

**Title of Research:**

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**Development of metabolic profiling system for in vitro evaluation of endocrine disruption by chemical substances**

**Principal Investigator:**

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**Summary of Research:**

The aim of this study was to apply metabolic profiling to phenotype analysis of cells exposed to chemicals, and to develop a system to evaluate endocrine disruptors by multimarker profiling based on chemical exposure-induced endogenous metabolite changes.

First, we constructed a platform to simultaneously analyze steroids in steroidogenesis pathways. To construct a versatile evaluation system, gas chromatography/mass spectrometry (GC/MS), which is known for its versatility, was used in this analysis. Seventeen steroids were simultaneously analyzed under optimized preparation and GC/MS conditions.

Next, we constructed a screening system for endocrine disruptors. Following the protocol of OECD TG 456, the human adrenocortical carcinoma cell line H295R exposed to nine compounds including forskolin and prochloraz were used as a positive control. Specific steroid profiles in each sample was successfully obtained.

Additionally, we performed the 28-day repeated dose oral toxicity experiment of ketoconazole in rat. The change of plasma steroid profiles was observed on day 1, 8, 15, 21 and 28 post-dose.

**Timeline:**

November 1, 2013 - February 28, 2015

**Topics:**

3<sup>rd</sup> New LRI Annual Conferences Symposium (Yaesu First Financial Building, August 31, 2014)  
“Development of *in vitro* screening endocrine disruptor by steroid profiling”

**Publications:**

1. Takeshi Bamba, Okuno Masashi, Yamashita Toshiyuki, Fukusaki Eiichiro  
Development of *in vitro* screening endocrine disruptor by steroid profiling  
62nd Annual Conference on Mass Spectrometry, HOTEL HANKYU EXPO PARK (Suita), May 14 (Wed)–16 (Fri), 2014
2. Takeshi Bamba, Okuno Masashi, Yamashita Toshiyuki, Fukusaki Eiichiro  
Simultaneous analysis of steroids by GC/MS for evaluation of endocrine disruption  
The 41<sup>st</sup> Annual Meeting of the Japan Society of Toxicology, Kobe Convention Center (Kobe), July 2 (Wen) - 4 (Fri), 2014
3. Sasano Ryoichi, Yamashita Toshiyuki, Okuno Masashi, Uchida Shigeru, Fukusaki Eiichiro, Takeshi Bamba  
Measurement of 17 $\beta$ -estradiol and testosterone released from H295R cells by the on-line derivatization LC-GC/MS system  
The 41<sup>st</sup> Annual Meeting of the Japan Society of Toxicology, Kobe Convention Center (Kobe), July 2 (Wen) - 4 (Fri), 2014