# Can currently available non-animal methods detect pre and pro-haptens?

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Predictive testing to identify and characterise substances for their skin sensitisation potential has historically been based on animal tests such as the Local Lymph Node Assay (LLNA). In recent years, regulations in the cosmetics and chemicals sectors has provided a strong impetus to develop and evaluate non-animal alternative methods. The AOP for skin sensitisation provides a framework to anchor non-animal test methods to key events in the pathway to help identify what tests can be combined together to generate the potency information required for risk assessment. The 3 test methods that have undergone extensive development and validation are the direct peptide reactivity assay (DPRA), the KeratinoSens™ and the human Cell Line Activation Test (h-CLAT). Whilst these methods have been shown to perform relatively well in predicting LLNA results (accuracy ~ 80%), a particular concern that has been raised is their ability to predict chemicals that

need to be activated to act as sensitisers (either abiotically on the skin (pre-hapten) or metabolically in the skin (pro-hapten)). The DPRA is a cell free system whereas the other two methods make use of cells that do not fully represent the *in vivo* metabolic situation. Based on previously published datasets of LLNA data, it has been found that approximately 25% of sensitisers are pre- and/or pro-haptens. This study reviewed an EURL ECVAM dataset of 127 substances for which information was available in the LLNA and the three non-animal test methods and found that 22% of sensitisers needed to be activated. The majority of these sensitisers were pre-haptens and were generally correctly identified by the 1 or more of the 3 test methods. Only 6 substances were categorised exclusively as pro-haptens but these were correctly identified by one of the cell based assays with the h-CLAT detecting the majority. The analysis showed that skin metabolism is not a major consideration for assessing skin sensitisation potential and that sensitisers requiring activation can still be identified correctly using one or more of the non-animal test methods currently available.