Overseas Practice on （Field Epidemiology・Collaborative Research）

report form（For Student）　　2019/07/05 (Year/Month/Day)

Name	Kraisiri KHIDKHAN
Laboratory	Toxicology
Year (Grade)	D3
Place of practice	1.Department of Science and Technology for Biological Resources and Environment, Graduate School of Agriculture, Ehime University, Japan. 2.Center for Marine Environmental Studies (CMES), Ehime University, Matsuyama, Japan.
Period of practice	09/05/2019-28/06/2019
Purpose	Collaborative research

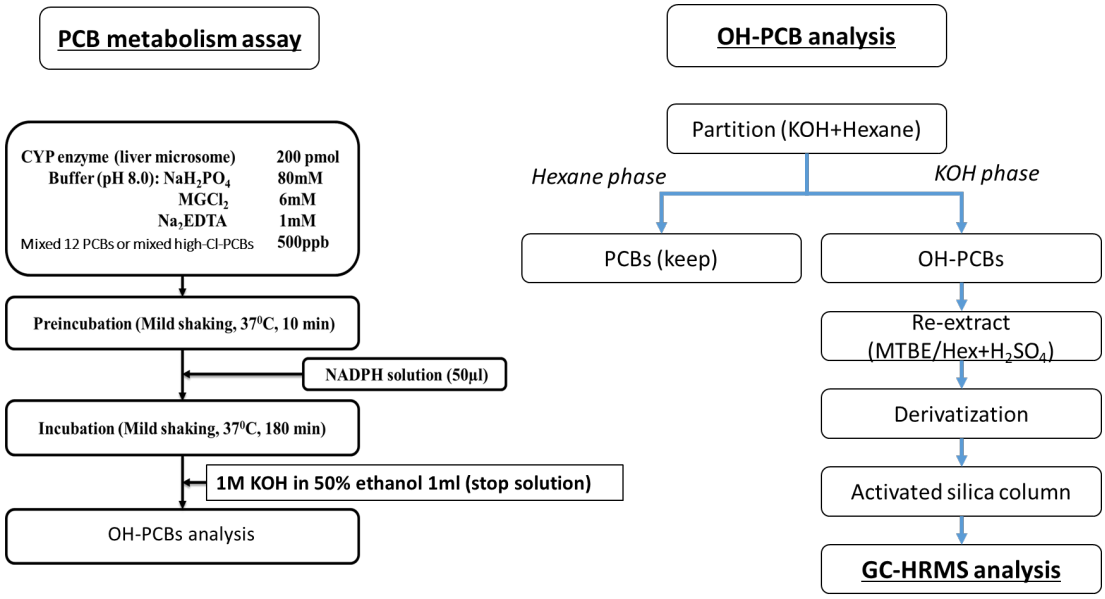
Summary of activities（about 800 words, provide photos, tables and figures that clearly show the activities during the period）

My study focuses on metabolism of polychlorinated biphenyls (PCBs) by cytochrome P450 (CYP) in cat compared to dog, therefore the objectives in this collaboration are (1) to learn the techniques of *in vitro* PCBs metabolism assay and the analytical techniques for detecting OH-PCBs (2) to perform PCBs metabolism assay using my samples (dog and cat microsomes) and analyze OH-PCBs levels after PCB metabolism assay.

After I arrived at Matsuyama, Associate Professor Hazuki Mizukawa, my host in Ehime, took me from the airport to the Graduate School of Agriculture at Ehime University for keeping my samples in the freezer and introducing me to her laboratory members. Then I checked-in at Ehime University' guesthouse. In the next day, I cleaned all equipment and prepared reagents such as hexane, acetone, and buffer for my experiment. In afternoon, I visited to the Center for Marine Environmental Studies (CMES) to introduce myself to Associate Professor Kei Nomiyama and laboratory members in CMES.

Graduate School of Agriculture (left), Center for Marine Environmental Studies (CMES) (right)

First week, I planned all my experiments with Associate Professor Mizukawa. She taught me for using the equipment and chemicals in the laboratory. We also tried the trial experiment together because this was my first time for doing the chemical analysis. I got new knowledge and good technique for chemical analysis and clean-up method form her directly. After that, I did my experiments by myself. The methods for my experiment are including (1) PCB metabolism assay; the PCBs substrate was incubated with microsome sample, which is mainly contained CYP, and this reaction could generate OH-PCBs, (2) OH-PCBs extraction; solution sample was cleaned-up to remove the unwanted matrix and to purify OH-PCBs in the final solution, (3) GC-HRMS analysis; the purified sample was injected in the GC-HRMS and this machine could detect and quantify the levels of OH-PCBs in our samples.

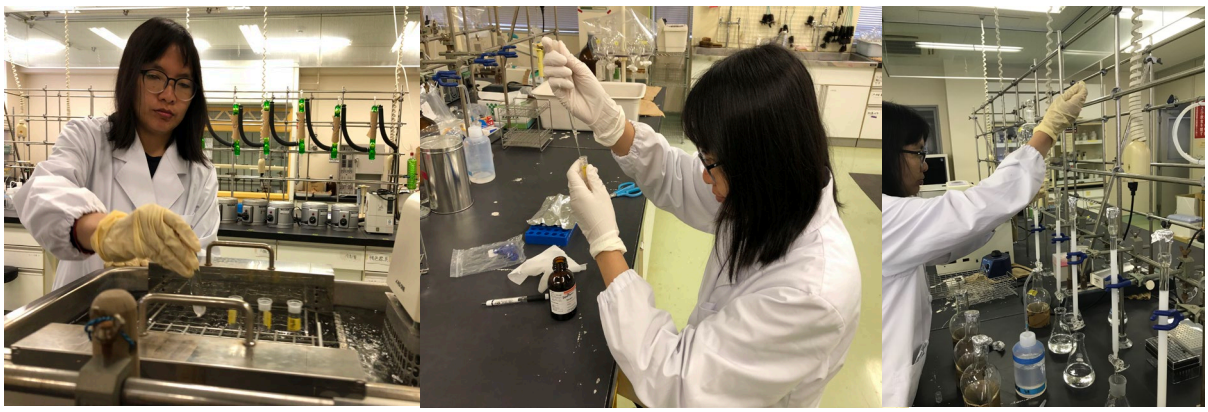


The outline protocol of experiment

According to my plan, the experiments were divided into three section periods including first, the trials for conditions of PCB substrate dose and the incubation time of PCB metabolism assay, second, the analysis of all real samples, and third, the conclusion and discussion for some data and preliminary results.

First section, I spent two weeks and half to adjust the best condition for dose and incubation time of PCBs metabolism assay, to learn the technique for using gas chromatography–high-resolution mass spectrometry (GS-HRMS) machine and to analyze the data with Miss Shimasaki Makoto, the 2nd year master student in CMES. Although the GC-HRMS was complicated machine and it's was difficult to understand for the machine operation, she kindly trained me from beginning until getting the results. She assigned the chromatogram peaks masterfully. Therefore, within these two weeks and half, I could get the appropriate dose and the best incubation time for doing the real experiment.

Second, I continuously did the experiments using real samples including cat and dog samples and used the same method as the trials. After the first section period, my skills for PCB metabolism assay, sample extraction and clean-up, and chemical analysis were improved and I could do each step faster than the first section. All 72 samples were completely analyzed within two weeks and half. During these two weeks, I also contacted with my professors in the laboratory of toxicology (Graduate School of Veterinary Medicine, Hokkaido University) to discuss my results and report my progress experiments.



PCB metabolism assay and sample extraction

Last week, I washed all equipment, managed the PCB, discarded the reagent wastes, and cleaned the laboratory room. Some glassware was baked at 400 degrees Celsius for 24 hours since PCB cannot degrade at normal temperature. After that, I summarized all methods, analyzed data, and discussed some results with Associate Professor Mizukawa and Associate Professor Nomiyama. They suggested me for interpreting the GC-HRMS data and making the interesting discussion. After data analysis, the preliminary results were found that cat has very low capacity for metabolizing some PCB congeners compared to dog, that was very remarkable and valuable result for my study.

GC-HRMS (left), Learning GC-HRMS machine with Miss Shimasaki Makoto (right)

During this time, I had the opportunity to introduce my research field and explain the study concept to professors and students in the Department of Science and Technology for Biological Resources and Environment, Graduate School of Agriculture. They asked me some considerate questions and gave me suggestions for my future work. I additionally attended the seminar about the developmental neurobehavioral toxicity by non-coplanar PCB and PBDE in zebrafish, and the cytochrome P450 in cat: structure, expression, enzymatic activity and polymorphism. This seminar was provided by CMES and the presenter was Professor Hiroki Teraoka, the feline CYP expert. After seminar, I asked him about his work and discussed with him about my future experiments. In addition, I got opportunity to talk with Professor Hisato Iwata, the specialist for docking simulation, and ask him for collaboration about the docking simulation of PCBs and CYP in cat compared to dog. I got many useful ideas and good suggestions from them, as well as the great opportunity to have dinner with them.

After dinner with professors (left), Professors and students in laboratory of Department of Science and Technology for Biological Resources and Environment (right)

In conclusion, this collaboration is very beneficial and effective for me because I can get not only valuable knowledge and the data supported my research project, but also I can get the good relationship with professors, experts, and many students. More significantly, I will transfer this knowledge gained through this collaboration to the junior students in our school who are interested in PCB metabolism and chemical analysis by GC-HRMS.

（Field Epidemiology・Collaborative Research） Evaluation by supervisor

Institution・Official title・Name	Laboratory of Toxicology・Professor・Mayumi ISHIZUKA　印
Describe overall evaluation on the applicant's activity in overseas practice.	

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