 2016).

However, one should also be aware of the reductionist nature of in vitro methods (Hartung, 2007). The physiological conditions in such a system may be quite different from the in vivo situation. Moreover, it is also clear that the exposure of the toxicological target might be different and for this reason a proper evaluation of the biokinetics in the in vitro system and the differences therein with the in vivo conditions should be taken into account (Groothuis et al., 2015; Kramer et al., 2015).

This development of in vitro toxicology has a history that goes hand in hand with the vast development of cellular and molecular biology (Blaauboer, 2015). Thus, since this is not a new development (the journal Toxicology in Vitro had its first issue in 1987) one might expect that the term in vitro toxicology would be well established. However, it appears that there is some confusion regarding the terminology used for in vitro toxicology experimentation. One term that is also used and adds to the confusion is “ex vivo”. Therefore, some clarity is needed and it is my intention to give clear descriptions in this short paper.

It may be good to define the terms in the context of two different processes:

- 1) the process of the toxic interaction of the chemical under study; and
- 2) the process of the measurement of a phenomenon.

In vivo

When performing an in vivo experiment, it is clear that the toxic interactions take place in an intact organism, and thus in the context of the physiology of that organism. The measurement of the adverse effect(s) (by determining the value of a biomarker, e.g. physiologically, biochemically, haematologically or histologically) can take place either inside the organism (in vivo measurement: e.g. blood pressure, neuronal activity, etc.) or on samples taken from the organism (blood or tissue samples). The latter can be considered an ex vivo measurement.

In vitro

When the experiment is performed in vitro, this will mean that the toxic interaction appears in the physiological context of the in vitro system. As mentioned above, there most probably will be wide differences when compared to the in vivo physiology. Thus, this context defines the in vitro system.

If we now consider the following case: the treatment of animals with an inducer of one or more cytochrome P450 enzymes, followed by the isolation of hepatocytes from these animals and the use of these cells to determine the effect of a drug. Following the reasoning that the physiological context is the leading principle, this case in effect describes two different experiments: one in vivo - the effect of the inducer - and one in vitro experiment - the effect of the drug.

When evaluating (or validating) the in vitro system for its meaningful value in determining the relevant toxicological effects, one needs to take these differences into account. It will be clear that the relevance of an in vitro study will be more optimal if the physiology as well as the exposure conditions for the toxicological target (now often described in terms of the molecular initiating event) will be as similar as possible when compared to the in vivo situation. Indeed, this would better allow the interpretation of the in vitro data in the in vivo context, in a quantitative in vitro-in vivo extrapolation (QIVIVE; Yoon et al., 2015).

A good example is the evaluation of compounds affecting the thyroid system, as done by Murk et al. (2013). For most of the different steps of possible interaction with the complete thyroid system this study showed that there are well-described in vitro systems available. It was also

acknowledged that these in vitro systems in isolation could not give the full picture of a possible effect, including its quantification, given the fact that any feedback loops are not taken into account. The possibility of modelling these in vivo-related conditions in a physiologically-based dynamic computer model is a vast challenge for the future. The development of so-called ontology descriptions of biological systems (Hardy et al., 2012), including descriptions of Adverse Outcome Pathways can be of help here.

In summary, I would argue that in vitro toxicological experiments are defined by the fact that the toxic interaction takes place in the physiological conditions of the in vitro system. This then implies that there is no such thing as an ex vivo experiment, only ex vivo measurements of biomarkers after the toxic interaction has taken place in an in vivo situation.

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Conflict of interest

none.

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