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IIVS WEBINAR SERIES

Integrated Testing Strategies for *In Vitro* Skin Sensitization Testing

Answers to Questions Received During the November 14th Webinar

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Q: If I need a quick screen on my ingredients, what approach should I take to testing them?

A: ***AN:*** One option is to do in silico assessment and a prescreen with KeratinoSens and DPRA, which are the cheaper two assays, and only go into more expensive h- CLAT in case the first two tests are contradictory.

SK: The design of a screening approach depends on the purpose. I.e., the number of test substances to be compared, the nature of test substances to be compared (e.g., any sensitization data on related substances available; consequence of positive test, etc). E.g., of certain compound classes it may be OK to have a sensitizer, but for others this may result in the termination of development.

Q: For the Keap 1 assay, you're testing the reactive molecules. How can you make sure it is not the free radicals? That might create false positive results. Because free radicals will not covalently bind to the protein.

A: ***AN:*** In some cases false-positive due to generation of oxidative stress can be anticipated. If the free radicals are exogeneous organic molecules, it is likely that they act as true sensitizers. See work of Kao et al. on radicals formed from Linalool.

Q: In the h-CLAT is detection by flow cytometry required? Can an ELISA type test detection also be used?

A: ***SK:*** The dendritic cell line activation tests that have been included in validation programs, are all based on flow cytometric analysis cell surface markers. Although it may be technically possible to use detect CD54 and CD86 using an ELSIA based method, this would change the assay and most likely prediction model. In particular disintegration of the cells and cell surface markers released would require special attention.

Q: Are efforts in place to extend testing to hydrophobic molecules? What strategies are being utilized?

A: ***AN:*** A certain hydrophobicity is possible, and then chemicals can be tested at somewhat lower concentrations. Thus many hydrophobic materials can still be tested at 0.5 mM in the 1%DMSO/1%FCS medium used in KeratinoSens. The 25% Acetonitrile in DPRA also allows testing many molecules. Above a certain cLog P (around5) it gets more difficult.

Q: Can RHE models be utilized for KeratinoSens type testing? What is the state of this art?

A: ***AN:*** Several laboratories have proposed RT-PCR based testing of Nrf2-genes in RHE models. A detailed comparison on benefit of this approach vs. monolayer approach has not been published. It is generally assumed that it gives better potency information due to topical application and that it may allow testing of non-soluble materials. However, the detailed analysis has not been made / published.

Q: What is the strategy for non-water soluble materials (including mixtures)?

A: ***AN:*** For Mixtures I would propose to make a fractionation, characterize the fractions and test them at lower concentrations.

SK: In addition to using different vehicles and dilutions of a poorly water soluble test substance in the aqueous systems, the RhE models currently under development may prove useful for poorly water soluble test substances.

Q: What is the availability of these tests? By that I mean who is actually providing these testing?

A: ***AN:*** for KeratinoSens there is currently transfer being made to 2 -3 European contract labs which will set up the test in early 2014. In the US IIVS is offering.

SK: Without having a complete list at hands, h-CLAT is offered by Bioassay (Heidelberg, Germany). Depending on lab capacities BASF conducts toxicological studies (including the in vitro sensitization tests) for external partners. Further a ring trial study for the LuSens is currently being conducted with the participation of several CROs. Hence this assay will be available shortly.

KN: IIVS currently offers the KeratinoSens assay and plans to offer the DPRA upon successful completion of assay transfer including an analysis of within lab and between lab reproducibility for the chemicals that were evaluated as part of the ECVAM pre-validation study. It is expected that the DPRA will be available in the first quarter of 2014. IIVS is also considering adopting the h-CLAT assay. As a comment during the webinar Q & A Charles River (UK) noted that they plan to offer the DPRA and PPRA (a modified version of the DPRA) in 2014.

Q: What are the cost comparisons between the integrated in vitro strategy and the LLNA

A: ***AN:*** Detailed costing can be done by IIVS. One option is to do in silico assessment and a prescreen with KeratinoSens and DPRA, which are the cheaper two assays, and only go into more expensive hClat in case the first two tests are contradictory. While LLNA tests are not becoming cheaper with scale, these assays will especially give cost advantage if e.g. SAR is made on multiple chemicals, as the assays inherently are designed for multiplexing.

SK: Based on the costs of the individual assays, the costs of the three in vitro tests used in the weight of evidence approach are about comparable to that of the LLNA.

KN: Based on costs of KeratinoSens and estimated costs for DPRA and h-CLAT the cost of running the 3 assays would be similar (perhaps a little lower) than the standard LLNA

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Q: Do you know the status of the OECD guidelines?

A: ***AN:*** Both the DPRA and KeratinoSens guidelines have been drafted and are being submitted to OECD.

SK: The h-CLAT has been included in the OECD work plan for the test guidelines program in 2013.

Q: Do you know of the progress using IL1-8 ELISA assay in RHEs for sensitization

A: ***AN:*** Up to date a small set of chemicals have been tested. These are the classical chemicals which are also nicely predicted by DPRA, KeratinoSens and h-CLAT. There is now a need to determine whether the RHE – IL-18 approach successfully can detect poorly soluble materials and the big question is how much it adds to potency assessment. A large database of chemicals needs to be tested to make the statistical assessment on the latter question.

Q: It is reasonable that at the low boiling point, the log p will not play a role. Have you ever tried to categorize the boiling points, such as low, medium and high? If the boiling point is high, the log P should play a role in the bioavailability

A: ***AN:*** This has not been done so far. Of course cLofP has a relation to bioavailability – question which is still under debate is how much bioavailability affects potency.

Q: Are these assays utilizable for use with chemicals/powders that really aren't soluble?

A: ***AN:*** Completely non-soluble materials cannot be tested. However, if it is about testing of impurities (e.g. residual monomers in unsoluble polymers, for example in medical device materials) there should be a window of opportunity.

SK: Bioavailability may be too poor in the cell based assays. Also, interferences may be observed with the detection systems (e.g. when testing particles in the flow cytometry based assays). When obtaining negative results with this kind of materials (or extracts as done for medical devices), it will be critical to show that the test substance was tested at sufficiently high concentrations.

Please contact Kimberly Norman to place a study at IIVS or
for information on the skin sensitization assays.
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