LRI

Long-range Research Initiative

Annual Report 2015

2015

Japan Chemical Industry Association

Annual Report 2015

Title of Research:

12_S01-01-3

Probabilistic Exposure Evaluation Model for Relatively Small-scale Occupational Workplace

Principal Investigator:

Akihiro Tokai, Dr. Eng. (Professor, Osaka University, Division of Sustainable Energy & Environmental Engineering, Laboratory of Environmental Management)
Yamadaoka 2-1, Suita, Osaka 565-0871, JAPAN
(tel)+81-6-6879-7676, (e-mail) tokai@see.eng.osaka-u.ac.jp

Collaborators:

Zhou Liang, Dr. Eng. (Project researcher, Osaka University, Division of Sustainable Energy & Environmental Engineering, Laboratory of Environmental Management)
Yamadaoka 2-1, Suita, Osaka 565-0871, JAPAN
(tel)+81-6-6879-7677, (e-mail) zhou@em.see.eng.osaka-u.ac.jp

Summary of Research:

The purpose of this study is to develop an exposure assessment model that assists voluntary chemicals management by Japanese downstream industries for occupational exposure which seem to have relatively higher risk in the industrial supply chain. We have developed a probabilistic assessment tool for the occupational exposure in Japan based on the framework of Advanced REACH Tool (ART) constructed in European ART consortium. We named this tool as SWEEs (integrated Score-based Workplace Exposure system) and validated the exposure estimating algorithm by using limited amount of some available data of the organic solvent in Japanese industries. What we have done were model validation and building pathway of practical application by industrial sectors.

Improvement of function for combined exposure was added and also flexibility for data handling of user interface were improved, then SWEEs ver. 1.0 was developed.

Timeline:

July 2015 – May 2016

Topics: N/A

Poster presentation at 3rd LRI annual meeting for presenting research output, Tokyo.

Publications:

Haruko Yamaguchi, Asato Itoh and Akihiro Tokai (2015) Development of Occupational Exposure Assessment Tool Assisting Voluntary Risk Management of Industrial Sectors, World Congress of Risk, Singapore 2015/7/20.

Akihiro Tokai (2016) Exposure Evaluation Model for Relatively Small-scale Occupational Workplace for assisting voluntary action, 89th annual meeting Japan society of occupational health, Fukushima, 2016/5/25

Akihiro Tokai, Haruko Yamaguchi, and Liang Zhou (2016) Development of Occupational Exposure Assessment Tool Assisting Voluntary Risk Management of Industrial Sectors, Intl. Journal of Risk Theory, accepted.

Development and assessment of new risk assessment methods

Title of Research:

12_S01-02-3

Human physiologically-based pharmacokinetic modeling of industrial chemicals with chimeric mice with humanized liver

Principal Investigator: Prof. Hiroshi Yamazaki, PhD (Showa Pharmaceutical University, Laboratory of Drug Metabolism and Pharmacokinetics), 3-3165, Higashi-tamagawa Gakuen, Machida, Tokyo 194-8543, Japan. (phone) +81-42-721-1406, (e-mail) hyamazak@ac.shoyaku.ac.jp.

Collaborators: Norie Murayama, ibid, (e-mail) muraya_n@ac.shoyaku.ac.jp; Makiko Shimizu, ibid, (e-mail) shimizu@ac.shoyaku.ac.jp; Hiroshi Suemizu, CIEA, (e-mail) suemizu@ciea.or.jp. Ryoji Takano, Fujitsu Kyushu Systems, (e-mail) takano.r@jp.fujitsu.com

Summary of Research: A simplified physiologically based pharmacokinetic (PBPK) model was defined in humans using humanized-liver mice, in which the liver has been repopulated with human hepatocytes is one of the challenge for evaluation of species differences. In order to overcome limitation of available human hepatocytes, the human hepatic cell line HepaRG were evaluated as promising donor cells for liver reconstitution in the TK-NOG mouse model. Taken together, the utility of this simplified PBPK model with humanized mice could be also expanded to the industry researchers and regulatory authorities to investigate a variety of chemicals.

Timeline: From March 1, 20145 to February 28, 2016

Topics: The principal Investigator was the leading organizer for 19th International Conference on Cytochrome P450 (Tokyo, 2015).

Publications:

1. Adachi,K., Suemizu,H., Murayama,N., Shimizu,M., and Yamazaki,H. (2015) Human biofluid concentrations of mono(2-ethylhexyl)phthalate extrapolated from pharmacokinetics in chimeric mice with humanized liver administered with di(2-ethylhexyl)phthalate and physiologically based pharmacokinetic modeling. *Environ.Toxicol.Pharmacol.*, **39**, 1067-1073.
2. Miyaguchi,T., Suemizu,H., Shimizu,M., Shida,S., Nishiyama,S., Takano,R., Murayama,N., and Yamazaki,H. (2015) Human urine and plasma concentrations of bisphenol A extrapolated from pharmacokinetics established in in vivo experiments with chimeric mice with humanized liver and semi-physiological pharmacokinetic modeling. *Regul.Toxicol.Pharmacol.*, **72**, 71-76.
3. Nishiyama, S., Suemizu, H., Shibata, N., Guengerich, F. P., and Yamazaki, H. (2015) Simulation of human plasma concentrations of thalidomide and primary 5-hydroxylated metabolites explored with pharmacokinetic data in humanized TK-NOG mice. Chem. Res.Toxicol., **28**, 2088-2090.
4. Yamazaki, H., Kunikane, E., Nishiyama, S., Murayama, N., Shimizu, M., Sugiyama, Y., Chiba, K., and Ikeda, T. (2015) Human plasma concentrations of tolbutamide and acetaminophen extrapolated from in vivo animal pharmacokinetics using in vitro human hepatic clearances and simple physiologically based pharmacokinetic modeling for radio-labeled microdose clinical studies. *Radioisotopes*, **64**, 509-519
5. Murayama,N., Usui,T., Slawny,N., Chesne,C., and Yamazaki,H. (2015) Human HepaRG cells can be cultured in hanging-drop plates for cytochrome P450 induction and function assays. *Drug Metab.Lett.*, **9**, 3-7.
6. Higuchi, Y., Kawai, K., Kanai, T., Yamazaki, H., Chesne, C., Guguen-Guillouzo, C., and Suemizu, H. Functional polymer-dependent 3D culture accelerates the differentiation of HepaRG cells into mature hepatocytes. *Hepatol.Res*., in press
7. Shida, S. and Yamazaki, H. Human plasma concentrations of five cytochrome P450 probes extrapolated from pharmacokinetics in dogs and minipigs using physiologically based pharmacokinetic modeling. *Xenobiotica*, in press.
8. Utoh, M., Suemizu, H., Mitsui, M., Kawao, M., Toda, A., Uehara, S., Uno, Y., Shimizu, M., Sasaki, E., and Yamazaki, H. Human plasma concentrations of cytochrome P450 probe cocktails extrapolated from pharmacokinetics in mice transplanted with human hepatocytes and from pharmacokinetics in common marmosets using physiologically based pharmacokinetic modeling. *Xenobiotica*, in press.

Title of Research:

12_S01-03-3

Establishment of animal testing for the prediction of respiratory sensitizing potential of chemicals

Principal Investigator:

Kohji Aoyama, PhD (Assistant Professor, Department of Hygiene and Health Promotion Medicine, Graduate School of Medicine and Dental Sciences, Kagoshima University,)
8-35-1 Sakuragaoka, Kagoshima, 890-8544, Japan
(tel)+81-99-275-5291 (e-mail) aoyama@m.kufm.kagoshima-u.ac.jp

Collaborators:

Hiroaki Kawaguchi (Associate Professor, Department of Pathology, Graduate School of Medicine and Dental Sciences, Kagoshima University)
8-35-1 Sakuragaoka, Kagoshima, 890-8544, Japan
(tel)+81-99-275-5263 (e-mail) k3038952@kadai.jp

Kunihiko Yamashita (Senior Researcher, Corporate Research Center, R & D Management, Daicel Corporation)
1239 Shinzaike, Aboshi-ku, Himeji, 671-1283, Japan
(tel)+81-79-274-4061 (e-mail) ku_yamashita@daicel.jp

Tomoko Muto (Senior Researcher, Safety Research Division, Safety Research Institute for Chemical Compounds Co.,LTD.)
363-24 Shin-ei, Kiyota-ku, Sappro, 004-0839, Japan
(tel)+81-11-885-5031 (e-mail) muto-tomoko@ka-anken.co.jp

Summary of Research:

The purpose of this project was to establish an animal model for predicting respiratory sensitizers and for evaluating their relative respiratory sensitizing potency. To this end, we developed a respiratory sensitization test using an intratracheal administration method in mice. The degree of Th2 type-allergic response in the lungs was determined using allergic inflammation scores based on histopathological grading. With this method, mice were sensitized with three concentrations of a test substance 5 days per week for 3 weeks. Three days following the last administration, mice were challenged with one concentration for three days, and were sacrificed 2 days later.

Next, we investigated the differences resulting from the different sensitization and elicitation treatment regimens in order to determine conditions that cause obvious allergic reactions. Interestingly, we found that allergic reactions tended to be reduced in mice sensitized with the highest concentration of a test substance. Therefore, we examined the influence of lower substance concentrations on eliciting responses based on the hypothesis that overdosing of the test substances in the sensitization and elicitation treatments suppressed allergic reactions. We also investigated the influence of the frequency of sensitization and elicitation treatments on those reactions. We found that toluene diisocyanate (TDI) and trimellitic anhydride (TMA) elicited allergic responses at lower concentrations than those of conventional elicitation. Additionally, both test substances resulted in allergic inflammation scores that were greater than or equal to conventional scores when eliciting concentrations were lower than those of conventional elicitation.

These results indicate that lower elicitation concentrations contributed to enhanced allergic reactions in our model system, which could be used to obtain obvious inflammatory responses by excluding primary irritation. Upon examining the influence of lowered sensitization and elicitation treatment frequency on inflammatory responses, we found that multiple elicitation treatments were required similar to the conventional elicitation study design. we will proceed with detailed studies to elucidate the influence of conditions such as frequency and duration on sensitization.

Timeline:

March 1, 2015 – February 29, 2016

Topics:

Publications:

Title of Research:

13_S01-01-2

Development of novel method to assess chemical compounds for the risk of cancer stem cell induction with iPS cells

Principal Investigator:

Masaharu Seno, Graduate School of Natural Science and Technology Okayama University

Collaborators:

Tomonari Kasai, Graduate School of Natural Science and Technology Okayama University
Shuichi Furuya, Okayama University Research Administration office
Akifumi Mizutani, Graduate School of Natural Science and Technology Okayama University
Junko Masuda, Graduate School of Natural Science and Technology Okayama University

Summary of Research:

Cancer stem cells are considered to be significantly responsible for growth, metastasis, invasion and recurrence of all cancer. We propose the risk assessment of chemical compounds for their potential of induction of cancer stem cells, while those for carcinogenic activity have been evaluated by mutagenicity test, repeated dose toxicity study, estimating with statistical analysis, and so on.

Cancer stem cells are typically characterized by continuous proliferation self-renewal as well as by differentiation potential, while stem cells are considered to differentiate into tissue specific phenotype of mature cells under the influence of microenvironment. Cancer stem cells can be traced back to the stem cells under specific influences of microenvironment, so called 'cancerous niche', which induces malignant tumors. We have very recently demonstrated the induction of cancer stem cells from mouse iPS cells culturing in the conditioned medium derived from cancer cells, although the details of the mechanisms of differentiation is not very well known as of yet.

In this study, we aim at the development of novel method to evaluate the risk of chemical compounds for the potential to induce cancer stem cells from iPS cells in vitro in a short period. Briefly, mouse iPS cells are suspended in the conditioned medium. The cells are further replenished with the growth medium with the compounds to be assessed.

We are currently observing the fluorescence intensity of GFP, of which expression is under the control of Nanog promoter, and the shape of colonies everyday for a period of 8 days. Based on these observations, 20 from 75 compounds assessed in the procedure were selected as prospectively positive candidates for converting mouse iPS cells to cancer stem cells. The modification of the method for efficient availability and high sensitivity is under way. We are planning further assessment for the mechanism through which the compounds are inducing the generation of cancer stem cells. The mechanism inducing cancer stem cells is needed to be studied in detail.

Timeline:

March 2012-February 2015

Topics:

Development of novel method to evaluate chemical compounds as the possible inducer of cancer stem cells using iPS cells. The 28th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments. (Dec. 2015, Yokohama)

Publications:

Analysis of the mechanism in the induction of cancer stem cells by signaling inhibitors. BMB 2015 (Dec. 2015, Kobe)

Development of a simple risk assessment of chemical compounds in the induction of cancer stem cells *in vitro*. The 28th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments. (Dec. 2015, Yokohama)

Title of Research: 12_PT01-02-3

Development of a user-friendly risk assessment tool for voluntary environmental risk assessment and management by business operators

Principal Investigator: Bin-Le Lin, Chief Senior Researcher, Research Institute of Science for Safety and Sustainability, National Institute of Advanced Industrial Science and Technology. 16-1 Onogawa Tsukuba City, Ibaraki, 305-8569 Japan. (tel) +81-29861 8844, (e-mail) binle-lin@aist.go.jp

Collaborators: Masashi Kamo (same as above). (tel) +81-29861 8029, (e-mail) masashi-kamo@aist.go.jp; Wataru Naito (same as above). (tel) +81-29861 8299, (e-mail) w-naito@aist.go.jp

Summary of Research: There is an urgent social need for the implementation of accurate and simple risk assessment and management of chemicals toward 2020 WSSD gogal. In order to address this need, during this project term we mainly focused on the improvement and enhancement of the English-and-Japanese versions of AIST-MeRAM 1.0 (released in Dec, 2014), implementation of function of risk management options based on the risk assessment results, expansion of the reliable databases. These achievements will be released as updated versions of “AIST-MeRAM 2.0” (English and Japanese) in May 2016. At the same time, in order to support the Asian strategy of JCIA and the promotion of Chemical Substances Control Law in Asian countries, a series of dissemination activities were performed such as 1) organize FTI-JCIA MeRAM workshop and joint workshop of the AIST and the Thailand National Laboratory, 2) support the workshop of bilateral cooperation of METI and the Thailand Ministry of Industry, 3) cooperate with JCIA member company to investigate the assessment of multiple substances by using MeRAM and to present on academic meeting. In addition to these achievements, research proposal based on agreements from plural meeting was developed and we have successfully proposed a win-win collaboration JCIA-LRI-project between ToxCalc and MeRAM.

Timeline: From March 2015 to February 2016

Topics: None

Publications: 1) (Presentation at Domestic Meeting) The 24th Environmental Chemistry Debate (24-26 June 2015), Sapporo Convention Center, Hokkaido, “Ecological risk assessment of surfactant by using AIST-MeRAM: case study of LAS”.
2) (Invited Speech at Domestic Workshop) YNU International Symposium 2015 on Management of Water Environment, “Dissemination of methodologies of chemical risk assessment and management to Asia: Introduction of AIST-MeRAM”. 3rd Aug 2015, Yokohama
3) (Paper Publication) JETOC Information B, 15-21 August 2015, “Introduction of a general tool for chemical ecological risk assessment and management: AIST-MeRAM”.
4) (Presentation at International Meeting) The 9th AIST-TISTR-NSTDA Conference, AIST Waterfront, Tokyo Japan, 28th April 2015. “AIST-MeRAM: a user-friendly software for ecological risk assessment and management of chemicals”.
5) (Presentation at METI-DIW Workshop) The 4th Workshop on Japan-Thailand Bilateral Cooperation on Chemical Management, 3rd -4th June 2015, DIW Office, Bangkok, Thailand.
6) (Organization of MeRAM Workshop at Thailand) 'MeRAM' Multi-purpose Ecological Risk Assessment and Management Tool Workshop. Queen Sirikit National Convention Center (QSNCC), Bangkok, Thailand. 8-9 July 2015..
7) (Research Outcome Exhibition) AIST Techno Bridge Fare, 22-23 October 2015, Tsukuba. To support an efficient environmental risk assessment and management of chemicals by "AIST-MeRAM" with the increase in the complexity.
8) (Presentation at International Meeting) The SETAC North America 36th Annual Meeting, Salt Lake City, United States. 1st-5th November 2015. Oral presentation: An all-in-one ecological risk assessment (ERA) tool: AIST-MeRAM.

Title of Research:

12_PT01-06-3

Sophisticated hazard prediction by active QSAR modeling

Principal Investigator:

Yoshimasa Takahashi, PhD (Professor, Dept. Comp. Sci. Eng., Toyohashi Univ. Tech.)
1-1 Hibarigaoka, Tempaku-cho, Toyohashi 441-8580 JAPAN
(tel)+81-0532-44-6878, (email)taka@cs.tut.ac.jp

Collaborators:

Yuji Ikegami (Graduate student, Toyohashi Univ. Tech.)
Yoshitaka Inagaki (Graduate student, Toyohashi Univ. Tech.)
Asumi Iwamoto (Graduate student, Toyohashi Univ. Tech.)

Summary of Research:

For this year, we have continued to develop the desk-top software tool, ToxCalc, which can be used for the eco-toxicity prediction of chemicals by means of the active QSAR modelling reported in our preceding work. Our points of the development of this tool are as follows; 1) System that the user wants to use, 2) system that is easy-to-use for the user, 3) system that is useful for the user. The user can use the Tox-Calc without any keyboard input. The system fully automated all the process of input a query structure, the active sampling of training compounds, making a QSAR model and the prediction of the specified toxicity. We have prepared the databases of three different endpoints, fish acute toxicity (96h-LC50), daphnia acute toxicity (48h-EC50) and algae growth rate (72h-EC50), which are used to collect the training compounds similar to the query and to make a QSAR model with those data. We have also implemented several additional functions to the system; searching for the experimental toxicity data by CAS number, referring to the QSAR information of the current prediction model, and setting the user preference of the system parameters for the prediction.

Timeline:

1st Mar. 2015 – 29th Feb. 2016

Topics:

Tox-Calc system demonstration at LRI Workshop 2015, Tokyo, Aug., 2015

Publications:

1）Yoshitaka Inagaki, Tomoya Yamazaki, Yoshimasa Takahashi, “Environmental toxicity prediction using active QSAR modelling”, The 24rd Symposium on Environmental Chemistry, Sapporo, Jun., 2015.<in Japanese>

2）Yoshitaka Inagaki, Tomoya Yamazaki, Yoshimasa Takahashi, “Development of a desk-top tool ToxCalc for eco-toxicity prediction of chemicals”, The 43rd Symposium on Structure-Activity Relationships and The 10th Japan-China Joint Symposium on Drug Discovery and Development、Niigata、Sep., 2015.

3）Yoshitaka Inagaki, Tomoya Yamazaki, Yoshimasa Takahashi, “Desk-top tool for eco-toxicity prediction of chemical substances”, The International Chemical Congress of Pacific Basin Societies 2015, Honolulu, Dec, 2015.

Title of Research:

13_PT01-01-2

Development of in vivo fluorescent imaging of neuronal differentiation in zebrafish for developmental neurotoxicity testing

Principal Investigator:

Yuhei Nishimura (Department of Pharmacogenomics, Mie University Graduate School of Medicine)

Collaborators:

Toshio Tanaka and Reiko Kawase (Department of Pharmacogenomics, Mie University Graduate School of Medicine)

Summary of Research:

Various chemicals may affect the differentiation of neurons, oligodendrocytes and astrocytes, which may cause neurodevelopmental disorders such as autism and attention deficit hyperactive disorder. *In vivo* models that can be used in both visualization of the neuronal differentiation and quantitative assessment of behavior are highly valuable for developmental neurotoxicity testing. Recently, zebrafish has emerged as an alternative non-mammalian animal model that allows testing of large numbers of subjects while reducing expenses and minimizing the use of mammalian subjects. In this study, we have developed three-color zebrafish exhibiting blue, yellow and red fluorescence in neurons, oligodendrocytes and astrocytes, respectively. Using the zebrafish, we demonstrated that anti-thyroid drugs and thyroid hormone inhibited and stimulated, respectively, the differentiation of oligodendrocytes, which is well consistent with the effects in mammals. Using *in silico* analysis of transcriptome data of mammalian stem cells, we identified sterol regulatory element binding transcription factors (SREBFs) as the key transcription factor in oligodendrocyte differentiation. Using the zebrafish, we were able to demonstrate that chemicals that could inhibit and activate SREBFs impaired and stimulated, respectively, the differentiation of oligodendrocytes. These results suggest that integration of *in silico* prediction and *in vivo* fluorescent imaging of neuronal differentiation in zebrafish can be useful to examine the developmental neurotoxicity of many chemicals and provide a sound basis for human risk assessments.

Timeline:

Mar 2015 ~ Feb 2016

Topics:

Publications:

1) **DNA damage response is involved in the developmental toxicity of mebendazole in zebrafish retina.** Sasagawa S, Nishimura Y, Kon T, Yamanaka Y, Murakami S, Ashikawa Y, Yuge M, Okabe S, Kawaguchi K, Kawase R, Tanaka T. *Frontiers in Pharmacology* 7:257 (2016)
2) **In vivo detection of mitochondrial dysfunction induced by clinical drugs and disease-associated genes using a novel dye ZMJ214 in zebrafish.** Sasagawa S, Nishimura Y, Koiwa J, Nomoto T, Shintou T, Murakami S, Yuge M, Kawaguchi K, Kawase R, Miyazaki T, Tanaka T. *ACS Chemical Biology* 11(2):381-8 (2016)
3) **Using zebrafish in systems toxicology for developmental toxicity testing.** Nishimura Y, Inoue A, Sasagawa S, Koiwa J, Kawaguchi K, Kawase R, Maruyama T, Kim S, Tanaka T. *Congenital Anomalies (Kyoto)*. 56(1):18-27 (2016)
4) **Pharmacological profiling of zebrafish behavior using chemical and genetic classification of sleep-wake modifiers.** Nishimura Y, Okabe S, Sasagawa S, Murakami S, Ashikawa Y, Yuge M, Kawaguchi K, Kawase R, Tanaka T. *Frontiers in Pharmacology* 6:257 (2015)

Title of Research:

13_PT01-02-2

Applied research of a novel in vitro method for developmental toxicity to facilitate the industrial utilization

Principal Investigator:

Koji Yamakage, PhD (Director, Hatano Research Institute, Food and Drug Safety Center)
Ochiai 729-5, Hatano-shi, Kanagawa 257-8523, Japan
(tel) +81-(0)463-82-4751, (e-mail) yamakage.k@fdsc.or.jp

Collaborators:

Noriho Hanaka (Hatano Research Institute, Food and Drug Safety Center) ; Hajime Kojima (National Institute of Health Sciences, Japan); Koichi Saito, Noriyuki Suzuki (Sumitomo Chemical, Co., Ltd); Yuichi Ito (Kao Corporation); Kazunori Yanagi (Sumika Chemical Analysis Service, Ltd.); Takashi Omori (KOBE University)

Summary of Research:

The purpose of the present research is to lead the Hand1-Luc EST, test using ES cells transfected with a reporter gene to a wide use in the chemical industry. To achieve this goal, the Hand1-Luc EST is currently under validation process. According to experts, to satisfy objective valuation and reliability of the test, it is necessary to gradually gather data, and verify the robustness, the predictability and the inter- and intra- laboratory reproducibility of the method. Before adoption of the test in 2013 by the Ministry of Economy, Trade and Industry, the validation management team, composed of Japanese and international expert on developmental toxicity or alternative test methods, has been organized. The international Validation was started to verify, with the aid of Japanese collaborating labs, the improvement of the protocol, the satisfaction of the established acceptance criteria, the technical transfer and inter- and intra- laboratory reproducibility. Until now, the technical transfer and high intra-laboratory reproducibility has been cleared by tests done with phase 0 and phase 1 study. With the support of LRI, by gathering the results from phase 2a, 2b and 2c, the inter- laboratory reproducibility reached 83.3% (10/12 chemicals were consistent with the in vivo data) and the intra-laboratory reproducibility was higher than 75% in each laboratory.
This semester, firstly, when phase2 was finished, we improved the way of analyzing the data after considering the advice from experts. Concretely, the way of fitting curves was changed from a 2 parameter curve fitting to a 3 parameter curve fitting. Thanks to this revision, the relation between the IC_{50} and ID_{50} ratio was improved and thus, it was decided that the 3 parameter curve fitting shall be used for further experiments. The IC_{50} and the ID_{50} values being changed by the new analysis, the prediction model was revised. The phase 3 consisted in 16 chemicals tested in 3 participant laboratories to test the within laboratory reproducibility of the Hand1-Luc EST. One dose finding study and two or three definitive studies were carried out according to the results given by the introduction of the data (IC_{50}, ID_{50}, Maximum dose) in the prediction model. 4 chemicals did not meet the requirements of the within laboratory reproducibility (1 laboratory had a different prediction from the 2 other labs). By analyzing more in details the data obtained, some explanations were found. The first point was the determination of the maximum dose of some chemicals that precipitated at this concentration. The second point was a high dilution ratio chosen by a laboratory leading to a lesser accuracy for the IC_{50} and ID_{50} determination. Facing those facts, it was acknowledged that the reproducibility would be significantly improved by minor modification of the protocol concerning those points. The phase 3 satisfied the criteria fixed in the study plan (within laboratory reproducibility of 75%, 12/16 chemicals).The predictivity and the applicability of Hand1-Luc EST were also discussed. On the whole, it was concluded that the positive predictive value was very high and thus this test would be useful to detect positive embryotoxicants by a Top-Down approach. With a common agreement about the end of the validation, the validation report will be prepared so as to, then, submit the Hand1-Luc EST to the OECD to become a test guideline.

Timeline: March, 2015-

Topics: Presented at the 3rd Annual Conferences of New LRI (28th August, 2015, Tokyo)

Title of Research:

13_PT01-03-2

Development of a combined in vitro/in silico system to predict and evaluate the complex hepatotoxicity of chemical compounds.

Principal Investigator:

Kouichi Yoshinari (University of Shizuoka)

Collaborators:

Hiroyuki Kojima (Hokkaido Institute of Public Health)

Summary of Research:

The aim of this study is to develop an in vitro/in silico system for toxicity evaluation and prediction, using the chemicals' biological properties based on the in vitro reactivity to xenobiotic-responsive nuclear receptors in combination with the physicochemical properties shown as chemical descriptors. Especially, we have developing an prediction system for rat repeated dose toxicity (RDT) using a HESS database, which contains RDT test data of Chemical Substances Control Law-related substances and is available from National Institute of Technology and Evaluation, and a rat RDT test database of pesticides, which has been constructed by our group. In this system, we are using a grouping (read-across) method based on hierarchical clustering. In this research period, with HESS database compounds we have found that groupings only with chemical descriptors show relatively high accordance with groupings with RDT test data (Rand index: ~0.8). Meanwhile, some concerns and problems have been raised including the importance of the selection of descriptors and toxicity findings and the insufficiency of in vitro assay data. These points need to be solved and cleared in the next year. For the analysis with pesticides, the database construction and in vitro reporter assays of 4 nuclear receptors were completed. After calculation of descriptors, grouping analysis with these toxicity data, in vitro assay data and descriptors will be performed in the next year.

Timeline:

March 2015 – February 2016

Topics:

None

Publications:

Invited lecture

1. K. Yoshinari: Development of a combined in vitro/in silico system to predict and evaluate the complex hepatotoxicity of chemical compounds. in "What Will Work? Future Approaches and Technologies for Assessing Chemical Safety", 2015 ICCA-LRI & U.S. EPA Workshop. June, 2015. New Orleans, LA, USA.
2. K. Yoshinari: Role of nuclear receptors in chemical toxicity and its application to toxicity prediction. 2015 LRI Annual Meeting, August, 2015. Tokyo.
3. K. Yoshinari: Development of an in vitro/in silico combined system for the toxicity prediction. CBI Annual Meeting 2015. October, 2015. Tokyo.
4. K. Yoshinari: A new approach to the prediction of repeated dose toxicity. The 28th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments. December, 2015. Yokohama.
5. K. Yoshinari: Role of xenobiotic-responsive nuclear receptors in hepatotoxicity. 13th Meeting of the Asia Pacific Federation of Pharmacologists. February, 2016. Bangkok, Thailand.

Oral/poster presentation

1. M. Masuda, K. Yoshinari: Toxicological characterization of hepatocyte hypertrophy using public data of in vivo toxicity tests. The 135th Annual Meeting of the Pharmaceutical Society of Japan. March, 2015. Kobe.

2. K. Yoshinari, M. Masuda, A. Masuda, M. Sekimoto, K. Nemoto: Toxicological characterization of hepatocyte hypertrophy using rat 2-year repeated dose toxicity/carcinogenicity tests. The 42nd Annual Meeting of the Japanese Society of Toxicology. June, 2015. Kanazawa.
3. A. Masuda, M. Masuda, M. Sekimoto, K. Nemoto, K. Yoshinari: Construction of the database of rat 90-day repeated dose toxicity tests and its application to toxicological characterization of hepatocyte hypertrophy. The 42nd Annual Meeting of the Japanese Society of Toxicology. June, 2015. Kanazawa.
4. K. Yoshinari, A. Masuda, M. Masuda: Construction of toxicity database of rat repeated dose toxicity tests and its application to toxicological characterization of hepatocyte hypertrophy. Forum 2015 Pharmaceutical Health Science & Environmental Toxicology. September, 2015. Kobe.
5. A. Masuda, M. Masuda, K. Yoshinari: Toxicological characterization of hepatocyte hypertrophy for the safety evaluation of food contaminants: Analysis using in vivo toxicity test data of pesticides. The 1st Forum of Regulatory Sciences for Young Scientists. September, 2015. Funabashi.
6. K. Yoshinari, H. Nakajima: In vitro and in silico analyses of the association of hepatocyte hypertrophy with nuclear receptor activation and enzyme induction. 30th the Japanese Society for the Study of Xenobiotics Annual Meeting. November, 2015. Tokyo.

Title of Research:

15_PT01-01

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

Principal Investigator:

Tsuyoshi Nakanishi (Laboratory of Hygienic Chemistry and Molecular Toxicology, Gifu Pharmaceutical University)

Collaborators:

Hisamitsu Nagase (Laboratory of Hygienic Chemistry and Molecular Toxicology, Gifu Pharmaceutical University)

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 to detect the tissue-specific 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 for 7 days. And then, mice were fed phytoestrogen-free purified diet containing estradiol (0, 0.01, 0.1, 1, 10 ppm) for another 7 days. Mice were dissected and then the estrogenic effects were evaluated by measurement of uterine weight, detection of Luc activity, or detection of c-Myc tag production by using immunohistochemical analysis.

Results: Luc activity in the uterus increased significantly when estradiol was administered at 0.1 ppm or greater. In contrast, the uterine weight rose significantly only when estradiol was administered at 1 ppm or greater, suggesting that the E-Rep mice provide a more sensitive assay of estrogenic activity than the uterotrophic bioassay. In addition, estradiol at 10 ppm caused a significant increase in Luc activity of the all tested organs. Furthermore, estradiol at above 1 ppm made clearly detection of estrogen-dependent production of Luc with c-Myc in the mammary gland by immunohistochemical staining.

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

Timeline: April, 2015 – March, 2016

Topics: None

Publications: None

Title of Research:

13_S02-01-2

Study on the cellular and environmental effect related with solubility of industrial nanomaterials

Principal Investigator:

Hitoshi Iwahashi
Gifu University

Collaborators:

Satoshi Iwamoto
Gifu University

Takehiro Himaki
Gifu University

Hideto Fukushi
Gifu University

Unko Takahashi
National Institute of Advanced Industrial Science and Technology

Masanori Horie
National Institute of Advanced Industrial Science and Technology

Summary of Research:

A nano-object is defined as an object with one or more external dimensions being nanoscale (1-100 nm). Nano-objects have possible impacts on cellular and environmental effects and are of significant concern. However, the accumulation of toxicity evaluations under the strictly controlled experiments teaches us the essential factor that is concern to solubility of nano-object.
To confirm the solubility contribute the toxicity of nano-object, we focused on the following five issues.
1. Characterization of solubility by nano-object in the solvents.
2. Evaluation of nano-object that may cause environmental effects using microbes.
3. Studies on solubility of nano-object in the cells.
4. Evidences that solubilized yttrium ions from yttrium oxide nanoparticles cause toxicity.
5. Evaluation of cellular toxicity caused by silica nanoparticles.

Timeline: From November 2013

Topics:

Publications:

Toxicity caused by yttrium oxide nanoparticle is due to yttrium ions
Akihiro Moriyama, Ikuho Yamada, Youta Mizuno, Hitoshi Iwahashi, Junko Takahashi and Masanori Horie
The 21th Meeting of THE JAPANESE SOCIETY OF ENVIRONMENTAL TOXICOLOGY
2015 Sept. 2nd-3rd Tokyo, Japan

The effect of titanium dioxide (TiO2) nanoparticles to microbes under Ultra Violet (UV) irradiation.
Ikuho Yamada, Youta Mizuno, Hitoshi Iwahashi, Junko Takahashi and Masanori Horie
The 21th Meeting of THE JAPANESE SOCIETY OF ENVIRONMENTAL TOXICOLOGY
2015 Sept. 2nd-3rd Tokyo, Japan

Importance of the evaluation of ion solubility from nanoparticles to cellular toxicity.
Masamitsu Fujita, Ikuho Yamada, Hitoshi Iwahashi, Masanori Horie
The 21th Meeting of THE JAPANESE SOCIETY OF ENVIRONMENTAL TOXICOLOGY
2015 Sept. 2nd-3rd Tokyo, Japan

Yamada, I. Nomura, K. Iwahashi, H. Horie, M. The effect of titanium dioxide (TiO2) nano-objects, and their aggregates and agglomerates greater than 100 nm (NOAA) on microbes under UV irradiation. Chemosphere 143, 123-7, 2016

Title of Research:

15_PT02-01

Construction of the novel *in vitro* evaluation systems based on the genotoxic mechanisms of nanomaterials

Principal Investigator: Yukari Totsuka, Ph.D., Natl. Cancer Centr. Res. Inst.

Collaborators:

Dai Nakae, M.D., Ph.D. (Professor, Laboratory of Food Safety Assessment Science, Department of Nutritional Science and Food Safety, Faculty of Applied Biosciences, Tokyo University of Agriculture),
Masatoshi Watanabe, M.D., Ph.D. (Professor, Yokohama National University, Graduate School of Engineering),
Koichiro Hayashi (Division on Materials Research, Institute of Materials and Systems for Sustainability, Nagoya University)

Summary of Research: To establish new in vitro evaluation systems for lung/skin toxicity of nanomaterials, we tried to establish assay models as listed below.

① A novel in vitro genotoxicity assay model to asses lung toxicity using a co-culture system.
② A novel in vitro assay model to assess skin toxicity using 3D human skin reconstitution models.
③ A novel in vitro assay model using 3D culture techniques such as tissue-slice and spheroid.

For the lung toxicity test, we used a co-culture system of fibroblast cells established from mice lungs (GDL1 cells) and murine macrophage cells (RAW264.7). Mutation frequencies induced in GDL1 by both MGT and MWCNT were significantly greater in the coexistence of RAW264.7 than in its absence. Mutation spectra observed in GDL1 co-cultureed with RAW264.7 were distinguished from those seen in single-cultured GDL1, and similar to those observed in mice lungs exposed to these nanomaterials in vivo. Moreover, the levels of oxidative- and inflammatory-related DNA adducts, 8-oxo-dG and ε dC, in GDL1 exposed to MGT or MWCNT exposure were greater in the co-culture condition than in the single-culture condition. Therefore, it is suggested that the co-culture assay model can be considered as a suitable evaluation system for lung toxicity of nanomaterials.

As a 3D skin model, we selected the LabCyte EPI model. The cytotoxicity of gold nanoparticles was assessed by measuring the activity of lactate dehydrogenase leaking into the culture medium. Significant cell death was induced in a clear dose-dependent manner. On the other hand, the same gold nanoparticles showed more potent cytotoxicity against monolayered cultured HepG2 cells than the 3D skin model. It is thus suggested that the LabCyte EPI model may be useful as a novel in vitro system to assess skin toxicity of nanomaterials, and that in this model reconstituted skin may possess a barrier function similarly to human and animal in vivo skin.

Timeline: March 1st, 2015 – February 29th, 2016

Topics: “Construction of the novel in vitro evaluation systems based on the genotoxic mechanisms of nanomaterials “ Presented at the poster session of the Annual Conference of New JCIA-LRI

Publications:

Journals:

1. Ishino Kousuke, Kato Tatsuya, Kato Mamoru, Shibata Tatsuhiro, Watanabe Masatoshi, Wakabayashi Keiji, Nakagama Hitoshi, Totsuka Yukari. “Comprehensive DNA adduct analysis reveals pulmonary inflammatory response contributes to genotoxic action of magnetite nanoparticles.” International Journal of Molecular Sciencs. 2015;16:3474-92.
2. Koichiro Hayashi, Wataru Sakamoto, Toshinobu Yogo “Smart Ferrofluid with Quick Gel Transformation in Tumors for MRI-Guided Local Magnetic Thermochemotherapy” Advanced Functional Materials 2016; 26: 1708–1718.

Meetings:

1. Saho Hashimoto, Sou Yamaguchi, Kanako Kojima, Nao Furuta, Tadashi Nittami, Kazuaki Kawai, Hiroshi Kasai, and Masatoshi Watanabe. “Cellular effects of magnetic nanoparticles as determined by cell type and surface coating.” The 74th Annual Meeting of the Japanese Cancer Association, Nagoya, Oct.8-10, 2015.
2. Nao Furuta, Saho Hashimoto, Jieun Seo, Kanako Kojima, Sou Yamaguchi, Tadashi Nittami, and Masatoshi Watanabe. “Effect of magnetic nanoparticles on cancer stem-like cells from human lung and prostate cancer cell lines.” The 74th Annual Meeting of the Japanese Cancer Association, Nagoya, Oct.8-10, 2015.
3. Koichiro Hayashi, Yoshitaka Sato, Wataru Sakamoto, Toshinobu Yogo “Multifunctional Nanoparticles for MRI-Guided Magnetic Thermochemotherapy” BIT’s 2nd Annual World Congress of Smart Materials-2016, Singapore, Mar. 2016 (invited).
4. Koichiro Hayashi, Yoshitaka Sato, Wataru Sakamoto, Toshinobu Yogo “Core-Shell Nanoparticles for the Combination of Magnetic Hyperthermia and Chemotherapy” ISETS '15 International Symposium on EcoTopia Science 2015 - Innovation for Smart Sustainable Society -, Nagoya, Nov. 2015.
5. Koichiro Hayashi “Clustered Magnetic Nanoparticles-Polymer Core-Shell Nanoparticles for Magnetic Thermochemotherapy” The 2015 Energy, Materials, and Nanotechnology (EMN) Meeting, Phuket, Thailand, May, 2015 (invited).
6. Koichiro Hayashi, Yusuke Sato, Takuma Maruhashi, Wataru Sakamoto, Toshinobu Yogo “Synthesis of multifunctional hybrid nanoparticles for imaging and therapy” Tokyo, The Ceramic Society of Japan Annual Meeting 2016, Mar. 2016 (invited).
7. Koichiro Hayashi, Yoshitaka Sato, Wataru Sakamoto, Toshinobu Yogo “Alternating Magnetic Field-Responsive Smart Core-Shell Nanoparticles for Magnetic Thermochemotherapy” Toyama, The Ceramic Society of Japan The 28th Fall Meeting, Sep. 2015.

Title of Research:

13_S03-01-2

Study on the mechanism of sick building syndrome and development of risk assessment system utilizing Drosophila

Principal Investigator:

Kaeko Kamei, PhD (Professor, Kyoto Institute of Technology, Department of Biomolecular Engineering)
Matsugasaki, Sakyo-ku, Kyoto, 606-8585, Japan
(tel) +81-75-724-7553, (e-mail) kame@kit.ac.jp

Collaborators:

Summary of Research:

The aim of this study is to reveal the mechanism of sick building syndrome and develop risk assessment system, utilizing Drosophila. We exposed Drosophila to formaldehyde gas or xylene gas for analyzing proteins of which concentrations were changed by exposing. The 2-D electrophoresis and quantitative RT-PCR of Drosophila individual revealed that the expression of triose phosphate isomerase, one of sugar metabolism enzymes, was enhanced by xylene treatment. We analyzed the relation between exposure period and expression level of triose phosphate isomerase. The quantitative RT-PCR of all enzymes involved in glycolysis and gluconeogenesis demonstrated that the expression of the glucose metabolism-related enzymes varies greatly by xylene exposure. In contrast, the expression of α-actinin and actin 5C, were reduced greatly by formaldehyde treatment. The reduction was largest at the early exposure to formaldehyde. These demonstrated that different causative agents affected the expression of different proteins. Therefore, the sick building syndrome is likely to develop at a different mechanism by causative agent.

Timeline:

March 1, 2015 – February 29, 2016

Topics:

The 3rd Research Meeting of the New LRI of Japan Chemistry Industry Association, Tokyo, August, 2014. “Study on the mechanism of sick building syndrome and development of risk assessment system utilizing *Drosophila*”, Poster presentation.

Title of Research:

15_S03-01-1

Establishment of in vitro evaluation system for the prediction of respiratory sensitizing potential of chemicals

Principal Investigator:

Takayuki Yoshimoto, PhD (Professor, Tokyo Medical University, Institute of Medical Science)
6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan
(tel)+81-3-3351-6141 Ext. 431, (e-mail) yoshimot@tokyo-med.ac.jp

Collaborators:

Izuru Mizoguchi, PhD (Research Associate, Tokyo Medical University, Institute of Medical Science) 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan
(tel) +81-3-3351-6141 Ext. 448, (e-mail) imizo@tokyo-med.ac.jp

Summary of Research:

Although the number of patients with occupational asthma has recently been estimated to be as high as approximately 15-18% of that with adult asthma, there is no established diagnostic methods for it and no standard, validated and regulatory-accepted methods to identify potential respiratory sensitizers and distinguish them from skin sensitizers. This is in marked contrast to the situation of contact hypersensitivity, where there are highly sophisticated, established diagnostic methods. Testing assays for assessment of chemical sensitizers including GPMT and LLNA, which utilize guinea pigs and mice, respectively, were previously developed. However, the use of animal models in safety testing will be significantly limited due to introduction of 3Rs principles of refinement, replacement, and reduction of animal experimentations in research wherever possible. Therefore, several in vitro assays including DPRA, KeratinoSense, h-CLAT, and IL-8 Luc Assay have been developed. However, these alternative methods cannot distinguish chemical respiratory sensitizers and chemical skin sensitizers, although the risk-management countermeasure is markedly different between them.

Therefore in the present study, we aim at developing a novel in vitro assay, which can discriminate chemical respiratory sensitizers from chemical skin sensitizers by taking advantage of the fundamental differences between their modes of function (AOP: Adverse outcome pathway); development of type 2 helper T (Th2) immune responses, which is critical for respiratory sensitization.

Using the Alvetex® Scaffold from Reinnervate, we have established an immunocompetent 3D co-culture system of human upper airway. It consists of a human airway epithelial cell line, human immature dendritic cells (DCs) derived from peripheral blood $CD14^+$ monocytes, and a human fibroblast cell line cultured in individual scaffolds and then assembled into a 3D multi-cell tissue model, which more closely mimics the in vivo physiological situation. The present results indicate that this 3D co-culture system has great potential to contribute to the establishment of in vitro evaluation system for the prediction of respiratory sensitizing potential of chemicals by means of enhanced expression of molecules critical for induction of Th2 differentiation and immune responses such as OX40L and IL-10 as markers.

Timeline:

March 1, 2015-Feberary 29, 2016

Topics:

The 3th Annual Meeting of The Japan Chemical Industry Association LRI, poster presentation “Development of a novel method for in vitro assessment of chemical respiratory sensitizers” (Tokyo, August 28, 2015)

Publications:

The 28th Annual Meeting of The Japanese Society for Alternatives to Animal Experiments, Japan Chemical Industry Association LRI Symposium, oral presentation “Development of a novel method for in vitro assessment of chemical respiratory sensitizers” (Yokohama, December 11, 2015)

Title of Research:

12_PT03-01-3

Development of high sensitivity in vitro assessment system of chemical-mediated hypersensitivity by using serine protease inhibitor-deficient cells
Subtitle: Analysis of in vitro assessment for respiratory allergy

Principal Investigator:

Akira Nakamura, Department of Immunology, Kanazawa Medical University.
1-1 Daigaku, Uchinada, Ishikawa, Japan 920-0293 (tel) +81-76-218-8120
(e-mail) aki-n@kanazawa-med.ac.jp

Summary of Research:

The allergic disorders triggered by various chemicals are separated into two types of diseases, IgE-dependent immediate allergy and IgE-independent chronic allergy. Mast cells, basophils and eosinophils act as the effector cells in both allergic reactions. In particular, basophils and eosinophils rapidly infiltrate into respiratory tissue, and cause airway hyper-responsiveness and airway obstruction. These cells secrete abundant serine proteases as well as chemical mediators and cytokines. Serine proteases, such as elastases, disrupt the basement membrane, leading to the infiltration of inflammatory cells. The serine proteases are repressed by the serine protease inhibitors. In the previous LRI research, we found that a serine protease inhibitor, basophils and eosinophils lacking a secretory leukoprotease inhibitor SLPI produced more cytokines than wild type cells after stimulation with IgE or LPS. Therefore, we have planed to establish the highly sensitive cell line by the deletion of serine protease inhibitors using a human basophilic cell line, KU812-F. Because KU812-F expresses high levels of Serpin b1 and b6, and we have generated stable Serpin b1 knockdown KU812- F cell lines by using lentiviral-delivered sh (short hairpin) RNA. The knockdown KU812-F produced IL-6 about 2 times higher than KU812-F after administration with toluene diisocyanate (TDI). The knockdown cells also secreted IL-13 upon stimulation with TDI. In contrast, scramble and wild type KU812-F cells did not produce IL-13. In the present LRI research, we investigated mRNA expressions of cytokines that contribute to allergic responses upon stimulated with respiratory sensitizers, TDI and formaldehyde, and contact sensitizers, methyl salicylate and DNCB. KU812-F cells showed high levels of IL-4, 6, 13, and TSLP mRNAs upon TDI stimulation. Although there were no significant differences of mRNA expressions between knockdown and control scramble cells, ELISA assays showed that the knockdown cells, but not scramble cells, produced IL-13, and also demonstrated that the knockdown cells produced more IL-13 than control cells after administration with formaldehyde. The knockdown cells did not secrete IL-6 and 13 when stimulated with contact sensitizers. These results suggested that IL-13 ELISA assay is a suitable application for the detection of respiratory sensitizes using Serpin b1 knockdown KU812-F cells.

Timeline:

2015/3/1-2016/2/28

Topics:

Poster presentation entitled “Development of high sensitivity *in vitro* assessment system of chemical-mediated hypersensitivity by using serine protease inhibitor-deficient cells.” at the 4th annual conference of new LRI, Tokyo, Japan, August 2015

Title of Research:

13_PT03-01-2

Quantitative prediction of the pharmacokinetics of chemical substances by the use of mathematical model with considering the age-dependent functional changes of metabolic enzymes and transporters

Principal Investigator:

Hiroyuki Kusuhara, PhD (Professor, Lab. of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo)
7-3-1 Hongo, Bunkyo-ku Tokyo 113-0033 JAPAN
(tel)+81-3-5841-4770, (e-mail) kusuhara@mol.f.u-tokyo.ac.jp

Collaborators:

Kazuya Maeda, PhD (Associate Professor, Lab. of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo)
7-3-1 Hongo, Bunkyo-ku Tokyo 113-0033 JAPAN
(tel)+81-3-5841-4772, (e-mail) kmaeda@mol.f.u-tokyo.ac.jp

Yuichi Sugiyama, PhD (Head, Sugiyama Laboratory, RIKEN Innovation Center, RIKEN Cluster for Industry Partnerships, RIKEN)
1-6 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045 JAPAN
(tel)+81-45-503-9211, (e-mail) ychi.sugiyama@riken.jp

Ichiro Ieiri, PhD (Professor, Dept. of Clinical Pharmacokinetics, Graduate School of Pharmaceutical Sciences, Kyushu University)
3-1-1 Maidashi, Higashi-ku Fukuoka 812-8582 JAPAN
(tel)+81-92-642-6656, (e-mail) ieiri-ttr@umin.ac.jp

Summary of Research:

To predict the influence of age on the pharmacokinetics of chemical substances, we performed a human clinical study for investigating the age-dependent functional changes of metabolism and transport processes of compounds with healthy adults and aged persons. The involvement of OCT family transporters in the membrane transport of cationic compounds was investigated both in vitro and in vivo. As a result, triptans especially sumatriptan and zolmitriptan can be used as good probe drugs for hepatic OCT1. We also found that OCT2 is responsible for the renal uptake of trimethylammonium N-oxide (TMAO).

Timeline:

March 1, 2015 -

Topics:

2015 JCIA LRI result presentation (poster) “Quantitative prediction of the pharmacokinetics of chemical substances by the use of mathematical model with considering the age-dependent functional changes of metabolic enzymes and transporters”

Publications:

Takeshi Miyake, Hiroyuki Kusuhara “Investigation of the transporters involving renal uptake and efflux of trimethylamine N-oxide (TMAO)”, 30th Japan Society of the Study of Xenobiotics (JSSX) Annual meeting, Tokyo, 2015.11
Takeshi Miyake, Hiroyuki Kusuhara “Organic Cation Transporter 2 (OCT2) is responsible for the renal influx of trimethylamine N-oxide (TMAO)”, 21st Forum for Pharmaceutical Technology Innovation Workshop for young scientists, Tokyo, 2015.11
Takeshi Miyake ”Organic Cation Transporter (Oct) is Responsible for the Renal Influx of Trimethylamine N-oxide (TMAO)”、Academy of Pharmaceutical Sciences and Technology, Japan (APSTJ) Global Education Seminar 15-3, Shizuoka, 2016.2

Title of Research:

12_PT04-01-3

Comprehensive evaluation methods for chemicals registered in PRTR.

Principal Investigator:

Norihide NAKADA (Research center for Environmental Quality Management, Graduate School of Engineering, Kyoto University)
520-0811, 1-2 Yumihama, Otsu, Shiga, +81-77-527-6220, nakada.norihide.8w@kyoto-u.ac.jp

Collaborators:

Shuhei TANAKA (Graduate School of Global Environmental Studies, Kyoto University)
606-8501, Yoshida-honmachi, Sakyo, Kyoto, +81-75-753-5151, t-shuhei@eden.env.kyoto-u.ac.jp
Yuji SUZUKI (Graduate School of Global Environmental Studies, Kyoto University)
606-8501, Yoshida-honmachi, Sakyo, Kyoto, +81-75-753-5168, suzuki.yuji.8s@kyoto-u.ac.jp

Summary of Research:

This research is aimed at establishing formation potential methods for toxic chemicals including trihalomethanes, aldehydes, nitrosamines and perfluorinated carboxylic acids. These formation potential methods were applied to chemicals registered in PRTR, and these chemicals and their formation potentials and precursors were monitored in sewage treatment plants and urban rivers. In addition, factors that governed the behavior in the environment were also quantified through batch experiments. The behaviors and factors were used to develop a method to estimate the behavior and concentration of the chemicals in the urban river stretch.

Timeline: March 1, 2015 – February 29, 2016

Topics:

1) Poster presentation at 3rd Annual Conference of LRI (poster)
2) Norihide NAKADA, “Removal and formation of emerging pollutant in wastewater treatment process and their ecological influences”, ICCL-LRI and EPA Workshop, New Orleans, LA, USA, 2015. (Oral)

Publications:

1) Norihide NAKADA, Shuhei ITAI, Seiya HANAMOTO, Hiroaki TANAKA, “Behavior of oxidation by-products and their formation potential in an urban river”, The 24th Annual Conference of Japan Society for Environmental Chemistry, Sapporo, June 2015. (Oral)
2) Yuji SUZUKI, Shuhei TANAKA, Shigeo FUJII, Norihide NAKADA, Norimitsu SAITO, “Biodegradation of Fluorotelomer Alcohols and Formation of Perfluorocarboxylic Acids in Biological Treatment Processes”, The 37th Annual Conference of the Association of Environmental & Sanitary Engineering Research, Kyoto, July 2015. (Oral)
3) Yuji SUZUKI, Shuhei TANAKA, Shigeo FUJII, Norimitsu SAITO, “Behavior of Perfluoroalkyl Carboxylates and Their Potential Precursors in Wastewater Treatment Processes”, The 18th Symposium of Japan Society of Water Environment, Sendai, Japan, September 2015. (Oral)
4) Shuhei ITAI, Norihide NAKADA, Hiroaki Tanaka, Formation potential test for individual chemicals during oxidation processes. The 36th Annual Conference of the Association of Environmental & Sanitary Engineering Research, Kyoto, July 2014. (Oral, Poster)

Title of Research:

13_PT05-01-2

Development and practical verification of novel comprehensive monitoring system for multiple contamination of environemntal pllutants in Mekong River basin

Principal Investigator:

Kazumasa Hirata, PhD (Professor, Osaka University, Pharmaceutical Sciences; 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan)
(tel) +81-06-6879-8238, (e-mail) hirata@phs.osaka-u.ac.jp

Collaborators:

Kazuo Harada, PhD (Associate Professor, Osaka University, Pharmaceutical Sciences; 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan); Hideyuki Matsuura, PhD (Assistant Professor, Osaka University, Pharmaceutical Sciences; same as above); Yoshinori Sumimura (Associate Professor, Osaka University, Global Collaboration Center; 2-7 Yamadaoka, Suita, Osaka 565-0871, Japan); Nguyen Quang Trung (Institute of Environmental Technology, Department of Environmental Toxic Analysis; 18 Hoang Quoc Viet Rd., Cau Giay Dist. Hanoi); Misa Muraoka (Specially Appointed Assistant Professor [Full time], Osaka University, Pharmaceutical Sciences; same as above); Tran Thi My Duyen (Lecturer, Can Tho University, College of Aquaculture and Fisheries, Department of Aquatic Pathogen; Campus II, 3/2 St., Xuan Khanh Ward, Ninh Kieu Dist., Can Tho city, Vietnam.: *Current title: ph.D. student, Osaka University, Pharmaceutical Sciences; same as above); Jun-ichi Nishikawa, PhD (Professor, Mukogawa Women's University, School of Pharmacy and Pharmaceutical Sciences; 11-68 Koshien Kyuban-cho, Nishinomiya 663-8179, Japan); Taku Yamashita (Associate Professor, Mukogawa Women's University, School of Pharmacy and Pharmaceutical Sciences; same as above)

Summary of Research:

Due to the rapid industrialization and population concentration, serious environmental pollution has been caused by multiple contaminations of pollutants, such as agricultural chemicals, endocrine disruptors, heavy metals, and antibiotics for the international river basin of Southeast Asia, like Mekong River basin. These environmental pollutions lead to the potential for serious health damage in ASEAN countries and for high-cost and careful inspection for imported foods from the corresponding area in Japan. To avoid these risks, it is important to construct a monitoring system of food and environmental samples for multiple contaminations of environmental pollutants. The aim of our project is to develop a novel comprehensive monitoring system for multiple contaminations as an appropriate technology for ASEAN countries. For this purpose, we plan to tackle the following topics; 1. Gathering of information and investigation of situation of pollution in Mekong River basin, 2. Development of bioassay system for detecting four group pollutants (agricultural chemicals, endocrine disruptors, heavy metals, and antibiotics), 3. Verification of the validity and conformity of bioassay for preliminary analysis applicable to Mekong River basin and ASEAN countries, 4. Improvement and establishment of the bioassay system, 5. Investigation on how appropriate for ASEAN countries the bioassay system is. In the present term, we have tackled a construction of biosensors for sulfonamides and antibiotics that inhibit bacterial protein synthesis. We proposed a new principle for each biosensor and performed proof-of-concept experiments.

Timeline:

March 1, 2015-

Topics:

The New LRI Research Meeting (Tokyo, August 2015; Poster presentation)

Publications:

Annual Report 2015

Japan Chemical Industry Association

1-4-1 Shinkawa, Chuo-ku Tokyo, 104-0033, Japan
TEL. +81-3-3297-2575　FAX. +81-3-3297-2612
URL：http://www.nikkakyo.org/
URL for LRI : http://www.j-lri.org/