Letter to the editor

The ‘μEST’ in vitro test for embryotoxicity – Validated and endorsed or not?

I feel bound to draw the attention of readers of Toxicology In Vitro to inconsistencies in publicly available information with regard to the validation status of the commercially-available in vitro ‘μEST’ test for screening chemicals for embryotoxicity. This test was developed and is marketed by a German-based biotechnology company, Axiogenesis A.G.

The following information about the validation status of ‘μEST’ is in the public domain. The European Centre for the Validation of Alternative Methods (ECVAM) has assessed the validity of ‘μEST’ (although, at the time, the test was referred to as R.E.Tox®) as a catch-up test to the embryonic stem cell test (EST) (www.axiogenesis.com), which itself had been previously validated, and independently endorsed by the ECVAM Scientific Advisory Committee (the ESAC; (Scholz et al., 1999; Genschow et al., 2004a; ESAC, 2002). However, following validation of ‘μEST’, the ESAC decided that the method and its validation could not be endorsed following an initial review of submitted information (ESAC, 2007a), and again, after further consideration, stating that: ‘on the basis of the data submitted for peer review, additional data provided and a careful re-examination of all the submitted documents, the ESAC has not endorsed the scientific validity of the RE Tox assay’ (ESAC, 2008).

Nevertheless, at the time of writing, and for the previous several months, the Axiogenesis website states: ‘μEST is a catch-up development to the “Embryonic Stem Cell Test” that has been approved as scientifically valid by the European Center for the Validation of Alternative Methods (EVCAM).’ In support of this claim, the website cites the ESAC statement for the EST (ESAC, 2002). In view of the close similarity between the acronyms for the two test methods, it is my personal view that, while it cannot be claimed that Axiogenesis is intent on mis-representing the facts about the validation status of its test, the uninitiated reader of the Axiogenesis website might be forgiven for believing that ‘μEST’ is a test that is sufficiently similar to the EST as to be considered valid for embryotoxicity testing. Clearly, however, the later, negative ESAC statement on R.E.Tox® (given above) applies to ‘μEST’ because they are actually the same assay.

What is one to make of this apparently conflicting information? The facts are simple in themselves: ‘μEST’ was subjected to a validation study, but the study was not endorsed by the ESAC. On the other hand, the website of the company who developed the test includes a statement that, while literally not being incorrect, is sufficiently vague as to be potentially open to the interpretation that the test has been accepted by ECVAM and the ESAC as being validated. In other words, the website statement could be interpreted as saying: ‘μEST has been approved as scientifically valid by the European Center for the Validation of Alternative Methods (EVCAM).’ Although, it is true that: (a) ‘μEST’ was subjected to a validation study as a catch-up method to the EST; and (b) the validation study of the EST was endorsed by the ESAC, the corollary that the validation study of ‘μEST’ can therefore be considered as also independently endorsed does not follow.

This situation is a serious one since independent ESAC endorsement of a validation study for a new test method is likely to facilitate its subsequent regulatory acceptance and adoption by the scientific community. This is because one of the roles of the ESAC is to provide impartial scientific advice to ECVAM. Independent peer review and endorsement is a crucial and integral part of the validation process, designed to avoid any undue influence on the outcome of studies by vested interests. However, further discussion concerning the scientific merits and validation status of ‘μEST’ is stifled by the fact that, unfortunately, there is very little information relating to the test in the public domain. Thus, I can only find one reference in the literature concerning the test method (see Schwenberg et al., 2005). In addition, the results and discussions of neither the validation study nor the independent ESAC peer review have been published, whereas publication is commonplace in the case of successful validation studies, as indeed the pages of this journal amply demonstrate.

As someone who was closely involved in the ESAC peer review of ‘μEST’, I might well be considered to be biased. I know, for example, that the peer review panel appointed by the ESAC for the assay considered the test sufficiently different from the EST as to question the justification for treating it as a catch up study. I also know the reasons why the validation study was not endorsed on scientific and procedural grounds, but I cannot divulge these reasons since the information remains confidential. In addition, I am aware that ECVAM was advised to suggest to Axiogenesis that it publish the methodology involved, and the results obtained with, the ‘μEST’. However, this does not appear to have happened.

Not unnaturally, I should be prepared to defend the decision of the ESAC peer review panel. However, I should much rather there be a situation in which others could judge for themselves the test method, as well as the design, analysis and outcome of the validation study, and the ESAC peer review itself. To this end, I strongly recommend that all validation studies are published, as well as peer reviews. This would also help companies and test developers to make clear and unambiguous claims concerning the validation status of their test methods. Usually, the results of validation studies are published either before, or shortly following the peer review process. A recent example of this is the validation study of the in vitro methods for skin irritation (ESAC, 2007b; Spielmann et al., 2007). Moreover, it is the practice of ICCVAM in the USA to publish the detailed results of peer review studies, and to identify those involved (see, for example ICCVAM, 1999).

It is unusual for a validation study not to be endorsed. However, when endorsement is withheld, I firmly believe that publication of
studies and peer reviews should still occur, for the sake of transparency, and to safeguard the validation process itself. Surely, this is in the best interests of all concerned, particularly test developers, regulators and bodies such as ECVAM, charged with assessing new test methods. In this regard, I am disappointed that a recent workshop on optimizing the post-validation process (Bottini et al., 2008) failed to fully address this problem, merely stating in one of its recommendations what is already usually the case, that: ‘the outcomes of validation studies should be widely disseminated...’ The current situation in which validation studies and their peer reviews are not published can lead to misinterpretation regarding the validation status of a test, and this should be avoided if at all possible. Lastly, it is important that the performance of validated tests is subjected to continuous review, in the light of new data. For example, the EST was recently shown to have a relatively high false-positive rate for a number of chemicals involved in the pharmaceutical industry (Paquette et al., 2008). These authors, however, used a slightly different protocol from the one that was validated for the EST, and the effects of these differences, as well as the predictivity of the test, need to be explored further.

References


ESAC, 2008. ESAC statement on the RETox assay for embryotoxicity testing. ATLA 36, 14.


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