LRI

Long-range Research Initiative

# Annual Report 2022

2022

Japan Chemical Industry Association

Annual Report 2022

***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, , The University of Tokyo) 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, 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:***

When the actin-binding protein drebrin which forms dendritic spines of neurons is eliminated by NMDA-type glutamate activity, the spines become thinner and eventually disappear. Loss of dendritic spines impairs learning and memory. In this study, we developed an image analysis algorithm that automatically counts the number of drebrin immunostained clusters in 3-weeks cultured rat fetal hippocampal-derived neurons. Next, we measured changes in the number of drebrin clusters caused by chemical substances. This research resulted in a proposal submitted an Adverse Expression Pathway (AOP) to OECD. The AOP started from the binding of the compound to the NMDA-type glutamate receptor as a molecular initiation event (MIE) , and end at the impairment of learning and memory as the adverse event (AO). The new proposal wad registered as Wiki475. We will collect evidence to complete AOP-Wiki475 in the future.


***Timeline:***

March 1, 2020 –

***Topics:***

Poster presentation in 2022 ICCA-LRI & NITE Workshop “Proposal of a new AOP for the neurotoxicity and developmental neurotoxicity assessment of glutamate receptor binding agonists that cause learning and memory impairment.”

***Publications:***


Mase S, Mitsuoka T, Koganezawa N, Shirao T, Sekino Y, "Drebrin cluster analysis using cultured hippocampal neurons"ISRN-2022, Web, March 2022

Sekino Y., "The neurochemistry of addiction and strategies for substance abuse prevention" 69th Annual meeting of Hokkaido Pharmaceutical Sciences, Web, May 2022

Shogo Mase, Toshinari Mitsuoka, Noriko Koganezawa, Hiroyuki Yamazaki, Yuuichi Kato, Izuo Tsutsui, Hiroshi Kawabe, Tomoaki Shirao, Yuko Sekino "Quantitative analysis of developmental neurotoxicity using drebrin immunocytochemical images of cultured rat hippocampal neurons" The 49th Annual Meeting of the Japanese Society of Toxicology, Sapporo, June 2022

Shogo Mase, Toshinari Mitsuoka, Noriko Koganezawa, Hiroyuki Yamazaki, Yuuichi Kato, Izuo Tsutsui, Hiroshi Kawabe, Tomoaki Shirao, Yuko Sekino, "High-content analysis using drebrin immunocytochemical images of cultured rat hippocampal neurons" Japan Basic and Clinical

Pharmacology Week 2022, Dec 2022

Yuko Sekino, "Collecting Evidence for Enlightenment Activities to Prevent Illegal Drug Use by Youth" Research Report on Subsidies for the Ministry of Health, Labor and Welfare Administration Promotion Research Project (FY2022)

## *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 developmental neurotoxicity potential.


## *Principal Investigator:*

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



## *Summary of Research:*

As thyroid hormones (TH) are essential for fetal brain development, the chemicals which induce reduction of serum TH levels may potentially interfere with the developing brain. Since standardized studies to identify developmental neurotoxicity (DNT) require significant resources, a simple screening test is eagerly awaited for sorting chemicals to be examined in the DNT studies. Recently, we began verifying the feasibility of a modified Comparative Thyroid Assay (CTA) by downsizing the number of rats but with the addition of examination of the brain TH levels and brain histology. The 1st study showed that the modified CTA could detect 6-propylthiouracil (6-PTU, 10 ppm)-induced severe (>70%) reduction of serum TH in dams, with >50% suppressed serum/brain TH levels and brain abnormality (heterotopia) in offspring. The modified CTA also detected sodium phenobarbital (NaPB, 1000 ppm)-induced mild (<35%) reduction of serum TH levels in dams, with mild (<35%) reduction of serum/brain TH levels in fetuses but not in pups, and without increased brain heterotopia. The 2nd study showed that the findings by NaPB at 1000 ppm were generally reproducible in dams and offspring. To investigate what degree of serum/brain TH disruptions will be adverse to brain morphology, we performed a 3rd study focusing on heterotopia formation by using treatment with a wider range of doses of 6-PTU (0, 1, 3, and 10 ppm in feed) during the critical time window for heterotopia formation (GD19-LD2). 6-PTU dose-dependently reduced serum TH in dams and serum/brain TH in offspring, and induced heterotopia formation in PND21 pups. Although detailed analysis is still in progress, our findings suggest that the modified CTA can be a potential short-term *in vivo* assay for sorting offspring TH disruptors.


## *Timeline:*

March 1, 2022 – February 28, 2023.

## *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 26th, 2022)

## *Publications:*

Kenta Minami[1], Hidenori Suto[1], Akira Sato[2], Keiko Ogata[1], Tadashi Kosaka[2], Hitoshi Hojo[2], Naofumi Takahashi[2], Naruto Tomiyama[2], Takako Fukuda [1], Katsumasa Iwashita [1], Hiroaki Aoyama[2] and Tomoya Yamada[1] ([1] Sumitomo Chemical Company, Ltd. [2] The Institute of Environmental Toxicology), 2023. Feasibility study for a downsized comparative thyroid assay with measurement of brain thyroid hormones and histopathology in rats: case study with 6-propylthiouracil and sodium phenobarbital at high dose. Regulatory Toxicology and

Pharmacology. 137, 105283.

Kenta Minami[1], Akira Sato[2], Naruto Tomiyama[2], Keiko Ogata[1], Tadashi Kosaka[2], Hitoshi Hojo[2], Naofumi Takahashi[2], Hidenori Suto[1], Hiroaki Aoyama[2] and Tomoya Yamada[1] ([1] Sumitomo Chemical Company, Ltd. [2] The Institute of Environmental Toxicology), 2023. Feasibility study for a downsized comparative thyroid assay with measurement of brain thyroid hormones and histopathology in rats: Part II. Within laboratory reproducibility for effects of sodium phenobarbital (submitted, under review)

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.

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.

○Keiko Ogata[1], Kenta Minami[1], Hidenori Suto[1], Hiroyuki Asano[1], Masahiko Kushida[1], Keiko Maeda[1], Akira Sato[2], Naofumi Takahashi[2], Hiroaki Aoyama[2], Tomoya Yamada[1] ([1] Sumitomo Chemical Company, Ltd. [2] The Institute of Environmental Toxicology)
“A method evaluating brain morphology in a screening study of low thyroid hormone-related developmental neurotoxicity”. The 39th Annual Meeting of the Japanese Society of Toxicologic pathology, Tokyo, Japan, January 2023.

○Tomoya Yamada[1] and Hiroaki Aoyama[2], ([1] Sumitomo Chemical Company, Ltd. [2] The Institute of Environmental Toxicology)
“A proposal for the use of a modified comparative thyroid assay with reduced number of animals and additional parameters”. The 62nd Annual Meeting of the Society of Toxicology, Nashville, United States, To be presented on March 2023.

○Kenta Minami[1], Hidenori Suto[1], Akira Sato[2], Keiko Ogata[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)
“Effects of sodium phenobarbital in a downsized comparative thyroid assay with additional examination of brain thyroid hormone levels and brain histology”. The 62nd Annual Meeting of the Society of Toxicology, Nashville, United States, To be presented on March 2023.

***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.
The third year of the study, mRNA expression changes encoding 12 molecules that play important roles in neurodevelopment, mainly acrylamide and tributyltin, were examined up to day 21 of culture. The results suggested that the expression of three genes, Dlg4, Syp, and Bdnf, changed at the same time as in the methylmercury-exposed group, although some genes showed different expression patterns from those in the methylmercury-exposed group. Considering the possibility that gene expression may be altered by other suspected developmental neurotoxicants, we examined the effects of suspected developmental neurotoxicants on Dlg4 gene expression in DIV10, 14, and 21, and found that Dlg4 gene expression was suppressed in some cases. Dlg4 expression in DIV14 was shown to be sensitive to chemical substances and may be a good indicator of developmental neurotoxicity. In addition to the 12 gene markers, we performed a comprehensive gene expression analysis using RNA-Seq to investigate the possibility that there are other genes that may be indicators of developmental neurotoxicity. The results revealed the existence of genes that were significantly up-regulated in common with the three developmental neurotoxicants and, conversely, genes that were significantly down-regulated in common with the three substances.

***Timeline:***
March 1, 2020-Februery 28, 2023

***Topics:***

***Publications:***

***Title of Research:***

20_PT03-08

# 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. In this study, we have developed a novel test method that can assess the DNT of chemicals on the morphology of microglia using a transgenic zebrafish line expressing mVenus, Cerulean, and mCherry in microglia, neurons, and astrocytes, respectively. Using the assay, we were able to detect the DNT of ethanol on the formation of microglia. We will apply this method to assess the DNT of other chemicals, such as valproic acid, ethinylestradiol, and chlorpyrifos. We will also analyze the correlation among the morphological change of microglia, neuroinflammation, and neurodifferentiation.


***Timeline:***

March 2020 ~ February 2023

***Topics:***

JCIA LRI Annual Workshop (2022)

***Publications:***


1. Komada M, Nishimura Y: **Epigenetics and Neuroinflammation Associated With Neurodevelopmental Disorders: A Microglial Perspective**. *Front Cell Dev Biol* 2022, **10**:852752.
2. Nishimura Y, Kurosawa K: **Analysis of Gene-Environment Interactions Related to Developmental Disorders**. *Frontiers in pharmacology* 2022, **13**:863664.

***Title of Research:***

20_PT03-05

# 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)
(e-mail) nakanishi@gifu-pu.ac.jp

***Collaborators:***

Daisuke Matsumaru (Laboratory of Hygienic Chemistry and Molecular Toxicology, Gifu Pharmaceutical University)
(e-mail) matsumaru-da@gifu-pu.ac.jp

***Summary of Research:***

Recently, the concern over the toxicity of environmental chemicals that induce hypothyroidism has been increased, since hypothyroidism during pregnancy can have a significant impact on the development of the fetal brain in human. However, the results of thyroid function disruption detected by toxicity testing have not been sufficiently utilized for risk assessment of chemical substances because the correlation between the degree of hypothyroidism during pregnancy and adverse effects on the development of the fetal brain remains unclear. In order to solve the 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 the past year, we have confirmed the usefulness of Tg mice in assessing developmental neurotoxicity and found the possibility of evaluating the effects of hypothyroidism from early pregnancy on the infant brain using the antithyroid drug propylthiouracil (PTU). In this fiscal year, we analyzed the correlation between the expression profile of *in vivo* imaging in the infant brain and maternal thyroid-related parameters when hypothyroidism was induced by exposure to various doses of PTU from early pregnancy.

PTU induced a dose-dependent decrease in triiodothyronine (T3)/thyroxine (T4) and an increase in thyroid-stimulating hormone (TSH) in maternal blood. In histological analysis of the thyroid gland, symptoms of hypothyroidism such as thickening of follicular cells were observed even at low-dose PTU exposure prior to significant changes in serum T3/T4/TSH levels. In addition, the expression of reporter molecules in the brain of the infants was also increased in a dose-dependent manner of PTU. Furthermore, similar to the results of thyroid histological analysis in the mother, the effects were observed even at low-dose without significant changes in the serum levels of thyroid-related hormones.

These results suggest that in chemical-induced hypothyroidism, thyroid histology is a more sensitive indicator for detecting their effects than serum thyroid-related hormones, and that some adverse effects on brain development in the child may occur prior to changes in maternal serum thyroid-related hormones.

***Timeline:*** April, 2022 – March, 2023

***Topics:***

1) 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 26th, 2022)
2) Report in the Yomiuri Shimbun Morning Edition “Environmental Chemicals and the Brain:A New Approach Methodology Using "Glowing Mice" - Gifu Pharmaceutical University, etc., Verify Effects on Children” (February 7th, 2023)

***Publications:***

1) Ishida K, Tatsumi K, Minamigawa Y, Mori K, Matsumaru D, Nagase H, Kanda Y, Takuma K, Nakanishi T, Neuronal differentiation reporter mice as a new methodology for detecting in vivo developmental neurotoxicity. ***Biochem. Pharmacol.***, 206:115332 (2022)
2) Ishida K, Matsumaru D, Shimizu S, Hiromori Y, Hisamitsu Nagase H, Nakanishi T, Evaluation of the estrogenic action potential of royal jelly by genomic signaling pathway *in vitro* and *in vivo*. ***Biol. Pharm. Bull.***, 45:1510–1517 (2022)
3) Ishida K, Furukawa M, Kunitani M, Yamagiwa R, Hiromori Y, Matsumaru D, Hu J, Nagase H, Nakanishi T, Novel, highly sensitive, *in vivo* screening method detects estrogenic activity at low doses of bisphenol A. ***J. Hazard. Mater.*** 445:130461 (2023).

***Conference Presentations:***

1) Ishida K, Minamigawa Y, Tatsumi K, Mori K, Matsumaru D, Nagase H, Kanda Y, Takuma K, Nakanishi T, Validation of brain neuronal differentiation reporter mice for improved developmental neurotoxicity evaluation, 2022 ICCA-LRI & NITE Workshop, Yokohama/Japan, June 2022
2) Tatsumi K, Ishida K, Minamigawa Y, Mori K, Matsumaru D, Nagase H, Kanda Y, Takuma K, Nakanishi T, Validation of brain neuronal differentiation tracer mice for improvement developmental neurotoxicity evaluation, The 49th Annual Meeting of the Japanese Society of Toxicology (Sapporo), 2022.
3) Mori K, Ishida K, Minamigawa Y, Tatsumi K, Matsumaru D, Murashima A, Nagase H, Kanda Y, Nakanishi T, Imaging analysis of the offspring brain in a model of maternal hypothyroidism, The 49th Annual Meeting of the Japanese Society of Toxicology (Sapporo), 2022.
4) Ishida K, Minamigawa Y, Tatsumi K, Mori K, Matsumaru D, Nagase H, Kanda Y, Takuma K, Nakanishi T, Establishment of *in vivo* neuronal differentiation tracing method for improved developmental neurotoxicity evaluation, The 62nd Annual Meeting of the Japanese Teratology Society (Kanazawa), 2022.
5) Ishida K, Matsumaru D, Nakanishi T, Perinatal hypothyroidism and DOHaD: Aiming for the risk assessment of neurodevelopmental disorders in offspring, Forum 2022 Pharmaceutical Health Sciences/Environmental Toxicology (Kumamoto), 2022.
6) Ishida K, Tatsumi K, Minamigawa Y, Mori K, Matsumaru D, Murashima A, Nagase H, Kanda Y, Takuma K, Nakanishi T, Neuronal differentiation reporter mice as a new methodology for detecting *in vivo* developmental neurotoxicity, *In vivo* imaging forum 2022 (Tokyo), 2022.

***Title of Research:***

XXXX

# 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 FY2022, we established methods for estimating MP loadings from major sources, which is indispensable for considering priorities for source control measures, etc. We estimated loadings for product-containing MP (PCP), laundry-derived fiber, and tire-wear particles (TWP) entering Tokyo Bay, and showed the range of possible values for the estimates. We investigated to understand the MP monitoring levels along the Japanese coast (especially in Tokyo Bay) and a method for estimating the distribution of MP concentrations in the unmeasured range and estimated the distribution of the number of unmeasured MP concentrations from available data. We derived an SSD based on hierarchical Bayes using toxicity values listed in the new MP ecotoxicity database. Furthermore, as a trial ecological risk assessment in Tokyo Bay, we compared the corrected MP particle concentrations in Tokyo Bay with threshold values using Food Dilution as an effect index. Through the environmental load estimation, analysis of the monitoring data, consideration of data correction methods, and trial risk assessment in Tokyo Bay, we were able to clarify items with large uncertainties and future research issues. In the future, we plan to establish an assessment framework that enables us to predict the future environmental burden and risk of MP by improving the accuracy of the estimation method and understanding the particle size distribution of environmental MP that matches the actual situation.

***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 2022 (Online)

***Publications:***

1. Naito W "Risk Assessment in Practice : Opportunities, Challenges and Evolving Roles for Complex Environmental Issues" SETAC AP 2022, Plenary Speech, Sep. 2022（Virtual Meeting）
2. Naito W "Development of a conceptual model for environmental risk assessment of microplastics and a trial risk assessment in Tokyo Bay" MARII Workshop on "Advancements and steps towards a holistic, quantitative risk assessment on microplastics" Oral presentation、 Oct. 2022、Barcelona, Spain
3. Ueda K, Iwasaki Y, Uesaka M, Naito W "Which concentration unit should be used for environmental risk assessment of microplastics?" 57th Annual Meeting of Japanese Society of Water Environment, Poster Presentation, March 2023, (Univ. Ehime, Japan)
4. Naito W, Iwasaki Y, Ono K, Ogura I "Framework of environmental risk assessment for microplastics and an illustrative example" 57th Annual Meeting of Japanese Society of Water Environment, Oral Presentation, March 2023, (Univ. Ehime, Japan)

***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 Medical University, School 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 Medical University)
Kazufumi Matsushita (Hyogo Medical University)
Masakiyo Nakahira (Hyogo Medical University)
Takumi Adachi (Hyogo Medical University)
Hinata Inoue (Hyogo Medical University)
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, and it is suggested 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. In general, inhaled particles are thought to be engulfed by alveolar macrophages and then excreted. Therefore, in this study we aim to develop methods of evaluation of inflammatory particles focusing on alveolar macrophage functions in response to particles. In addition, animal-free toxicity testing is recommended in these days, so we also aim to develop in vitro evaluation methods using alveolar macrophage cell lines which is useful tools for detecting inflammatory particles.

Freshly isolated alveolar macrophages were immortalized by SV40 large T gene transfer using lentiviral vector system, and then cultured with GM-CSF and PPAR-$\gamma$ agonist to maintain their characteristics of alveolar macrophage. After screening of obtained cells, and we established alveolar macrophage cell line, ALV-3. The same as primary alveolar macrophages, ALV-3 cells responded to inflammatory particles and induced cell death and subsequently IL-1$\alpha$ release. Furthermore, ALV-3 cells expressed Siglec F and CD11c antigens, which are unique antigens for alveolar macrophages, on their surface. MH-S cells, which are commercially available alveolar macrophage cell line, did not expressed Siglec F antigen and did not induce IL-1$\alpha$ release in response to inflammatory particles. These results suggest that ALV-3 cells might be useful cell line to evaluate inflammatory particles, as an alternative in vitro method to animal testing. Now we are further investigating the characteristics of ALV-3 cells, as alternative to primary alveolar macrophages.

***Timeline:***

March 1, 2022—February 28, 2023

***Topics:***

Research meeting of LRI by JCIA, oral, “Development of evaluation method of inflammatory particles based on alveolar macrophage function”, online, August 26, 2022.

***Publications:***

Kuroda Etsushi “Analysis of alveolar macrophage functions in response to fine particles" The 127th Annual meeting of the Japanese Association of Anatomists, Online Symposium, 2022, March 28.

Kuroda Etsushi “DAMPs release and allergic airway inflammation caused by inhaled fine particles” SOT 61st Annual Meeting, Online Symposium, 2022, March 29.

***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)
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)
Hiroyuki Teramae, PhD (Josai University)

***Summary of Research:***

Purpose: The skin is exposed to many chemical substances, including pharmaceuticals, cosmetics, and fragrances, and is inadvertently exposed to many chemical substances in daily life. Therefore, it is extremely important to evaluate the skin permeability of chemical substances that may contact the skin. Next-generation risk assessment (NGRA), a risk assessment method that combines various methods without using animals, has attracted much attention in recent years. In the present study, development of in silico model that predict dermal absorption after chemical exposure under real-world condition, finite dose, was investigated by considering changes in concentration of the applied substance on the skin surface.
Methods: Prediction of skin permeation parameters: Physicochemical parameters of various chemical substances were calculated using Scigress software. Diffusion and distribution coefficients, which are skin permeation parameters of chemicals, were predicted by random forest regression analysis, an ensemble learning algorithm, using the statistical software JMP Pro16 with physicochemical parameters as descriptors.
Results and conclusion: The study using random forest regression analysis showed that all of the skin permeation parameters were well predicted when regression analysis was performed using M.W., ClogP, HOMO, and LUMO as descriptors. Skin permeation profiles, calculated by two-layer diffusion model after chemical exposure under finite dose condition, showed good perdition results when water volatilization rate constant was considered. There were, however, some deviations between the predicted and observed skin permeation profiles, the overall prediction results were good. These results suggest that the constructed model would be used to predict the dermal absorption of finite dose systems.

***Timeline:***

March 1st, 2021-

***Topics:***

2022 ICCA-LRI & NITE Workshop（June 20, 2022）
*In silico* estimation of skin permeation of chemicals with their diffusion and partition parameters
2022 年 LRI Annual Workshop（August 26, 2022）
Establishment of in silico model to predict skin absorption of chemical compounds with two-layered diffusion model

***Title of Research:***

XX-XX-XX

# Establishment of Human iPSC Reporter-Based Developmental Toxicity Assay that Detects FGF Signal Disruption


***Principal Investigator:***

Junji Fukuda (PhD, Professor, Yokohama National University, Faculty of Engineering)
79-5 Tokiwadai, Hodogaya Ward, Yokohama, Kanagawa 240-8501, Japan

***Collaborators:***

Yusuke Okubo (PhD, Principle Investigator, National Institute of Health Sciences)
3-25-26 Tono-machi, Kawasaki-ku, Kawasaki, Kanagawa 210-9501, Japan

Yoshihiro Nakajima (PhD, Principle Investigator, National Institute of Advanced Industrial Science and Technology)
2217-14 Hayashi-cho, Takamatsu, Kagawa, 761-0395, Japan



***Summary of Research:***

In the 10th period, we reported that the compounds listed in ICH (S5), including thalidomide, could be detected by using RTK-SRF signal reporter cells (S. Kanno, et al., Journal of Bioscience and Bioengineering, 133, 3, 291- 299 (2022)). As a first step to spread this approach, we also reported a detailed experimental protocol in a scientific paper (S. Kanno, et al., Star Protocols, 3, 2, 101439 (2022)). As for the experiments, a real-time luminescence measurement system was introduced to improve the data accuracy of the kinetic assay, which was a feature of this method. In other words, the amount of luminescence was measured manually every few hours in the past, but the introduced device made it possible to perform real-time measurement. When measurements were continued for three days instead of the conventional one day using this device, it was also confirmed that the luminescence peak occurred not once, but at least twice. This is a fact that had been overlooked manually. In the 11th period, we would like to add a discussion on the biological significance of this phenomenon and the measurement mechanism.


***Timeline:***

March 1, 2022 -

***Topics:***

Poster presentation at 2022 ICCA-LRI and NITE Workshop, “Establishment of developmental toxicity test based on the integration of FGF signal disruption effects for safety evaluation of drugs and chemicals using human iPS cells”

***Publications:***


Seiya Kanno, Kashu Mizota, Yusuke Okubo, Tatsuto Kageyama, Lei Yan, Junji Fukuda, “Luciferase assay system to monitor fibroblast growth factor (FGF) signal disruption in human iPSCs”, Star Protocols, 3, 2, 101439, 2022

Seiya Kanno, Yusuke Okubo, Tatsuto Kageyama, Lei Yan, Junji Fukuda, “Integrated FGF signal disruptions in human iPS cells for prediction of teratogenic toxicity of chemicals”, Journal of Bioscience and Bioengineering, 133, 3, 291-299, 2022

***Title of Research:*** 22-3-01

## Prediction of internal concentrations of chemicals orally administered using data-driven pharmacokinetic modeling

***Principal Investigator:*** Prof. Hiroshi Yamazaki, PhD (Showa Pharmaceutical University, Laboratory of Drug Metabolism and Pharmacokinetics), 3-3165, Higashi-tamagawa Gakuen, Machida, Tokyo 194-8543, Japan. (phone) +81-42-721-1406, (e-mail) hyamazak@ac.shoyaku.ac.jp.

***Collaborator:*** Makiko Shimizu, ibid, (e-mail) shimizu@ac.shoyaku.ac.jp

***Summary of Research:*** To evaluate internal exposures in humans without any reference to experimental data, physiologically based pharmacokinetic (PBPK) modeling could be used. The input parameters for PBPK models (i.e., fraction absorbed x intestinal availability, absorption rate constants, volumes of the systemic circulation, and hepatic intrinsic clearances) were estimated for a panel of 355 chemicals using a light gradient boosting machine learning algorithms (LightGBM) based on between 11 and 29 *in silico*-calculated chemical descriptors. Parameters for human PBPK models for a diverse range of compounds could be successfully estimated using chemical descriptors. This approach to pharmacokinetic modeling has potential for application in computational toxicology and in the clinical setting for assessing the potential risk of general chemicals.

***Timeline:*** From March 1, 2022 to February 28, 2023

***Topics:*** The principal Investigator has been the recipient of Scientific Achievement Award from the International Society of Study of Xenobiotics (ISSX) for major contributions to the field of xenobiotics in the Asia Pacific Region (Bangalore, India, 2023).

***Publications:***

(1) Adachi, K., Shimizu, M., and Yamazaki, H. (2022) Updated *in Silico* Prediction Methods for Fractions Absorbed and Key Input Parameters of 355 Disparate Chemicals for Physiologically Based Pharmacokinetic Models for Time-Dependent Plasma Concentrations after Virtual Oral Doses in Humans. *Biol Pharm Bull 45*, 1812-1817.
(2) Kamiya, Y., Handa, K., Miura, T., Ohori, J., Kato, A., Shimizu, M., Kitajima, M., and Yamazaki, H. (2022) Machine Learning Prediction of the Three Main Input Parameters of a Simplified Physiologically Based Pharmacokinetic Model Subsequently Used to Generate Time-Dependent Plasma Concentration Data in Humans after Oral Doses of 212 Disparate Chemicals. *Biol Pharm Bull 45*, 124-128.
(3) Shimizu, M., Hayasaka, R., Kamiya, Y., and Yamazaki, H. (2022) Trivariate Linear Regression and Machine Learning Prediction of Possible Roles of Efflux Transporters in Estimated Intestinal Permeability Values of 301 Disparate Chemicals. *Biol Pharm Bull 45*, 1142-1157.
(4) Kamiya, Y., Handa, K., Miura, T., Ohori, J., Shimizu, M., Kitajima, M., Shono, F., Funatsu, K., and Yamazaki, H. (2022) Correction to "An Updated In Silico Prediction Method for Volumes of Systemic Circulation of 323 Disparate Chemicals for Use in Physiologically Based Pharmacokinetic Models to Estimate Plasma and Tissue Concentrations after Oral Doses in Rats". *Chem Res Toxicol 35*, 1433.
(5) Adachi, K., Shimizu, M., and Yamazaki, H. (2022) Updated in silico prediction methods for fractions absorbed and absorption rate constants of 372 disparate chemicals for use in physiologically based pharmacokinetic models for estimating internal concentrations in rats. *J Toxicol Sci 47*, 453-456.
(6) Miura, T., Uehara, S., Shimizu, M., Suemizu, H., and Yamazaki, H. (2022) Forward and reverse dosimetry for aniline and 2,6-dimethylaniline in humans extrapolated from humanized-liver mouse data using simplified physiologically based pharmacokinetic models. *J Toxicol Sci 47*, 531-538.

*Title of Research:*

22-5-03

Constructing a model for estimating a vector effect of microplastics using an artificial bioconcentration device and predicting impact in the real environment

*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 daily life due to its low cost, lightweight, and rigid decomposing. Due to their high production and slow degradation, plastic pollution has expanded worldwide in aquatic environments. Plastic pollution from large items to small particles (microscopic plastic MP, < 5mm) emerged. The effect of MP and its vector effect on pollutants are of concern. Many studies have examined these topics. However, most of the study were used pristine MPs. Thus, a study on the effect of aging on the vector effect of MP is required.

This fiscal year, freshwater and marine medaka were co-exposed to microplastics (MP) with chlorobenzenes (CBs) and anthracene (ANT) under various conditions, and vector effects were investigated. No vector effect could be observed in CBs (logPow 4.19-5.73). However, a vector effect was confirmed with anthracene (logPow4.45). Therefore, it was concluded that the vector effect differs greatly depending on the character of the chemicals, even if the LogPow is close. Further studies may be required on PAHs, POPs, etc., concerned about the influence of vector effects. The details are described below.

1) Freshwater medaka-CBs + degraded crushed PE-MP 3-day co-exposure experiment: CBs group (CBs; TriCB: 10 μg/L, TetCB: 10 μg/L, PentCB: 10 μg/L, HexCB: 8 μg/L), CBs + aged fragmented PE-MP (1 mg/L) co-exposure group, CBs + unaged fragmented PE-MP (1 mg/L) co-exposure group were set, and after equilibrating with MP for one day, medaka ( 3 fish/group) were exposed for 3 days. As a result of the analysis, CBs in the unaged group was 1.6 times higher than in the CBs group. Moreover, there was no effect on aging.

2) 7-day co-exposure experiment with different concentrations of freshwater medaka-CBs+PE-MP : CBs group (CBs; TriCB: 10 μg/L, TetCB: 10 μg/L, PenCB: 10 μg/L, HexCB:

8 μg/L ), CBs + low-concentration fragmented PE-MP (0.1 mg/L) Co-exposure group, and CBs+ high-concentration crushed PE-MP (1.0 mg/L) group were set. They were exposed to Japanese medaka (33 fish/section) for 7 days and excreted for 5 days. Sampling was performed on the 3rd, 5th, 7th, 9th, and 12th days. The water exchange was semi-static (once a day, full exchange). There was no significant difference in CBs concentrations compared with the water-exposed group, indicating no vector effect.

3) Freshwater medaka-CBs + PS-MP co-exposure experiment with different particle sizes: CBs (CBs; TriCB: 10 μg/L, TetCB: 10 μg/L, PenCB: 10 μg/L, HexCB: 8 μg/L) exposure group, CBs+10-μm PS-MP co-exposure group,and a CBs+45-μm PS-MP co-exposure group were set.. After 7 days of exposure, they were excreted for 5 days and sampled on the 7th, 8th, 9th, 10th, and 12th days. The water exchange was semi-static (once a day, full exchange). As a result, the CBs concentration in the co-exposed groups did not differ from that in the CBs-exposed group and somewhat decreased, indicating no vector effect.

4) Freshwater medaka-ANT+58-μm PE-MP co-exposure experiment with different MP concentrations: ANT (200 μg/L) group, ANT+ low-concentration PE-MP (0.6 mg/L) co-exposure group, ANT+ middle-concentration PE-MP ( 6 mg/L) co-exposure group, and ANT+ high-concentration PE-MP (60 mg/L) co-exposure group were set. After 10 days of exposure, fish were excreted for 4 days and sampled on the 10th, 11th, 12th, 13th, and 14th days. The water exchange was semi-static (once a day, full exchange). The vector effect was confirmed from the obtained concentrations of ANT in the medaka. A twin-compartment vector model was used to analyze the effect of MP concentration, and as a result, the vector coefficient decreased as the MP concentration increased.

5) Prediction of ANT-MP vector effect in the real environment: The data set (Takai, PhD. thesis, Kyushu University, 2023.3) was used obtained from marine Java medaka-anthracene (ANT) + different size (2, 10-μm) polystyrene MP (PS-MP) exposure studies. As a result of evaluating the vector effect from the different concentrations of PS-MP using a twin-compartment vector model, it was estimated that the vector effect would hardly occur in the real environment.

6) Effect of PS-MP particle size on ANT adsorption: As a result of examining the adsorption of ANT to PS-MP with particle sizes of 10, 45, and 90 μm. The smaller the particle size PS-MP show a larger surface area and adsorption potency to ANT, suggesting a vector effect. However, it was expected that the larger the particle size PS-MP has minor adsorption potency and the weaker the vector effect.

*Timeline:*

March 1, 2022- Feb28, 2023

*Topics:*

*Publications:*

1. Takai Y., Tokusumi, H., Sato, M., Inoue D., Chen K., Takamura T., Enoki S., Ueno Y., Kang I. J., Shimasaki Y., Qiu X., Oshima Y., Combined effect of diazepam and polystyrene microplastics on the social behavior of medaka (*Oryzias latipes*), Chemosphere, 299 134403-134403.

2. Takai Y., Tokunaga M., Honda M., Qiu X, Shimasaki Y., Kang I.J., Oshima Y., Size effect of polystyrene microplastics on the accumulation of anthracene for Java medaka (*Oryzias javanicus*). Chemosphere (Under reviewing)

3. Takai Y., Tominaga A., Honda M, Qiu X., Shimasaki Y., Kang I.J, Oshima Y., Combined effect of anthracene and polyethylene microplastics on Java medaka (*Oryzias javanicus*). Ecotoxicology. (2023.3.31, submit)

4. Takai Y., Honda M., Qiu X., Shimasaki Y., Kang I.J., Oshima Y., Concentration effect of polystyrene microplastics on the accumulation of anthracene for Java medaka (*Oryzias javanicus*). Mar poll bull (2023.4, submit)

***Title of Research:***

22-6-02

# Development of an objective read-across method based on statistical and mathematical sciences for evaluation of repeated-dose toxicity


***Principal Investigator:***

Kouichi Yoshinari (School of Pharmaceutical Sciences, University of Shizuoka)
52-1 Yada, Suruga-ku, Shizuoka 422-8526 Japan. E-mail: yoshinari@u-shizuoka-ken.ac.jp

***Collaborators:***

Jun-ichi Takeshita (Research Institute of Science for Safety, National Institute of Advanced Industrial Science and Technology (AIST))
Tomomichi Suzuki (Department of Industrial Administration, Tokyo University of Science)



***Summary of Research:***

Repeated-dose toxicity (RDT) test is important for chemical safety evaluation. Currently, it is highly recommended to develop alternatives to rat RDT tests, but it remains very difficult to develop such a method largely due to the diversity and complexity of the toxicity. The aim of this project is to develop an objective read-across method, in which the toxicity of an untested substance is predicted from the toxicity information of similar substances (called “source substances”), using chemical information and in vitro assays as an alternative to rat RDT tests. In this year, we selected test substances from HESS and REACH databases and set 8 group endpoints (6 hepatotoxicity- and 2 hematotoxicity-related) as targets. Studies were conducted on criteria for judging the presence or absence of toxicity of test substances from source substances, molecular descriptors to be used for similarity assessment, and methods for selecting source substances. Results obtained indicate that the criteria for judging the toxicity of test substances should be adjusted based on the positive rate of the group endpoints, source substances differ depending on the type of inter-substance distances used, the descriptors should be selected by an appropriate method, and the accuracy of toxicity prediction is improved by appropriately selecting descriptors.


***Timeline:***

March 1, 2022 - February 28, 2023

***Topics:***

Publication #1 (Yu Harakawa) received Presentation Award for Students at the 35th annual meeting of the Japanese Society for Alternatives to Animal Experiments.

***Publications:***


1. Y Harakawa, N Omura, J Takeshita, R Shizu, T Hosaka, Y Kanno, K Yoshinari. Development of a read-across method for evaluating repeated-dose toxicity using molecular descriptors: Examination of methods of selecting molecular descriptors for better prediction. The 35th annual meeting of the Japanese Society for Alternatives to Animal Experiments. Shizuoka, Japan, 2022.11.18-20.
2. K Yoshinari. Evaluation of developmental neurotoxicity of chemical substances by a read-across method using chemical structure information. The 35th annual meeting of the Japanese Society for Alternatives to Animal Experiments. Shizuoka, Japan, 2022.11.18-20.
3. K Yoshinari. Safety assessment of chemical compounds by in vitro and in silico methods: challenges and future prospects. ILSI Japan symposium: Alternatives to animal experiments in the food science area -current situation, trends and initiatives for the future. Online, 2023.2.3.
4. Y Harakawa, N Omura, R Shizu, T Hosaka, J Takeshita, K. Yoshinari. Examination of the selection method of molecular descriptors for the evaluation of repeated-dose toxicity using a read-across approach. The Society of Toxicology 62nd Annual Meeting and ToxExpo. Nashville, TN, USA. 2023.3.19-23.

***Title of Research:***

22-D-01 Elucidation of the mechanism and rate of microplastic formation contributing to risk assessment and preparation of standard 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:***

Kuriyama Takashi (Graduate School of Organic Materials and Systems Science, Yamagata University), 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) , Ohtani Hajime (Graduate School of Engineering, Nagoya Institute of Technology), Kouzai Hiroaki (Department of Science and Engineering, Kanto Gakuin University), Kawai Takahiko (Graduate School of Science and Engineering, Gunma University)

***Summary of Research:***

MP collected in the lower reaches of the Arakawa River were sorted and observed. The MP pellets were ball-milled with glass beads. The results showed that as the size of the MPs decreased, they became more spherical in shape. All MPs that were surface peeled off in the ball milling test were less than 1 mm in diameter, indicating that the MPs changed from flaky MPs to particle-like MPs. This is interpreted to indicate that the formation mechanism of MPs is similar to that of rocks converted to gravel by river transport (i.e., collapse by mechanical action).

The iPP sheets were exposed to high temperatures, and the results showed that the thermally deteriorated sheets had localized bleaching and yellowing in the peripheral areas, while a healthy area remained in the central part. In the bleached areas, cracks were formed due to shrinkage caused by chemical crystallization and propagated in one direction, forming a columnar structure. In the yellowing area, chemical crystallization progressed further and some of the cracks collapsed to form fine powder of about 0.2 mm in diameter.

Dumbbell pieces punched from PET bottles, PE sheets, and PP sheets were exposed to a xenon weather meter, and the results showed that photo-oxidation degradation progressed significantly in PP, with a marked decrease in molecular weight and mechanical properties, and cracks appeared and developed. The molecular weight of PET also decreased, and cracks appeared on the surface in a mosaic-like pattern, and some of the cracks appeared to be peeling off and collapsing.

Based on the above findings, it is considered that the collapse and exfoliation of the "weathering layer" generated by the cracks that occur with the degradation of plastics are deeply related to the generation of MP. The formation mechanism of this "weathering layer" can be explained by a pattern formation in which shrinkage propagates, as is the case with dry cracks.

The aerosols were classified and collected for analysis by pyrolysis GC/MS, and the pyrolysis GC/MS allowed the identification and quantification of atmospheric MPs without any special pretreatment. The fact that no pretreatment is required is also important to prevent contamination.

On the other hand, the use of a blade-type jet mill with many blades in a swirling stream of compressed air, which is an improvement over conventional jet mills, has made it possible to reduce various plastics to near-spherical particles of approximately 10 μm in diameter. Furthermore, UV irradiation of atomized plastics in a mixed solvent of acetone and alcohol enabled efficient surface oxidation.

***Timeline:*** March 2022-.

***Topics:*** Online presentation at the 2022 LRI Research Report Meeting

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

1) Shinichi Kuroda, ” Elucidation of the formation mechanism of microplastics”, Microplastics Advance Research and Innovation Initiative (MARII) Workshop on “Advancements and steps towards a holistic, quantitative risk assessment on microplastics”, 12 - 13 October 2022 (Online).

# LRI Annual Report 2022


**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