LRI

Long-range Research Initiative

Annual Report 2021

2021

Japan Chemical Industry Association

Annual Report 2021

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:

Hideyuki Mizumachi, PhD (Safety Science Research Laboratory, Kao Corporation)
2606, Akabane, Ichikai-Machi, Haga-Gun, Tochigi 321-3497, Japan
(tel) +81-70-3297-2574, (e-mail) mizumachi.hideyuki@kao.com

Collaborators:

Sho Suzuki (Safety Science Research Laboratory, Kao Corporation)
Makoto Mizuno, Megumi Sakuma, PhD, Momoko Shibata (KOSÉ Corporation)
Mika Watanabe, Michiyo Yasuda, Michika Kajiwara (Hatano Research Institute, Food and Drug Safety Center)
Shin-ichi Watanabe, Junko Ueno (LION 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. We have 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.

For evaluation of within laboratory reproducibility (WLR), three laboratories tested 15 coded chemicals in three independent experiments during Phase I study, and target criteria was established at 85% by the VMT. Several protocol modifications and additional experiments were performed as appropriate, and the mean WLR resulted in 91% and satisfied the target criteria. The VMT concluded that the Phase I study was successful. Regarding subsequent Phase II study for evaluation of between laboratory reproducibility (BLR), three laboratories tested 12 coded chemicals once, and target criteria was established at 80%. The BLR was calculated using 27 test chemicals including Phase I and Phase II chemicals, and the final prediction for the chemicals that were tested 3 times in each laboratory was based on the median classification. As a result, the BLR was 89% and satisfied the target criteria. In addition, protocol modification was proposed to avoid the potential cross-contamination effect of volatile strong sensitizers. Furthermore, hazard predictive performances at participating laboratories were evaluated using 27 test chemicals, the performances were similar to that of the lead laboratory. From these results, the VMT concluded that the Phase II study was successful, and the protocol modification was accepted.

Timeline:

April 1, 2018-

Topics:

Presentations at 2018,2019, 2020, and 2021 JCIA-LRI Workshop

Publications:

The 34th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments, Okinawa and On-line, Japan, Nov. 2021.

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:

Although several *in vitro* assays that predict the sensitizing potential of chemicals have been developed, none can distinguish between chemical respiratory and skin sensitizers. Previously, we established a new three-dimensional dendritic cell (DC) coculture system consisting of a human airway epithelial cell line, immature DCs derived from human peripheral monocytes, and a human lung fibroblast cell line. In this coculture system, compared to typical skin sensitizers, typical respiratory sensitizers showed enhanced mRNA expression in DCs of the key costimulatory molecule OX40 ligand (OX40L), which is important for T helper 2 (Th2) cell differentiation. Herein, we established a new two-step DC/T cell coculture system by adding peripheral allogeneic naive $CD4^+$ T cells to the DCs stimulated in the DC coculture system. In this DC/T cell coculture system, typical respiratory sensitizers but not skin sensitizers enhanced mRNA expression of the predominant Th2 marker IL-4 and its transcription factor GATA-3. To improve the versatility, in place of peripheral monocytes, monocyte-derived proliferating cells called CD14-ML were also used in the DC coculture system. Similar to peripheral monocytes, enhanced mRNA expression of OX40L was observed by typical respiratory sensitizers compared to skin sensitizers. In the 9th term, these cell lines were applied to the DC/T cell coculture system with peripheral allogeneic naive $CD4^+$ T cells, and it was revealed that typical respiratory sensitizers but not skin sensitizers enhance the mRNA expression of IL-4. When allogeneic Th2 cell line was also applied to it, typical respiratory sensitizer enhanced IL-4 mRNA expression, as well as IL-4 secretion. Thus, this DC/T cell coculture system might be useful for discriminating between respiratory and skin sensitizers by differential upregulation of IL-4 in T cells.

Timeline:

March 1, 2019-Feberary 28, 2022

Topics:

2021 Annual Meeting of The Japan Chemical Industry Association LRI, oral 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)” (Web meeting, Aug. 20, 2021)

Publications:

1. Mizoguchi I, et al. A novel coculture system for assessing respiratory sensitizing potential by IL-4 in T cells. ALTEX in press.
2. Yoshimoto et al. Development of a novel alternative method for evaluation of sensitizing potential and allergenicity by measuring human T cell activation. The 34th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments, Web Oral Presentation (Nov. 11-13, 2021, Okinawa)
3. Mizoguchi et al. Development of a novel alternative method for evaluation of sensitizing potential and allergenicity by measuring human T cell activation and differentiation. The 34th

Annual Meeting of the Japanese Society for Alternatives to Animal Experiments, Web Oral Presentation (Nov. 11-13, 2021, Okinawa) President's Special Award

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 extensive use in our daily life due to its low cost, lightweight, and hard to decompose. Due to their high production and slow degradation, plastic pollution has expanded worldwide in aquatic environments. Pollution of plastic from large items to small particles (microscopic plastic MP, < 5mm) emerged. The effect of MP and its vector effect with pollutants are of concerns. Many researchers have studied these topics. However, most of study were used pristine MPs. Thus, the study on effect of aging on vector effect of MP is required.

First, commercially available polyethylene pellets were used to produce grind polyethylene microplastics (cgPE-MP, 200 μm), and then this MP was aged by exposure to UV irradiation equivalent to 5 years in a real environment (acgPE-MP).

Next, effect of aging on anthracene (ANT) uptake on medaka fish was examined using the cgPE-MP and acgPE-MP. No apparent difference was observed in ANT concentrations of fish between cgPE-MP and acgPE-MP co-exposure treatments. This result might be attributed to decrease of ANT concentration in water phase caused by absorption of ANT to cgPE.

The results of a follow-up test using sphere PE-MP (sPE-MP) and cgPE-MP showed that the concentration of ANT in the water was reduced to 87% and 73%, respectively. Simulation results showed a vector effect of sPE-MP and cgPE-MP co-exposure group.

3-year studies have confirmed the vector effect on ANT for PE and PS-MP (approximately 200 μm, 40 mg/L). There seemed to be no significant difference in vector effect due to shape (sphere or grind) or aging. The cPE-MP also showed that it could strongly bind to ANT in water and reduce ANT concentration in the fish.

Furthermore, a PE-MP-ANT dual one-compartment model was constructed to predict vector effect in real environment, and it was estimated that the vector effect would be apparent at high MP concentrations (40 mg/L), but that the vector effect on ANT would be weak at low MP concentrations (0.1 mg/L, similar to the real environment). In the future work, it is necessary to construct vector effect prediction model that considers MP quality,

Assessment on the effects on ecosystems and the environment particle size, vector chemicals.

Timeline:

March 1, 2019 – Feb 1, 2022

Topics:

Publications:

1) Qiu, X., Saovany, S., Takai, Y., Akasaka, A., Inoue, Y., Yakata, N., Liu, Y., Waseda, M., Shimasaki, Y., Oshima, Y., 2020. Quantifying the vector effects of polyethylene microplastics on the accumulation of anthracene to Japanese medaka (Oryzias latipes). Aquatic Toxicology 228, 105643.
2) Assas, M., Qiu, X., Chen, K., Ogawa, H., Xu, H., Shimasaki, Y., Oshima, Y., 2020. Bioaccumulation and reproductive effects of fluorescent microplastics in medaka fish. Mar. Pollut. Bull. 158, 111446.
3) Liu, Y., Qiu, X., Xu, X., Takai, Y., Ogawa, H., Shimasaki, Y., Oshima, Y., 2021. Uptake and depuration kinetics of microplastics with different polymer types and particle sizes in Japanese medaka (*Oryzias latipes*). Ecotoxicol. Environ. Saf. 212, 112007.

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) , Kawai Takahiko (Graduate School of Science and Engineering, Gunma University)

Summary of Research:

The purpose of this study is to clarify the mechanisms by which secondary microplastics are generated from plastic products from the viewpoints of polymer science and polymer engineering. The study focused on analyzing microplastics (MP) in the environment and elucidating the mechanism of the miniaturization of the MP in the environment.

The use of deactivated SiO_2 as a diluent was effective in Py-APGC-MS measurements for pyrolysis analysis of plastics. An algorithm for analyzing MP in the environment was developed and its usefulness for analyzing unknown marine plastic samples was demonstrated.

Next, MP shapes recovered in the estuary were divided into pellets and flake fragments, with pellets recovered in greater numbers than flakes. In addition, crack patterns were observed in 13% of all PP pellets. Most of the spherical pellets showed an isotropic crackle pattern, indicating that these pellets were manufactured by hot cutting. In contrast, about 30% of the flakes developed parallel crack patterns, indicating the influence of molecular orientation during flow molding. In order to evaluate the mechanical collapsibility of the pellets, ball milling tests using glass beads were conducted, and it was found that the collapsibility can be evaluated by the actual MP surface degradation layer peeling off due to mechanical action.

Finally, a diamond disk grinder was installed and rotated in the flow path of the high-speed swirling airflow of the conventional jet mill system to increase the efficiency of accelerated MP formation. The PS elastomer was successfully milled to a fine particle size of approximately 5 μm in diameter using this improved jet mill system. It is expected that this improved jet mill can be used to grind any type of plastic into fine particles of a few microns in diameter, and is expected to be used as a standard MP for biotoxicity evaluation.

Timeline:

March 2021-.

Topics:

Online presentation "Elucidation of the mechanism of microplastic formation" at the 2021LRI Research Report Meeting

Publications:

Presentations: (Only the PI's presentation)
1) Shinichi Kuroda, "
Accelerated Production of Micro- and Nanoplastics Using Impact Pulverization", Academic Symposium on Marine Plastic Litter, March 3, 2021 (online)
2) Shinichi Kuroda, "Issues and Research Trends on Microplastics", Suga Weathering Webinar 2021, December 1, 2021 (online)

Title of Research:

20-1-11

Proposal of a new AOP for the neurotoxicity and developmental neurotoxicity assessment of glutamate receptor binding agonists that cause learning and memory impairment.

Principal Investigator:

Yuko Sekino, PhD (Project Professor, Endowed Laboratory of Human Cell-Based Drug Discovery, Graduate School of Pharmaceutical Sciences, The University of Tokyo) 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
(tel)+81-3-5841-4355, (e-mail) yukos@g.ecc.u-tokyo.ac.jp

Collaborators

Hiroyuki Yamazaki, PhD (Associate Professor, Faculty of Social Welfare, Gunma University of Health and Welfare) 191-1, Kawamagari-machi, Maebashi, Gunma, 371- 0852, Japan
(tel) 027-253-0294 (e-mail) yamazaki@shoken-gakuen.ac.jp
Yonehiro Kanemura, MD, PhD (Director, Department of Biomedical Research and Innovation Institute for Clinical Research, National Hospital Organization Osaka National Hospital) 2-1-14 Hoenzaka, Chuo-ku, Osaka 540-0006, Japan
(tel)+ 81-6-6942-1331, (e-mail) kanemura.yonehiro.hk@mail.hosp.go.jp
Daiju Yamazaki, PhD (Section Chief, Division of Pharmacology, National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki-ku, Kawasaki City, Kanagawa, 210-9501, JAPAN
(tel)+81-44-270-6642, (e-mail) daiju-y@nihs.go.jp
Noriko Koganezawa, PhD (Associate Professor, Department of Pharmacology, Gunma University Graduate School of Medicine) 3-39-22, Showa-machi, Maebashi, Gunma, 371- 8511, Japan
(tel) 027-220-8052, (e-mail) n-koganezawa@gunma-u.ac.jp

Summary of Research:

The purpose of this study is to propose an adverse outcom pathway (AOP) in which the molecular initiating event (MIE) is the binding of a compound to the glutamate receptors, resulting in the adverse outcome (AO) defined as learning and memory impairment. First, we have established an algorithm for high content imaging to detect dendritic spines with drebrin immunoreactivity, and an image analysis method by machine learning. Then, morphological changes of dendritic spines were quantitatively evaluated using frozen hippocampal nerve cells from rat embryos and neurons derived from human iPS cells. We will validate the possibility as an alternative method for developmental neurotoxicity/neurotoxicity testing, using known compounds with learning and memory impairment.

Timeline:

March 1, 2020 –

Topics:

Presentation in LRI Research Report Meeting 2021 “Establishment of an in vitro test method for predicting the neurotoxicity and developmental neurotoxicity of glutamate receptor-binding compounds that cause learning and memory impairment.”

Publications:

Shogo Mase, Izuo Tsutsui, Toshinari Mitsuoka, Noriko Koganezawa, Hiroyuki Yamazaki, Yuuichi Kato, Hiroshi Kawabe, Tomoaki Shirao, Yuko Sekino, “Developmental neurotoxicity assessment of glutamate receptor binding agonists that cause learning and memory impairment : analysis of drebrin immunoreactivity in rat hippocampal cultured neuron” The 48th Annual Meeting of the Japanese Society of Toxicology, Kobe (Web), July 2021

Shogo Mase, Toshinari Mitsuoka, Noriko Koganezawa, Hiroyuki Yamazaki, Yuuichi Kato, Izuo Tsutsui, Tomoaki Shirao, Hiroshi Kawabe, Yuko Sekino “Upregulation of drebrin in dendritic spines and neuronal death induced by a synthetic cannabinoid, CP55940, in cultured rat hippocampal neurons” The 44th Annual Meeting of the Japan Neuroscience Society, Kobe (Web), July 2021.

Shogo Mase, Toshinari Mitsuoka, Noriko Koganezawa, Hiroyuki Yamazaki, Yuuichi Kato, Izuo

Tsutsui, Tomoaki Shirao, Hiroshi Kawabe, Yuko Sekino “Effects of a synthetic cannabinoid, CP55940, on synaptogenesis of cultured hippocampal neurons: imaging analysis of drebrin immunocytochemistry” The 64th Annual Meeting of the Japanese Society for Neurochemistry, Nara (Web), September 2021

Title of Research:

20-3-02
Development of a short-term *in vivo* assay for thyroid hormone disrupting activity in maternal rats and their fetus/pups as prescreening for potential of developmental neurotoxicity.

Principal Investigator:

Tomoya Yamada, DVM, PhD (Fellow, Environmental Health Science Laboratory, Sumitomo Chemical Company, Ltd.)
3-1-98 Kasugadenaka, Konohanaku, Osaka 554-8558, Japan
(e-mail) yamadat8@sc.sumitomo-chem.co.jp

Collaborators:

Hiroaki Aoyama, PhD (Executive Director, Toxicology Division, The Institute of Environmental Toxicology)
4321 Uchimoriya-machi, Joso-shi, Ibaraki 303-0043, Japan
(e-mail) aoyama@iet.or.jp
Hidenori Suto, PhD (Research Associate, Environmental Health Science Laboratory, Sumitomo Chemical Company, Ltd.,)
3-1-98 Kasugadenaka, Konohanaku, Osaka 554-8558, Japan
(e-mail) sutoh@sc.sumitomo-chem.co.jp

Summary of Research:

As thyroid hormones (THs) are essential for brain development, concern has been raised that mild THs disrupting chemicals may have potential to interfere with the developing brain. Since guideline studies to evaluate developmental neurotoxicity require significant resources, a simple screening assay would be valuable. The Comparative Thyroid Assay (CTA) is a candidate screening test, but it requires several animals and advanced techniques of sampling and THs measurements. We are currently attempting to develop a downsized CTA that reduces the animal number, while adding extra parameters in offspring (brain THs levels and histology). We had shown that 6-propylthiouracil (PTU, 10 ppm) induced severe (> 50%) reduction of THs and offspring brain abnormalities (heterotopia), while phenobarbital (PB, 1000 ppm) induced mild (approx. 30%) reduction of THs but did not induce brain abnormalities. Recently, the previous findings at PB 1000 ppm were reproduced, and detection of heterotopia was improved by step section. These findings suggest that the modified CTA is feasible and reliable and mild suppression of THs may have little impact on offspring brain development. Further studies should be investigated.

Timeline:

March 1, 2021 – February 28, 2022.

Topics:

Oral presentation at JCIA LRI Annual Workshop 2021 “Development of a short-term *in vivo* assay for thyroid hormone disrupting activity in maternal rats and their fetuses/pups as prescreening for potential of developmental neurotoxicity.” (On-line, August 20th, 2021)

Publications:

○Hidenori Suto[1], Akira Sato[2], Keiko Ogata[1], Kenta Minami[1], Tadashi Kosaka[2], Hitoshi Hojo[2], Naofumi Takahashi[2], Naruto Tomiyama[2], Katsumasa Iwashita[1], Hiroaki Aoyama[2], Tomoya Yamada[1] ([1] Sumitomo Chemical Company, Ltd. [2] The Institute of Environmental Toxicology)
“Development of a short-term *in vivo* assay for thyroid hormone disrupting activity in maternal rats and their fetuses/pups as prescreening for potential developmental neurotoxicity: Propylthiouracil and phenobarbital examples. I. Findings in maternal rats and their fetuses”. The 48th Annual Meeting of the Japanese Society of Toxicology, Kobe, Japan, July 9th, 2021.

○Akira Sato[1], Hidenori Suto[2], Keiko Ogata[2], Kenta Minami[2], Tadashi Kosaka[1], Hitoshi Hojo[1], Naofumi Takahashi[1], Naruto Tomiyama[1], Katsumasa Iwashita[2], Hiroaki Aoyama[1], Tomoya Yamada[2] ([1] The Institute of Environmental Toxicology [2] Sumitomo Chemical Company, Ltd.)
“Development of a short-term *in vivo* assay for thyroid hormone disrupting activity in maternal rats

and their fetuses/pups as prescreening for potential developmental neurotoxicity: Propylthiouracil and phenobarbital examples. II. Findings in maternal rats and their pups". The 48th Annual Meeting of the Japanese Society of Toxicology, Kobe, Japan, July 9th, 2021.

Tomoya Yamada (Sumitomo Chemical Company, Ltd.)
"A short-term *in vivo* assay for thyroid hormone disrupting activity in maternal rats and their fetuses/pups as prescreening for potential developmental neurotoxicity". The 61st Annual Meeting of the Japanese Society Teratology, On-line, August 8th, 2021.

Tomoya Yamada (Sumitomo Chemical Company, Ltd.)
"Comparative Thyroid Assay: Current situation of a short-term *in vivo* assay for thyroid hormone disrupting activity in maternal rats and their offspring as prescreening for potential developmental neurotoxicity". The 49th Annual Meeting of the Japanese Society of Toxicology, Sapporo, Japan, July 2022.

Kenta Minami[1], ○Hidenori Suto[1], Akira Sato[2], Keiko Ogata[1], Kenta Minami[1], Tadashi Kosaka[2], Hitoshi Hojo[2], Naofumi Takahashi[2], Naruto Tomiyama[2], Hiroaki Aoyama[2], Tomoya Yamada[1] ([1] Sumitomo Chemical Company, Ltd. [2] The Institute of Environmental Toxicology)
"Feasibility and reliability of a downsized comparative thyroid assay for evaluating thyroid hormone disrupting activity in maternal rats and their offspring: reproducibility study with sodium phenobarbital". The 49th Annual Meeting of the Japanese Society of Toxicology, Sapporo, Japan, July 2022.

Title of Research:

20-3-06

Evaluation methods for toxicity using indices of developing neurons

Principal Investigator:

Yaichiro Kotake, Ph.D (Professor, Hiroshima University, Graduate School of Biomedical and Health Sciences)
1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan
(tel)+81-82-257-5325 (e-mail)yaichiro@hiroshima-u.ac.jp

Collaborators:

Yasunari Kanda, Ph.D (Division Head, National Institute of Health Sciences, Division of Pharmacology)
3-25-26 Tonomachi, Kawasaki-ku, Kawasaki 210-9501, Japan
(tel)+81-44-270-6640 (e-mail)kanda@nihs.go.jp

Summary of Research:

In recent years, basic research in the field of neuroscience has revealed many molecules involved in neurite outgrowth and neuronal reorganization, but there have been few attempts to evaluate the toxicity of chemical substances using these molecules as indicators. The purpose of this study was to identify better indicators of developmental neurotoxicity of chemicals from key molecules in neurodevelopment and to clarify their usefulness in assessing developmental neurotoxicity of chemicals.

In the second year of the project, a validation check of the experiment was conducted due to a change in the experimenter in order to eliminate the variation in gene expression data. By strictly standardizing the position of fetal cerebral cortex sections and the method of cell counting before seeding, we were able to obtain data with less variation than before. Next, changes in mRNA expression encoding 12 molecules that play important roles in neurodevelopment were examined up to day 21 of culture when exposed to acrylamide, a candidate positive control substance. The results suggest that the expression of three genes, *Dlg4*, *Syp*, and *Bdnf*, is altered at similar time points as during methylmercury exposure. Since the experimental conditions for human iPS neurons are now in place, we plan to increase the number of compounds evaluated in rat cortical neurons in the next year and compare them with human iPS neurons to clarify a better evaluation index.

Timeline:

March 1, 2021-Februery 28, 2022

Topics:

Publications:

Title of Research:

20-3-08

Development of a novel test for the assessment of neuroinflammation useful to elucidate adverse outcome pathways in developmental neurotoxicity

Principal Investigator:

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

2-174 Edobashi, Tsu, Mie 514-8507, Japan Tel: 81-59-231-5006 E-mail: yuhei@med.mie-u.ac.jp

Collaborators:

Takashi Shiromizu (Department of Integrative Pharmacology, Mie University Graduate School of Medicine)

Munekazu Komada (Department of Life Science, Faculty of Science and Engineering, Kindai University)

Summary of Research:

The incidence of neurodevelopmental disorders such as autism, attention deficit hyperactivity disorder, and learning disabilities is increasing year by year and has become a major social problem. Although chemical exposure during development has been suggested to increase the risk of developing these neurodevelopmental disorders, the detailed mechanisms underlying developmental neurotoxicity (DNT) remain largely unclear. Various molecular initiating events (MIEs) are involved in the developmental neurotoxicity mechanisms of chemical substances, but different MIEs often exert toxicity through a common key event (KE). Developing a test method that can evaluate such a common KE is an effective strategy that will lead to the elucidation of the adverse outcome pathway (AOP) of chemical substances. Impaired differentiation of neural stem cells into neurons and astrocytes, and neuroinflammation mediated by microglia, which are resident immune cells of the brain parenchyma, are attracting attention as the KE common to the developmental neurotoxicity of chemical substances. The purpose of this study is to develop a novel test method that can assess the DNT of chemicals focusing on neuroinflammation through microglia and the differentiation of neural stem cells into neurons and astrocytes. In 2021, we were able to generate a transgenic zebrafish line selectively expressing fluorescent proteins mVenus, Cerulean, and mCherry in microglia, neurons, and astrocytes. In 2021, we will try to establish a method to assess the DNT of chemicals on the microglial dynamics and the differentiation of neurons and astrocytes, which can be useful to elucidate the adverse outcome pathways related to neuroinflammation.

Timeline:

March 2020 ~

Topics:

JCIA LRI Annual Workshop (2021)

Publications: [1-4]

1. Nishimura Y, Kanda Y, Sone H, Aoyama H: **Oxidative Stress as a Common Key Event in Developmental Neurotoxicity**. *Oxidative Medicine and Cellular Longevity* 2021, **2021**:6685204.
2. Higuchi A, Wakai E, Tada T, Koiwa J, Adachi Y, Shiromizu T, Goto H, Tanaka T, Nishimura Y: **Generation of a Transgenic Zebrafish Line for In Vivo Assessment of Hepatic Apoptosis**. *Pharmaceuticals* 2021, **14**(11):1117.
3. Adachi Y, Higuchi A, Wakai E, Shiromizu T, Koiwa J, Nishimura Y: **Involvement of homeobox transcription factor Mohawk in palatogenesis**. *Congenital Anomalies* 2022, **62**(1):27-37.
4. Nishimura Y, Kurosawa K: **Analysis of gene-environment interactions related to developmental disorders**. *Front Pharmacol* 2022, **in press**.

Title of Research:

20-3-10

Establishment of an adverse outcome pathway for the evaluation of developmental neurotoxicity in chemical-induced hypothyroidism

Principal Investigator:

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

Collaborators:

Daisuke Matsumaru (Laboratory of Hygienic Chemistry and Molecular Toxicology, Gifu Pharmaceutical University)

Summary of Research:

Recently, the concern over environmental chemicals that induce hypothyroidism has been increased, since hypothyroidism in pregnancy can have a significant impact on the development of the fetal brain in human. However, detection of abnormalities in thyroid function of pregnant animals in developmental toxicity tests have not been sufficiently utilized for risk assessment of chemical substances because the detailed causal relationship between the thyroid function abnormalities and adverse effects on the development of the fetal brain remains unclear. In order to solve such a problem, we focused on differentiation markers of mature neurons and produced reporter transgenic mice (Tg mice) carrying luciferase (Luc2) and LacZ genes downstream of a neuronal differentiation marker promoter. In this fiscal year, we characterized the expression profile of *in vivo* imaging in the brain during the developmental stage of Tg mice and analyzed the expression profile when hypothyroidism was induced in pregnancy. The daily profiles of *in vivo* imaging in the brain of Tg pups consisted with those of *in vitro* reporter assay in the removed brain. In addition, when antithyroid drugs are administered to pregnant Tg mice under conditions that induce hypothyroidism, *in vivo* imaging was possible to capture their effects on the brain of Tg pups. These results suggest that *in vivo* imaging with our Tg mice may be able to noninvasively detect the effects of chemical exposure during pregnancy on the brain development in pups. In the next fiscal year, the effects of hypothyroidism during pregnancy on the brain development in pups will be examined in detail using the expression profile of reporter gene.

Timeline:

April, 2021 – March, 2022

Topics:

None

Conference Presentations:

1) Ishida K, Minamigawa Y, Mori K, Matsumaru D, Nakanishi T, Characterization of neuronal differentiation tracer mouse for novel developmental neurotoxicity evaluation system, The 48th Annual Meeting of the Japanese Society of Toxicology (Kobe), 2021.
2) Minamigawa Y, Ishida K, Mori K, Tatsumi K, Matsumaru D, Takuma K, Nakanishi T, Validation of neuronal differentiation tracer mice for a novel developmental neurotoxicity *in vivo* evaluation system, Forum 2021 Pharmaceutical Health Sciences/Environmental Toxicology (Chiba), 2021.

Title of Research:

20-6-04

Development of a conceptual model for environmental risk assessment of microplastics and a trial risk assessment in Tokyo Bay

Principal Investigator:

Wataru Naito. National Institute of Advanced Industrial Science and Technology (AIST), Research Institute of Science for Safety and Sustainability (RISS), Group Leader
w-naito@aist.go.jp

Collaborators:

Masashi Gamo, AIST, RISS
Kiyotaka Tsunemi, AIST, RISS
Hideo Kajihara, AIST, RISS
Kyoko Ono, AIST, RISS
Isamu Ogura, AIST, RISS
Bin-Le Lin, AIST, RISS
Xue Mianqiang, AIST, RISS
Hiroyuki Mano, AIST, RISS
Yuichi Iwasaki, AIST, RISS
Naohide Shinohara, AIST, RISS
Yuriko Ishikawa, AIST, RISS

Summary of Research:

The purpose of this study project is to review the existing domestic and international literature for environmental risk assessment of microplastics (MPs), to develop a conceptual model that concretely shows the risk assessment procedure of MPs, and to conduct a trial risk assessment for Tokyo Bay. In FY2021, a literature review was conducted to highlight the latest trends and parameters for emission estimation, exposure analysis, hazard assessment and risk assessment of MP. In order to conduct a trial risk assessment for Tokyo Bay, we attempted to estimate emissions from major sources, evaluate key parameters in exposure concentration analysis, gather and compile a dataset for the development of species susceptibility distributions (SSD), and calculate effect concentrations based on SSD. In addition, an online survey was conducted to investigate public perceptions of marine plastic litter and MP. In the next fiscal year, while verifying the methods and data that contribute to the environmental risk assessment of MPs studied in the current fiscal year, we will present a conceptual model (framework and approach) for environmental risk assessment and management that takes into account the characteristics of MPs by illustrating a trial risk assessment for Tokyo Bay. Furthermore, based on recent trends, we will update research issues and points in MP risk assessment and management, which are necessary for more realistic risk assessment.

Timeline:

March 2020-

Topics:

JCIA-LRI Annual Workshop “Development of a conceptual model for environmental risk assessment of microplastics and a trial risk assessment in Tokyo Bay”, August 2021 (Online)

Publications:

1. Iwasaki Y., Mano H., Lin BL, Naito W. “Current status and issues of hazard assessments focusing on the effects of microplastic particles on aquatic organisms” Jpn. J. Environ. Toxicol. 24: 53-61, 2021.
2. Takeshita KM., Iwasaki Y, Sinclair TM, Hayashi TI, Naito W “Developing a species sensitivity distribution for nano- and microplastic particles by using Bayesian hierarchical modeling” SETAC NA 42nd Annual Meeting、Oral Presentation, Nov. 2021 (Virtual Meeting)
3. Takeshita KM., Iwasaki Y, Sinclair TM, Hayashi TI, Naito W “Illustrating a Species Sensitivity Distribution for Nano- and Microplastic Particles Using Bayesian Hierarchical Modeling” Env. Tox. Chem. doi:10.1002/etc.5295, 2022
4. Ono K, Xue M, Naito W, Tsunemi K. “Preliminary source analysis of microplastics entering Tokyo Bay”, 56th Annual Meeting of Japanese Society of Water Environment, Poster Presentation, March 2022, (Univ. Toyama, On-line)

Title of Research:

21-2-01

Development of evaluation method of inflammatory particles based on alveolar macrophage function.

Principal Investigator:

Etsushi Kuroda, PhD (Professor and Chairman, Hyogo College of Medicine, Department of Immunology)
1-1 Mukogawa-cho, Nishinomiya, Hyogo 663-8501, Japan
tel: +81-798-45-6574, e-mail: kuroetu@hyo-med.ac.jp

Collaborators:

Koubun Yasuda (Hyogo College of Medicine)
Kazufumi Matsushita (Hyogo College of Medicine)
Masakiyo Nakahira (Hyogo College of Medicine)
Takumi Adachi (Hyogo College of Medicine)
Yasuo Morimoto (University of Occupational and Environmental Health)
Hiroto Izumi (University of Occupational and Environmental Health)
Yoshitaka Shirasaki (The University of Tokyo, Faculty of Pharmaceutical Siences)

Summary of Research:

The number of patients with allergic diseases have increased in developed countries. Many factors are thought to be involved in this issue, and multiple studies have demonstrated that particulate pollution such as PM2.5 might be one of factors of exacerbation of allergic inflammation. These particulates function as adjuvant and induce allergic immune responses. However, the mode of action of particulate and the mechanisms by which they induce allergic responses remain to be elucidated. In general, inhaled particles are engulfed by alveolar macrophages as sentinel cells of the lung immune systems, and then excreted. Recently we found that inflammatory particulate alum induced cell death in alveolar macrophages after phagocytosis and then IL-1α was released ad dead cell factor, and also that released IL-1α was involved in allergen-specific IgE production in vivo. These results suggest that responses of alveolar macrophages against particulates are a useful tool for detecting particulates that cause inflammation.

In this term, we performed four types of experiments, those are 1) study of in-vitro-differentiated alveolar macrophages and of establishment of alveolar macrophage cell line, 2) search for new factor(s) specifically induced in response to inflammatory particulates, 3) establishment of in vivo experiment systems to evaluate in vitro study, and 4) study of live cell imaging as a new method for monitoring alveolar macrophages in response to particulates. In these experiments, we found new factor for evaluating inflammatory particulates by comprehensive analysis of lipid mediator release from alveolar macrophages. This lipid mediator was released both in vitro and in vivo. We are currently investigating its efficacy for in vivo evaluation method, along with IL-1α. For live cell imaging analysis, we observed that IL-1α was released form alveolar macrophage death by alum. We will perform same experiments using other particulates such as Al2O3, ZnO, silica, TiO2, sand dust and so on to understand detailed mechanisms of alveolar macrophage function in response to particulates.

Timeline:

March 1, 2021—February 28, 2022

Topics:

Research meeting of LRI by JCIA, oral, “Development of evaluation method of inflammatory particles based on alveolar macrophage function”, online, August 31, 2021.

Publications:

Kuroda Etsushi “Lung immune responses and allergic inflammation induced by fine particles”, The 62nd Annual Meeting of Japan Society for Atmospheric Environment, special meeting, online, September 16, 2021.

Title of Research:

21-3-01

Establishment of in silico model to predict skin absorption of chemical compounds with two-layered diffusion model

Principal Investigator:

Hiroaki Todo, PhD (Associate Professor, Faculty of Pharmacy and Pharmaceutical Sciences, Josai University)
1-1 Keyakidai, Sakado, Saitama 350-0295, Japan
(tel)+81-049-271-7367

Collaborators:

Kenji Sugibayashi, PhD (Josai University)
Kozo Takayama, PhD (Josai University)
Shoko Itakura, PhD (Josai University)
Kenji Mori, PhD (Josai International University, 1 Gumyo, Tougane-shi, Chiba, 283-8555, Japan)
Takeshi Oshizaka, PhD (Josai International University)
Chihiro Takei (Josai International University)
Tetsuya Watanabe (Ohu University) 31-1 Koriyama-shi, Fukushima, 963-8611, Japan
Masahiro Sugino (Ohu University)

Summary of Research:

The skin is the site of exposure to various chemicals. Therefore, the prediction of the chemical's permeability to the ski and local concentration in the skin is very important to assure ensure the safety of the chemicals. Many *in silico* models for predicting these parameters are based on the prediction of the permeability coefficient (*P*) of the exposed chemical. On the other hand, the amount of chemicals absorbed in the body after dermal exposure is calculated from the sum of the amount of the chemical permeated through the skin and the amount of chemical in the viable epidermis and dermis (VED). The amount of chemical permeated through the skin can be calculated by the *P* value. However, the amount of chemical distributed in the VED layer, which is also related to the local safety of the exposed substance, cannot be predicted.

We have already reported that two-layer diffusion model based on Fick's second law of diffusion could predict blood concentration- and skin concentration-time profiles. However, this model has the drawback that skin permeation parameters (chemical diffusivity in stratum corneum (SC) and Ved layers, chemical partitioning into SC and VED layers) must be obtained from *in vitro* skin permeation experiment.

Therefore, this year, we aimed to develop an *in silico* model by clarifying the relationship between skin permeation parameters and characteristic values of chemical substances (molecular weight, fat solubility, molecular volume, energy difference between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of the molecule, etc.).

The approach with linear regression analysis, it was not possible to obtain a highly correlated relationship between skin permeation parameters and characteristic values of the chemical substance. On the other hand, the regression analysis using random forest regression analysis revealed that good correlations were obtained with all skin permeation parameters when molecular weight, lipophilicity, HOMO, and LUMO were selected. The permeability coefficients of 30 compounds were calculated based on the results using random forest regression analysis, and while two chemicals showed more than 5-fold difference in permeability coefficients, 22 chemicals showed a difference within 2-fold. In the future, we would like to evaluate the predictability of skin permeability of various compounds that listed in the EDETOX database to clarify the applicability of this method.

Timeline:

March 1st, 2021-

Topics:

Publications:

ICCA-LRI Workshop (June 20, 2022)

Annual Report 2021

Japan Chemical Industry Association

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