Title of Research:

13_PT01-02

Applied research of a novel *in vitro* method for developmental toxicity to facilitate the industrial utilization

Principal Investigator:

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Summary of Research:

We are developing the Hand1-Luc Embryonic Stem Cell Test (Hand1-Luc EST) which uses ES cells introduced the reporter gene into as a test method to detect embryonic toxicants. To familiarize the Hand1-Luc EST in the chemical industry, the Hand1-Luc EST is currently under validation process. To obtain objective evaluation and reliability of this test from experts, it is necessary to accumulate data and validate the test about the robustness, the predictability and the inter- and intra- laboratory reproducibility.

In 2013 as a project supported by the Ministry of Economy, Trade and Industry, the validation management team, composed of experts on developmental toxicity or alternative test methods from Japan and other counties, has been organized. The international validation study was started with the collaboration of Japanese three laboratories to optimize the test protocol, to establish acceptance criteria, and to verify the technical transfer and inter- and intra-laboratory reproducibility. In the phase 0 and phase 1 studies, the technical transfer and intra-laboratory reproducibility has been cleared respectively.

In Phase2a study, which was started as the project supported by LRI, the necessity of multiple experiments for prediction of the positivity or the negativity of chemicals was investigated because at the very beginning, we proposed that the prediction can evaluate with only one experiment. Experiments in each chemical were performed two or three times according to the revised protocol and the results of four coded chemicals were compared between three laboratories. In each laboratory, all of four chemical results were consistent with the in vivo results and the accuracy and the inter-laboratory reproducibility were 100%. Thus, the improved protocol was confirmed to be valid. Following those results, to verify the intra- and the inter-laboratory reproducibility, 24 coded chemicals (8 kinds of chemicals, 3 bottles per chemical were prepared) were distributed to the 3 laboratories and the phase 2b/2c studies were started (May 2014 – February 2015). By gathering the results from phase 2a, 2b and 2c studies, the inter-laboratory reproducibility reached 83.3% (10/12 chemicals were consistent with the *in vivo* data) and the intra-laboratory reproducibility was higher than 75% in each laboratory (the number of chemicals of which the results were consistent between three tests in each chemical, Lab A: 7/8 chemicals, Lab B: 7/8 chemicals, Lab C: 6/8 chemicals).

Timeline:

November 1, 2013-

Topics:

Presented at the 2nd Annual Conferences of New LRI (29th August, 2014, Tokyo)

Publications:

27th Annual Meeting, the Japanese Society for Alternatives to Animal Experiments, Yokohama,