Collaborative project in JCIA for testing strategy of skin sensitization using h-CLAT, DPRA and DEREK

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For the replacement of LLNA, the combination of several alternative methods is necessary. JCIA organized a working group to investigate testing strategies. In this study, considering the applicable domain, we demonstrated the utility of a testing strategy using the human Cell Line Activation Test (h-CLAT), Direct Peptide Reactivity Assay (DPRA) and the *in silico* system, DEREK. A total of 133 chemicals, some of which exhibt poor water-solubilities, were evaluated. For h-CLAT, THP-1 cells were exposed to each test chemical for 24 hours. The CD86 and CD54 expressions were analyzed by flow cytometry. For DPRA, model peptides were mixed with test chemical for 24 hours. The depletion of peptides was analyzed by HPLC. For DEREK, the alert of chemical structure was examined. For 133 chemicals, the accuracy of h-CLAT, DPRA and DEREK to predict LLNA results was 78, 74 and 74%, respectively. Next, we investigated 3 testing strategies: the Integrated Testing Strategy (A), a tiered approach (B), and a multiple regression analysis model (C). Each strategy had a high potential prediction (A:84%, B:81%, C:84%). Regarding false negative chemicals, some chemicals induced no cytotoxicity and other chemicals crystallized or separated in the medium and the buffer. The logP values of these chemicals were mostly above 3.5. If hydrophobic chemicals (logP>3.5) were removed from analysis, the accuracy was improved to A:88%, B:86%, C:87%. The sensitivity was improved to A:96%, B:97%, C:100%. All strategies provided an accuracy of 73% for the potency prediction. These results suggested that every strategy has high predictivity for the potential and potency.