



Long-range Research Initiative

**Annual
Report
2018**



2018

Japan Chemical Industry Association

Annual Report 2018

Title of Research:

15_PT01-01

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

Principal Investigator:

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Collaborators:

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

Summary of Research:

So far we have been developing a new short-term *in vivo* screening test method for xenoestrogens by using our transgenic mice carrying estrogen-responsive reporter gene (E-Rep mice) and systemic *in vivo* imaging analysis as an end point. This method has been shown the possibility of a high-superiority test method that can solve several problems of the uterotrophic bioassay which is the only *in vivo* screening test method standardized as an OECD test guideline No.440. In the current period, using our established test method, we evaluated estrogenic/anti-estrogenic potential of Bisphenol A (BPA) which is a typical xenoestrogens.

As a result, in the state of estrogen depletion, BPA showed estrogenic effect at high doses, of which action was not nonlinearity. On the other hand, in the state of maintaining the physiological estrogen level in the test animal, BPA showed anti-estrogenic effect.

These results indicate that it has been confirmed that BPA acts as a xenoestrogen in our new method. In addition, our results suggest that our new test method using systemic *in vivo* imaging analysis should perform the test in both the state of maintaining the physiological estrogen level and of estrogen depletion.

Timeline:

April 1, 2018 – March 31, 2019

Topics:

Poster presentation at JCIA LRI Annual Workshop 2018 “Development of new *in vivo* screening method to validate the low-dose effects induced by estrogenic chemicals using estrogen reporter mice” (Tokyo, August 31st, 2018)

Publications:

1. Törner K, Nakanishi T, Matsuura T, Kato Y, Watanabe H, Genomic integration and ligand-dependent activation of the human estrogen receptor α in the crustacean *Daphnia magna*, **PLOS One**, 13:e0198023 (2018)

Title of Research:

16_PT01-02-3

Construction of prediction models for toxicities induced by drugs and chemicals based on chemical structures and AOP**Principal Investigator:**

Yoshihiro Uesawa (Department of Clinical Pharmaceutics, Meiji Pharmaceutical University)

Summary of Research:

Quantitative structure–activity relationship (QSAR) analysis applies structural and quantum chemical, and physicochemical features, calculated from molecular geometry as explanatory variables, to predict physiological activity. In current studies, the applications of QSAR prediction models in a variety of adverse effects such as Delirium, pulmonary edema, nausea/vomiting, respiratory depression, somnolence, rash, diarrhea, loss of appetite, nephritis, uterine cancer ... etc., have been constructed by databases such as JADER and FAERS (JAPIC-AERS). Furthermore, data set that includes FAERS and Tox21-AOP/MIE data was constructed and applied to analyze the relationship between adverse effects and major MIEs such as nuclear receptors and stress response pathways.

Timeline:

March 1, 2018 – February 28, 2019

Topics:

1. Poster presentation at JCIA LRI Annual Workshop 2018 “Construction of prediction models for toxicities induced by drugs and chemicals based on chemical structures and AOP” (Tokyo, August 31st, 2018)
2. Uesawa, Y., inventor; Meiji Pharmaceutical University, assignee. Prediction systems, prediction methods, prediction programs, learning model input data generation systems, and learning model input data generation programs. Pending Japanese patent application 2018-122565(PTC). 2018.
3. Matsuzaka, Y., Uesawa, Y.; Excellent Poster, CBI Annual Meeting 2018.
4. Asato, S, Kagaya, H., Uesawa, Y.; Excellent Presenter, The 4th Regulatory science forum for young people. 2018.

Publications:

1. Uesawa Y, "Quantitative structure-activity relationship analysis using deep learning based on a novel molecular image input technique", Bioorg Med Chem Lett, 28(20):3400-3403 (2018)
2. Matsuzaka Y, Uesawa Y, "Optimization of a Deep-Learning Method Based on the Classification of Images Generated by Parameterized Deep Snap, a Novel Molecular-Image-Input Technique for Quantitative Structure–Activity Relationship (QSAR) Analysis", Frontiers in Bioengineering and Biotechnology, in press (2019)
3. Ayano Shiota, Hajime Kagaya, Yoshihiro Uesawa, “Analysis of drug-induced somnolence based on JADER with chemical structure information”, 138th annual meeting of the pharmaceutical society of Japan, Sendai Japan, 2018/3
4. Yuina Tatsuhiro, Hajime Kagaya, Yoshihiro Uesawa, “Analysis of structural and physicochemical features of drugs that cause non-cardiogenic pulmonary edema using Japanese Adverse Drug Event Report database (JADER)”, 138th annual meeting of the pharmaceutical society of Japan, Sendai Japan, 2018/3
5. Yoshiyasu Ozu, Hajime Kagaya, Yoshihiro Uesawa, “Classification of constipation-related diseases using Japanese Adverse Drug Event Report database (JADER)”, 138th annual meeting of the pharmaceutical society of Japan, Sendai Japan, 2018/3.
6. Sumino Asato, Hajime Kagaya, Yoshihiro Uesawa, “Construction of a prediction model for nausea and vomiting based on JADER with chemical structures”, 138th annual meeting of the pharmaceutical society of Japan, Sendai Japan, 2018/3
7. Yuka Seta, Hajime Kagaya, Yoshihiro Uesawa, “The analysis on drug-induced delirium using Japanese Adverse Drug Event Report database with chemical structure information”, 138th



Development and assessment of new risk assessment methods
annual meeting of the pharmaceutical society of Japan, Sendai Japan, 2018/3

Title of Research:

17_PT01-01

Chip device for in vitro systemic toxicology**Principal Investigator:**

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Summary of Research:

The aim of this study is to develop microdevices, so-called Body on a chip, for *in vitro* drug testing to evaluate systemic toxicity of chemicals. This device may enable to replicate *in vivo* kinetics and interactions between several tissues including the skin, the small intestine, and the lung for drug absorption, liver and myocardial tissues for drug metabolism and exposure. The point of our device is to engineer cell layer and spherical tissues on a chip as tissue models to acquire more physiological responses.

In the second year of the project, we improved functionalities of spherical tissues. Data we obtained last year indicated that when cells form tissues and sufficient oxygen is supplied, not only positive effects but also adverse effects were observed, probably due to generation of reactive oxygen species. To alleviate the negative effects, we examined antioxidants. Indeed, the addition of antioxidants in culture medium further improved insulin secretion on pancreatic spheroids. Permeability testing was performed with skin and intestine models that we fabricated last year. In addition, interactions between two model tissues, liver and breast cancer, were evaluated on a seesaw-like stage, with which culture medium were perfused in the microdevice without any external pump and air pressure supply. In this setup, a pro-drug was metabolized with the liver model and inhibited growth of the cancer cells. Furthermore, we demonstrated that the modified h-CLAT testing shows a positive reaction to a positive control substrate.

Timeline:

March 1, 2018 – February 28, 2019

Topics:

Poster presentation at JCIA LRI Annual Workshop 2018 “Chip device for in vitro systemic toxicology” (Tokyo, August 31st, 2018)

Publications:

1. D. Myasnikova, T. Osaki, K. Onishi, T. Kageyama, B. Zhang, J. Fukuda, Synergic effects of oxygen supply and antioxidants on pancreatic β -cell spheroids, *Scientific Reports*, 9, 1802 (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

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Takahiko Kato (Professor, Department of Public health, Graduate School of Medicine, Kumamoto University))

Summary of Research:

Detecting organ injury (e.g. by toxins or chemicals) at earlier stage is important to prevent the adverse outcomes. For such purpose, we have developed a novel screening system using serum. This system is simple and minimally invasive, because we utilized serum to detect cell free circulating DNA (cfDNA), whose origin may be identifiable if they harbor tissue-specific epigenetic modification. Furthermore, our system may have a potential to detect fetal organ injury if cfDNA of fetal origin in maternal serum is identifiable. Previously, we identified and selected several candidate regions as the adult and fetal organ-specific markers (specific un-methylated regions). In this term, first, to confirm whether these regions harbor tissue-specific epigenetic modification, we performed bisulfite amplicon sequence (BSAS) assay with genome DNA, derived from several fetal tissues. Second, we produced organ-specific injury mouse model using chemical exposure. We could detect cfDNA harboring each organ-specific nature. The level of such organ-specific cfDNAs increased with the increase in chemicals causing organ-specific injury. These results indicate that our system may be useful to detect specific injured organs just by using serum.

Timeline:

March 1, 2018 – February 28, 2019

Topics:

Poster presentation at JCIA LRI Annual Workshop 2018 "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 31st, 2018)

Publications:

1. Poster presentation at 21st Japan Society of Endocrine Disruptors Research Annual Meeting, title: Development of novel screening system to predict the effects of environmental factors in each organ by using cell-free DNA in serum
2. Oral presentation at 89th The Japan Society for Hygiene Annual Meeting, title: Development of a novel screening system to predict injured organs using cell-free DNA in serum

Title of Research:

18_S01-X01

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

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Katsuhiko Uno, PhD (Hatano Research Institute, Food and Drug Safety Center)
Shinichi Watanabe (LION Corporation)
Junko Ueno (LION Corporation)

Summary of Research:

The assessment of skin sensitization potential is a major content of safety evaluation for novel substances and was traditionally performed by animal tests. On the other hand, the development of non-animal tests for identifying the skin sensitization has been recently activated because of animal welfare and regulatory requirements. However, lipophilic chemicals are difficult to correctly evaluate, since 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 gene expression of four markers related to induction of skin sensitization. Based on the comparison with the results of animal test, we confirmed that EpiSensA had the great predictive performance for lipophilic test chemicals. 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 current study, the transferability to the three naive laboratories was evaluated based on three sensitizers and a non-sensitizer distributed from Kao Corporation with non-coded. As the results, the concurrent results of EpiSensA compared to animal test were observed with great reproducibility at all laboratories. From these results, we concluded that the technical transfer to three naive laboratories was successfully completed. From November 2018, the Phase I study for the evaluation of with-in laboratory reproducibility have been performed based on 14 test chemicals. After that, the Phase II study for between laboratory reproducibility will be performed from March 2020, and the validation study of EpiSensA will finish at early 2021.

Timeline:

March 1, 2018 – February 28, 2019

Topics:

Poster presentation at JCIA LRI Annual Workshop 2018 “The validation study of EpiSensA (Epidermal Sensitization Assay); the *in vitro* skin sensitization assay based on reconstructed human epidermis” (Tokyo, August 31st, 2018)



Research on the safety of new chemicals such as "Nano materials" etc.

Title of Research:

17_PT02-01

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

Principal Investigator:

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Summary of Research:

Purpose: To establish a method of evaluating the toxicity and carcinogenicity of respirable nanomaterials, especially carbon nanotubes, that is less expensive and easier to perform than conventional high-cost inhalation exposure. We developed transtracheal intrapulmonary spraying (TIPS) to administer test materials to the lungs of test animals. We tested several types of multi-walled carbon nanotubes (MWCNT): MWCNT-7 (Hodogaya, 40 layers), needle-like aggregates of MWCNTs; MWCNT-N (Nikkiso, about 30 layers), another type of MWCNT that forms needle-like aggregates; MWCNT-A (Company C, 150 layers), a third type of MWCNT that forms needle-like aggregates; MWCNT-B (Company C, 15 layers), which because of its much smaller number of layers is flexible and produces cotton-like aggregates. We have shown that these four types of MWCNTs are carcinogenic to the lung. We are currently testing two-layered carbon nanotubes (DWCNT) to determine if this type of carbon nanotube is also harmful to the lung and the pleural tissues as observed with the MWCNTs composed of several layers.

Methods: We conducted a preliminary dose setting trial using DWCNT from Sigma-Aldrich and from Company A. We determined that 0.5 mg per rat was an appropriate dose. We administered DWCNT (1-3 nm diameter) (Company A) to 10-week old male rats. The test materials were suspended in a solvent (saline + 0.5% dispersant PF68 PF copolymer) to give final concentrations of 0.125 mg/ml and 0.0625 mg/ml, and 0.5 ml of the suspended material was administered to the rats a total of 8 times, for final doses of 0.25 and 0.50 mg per rat. The control groups were (1) untreated and (2) administered vehicle alone. MWCNT-7, whose lung carcinogenicity was demonstrated using both a whole body exposure inhalation test and our TIPS administration procedure was used as the carcinogenic positive control. Rats were killed at experimental week 3 (1 week after the end of administration) and week 8 (6 week after the end of administration). Histopathological analysis of the lung and pleura and biochemical analysis of the lung tissue, tracheopulmonary lavage (BALF), and thoracic cavity lavage (PLF) was performed for each rat. Inflammatory markers in supernatants and lavage cell pellets were also evaluated.

Results: Real time PCR and ELISA analyses of the lung tissue, pathological and histopathological observation (distribution of sample, shape and degree of inflammation), and biochemical analysis (inflammatory cytokines, DNA damage, oxidative stress markers) indicated that damage of the lung tissue and visceral pleural mesothelium was absent or weak in the DWCNT treated rats. No



Research on the safety of new chemicals such as "Nano materials" etc. grossly recognized neoplastic lesions were observed in rats killed at one year (52 weeks) after the start of administration. Tissue samples from these rats are currently being prepared for histopathological analysis.

New Studies: Using TIPS administration, we have started a study of the pleural toxicity of two new types of materials. Both materials, like single walled carbon nanotubes, are constructed from a single carbon sheet. The 8-week interim sacrifice is scheduled for this April 15-16.

Timeline:

March 1, 2018 – February 28, 2019

Topics:

Poster presentation at JCIA LRI Annual Workshop 2018 "Establishment of mechanism based assay protocol for hazard and carcinogenic risk of carbon based nanomaterials" (Tokyo, August 31st, 2018)

Publications:

1. Elgazzar AM., Abdelgied M., Alexander D., Alexander W., Numano T., Iigo M., Naiki A., Takahashi S., Takase H., Hirose A., Kanno J., Elokke OM., Nasem AM., Tsuda H. Comparative pulmonary toxicity of a DWCNT and MWCNT-7 in rats, Arch. Toxicol., Oct. 10, <https://doi.org/10.1007/s00204-018-2336-3>, 2018
2. 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., Tsuda H. Potassium octatitanate fibers induce persistent lung and pleural injury and are possibly carcinogenic in male Fischer 344 rats, Cancer Sci., May 2018, <https://doi.org/10.1111/cas.13643>



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

Title of Research:

15_S03-01

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

Principal Investigator:

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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 sensitizers and skin sensitizers, although the risk management systems for them are quite different. Therefore, in the present study, we aim 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 is critically important for respiratory sensitization.

So far, we established a new immunocompetent 3D co-culture system consisting of human airway epithelial cells, immature dendritic cells (DCs) derived from human peripheral blood CD14⁺ monocytes, and human fibroblast cells. This co-culture system was demonstrated to predict the respiratory sensitizing potential of chemicals by means of more enhanced expression of key costimulatory molecule OX40Ligand (OX40L), which is important for Th2 differentiation, in DCs. In this term, to further improve the versatility, we utilized iPS cell-derived DCs based on published protocols, although their efficacy is not so good. Therefore, we initially tried to establish a novel method to generate infinitely growing myeloid progenitors by stimulation with various cytokines and/or lentiviral incorporation of genes related to cell cycle and survival. Finally, we have developed several types of immortalized myeloid progenitors. By using these cells-derived DCs, the co-culture system seems to be able to discriminate between skin and respiratory sensitizers as in the case of primary DCs. Moreover, we also tried to develop a novel DC/T co-culture system, which consists of sensitized DCs and allogenic naive CD4⁺ T cells. In this system, we have great advantage that IL-4 up-regulation can be used as a marker for the prediction of respiratory sensitizing potential. Indeed, our preliminary data suggest that selective up-regulation of IL-4 was observed by the stimulation with respiratory sensitizer as compared to that with skin sensitizer.

Timeline:

March 1, 2018 - February 28, 2019

Topics:

Poster presentation at JCIA LRI Annual Workshop 2018 Establishment of a novel in vitro evaluation system for the prediction of respiratory sensitizing potential of chemicals” (Tokyo, August 31st, 2018)

Publications:

1. Yoshimoto T, Ohashi M, Hasegawa H, Orii N, Xu M, Owaki T, Mizoguchi I. Development of a novel alternative method by using 3D co-culture system for evaluation of respiratory sensitizing potential. The 45th Annual Meeting of The Japanese Society of Toxicology, Workshop. (Osaka, Jul. 18-10, 2018)
2. Yoshimoto T, Hasegawa H, Orii N, Xu M, Mizoguchi I. Prediction of chemical respiratory sensitizers by OX40L expression in dendritic cells using a new 3D co-culture system. The 4th International Conference on Toxicity Testing Alternatives & Translational Toxicology and the 2nd



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

Asian Congress on Alternatives. (Guangzhou, Guangdong, China, Oct. 9-12, 2018)



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

Title of Research:

12_PT03-01-6

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. Because we found that basophils and eosinophils lacking a secretory leukoprotease inhibitor SLPI produced more cytokines than wild type cells after stimulation with IgE or LPS. Therefore, we have planned to generate the highly sensitive cell line by the deletion of serine protease inhibitors using a human basophilic cell line, KU812-F. Because KU812-F expresses high levels of Serpin b1 and b6, and we have generated stable Serpin b1 knockdown KU812-F cell lines by using lentiviral-delivered sh (short hairpin) RNA. The knockdown KU812-F cells (KD cells) produced IL-6 about 2 times higher than KU812-F after administration with toluene diisocyanate (TDI). KD cells also secreted IL-13 upon stimulation with TDI. In contrast, scramble and wild type KU812-F cells did not produce IL-13. We investigated gene alteration between KD cells and scramble KU812-F cells in responses to TDI by mRNA sequencing analysis. We found significant elevation of three genes as follows: CD69 (T cell activation marker), a chemokine receptor, CXCR4, and sphingosine-1-phosphate receptor 4. We evaluated the relative fluorescence intensities (RFI) in accordance with human Cell Line Activation Test (H-CLAT). KU812-F and KD cells showed higher RFI of CD69 than scramble cells upon TDI and formaldehyde stimulation, whereas all cell lines did not display any alteration of CD69 expression after DNCB and Methyl Salicylate. We also found that the levels of CD69 mRNA are significantly augmented in KD cells after TDI stimulation. In the present LRI research, we confirmed the high level of CD69 expression in KD cells. Moreover, to establish a simpler method for the screening, we analyzed the CD69 expression using wild type KU812-F cells in response to 12 kinds of chemicals since wild type cells are also sensitive to chemical stimulation. The RFI values displayed more than 150% in WT cells after administration with TDI and *p*-phenylenediamine. Collectively, our data suggested that the analysis of CD69 expression is a suitable application for the detection of respiratory sensitizers by using KU812-F cells in addition to Serpin b1 knockdown KU812-F cells.

Timeline:

March 1, 2018 – February 28, 2019

Topics:

Poster presentation at JCIA LRI Annual Workshop 2018 “Development of high sensitivity *in vitro* assessment system of chemical-mediated hypersensitivity by using serine protease inhibitor-deficient cells.” (Tokyo, August 31st, 2018)

Title of Research:

16_PT04-01

Development of the platform to manage the monitoring data of chemicals in environment, ChemTHEATRE

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Nobuaki Ohno (Graduate School of Simulation Studies, University of Hyogo)

Summary of Research:

We have launched a web platform to register and visualize the monitoring data of environmental contaminants, named 'ChemTHEATRE' (Chemicals in the THEATRE: Tractable and Heuristic E-Archive for Traceability and Responsible-care Engagement) at <https://chem-theatre.com/>. So far, the data of 83 projects have been registered to the database. The chemical concentration data in environmental and biological specimens can be found by sample type, chemical name, sampling date and location, or other metadata. Users can export the query results for further analyses.

The registered data to ChemTHEATRE are also available at the websites of National Bioscience Database Center, as follows,

Integbio Database Catalog: <https://integbio.jp/dbcatalog/>

Life Science Database Cross Search: <https://biosciencedbc.jp/dbsearch/>

Life Science Database Archive: <https://dbarchive.biosciencedbc.jp/en/chemtheatre/desc.html>

Timeline:

March 1, 2018 – February 28, 2019

Topics:

Poster presentation at JCIA LRI Annual Workshop 2018 "Development of the platform to manage the monitoring data of chemicals in environment, ChemTHEATRE" (Tokyo, August 31st, 2018)

Publications:

1. Seiichi Uno, Machi Kawano, Tomohiko Isobe, Itsuki C Handoh, Nobuaki Ohno, Tatsuya Kunisue, Kei Nakayama. "Monitoring of PAHs in shellfish after oil spill from the Sanchi tanker; comparison of polluted conditions with other spill accident using ChemTHEATRE." SETAC North America 39th Annual Meeting, Sacramento, CA, USA, November 4–8, 2018.
2. Itsuki C Handoh, Toru Kawai, Tomohiko Isobe, Nobuaki Ohno, Seiichi Uno, Tatsuya Kunisue, Kei Nakayama. "Bayesian uncertainties analysis of modelled chemical exposure of global fish community: How can ChemTHEATRE improve the performance of FATE?" SETAC North America 39th Annual Meeting, Sacramento, CA, USA, November 4–8, 2018.
3. Kei Nakayama, Binle Lin, Tomohiko Isobe, Seiichi Uno, Itsuki C Handoh, Nobuaki Ohno, Tatsuya Kunisue. "ChemTHEATRE, a platform to browse the published or public data of environmental monitoring and its use for risk assessment." SETAC North America 39th Annual Meeting, Sacramento, CA, USA, November 4–8, 2018.
4. Tomohiko Isobe, Kei Nakayama, Seiichi Uno, Itsuki C Handoh, Nobuaki Ohno, Tatsuya Kunisue. "ChemTHEATRE: Opendata platform for environmental monitoring of POPs and other contaminants." 11th SETAC Asia-Pacific Meeting, Daegu, South Korea, September

16–19, 2018.

5. Tatsuya Kunisue, Kana Egashira, Tomohiko Isobe, Kei Nakayama, Takashi Matsuishi, Yuko Tajima, Tadasu K Yamada, Shinsuke Tanabe. “Temporal trend analyses of POPs in three toothed whale species stranded along the Japanese coastal waters: importance of samples and data stored in es-BANK and ChemTHEATRE.” 38th International Symposium on Halogenated Persistent Organic Pollutants & 10th International PCB Workshop: DIOXIN 2018, Kraków, Poland, August 26–31, 2018.
6. Kei Nakayama, Tomohiko Isobe, Seiichi Uno, Itsuki C Handoh, Nobuaki Ohno, Tatsuya Kunisue. “Introduction to ChemTHEATRE: a platform to utilize the published or public data of environmental monitoring.” Water and Environment Technology Conference 2018 (WET2018), Matsuyama, Japan, July 14–15, 2018.

Title of Research:

18_S04-01

Exploring roles and simple estimation methods of species sensitivity distribution for deriving PNECs**Principal Investigator:**

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Collaborators:

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Summary of Research:

The aim of the present study is to develop a new methodology to determine a suitable predicted no effect concentrations (PNEC) by using a sensitivity distribution (SSD) in the ecological risk assessment. The study is carried out in two perspectives. One is to develop a statistical method to estimate SSD with limited number of toxicity data [1], and the other is to develop a method to quantify an uncertainty in the risk assessment through a comparison of using SSD and using conventional assessment factors (AF) [2] (termed SSD-method and AF-method, respectively). In [1], toxic data are collected to estimate SSD. Data selection are carried out based on reliability of toxicity tests and number of species tested in SSD. As a result, 28 chemicals were selected for SSD estimation. These SSD will be used as a learning set for the SSD predictions. Descriptors of 28 chemicals such as logKow, boiling (melting) points, toxic values of model species in toxicity tests are collected. At the first step, correlations between these descriptors and mean (μ)/standard deviation (σ) of the SSDs are investigated, respectively. The correlations between the descriptors and μ are relatively high, compared to those between the descriptors and σ . These results suggest that the prediction for σ is a harder task than the prediction for μ . In [2], we quantified the uncertainty which is inevitable for the SSD estimation and then developed the method to determine a magnitude of AF under the uncertainty. Using the AF, the performance abilities of SSD-method and AF-method are compared. Measurement of the performance ability is as follows. For a given toxicity data set, PNEC is determined, and if PNEC is greater than the protection goal, determination of PNEC is counted as failure. These processes are repeated many times and the number of failures is counted at various μ and σ of SSD. The performance ability of AF-method is σ sensitive. In lower σ , conventional AF-method had a lower failure probability compared to SSD-method. On the other hand, in higher σ , SSD-method has a lower failure probability. Our result suggests that it is important to understand the factors affecting the performance ability, and σ of SSD is surely one of them.

Timeline:

March 1, 2018 – February 28, 2019

Topics:

Poster presentation at JCIA LRI Annual Workshop 2018 “Exploring roles and simple estimation methods of species sensitivity distribution for deriving PNECs” (Tokyo, August 31st, 2018)

Publications:

1. Oral presentation at an annual meeting of the Japanese society of environmental toxicology, titled Comparison of performance ability of various assessment method by Masashi kamo (September 11th 2018 at satellite campus of Gifu university).

Title of Research:

17_S05-01

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

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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 hidden 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. In the 5th term, we carried out fine-tuning of these two.

As for 2DE, we have investigated intra-day deviation of profile data because it is indispensable for the construction of database. HeLa cells treated with or without bortezomib were lysed and the specimens were subjected to 2DE. The difference between days were smaller than that between treatments (bortezomib and DMSO as a control). We have written the detailed protocol of our 2DE method to minimize the dependency of the data on investigators.

As for data analysis, we have summarized the results so far and it was accepted by *Scientific Reports*. By this method, it is expected that profile data of samples subjected to multiple exposures can be separated in unsupervised manner, and it can be estimated what kind of action is combined. In this term, we searched for data subjected to combined exposure from Gene expression omnibus (GEO; <https://www.ncbi.nlm.nih.gov/gds>), and analyzed the data analysis with OLSA. Six combined exposure data were found (ID: GSE10466, GSE16816, GSE17589, GSE24065, GSE35230, GSE59882). Of these, combined exposure data of GSE16816, GSE24065, and GSE59882 were expressed in a linear combination of vectors obtained by OLSA.

Timeline:

March 1, 2018 – February 28, 2019

Topics:

Poster presentation at JCIA LRI Annual Workshop 2018 “Development of Novel Analytical System of Biological Responses to Chemicals by Deep Phenotyping Method” (Tokyo, August 31st, 2018)

Publications:

1. The 2nd workshop for Japan-Korea Young Scientists on Pharmaceutics, Seoul, 2018/7/9-11;
2. GPEN2018, Singapore, 2018/9/25-29



Other issues to be urgently addressed.

3. 33rd JSSX, Ishikawa, 2018/10/1-5
4. CBI 2018, Tokyo, 2018/10/9-10/11
5. 第40回生体膜と薬物の相互作用シンポジウム, 2018/10/18-19, Miyagi
6. THE 13th SUGIYAMA LABORATORY (RIKEN) OPEN SYMPOSIUM, 2019/2/22, Yokohama
7. Journal) Mizuno T, *Sci Rep*, 2019

Title of Research:

18_R05-01

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

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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. However, there are many unclarified questions such as abundance in the water, transfer ratio to organisms or sediment, lifetime and the mass balance of those chemical substances related with MPs in the environment. Our study will try to clarify whether the chemical substances adhered to MP are eluted, and absorbed / transferred / accumulated in the body of organisms.

In this fiscal year, we conducted quantitative analysis of the chemicals (13 kinds of polycyclic aromatic hydrocarbon) preparedly adhered to MP. The result showed that the concentration of forcibly adhered PAH to MP were correlated to molecular weight and hydrophobicity of PAH. The adhered concentration were different between the materials of MP. Also, the materials of MP differentiated the elution characteristics of water and seawater. Acute toxicity test of MP and the consideration of feeding condition to fish as preliminary examinations for the biological accumulation study were conducted. The medaka was not able to eat the MP which diameter was more than 1.5mm, but they preferably ate the MP covered with feed which diameter was around 1.0mm. Further examination is needed for the acute toxicity testing.

From next fiscal year, we will conduct the quantitative analysis of organisms, which have taken MPs and accumulated PAHs preparedly adhered to MP. The transition efficiency will be calculated when the concentration of internal chemical substance and the amount of total chemical intake is provided by this study.

Timeline:

June 1, 2018 – February 28, 2019

Topics:

Poster presentation at JCIA LRI Annual Workshop 2018 “Study on the contribution of microplastics to bioaccumulation and biological magnification towards fish” (Tokyo, August 31st, 2018)

Publications:

1. Norihisa Tatarazako, Yukiyo Okazaki, etc, 39th SETAC North America, Nov. 2018, Sacramento



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