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Summary and conclusions

We evaluated the utility of a combination of yeast growth inhibition and hemolysis testing as an alternative to phototoxicity testing. This method has been proposed by the Shiseido Quality Assessment Center, where trial testing was performed using 24 substances classified as fragrances, ultraviolet absorbing agents, drugs, antimicrobials, or dyes, which have exhibited phototoxicity in vivo. The results of these trials indicated both sufficient sensitivity and agreement with the results of in vivo testing. Thereafter, the Japanese Committee for the Academic Validation of Alternative Methods undertook a multi-laboratory validation to demonstrate the universality of these results. An initial validation using a total of nine substances was performed at six participating laboratories, which each tested six substances that were identified only by code numbers. Implementation of the test was preceded by technical training and the tests were performed following a protocol that conformed with Good Laboratory Practice. A look at intra-laboratory reproducibility reveals that Lab B exhibited poor reproducibility, which was attributed to having performed the test at a rented facility, where control of the experimental environment proved to be problematic. Inter-laboratory reproducibility was less than satisfactory, with poor agreement of results except for acridine, 4-t-buty1-4-methoxydibenzoylmethane (BMDM), chlorhexidine (CHD), and bithionol. In spite of the fact that CHD and bithionol tested negative for phototoxicity in vivo, the initial validation showed a positive result, and there were other substances that tested positive in vivo but gave pseudo-positive or negative results. When results from Lab B are excluded and pseudo-positive results are assumed to be positive, the primary validation exhibits a 70% agreement with in vivo testing.

Insofar as the results of the initial validation included inconsistencies such as pseudo-positive findings for positive substances, revisions were made to the criteria for positive findings in the yeast growth inhibition test as well as to the parameters for pre-incubation time in the test protocol for use in supplemental testing. The supplemental testing used the same test substances as the initial validation testing, and was conducted at five laboratories (Lab B having been excluded). There were fewer pseudo-positive findings in the supplemental test, and compared with the initial validation testing, both intra- and inter-laboratory reproducibility were satisfactory, indicating that the revised protocol was effective.

Although the revised protocol for this test does leave open the possibility that some negative substances might be found positive, there are no instances of positive substances being found negative, and in terms of safety, we find this test method to be useful for the in vitro screening of phototoxicity. The advantages of this test method include:

1. Useful for evaluating substances that are not easily soluble in water.
2. Does not require aseptic conditions and is easy to perform.
3. Is relatively inexpensive.

Disadvantages of this test method include:

1. Since it is a combination of two tests (battery), it requires more time than single trial methods such as 3T3-NRU.
2. There are substances that yield pseudo-positive findings.

In addition to these, the following points of concern also require further study until a solution is found.

1. Although this test method is touted as useful for evaluating substances that are not easily soluble in water, this has yet to be sufficiently verified. We would like to see a validation of at least four substances that are not easily soluble in water.
2. A total of only nine substances were tested under the final protocol, and the full extent of other substances that can be tested using this method has yet to be clarified. We would like to see additional studies done on the 24 substances that were previously tested by Shiseido (except for those substances that have already been validated).
3. No direct comparison with 3T3-NRU has been made, and the extent to which results based on data obtained from the revised protocol are equivalent or superior to 3T3-NRU testing has yet to be clarified.
4. The validation does not address the issue of consistency across results obtained when using different light sources.
5. Although mention is made of discrepancies in results due to rising temperature, no addition tests

were made to address that issue.

6. As with 3T3-NRU testing, no parameters for metabolic activation were established, and the validation did not address the safety of whole body exposure or issues other than transdermal administration.
7. There was no consideration given to whether or not the two tests comprising the battery should be performed in a particular sequence.
8. There is room for improvement in determining an optimal cut-off value for positive reactions in the yeast growth inhibition test.
9. There is room for improvement in determining an optimal selection of a photometric absorption spectra for the hemolysis test.

Based on the above, and with due consideration to the fact that the usefulness of this test method in screening test substances for phototoxicity has been recognized, it is our opinion that addition studies are necessary before this test method can be considered reliable enough for use.