Comment for ER/AR mediated transactivation assays

May 8, 2002

- ***Masahiro Takeyoshi, Ph.D., CERI-Japan***

I have read out the background documents concerning the ER/AR mediated transactivation assays.

Now I had a comment for the committee on the data analysis strategy for reporter gene assay. Most of all methods mentioned in the report employed EC50 or related parameters to express relative potency of hormonal activity of chemicals.

In the case for comparing the performance of assay systems by using a single chemical, EC50 values are acceptable because all response curves obtained by all assay systems would be similar ones.

In our experiences, however response curves obtained from deferent chemicals were varied from chemicals to chemicals. Namely, shapes of response curve were different from chemical to chemical the maximum induction levels with chemical were different from chemical to chemical. Moreover, EC50 values are usually calculated by the logistic equation, and the equation would be applicable in the case that the chemical induces typical sigmoidal dose responses. Therefore EC50 cannot become the universal index for measuring the intensity of transcriptional activity of the compound with the different properties.

We have proposed the PC values in the article: Yamasaki, K., Takeyoshi, M., Yakabe, Y., Sawaki, M., Imatanaka, N., and Takatsuki, M. (2002), ***Comparison of Reporter Gene Assay and Immature Rat Uterotrophic Assay of Twenty-Three Chemicals***. Toxicology, 170: 21-30. The PC50 and PC10 values are defined as the test chemical concentrations estimated to show 50% and 10%, respectively, of the transcriptional activity of positive control wells. These PC values were estimated by a simple linear regression using two variable data points in mean transcriptional activity. In our experiments, the positive control wells treated with natural ligands (100pM or 1 nM of 17β-estradiol) ordinary showed maximum response and it showed well reproducibility.

Calculation of EC50 using logistic equation require at least three variable data points except a bottom value, but that of PC10 and PC50 values require only two variables because these were calculated with a simple linear regression. We found a very good linear relationships between EC50 and PC10 (R2=0.9202) or PC50 (R2=0.9431) values in 6 chemicals possessing both parameters. To evaluate estrogenic potency in various types of chemicals in numerically, numerical parameter that could be calculated for wide range of estrogenic chemicals would be required. Our results demonstrate that PC values are preferable parameter to EC50 value for predicting the hormonal activities of chemicals, which may be ranged widely in potency.