LRI

Long-range Research Initiative

Annual Report 2020

2020

Japan Chemical Industry Association

Annual Report 2020

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

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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 test 15 coded chemicals in three independent experiments each. This Phase I was conducted in three parts (Phase I-A, I-B, and I-C), each including 5 chemicals. The Phase I-A and I-B were finished, and subsequent Phase I-C was performed. As a result, the WLR was not confirmed at one out of the five test chemicals in two laboratories, so the data analysis of the chemical was performed. In consequence, it was confirmed that the cross-contamination of volatile strong sensitizer was the cause of unreproducible result. Therefore, protocol modification was proposed to avoid the potential cross-contamination effect of volatile strong sensitizers, and approved by the VMT. Finally, the WLR using 15 coded test chemicals satisfied the target criteria or 85% in all laboratories. The validation management team concluded that the entire Phase I study was completed successfully.

Timeline:

March 1, 2020 - February 28, 2021

Topics:

Oral presentation at JCIA LRI Annual Workshop 2020 “The validation study of EpiSensA (Epidermal Sensitization Assay); the in vitro skin sensitization assay based on reconstructed human epidermis” (On-line, August 21st, 2020)

Publications:

The 33rd Annual Meeting of the Japanese Society for Alternatives to Animal Experiments, On-line, Japan, Nov. 2020.

Title of Research:

18_R04-01

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

Principal Investigator:

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Summary of Research:

We developed models to predict species sensitivity distribution (SSD) parameters from limited data (research 1), and quantified the uncertainties associated with SSD-based assessment (research 2). In the research 1, to predict mean and standard deviation (SD) of acute SSDs, we developed multiple linear regression models that included, in addition to readily obtainable descriptors, the mean and SD of the $\log_{10}$-transformed concentrations that are acutely toxic to one algal, one crustacean, and one fish species, as predictors. We also found that the means of chronic SSDs were, one average, 10 times lower than acute SSD means, and the SDs of chronic and acute SSDs were similar. In the research 2, a condition that we need uncertainty factor to determine a predicted no effect concentration (PNEC) by SSD approach was argued.

Timeline:

March 1, 2020 - February 28, 2021

Topics:

Oral presentation at JCIA LRI Annual Workshop 2020 “Exploring roles and simple estimation methods of species sensitivity distribution for deriving PNECs” (On-line, August 21st, 2020)

Publications:

1. Yuichi Iwasaki, Kiyan Sorgog (2021). Estimating species sensitivity distributions on the basis of readily obtainable descriptors and toxicity data for three species of algae, crustaceans, and fish. PeerJ 9: e10981 https://doi.org/10.7717/peerj.10981
2. Kyoshiro Hiki, Yuichi Iwasaki (2020). Can we reasonably predict chronic species sensitivity distributions from acute species sensitivity distributions? Environmental Science & Technology 54(20):13131-13136. doi: 10.1021/acs.est.0c03108.
3. Masashi Kamo, Kiyan Sorgog (2020). Response to: Quantifying the precision of ecological risk: Misunderstandings and errors in the methods for assessment factors versus species sensitivity distributions by Drs. Scott E. Belanger and Gregory J. Carr. Ecotoxicology and Environmental Safety. Vol.207, No. 111542
4. 加茂将史、岩崎雄一、Kiyan Sorgog、内藤航 (2021). 生態リスク評価における種の感受性分布の活用について. 第 55 回日本水環境学会年会. オンライン開催 (in Japanese)
5. 日置恭史郎、岩崎雄一（2021）. 慢性毒性に基づく種の感受性分布を急性毒性に基づく種の感受性分布から予測できるか？日本環境毒性学会第1回オンライン研究発表会、オンライン開催(in Japanese)
6. Kiyan Sorgog, Yuichi Iwasaki (2020). Developing models to estimate parameters of species sensitivity distribution by three species ecotoxicity data. 41st annual meeting on Society of Environmental Toxicology and Chemistry (SETAC) North America. virtual conference.

Title of Research:

18_R05-01

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

Principal Investigator:

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Summary of Research:

The microplastics (MPs) are known to adsorb chemical substances and there is a concern about those 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.

In previous report, the concentration analysis of the PAHs which is adsorbed to MP, and uptaken and bioaccumulated by fish were conducted presuming that comparison of the applied dose and chemical concentration of fish meat calculates the accumulation factor. It was found by analyzing the 13 kinds of PAHs that the amount of chemical substances adsorbed varies depending on the type of MP and environmental conditions, and that the more hydrophobic the substance, the more difficult it is to desorb once it is adsorbed on the MP surface.From the result of examining the uptake of MP, medaka did not affected by MPs of all sizes and Daphnia did not affected by MPs with a diameter of 1 ㎛ or more. By using the fluorescent plastics, the MP beads were observed to accumulate in the gastrointestinal tract but not transferred to the outside of the gastrointestinal tract. Also, no translocation of MP from Daphnia to medaka was observed by feeding the Daphnia which had exposed (and taken) MP, to medaka.

There were difficulties to explain the presence or absence of the vector effect only by analyzing the chemical concentration accumulated in the body, we employed new method to detect whether the toxicity changes under the condition of coexistence of the MP and chemical, and to confirm the vector effect indirectly. As a result, in the closed experimental system, the presence of MP with sizes of uningested by living organisms reduced the concentration of chemical substances by adsorption, and the toxicity was mitigated. In the case of medaka, the toxic effect was mitigated even with the sizes of ingested MP and any harmful effect due to the vector effect were not confirmed. Only Daphnia exhibited adverse effects when the intakes were high, but it was due to intestinal obstruction by MP.

In conclusion, the vector effect of MP exists in the theory, though it is estimated that the amount of MP in the environment has no harmfulness in reality.

Timeline:

March 1, 2020 - February 28, 2021

Topics:

Oral presentation at JCIA LRI Annual Workshop 2020 “Study on the contribution of microplastics to bioaccumulation and biological magnification towards fish” (On-line, August 21st, 2020)

Publications:

1. Norihisa Tatarazako, ”Ecotoxicity of microplastics”, Society of Environmental Hormone,News Leter 23-2（2020）

2. Norihisa Tatarazako, ”Ecotoxicity of microplastics”, Microfiber lecture（Japan Chemical Fiber Association）, on web, 27/11/2020
3. Norihisa Tatarazako, Yukiyo Okazaki, Ecotoxicity of microplastics, The 22nd UK-Japan Annual Scientific Workshop,Research into Environmental Endocrine Disrupting Chemicals & Chemicals of Emerging Concern, on web, 27/10/2020

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:

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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 year, we selected 9 hepatotoxins and 10 non-hepatotoxins as negative control for SVM. 1) We determined IC10 concentration for all chemicals and obtained gene expression data from 19 chemical exposure samples. As a results, 2) we can predict at more than 90 % accuracy.

Timeline:

March 1, 2020 - February 28, 2021

Topics:

Oral presentation at JCIA LRI Annual Workshop 2020 “Development of rapid, accurate, and low-cost AI drug hazard assessment method by human stem cell test” (On-line, August 21st, 2020)

Publications:

Panina, Y., Yamane, J., Kobayashi, K., Sone, H., Fujibuchi, W. 2021. Human ES and iPS cells display less drug resistance than differentiated cells, and naïve-state induction further decreases drug resistance. J. Toxicol. Sci. 46 (3), pp. 131-142.

Title of Research:

19_R03-01-2

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

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Summary of Research:

Several in vitro assays to predict the sensitizing potential of chemicals have been developed so far. 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 peripheral 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 mRNA expression of key costimulatory molecule OX40 ligand (OX40L), which is important for Th2 differentiation, in DCs (Mizoguchi et al. Front Immunol. 2017). In this project, we have been further 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 DC progenitor cell lines and T cell lines to apply for it.

In the 8th term, as a DC progenitor cell line, we eventually generated monocytic cell lines by introducing genes related to cell survival and cell cycle into peripheral $CD14^+$ monocytes. When these cell lines were applied for the 3D DC coculture system, the similar more enhanced mRNA expression of OX40L was observed by respiratory sensitizers than skin sensitizers. When these cell lines were then applied for the 3D DC/T coculture system using primary $CD4^+$T cells, the similar more enhanced mRNA expression of IL-4 was also observed by respiratory sensitizers. As the possible molecular mechanism to enhance IL-4 mRNA expression, augmented expression of the transcription factor GATA-3, that is important for Th2 differentiation, was also observed by respiratory sensitizers. Furthermore, we have generated allogenic Th1 and Th2 cells and syngeneic cedar pollen antigen (Cryj1)-specific Th2 cells, and are currently cloning them.

Timeline:

March 1, 2020 - February 28, 2021

Topics:

Oral presentation at JCIA LRI Annual Workshop 2020 “Development of a novel alternative method for evaluation of sensitizing potential and allergenicity by measuring human T cell activation and differentiation (Key event 4)” (On-line, August 21st, 2020)

Publications:

Yoshimoto T. Expectations for immune on-chip. Symposium on New Technology for Cell-based Drug Assay (Web meeting, Jan. 26, 2021)

Title of Research:

19_R05-01

Establishment of medaka kinetic model for aged microplastic and adsorbed chemical

Principal Investigator:

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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 (microscopic plastic MP, < 5mm) were emerged. Effect of MP and its vector effect with toxicants are of concerns. Many researchers have studied these topics. However, most of study were used pristine MPs. Thus, risk analysis of aged MP is required.

In this study, three kinds of aged plastics (sphere polyethylene (PE), grind PE, grind polystyrene (PS)) were prepared by exposure to ultraviolet for 180 and 460 hours. Changes on chemical characters were detected by aging. Decrease in sorption of anthracene on aged MPs were observed, especially aged grind PS showed 1/8 of sorption constant (ke) compared with that of pristine grind PS. Bioaccumulation studies were performed in medaka mix-exposed with anthracene and MPs, respectively. As results, no effect was observed in maximum concentration of anthracene in medaka body among aging, quality or shape of MPs. However, anthracene was detected in grind-aged PE and PS even at depuration periods. These results suggested that small particle of PE or PS in grind MPs may remained inside of body after depuration period. Further study on another type of aged MP is required.

Timeline:

March 1, 2020 - February 28, 2021

Topics:

Oral presentation at JCIA LRI Annual Workshop 2020 “Establishment of medaka kinetic model for aged microplastic and adsorbed chemical” (On-line, August 21st, 2020)

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:

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Summary of Research:

The following conclusions were obtained with using various methods to clarify the mechanisms by which secondary microplastics are formed from the viewpoint of polymer science and polymer engineering.

In the Py-APGC-MS measurement of plastics, by focusing on fragment ions characteristic of plastics and recording extracted ion chromatograms, we succeeded in extracting individual component information from plastic mixture samples of PP, PE, PVC, PS, and PET. We also succeeded in detecting PS taken up by Daphnia magna (adult).

As a result of comparative study of seawater degradation and photo-degradation of various plastics, it was found that the presence of seawater suppressed the progression of degradation of HDPE, PP, and PET by inhibiting the increase in sample temperature and oxygen diffusion, while the elongation at break of PA66 decreased significantly with irradiation time in seawater degradation. However, PA66 showed a significant decrease in elongation at break with irradiation time, suggesting that seawater was the main factor in the degradation.

In the case of PP, the surface of the micro-cut powder showed a brittle fracture surface and the "crack pattern" grew inside, suggesting that MP was generated from the consolidated growth of cracks, similar to the outdoor exposure. Crack patterns were also observed in the river MPs after pulverization by glass beads, but no brittle fracture surfaces were observed in most of the leaking marine MPs, suggesting that the MPs have smooth surfaces and are less prone to collapse-type weathering mechanisms.

On the other hand, the PET bottles recovered from the river retained their shape despite being left in the environment for nearly 20 years. The outer surface of the PET bottles showed no signs of becoming microplastics, although some degradation such as a decrease in molecular weight was observed.

Finally, it was confirmed that all types of plastics, including elastomers, could be miniaturized to a spherical shape of less than 1 mm in a short time by treatment with a new type of jet mill device with multiple blades arranged in an air flow channel and additional treatment with a regular jet mill. In addition, it was confirmed that the bead-mill treatment efficiently progressed the plastic refinement in a water atmosphere.

Timeline:

March 1, 2020 - February 28, 2021.

Topics:

Oral presentation at JCIA LRI Annual Workshop 2020 “Elucidation of the formation mechanism of microplastics” (On-line, August 21st, 2020)

Publications: Presentations: (Only the PI's presentation)

1. Shinichi Kuroda, "Elucidation of the formation mechanism of microplastics," 69th Symposium on Macromolecules, September 16, 2020 (online)

2. Shinichi Kuroda, "Elucidation of the formation mechanism of microplastics," MICRO 2020, November 25, 2020 (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:

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Summary of Research:

This study proposes an adverse outcome pathway (AOP) in which the molecular initiating event (MIE) is the binding of compounds to glutamate receptors and the key event (KE) is drebrin loss. The adverse event (AO) is the learning and memory impairment caused by morphological changes in dendritic spines. Drebrin is an actin-binding protein that governs dendritic spine formation of CNS neurons and is responsible for the morphological plasticity of dendritic spines associated with learning and memory. The subcellular localization of drebrin is determined by glutamate receptor activity, and when drebrin is lost, the learning and memory mechanism does not function properly. In this study, we have established an experimental system using frozen hippocampal neurons prepared from rat embryo. We will construct an in vitro method to evaluate compounds that cause learning and memory impairment, and will replace animal experiments for neurotoxicity. We have developed an image processing algorithm for quantitative analysis of neuron count, dendrite length, and drebrin clusters from high-content image data using a confocal image cytometer. In particular, the brightness distribution analysis of drebrin clusters is highly sensitive. We have started to develop a machine learning platform for AI. From the images of immunocytochemical staining, we will clarify the indices for quantitatively evaluating the structural changes of neurons, and provide SOPs for culture techniques and analysis methods. In the future, we are planning to build an experimental system using neurons derived from human iPS cells.

Timeline:

March 1, 2020 - February 28, 2021

Topics:

Oral presentation at JCIA LRI Annual Workshop 2020 “Proposal of a new AOP for the neurotoxicity and developmental neurotoxicity assessment of glutamate receptor binding agonists that cause learning and memory impairment.” (On-line, August 21st, 2020)

Publications:

1. Shogo Mase, Yuko Sekino, Tomoaki Shirao, Toshinari Mitsuoka, “Optimization of algorithms to quantify changes in drebrin distribution induced by glutamate stimulation of cultured neurons for a confocal quantitative image cytometer.” The 63rd Annual Meeting of the Japanese Society for Neurochemistry, Web, September, 2020

2. Toshinari Mitsuoka, Shogo Mase, Noriko Koganezawa, Yuichi Kato, Tomoaki Shirao, Yuko Sekino “Assessment of CB agonist CP55940 in maturity for rat hippocampal neurons using a high-throughput immunocytochemical assay and image digital analysis.” The 94th Annual Meeting of the Japanese Pharmacological Society, Web＋On Site, Sapporo, March 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.

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Summary of Research:

While thyroid hormones (THs) are essential for brain development, effects of mild suppression of maternal blood THs by hepatic enzyme inducers on the infant brain developments is not fully understood. Conducting guideline study to identify of developmental neurotoxicity induced by chemicals requires significant resources (animals, time and costs). A simple screening test for investigating whether maternal chemical exposure reduces brain THs in fetal and neonatal rats would be valuable. To verify reliability of the Comparative Thyroid Assay with additional examination of the brain, THs and histology, propylthiouracil (PTU, 10 ppm) and phenobarbital (PB, 1000 ppm) were dosed by feeding. Clearly suppressed brain THs in rat fetuses and pups and brain abnormality (heterotopia) in pups were noted with PTU but not with PB. Reproducibility and effects of PB at higher dose level will be examined in further study.

Timeline:

March 1, 2020 – February 28, 2021.

Topics:

Oral presentation at JCIA LRI Annual Workshop 2020 “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 21st, 2020)

Publications:

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], 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 2021.

2. 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 2021.

Title of Research:

20-3-06

Evaluation methods for toxicity using indices of developing neurons

Principal Investigator:

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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.

First, we established culture conditions for primary cultured neurons of the rat cerebral cortex and examined changes in mRNA expression of 12 key molecules which play an important role in neurodevelopment until days in vitro 21. The results showed three major patterns: genes whose expression was low in early culture and increased with culture, genes whose expression was maximum in mid-culture, and genes whose expression was maximum in early culture and decreased with culture. Next, we used methylmercury, a known developmental neurotoxin, to investigate the effects of these molecules on gene expression. We found that methylmercury suppressed the expression of three genes, *Dlg4*, *Syp* and *Bdnf*. In the next year, we will investigate whether these gene expression changes are also observed with other developmental neurotoxicants and whether these phenomena are reproduced in human iPS neurons, which will provide clues for the development of evaluation indices for developmental neurotoxicity.

Timeline:

March 1, 2020 - February 28, 2021

Topics:

Oral presentation at JCIA LRI Annual Workshop 2020 “Evaluation methods for toxicity using indices of developing neurons” (On-line, August 21st, 2020)

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:

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Collaborators:

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

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 the developmental neurotoxicity 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 macrophages resident in the central nervous system, 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 developmental neurotoxicity of chemicals focusing on neuroinflammation through microglia and the differentiation of neural stem cells into neurons and astrocytes. In 2020, we were able to produce a one-color zebrafish that selectively expressed a fluorescent protein mVenus in macrophages/microglia. We also produced two-color zebrafish that expressed fluorescent proteins Cerulean and mCherry in neurons and astrocytes, respectively. In addition, we decided the optimal concentrations of valproic acid, chlorpyriphos, ethinylestradiol, and ethanol, which are positive chemical substances that induce neuroinflammation, to assess the developmental neurotoxicity related to the neuroinflammation in zebrafish. In 2021, we will try to produce a three-color zebrafish that expresses mVenus, Cerulean, and mCherry in macrophages/microglia, neuron, and astrocytes, respectively.

Timeline:

March 1, 2020 - February 28, 2021

Topics:

Oral presentation at JCIA LRI Annual Workshop 2020 “Development of a novel test for the assessment of neuroinflammation useful to elucidate adverse outcome pathways in developmental neurotoxicity” (On-line, August 21st, 2020)

Publications:

Wakai E, Suzumura Y, Ikemura K, Mizuno T, Watanabe M, Takeuchi K, Nishimura Y: An Integrated In Silico and In Vivo Approach to Identify Protective Effects of Palonosetron in Cisplatin-Induced Nephrotoxicity. *Pharmaceuticals* 2020, 13(12):480.

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:

In humans, environmental chemicals that induce hypothyroidism can be a significant risk during pregnancy, since fetal brain development in early pregnancy depends on thyroid hormones supplied by the mother. However, the detailed causal relationship between abnormalities in thyroid function during pregnancy and adverse effects on the development of the baby's brain is unknown. 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 differentiation marker promoter. In this fiscal year, we verified the usefulness of Tg mice by characterizing the expression of reporter genes in Tg mice. Among the three lines obtained, the highest Luc2 activity was observed in the mature males and females of line #1 at the sites responsible for higher brain functions, including the cerebral cortex. Although, in males, Luc2 activity was also observed in the testes as in brain tissue, Luc2 activity was rarely detected in other organs. In the brain, Luc2 expression peaked immediately after birth and dropped sharply as the age progressed. When the brain slices were X-gal stained to confirm the expression of the LacZ gene, lacZ also showed the same expression pattern as Luc2. These results indicate that tracing luc2 and LacZ expression may provide some understanding of neuronal differentiation. In the future, *in vivo* imaging immediately after birth of this Tg mouse will be performed to verify the usefulness of this mouse, and the adverse effects induced during hypothyroidism during pregnancy will be examined by tracing the expression fluctuations of the reporter genes.

Timeline:

April 1, 2020 – March 31, 2021

Topics:

Oral presentation at JCIA LRI Annual Workshop 2020 “Establishment of an adverse outcome pathway for the evaluation of developmental neurotoxicity in chemical-induced hypothyroidism” (On-line, August 21st, 2020)

Publications:

Ishida K, Minamigawa Y, Mori K, Matsumaru D, Nakanishi T, Establishment of neuronal differentiation tracer mouse for evaluation of developmental neurotoxicity, The 141st Annual Meeting of the Pharmaceutical Society of Japan (Hiroshima), 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:

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Collaborators:

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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 FY2020, we have reviewed a couple of assessment documents published by international organizations and the latest literature, and presentation materials found in academic conferences (SETAC Europe, SETAC North America, Water Environment Society of Japan). We have exchanged opinions with MPs experts to understand current research challenges. After reviews and discussions among co-researchers, current problems and challenges in conducting environmental risk assessment of MPs were identified under six categories, i.e., MPs in the ocean, emission estimates, exposure analysis, analysis and measurement methods of MPs, hazard assessment, and environmental risk assessment case studies and critical reviews. The key elements identified include lack of knowledge on the origin and formation mechanism of MPs in the ocean, determination and validation of emission factors necessary for estimating environmental emissions of various MPs, characterization of MP types, sizes, shapes, lack of information on vertical distribution of MPs in water and changes in density of MPs, lack of standard measurement methods for MPs, and lack of ecological relevance between toxicity tests and the real environment, and the need to clarify the purpose and target of the assessment. In addition, a draft framework for the environmental risk assessment of MPs was developed in order to propose a conceptual model that shows specific procedures for risk assessment considering the characteristics of MPs. In the next fiscal year, while reviewing the latest findings that will contribute to the environmental risk assessment of MPs, we will examine the methodology at each stage of the risk assessment, determine the important parameters, and start the trial risk assessment for Tokyo Bay.

Timeline:

March 1, 2020 - February 28, 2021

Topics:

Oral presentation at JCIA LRI Annual Workshop 2020 “Development of a conceptual model for environmental risk assessment of microplastics and a trial risk assessment in Tokyo Bay” (On-line, August 21st, 2020)

Publications:

Wataru Naito (2020) Challenges to Environmental Risk Assessment of Marine Plastics and Microplastics. BIOINDUSTRY 37(9): 40-49 (In Japanese)

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