Title of Research:

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

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Summary of Research:

The purpose of the present research is to lead the Hand1-Luc EST, test using ES cells transfected with a reporter gene to a wide use in the chemical industry. To achieve this goal, the Hand1-Luc EST is currently under validation process. According to experts, to satisfy objective valuation and reliability of the test, it is necessary to gradually gather data, and verify the robustness, the predictability and the inter- and intra- laboratory reproducibility of the method. Before adoption of the test in 2013 by the Ministry of Economy, Trade and Industry, the validation management team, composed of Japanese and international expert on developmental toxicity or alternative test methods, has been organized. The international Validation was started to verify, with the aid of Japanese collaborating labs, the improvement of the protocol, the satisfaction of the established acceptance criteria, the technical transfer and inter- and intra- laboratory reproducibility. Until now, the technical transfer and high intra-laboratory reproducibility has been cleared by tests done with phase 0 and phase 1 study. With the support of LRI, by gathering the results from phase 2a, 2b and 2c, 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.
This semester, firstly, when phase2 was finished, we improved the way of analyzing the data after considering the advice from experts. Concretely, the way of fitting curves was changed from a 2 parameter curve fitting to a 3 parameter curve fitting. Thanks to this revision, the relation between the IC_{50} and ID_{50} ratio was improved and thus, it was decided that the 3 parameter curve fitting shall be used for further experiments. The IC_{50} and the ID_{50} values being changed by the new analysis, the prediction model was revised. The phase 3 consisted in 16 chemicals tested in 3 participant laboratories to test the within laboratory reproducibility of the Hand1-Luc EST. One dose finding study and two or three definitive studies were carried out according to the results given by the introduction of the data (IC_{50}, ID_{50}, Maximum dose) in the prediction model. 4 chemicals did not meet the requirements of the within laboratory reproducibility (1 laboratory had a different prediction from the 2 other labs). By analyzing more in details the data obtained, some explanations were found. The first point was the determination of the maximum dose of some chemicals that precipitated at this concentration. The second point was a high dilution ratio chosen by a laboratory leading to a lesser accuracy for the IC_{50} and ID_{50} determination. Facing those facts, it was acknowledged that the reproducibility would be significantly improved by minor modification of the protocol concerning those points. The phase 3 satisfied the criteria fixed in the study plan (within laboratory reproducibility of 75%, 12/16 chemicals).The predictivity and the applicability of Hand1-Luc EST were also discussed. On the whole, it was concluded that the positive predictive value was very high and thus this test would be useful to detect positive embryotoxicants by a Top-Down approach. With a common agreement about the end of the validation, the validation report will be prepared so as to, then, submit the Hand1-Luc EST to the OECD to become a test guideline.

Timeline: March, 2015-

Topics: Presented at the 3rd Annual Conferences of New LRI (28th August, 2015, Tokyo)