Proposing alerts for 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 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)). This study reviewed an EURL ECVAM dataset containing 271 substances for which information was available in the LLNA and for one or more of the three non-animal test methods. The chemical structures of the substances were inspected and each assigned to a reaction mechanistic domain. Fifty-three substances were expected to require activation. Plausible reaction pathways were considered for each of the substances from which three structural alerts were hypothesised: autoxidation to hydroperoxides, aromatic ortho and para-diamino or di phenol derivatives, and aromatic meta-diamino/hydroxy derivatives. For each alert, the available non-animal test data was compared with the LLNA results to understand whether one or other test method was more predictive for these specific substances. Eleven substances were identified as likely to undergo autoxidation resulting in the formation of hydroperoxides. The performance of the 3 methods for these substances was very mixed with no clear pattern. This was anticipated since the test results are very dependent on the actual test sample and similar mixed findings have been found with LLNA data. Twelve substances that fell within the scope of being an aromatic ortho

and para-diamino or diphenol derivative were identified. They all were categorised as pre and/or pro-Michael acceptors. All were correctly identified as sensitisers by any of the test methods. There were 12 substances within the Aromatic meta: diamines, aminophenols, di-phenols, and aromatic monoamines alert. This alert comprised 4 aromatic meta amino/hydroxy derivatives and 8 aromatic monoamines. The h-CLAT was found to perform better than either of the other test methods. The ability to extract structural alerts information based on reaction domain and type of activation can be helpful in directing which key event and its associated non-animal test method might be most effective in predicting skin sensitisation potential.