ISSN 2189-0137

ICAEC2014

International Conference of Asian Environmental Chemistry 2014

Programs and Abstracts

Venue : Convention Center, Chulabhorn Research Institute
Bangkok, Thailand

Date : November 24th – 26th, 2014

JEC Japan Society for Environmental Chemistry

Outcome of ICAEC 2014

The expected outcomes are as follows;

- To declare importance to prevent pollution by micro-pollutants (declare a statement)
- To make clear environmental pollution by micro-pollutants in South East Asia, East Asia and South Pacific region
- To strengthen relationship of researchers, particularly young researchers between Thailand and Japan

Executive Chair

Takeshi Nakano	Osaka University

Advisory Board Members

Kiwao Kadokami	The University of Kitakyushu
Masatoshi Morita	Ehime University
Noriyuki Suzuki	National Institute For Environmental Studies
Susumu Kawahara	Japan Society For Environmental Chemistry
Takumi Takasuga	Shimadzu Techno-Research Inc.
Yoshinori Fujimine	Otsuka Pharmaceutical Co., Ltd

Scientific Committee

Chisato Matsumura	Hyogo Pref. Institute of Environmental Sciences
Daisuke Nakajima	National Institute for Environmental Studies
Daisuke Ueno	Saga University
Haruhiko Nakata	Kumamoto University
Hideshige Takada	Tokyo University of Agriculture And Technology
Hiroshi Yamamoto	The University of Tokushima
Izumi Watanabe	Tokyo University of Agriculture And Technology
Kazuhiko Akutsu	Osaka Pref.Institute of Public Health
Koji Arizono	Prefectural University of Kumamoto
Norihisa Tatarazako	National Institute for Environmental Studies
Seiji Watanabe	National Research And Training Institute, Japan
Shin Takahashi	Ehime University
Shoji Nakayama	National Institute for Environmental Studies
Takanori Sakiyama	Osaka city Institute of Environmental Sciences
Takashi Amagai	University of Shizuoka
Tatsuya Kunisue	Ehime University
Tomohiko Isobe	National Institute for Environmental Studies
Yasuyuki Shibata	National Institute for Environmental Studies
Yoshiharu Shirane	SOWA Consultants Inc.
Yuichi Horii	Center for Environmental Science in Saitama
Amornrat Lertworasirikul	Kasetsart University
Aphichart Rodchanarowan	Kasetsart University
Apirat Laobuthee	Kasetsart University
Chart Chiemchaisri	Kasetsart University
Cheema Soralump	Kasetsart University
Daam Settachan	Chulabhorn Research Institute
Gautier Landvot	Kasetsart University

Jutamaad Satayavivad	Chulabhorn Research Institute
Panida Navasumrit	Chulabhorn Research Institute
pongsak noophan	Kasetsart University
Sanya Sirivithayapakorn	Kasetsart University
Wilai Chiemchaisri	Kasetsart University

Japan Committee

Akira Toriba	Kanazawa University
Hatsue Braatz	SheGoTec Japan, Inc.
Hideo Yamazaki	Kinki University
Hiroshi Hoshino	Center for Environmental Science in Saitama
Jun Sekizawa	Communication Center for Food and Health Sciences, Japan
Jun Yoshinaga	The University of Tokyo
Kanji Iwamoto	Nihon BUCHI K.K.
Katsuhiro Nakagawa	Shimadzu Corporation
Kei Nomiyama	Ehime University
Kensaku Kakimoto	Osaka Prefectural Institute of Public Health
Kyoichi Komori	Japan Analytical Instruments Manufacturers' Association
Mari Asami	National Institute of Public Health
Masayuki Kunugi	Kanazawa University
Muneko Nishijo	Kanazawa Medical University
Shinsuke Tanabe	Ehime University
Shunji Hashimoto	National Institute for Environmental Studies
Takeshi Enomoto	JEOL. Ltd.
Takuya Shiozaki	Japan Environmental Sanitation Center
Teruhiko Kido	Kanazawa University
Toru Matsumura	UAE-IDEA Advance Analytical Company Limited
Yasuhiro Hirai	Kyoto University
Yoko Fujikawa	Kyoto University
Yoshitoku Yoshida	Nagoya University
Youko Takagi	Kyoto Electronics Manufacturing Co., Ltd.
Yuji Kunimi	Nagao Clinic

Thai committee

Ministry of Natural Resources and Environmental
Department of Environmental Quality Promotion
Environmental Research and Training Center
Chulabhorn Research Institute
Pollution Control Department
Department of Science Service
Department of Agriculture
Department of Industrial Works
Department of Medical Sciences
Chemical Society of Thailand
Polymer Society of Thailand
Kasetsart University
Center of Excellence on Hazardous Substance Management

P-83	24	Comparative Study on Human and Mouse Nuclear Receptor Activities of Hepatomegaly-Inducing Perfluorooctanoic acid and its Related Compounds	Hiroyuki KOJIMA[1]*, Shinji TAKEUCHI[1], Fumihiro SATA[2], Hiroyuki NAKAJIMA[3], Chise NOOMOTE[3], Chieri FUJINO[4], Naoto URAMARU[4], Shigeyuki KITAMURA[4], Kouichi YOSHINARI[3,5] [1]Hokkaido Institute of Public Health, Japan; [2]National Institute of Public Health, Japan; [3]Graduate School of Pharmaceutical Sciences, Tohoku Univ., Japan; [4]Nihon Pharmaceutical Univ., Japan; [5]School of Pharmaceutical Sciences, Univ. of Shizuoka, Japan
P-84	107	Interaction of PFOS, PFOA and 8:2 FTOH with Human, rat and Microbial Cytochrome P450s : Similarities and Differences	Vladimir BEŠKOSKI[1,2,3], Takeshi NAKANO[4], Chisato MATSUMURA[5], Katsuya YAMAMOTO[5], Atsushi YAMAMOTO[6], Mamoru MOTEGI[7], Hideo OKAMURA[8], Hideyuki INUI[2] [1]Faculty of Chemistry, Univ. of Belgrade, Serbia; [2]JSPS Invitation Fellowship Program for Research in Japan; [3]Research Center for Environmental Genomics, Kobe Univ., Japan; [4]Research Center for Environmental Preservation, Osaka Univ., Japan; [5]Hyogo Prefectural Institute of Environmental Sciences, Japan; [6]Osaka City Institute of Public Health and Environmental Sciences, Japan; [7]Center for Environmental Science in Saitama, Japan; [8]Graduate School of Maritime Sciences, Kobe Univ., Japan
P-85	108	Biotransformation of Perfluorinated Compounds by the Action of Microbial Community Isolated from Polluted Environment - Road to Successful Bioremediation	Vladimir P. BEŠKOSKI[1,2,3], Takeshi NAKANO[4], Atsushi YAMAMOTO[5], Chisato MATSUMURA[6], Katsuya YAMAMOTO[6], Mamoru MOTEGI[7], Hideo OKAMURA[8], Hideyuki INUI[3] [1]Faculty of Chemistry, Univ. of Belgrade, Serbia; [2]JSPS Invitation Fellowship Program for Research in Japan; [3]Research Center for Environmental Genomics, Kobe Univ., Japan; [4]Research Center for Environmental Preservation, Osaka Univ., Japan; [5]Osaka City Institute of Public Health and Environmental Sciences, Japan; [6] Hyogo Prefectural Institute of Environmental Sciences, Japan; [7] Center for Environmental Science in Saitama, Japan; [8]Graduate School of Maritime Sciences, Kobe Univ., Japan
P-86	102	Blood Cholinesterase Activity Levels of Farmers from Mae Taeng District, Chiang Mai Province, Thailand	Surat HONGSIBSONG[1]*, Tanyaporn KERDNOI[1], Niphan SRINUAL[1], Vanvimol PATARASIRIWONG[2], Tippawan PRAPAMONTOL[1] [1]Research Institute for Health Sciences, Chiang Mai Univ., Thailand; [2]Dept. of Environmental Quality Promotion, Ministry of Natural Resources and Environment, Thailand
P-87	142	Health Effects of Exposure to Carcinogenic Compounds Emitted from Incense Smoke in Temple Workers	Varabhorn PARNLOB[1], Panida NAVASUMRIT[1], Jeerawan PROMVIJIT[1], Suppachai CHOONVISASE[1], Samroeng CHANTCHAEMSAI[1], Netnapa NAKNGAM[1], Mathuros RUCHIRAWAT[1] [1]Laboratory of Environmental Toxicology, Chulabhorn Research Institute, Thailand
P-88	M-19	Toxicity Evaluation of 4-Nonylphenol Isomers with Ecotoxicological Bioassay using Delayed Fluorescence in the Green Alga *Pseudokirchneriella subcapitata*	Ryo OMAGARI[1]*, Masakazu KATSUMATA[2], Koji ARIZONO[1], Yasuhiro ISHIBASHI[1] [1]Prefectural Univ. of Kumamoto, Japan; [2]Hamamatsu photonics K.K., Japan
P-89	M-4	Effect of Excessive Doses of Oxytetracycline on Antioxidative Capacity in Coho Salmon	Toshiki NAKANO[1]*, Satoshi HAYASHI[1], Norimi NAGAMINE[1], Toshiyasu YAMAGUCHI[1], Minoru SATO[1] [1]Marine Biochem. Lab., Graduate School of Agriculture Science, Tohoku Univ., Japan

P-84

INTERACTION OF PFOS, PFOA AND 8:2 FTOH WITH HUMAN, RAT, AND MICROBIAL CYTOCHROME P450s: SIMILARITIES AND DIFFERENCES

Vladimir BEŠKOSKI[1,2,3], Takeshi NAKANO[4], Chisato MATSUMURA[5], Katsuya YAMAMOTO[5], Atsushi YAMAMOTO[6], Mamoru MOTEGI[7], Hideo OKAMURA[8], Hideyuki INUI[2]

[1]Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, Belgrade, Serbia; [2]JSPS Invitation Fellowship Program for Research in Japan (Long term); [3]Research Center for Environmental Genomics, Kobe University, 1-1 Rokkodaicho, Nada-ku, Kobe, Hyogo 657-8501, Japan; [4]Research Center for Environmental Preservation, Osaka University, Yamadaoka 2-4, Suita, Osaka, 565-0871 Japan; [5]Hyogo Prefectural Institute of Environmental Sciences, 3-1-27 Yukihira-cho, Suma-ku, Kobe 654-0037, Japan; [6]Osaka City Institute of Public Health and Environmental Sciences, 8-34 Tojocho, Tennoji-ku, Osaka 543-0026, Japan; [7]Center for Environmental Science in Saitama, 914 Kamitanadare, Kazo, Saitama 347-0115, Japan; [8]Graduate School of Maritime Sciences, Kobe University, Fukaeminami 5-1-1, Higashinada-ku, Kobe 658-0022, Japan

Keywords : PFOS, PFOA, 8:2 FTOH, mammalian and microbial cytochrome P450

Abstract : Chemicals known as perfluoroalkyl and polyfluoroalkyl substances (PFASs) do not occur naturally, but because of wide use in chemical production since the 1950s, they can be found in the environment. Currently, the two most persistent and toxic PFASs are perfluorooctanoic acid (PFOA, $C_7F_{15}COOH$) and perfluorooctane sulfonic acid (PFOS, $C_8F_{17}SO_3H$). PFOS including the form of salts and its precursor, perfluorooctane sulfonyl fluoride, are classified as persistent organic pollutant (POPs) according to Stockholm Convention on POPs. A number of studies have reported the ubiquitous distribution of PFASs in wildlife and humans [1-3] and thus it is important to determine mammalian and microbial metabolisms of PFOS and PFOA with cytochrome P450 (CYP) monooxygenases to understand distribution and impact of PFASs on human and wildlife. In the detoxification of xenobiotics, P450 enzymes play important physiological roles. For example, CYP1A1 proteins can metabolize some dioxins and PCBs by hydroxylation, but the activities of human and rat CYP1A1 proteins are very different. Recently it has been demonstrated [4] that rat CYP1A1 metabolized 3,3',4,4',5-pentachlorobiphenyl (PCB126) into 4-OH-3,3',4',5-tetrachlorobiphenyl and 4-OH-3,3',4',5,5'-pentachlorobiphenyl, but human CYP1A1 did not metabolize. Furthermore, indigenous microorganisms are a key for biotransformation and biodegradation of organic molecules such as xenobiotics, PCBs and pesticides as well as oil hydrocarbons [5-7]. Possible mechanisms of aerobic microbial biotransformation/biodegradation of fluorinated organic compounds in the polluted environment may include catalysis by fungal or bacterial CYP monooxygenases.

In order to determine behavior and metabolism of PFOS, PFOA and 8:2 FTOH, *in vitro* oxidation experiments of PFASs with rat and human CYP monooxygenases were conducted. Herbicide chlortoluron was used as positive control for CYP binding. Furthermore, indigenous microbial consortium was enriched and isolated from the environment polluted by PFASs. *In vitro* interaction of PFASs and total microbial CYP were also analyzed. These results give an insight in similarities and differences between mammalian and microbial metabolisms of PFOS, PFOA and 8:2 FTOH with CYP monooxygenases, and how ubiquitous presence of PFASs in the environment and biota affects on the metabolism.

1. Haug, L.S., et al., Environ. Int. 37, (2011) 687-693;
2. Croes K., et al., Chemosphere 89, (2012) 988-994;
3. Domingo J.L., Environ. Int. 40, (2012) 187-195;
4. Yamazaki K., et al., J. Biochem. 149, (2011) 487–494;
5. Winogradsky, S., C. R. Acad. Sci. Hebd. Seances Acad. Sci. D. 178 (1924) 1236-1239;
6. Gojgic-Cvijovic, G., et al., Biodegradation 23, (2012) 1-14;
7. Dionisi, D., ChemBioEng Rev 1, (2014) 67–82;

P-84

INTERACTION OF PFOS, PFOA AND 8:2 FTOH WITH HUMAN, RAT, AND MICROBIAL CYTOCHROME P450s: SIMILARITIES AND DIFFERENCES

Vladimir BEŠKOSKI[1,2,3], Takeshi NAKANO[4], Chisato MATSUMURA[5], Katsuya YAMAMOTO[5], Atsushi YAMAMOTO[6], Mamoru MOTEGI[7], Hideo OKAMURA[8], Hideyuki INUI[2]

[1]Faculty of Chemistry, University of Belgrade, Belgrade, Serbia; [2]JSPS Invitation Fellowship Program for Research in Japan; [3]Research Center for Environmental Genomics, Kobe University, Kobe, Japan; [4]Research Center for Environmental Preservation, Osaka University, Osaka, Japan; [5]Hyogo Prefectural Institute of Environmental Sciences, Kobe, Japan; [6]Osaka City Institute of Public Health and Environmental Sciences, Osaka, Japan; [7]Center for Environmental Science in Saitama, Saitama, Japan; [8]Graduate School of Maritime Sciences, Kobe University, Kobe, Japan

Introduction

Chemicals known as perfluoroalkyl and polyfluoroalkyl substances (PFASs) do not occur naturally, but because of wide use in chemical production since the 1950s, they can be found in the environment. Currently, the two most persistent and toxic PFASs are perfluorooctanoic acid (PFOA, $C_7F_{15}COOH$) and perfluorooctane sulfonic acid (PFOS, $C_8F_{17}SO_3H$). PFOS including the form of salts and its precursor, perfluorooctane sulfonyl fluoride, are classified as persistent organic pollutant (POPs) according to Stockholm Convention on POPs. A ubiquitous distribution of PFASs in wildlife and humans is confirmed [1-3] and thus it is important to determine mammalian and microbial metabolisms of PFOS and PFOA with cytochrome P450 (CYP) monooxygenases to understand distribution and impact of PFASs on human and wildlife.

P450 enzymes play important physiological roles in the detoxification of xenobiotics. For example, CYP1A1 proteins can metabolize some dioxins and PCBs by hydroxylation, but the activities of human and rat CYP1A1 proteins are very different. Recently it has been demonstrated [4] that rat CYP1A1 metabolized 3,3',4,4',5-pentachlorobiphenyl (PCB126) into 4-OH-3,3',4',5-tetrachlorobiphenyl and 4-OH-3,3',4',5,5'-pentachlorobiphenyl, but human CYP1A1 did not metabolize. Furthermore, indigenous microorganisms are a key for biotransformation and biodegradation of organic molecules such as xenobiotics, PCBs and pesticides as well as oil hydrocarbons [5-7]. Possible mechanisms of aerobic microbial biotransformation/biodegradation of fluorinated organic compounds in the polluted environment may include catalysis by fungal or bacterial CYP monooxygenases.

Material and methods

In order to determine behavior and metabolism of PFOS, PFOA and 8:2 FTOH, *in vitro* oxidation experiments of PFASs with rat CYP monooxygenases were conducted. Yeast with transformed rat CYP1A1 gene and human CYP1A1 gene were grown in SD media (N-base 6.7 g/L; Glucose 80 g/L and Histidine 160 mg/L) and YPAD media (yeast extract 10 g/L; BactoPeptone 20 g/L; Adenin sulphate 40 mg/L and Glucose 80 g/L). Cytochrome from *Bacillus megaterium* CYP102A1 (also referred to as P450 BM-3), were used for studying interaction of PFASs with microbial cytochromes.

Hydroxylation of PFOS and PFOA with rat CYP1A1 microsomal fraction was studied: Microsomal fraction (protein concentration 40pmol), PFOS and PFOA (100 ppb) and NADPH were used. Concentration of human CYP1A1 was to low to analyze interactions.

The extracts were loaded to Solid Phase Extraction cartridge (Presep PFC-II, Wako Pure Chemical Industries) and MeOH eluates were analyzed using LC/MS/MS.

Competition for CYP binding between chlorotoluron (CT) and PFAS were studied. Herbicide chlortoluron was used as positive control for CYP binding.

Results and discussion

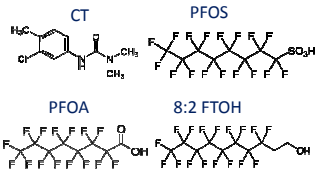
The concentration of microsomal fraction based on CO difference spectroscopy of rat CYP1A1 was 0.3pmol/µL (Fig 1).

1. Products of possible hydroxylation of PFAS after incubation with CYP1A1 were studied using 6 model systems (Table 1).

Table 1. Model systems and recovery rates

	MF	NADPH	PFOS	PFOA	ppb	%
1.	+	+	+	-	80.1	80
2.	+	+	-	+	81.8	82
3.	+	-	+	-	80.6	81
4.	+	-	-	+	96.2	96
5.	-	+	+	-	82.3	82
6.	-	+	-	+	99.5	99

MF-microsomal fraction



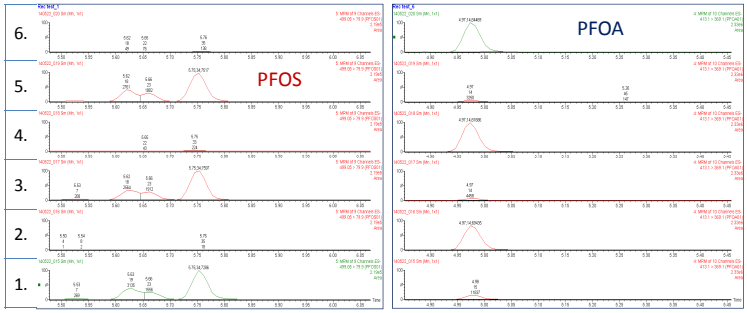


Fig 1. LC/MS chromatograms of 6 model systems extracts

However, recovery rate is suggesting that **PFOS and PFOA interact differently with rat CYP1A1 binding place.** Recovery rate of PFOS from solutions 3 and 5 indicate reduction because of abiotic factors.

High recovery rate in 4 and 6 of PFOA indicate that decrease in solution 2 is possible consequences of interaction of PFOA with MF and NADPH.

Oxidation of PFOA could lead to change of polarity and reduction of recovery rate. However, new peaks, **hydroxylation products were not detected using LC/MS/MS.**

2. Binding interaction of CT, PFOS, PFOA and 8:2 FTOH with rat CYP1A1 and microbial CYP102A1

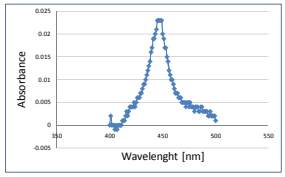


Fig 2. CO difference spectrum of rat CYP1A1

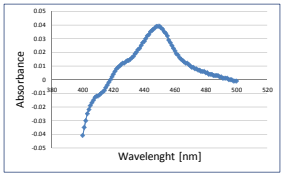
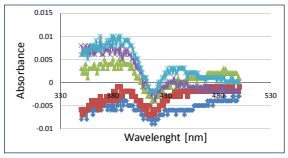


Fig 3. CO difference spectrum of microbial CYP102A1



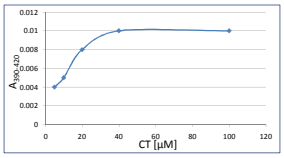
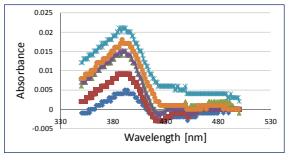


Fig 4a-b. Rat CYP1A1 spectrum of CT binding



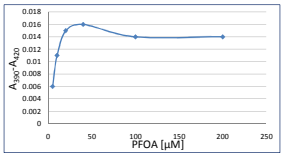


Fig 5a-b. Microbial CYP102A1 spectrum of PFOA binding

Table 2. Kd values of CT, PFOS, PFOA and 8:2 FTOH binding to rat CYP1A1 and microbial CYP102A1

Kd - rat CYP 1A1 [µM]						
CT	PFOS	PFOA	8:2 FTOH	CT & PFOS	CT & PFOA	CT & 8:2FTOH
10.2	44.2	3.94	-42.6	-1.47	0.164	-4.76
Kd - microbial CYP102A1 [µM]						
CT	PFOS	PFOA	8:2 FTOH	CT & PFOS	CT & PFOA	CT & 8:2FTOH
8.62	37.9	1.61	-0.00986	10.3	7.50	-2.32

Rat CYP1A1 Kd for CT is 10.2 µM, for PFOS is 44.2 µM and for PFOA is 3.94 µM. Microbial CYP102A1 Kd for CT is 8.62 µM, for PFOS is 37.9 µM and for PFOA is 1.61 µM. These results suggest that these three substrates are capable to enter binding pocket of P450 and to interact with this enzyme although CT is structurally not similar to PFOS and PFOA. Furthermore, Kd values suggest that **binding affinity increase in the following order: PFOS<CT<PFOA both in mammalian and microbial cytochroms tested**.

During incubation with 8:2 FTOH binding in the binding pocket was not determined in Rat CYP1A1 nor microbial CYP102A1. However, during incubation of Rat CYP1A1 and Microbial CYP102A1 cytochromes with CT & 8:2 FTOH it was observed that binding of CT was inhibited. In model systems with CT + PFOS and CT + PFOA it was observed that kinetics of the reaction did not followed the hyperbolic relationship between the rate of reaction and the concentration of substrate. This data suggest that there is a **competition between CT and PFOS as well as CT and PFOA for the binding pocket of P450**.

Acknowledgements: This research was supported by Japan Society for the Promotion of Science. Microbial CYP102A1 was kindly provided by Dr. Osami Shoji, Nagoya University.

References

1. Haug, L.S., et al., Environ. Int. 37, (2011) 687-693;
2. Croes K., et al., Chemosphere 89, (2012) 988-994;
3. Domingo J.L., Environ. Int. 40, (2012) 187-195;
4. Yamazaki K., et al., J. Biochem. 149, (2011) 487–494;
5. Winogradsky, S., C. R. Acad. Sci. Hebd. Seances Acad. Sci. D. 178 (1924) 1236-1239;
6. Gojgic-Cvijovic, G., et al., Biodegradation 23, (2012) 1-14;
7. Dionisi, D., ChemBioEng Rev 1, (2014) 67–82;