***Title of Research:***

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# Development of new *in vivo* screening method to validate the low-dose effects induced by estrogenic chemicals using estrogen reporter mice

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***Summary of Research:***

**Objective:** The uterotrophic bioassay has been proposed as a short-term *in vivo* screening test to detect the estrogenic properties of potentially endocrine disrupting chemicals by the Organisation for Economic Co-operation and Development (OECD). Although the test is already standardized as a OECD test guideline (Test No.440), there is a possibility that the test might overlook a certain estrogenic properties which has no effect to the uterus. On the other hand, we recently generated a new transgenic mouse carrying “E-Rep” reporter gene which consisted of the luciferase (Luc) with c-Myc tag under the control of estrogen-responsive elements coupled to a minimal promoter. E-Rep mouse is a potential powerful tool for assessing the kinetics of gene activation by estrogenic properties *in vivo*. We here established a new *in vivo* screening assay using E-Rep mice with *in vivo* bioluminescence imaging to detect the estrogenic effects of xenobiotics and validated the assay to compare with the uterotrophic bioassay.

**Methods:** The experimental protocols of the new assay using E-Rep mice are based on the uterotrophic bioassay with some modifications. Briefly, E-Rep female mice (8 weeks of age) were ovariectomized and then fed phytoestrogen-free purified diet during the experimental period. After 7 days of ovariectomy, the mice were orally gaveged with 17$\alpha$-ethinylestradiol (EE) for 7 consecutive days. The Luc activity was monitored in living animals with IVIS imaging at Day 1, 2, 4, 7. After last detection of *in vivo* bioluminescence imaging, mice were dissected and then the estrogenic effects were evaluated by measurement of uterine weight or detection of Luc activity at Day 7.

**Results:** Luc activity in the uterus increased significantly when EE was administered at 3 $\mu$g/kg/day or greater at Day 7. In contrast, the uterine weight rose significantly only when estradiol was administered at 10 $\mu$g/kg/day. In addition, *in vivo* bioluminescence imaging analysis could detect significant increment of Luc activity at above 0.3 $\mu$g/kg/day of EE, suggesting that detection of Luc activity in E-Rep mice by *in vivo* bioluminescence imaging provide a more sensitive assay of estrogenic activity than the uterotrophic bioassay.

**Conclusion:** E-Rep mice with *in vivo* bioluminescence imaging provide a potential new assay system to measure estrogenic activity of endocrine disrupting chemicals in multiple organs with high sensitivity.

***Timeline:*** April, 2016 – March, 2017

***Topics:***

1) Our research paper “Fluorene-9-bisphenol is anti-oestrogenic and may cause adverse pregnancy outcomes in mice” published in *Nature Communications* was highlighted in natureasia.com (http://www.natureasia.com/en/research/highlight/11678).
2) Our research paper “Fluorene-9-bisphenol is anti-oestrogenic and may cause adverse pregnancy outcomes in mice” published in *Nature Communications* featured as a daily news

entitled “BPA-free water bottles may contain another harmful chemical” in *New Scientist* (https://www.newscientist.com/article/2123098-bpa-free-water-bottles-may-contain-another-harmful-chemical/).

***Publications:***

1) Zhang Z, Hu Y, Guo J, Yu T, Sun L, Xiao X, Zhu D, Nakanishi T, Hiromori Y, Li J, Fan X, Wan Y, Cheng S, Li J, Guo X, Hu J, Fluorene-9-bisphenol is anti-oestrogenic and may cause adverse pregnancy outcomes in mice, *Nature Communications* 8:14585 (2017)