LRI

Long-range Research Initiative

Annual Report 2013

2013

Japan Chemical Industry Association

Annual Report 2013

Research Field 1: Development and assessment of new risk assessment methods

Development of human exposure assessment methods with the use of the probabilistic approach assisting voluntary risk management by industrial sector

Akihiro Tokai, PhD (Professor, Osaka University, Graduate School of Engineering, Division of Sustainable Energy and Environmental Engineering)

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

Hiroshi Yamazaki, PhD (Professor, Showa Pharmaceutical University, Laboratory of Drug Metabolism and Pharmacokinetics)

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

Kohji Aoyama (Department of Hygiene and Health Promotion Medicine, Graduate School of Medical and Dental Sciences, Kagoshima University)

Multi-functional testing method for attention ability by elaborating the target detection method and application to developmental neurotoxicology : testing for selective, sustained, shifted, and bisectional attention

Hiromi Wada (Hokkaido University, Graduate School of Letters, Division of Psychology)

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

Bin-Le Lin (Research Institute of Science for Safety and Sustainability, National Institute of Advanced Industrial Science and Technology)

Research for establishing new method for risk assessment of chemical toxicity using gene-modified ES cells

Takashi Morita（Professor of Department of Molecular Genetics, Osaka City University, Graduate School of Medicine）

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

Nobuhiro Tanuma (Div. Cancer Chemother., Miyagi Cancer Ctr. Res. Inst.)

Development of *in vitro* screening endocrine disruptor by steroid profiling

Takeshi BAMBA (Department of Biotechnology, Graduate School of Engineering, Osaka University)

Sophisticated hazard prediction by active QSAR modeling

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

Title of Research:

12_S01-01

Development of human exposure assessment methods with the use of the probabilistic approach assisting voluntary risk management by industrial sector

Principal Investigator:

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Summary of Research:

Risk management throughout life cycles of chemical substances becomes increasingly implemented, as enactment of laws and regulations on chemicals management and voluntary chemicals management in the industrial sector are advanced. For further development of the chemicals management, a probabilistic exposure assessment tool that estimates exposure levels and their spatial/temporal variability or uncertainty on estimated error would facilitate an effective risk management.

Thus, the purpose of this study is to develop an exposure assessment model that assists industrial voluntary chemicals management for occupational exposure which is expected to have the highest risk in the supply chain. With a reference to the Advanced REACH Tool (ART) constructed in Europe, we have developed a probabilistic assessment tool for the occupational exposure in Japan, named SWEEs (integrated Score-based Workplace Exposure system) and validated the algorithm by using some data in Japanese industries.

Timeline: November 1, 2012 –

Topics:

The 2nd Research Meeting of the New LRI of Japan Chemical Industry Association, Tokyo, August, 2013. “Development of human exposure assessment methods with the use of the probabilistic approach assisting voluntary risk management by industrial sector”, Oral and Poster presentation.

Publications:

Ishimaru, T., Yamaguchi, H., Tokai, A. and Nakakubo, T.（2013） Development of quantifying method for the effect of metabolic inhibition during co-exposure by applying PBPK model: A case of toluene and n-Hexane， Proceeding of the SRA-JAPAN 26th annual meeting, B-6-3 (in Japanese).

Ichikawa, J., Yamaguchi, H., Tokai, A. and Nakakubo, T.（2013）Impact of lack of knowledge about model parameter on reduction of uncertainty factors by applying PBPK/PD model: A case of chlorpyrifos， Proceeding of the SRA-JAPAN 26th annual meeting, B-3-2 (in Japanese).

Ishimaru,T., Yamaguchi, H., Tokai， A. and Nakakubo, T., (2013)， Development of practical quantifying method applicable for risk assessment of metabolic inhibition during co-exposure in workplaces by applying a PBPK model in humans, Society for Risk Analysis, Annual meeting, (2013.12.7-13,Baltimore) ※

※student merit award for the Dose-Response Specialty Group (DRSG) of the SRA.

※travel award.

Title of Research:

12_S01-02

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

Principal Investigator:

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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, melengestrol acetate (animal drug) and molinate (pesticide), were multi-dosed apparently accumulated 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.

Timeline: November 1, 2012 –

Topics:

An invited presentation in 2013 ICCA-LRI & NCATS Workshop, “What Is Normal? Implications for Chemical Safety Assessment”, Santa Fe, New Mexico, USA

Publications:

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,* in press (doi:10.3109/00498254.2013.836257)

A. Tsukada, H. Suemizu, N. Murayama, R. Takano, M. Shimizu, M. Nakamura, and H. Yamazaki. Plasma concentrations of melengestrol acetate in humans extrapolated from the pharmacokinetics established in in vivo experiments with rats and chimeric mice with humanized liver and physiologically based pharmacokinetic modeling. *Regul.Toxicol.Pharmacol.* 65:316-324, 2013.

H. Yamazaki, H. Suemizu, N. Murayama, M. Utoh, N. Shibata, M. Nakamura, and F. P. Guengerich. *In vivo* drug interactions of the teratogen thalidomide with midazolam: Heterotropic cooperativity of human cytochrome P450 in humanized TK-NOG mice. *Chem.Res.Toxicol.* 26:486-489, 2013.

Title of Research:

12_S01-03

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

Principal Investigator:

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Summary of Research:

Respiratory allergy caused by occupational chemicals remains as a significant health concern for industrial workers. However, to date there are no proper animal models for effectively predicting their respiratory sensitizing potentials. The goals of this project are: (1) to develop the animal models for evaluating the relative hazard of respiratory sensitizing chemicals; and (2) to provide the useful information required for hazard assessment of these sensitizers in the workplaces. we developed a small device that enables intratracheal administration of testing substances in small animals such as mice. In this project, TDI, TMA and GA, three well-known respiratory sensitizing chemicals and chicken ovalbumin (OVA), were evaluated. Known contact sensitizing DNCB served as the negative control for respiratory allergens. Under light anesthesia with diethyl ether, mice were sensitized by administering individual agents, each at 4 doses including vehicle control, 5 times a week for a total of 3 weeks using a mouse intra-tracheal installation device. Three days following the last installation, these mice were daily challenged intratracheally with the corresponding agents for 3 days, and sacrificed 2 days thereafter. The degree of airway inflammation was evaluated using semi-quantitative histological grading systems. We found that histological scores, representing the degree of airway inflammation, increased with increasing doses of OVA and TDI used for sensitization. Similar trends were also noted for TMA- or GA-sensitized mice. They were also true for PAS staining in airway epithelial cells. In contrast, such changes were not observed in mice treated with DNCB. Thus, our testing system confirmed the relative sensitizing potency of these known respiratory sensitizers as previously reported in case-studies or epidemiologic studies. As the strategy for assessing the risk for respiratory sensitizing agents in workplace, although the relationship between airway sensitization onset (as determined by various endpoints) and exposure dose/concentration should be first investigated, these evidence for setting exposure limit vary in substances and exposure conditions. Thus, multi-factors, including experimental evidence, workplace exposure and clinical signs should be considered for properly and effectively controlling occupational respiratory allergy.

Timeline: November 1, 2012 –

Topics:

Presented at the 2th Annual Conferences of New LRI (31th August, 2013, Tokyo)

Title of Research:

12_PT01-01

Multi-functional testing method for attention ability by elaborating the target detection method and application to developmental neurotoxicology: testing for selective, sustained, shifted, and bisectional attention

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Summary of Research:

Pregnant rats were treated with 0.01% or 0.015% Methimazole. The resulted pups were trained with the selective, sustained, shift, and divided attention tasks. The hypothyroid rats displayed longer reaction times than the control rats in the selective attention task. In the sustained attention task, the hypothyroid rats exhibited increased variability of reaction times compared with the control rats. In the shift attention task, the hypothyroid rats exhibited increased reaction times when they were required to shift attention from one target to the other target. We conclude that the hypothyroid rats were difficult to pay attention to a target quickly, to maintain attention to a target, to shift attention from a target to another target. The hypothyroid rats reduced total T4 to 49-55% and 35-38% of control levels at age of 21-22 in the treatments of 0.01% and 0.015% Methimazole, respectively. Total T3 and TSH did not change at all. At age of 55-56, the hypothyroid rats displayed increased TSH but normal levels of total T4. It is necessary to maintain total T4 level more than 55% of the control level.

Timeline: November 1, 2012 –

Topics:

2nd Meeting of JCIA-new LRI Poster presentation ” The testing methods for selective, sustained, shifted, and divided attention by elaborating the target detection method”

Publications:

1, Wada Hiromi and Seto Yukina “Effects of perinatal hypothyroidism on shift attention in rats.” Organohalogen Compounds, 74：1385-1388, 2012 (http://www.dioxin20xx.org/).
2, Wada Hiromi and Seto Yukina “Developmental effects of perinatal hypothyroidism on cognitive functions: Focused on attention.” Psychiatria et Neurologia Japonica 114(8)：949－956, 2012
3, Wada Hiromi and Seto Yukina “Effects of perinatal hypothyroidism on divided attention in rats.” Scientific Committee on Neurotoxicology and Psychophysiology, Cape Town, South Africa, March 2013.
4, Seto Yukina and Wada Hiromi “Effects of perinatal hypothyroidism on shift attention in rats.” 73nd Annual Meeting of the Japanese Society for Animal Psychology, Tsukuba, August 2013
5, Wada Hiromi, Yumoto Syoko, and Iso Hiroyuki. “Irreversible damages to auditory system functions caused by perinatal hypothyroidism in rats.” Neurotoxicology and Teratology, 37: 18-22, 2013.
3. Innovation Kansai, Poster Announcement "Toxic judgment method of using pluripotent stem cell" December 6, 2012, Osaka International Conference Hall

Title of Research:

12_PT01-02

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

Principal Investigator:

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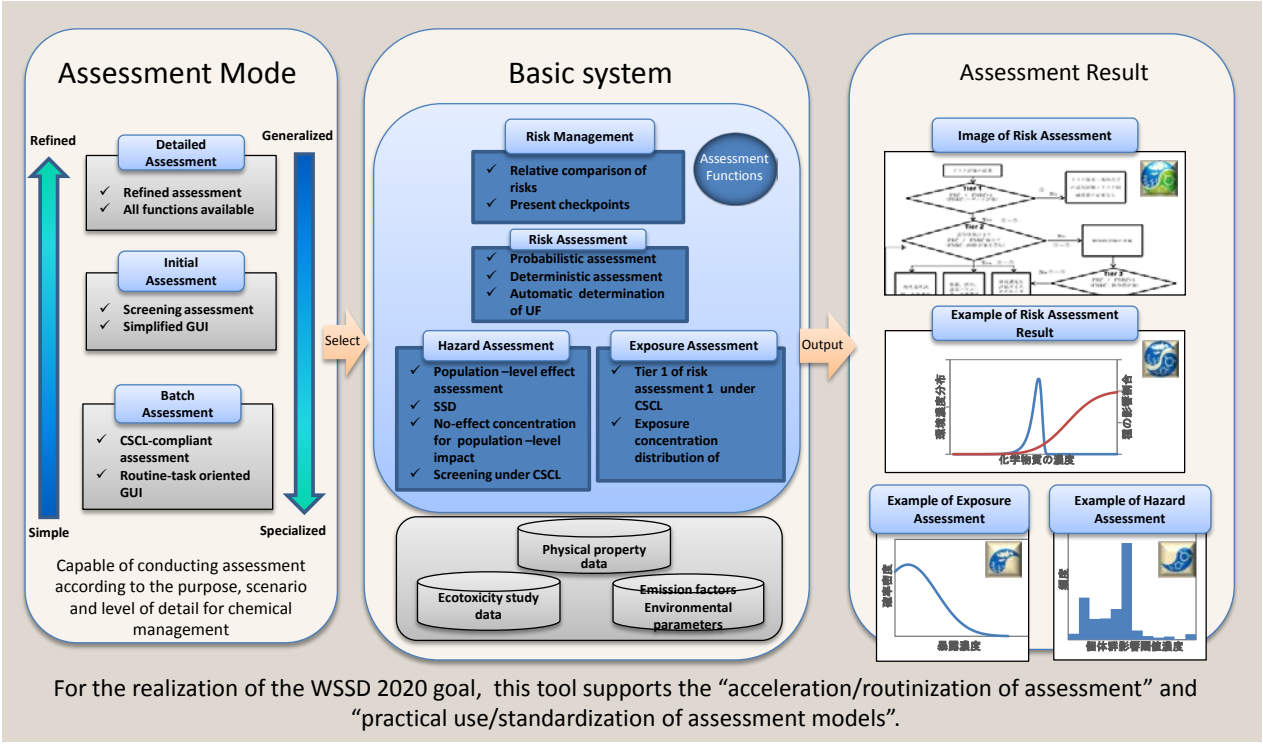

Figure 1. Features and Functions of AIST-MeRAM

Summary of Research:

In the midst of the growing social needs for environmental risk assessment to address new national/international regulations, this study has been promoting the development of the Multi-purpose Ecological Risk Assessment and Management Tool (AIST-MeRAM) that allows business operators to select an assessment model on their own, and to perform a variety of assessments easily and efficiently in order to meet their purpose.(Fig.1)

During the first term of New LRI, we made some improvements to the algorithm and interface, based on the hearings from the industry and government, and undertook the development of a standalone application. As a result, we released the standalone version of AIST-MeRAM (Japanese version: 0.9.12) that contains large amounts of toxicity data and is capable of conducting many different types of ecological risk assessments as well as *Kashinhou*-compliant screening assessment.

Timeline: November 1, 2012 –

Publications:

1) Newspaper coverage: "'New LRI': JCIA started taking actions. 130,000 ecotoxicity data", *The Chemical Daily*, 26 February 2013, p.10 (section of environment).
2) Newspaper coverage: "Development of a user-friendly tool for ecological risk assessment", *Business Line*, 26 April 2013, p.27.
3) Newspaper coverage: "AIST's green innovation for future sustainable society: Ecological risk assessment and management of chemicals – AIST-MeRAM", *Business Line*, 25 July 2013, p.30.
4) AIST-RISS Newsletter No.18. "AIST-MeRAM: Industry-academia-government needs adaptive tool for ecological risk assessment and management".
5) Lin, Naito and Kamo (October 2013) AIST OpenLab 2013: "AIST-MeRAM: Tool for dissemination, optimization and standardization of ecological risk assessment of chemicals.
6) Lin, Hirata, Naito, Kamo (June 2013). "AIST-MeRAM: an easy-to-use tool for aquatic environmental risk assessment and management of chemicals", Water and Environment Technology Conference, June 15-16 2013, Kaganei Campus TUAT.
7) Lin, Kamo and Naito (August 2013). Lecture and poster presentation at the 2nd Annual Meeting of New LRI. "AIST-MeRAM: Development of an industry needs adaptive tool for risk assessment and management".

Title of Research:

12_PT01-03

Research for establishing new method for risk assessment of chemical toxicity using gene-modified ES cells

Principal Investigator:

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Summary of Research:

We will establish a new method of assessment of many chemical risks by using embryonic stem cells and iPS cells with gene-modification. We will examine growth retardation, chromosome aberration and developmental defects of stem cells. We cultured mouse ES cells in 96-well plates. After adding carcinogenic chemicals (DEN, MNU, MMS), we examined the cell growth by MTT assay. We detected cytotoxicity and difference of effect depending on the status of p53. Our experiment revealed that (1) difference of cytotoxicity by chemical species, (2) that p53 mutation resulted in resistance to the cells, (3) and that high frequencies of chromosome aberration in p53-deficient ES cells. These result suggested that risk assessment using ES cells will become high through-put system capable of characterizing mechanisms of toxicity and defense.

Timeline: November 1, 2012 –

Topics:

1. First new LRI Annual research Report 2012 Aug,31
2. Chemical Industrial News Paper 2013Apr2
3. Second new LRI Annual research Report 2013 Aug,30

Publications:

1. Patent Application
   Patent number: 2012-110115;
   Inventors: Takashi Morita and Kayo Yoshida
   Invention: The assessment method of the toxicological risk using a pluripotent stem cell
   Applicant: Osaka City University,
   Filing date: May 11, 2012

2. JST new technology, Oral presentation "Toxic judgment method of using pluripotent stem cell" October 19, 2012, Tokyo JST headquarters

3. Innovation Kansai, Poster Announcement "Toxic judgment method of using pluripotent stem cell" December 6, 2012, Osaka International Conference Hall

Title of Research:

12_PT01-04

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

Principal Investigator:

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Summary of Research:

Increased flux of glycolysis is a common feature of tumors, 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. This PKM-switch (from M1 to M2) is shown to be essential to organize Warburg effect. We have developed a reporter-gene system, which enable us to visualize PKM-switch by cell-autonomous fluorescence derived from the reporter gene. In this study, the reporter-gene was introduced into mouse genome by BAC-transgenic method. We established three independent lines of transgenic mice harboring the reporter gene. These transgenic mice are expected to visualize "Metabolic reprograming" at early phase of tumorigenesis, and to be applied to caricinogenicity tests.

Timeline: November 1, 2012 –

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

Title of Research:

12_PT01-05

Development of *in vitro* screening endocrine disruptor by steroid profiling

Principal Investigator:

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Summary of Research:

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

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

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

Timeline: November 1, 2012 -

Topics:

2nd New LRI Annual Conferences (Yaesu First Financial Building, August 31, 2013) “Development of *in vitro* screening endocrine disruptor by steroid profiling”

Publications:

1. Bamba Takeshi, Masashi Okuno, Toshiyuki Yamashita, Eiichiro Fukusaki
   Simultaneous analysis of steroids by GC/MS for evaluation of endocrine disruption
   65th SBJ Annual Meeting, International Conference Center Hiroshima (Hiroshima), September 20, 2013

2. Masashi Okuno, Toshiyuki Yamashita, Eiichiro Fukusaki, Bamba Takeshi
   Development of *in vitro* screening endocrine disruptor by steroid profiling
   Metabolome Symposium 2013, Kyushu University School of Medicine Centennial Hall (Fukuoka), October 3 – 4, 2013

Title of Research:

12_PT01-06

Sophisticated hazard prediction by active QSAR modeling

Principal Investigator:

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

Collaborators:

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Tomoya Yamazaki (Master student, Toyohashi Univ. Tech.)
Keisuke Saito (Master student, Toyohashi Univ. Tech.)

Summary of Research:

In preceding work, we proposed a technique for active QSAR Modeling that is based on active sampling of a temporary training data set, which gives us higher performance in the prediction. However, the prediction result depends on the number of compounds for the temporary training set used for making the model. And, it is difficult to know the optimal number of the neighbors to be used, in advance. In the present work, we employed a threshold of the similarity at exploring the neighbors but not the number of neighbors to be searched. Computer experiment showed us that the method with the threshold of similarity gives a better performance. Besides, it was shown that a QSAR model obtained from the whole data could give us better prediction when the appropriate neighbors are not available enough. Alternatively the performance of the method also depends on the local data structure around a query of interest. But, it is impossible to see a data structure of the local space around the query in advance. We employed kNN, RMSST (Rooted Minimum Spanning Sub-Tree) and Centroid method to explore the neighbors. Once, we generated the QSAR models with the different training sets that are obtained by the different neighbor searching methods, and we evaluated the statistical performance of the models. We used the best approximation model among them. Computational experiment with a data set of toxic chemicals suggested that the current approach can provide us much better predictions for the case.

Timeline: November 1, 2012 -

Topics:

The Secondt New LRI Workshop, Tokyo, Aug., 2013

Publications:

1) Tomoya Yamazaki and Yoshimasa Takahashi, Toxicity prediction of chemical substances by Active Sampling :Introduction of cutoff value by similarity, Proc. of the 22nd Symposium on Environmental Chemistry, 2013, July, Tokyo.
2) Tomoya Yamazaki and Yoshimasa Takahashi, Toxicity prediction of chemical substances by Active Sampling: Neighbor search method and model selection, Proc. of the 41th Symposium on Structure-Activity Relationships, 2013, November, Osaka.

Research Field 2: Research on the safety of new chemical substances including nanomaterials

Evaluation of the relationship between physicochemical properties, bio-distribution, and safety of nanomaterials

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

Title of Research:

12_S02-01

Evaluation of the relationship between physicochemical properties, bio-distribution, and safety of nanomaterials

Principal Investigator:

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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 1000 nm (NP1000) or less than 100 nm (NP100). Nickel particles are generally used for industrial products, such as a ceramic capacitor. In addition, nickel ion is known to induce metal allergy to the skin exposure. Our results showed that the ion and the nickel particles used in this study could not penetrate the skin in vivo and in vitro, although more precise investigation is needed. The data could provide information to ensure the safety of nanomaterials.

Timeline: November 1, 2012 –

Topics:

Poster presentation at The 2nd LRI Annual Conferences “Evaluation of the relationship between physicochemical properties, biodistribution, and safety of nanomaterials”

Research Field 3: Research on the effects of chemical substances on children, elderly people, and those with gene disorders

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

Nakamura Akira (Department of Immunology, Kanazawa Medical University)

Title of Research:

12_PT03-01

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:

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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) are expressed in murine basophils and eosinophils. Both *Slpi* $^{-/-}$ (SLPI-deficient) basophils and eosinophils produced more cytokines than wild type (WT) 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 2012LRI study, we firstly examined the cytokine responses of murine *Slpi* $^{-/-}$ basophils and eosinophils. As in the case of 2011LRI research, *Slpi* $^{-/-}$ basophils were susceptible for the cell toxicity of the chemicals, demonstrating that murine *Slpi* $^{-/-}$ basophils seemed to be inadequate for the screening of the sensitizers. Conversely, *Slpi* $^{-/-}$ eosinophils showed high viabilities after administration with various chemicals. In addition, IL-6 production stimulated with beryllium sulfate was augmented in *Slpi* $^{-/-}$ eosinophils, suggesting that eosinophils lacking serine protease inhibitors are suitable for the screening of some chemicals. We next investigated the expression profile of the serine protease inhibitors in mast cells, basophils, and eosinophils by DNA microarray analysis. The array studies showed the results as follows. : Mast cells express Serpin b1a and b6a. Basophils express Serpin b1a, b2, and SLPI. Therefore, these serine proteases would be the target genes for the generation of cell lines. We also induced the toluene diisocyanate (TDI)-induced respiratory inflammation. However, since the cellular infiltration was comparable between WT and *Slpi* $^{-/-}$ mice, the methods should be improved. Finally, we tested the cell viability and cytokine responses in the basophilic cell line KU812. KU812 showed high viabilities against various chemicals. KU812 also secreted cytokines in response to several chemicals, suggesting that KU812 lacking serine protease inhibitors would be an ideal risk evaluation model against chemical substances.

Timeline: November 1, 2012 -

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 2th annual conference of new LRI, Tokyo, Japan, August 2012

Research Field 4: Assessment on the effects on ecosystems and the environment

Development of *in silico* Prediction Model on Environmental Fate of Chemical Substances

Tatsuya Takagi (Graduate School of Pharmaceutical Sciences, Osaka University; Genome Information Research Center, Research Institute for Microbial Diseases, Osaka Univ.)

Comprehensive evaluation methods for chemicals registered in PRTR.

Norihide Nakada (Research center for Environmental Quality Management, Graduate School of Engineering, Kyoto University)

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

Yuhei INAMORI (Fukushima University)

Title of Research:

12_S04-01

Development of *in silico* Prediction Model on Environmental Fate of Chemical Substances

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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.

The experimental results of the direct photolysis and hydrolysis under certain conditions were collected and then the datasets of hydrolysis and direct photolysis consisted of the results of 143 and 106 compounds, respectively. Although the experimental results of indirect photolysis were also checked, since there were few results whose experimental conditions were under the EPA guideline, we decided that the prediction on photolysis of this project was focused on direct one. We are intended to collect the information on photolysis and hydrolysis and to add it into the dataset continuously. The quantitative model on hydrolysis of “unstable” compounds whose half-lives are less than a year was built by using publicly available software, and its prediction performance was adequate. We are preparing to calculate an excited state that is regarded as a key factor for the prediction of direct photolysis of a compound using publicly available software. The trial system which provides the prediction models through the WWW was constructed. We are adding several functions and improving usability of this web site.

Timeline: November 1, 2012 –

Topics:

The 2th Annual Conference of New LRI, “Development of new tool for in silico prediction of environmental fate of compounds”, Aug 30, 2013

Publications:

The 40th Symposium of Structure-Activity Relationships, Aichi, Japan, Nov 2012

Title of Research:

12_PT04-01

Comprehensive evaluation methods for chemicals registered in PRTR.

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Summary of Research:

Chemicals are widely used in chemical industries. Huge amount of the chemical usage and consequent discharge might result in their entering into the wastewater and even water treatment units such as the biological and/or physicochemical processes. This research is aimed at establishing the formation potential methods for toxic chemicals during those treatments above, and proposing new risk assessment methods.

In the first term, four analytical methods were established for each group including four trihalomethanes (THMs), four aldehydes, eight nitrosamines and six perfluorinated carboxylic acids (PFCAs), respectively. Moreover, four fluorotelomer alcohols (FTOHs) as the precursors of PFCAs were established. Besides, according to the reported mechanisms for forming the THMs, aldehydes and nitrosames, the predicted precursors were selected among the chemicals registered in PRTR. And their concentrations in wastewater were calculated based on the PPTR data and annual wastewater in Japan. Three assessment methods were established for formation potential tests, the ozonation treatment (two conditions: high and low dissolved ozone dose), chloramination treatment, and biological treatment (two conditions: aerobic and anaerobic), respectively. Samples covered the chemicals in PPTR and actual wastewater samples. Five chemicals were firstly selected as the potential precursors according to the high potential of exposure in wastewater (calculated concentration in wastewater) and reported precursors in literatures. The formation potential for the aldehydes and nitrosamines during the ozonation and chlorination treatments were investigated. The results showed during the ozonation treatment, the dimethylhydrazine formed significant formaldehyde and N-nitrosodimethylamine. Further, more chemicals will be tested and quantified for the formation potential. In the case of PFCAs formation potential during the biological treatment, the formation and degradation rates were studied under the anaerobic (10, 20 day, 1, 2, and 3 month) and the aerobic condition (10, 20 day, 1 month). The results found the PFCAs formation was correlated with the degradation of FTOHs predictor.

Timeline: November 1, 2012 –

Topics:

Poster presentation at 2nd Annual Conference of LRI

Publications:

48th Annual Conference of Japan Society of Water Environment, Sendai, Japan, March 2014.

Title of Research:
12_PT04-02

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

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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 is 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 is not established by problems, such as stability, high cost nature and reproducibility. In addition, the fundamental manual concerning test operation is already built (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 turned to carry out to establish as the OECD standard test method.

Timeline: November 1, 2012 –

Topics:
2nd New LRI Research Report Meeting: "Development of Ecosystem Risk Impact Assessment System Methods for Chemicals using Microcosm Systems"

Publications:
Kunihiko Kakazu, Hideto Usui, Jun Kumada, Katsura Sugiura, Ryuhei Inamori, Yuhei Inamori : "Ecotoxicity Assessment of SDS with P,R (Production, Respiration) about Aquatic Microcosm", *Journal of Water and Waste*, Vol.54, No.9, pp.683-690 (2012).

Annual Report 2013

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