LRI

Long-range Research Initiative

Annual Report 2014

2014

Japan Chemical Industry Association

Annual Report 2014

Title of Research:

12_S01-01-2

Probabilistic Exposure Evaluation Model for Relatively Small-scale Occupational Workplace

Principal Investigator:Akihiro Tokai

Collaborators:Haruko Yamaguchi, Asato Itoh, ShunKimura

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 seems 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.

First, for a validation of SWEEs, we collected the observed exposure data of the organic solvent measured in some workplaces related to the automobile manufacturing industry in Japan. The result indicated that the exposure estimate by SWEEs was the 0.1-873 times larger than the measured data as a whole. When looking at each task, the 93% of estimated exposure estimate on the paint production were within the factor of 3. These suggest that continuous effort to collect relevant information on workplace exposure are strongly required. This also needs cooperative actions from stakeholders.

Second, to prioritize the required collected data for SWEEs, we tried to estimate probabilistically estimated the exposure concentration stochastically by assuming providing the probability distribution to each of mechanistic model parameters used in of ART and performed sensitive analysis with the case of workers exposed to toluene in gravure printing industry in Japan. As the result of this analysis, the average of estimated concentration of toluene in the painting industry was 30.8 ppm, the 0.66 times of observed concentration (46.7ppm). In addition, modified factors such as related to local control measurement and action of workers in workplace were identified as a key parameters that contributed largely to the estimated concentration by this sensitive analysis. In parallel with this modeling study, we continue to improve model framework applicable under limited data availability condition, up to now we have examined introduction of the model for multi chemical behavior in the workplace, validation through data rich case studies. We also continuously do socio-economic needs assessment of risk and exposure assessment of industry sectors. Through integrating these tasks, final goal will be the participatory evolving systems of voluntary risk management system in industrial sectors in Japan.

Timeline: 2014.4 – 2015.6

Topics: N/A

Publications:

Yamaguchi, Haruko; Hamada, Hayato; Ito, Asato; Tokai, Akihiro、Development of probabilistic occupational exposure assessment tool assisting voluntary chemical risk management of industrial sector in Japan, 2014 annual meeting of Society for Risk Analysis-Europe

Title of Research:

12_S01-02-2

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 based on metabolic parameters determined experimentally *in vitro* and/or *in vivo* and physiological parameters derived from the literature. In this study, the PBPK model basically consists of a chemical absorption compartment, a metabolizing compartment, and a central compartment for a wide of academic, regulatory, and industrial users. Test chemicals and primary metabolites, organophosphorus pesticides acephate and chlorpyrifos, herbicidal carbamate molinate, di(2-ethylhexyl)phthalate, and bisphenol A were evaluated in human bodies by the present PBPK modeling. 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 November 1, 2013 to February 28, 2015

Topics: The principal Investigator was awarded by Japanese Society for the Study of Xenobiotics.

Publications:

1. Y. Higuchi, K. Kawai, H. Yamazaki, M. Nakamura, F. Bree, C. Guillouzo, and H. Suemizu. The human hepatic cell line HepaRG cells, possible cell source for steady generation of humanized liver TK-NOG mice. *Xenobiotica* 44:146-153, 2014.
2. N. Murayama, R. van Beuningen, H. Suemizu, Guguen-Guillouzo, C., N. Shibata, K. Yajima, M. Utoh, M. Shimizu, C. Chesne, M. Nakamura, F. P. Guengerich, R. Houtman, and H. Yamazaki. Thalidomide increases human hepatic cytochrome P450 3A enzymes by direct activation of pregnane X receptor. *Chem.Res.Toxicol.* 27:304-308, 2014.
3. M. Yamashita, H. Suemizu, N. Murayama, S. Nishiyama, M. Shimizu, and H. Yamazaki. Human plasma concentrations of herbicidal carbamate molinate extrapolated from the pharmacokinetics established in *in vivo* experiments with chimeric mice with humanized liver and physiologically based pharmacokinetic modeling. *Regul.Toxicol.Pharmacol.* 70:214-221, 2014.
4. H. Suemizu, S. Sota, M. Kuronuma, M. Shimizu, and H. Yamazaki. Pharmacokinetics and effects on serum cholinesterase activities of organophosphorus pesticides acephate and chlorpyrifos in chimeric mice transplanted with human hepatocytes. *Regul.Toxicol.Pharmacol.* 70:468-473, 2014.
5. K. Adachi, H. Suemizu, N. Murayama, M. Shimizu, and H. Yamazaki. 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.* in press (doi:10.1016/j.etap.2015.02.011)
6. T. Miyaguchi, H. Suemizu, M. Shimizu, S. Shida, S. Nishiyama, R. Takano, N. Murayama, and H. Yamazaki. 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.* in press (doi: 10.1016/j.yrtph.2015.03.010).

Title of Research:

12_S01-03-2

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

Principal Investigator:

Kohji Aoyama, PhD (Assistant Professor, Kagoshima University, Department of Hygiene and Health Promotion Medicine, Graduate School of Medicine and Dental Sciences)
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 (Laboratory of Veterinary Histopathology, Joint Faculty of Veterinary Medicine, Kagoshima University)
1-21-1 Korimoto, Kagoshima, 890-8580, Japan
(tel)+81-99-285-8720 (e-mail) k3038952@kadai.jp
Kunihiko Yamashita (Corporate Research Center, R & D Management, Daicel Corporation)
1239 shinazaike, aboshi-ku, Himeji, 671-1283, Japan
(tel)+81-79-274-4061 (e-mail)ku_yamashita@daicel.jp

Summary of Research:

Currently, there is no test for evaluating the respiratory sensitizing potential of chemicals. The purpose of this project is the establishment of animal testing methods for predicting respiratory sensitizers and for evaluating their relative respiratory sensitizing potency. We have developed a respiratory sensitization testing method using intratracheal administration.
In the second stage, we verified the effectiveness of the testing method using several new but known respiratory sensitizers. Test substances used were phthalic anhydride (PA), methyl tetra hydrophthalic anhydride (MTHPA), maleic anhydride (MA), and hexamethylene diisocyanate (HDI), which are well-known respiratory sensitizers. We used oxazolone (OX) and chlorobenzene (CB), which are a skin sensitizer and non-sensitizer, respectively. Mice were sensitized by intratracheal instillation of individual chemicals, each at three concentrations, on 5 days per week for 3 weeks. Three days following the last installation, mice were challenged with the corresponding agents for three days, and were sacrificed 2 days later. The degree of Th2 type-allergic inflammation in lungs was determined using an allergic inflammation score based on the histopathological grading.
PA and MTHPA elicited weak Th2-type allergic inflammatory responses in a dose-related manner. It was difficult to identify a respiratory sensitizing potency for MA under the test conditions, although Th2-type allergic inflammatory responses were elicited in the lungs of mice sensitized with MA. With HDI, Th2-type allergic inflammatory responses were elicited in elicitation-control and low-concentration groups; the reason for this is unknown. OX showed the same levels of allergic inflammation scores as the vehicle-control group. No allergic inflammatory reaction to CB was found at non-sensitizing potency. The relative sensitizing potency, based on the relationship between sensitizing concentrations and allergic inflammation scores, could be used to compare the potency of PA, MTHPA, OX, and CB. Thus, the present test method could separate PA and MTHPA from OX and CB, and confirmed the usefulness of evaluating relative sensitizing potency. Further studies should be conducted on enhancing the elicitation reaction for weak respiratory sensitizers.

Timeline:

November 1, 2013 – February 28, 2015

Topics:

Presented at the 3th Annual Conferences of New LRI

Publications:

88th Annual Meeting of Japan Society for Occupational Health, Osaka, May 2015

Title of Research:

13_S01-01

Development of novel method to evaluate the indicibility of cancer stem cells from iPS cells in chemical compounds

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
Jyunko 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 for 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, seeded at a density of 1,000 or 2,000 cells/well in 96-well plates and incubated for 24 hrs. The cells are further replenished with the growth medium with the compounds to be assessed.

We are currently observing the fluorescence intensity of GFP, which corresponds to the active 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 for usable method and high sensibility is under way. We plan to further assess the mechanism through which the compounds are inducing the cancer stem cells generation. The clarification of the mechanisms of cancer stem cells derivation is needed.

Timeline:

March 2014-February 2015

Topics:

Japanese Patent Application No. 2014-246457

Publications:

Masaharu Seno. Development of novel method to evaluate chemical compounds as the possible inducer of cancer stem cells using mouse iPS cells. The 27th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments. (Dec. 2014, Yokohama)

Title of Research:

12_PT01-02-2

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

Principal Investigator:

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

Collaborators:

1) Masashi Kamo (Research Institute of Science for Safety and Sustainability, National Institute of Advanced Industrial Science and Technology). 16-1 Onogawa Tsukuba-shi, Ibaraki, 305-8569 Japan. (tel) +81-29861 8029, (e-mail) masashi-kamo@aist.go.jp;
2) Wataru Naito (Research Institute of Science for Safety and Sustainability, National Institute of Advanced Industrial Science and Technology). 16-1 Onogawa Tsukuba-shi, Ibaraki, 305-8569 Japan. (tel) +81-29861 8299, (e-mail) w-naito@aist.go.jp

Summary of Research:

Risk assessment and management of chemicals have become increasingly cumbersome due to reinforcements and revisions of national/international laws and regulations. For this reason, there is an urgent need for the implementation of accurate and simple risk assessment and management of chemicals. In order to address this need, we have focused on the improvement and enhancement of the Japanese version of the risk assessment tool, AIST-MeRAM 0.9.12 (released in Jul., 2013) during this project term, and eventually released its updated version, “AIST-MeRAM 1.0.1” in Dec., 2014 as a research outcome (Figure 1). At the same time, we have been also engaged in the development of the English version of the tool to promote international business operations of the Japanese chemical industry and its strategies for Asia, as well as to support the dissemination of risk assessment scheme described in Chemical Substances Control Law of Japan in Asian countries. The English version, “AIST-MeRAM 1.0.0”, was also released in Dec., 2014 (Figure 1) as scheduled. In addition to these achievements, we have developed AIST-MeRAM web pages to encourage the use of these tools, lectured at the chemical risk forum hosted by Japan Chemical Industry Association, and conducted dissemination activities in Thailand, Vietnam, etc.

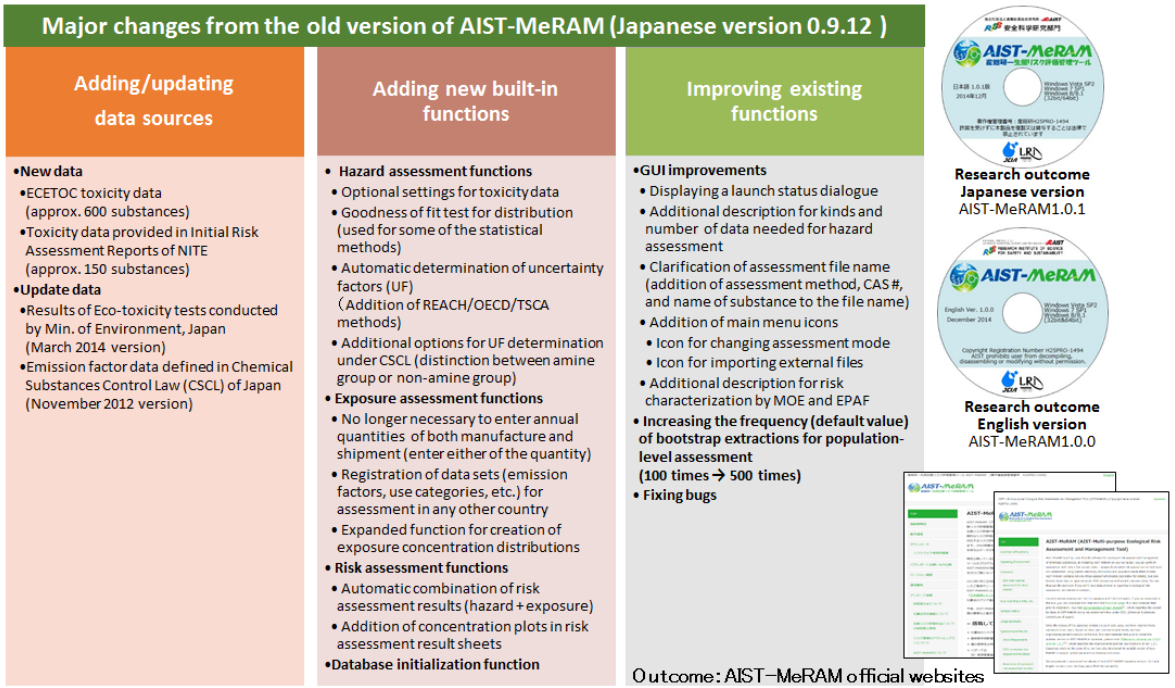

Figure 1 List of major research achievements during the project term

Timeline:

From November, 2013 to February, 2015

Topics:

None

Publications:

1) (Presentation at domestic meeting) The 26th Annual Meeting of the Society for Risk Analysis Japan (Nov. 15-17, 2013), Chuo Univ., Korakuen Campus, “Development of a social need-oriented tool for ecological risk assessment and management, AIST-MeRAM”.
2) (Invited speech at domestic workshop) NIAES 30th Anniversary Workshop: the 4th Agro-environmental Inventory Workshop, “Establishment of inventories for the efficient risk assessment of chemical substances, including pesticides”. Feb. 27, 2014, Tsukuba International Congress Center (Epochal Tsukuba)
3) (Paper publication) Journal of Japan Society for Safety Engineering vol.53 No.2, 82-88, 2014, “Development of models for ecological risk assessment of chemicals and support practical use of the models in society”.
4) (Presentation at international meeting) 2014 ISEH, 2014/7/1-6, Beijing China, “AIST-MeRAM: an easy-to-use tool for aquatic environmental risk assessment and management of chemicals”.
5) (Presentation at international meeting) SETAC North America 35th Annual Meeting, 2014/11/9-13, Vancouver Canada, “AIST-MeRAM: an easy-to-use tool for aquatic environmental risk assessment and management of chemicals”.
6) (Research outcome exhibition) AIST Techno-bridge Salon, Oct. 23-24, 2014, Tsukuba, “AIST-MeRAM: a tool for facilitating increasingly cumbersome tasks of risk assessment in the context of regulatory reinforcements”.
7) (Invited speech at domestic forum) Chemical risk forum organized by Japan Chemical Industry Association, Jan. 30, 2015, Tokyo, “AIST-MeRAM, an all-in-one ecological risk assessment tool for complying the risk assessment procedures defined in Chemical Substance Control Law”.

Title of Research:

2012 PT1-04

Development of mice visualizing "Metabolic reprograming" at early phase of tumorigenesis, and its application to caricinogenicity tests

Principal Investigator:

Nobuhiro Tanuma (Div. Cancer Chemother., Miyagi Cancer Ctr. Res. Inst.)
47-1 Noda-Yama, Medeshima-Shiode, Natori 981-1293, Japan
(tel):+81-022-381-1165, (e-mail) ntanuma@med.tohoku.ac.jp

Collaborators:

Toshio Watanabe (Grad. Sch. Humanities & Sci., Nara Women's Univ.)
Kitauoya-Nishimachi, Nara 630-8506, Japan
(tel) +81-0742-203413, (e-mail) toshiwatana@cc.nara-wu.ac.jp
Gen Kondoh (Inst. Frontier Med. Sci., Kyoto Univ.)
53 Kawara-Machi, Shogo-In, Sakyo-Ku, Kyoto 606-8507, Japan
(tel) +81-075-7514860, (e-mail) kondohg@frontier.kyoto-u.ac.jp
Hiroshi Shima (Div. Cancer Chemother., Miyagi Cancer Ctr. Res. Inst.)
(e-mail) shima@med.tohoku.ac..jp

Summary of Research:

Increased flux of glycolysis is a common feature of cancer cells, and known as Warburg effect. Together with alterations of other pathways, it mediates metabolic reprograming, now recognized as a core hallmark of cancer. One of key molecules in such a reprograming is pyruvate kinase M (PKM) that exists as two isoforms, M1 and M2, generated by alternative splicing. Expression of these isoforms switches from M1- to M2-type during tumorigenesis so that normal differentiated and proliferating/tumor cells express M1 and M2, respectively. In this study, a reporter-gene system, enabling us to visualize PKM-switch by cell-autonomous fluorescence, was developed. Using the reporter-gene, we generated transgenic mice, and examined those for lung tumor model. Unfortunately, any fluorescent signals except for auto-fluorescence were detected in tissues examined including tumor of the Tg-mice. More improvement(s) of the Tg-construct and/or alternative methods for introducing it into the mouse genome might be needed.

.

Timeline:

1 Nov. 2012 – 28 Feb. 2015

Topics:

Reported in "1st Annual meeting of JICA New LRI: Development of mice visualizing "Metabolic reprograming" at early phase of tumorigenesis, and its application to caricinogenicity tests

Publications:

Title of Research:

12_PT01-05-2

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

Principal Investigator:

Takeshi BAMBA (Department of Biotechnology, Graduate School of Engineering, Osaka University)
2-1 Yamadaoka Suita, Osaka 565-0871 Japan
phone/fax: +81-6-6879-7418
e-mail: bamba@bio.eng.osaka-u.ac.jp

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:

3rd 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 41st 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 17β-estradiol and testosterone released from H295R cells by the on-line derivatization LC-GC/MS system
   The 41st Annual Meeting of the Japan Society of Toxicology, Kobe Convention Center (Kobe), July 2 (Wen) - 4 (Fri), 2014

Title of Research:

12_PT01-06-2

Sophisticated hazard prediction by active QSAR modeling

Principal Investigator:

Yoshimasa Takahashi (Professor, Dept. Comp. Sci. Eng., Toyohashi Univ. Tech.)

Collaborators:

Tomoya Yamazaki (Master student, Toyohashi Univ. Tech.)
Yuji Ikegami (Master student, Toyohashi Univ. Tech.)
Yoshitaka Inagaki (Master student, Toyohashi Univ. Tech.)

Summary of Research:

In the preceding study, we obtained successful results about data predictions by the PLS modeling with the active sampling which was based on structure similarity for making a set of training data. In this study, according to the active QSAR modeling approach to the data prediction, we have developed a computer system for the eco-toxicity prediction of chemicals, which automated a whole process of the input of query structure , active sampling of training compounds that have similar structures to the query, computation of the PLS model, prediction and display of the toxicity of interest. The user can use the system without the use of keyboard input. Following three aspects is an important basic concept in the development of this system; 1) System that the user wants to use, 2) that is easy-to-use for the user, 3) that is useful for the user. In this study, in addition to the automation of the whole process described above, we have developed a structure editor, and additional tools for calculation of molecular formula and molecular weight calculation. We have implemented a computer program for logP estimation which is based on the group contribution method, to the system. The performance of the system that was developed in the study was evaluated through the prediction experiment with external data. These results were also compared with the results of other systems (ECOSAR and KATE).

Timeline:

1st Nov. 2013 – 28th Feb. 2015

Topics:

Poster presentation and system demonstration at The Second New LRI Workshop, Tokyo, Aug., 2014

Publications:

1) Yuji Ikegami, Yoshimasa Takahashi, **Prediction of eco-toxicity of chemicals using atomic fragments: Prediction of fish toxicity (96h-LC50)**, Proc. of the 23rd Symposium on Environmental Chemistry, 2014, May, Kyoto.
2) Yoshimasa Takahashi , Mika Ohyama and Tomoya Yamazaki, **Active QSAR modelling for environmental toxicity prediction of chemical substances**, 20th EuroQSAR, Saint-Petersburg, Russia, Sep. 2014.
3) Yuji Ikegami, Yoshimasa Takahashi, **Fish toxicity prediction of chemicals using atomic fragment method: Grobal parameters and chemical group parameters**, Proc. of the 42nd Symposium on Structure-Activity Relationships, 2014, November, Kumamoto.
4) Yoshimasa Takahashi, Tomoya Yamazaki, Mika Ohyama, Yuji Ikegami, **Sophisticated hazard prediction by active QSAR modeling**, Proc. of the 27th annual meeting of the Japanese Society for Alternatives to Animal Experiments, 2014, December, Yokohama.

Title of Research:

13_PT01-01

Development of in vivo fluorescent imaging of neuronal differentiaon in zebrafish for developmental neuotoxicity 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:

The developing brain is extremely sensitive to many chemicals. Exposure to neurotoxicants during development has been implicated in various neuropsychiatric and neurological disorders, including autism spectrum disorder and attention deficit hyperactive disorder. Although rodents have been widely used for developmental neurotoxicity testing, experiments using large numbers of rodents are time-consuming, expensive, and raise ethical concerns. Using alternative non-mammalian animal models may relieve some of these pressures by allowing testing of large numbers of subjects while reducing expenses and minimizing the use of mammalian subjects.

In this study, we have tried to make the three-color zebrafish in which cerulean, a fluorescent protein with maximum excitation (Ex) /emission (Em) at 433/475 nm, would express in neurons, mCitrine, a fluorescent protein with Ex/Em at 516/529 nm, would express in oligodendrocytes, and mCherry, a fluorescent protein with Ex/Em at 587/610 nm, would express in astrocytes. We were able to make one-color zebrafish expressing cerulean in neurons, mCitrine in oligodendrocytes, or mCherry in astrocytes. We were also able to confirm that the zebrafish expressing cerulean in neurons and the zebrafish expressing mCitrine in oligodendrocytes could transmit the phenotype to their offspring. We will cross these two zebrafish to make two-color zebrafish. We will also examine whether zebrafish expressing mCherry in astrocytes could transmit the phenotype to their offspring. Finally, we will cross the two-color zebrafish and the mono-color zebrafish to make three-color zebrafish expression cerulean in neurons, mCitrine in oligodendrocytes, and mCherry in astrocytes.

It has been demonstrated that various developmental neurotoxicants can affect the differentiation of neuron, oligodendrocyte, and astrocytes and that the dysregulated differentiation may cause various neuropsychiatric disorders. The tricolor zebrafish we will develop would make it possible to examine the developmental neurotoxicity of many chemicals and provide a sound basis for human risk assessments.

Timeline:

Nov 2013 ~ Feb 2015

Topics:

Publications:

Zebrafish as a systems toxicology model for developmental neurotoxicity testing.
Nishimura Y, Murakami S, Ashikawa Y, Sasagawa S, Umemoto N, Shimada Y, Tanaka T.
Congenital Anomalies 55(1):1-16 (2015)

In vivo fluorescent imaging of blood-brain barrier disruption in zebrafish using a novel dye.
Nishimura Y, Murakami S, Ashikawa Y, Sasagawa S, Umemoto N, Shimada Y, Tanaka T.
DNT4, Philadelphia, May 2014

Evaluation of developmental neurotoxicity of nicotine using gene knockout in zebrafish
Nishimura Y, Murakami S, Ashikawa Y, Sasagawa S, Kawabata M, Umemoto N, Ariyoshi M, Zhang B, Shimada Y, Tanaka T. The 41st Annual Meeting of Japanese Society of Toxicology, Kobe, July 2014

Title of Research:

13_PT01-02

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

Principal Investigator:

Kohji Yamakage (Division of Alternative Toxicology Test, Hatano Research Institute, Food and Drug Safety Center)
Ochiai 729-5, Hadano-shi, Kanagawa 257-8523, Japan
(tel) +81-(0)463-82-4751, (e-mail) yamakage.k@fdsc.or.jp

Collaborators:

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

Summary of Research:

We are developing the Hand1-Luc Embryonic Stem Cell Test (Hand1-Luc EST) which uses ES cells introduced the reporter gene into as a test method to detect embryonic toxicants. To familiarize the Hand1-Luc EST in the chemical industry, the Hand1-Luc EST is currently under validation process. To obtain objective evaluation and reliability of this test from experts, it is necessary to accumulate data and validate the test about the robustness, the predictability and the inter- and intra- laboratory reproducibility.

In 2013 as a project supported by the Ministry of Economy, Trade and Industry, the validation management team, composed of experts on developmental toxicity or alternative test methods from Japan and other counties, has been organized. The international validation study was started with the collaboration of Japanese three laboratories to optimize the test protocol, to establish acceptance criteria, and to verify the technical transfer and inter- and intra-laboratory reproducibility. In the phase 0 and phase 1 studies, the technical transfer and intra-laboratory reproducibility has been cleared respectively.

In Phase2a study, which was started as the project supported by LRI, the necessity of multiple experiments for prediction of the positivity or the negativity of chemicals was investigated because at the very beginning, we proposed that the prediction can evaluate with only one experiment. Experiments in each chemical were performed two or three times according to the revised protocol and the results of four coded chemicals were compared between three laboratories. In each laboratory, all of four chemical results were consistent with the in vivo results and the accuracy and the inter-laboratory reproducibility were 100%. Thus, the improved protocol was confirmed to be valid. Following those results, to verify the intra- and the inter-laboratory reproducibility, 24 coded chemicals (8 kinds of chemicals, 3 bottles per chemical were prepared) were distributed to the 3 laboratories and the phase 2b/2c studies were started (May 2014 – February 2015). By gathering the results from phase 2a, 2b and 2c studies, 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 (the number of chemicals of which the results were consistent between three tests in each chemical, Lab A: 7/8 chemicals, Lab B: 7/8 chemicals, Lab C: 6/8 chemicals).

Timeline:

November 1, 2013-

Topics:

Presented at the 2nd Annual Conferences of New LRI (29th August, 2014, Tokyo)

Publications:

27th Annual Meeting, the Japanese Society for Alternatives to Animal Experiments, Yokohama,

Title of Research:

13_PT01-03

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

Principal Investigator:

Kouichi Yoshinari, Ph.D., School of Pharmaceutical Sciences, University of Shizuoka

Collaborators:

N/A

Summary of Research:

Nuclear receptors are the group of transcription factors that are activated by a wide variety of chemicals and are involved in hepatotoxicity. The aim of this study is to develop a new method for the prediction of chemical-induced hepatotoxicity, based on a combined use of data analysis technology and the chemical properties for nuclear receptor activation, which can be assessed by in vitro assays. In this research term, we extracted in vitro toxicity data (liver, blood chemistry) from a publicly available database, HESS-DB (NITE, Japan) to build a database for the association study. In addition, we performed reporter assays of 5 rat nuclear receptors (AHR, CAR, PXR, PPARα, LXRα) for 190 chemical compounds selected from HESS-DB. To obtain physicochemical properties of these compounds, 3764 molecular descriptors from 19 different categories were calculated using the software Dragon. We are currently performing association studies among in vivo toxicity, the results of in vitro assays and chemical properties obtained in silico. Moreover, we have established a metabolism-integrated in vitro reporter gene assay to improve the in vivo and in vitro correlation.

Timeline:

November 1st, 2013 – February 28th, 2015

Topics:

N/A

Publications:

Invited lectures:

1. Mechanistic analysis, assessment and prediction of chemical-induced hepatotoxicity using a database of in vivo toxicity and in vitro assays. Yoshinari, K., CBI 2013 Annual Meeting. October 2013, Tokyo.
2. In vitro and in silico studies of nuclear receptors toward the prediction of chemical-induced hepatotoxicity. Yoshinari, K., 2013 Seminar for QSAR for chemicals. March 2014, Tokyo.

Meeting presentations:

1. Development of metabolism-integrated in vitro reporter gene assay. Yoshinari, K., Nakajima, H., Noomote, C. The 26th Annual Meeting of Japanese Society for Alternatives to Animal Experiments. December 2013, Kyoto, Japan.
2. Establishment of reporter gene assay equipped with metabolic system. Noomote, C, Nakajima, H., Yoshinari, K. The 134th Annual Meeting of the Pharmaceutical Society of Japan. March 2014, Kumamoto, Japan.

Title of Research:

12_S02-01-2

Evaluation of the relationship between physicochemical properties and biodistribution of nanomaterials

Principal Investigator:

Yasuo Yoshioka (Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University)

1-6 Yamadaoka, Suita, Osaka 565-0871, Japan. (tel) +81-6-6879-8233 (e-mail) yasuo@phs.osaka-u.ac.jp

Collaborators:

Makiko Kuwagata (Laboratory of Pathology, Toxicology Division, Hatano Research Institute, Food and Drug Safety Center)

729-5 Ochiai, Hadano, Kanagawa 257-8523, Japan. (tel) +81-463-82-4751 (e-mail) kuwagata.m@fdsc.or.jp

Summary of Research:

Advances in nanotechnology have led to the recent development of many nanomaterials. However, the increasing use of nanomaterials has prompted public concern regarding their potential safety. Because nanomaterials have great potential to improve the quality of human life, it is essential to ensure the safety of nanomaterials for the development of safety-assessed products. The safety of nanomaterials is related to the dose, concentration, and duration of the exposure and their abundance and persistence in tissue. Accordingly, a systematic and thorough analysis of the Absorption, Distribution, Metabolism, and Excretion (ADME) of nanomaterials is essential as the basis for determining the potential for risk to human health. In addition, understanding of the ADME of nanomaterials is necessary in regard to their tissue toxicity. In this study, we examined the absorption of nanomaterials via skin route. We used nickel particles with the diameter of 3 nm and gold nanoparticle with the diameter of 10 nm. Our results showed that the penetration ability of these nanoparticles via skin was 0.01% or less. The data could provide information to ensure the safety of nanomaterials.

Timeline:

2013/11/1 -2015/2/28

Topics:

None

Publications:

In preparation

Title of Research:

13_S02-01

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 three 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.

Timeline: From November 2013

Topics:

Publications:

The effect of titanium dioxide (TiO2) nanoparticles to microbes under Ultra Violet (UV) irradiation.
Ikuho Yamada, Kazuki Nomura, Hitoshi Iwahashi, and Masanori Horie
The 10th International Symposium on Advanced Environmental monitoring and modeling
August 11-13, 2014 Doubletree by Hilton Berkeley Marina Berkeley, California, USA

Solubility of nano-particles.

Research on the safety of new chemical substances including nanomaterials
Masamitsu Fujita, Ikuho Yamada, Hitoshi Iwahashi
The 10th International Symposium on Advanced Environmental monitoring and modeling
August 11-13, 2014 Doubletree by Hilton Berkeley Marina Berkeley, California, USA

Standard and essential protocols before starting in vitro toxicity tests for nano-objects
Hitoshi Iwahashi, Haruhisa Kato, Shigehisa Endoh, and Masanori Horie
The 10th International Symposium on Advanced Environmental monitoring and modeling
August 11-13, 2014 Doubletree by Hilton Berkeley Marina Berkeley, California, USA

Essential protocols before starting in vitro toxicity tests for nano-objects
Hitoshi Iwahashi, Haruhisa Kato, Shigehisa Endoh, and Masanori Horie
The 2oth Meeting of THE JAPANESE SOCIETY OF ENVIRONMENTAL TOXICOLOGY
2014 Sept. 10th-11th Toyama Japan

The effect of titanium dioxide (TiO2) nanoparticles to microbes under Ultra Violet (UV) irradiation.
Ikuho Yamada, Kazuki Nomura, Hitoshi Iwahashi, and Masanori Horie.
Chemoshere (under communication)

Title of Research:

13_S03-01

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:

Masamitsu Yamaguchi, PhD (Professor, Kyoto Institute of Technology, Department of Applied Biology)
Matsugasaki, Sakyo-ku, Kyoto, 606-8585, Japan
(tel) +81-75-724-7781, (e-mail) myamaguc@kit.ac.jp

Yoshihiro Inoue, PhD (Associate Professor, Kyoto Institute of Technology, Insect Biomedical Research Center)
Matsugasaki, Sakyo-ku, Kyoto, 606-8585, Japan
(tel) +81-75-724-7876, (e-mail) yhinoue@kit.ac.jp

Summary of Research:

The genome of *Drosophila melanogaster* has orthologues for 60% of human genes and 80% of human disease related genes, and the conservation of amino acid sequences between *Drosophila* and human is much higher than those of other model organisms like nematode and yeast. Therefore, *Drosophila* is believed to be a useful model organism of mammalian. The aim of this study is to reveal the mechanism of sick building syndrome and develop the risk assessment system utilizing *Drosophila.*

The proteins extracted from *Drosophila* after exposure to sick building syndrome causative agent were comprehensively analyzed by using two-dimensional electrophoresis followed by silver staining. The intensities of proteins on gel indicated that the exposure to formaldehyde or *o*-xylene caused the concentration changes of several proteins in *Drosophila*. The same tendency of the concentration change of proteins was observed between exposure to formaldehyde or xylene and aging. This may suggest the existence of the common mechanism between sick building syndrome and aging. Formaldehyde and *o*-xylene caused the concentration change in some common proteins, while concentrations of some proteins were changed by only exposure to xylene.

For identification, the proteins of which concentrations were changed by exposure to formaldehyde were analyzed by mass spectrometry after trypsin digestion. The candidates obtained by database search include the energy metabolism-related proteins. This might suggest the onset mechanism of sick building syndrome.

Timeline:

March 1, 2014 – February 28, 2015

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:

12_PT03-01-2

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 2011 LRI research, we found that a serine protease inhibitor, secretory leukoprotease inhibitor SLPI-deficient (*Slpi* $^{-/-}$) basophils and eosinophils produced more cytokines than wild type cells after stimulation with IgE or LPS. Therefore, we have planed to establish the highly sensitive cell lines of chemical-mediated hypersensitivity by the deletion of serine protease inhibitors including SLPI. In 2012 LRI research, we found that *Slpi* $^{-/-}$ eosinophils highly produced IL-6 stimulated with beryllium sulfate. The DNA microarray analyzes revealed the expression profile of serine protease inhibitors in mast cells, basophils and eosinophils. Moreover, a human basophilic cell line KU812 secreted cytokines in response to several chemicals. In the present 2013 LRI study, we examined the cytokine responses by a human eosinophilc cell line EoL-1. EoL-1 produced IL-6 after stimulation with Nickel compounds among various chemicals. We next investigated the expression of serine protease inhibitors in KU812 and KU812 substrain, KU812-F. KU812-F expresses higher levels of Serpin b1 and b6 than KU812. In addition, IL-6 production was augmented in KU812-F than KU812 cells. Finally, we 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 produced IL-6 in response to 9 kinds of chemicals that KU812-F showed no responses. The knockdown cells also secreted IL-13 upon stimulation with TDI that KU812-F did not response. These results suggested that Serpin b1 knockdown KU812-F cell would be an ideal risk evaluation tool against chemicals.

Timeline:

2013/1/1-2015/2/28

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 3th annual conference of new LRI, Tokyo, Japan, August 2014

Research on the effects of chemical substances on children, elderly people,and those with gene disorders

Title of Research:

13_PT03-01

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, Laboratory 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, Laboratory 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 (Laboratory Head, Sugiyama Laboratory, RIKEN Innovation Center, RIKEN Research Cluster for Innovation, RIKEN)
1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama City, Kanagawa 230-0045, Japan
(tel) +81-45-503-9211 (e-mail) ychi.sugiyama@riken.jp

Ichiro Ieiri, PhD (Professor, Department 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 effect of age on the pharmacokinetics of chemical substances, a clinical study will be performed to evaluate the molecular functions of metabolic enzymes and transporters of chemical substances with probe drugs. By mathematical modeling, pharmacokinetic data in elder people can be predicted based on PKs in healthy volunteers. In this year, Cluster Newton Method was created for rational settings of model parameters and its effectiveness was proven. Moreover, we performed in vitro experiments to characterize the property of probe drugs for various molecules such as CYP2E1 and OCT1 and also prepared for the future clinical study.

Timeline: November 1, 2013-

Topics: None

Publications:

Kazuya Maeda, “Quantitative prediction of the impact of drug transporter function on human in vivo pharmacokinetics”, 7th Young Investigators Symposium on Clinical Pharmaceutical Sciences, Sendai, 2013.11

Kazuya Maeda, Kenta Yoshida, Akihiko Konagaya, Hiroyuki Kusuhara, “Integrative analyses of the drug –drug interactions with Cluster Newton Method”, 29th APSTJ Annual Meeting, Saitama, 2014.5

Xuan Zhang, Kazuya Maeda, Hiroshi Suzuki and Hiroyuki Kusuhara, “Contribution of hepatic OCT1 to the pharmacokinetics of triptans”, 29th APSTJ Annual Meeting, Saitama, 2014.5

Kazuya Maeda, Kenta Yoshida, Yuichi Sugiyama, Akihiko Konagaya and Hiroyuki Kusuhara, “Precise estimations of the inhibition constants by PBPK analyses of metabolites’ pharmacokinetic alterations using Cluster Newton Method”, 19th North American ISSX Meeting/29th JSSX Meeting, San Francisco, CA, USA, 2014.10

Xuan Zhang, Kazuya Maeda, Hiroshi Suzuki and Hiroyuki Kusuhara, “Hepatic Organic Cation Transporter 1 (OCT1) Regulates the Hepatic Clearance of Triptans, But Not Beta-blockers”, 19th North American ISSX Meeting/29th JSSX Meeting, San Francisco, CA, USA, 2014.10

Title of Research:

12_S04-01-2

Development of in Silico Prediction Model on Environmental Fate of Chemical Substances

Principal Investigator:

Tatsuya Takagi, Graduate School of Pharmaceutical Sciences, Osaka University; Genome Information Research Center, Research Institute for Microbial Diseases, Osaka Univ.
1-6, Yamadaoka, Suita, Osaka, Japan, 565-0871, (tel) +81-6-6879-8243, (e-mail) ttakagi@phs.osaka-u.ac.jp

Collaborators:

Norihito Kawashita, Graduate School of Pharmaceutical Sciences, Osaka University; Genome Information Research Center, Research Institute for Microbial Diseases, Osaka Univ.
1-6, Yamadaoka, Suita, Osaka, Japan, 565-0871, (tel) +81-6-6879-8244, (e-mail) kawasita@gen-info.osaka-u.ac.jp
Kousuke Okamoto, Graduate School of Pharmaceutical Sciences, Osaka University;
1-6, Yamadaoka, Suita, Osaka, Japan, 565-0871, (tel) +81-6-6879-8242, (e-mail) okamotok@phs.osaka-u.ac.jp

Summary of Research:

In 2009, since the Act on the Evaluation of Chemical Substances and Regulation of Their Manufacture, etc., was revised, chemical substances which are readily degradable have become subject to control under the law. Chemical industries are required to examine various physical, chemical, and biological properties of compounds such as toxicity, accumulation behavior, and degradation property. If there is a procedure which enables us to predict any properties of new or existing chemical substances without experiments, we are able to plan the efficient order of the experiments based on the ranking of the predicted properties. Because most existing prediction models on abiotic degradation, especially photolysis and hydrolysis are targeted to similar chemical substances, the development of the prediction models on the photolysis and hydrolysis of more various chemical substances would be contributed to obtain the effective ranking of candidates for experimentation.

In this term, the qualitative models for predicting whether a compound is stable to hydrolysis or not were developed. In our procedures for predicting hydrolysis, a predicted compound is classified to binary evaluation for hydrolysis by these qualitative models at first. If the compound is not stable to hydrolysis then its half-life is predicted by the quantitative models. and thus a sequence of our procedures for hydrolysis were completed. Additionally, availability domain in each prediction model was defined. The ground state and excitation states were calculated by DFT and TD-DFT in all compounds included in the dataset for predicting direct photolysis, and then five indices were calculated from excitation energies and oscillator strength of the excitation states. The plots between the index and the category based on half-life of the compounds suggested the correlation between the two variables and the area under the curve (AUC) values of the binary predictions of direct photolysis half-life based on the index were around 0.75. The web site for providing our prediction models of the hydrolysis was limitedly opened and the prototype of web site which provides the prediction models of the direct photolysis was constructed.

Timeline: Nov 1, 2013 - Feb 28, 2015

Topics:

The 3rd Annual Conference of New LRI, “Development of new tool for in silico prediction of environmental fate of compounds”, Aug 29, 2014

Publications:

5th FIP Pharmaceutical Sciences World Congress, Melbourne, Australia April 2014 (Oral session)
The 42th Symposium of Structure-Activity Relationships, Kumamoto, Japan, Nov 2014 (Oral session)

Title of Research:

12_PT04-01-2

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

Summary of Research:

For chemicals registered in PRTR, four test methods were established to evaluate formation potentials of trihalomethanes, aldehydes, and nitrosamines during oxidation processes using ozone, chlorine or chloramine. In addition, a quick and robust oxidation method was developed to evaluate formation potential of perfluorocarboxylic acids (PFCAs) from their precursors in wastewater samples. These methods were applied to wastewater and river water samples.

Timeline: November 1, 2012 – February 28, 2015

Topics: Poster presentation at 2nd Annual Conference of LRI

Publications:

1) Yuji SUZUKI, Shuhei TANAKA, Shigeo FUJII, Norihide NAKADA, Kazuma ISHIKAWA, Kongpran JIRA, and Norimitsu SAITO, “Study on Behavior of Perfluorocarboxylic Acids in Wastewater Treatment Plants in Consideration of the Formation Potential from Their Precursors”, Journal of Japan Society of Civil Engineers, Vol.70 (7): p.III55-p.III64, 2014.
2) Yuji SUZUKI, Shuhei TANAKA, Shigeo FUJII, Norihide NAKADA, Kongpran JIRA, Kazuma ISHIKAWA, and Norimitsu SAITO, “Study on Formation Behavior of Perfluorinated Compounds from Their Precursors in Wastewater Samples”, The 48th Annual Conference of Japan Society of Water Environment, Sendai, Japan, March 2014.
3) Yuji SUZUKI, Shuhei TANAKA, Shigeo FUJII, Norihide NAKADA, Kongpran JIRA, Kazuma ISHIKAWA, and Norimitsu SAITO, “Study on the examination methods of formation potential of perfluorinated compounds from their precursors in wastewater samples by preservation and oxidation processes”, The 23th Annual Conference of Japan Society for Environmental Chemistry, Kyoto, May 2014.
4) Shuhei ITAI, Norihide NAKADA, Yongkui YANG, Hiroaki TANAKA, “Development of by-products formation potential test for PRTR chemicals during oxidation processes”, The 23th Annual Conference of Japan Society for Environmental Chemistry, Kyoto, May 2014.
5) Yuji SUZUKI, Shuhei TANAKA, Shigeo FUJII, Norihide NAKADA, Kongpran JIRA, Kazuma ISHIKAWA, and Norimitsu SAITO, “Study on formation potential of perfluorinated compounds from their precursors by oxidation processes and determination of intermediate products”, The 36th Annual Conference of the Association of Environmental & Sanitary Engineering Research, Kyoto, July 2014.
6) 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.
7) Yuji SUZUKI, Shuhei TANAKA, Shigeo FUJII, Norihide NAKADA, Jira KONGPRAN, Kazuma ISHIKAWA, and Norimitsu SAITO, “Study on Oxidation Conditions in Evaluating Formation Potential of Perfluorinated Compounds from their Precursors”, The 17th Annual Symposium of Japan Society of Water Environment, Hikone, Japan, September 2014.
8) Yuji SUZUKI, Shuhei TANAKA, Shigeo FUJII, Kazuma ISHIKAWA, Norihide NAKADA, Tsz Kit LIU, and Norimitsu SAITO, “Stuvey on Occurrences of Perfluorinated Compounds and their Formation Potentials in Wastewater Samples in Dissolved and Particulate Phases”, The 49th Annual Conference of Japan Society of Water Environment, Kanazawa, March 2015.

Title of Research:

12_PT04-02-2

Development of Ecosystem Risk Impact Assessment System Methods for Chemicals using Microcosm Systems

Principal Investigator:

Yuhei INAMORI (Foundation for Advancement of International Science (FAIS))
3-24-16 Kasuga, Tsukuba, Ibaraki 305-0821 Japan
(tel) 029-860-3333 (e-mail) y_inamori@fais.or.jp

Collaborators:

Kazuhito MURAKAMI (Chiba Institute of Technology)
2-17-1 Tsudanuma, Narashino, Chiba 275-0016 Japan
(tel)047-478-0455 (e-mail) kaz_murakami@sky.it-chiba.ac.jp
Takashi AMEMIYA (Yokohama National University)
79-1 Tokiwadai, Hodogaya-ku, Yokohama, Kanagawa 240-8501 Japan
(tel) 045-339-4353 (e-mail) amemiyat@ynu.ac.jp
Ken-ichi SHIBATA (Yokohama National University, Toyo University)
79-1 Tokiwadai, Hodogaya-ku, Yokohama, Kanagawa 240-8501 Japan
(tel) 0276-82-9337 (e-mail) shibata091@toyo.jp
Ryuhei INAMORI (Foundation for Advancement of International Science)
(tel) 029-860-3333 (e-mail) r_inamori@fais.or.jp
Katsura SUGIURA (Sagami Women's University), Kunihiko KAKAZU, Yuuki KANZO (Foundation for Advancement of International Science), Saki AGATSUMA (Chiba Institute of Technology)

Summary of Research:

In this research, research and development in the environmental impact risk assessment of a chemical substance was conducted paying attention to microcosm system which is an aquatic model ecosystem which consists of a producer (phytoplankton), a predator (zooplankton), and a decomposer (bacteria). The enactment as international guideline as OECD test of the general-purpose microcosm test method from Japan was aimed at by making the P/R (quantity of production/respiration) ratio which can indicate the change of the whole ecosystem into an assessment index. Although the environmental impact risk assessment examination of a chemical substance has been conventionally carried out using a single species creature, as for the model ecosystem examination which imitated the nature, the general-purpose standard test method has not been established by problems, such as stability, reproducibility and high cost. In addition, the fundamental manual concerning test operation has been already established (Funds for the Overall Promotion of Environmental Research -Ministry of Environment in FY2009-2011), and the ring test etc. which carried out the test between several research institutions for wide use, are planned to carry out to establish as the OECD standard test method.

Timeline: 2013.11.1. - 2015.2.28.

Topics:

3rd New LRI Research Report Meeting: "Development of Ecosystem Risk Impact Assessment System Methods for Chemicals using Microcosm Systems"

Publications:

Yuhei Inamoro, Kazuhito Murakami, Kunihiko Kakazu, Ryuhei Inamoro, Yuuki Kanzo, Ken-ichi Shibata, Takashi Amemiya Katsura Sugiura : "Development of Environmental Impact Risk Assessment Method using Microcosm for OECD Standard Test", *20th Symposium on Japan Society of Environmental Toxicology in Toyama* (2014.9)

Title of Research:

13_PT05-01

Development and practical verification of novel comprehensive monitoring system for multiple contaminations of environmental pollutants 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 Biology and Pathology; 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.

Timeline: November 1, 2013-

Topics:

The 3rd New LRI Research Meeting (Tokyo, August 2014; Poster presentation)

Publications:

- Newspaper coverage: "Development of monitoring system for multiple contaminations of environmental pollutants", The Nikkei, March 11, 2014
- Matsushima K., Kaneda H., Harada K., Matsuura H., Hirata K., Immobilization of enzymatic extracts of Portulaca oleracea cv. roots for oxidizing aqueous bisphenol A., Biotechnology Letters (in press)

Annual Report 2014

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/