LRI

Long-range Research Initiative

Annual
Report
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Japan Chemical Industry Association

Title of Research:
17_PT01-01
Chip device for in vitro systemic toxicology

Principal Investigator:
Junji Fukuda, PhD (Professor, Yokohama National University, Engineering)
79-5 Tokiwadai, Hodogaya, Yokohama, Kanagawa, 240-8501, Japan
(tel) +81-045-339-4008, (e-mail) fukuda@ynu.jp

Collaborators:
Kazutomo Iijima, PhD (Associate Professor, Yokohama National University, Engineering)
79-5 Tokiwadai, Hodogaya, Yokohama, Kanagawa, 240-8501, Japan
Yasunari Kanda, PhD (Head of Cardiac pharmacology, National Institute of Health Sciences, Division of Pharmacology)
3-25-26 Tonomachi, Kawasaki-ku, Kawasaki, Kanagawa, 210-9501, Japan
Yusuke Okubo, PhD (Senior staff scientist, National Institute of Health Sciences, Division of Cellular and Molecular Toxicology)
3-25-26 Tonomachi, Kawasaki-ku, Kawasaki, Kanagawa, 210-9501, Japan
Kiyomi Ohmori, PhD (Adjunct Professor, Yokohama National University, Research Initiatives and Promotion Organization)
79-5 Tokiwadai, Hodogaya, Yokohama, Kanagawa, 240-8501, Japan

Summary of Research:
  Body-on-a-chip has emerged as a novel approach for Alternatives to Animal Testing. The aim of this study is to develop a body-on-a-chip for *in vitro* drug testing to evaluate systemic toxicity of chemicals. In our chip devices, several tissues including the liver, the cardiac tissue, and the small intestine were fabricated as either cell layer tissues or spherical tissues to replicate *in vivo* kinetics and interactions. One of our results showed that improved oxygen supply and a selection of energy metabolisms significantly matured human iPS-derived cardiomyocyte spheroids. Changes in beating of these spheroids were characterized using cell motion imaging on the chip device, indicating that this can be an important toxicity testing. In addition, human iPS cell-based signaling reporter system was also developed for prediction of chemical teratogenicity.

Timeline:
March 1, 2019 - February 29, 2020

Topics:
Poster presentation at JCIA LRI Annual Workshop 2019 "Chip devices for *in vitro* systemic toxicology" (Tokyo, August 30th, 2019)

Publications:
1. Y. Sawada, Y. Miura, E. Kobayashi, K. Iijima, J. Fukuda, H. Itagaki, "Evaluating immunotoxicity in co-culture system of THP-1 and undifferentiated / keratinized NHEK", The 46th Annual Meeting of The Japanese Society of Toxicology, Tokushima, Japan, June 2019
2. T. Shinagawa, J. Fukuda, S. Toyama, J. Fujita, K. Fukuda, Y. Kanda, A. Satsuka, "Maturation of human iPS-derived cardiomyocytes on oxygen-permeable spheroid culture device", Yokohama Branch Meeting of Society for Chemical Engineers, Yokohama, Japan, August 2019
3. T. Usui, D. Qin, J. Fukuda, "Co-culture of CACO-2 cell sheets and E. coli-encapsulated microbeads for in vitro intestine model", APCChE2019, Sapporo, September 2019
4. Y. Yamamura, J. Fukuda, "Drug evaluation chip device using multiple 3D tissue models", The 32nd Annual Meeting of the Japanese Society for Alternatives to Animal Experiments, Tsukuba, Japan, November 2019
5. D. Qin, T. Usui, J. Fukuda, "Co-culture of intestinal epithelial cells and bacteria-embedded beads for *in vitro* small intestine model", The 32nd Annual Meeting of the Japanese Society for Alternatives to Animal Experiments, Tsukuba, Japan, November 2019

Title of Research:

17_PT01-02

Development of novel screening system to predict the effects of environmental factors in each organ by using cell-free circulating DNA in serum -- Advanced system for prediction of hazards from environmental factors in fetus using cell free fetal DAN in mother's blood

Principal Investigator:

Wataru Miyazaki, PhD (Professor, Department of Bioscience and Laboratory Medicine, Graduate School of Health Sciences, Hirosaki University)
66-1 Hon-cho, Hirosaki, Aomori 036-8564, Japan
(tel) +81-172-39-5955, (e-mail) miya@hirosaki-u.ac.jp

Collaborators:

Noriyuki Koibuchi (Professor, Department of Integrative Physiology, Graduate School of Medicine, Gunma University)
Izuki Amano (Assistant Professor, Department of Integrative Physiology, Graduate School of Medicine, Gunma University)
Takahiko Katoh (Professor, Department of Public health, Graduate School of Medicine, Kumamoto University))

Summary of Research:

To prevent the adverse effects of several toxic chemicals at an early stage, it is important to develop a novel screening system for detecting organ injury. For such purpose, we have focused on the cell-free circulating DNA (cfDNA), whose origin may be identifiable if they harbor tissue-specific epigenetic modification. This system is simple and minimally invasive because we utilized cfDNA in serum to detect the injuries. Furthermore, our system may have the potential to detect fetal organ injury using cfDNA of fetal origin in maternal serum. Previously, we identified several candidate regions as the adult and fetal organ-specific markers (specific un-methylated regions). We performed bisulfite amplicon sequence assay with cfDNA derived from organ-specific injury mouse model, and detect the organ injuries. In this term, we have continued the assessment of the novel system using cfDNA from several chemical-exposure mouse models (nicotine, ethanol, high fat). The injuries in the liver and cerebellum were detected in ethanol and/or high fat exposed groups using our system. On the other hand, we could not detect the injuries of fetal organs from maternal cfDNA. To improve the labor, time, and cost of our system, we have attempted an improved method with Oligoribonucleotide Interference-PCR (ORNi-PCR). Both the cost and time of the improved method were smaller than the previous. These results indicate that our system may be useful to detect specific injured organs just by using serum, although further experiments are needed to detect the injuries of fetal organs using maternal blood.

Timeline:

March 1, 2019 – February 29, 2020

Topics:

Poster presentation at JCIA LRI Annual Workshop 2019 "Development of novel screening system to predict the effects of environmental factors in each organ by using cell-free circulating DNA in serum" (Tokyo, August 30th, 2019)

Publications:

1. Poster presentation at 9th Federation of Asian and Oceanian Physiological Societies Congress: FAOPS2019, title: A novel screening system to predict injured organs using cell-free DNA in serum

Title of Research:

18_S01-01

The validation study of EpiSensA (Epidermal Sensitization Assay); the *in vitro* skin sensitization assay based on reconstructed human epidermis

Principal Investigator:

Masaaki Miyazawa, PhD (Safety Science Research Laboratory, Kao Corporation)
2606, Akabane, Ichikai-Machi, Haga-Gun, Tochigi 321-3497, Japan
(tel) +81-80-8466-6147, (e-mail) miyazawa.masaaki@kao.com

Collaborators:

Hideyuki Mizumachi, PhD (Safety Science Research Laboratory, Kao Corporation)

Summary of Research:

Lipophilic chemicals are difficult to correctly evaluate by existing in vitro tests because these tests employ aqueous-phase systems. To overcome the limitation, we focused on a reconstructed human epidermis (RhE) and developed the Epidermal Sensitization Assay (EpiSensA) based on the expression of four marker genes related to induction of skin sensitization. Based on the comparison with the results of animal test, we confirmed that EpiSensA has better predictive performance for a variety of chemicals including lipophilic chemicals than existing in vitro tests. Therefore, the validation study of EpiSensA was started from July 2018 at JaCVAM (Japanese Center for the Validation of Alternative Methods) to adopt it for OECD test guideline. At the last year, we reported that the validation management team (VMT) concluded the technical transfer from the lead laboratory to three participating laboratories (Food and Drug Safety Center, KOSÉ Corporation and LION Corporation) was successfully completed.

Therefore, For the evaluation of with-in laboratory reproducibility (WLR), the Phase I study was started from November 2018. The Phase I study using 15 coded test chemicals is divided into three parts (Phase I-A, I-B, and I-C) and each part includes three repetition tests of 5 chemicals. Regarding the Phase I-A, two out of three laboratories didn't meet the target criteria of WLR (>85%) established by the VMT. However, the following points of the protocol were revised and agreed by the VMT; i) statistically determined acceptance criteria of vehicle control, ii) clarified criteria of retesting at a low dilution rate and iii) retesting when internal control gene fails to meet the criteria. After reanalysis and retesting based on the revised protocol, all laboratories met the target criteria. In the subsequent Phase I-B, all laboratories showed 100% WLR according to the revised protocol. Finally, the mean WLR using 10 coded test chemicals was 93% and satisfied the target criteria. Currently, the Phase I-C study is under-evaluation.

Timeline:

March 1, 2019 – February 29, 2020

Topics:

Poster presentation at JCIA LRI Annual Workshop 2019 "The validation study of EpiSensA (Epidermal Sensitization Assay); the in vitro skin sensitization assay based on reconstructed human epidermis" (Tokyo, August 30th, 2019)

Title of Research:

19_R01-01

Development of rapid, accurate, and low-cost AI drug hazard assessment method by human stem cell test

Principal Investigator:

Wataru Fujibuchi, Ph.D.
Professor, Center for iPS Cell Research and Application (CiRA), Kyoto University
53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507
(tel) 075-366-7012, (e-mail) fujibuchi-g@cira.kyoto-u.ac.jp

Summary of Research:

We developed a new method "hEST", which has more than 95% accuracy predicting neurotoxicity, and genotoxic/non-genotoxic carcinogen categories, using machine learning of gene network data obtained from exposure of human embryonic stem cells to chemicals. This first year, we 1) selected 4 Japanese male/female ES/iPS cell lines as the test cells for 10 hepatotoxic substances. Surprisingly, cell death occurred with 8 hepatotoxic substances when ES cells were used, however, the sensitivity of iPS cells to chemical substances was low. In order to improve iPS cells' sensitivity and standardize the test cell lines, we 2) tested 24 existing naïve cell transformation methods, and 3) developed our own culture method. Although iPS cells' sensitivity increased, it has decreased again as cells were passaged. We will continue to develop more stable, high sensitivity iPS cells, and will obtain gene expression data from ES cells as well.

Timeline:

March 1, 2019 - February 29, 2020

Topics:

Poster presentation at JCIA LRI Annual Workshop 2019 "Development of rapid, accurate, and low-cost AI drug hazard assessment method by human stem cell test" (Tokyo, August 30th, 2019)

Publications:

1. Wataru Fujibuchi "代替法としてのヒト幹細胞への大きな期待-その驚くべき潜在能力" The 32nd Annual Meeting of the Japanese Society for Alternatives to Animal Experiments (Tsukuba, November, 2019)
2. Panina Yulia, Junko Yamane, Kenta Kobayashi, Wataru Fujibuchi "Development of a fast, reliable and low-cost toxicity prediction system using AI and reset iPS cells." Kyoto University Medical Innovation Graduate School Program Medical research exchange salon, Poster presentation (Kyoto, January, 2020)

Title of Research:

17_PT02-01

Establishment of mechanism based assay protocol for hazard and carcinogenic risk of carbon based nanomaterials

Principal Investigator:

Hiroyuki Tsuda, MD, PhD (Professor, Nagoya City University, Nanotoxicology Project Lab)
3-1, Tanabedohri, Mizuho-ku, Nagoya, 467-8603, Japan
(tel)+81-52-836-3496, (e-mail) htsuda@phar.nagoya-cu.ac.jp

Summary of Research:

To evaluate the toxicity and carcinogenicity of carbon nanotubes (CNT), we are establishing an inexpensive intratracheal intrapulmonary spraying (TIPS) method for administration of CNTs via the airway. TIPS will greatly expand research into the mechanism of CNT-mediated tissue injury and carcinogenesis beyond what is currently possible using conventional expensive inhalation exposure testing. Work in our laboratory has shown that among multi-walled carbon nanotubes (MWCNT), MWCNT-7 (M-H company, 40 wall layers) and MWCNT-N (N company, about 30-50 layers), which form needle-like aggregates, are carcinogenic to the lung and pleura of rats. More recently, work in our laboratory and by others using TIPS have shown that MWCNT-A (Company C, 150 layers) and MWCNT-B with fewer layers (Company C, 15 layers) are also lung carcinogens. In the current study, double-walled carbon nanotubes (DWCNT) (diameter 1-3 nm) (Company A) were compared with the carcinogenic CNT, MWCNT-7, using the TIPS method: acute / subchronic / chronic toxicity / carcinogenicity was analyzed.

Methods: 10-week-old male rats were administered a total dose of 0.25 and 0.5 mg DWCNT and MWCNT-7 per rat for the short-term test, and 10-week-old male rats were administered a total dose of 0.125, 0.25, and 0.5 mg DWCNT or 0.5 mg for MWCNT-7 per rat for the long-term test. CNTs were suspended in a solvent (saline + 0.5% dispersant PF68PF copolymer) and administered every other day over the course of 15 days (total of 8 TIPS administrations). The control groups were untreated rats and rats administered the solvent. MWCNT-7, which is known to be carcinogenic in the lung and pleural mesothelium, was used as a positive control.

Short-term test: 3 and 8 weeks after beginning administration (1 and 6 weeks after the final TIPS administration): The distribution and shape of the administered CNTs was examined. Histopathological analysis of lung tissue was performed to determine lung tissue pathology. Tracheal and lung lavage fluid, pleural lavage fluid supernatant, inflammatory markers in the cell pellets, and biochemical material prepared from lung tissue were analyzed to determine the degree of inflammation. Inflammatory cytokines, DNA damage, and oxidative stress markers revealed that DWCNT was less toxic than MWCNT-7.

Subchronic test (52W): The number of granules and alveolar Mφ of the encapsulated granulations in lung specimens was greater in the DWCNT treated rats compared to the MWCNT-7 treated rats. Notably, the number of PCNA-positive alveolar epithelial cells was less in the DWCNT treated rats compared to the MWCNT-7 treated rats. Many MWCNT-7 fibers were not encased in granulations and were often deposited in the alveoli. No neoplastic lesions were observed in either group.

Chronic toxicity. Carcinogenicity test (104w):

Lung tumor incidence (adenoma + adenocarcinoma) was significantly higher in the 0.5mg DWCNT group, 7/24, (29.2%) compared to vehicle group, 1/25, (P <0.048). The incidence of pleural malignant mesothelioma was significantly higher in the MWCNT-7 group, 16/25, (64%) compared to the vehicle group, 0/25, (P <0.0001).

Less acute toxicity and more pronounced granulation encapsulation in the lungs of rats administered DWCNT compared to rats administered MWCNT-7 suggests that malignant pleural mesothelioma developed relatively early in the MWCNT group, resulting in death before lung tumors were able to develop, however, this is a topic that requires further study. We are also studying carbon nanohorns and nanobrushes containing SWCNT structures using this same method (currently 46w).

Timeline:

March 1, 2019 – February 29, 2020

Topics:

Research on the safety of new chemicals such as "Nano materials" etc.
Poster presentation at JCIA LRI Annual Workshop 2019 "Establishment of mechanism based assay protocol for hazard and carcinogenic risk of carbon based nanomaterials" (Tokyo, August 30th, 2019)

Publications:

1. Abdelgied M., Elgazzar AM., Alexander D., Alexander W., Numano T., Iigo M., Naiki-Ito A., Takase H., Abdou KB., Hirose A., Taquahashi Y., Kanno J., Abdelhamid M., Tsuda H., Takahashi S.   Pulmonary and pleural toxicity of potassium octatitanate fibers, rutile titanium dioxide nanoparticles, and MWCNT-7 in male Fischer 344 rats, Arch. Toxicol., 93(4): 909-920, 2019

2. Abdelgied M., Elgazzar AM., Alexander TW., Numano T., Iigou M., Naiki-Ito A., Takase H., Hirose A., Taquahashi Y., Kanno J., Abdelhamid M., Khaled AA., Takahashi S., Alexander BD, Tsuda H. Carcinogenic effect of potassium octatitnate (POT) fibers in the lung and pleura of male Fischer 344 rats after intrapulmonary administration, Particle and Fibre Toxicology, https://doi.org/10.1186/s12989-019-0316-2 2019

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

Title of Research:

19_R03-01

Development of a novel alternative method for evaluation of sensitizing potential and allergenicity by measuring human T cell activation and differentiation (Key event 4)

Principal Investigator:

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

Collaborators:

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

Summary of Research:

  Recently, several in vitro assays to predict the sensitizing potential of chemicals have been developed. However, these animal testing alternatives cannot distinguish chemical respiratory and skin sensitizers, although the risk management systems for them are quite different. Therefore, we have been aiming at developing a novel in vitro assay, which can discriminate them by taking advantage of the fundamental differences between their modes of function; development of helper T (Th) 2 immune responses, which are critically important for respiratory sensitization. Recently, we established a new 3-dimentional (3D) dendritic cell (DC) coculture system consisting of human airway epithelial cell line, immature DCs derived from human monocytes, and lung fibroblast cell line. This coculture system was shown to successfully discriminate respiratory sensitizers from skin sensitizers using 6 representative chemical sensitizers by more enhanced expression of key costimulatory molecule OX40 ligand (OX40L), which is important for Th2 differentiation, in DCs. In the present study, we are currently trying to establish a new 2-step DC/T coculture system by introducing T cells in the DC coculture system, in which the Key event 4, that is T cell, in the adverse outcome pathway of sensitization can be used as a marker. To increase the versatility, we are also trying to generate iPS-derived DC progenitors and T cell lines to apply for it.

  In the 7th term, DCs stimulated by 6 representative sensitizers in the DC coculture system were further reacted with allogeneic naive CD4$^+$ T cells in the 2-step DC/T coculture system. As a result, selective up-regulation of IL-4, Th2 differentiation marker, by respiratory sensitizers could be seen after 5-day culture. Furthermore, we generated several iPS-derived DC progenitor lines by transduction of genes related to cell survival and cell cycle. Some of them showed a tendency to selectively up-regulate OX40L by respiratory sensitizer in the 3D DC coculture system as primary monocyte-derived immature DCs did. We are currently still continuing to investigate them.

Timeline:

March 1, 2019 - February 29, 2020

Topics:

Oral presentation "Establishment of a novel in vitro evaluation system for the prediction of respiratory sensitizing potential of chemicals" and poster presentation "Development of a novel alternative method for evaluation of sensitizing potential and allergenicity by measuring human T cell activation and differentiation (Key event 4)" at JCIA LRI Annual Workshop 2019 (Tokyo, August 30th, 2019)

Publications:

1. Mizoguchi I, Yoshimoto T, et al. Development of a novel 3D co-culture system to evaluate sensitizing potential of chemicals. The 32th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments. (Tsukuba, Nov. 20-22, 2019)
2. Mizoguchi I, Yoshimoto T, et al. Development of a novel alternative method for evaluation of sensitizing potential and allergenicity by measuring human T cell activation and differentiation

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

in vitro. The 48th Annual Meeting of the Japanese Society for Immunology. (Hamamatsu, Dec. 12-13, 2019)

Assessment of the effects on ecosystems, ecotoxicity and environment.

Title of Research:

18_S04-01

Exploring roles and simple estimation methods of species sensitivity distribution for deriving PNECs

Principal Investigator:

Masashi Kamo (National Institute of Advanced Industrial Science and Technology. Research Institute of Science for Safety)
(e-mail) masashi-kamo@aist.go.jp

Collaborators:

Yuichi Iwasaki. National Institute of Advanced Industrial Science and Technology. Research Institute of Science for Safety
Wataru Naito. National Institute of Advanced Industrial Science and Technology. Research Institute of Science for Safety

Summary of Research:

Species sensitivity distribution (SSD) has been internationally used to derive predicted no effect concentration (PNEC) in ecological risk assessment. In this study, we aimed to (1) develop statistical models to estimate log-normal SSD parameters (i.e., mean and standard deviation of SSD) based on limited data and (2) quantify the uncertainties associated with risk assessments by using SSD. For the former study (1), we first collected acute ecotoxicity data, and estimated SSDs for 64 chemicals. We developed best models for estimating those SSD parameters based on Akaike information criterion. In addition, we collected chronic ecotoxicity data, derived SSDs for 15 chemicals, and performed some preliminary analysis. For the latter study (2), we published a peer reviewed paper based on the results obtained last year and presented the results at the SETAC Europe 29th Annual Meeting. Based on standard deviations of acute SSDs estimated in the study (1), we also determined the magnitude of assessment factors required to determine more accurate PNECs for the ecosystem protection.

Timeline:

March 1, 2019 – February 29, 2020

Topics:

Poster presentation at JCIA LRI Annual Workshop 2019 "Exploring roles and simple estimation methods of species sensitivity distribution for deriving PNECs" (Tokyo, August 30th, 2019)

Publications:

1. Kiyan Sorgog and Masashi Kamo. Quantifying the precision of ecological risk: Conventional assessment factor method VS. species sensitivity distribution method. Ecotoxicology and Environmental Safety. Volume 183, 15, November 2019.
   https://doi.org/10.1016/j.ecoenv.2019.109494
2. Poster presentation at the 29th SETAC Europe Annual Meeting at Helsinki, Finland from the 26th till the 30th of May 2019, titled Assessment factor and SSD methods: which one provides more protective threshold for the ecosystem against adverse effects of toxicants? by Kiyan Sorgog, Masashi Kamo, Yuichi Iwasaki, Wataru Naito.
3. Poster presentation at 25th annual meeting of the Japanese society of environmental toxicology, Tsukuba, Ibaraki, September 2019, titled Developing a new approach to predicting the species sensitivity distribution by limited data by Kiyan Sorgog, Yuichi Iwasaki.
4. Poster presentation at 54th Annual Conference of Japan Society on Water Environment, titled Comparison of performance ability of various assessment method by Kiyan Sorgog, Yuichi Iwasaki (March 16-18th 2020 at campus of Iwate university).

Title of Research:

17_S05-01

Development of Novel Analytical System of Biological Responses to Chemicals by Deep Phenotyping Method

Principal Investigator:

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

Collaborators:

Tadahaya Mizuno (Assistant Professor, University of Tokyo, Graduate School of Pharmaceutical Sciences, Department of Molecular Pharmacokinetics)
7-3-1 Hongo, Bunkyo, Tokyo, 113-0033, Japan
(tel) +81-3-5841-4771, (e-mail) tadahaya@mol.f.u-tokyo.ac.jp

Setsuo Kinoshita (Contract Research Student, University of Tokyo, Graduate School of Pharmaceutical Sciences, Department of Molecular Pharmacokinetics)
7-3-1 Hongo, Bunkyo, Tokyo, 113-0033, Japan

Summary of Research:

  Today, organisms are always exposed to chemicals in every situation to some extent. Wide variety of chemicals exist around us and the chemicals affect organisms not one by one but simultaneously like a complex. The fact prompts us to care "multiple exposure". To achieve the appropriate and correct prediction of multiple exposure of chemicals, it is necessary to comprehend the effects of chemicals even if those are unknown and difficult to be detected. In this study, we tackle with the theme by omic approaches with innovative techniques in both data acquisition and data analysis method.

  This study consists of two pillars: (1) a novel acquisition method of proteome profile data by 2-dimensional electrophoresis (2DE) and (2) a new analysis method of profile data, orthogonal linear separation analysis (OLSA). In this 7th term, the last term, we tested the performance of 2DE in detection of chemical features and showed capacity of OLSA to uncover novel aspects of a natural product.

  We have carried out 2DE of HeLa cells treated with 6 different anti-cancer drugs under several conditions obtained total 33 proteome profile data. Clustering analysis revealed good separation of each drug group composed of three biological replicates except for Cisplatin and doxorubicin. Of note, combinatorial treatment of a MEK1/2 inhibitor (PD184352) and a microtubule inhibitor (vincristine) constructed a specific cluster and OLSA successfully extracted the combinatorial effect as a specific vector that was not observed in each inhibitor alone.

  As for data analysis, we have extended our profile data analysis method to achieve fully unbiased analysis by implementation of parallel analysis. This extension enabled us to estimate the appropriate number of effects to be decomposed, without existing body of biological knowledge. In fact, the basic effects generated by the renewed method were well consistent with gene groups defined in gene ontology, compared with the original one, when Gaussian noise were added to the data. To test the performance of our decomposition analysis approach, Rescinnamine, a natural product was subjected to OLSA. We detected four main effects of the natural product and two of them were actually confirmed in vitro analysis such as luciferase assay and western blotting analysis. All of these data suggest that our platform has the potential to boost deep understanding of combinatorial exposure of chemicals.

Timeline:

March 1, 2019 - February 29, 2020

Topics:

1. Poster presentation at JCIA LRI Annual Workshop 2019 "Development of Novel Analytical

Other issues to be urgently addressed.
System of Biological Responses to Chemicals by Deep Phenotyping Method" (Tokyo, August 30th, 2019)

Publications:
1. Akademi) ICCA-LRI workshop, June 19-20, 2019, Stresa, Italy
2. Journal) Kinoshita S, *Biol Pharm Bull*, 2019

Other issues to be urgently addressed.

Title of Research:
19_R05-01

Study on the contribution of microplastics to bioaccumulation and biological magnification towards fish

Principal Investigator:
Tatarazako Norihisa（Professor, Ehime University, Graduate School of Agriculture）
3-5-7 Tarumi, Matsuyama, Ehime, 790-8566 Japan

Collaborators:
Tatsuhiro Niino (LSI Medience Corporation)
1000 Kamoshidacho, Aoba-ku, Yokohama-shi, Kanagawa 227-0033, Japan
Shin Takahashi（Ehime University, Graduate School of Agriculture）
3-5-7 Tarumi, Matsuyama, Ehime, 790-8566 Japan
Yoshifumi Horie (Akita Prefectural Univesity)
241-438 Kaidobata-Nishi Nakano Shimoshinjo Akita City 010-0195 Japan

Summary of Research:
  The microplastics (MPs) are detected from sewage treated water or sea water, and harmful chemical substances are known to adsorbed to those MPs. And there is a concern about those adsorbed chemicals may be taken more efficiently to organisms intermediated by MP and that biological concentration or biological magnification is accelerated. Our study will try to clarify whether the chemical substances adhered to MP are eluted, and absorbed / transferred / accumulated in the body of organisms.
  This year, we reviewed the kind of solvent to be adsorbed on MP of PAHs and the quantitative analysis procedure, and when the amount of adsorption was re-measured under new procedure, the result was PE> PP> TF. A significant correlation was observed between the amount of PAHs adsorbed and the octanol / water partition coefficient. Furthermore, the amount of PAHs adsorbed on MP was higher in seawater than in pure water. It is necessary to confirm the acute toxicity of MPB itself in medaka and daphnia in order to determine the set concentration for the accumulation test. Medaka did not die with MPB of all sizes, whereas daphnia showed acute toxicity with MPB of 0.2 and 0.5 μm in diameter. Medaka was toxic to benzo [a] pyrene and pyrene in an extended acute toxicity test. When larvae and Daphnia larvae were fed 5 μm-diameter fluorescent MPB and observed under a microscope, both were confirmed to accumulate MPB in the digestive tract, but no transfer to other internal organs and muscle tissues was observed. In addition, in order to examine biomagnification, it was examined whether fluorescent MP was transferred to juvenile medaka through daphnia. Although water flea was eaten by medaka, MPB was transferred, but no accumulation of MPB was detected.
  In the next fiscal year, we will determine the concentration of chemicals accumulated in fish by feeding MPs with PAHs adsorbed. The transfer efficiency is determined from the total intake and the amount of chemicals in the fish.

Timeline:
March 1, 2019 - February 29, 2020

Topics:
Poster presentation at JCIA LRI Annual Workshop 2019 "Study on the contribution of microplastics to bioaccumulation and biological magnification towards fish" (Tokyo, August 30th, 2019)

Publications:
1. Norihisa Tatarazako, Kazufumi Misawa, Yukiyo Okazaki, SETAC Europe 29th Annual Meeting 26−30 May 2019 | Helsinki, Finland,poster
2. Yukiyo Okazaki, Takahiro Ishizaka, Norihisa Tatarazako, SETAC Europe 29th Annual Meeting 26−30 May 2019 | Helsinki, Finland,poster
3. Norihisa Tatarazako, Yukiyo Okazaki, SETAC North America 40th Annual Meeting 3–7 November 2019, Metro Toronto Convention Centre, Toronto, Ontario

Other issues to be urgently addressed.
4.   Yukiyo Okazaki, Norihisa Tatarazako, Shin Takahashi, Harumi Kasahara, Yaeko Sera, Tatsuhiro Niino, SETAC North America 40th Annual Meeting 3–7 November 2019, Metro Toronto Convention Centre, Toronto, Ontario

Other issues to be urgently addressed.

Title of Research:
19_R05-01

Establishment of medaka kinetic model for aged microplastic and adsorbed chemical

Principal Investigator:
Yuji Oshima (Kyushu University, Faculty of Agriculture, Laboratory of Marine Environmental Science Professor)
744 Motooka, Nishi-ku, Fukuoka 8020345, Japan
(tel&fax)+81-092-802-4607

Collaborators:
Yohei Shimasaki (Faculty of Agriculture, Kyushu University, Associated Professor)
Kang Ik Joon (School of Interdisciplinary Science and Innovation, Kyushu University, Associated Professor)

Summary of Research:
  Plastic has an extensive use in our daily life due to its low cost, lightweight and hard to decompose. Due to their high production and a quite slow degradation, plastic pollution has been expanded worldwide in aquatic environments. Pollution of plastic from large items to small particle (i.e. microscopic plastic MP, < 5mm) were emerged. Thus, effect of MP and its vector effect with toxicants are of concern. Many researchers have been performed in these topics. However, most of study were performed using pristine MP. Thus, risk analysis of aged MP is required.
  In this study, two kinds of aged PE plastics were prepared by exposure of ultraviolet for 180 and 460 hours. Weak changes on chemical characters were detected. No aging effect on sorption and adsorption of anthracene to MP was observed. Bioaccumulation factor, and elimination and absorption constants were not changed in medaka fish co-exposed with anthracene and aged MP. From results of one compartment analysis and simulation, vector effect of MP on accumulation of anthracene on medaka fish may suggest. Further study on another type of aged MP is required.

Timeline:
March 1, 2019 - February 29, 2020

Topics:
Poster presentation at JCIA LRI Annual Workshop 2019 "Establishment of medaka kinetic model for aged microplastic and adsorbed chemical" (Tokyo, August 30th, 2019)

Title of Research:

19_D08-01

Elucidation of the formation mechanism of microplastics

Principal Investigator:

Kuroda Shinichi (Graduate School of Science and Engineering, Gunma University)
29-1 Honmachi, Ota City, Gunma Prefecture 373-0057
(tel) 0276-50-2434 (e-mail) skuroda@gunma-u.ac .jp

Collaborators:

Kouzai Hiroaki (Department of Science and Engineering, Kanto Gakuin University)
Kuriyama Takashi (Graduate School of Organic Materials and Systems Science, Yamagata University)
Ohtani Hajime (Graduate School of Engineering, Nagoya Institute of Technology)
Igarashi Toshio (Research Division for Science and Technology, Kanazawa University)
Hiejima Yusuke (Research Division for Science and Technology, Kanazawa University)
Tokumitsu Katsuhisa (Materials Science Division, Faculty of Engineering, University of Shiga Prefecture)
Nakatani Hisayuki (Department of Chemistry and Materials Engineering, Nagasaki University)

Summary of Research:

The purpose of this study is to clarify by what mechanism and from what kind of plastic products the secondary microplastics are generated from the perspective of polymer science and polymer engineering. Various approaches of us lead the following results.

In the analysis using the pyrolysis method, individual component information was successfully extracted from the plastic mixture samples by means of Py-APGC-MS measurements with selecting the ions characteristic of the object polymer and recording the ion chromatogram with high mass resolution.

In the morphological analysis of secondary microplastics, the microplastics recovered from rivers and beaches showed cracks as in the outdoor-exposed polymers, where light-induced oxidation takes place. On the other hand, no crack was observed in the microplastics recovered from marine.

In a study considering microbial corrosion, the "ABC degradation" mechanism was revealed: it was found that a complex degradation takes place where the hydrophilization of polymer proceeds owing to the photo-oxidative degradation (Abiotic) and microbial corrosion (Biotic), followed by the micro-flake detachment (Cutting) due to the dissociation of the hydrophilic zone caused by water ingress.

Chemiluminescence analysis shows that hydrophobic polyolefins such as HDPE and PP tends to decelerate the degradation owing to the suppression of temperature increase and oxygen diffusion due to the presence of seawater.

Focusing on mechanical effects, it is was found that fatigue may cause the fragmentation of plastic materials and that the jet mill could efficiently fine-grind plastics.

In the future, a database of literature and research results on the formation mechanism of microplastics will be build. In addition, the microplastic formation behavior through long-term outdoor exposure will be observed and the microplastic accelerated generation test methods will be developed.

Timeline:

March 1, 2019 - February 29, 2020

Topics:

Poster presentation at JCIA LRI Annual Workshop 2019 "Elucidation of the mechanism of microplastic formation" (Tokyo, August 30th, 2019)

Publications:

1. Kuroda Shinichi, "Trends in Research on Microplastics", Materiaru Raifu Gakkaishi, 31 (1): 10-14, 2019

2. Kuroda Shinichi, "Microplastics - its problems and research trends", Jisedai Poriorefin Sogokenkyu, 13: 17-22, 2019

3. Igarashi Toshiro, "Resource recycling of PE from the perspective of microplastics", Jisedai Poriorefin Sogokenkyu, 13: 23-30, 2019

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