Evaluation Report on an Alternative Test Method for Photosafety: Reactive Oxygen Species Assay

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The Reactive Oxygen Species (ROS) assay was proposed as a simplified means for assessing the photosafety of a large number of test chemicals by measuring their photochemical characteristics. As a photoreactive test for assessing the production of either singlet oxygen or super oxide anion after a test chemical is irradiated with artificial sunlight, it has been shown to be an effective means of predicting the potential for photodermatosis. The ROS assay reached Step 5 in 2014 and was described in the ICH (The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use)S10 guidelines as a test for which the "data suggest that this assay has high sensitivity for predicting direct in vivo phototoxicants."

The usefulness of the ROS assay was evaluated in JaCVAM (Japanese Center for the Validation of Alternative Methods)-sponsored validation study performed at multiple laboratories, and the results showed the ROS assay to possess a high degree of both intra- and inter-laboratory reproducibility as well as excellent versatility. Also, if data from test substances that could not be tested due to poor solubility are excluded, the ROS assay demonstrates a positive prediction rate of 100% for phototoxic test chemicals. Since there were no false negatives, we consider the ROS assay to be an effective means of evaluating phototoxic potential. An independent peer review of the validation report concurred with this evaluation.

Given this background and in accordance with OECD (Organisation for Economic Co-operation and Development) standards established in 2005, JaCVAM organized an independent peer review panel to perform a scientific evaluation and validation of the ROS assay. This panel was first convened in February 2013 and went on to hold four further meetings during which additional revisions were made. This assessment report comprises a summary of the evaluation made and conclusions reached by the peer review panel.

The panel's conclusion was that the ROS assay demonstrated reproducibility and predictive capacity that was well suited for the comprehensive assessment of the phototoxic potential of chemical substances. In the future, although it is recommended that chemical substances yielding a positive result in the ROS assay undergo further testing with the 3T3 NRU Phototoxicity test (OECD Test Guideline 432) or other in vitro test method, we can conclude that substances yielding negative results will require neither animal tests nor any other type of test, and that this can be expected to reduce not only the time and cost required to assess phototoxicity but the use of laboratory animals as well.

Also, the reproducibility and predictive capacity of this assay mean that it will be a useful supporting element in both the early stages of drug development and subsequent decision-making as part of the phototoxicity testing of pharmaceutical products. Additionally, recent studies have suggested that the ROS assay can be applied to mixtures and substances of an unknown molecular weight, which heightens expectations that it could be useful for identifying photosafety hazards in cosmetic ingredients, general-purpose chemical substances, and agrochemicals.

Key words: *Reactive oxygen species, ROS, ICH S10, photsafety, OECD, 3Rs*