LRI

Long-range Research Initiative

Annual Report 2025

2025

Japan Chemical Industry Association

Annual Report 2025

Reports of the 14th period researches

LRI
Long-Range
Research Initiative

Title of Research: ***23-1-03***

Development of an alternative method for teratogenicity using zebrafish

Principal Investigator:

Hiromi Hirata, PhD (Professor, Department of Chemistry and Biological Science, College of Science and Engineering, Aoyama Gakuin University)
5-10-1 Fuchinobe, Sagamihara 252-5258, Japan
(tel) 042-759-6235 (email) hihirata@chem.aoyama.ac.jp

Collaborators:

Chitose Taya (Research Associate, Aoyama Gakuin University)
Kota Ujibe (Graduate Student, Aoyama Gakuin University)
Kenichiro Sadamitsu (Graduate Student, Aoyama Gakuin University)
Makoto Kashima (Lecturer, Toho University)
Junichi Tasaki (Kao Corporation)
Shujie Liu (Kao Corporation)

Summary of Research:

The development of alternatives to animal testing based on animal welfare principles is an urgent global priority. Zebrafish embryos before the onset of feeding are promising models because they fall outside many regulatory restrictions while retaining developmental mechanisms conserved with humans. This study established a reproducible teratogenicity testing platform using zebrafish embryos. A simplified exposure protocol, including embryo selection at 5 hours post-fertilization and a 24-well plate assay without medium replacement, reduced natural mortality and enabled stable results. Comparative genomic and transcriptomic analyses across multiple strains showed that strain-dependent differences in teratogenic sensitivity were minimal. Single-embryo RNA sequencing revealed early transcriptomic responses before visible malformations, identifying hypoxia and oxidative stress as key events in developmental toxicity.

Timeline:

March 2025 – February 2026

Topics:

Presented at the LRI annual research meeting of JCIA. August 29, 2025.

Publications:

1. Sadamitsu, K., Velilla, F., Shinya, M., Kashima, M., Imai, Y., Kawasaki, T., Watai, K., Hosaka, M., Hirata, H. and Sakai, N. (2024) Establishment of a zebrafish inbred strain, M-AB, capable of regular breeding and genetic manipulation. Sci. Rep. 14(1): 7455.
2. Mori, K., Aoki, Y., Mikashima, F., Maki, K., Tanaka, T., Hayashi, M., Sugimoto, W., Ono, M., Umekita, S., Niino, T., Fujiwara, M., Ebata, T., Hirata, H. and Kojima, H. (2024) Validation of a new protocol for a zebrafish MEFL (malformation or embryo-fetal lethality) test method that conforms to the ICH S5 (R3) guideline. J. Toxicol. Sci. 49(8): 337-348.
3. Sadamitsu, K., Kashima, M., Wada, S., Ishioka, A., Nakayama, S., Nakayama, R., Okamoto, H. and Hirata, H. (2025) Establishment and genetic characterization of zebrafish RW line. Sci. Rep. 15: 14512.
4. Mori, K., Aoki, Y., Hayashi, M., Sugimoto, W., Ono, M., Umekita, S., Niino, T., Ebata, T., Mikashima, F., Maki, K., Tanaka, T., Hirata, H. and Kojima, H. (2025) Variation and classification of chemically-induced zebrafish malformations for the ICH S5 (R3) guideline: an atlas for zebrafish teratogenesis. J. Toxicol. Sci. 50(8): 431-444.
5. Taya, C., Ujibe, K., Shimodaira, S., Sakamoto, A., Wada, S., Kashima, H. and Hirata, H. (2025) Comparative analysis of teratogen-induced malformations and gene expression across zebrafish strains in early development. Toxicol. Rep. 15: 102117.

Title of Research: **25-4-06**

Characterization of Microplastic Effects on Aquatic Organisms and Proposal of New Guidelines for Ecotoxicity Testing

Principal Investigator:

Norihisa Tatarazako Professor
Department of Environmental Science and Technology
Graduate School of Agriculture, Ehime University

3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan
Tel: +81-89-946-9902 (Direct) / Ext. 389
E-mail: tatarazako.norihisa.wn@ehime-u.ac.jp

Collaborators:

Yukiyo Okazaki Research Fellow
Department of Environmental Science and Technology
Graduate School of Agriculture, Ehime University

Summary of Research:

Microplastics (MPs) have been detected in aquatic environments, the atmosphere, and living organisms, raising concerns about their ecological impacts. However, current ecotoxicity tests were designed mainly for water-soluble chemicals and are not well suited for particulate materials such as MPs. In particular, testing methods that consider particle-specific properties—including particle size, surface characteristics, sedimentation behavior, and ingestion dynamics—are still insufficiently developed.

In this study, potential physical effects of MPs and the hypothesized mechanism of nutritional inhibition due to gastrointestinal blockage were examined using Daphnia magna. Polystyrene particles with diameters of 31.4, 10.5, 3, and 1 μm were tested. A modified extended acute toxicity test was conducted: the first 48 h followed OECD Test Guideline 202 under non-feeding conditions, after which algae were supplied and observations continued to 96 h. Toxicity of the supernatant from centrifuged commercial MP suspensions was also evaluated.

No significant effects were observed for 31.4 μm and 10.5 μm particles, whereas increased mortality occurred for 3 μm and 1 μm particles after 48 h despite feeding. Microscopy showed accumulation of small particles in the digestive tract, although MPs were also excreted, indicating that complete intestinal blockage did not occur. The results suggest that the observed effects are more likely caused by competition between food particles and MPs during ingestion, leading to reduced nutritional uptake. Adhesion of 1 μm particles to the body surface was also observed, suggesting possible physical interference with body surfaces or gill structures.

Strong acute toxicity was detected in the supernatant of commercial MP suspensions within 48 h, indicating that additives such as dispersants or preservatives may contribute to the observed toxicity. These findings suggest that studies using untreated MP suspensions may confound additive toxicity with the intrinsic effects of MPs.

Overall, MP toxicity assessment should consider both particle properties and additives in test materials. The results indicate that MP effects are better explained by nutritional inhibition due to competition with food particles rather than intestinal blockage.

Timeline:

March 2025 – February 2026

Topics:

Presented at the LRI annual research meeting of JCIA. August 29, 2025.
”

Publications:

Society of Environmental Toxicology Asia Pacific (SETAC Asia-Pacific)
September 2026 (planned)

Title of Research: ***25-5-04***

Development of a comprehensive risk screening method for substances in recycled-plastic materials using quantitative non-target analysis

Principal Investigator:

Masahiro Tokumura, PhD (Assistant Professor, University of Shizuoka)
52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
(tel)+81-054-264-5783, (e-mail) tokumura@u-shizuoka-ken.ac.jp

Collaborators:

Takeshi Enomoto, PhD (Visiting Research Fellow, University of Shizuoka)
52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
(tel)+81-054-264-5783, (e-mail) gp2201@u-shizuoka-ken.ac.jp

Summary of Research:

From the perspective of the circular economy, the use of recycled plastics is expected to increase rapidly in the coming years. Recycled plastics may contain a wide variety of chemical substances, including additives originating from the raw materials as well as unintentionally formed by-products generated during recycling processes. In the case of post-consumer materials, it is also expected that unpredictable chemicals may be present. Non-targeted analysis is a powerful approach for comprehensively detecting chemical substances. However, quantitative evaluation of identified compounds generally requires the development of individual analytical methods for each compound, similar to conventional targeted analysis. Method development typically requires authentic standards, but it is unlikely that standards are commercially available for all substances detected by non-targeted analysis, particularly for unintentionally generated by-products. In addition, developing analytical methods for each compound individually is difficult from both time and economic perspectives. In this study, a comprehensive risk screening method for chemical substances derived from recycled plastics is developed by combining quantitative non-targeted analysis (qNTA) using post-column reaction gas chromatography with dual detection by mass spectrometry and flame ionization detection (GC-MS/PR-FID) with toxicity prediction approaches.

Timeline:

March 2025 – February 2026

Topics:

Presented at the LRI annual research meeting of JCIA. August 29, 2025.

Publications:

Sota Sakai, Masahiro Tokumura, Takeshi Enomoto, Kouichi Tatsu, Naohide Shinohara, Takeshi Fujimoto, Noe Koto, Satomi Morizumi, Masakazu Makino, “Development of a Risk Screening Method for Chemical Substances in Recycled Plastic Feedstock Based on qNTA Using Waste Fishing Nets as a Case Study,” 2025 Annual Meeting of the Society of Indoor Environment, Kokura, Japan, December 2025【Recipient of the Conference Chair’s Encouragement Award】

Sota Sakai, Masahiro Tokumura, Takeshi Enomoto, Kouichi Tatsu, Naohide Shinohara, Takeshi Fujimoto, Noe Koto, Satomi Morizumi, Masakazu Makino, “Development of a Quality Evaluation Scheme for Recycled Plastic Feedstock Toward a Safe and Secure Circular Economy,” 2025 Annual Meeting of the Society for Environmental Science, Hiroshima, Japan, September 2025.

Masahiro Tokumura, Sota Sakai, Takeshi Enomoto, Kouichi Tatsu, Naohide Shinohara, Masakazu Makino, “Environmental Risk Screening of Recycled Plastics Using a Quantitative Non-Targeted Analytical Approach”, 12th IWA International Symposium on Waste Management Problems in Agro-Industries—AGRO'2025, Istanbul, Turkey, September 2025.

Sota Sakai, Masahiro Tokumura, Takeshi Enomoto, Kouichi Tatsu, Naohide Shinohara, Takeshi Fujimoto, Noe Koto, Masakazu Makino, “Quality Evaluation Scheme for Fishing Nets Toward Plastic Recycling Based on qNTA,” The 4th Joint Conference on Environmental Chemical Substances, Yamagata, Japan, July 2025【Recipient of the American Chemical Society Award】

Title of Research: *23-5-06*

Ecotoxicological risk assessment of microplastics -as a model case of Osaka Bay-

Principal Investigator:
Yoshifumi Horie, PhD (Professor), Kobe University, Research Center for Inland Seas
5-1-1 Fukaeminami-machi, Higashinada-ku, Kobe 658-0022, JAPAN

Collaborators:
Hideo Okamura (Professor), Research Center for Inland Seas, Kobe University
Gomez Christopher (Professor), Graduate School of Maritime Sciences, Kobe University
Akira Ijiri (Professor), Graduate School of Maritime Sciences, Kobe University
Kazuyo Yamaji (Professor), Graduate School of Maritime Sciences, Kobe University
5-1-1 Fukaeminami-machi, Higashinada-ku, Kobe 658-0022, JAPAN

Summary of Research:
In the third year of this study, we focused on polypropylene particles, which were the most frequently detected microplastics in the surface waters of Osaka Bay during the first year of investigation. Virgin polypropylene particles were continuously exposed to medaka (*Oryzias latipes*) over a long period in order to evaluate their potential toxicity.

The exposure period lasted a total of 19 weeks and included three generations: the F0 generation (from 3 months after hatching for 3 weeks), the F1 generation (from immediately after fertilization for 14 weeks), and the F2 generation (from fertilization to hatching for 2 weeks). Continuous exposure was conducted throughout these generations. Seven endpoints were examined: (1) embryonic development, (2) growth, (3) secondary sexual characteristics, (4) histological observation of the gonads, (5) microplastic residues in the digestive tract, (6) reproduction, and (7) survival. The results showed no statistically significant differences between the exposure group and the control group for embryonic development, growth, secondary sexual characteristics, gonadal histology, reproduction, or survival. Even under long-term exposure to polypropylene particles at concentrations much higher than those found in the natural environment, no clear adverse effects were observed. These findings suggest that the toxicity of microplastics as particles alone is limited.

In addition, environmental samples were analyzed using pyrolysis gas chromatography–mass spectrometry (Py-GC/MS). The results revealed the presence of multiple types of microplastics in both sediments and surface waters in the coastal area of Osaka Bay. In the sediment samples, the polymer composition varied considerably among sampling locations. Only polyvinyl chloride (PVC) was detected around Wada Misaki, whereas polypropylene (PP) and PVC were detected around Tsubasa Port and Iwaya Port. In particular, relatively high concentrations of PVC, PP, and polyethylene terephthalate (PET) were detected around Iwaya Port. These results suggest that even polymers with a specific gravity lower than 1 may sink and accumulate in sediments due to processes such as the formation of attached materials and particle aggregation. In the surface water samples, several polymers including PP, PVC, polystyrene (PS), polymethyl methacrylate (PMMA), polyethylene (PE), and acrylonitrile butadiene styrene (ABS) were detected. Consistent with the results obtained in the first year using micro-FTIR analysis, PP was detected at many sampling locations.

Timeline:
March 2025 – February 2026

Topics:
Presented at the LRI annual research meeting of JCIA. August 29, 2025.
October 15-17, 2025 Presented at MARII Summit ”Ecotoxicity of Microplastics Using Fish”

Publications:

Title of Research: 23-5-08

Assessing sources, emissions and environmental risk of microplastics in support of effective risk reduction strategies

Principal Investigator:

Wataru Naito (National Institute of Advanced Industrial Science and Technology (AIST), Integrated Research Center for Nature Positive Technology (NPRC))

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), Yuichi Iwasaki (AIST, NPRC), Yuriko Ishikawa (AIST, RISS), Yutaka Kameda (Chiba Institute of Technology)

Summary of Research:

The objective of this research project is to facilitate realistic and effective risk management strategies against microplastic (MP) pollution by analyzing the load and sources of MP, and conducting practical risk assessments focused on Tokyo Bay. Specifically, leveraging material flow analysis and precise field data, our goal is to quantitatively assess the sources of MP pollution in marine environments and their respective contributions. Additionally, we aim to quantify the temporal changes in MP-related environmental risks and the efficacy of various mitigation measures. Moreover, we propose an environmental risk assessment methodology tailored to the unique characteristics of MP, drawing from practical case studies in Tokyo Bay and the latest insights from both domestic and international sources.

Timeline:

March 2025 – February 2026

Topics:

Presented at the LRI annual research meeting of JCIA. August 29, 2025.

Publications:

I Ueda K, Kameda Y, Fujita E, Fujimura G, Fukazawa E, Numazawa Y, Iwasaki Y, Naito W. (2025). Size-specific distribution, morphology, and polymer composition of microplastic particles in surface water and sediments of Tokyo Bay. *Microplastics and Nanoplastics*, 5(1), 47.
https://doi.org/10.1186/s43591-025-00168-z

Iwasaki Y, Takeshita K, Ueda K, Naito W. Bayesian species sensitivity distribution modeling for microplastic particles: integrating particle characteristics and intra-species variation. *Environmental Toxicology and Chemistry (ET&C)*, Accepted (10 March, 2026)

Naito W, Ueda K, Iwasaki Y. Ueda K., Comparison of species sensitivity distribution modeling approaches for assessing risk of microplastic particles using monitoring data from Tokyo Bay. SETAC Europe 35th Annual Meeting, 11-15 May 2025, Vienna, Austria

Title of Research:23-6-01

Development of risk assessment method based on the concept of the bioavailability with a model predicting the toxicities for difficult-to-test substances.

Principal Investigator:

Masashi Kamo, Dr. of Science (National institute of advanced industrial science and technology (AIST), Research center for nature positive technology)
16-1 Onogawa, Tsukuba, Ibaraki, 305-8569, Japan
(tel)+81-80-2191-6656, (e-mail) masashi-kamo@aist.go.jp

Collaborators:

Yuriko Ishikawa[1)], Hiroyuki Mano[1)], Akifumi Ogawa[2)], Tetsuro Okamura[2)], Atsushi Sawai[2)], Kasumi Yamaguchi[2)], Jun Yamamoto[2)]
1) AIST, Research institute of science for safety and sustainability
2) IDEA Consultant, Inc. Institute of Environmental Ecology

Summary of Research:

Chemical risk assessments in Japan are conducted under the Chemical Substances Control Law (CSCL), yet progress remains limited for substances categorized as difficult-to-evaluate chemicals. This study developed an ecological risk assessment approach using monoalkyl cation C16 (HTAC), a cationic surfactant designated as a CSCL Priority Assessment Chemical. HTAC toxicity is known to decrease with increasing dissolved organic carbon (DOC), and discrepancies frequently arise between nominal and measured concentrations, hindering reliable exposure assessment. Algal growth inhibition tests were conducted at DOC concentrations of 0, 0.5, 2.5, and 5 mg/L. The resulting no-observed-effect concentrations (NOECs) based on nominal concentrations were 5, 55, 63, and 130 µg/L, respectively, confirming reduced toxicity with higher DOC. Time-course measurements revealed that the decline in measured concentration occurred predominantly within the first hour, reaching near-equilibrium far more rapidly than previously assumed. Additional decreases commonly observed at test termination were attributed to centrifugation during algal removal rather than to adsorption losses. These findings suggest that the 72-hour concentration measured without centrifugation is the most appropriate indicator of exposure. Using these data, we constructed a mathematical model to predict toxicity across varying DOC levels. A preliminary risk assessment combining the model with AIST-SHANEL exposure estimates indicated that ecological risk of HTAC is likely low. The applicability of this method to other difficult-to-evaluate substances is also discussed.

Timeline:

March 2025 – February 2026

Topics:

Presented at the LRI annual research meeting of JCIA. August 29, 2025.

Publications:

Masashi Kamo, Hiroyuki Mano, Tetsuro Okamura, Jun Yamamoto, Kasumi Yamaguchi, Akifumi Ogawa, Atsushi Sawai. “Toxicity of Cationic Surfactants Varies with Water Quality: Its Verification and Predictive Modeling”. 4th Joint Conference on Environmental Chemicals. July 15th, 2025, Yamagata

Title of Research:23-6-03

A Validation Study for approval of AOP475 that proposes a New Approach Method for OECDTG on Neurotoxicity and Developmental Neurotoxicity

Principal Investigator:

Yuko Sekino, PhD (Project Professor, Grad Sch Agri and life Sci, Univ of Tokyo) 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan (tel)+81-3-5841-5390, (e-mail) yukos@g.ecc.u-tokyo.ac.jp

Collaborators:

Izuo Tsutsui, PhD (Researcher, Grad Sch Agricultural and Life Sciences, The University of Tokyo) 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan (tel)+81-3-5841-5390, (e-mail) iztsutsui@g.ecc.u-tokyo.ac.jp

Yonehiro Kanemura, MD, PhD (Director, Dep. Biomedical Research and Innovation Institute for Clinical Research, NHO 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

Shihori Tanabe, PhD (Senior Researcher, Div. Risk Assessment, National Institute of Health Sciences) 3-25-26 Tonomachi, Kawasaki-ku, Kawasaki City, Kanagawa, 210-9501, Japan (tel)+81-44-270-6600, (e-mail) stanabe@nihs.go.jp

Sachiko Yoshida, PhD (Graduate School of Engineering, Toyohashi University of Technology) 1-1 Hibarigaoka, Tenpaku-cho, Toyohasi-city, Aichi, 441-8580, Japan (tel) +81-532-81-5106, (e-mail) syoshida@tut.jp

Satoshi Yokota, PhD (Head of Laboratory, Div. Cellular and Molecular Toxicology, National Institute of Health Sciences) 3-25-26 Tonomachi, Kawasaki-ku, Kawasaki City, Kanagawa, 210-9501, Japan (tel)+81-44-270-6600, (e-mail) stanabe@nihs.go.jp

Summary of Research:

The aim of this research is to further validate and finalize the AOPwiki entry for AOP475 proposed to the OECD. During this term, we continued strengthening the scientific basis of the Key Events (KEs) and Key Event Relationships (KERs) through literature analysis and experimental validation. In addition to the previously described events, we incorporated new KEs including **Loss of drebrin** and **dendritic spine abnormality**, which describe a pathway leading to impairment of learning and memory without neuronal cell death. Experiments using frozen rat hippocampal neurons for primary culture were conducted to examine the relationship between receptor activation, intracellular calcium signaling, and drebrin loss. Activation of NMDA receptors and kainate receptors induced a reduction of drebrin clusters in dendritic spines, even under conditions that did not necessarily cause neuronal cell death. Furthermore, experiments using a calcium ionophore demonstrated that intracellular calcium overload can trigger drebrin loss independently of calpain-mediated neuronal death. In addition, a clinical study using the ELISA-based drebrin quantification method (Syncheck™) revealed that drebrin degradation products in human plasma are significantly decreased in patients with mild cognitive impairment. These findings support the human relevance of AOP475 and suggest that changes in drebrin localization and expression can serve as useful indicators for the risk assessment of compounds affecting learning and memory.

Timeline:

March 2025 – February 2026

Topics:

Presented at the LRI annual research meeting of JCIA. August 29, 2025.
Three at Society for Neurochemistry (11th-12th Sept.2025),
One at Society for Regulatory Science (5th-6th Oct.2025)
Press release on the web site of Univ. Tokyo on 7th Jan. in 2026

Publications:

[Original paper] Shoji M, Kawarabayashi T, Nakamura T, Sugawara T, Ishizawa K, Amari M, Takahashi R, Kasahara H, Koganezawa N, Higa A, Takatama M, Ikeda Y, Sekino Y, Shirao T. Drebrin is a novel biomarker of cognitive deterioration in Alzheimer's disease. J Alzheimers Dis. 2026 Feb;109(4):1824-1833.

Title of Research: ***24-5-08***

Study on Risk-Based Grading of Recycled Plastic and its Application for Enhancing Plastic Recycling

Principal Investigator:

Ono Kyoko Ph.D. (Duputy Director, Integrated Research Center for Circular Technology, National Institute of Advanced Industrial Science and Technology (AIST))
16-1 Onogawa, Tsukuba, Ibaraki, 305-8569, JAPAN
(tel) +81-50-3522-5897, (e-mail) kyoko.ono@aist.go.jp

Collaborators:

Masashi Gamo (Research Institute of Science for Safety and Sustainability (RISS), Hitachi-AIST CE Laboratory, National Institute of Advanced Industrial Science and Technology (AIST)) masashi-gamo@aist.go.jp

Isamu Ogura (Research Institute of Science for Safety and Sustainability (RISS), National Institute of Advanced Industrial Science and Technology (AIST)) i-ogura@aist.go.jp

Naohide Shinohara (Research Institute of Science for Safety and Sustainability (RISS), National Institute of Advanced Industrial Science and Technology (AIST)) n-shinohara@aist.go.jp

Naoya Kojima (Integrated Research Center for Circular Technology, National Institute of Advanced Industrial Science and Technology (AIST)) kojima-naoya@aist.go.jp

Summary of Research:

Promoting plastic recycling is an increasingly important issue from the perspective of resource recycling and the prevention of plastic pollution. Determining the risk of chemicals contained in recycled plastic is difficult because ‘recycled plastic’ is made from a variety of plastic waste, such as used plastics and residue from the production of plastic products. This study aims to develop an approach to risk-based grading of recycled plastics, which will contribute to the implementation of risk assessment and management of plastic recycling in the supply chain. We organized the conceptual framework for establishing grades of recycled plastics and clarified the information required for such classification. We collected and organized information on exposure factors related to plastic products used in various applications. As a case study for setting a separator of each grade, the exposure potential to a plastic chemical by use of five plastic products. The results confirmed that exposure potential vary across their use, suggesting that, by appropriately selecting uses that differ in use patterns and contact frequency, it may be possible to establish meaningful separator of the grades.

Timeline:

March 2025 – February 2026

Topics:

Presented at the LRI annual research meeting of JCIA. August 29, 2025.

Publications:

Kyoko Ono, Masashi Gamo, Isamu Ogura, Naohide Shinohara, Naoya Kojima
"An attempt to establish risk-based recycled plastic grades to enable appropriate plastic recycling", SETAC Europe 35th Annual Meeting, Wien, Austria (2025/05), Poster presentation

Kyoko Ono, Masashi Gamo, Isamu Ogura, Naohide Shinohara, Naoya Kojima
"Establishing risk-based grading of recycled plastics focusing on plastic additives to enable appropriate recycling", SafePlastChem Conference, Ascona, Switzerland (2026/01), Oral presentation

Title of Research: ***24-6-01***

Development of a coculture system to assess respiratory sensitization using IL-4 production from human Th2 cells as a marker

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:

The development of alternative methods for animal testing of respiratory sensitization has been the subject of an OECD Detailed Review Paper since the year before last, and the final version was recently completed. Our dendritic cell (DC) coculture system (DCsens) and the two-step DC/T coculture system (DC/Tsens), which adds T cells to DCsens, are also included. DCsens is a coculture system using human bronchial epithelial cell line BEAS-2B and human monocyte cell line CD14-ML cells with a scaffold. DC/Tsens is a coculture system where T cells are added to the DC layer stimulated in the DCsens. DC/Tsens uses IL-4 production, a powerful inducer of Th2 differentiation from T cells and an effector molecule of Th2 cells, as its indicator.

Based on studies conducted up to last year, using an allo-specific Th2 cell line enabled the measurement of IL-4 production via ELISA. For a total of 13 chemicals, including 6 skin sensitizing chemicals and 7 respiratory sensitizing chemicals, Evaluation method 1 yielded predictive sensitivity, specificity, and accuracy of approximately 85% each. However, as the number of chemicals increased, sensitivity—the ability to identify respiratory sensitization—began to decline. This evaluation method 1 calculated the cytokine production ratios for IL-2, IFN-γ, and IL-4 using the maximum concentration at which each cytokine showed peak production, even if this maximum concentration differed across the tested concentrations. Therefore, as evaluation method 2, we first calculated the ratio of cytokine production values between concentrations of the same chemical substance and used the maximum value. In this case, since abnormally large values could occur depending on the concentration, we decided to raise the cell survival rate threshold from 20% to 40%. As a result, we evaluated only those chemicals meeting this cell survival rate criterion. This increased sensitivity to 100% for a total of 15 chemicals, including 8 skin sensitizing chemicals and 7 respiratory sensitizing chemicals. We plan to collect data using this survival rate criterion going forward.

Timeline:

March 2025 – February 2026

Topics:

Presented at the LRI annual research meeting of JCIA. August 29, 2025.

Publications:

1. Yoshimoto T, Katahira Y, Hasegawa H, Qu N, Yamaguchi N, Horio E, Ashikaga S, Mizoguchi I. Prediction of the Respiratory Sensitizing Potential of Chemicals Using DC Coculture and DC/T Cell Coculture Systems. SOT 2025（2025.3.20-24）Orlando, FL, USA
2. Yoshimoto T. Predictive potential of chemical respiratory sensitization using DC coculture (DCsens) and two-step DC/T cell coculture (DC/Tsens) systems. RIFM Respiratory Research Workshop: Future Forward（2025.7.15-16）Mahwah, NJ, USA
3. Mizoguchi I. Development of a 3D coculture system enabling accurate prediction and evaluation of respiratory sensitizing chemicals. The 32nd Annual Meeting of The Japanese Society of Immunotoxicology（2025.9.4-5）Gifu, Japan

Title of Research: ***25-1-12***

Elucidation of a New AOP Mechanism for Developmental Neurotoxicity Mediated by Oxidative Stress and Neuroinflammation

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:

Mizuki Yuge, Takashi Shiromizu, Junko Koiwa (Department of Integrative Pharmacology, Mie University Graduate School of Medicine)
Makoto Kashima (Faculty of Science Department of Biomolecular Science, Toho University)

Summary of Research:

To assess the effects of chemical exposure on children's neurodevelopment (developmental neurotoxicity, DNT), the OECD established new guidelines (No. 377) in 2023, combining 17 types of in vitro tests. These in vitro tests are expected to contribute significantly to elucidating molecular initiating events, such as receptor inhibition, and key events at the cellular level, such as neural differentiation, within Adverse Outcome Pathways (AOPs). However, it is increasingly recognized that existing test methods alone have limitations in elucidating AOPs. For example, the morphology and function of neurons and glial cells that make up the brain vary depending on the region and environment, but it is not easy to evaluate these changes in in vitro tests. On the other hand, while these aspects can be evaluated using mammalian models such as rats, issues arise regarding cost, effort, and animal welfare. Developing new approach methods that complement the weaknesses of existing methods and accelerating the elucidation of the AOP of DNT can contribute to accurately assessing the impact of chemicals on children's neurodevelopment and to realizing a sustainable society through the appropriate use of chemicals. In recent years, it has been established that oxidative stress and neuroinflammation are key events in the AOP of DNT, and that the oxidative stress and neuroinflammation induced by chemical exposure during development vary depending on the brain region. Furthermore, zebrafish, a vertebrate, are recognized as a useful model for chemical toxicity assessment because they are not considered animals during the larval stage (up to approximately 5 days post-fertilization) due to their inability to feed independently, thus raising few ethical concerns, and because the development of various organs is similar to that in mammals. The objective of this study is to elucidate the AOP by creating zebrafish that allow for the evaluation of the morphology and function of microglia, which are closely involved in oxidative stress and neuroinflammation in the brain,in each brain region, and by establishing a new DNT test using these zebrafish larvae. This year, we established a method for quantifying the morphology of microglia in the brain regions of zebrafish larvae by performing live imaging using two-photon excitation microscopy on transgenic zebrafish that express a fluorescent protein under the control of the mpeg1 promoter, a microglia- and macrophage-specific gene. We also used this method to evaluate the DNT of ethanol and valproic acid exposure. Furthermore, we generated transgenic zebrafish to visualize oxidized glutathione and hydrogen peroxide, markers of oxidative stress, in microglia. Next fiscal year, we will expose these zebrafish to compounds that are positive and negative for DNT and evaluate changes in oxidized glutathione and hydrogen peroxide in microglia for each brain region.

Timeline:

March 2025 ~ February 2026

Topics:

Presented at the LRI annual research meeting of JCIA. August 29, 2025.

Publications:

Mizuki Yuge, Junko Koiwa, Takashi Shiromizu, Eri Wakai, Akira Migoguchi, Yuhei Nishimura. **Application to developmental toxicity testing of a novel method for whole-brain imaging of microglia in zebrafish.** *Current Research in Toxicology* 10:100276 (2025)

Title of Research: 25-4-01

Quantitative assessment of the pharmacokinetics of MP as vectors in fish: Implementation of a novel digestive tract administration technique

Principal Investigator:

Takeshi Hano, PhD (Group leader, National Research and Development Agency, Japan Fisheries Research and Education Agency, Fisheries Technology Institute, Environment and Fisheries Applied Techniques Research Department, Environmental Conservation Division, Chemical substance group)

Collaborators:

Chisato Kataoka, Mana Ito, Kumiko Kono, Nobuyuki Okubo, Md. Khorshed Alam
(National Research and Development Agency, Japan Fisheries Research and Education Agency, Fisheries Technology Institute, Environment and Fisheries Applied Techniques Research Department, Environmental Conservation Division, Chemical substance group)

Summary of Research:

The array of microplastic (i.e., MP) pollution in marine ecosystems has been expanding due to greater bioavailability, leading to frequent detection in organisms, including fish, in natural habitats. Recent detection of MPs in the gastrointestinal tracts of marine fish raises concerns of possible translocation within the blood, internal organs, and tissues. Although a few reports claim the presence of MPs in various fish organs, understanding the quantitative translocation, expansion, and mechanisms remains limited. Challenges arise in laboratory-based analysis of quantitative expansion and translocation of MPs within the body, as volunteer feeding often accompanies baseline variability. Thus, to address the knowledge gap and create a robust and reproducible database, the current study aimed to develop a tube-feeding method that enables MPs to control oral administration in the digestive tract, thereby reducing variability in ingestion rate. The prototype was constructed prior to measuring changes in mouth diameter, length from the mouth to the stomach, and esophageal diameter in red sea bream juveniles (i.e., *Pagrus major*; approximately 10–30 g and 8–12 cm of total length). Then, the feed viscosity was evaluated for the conventional feed mixtures (e.g., Otohime EP2; Nissin Marubeni Feed) by inserting a syringe into each feed composition and measuring the force required to move the feed using a digital force gauge. After that, the tolerable volume of feed and MPs was measured based on the stomach's bearing capacity to design the tube-feeding device. Consequently, the tube-feeding device was constructed using a 1 ml syringe and a Teflon tubing (inner × outer diameter = 3 × 4 mm).

The red sea bream juveniles were chosen as the model species based on their ability to tolerate handling stress during our initial trial in the current study. Initially, the effectiveness of the design was tested by administering MPs with a diameter of 250–300 μm (spherical, fluorescent, yellow) to red sea bream juveniles weighing 10 and 30 g (n = 3). The excreted MP particles were counted, and the recovery rate consistently exceeded 90%, demonstrating the technique's higher reproducibility. Additionally, the retention time of MPs (e.g., 250–300 and 10–20 μm diameter spherical fluorescent; green) in the digestive tract was assessed in red sea bream juveniles, and the temporal pattern of MP excretion was recorded. The results demonstrated that 90% of the administered dose was excreted within 30 hours, regardless of MP size or fish body weight, indicating the suitability and reliability of the established technique for assessing the pharmacokinetics of MP and related vector effects.

Timeline:

March 2025 – February 2026

Topics:

Presented at the LRI annual research meeting of JCIA. August 29, 2025.

Publications:

N/A

Annual Report 2025

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/

一般社団法人 日本化学工業協会
Japan Chemical Industry Association