LRI

Long-range Research Initiative

Annual Report 2023

2023

Japan Chemical Industry Association

Annual Report 2023

Title of Research:

21-2-01 Development of evaluation method of inflammatory particles based on alveolar macrophage function.

Principal Investigator:

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Collaborators:

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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. In this study, we were focused on alveolar macrophages that engulf and excrete inhaled particles, and we aim to develop methods of evaluation of inflammatory particles focusing on alveolar macrophage functions in response to particles.

Recently, animal-free toxicity testing is recommended in these days, so we tried to develop in vitro evaluation methods using alveolar macrophage cell lines, and cell line ALV3 was we successfully obtained. Then single cell cloning was performed and a stable cell line, ALV3.7, was generated. Next, we examined the expression of surface marker, the responses to inflammatory particles, and comprehensive analysis of gene expression, and found that enhanced gene expression regarding DNA replication and cell cycle were observed. However, ALV3.7 was thought to be maintained the characteristics of alveolar macrophages. In vitro analysis revealed that inflammatory particles stimulated ALV3.7 to induce cell death and subsequent release of intracellular IL-1α, confirmed by live cell imaging analysis. Furthermore, these particles induced allergic inflammation in mice after the airway sensitization, suggesting that in vitro responses of ALV3.7 reflect to the biological responses in vivo, especially allergic responses.

In conclusion, we have established the alveolar macrophage cell line, ALV3.7 as a useful tool for in vitro assessment of inflammatory particles. In addition, ALV3.7 can be expected to be applied to various chemical evaluation methods without using animals.

Timeline:

March 1, 2023—February 29, 2024

Topics:

Research meeting of LRI by JCIA, oral, “Development of evaluation method of inflammatory particles based on alveolar macrophage function”, on-site/online, August 25, 2023.

Publications:

Adachi, T., Inoue, H., Izumi, H. and Kuroda E. “Establishment of a functional alveolar macrophages cell line” The 30th Annual Meeting of the Japanese Society of Immunotoxicology, Kanagawa, 2023.9.11.

Kuroda Etsushi “The effect of chemical properties of particulate matter on alveolar macrophage activation" Consortium of Metal Biosciences 2023 Symposium, Gifu, 2023.10.

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:

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Summary of Research:

The skin is an exposure site for various chemical substances, and predicting the skin permeation rate of a chemical substance is very important for evaluating the safety of the exposed chemical substances. In general, in vitro skin permeation test with excised skin have been applied to estimate skin permeation rates and skin concentrations based on exposure scenarios. On the other hand, since animal testing of all cosmetic products has been banned internationally, it is necessary to establish animal-free testing methods, including systemic toxicity evaluation. Thus, there has been increasing interest in the development of methods to predict transdermal absorption using in silico models. Skin permeability can be expressed by Fick's diffusion law. Therefore, it will be possible to estimate skin permeability when parameters such as diffusion coefficient in skin and partition coefficient to skin of chemical substances can be predicted. In this study, we developed a method to estimate permeation parameters obtained from human skin based on physicochemical properties of chemical substances. Further developed an in silico skin permeation model based on scenarios of dermal exposure to chemical substances and cosmetic formulations. In addition, a software was developed that can easily predict skin permeation by simply entering the structure of the chemical substance with SMILS, the area of application, the concentration applied, and the thickness of the skin. As a result, constructed model was able to accurately predict the amount of chemical substances permeated through the skin with simple web browser-based software.

Timeline:

March 1, 2023- February 29, 2024

Topics:

LRI Annual Workshop（August 25, 2023）
Establishment of in silico model to predict skin absorption of chemical compounds with two-layered diffusion model

LRI
Long-Range
Research Initiative

Publications:

Title of Research:
XX-XX-XX

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

Principal Investigator:
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Summary of Research:
In this study, a real-time luminescence measurement system was introduced to improve the accuracy of the kinetic assay, which has been a hallmark of the signal disruption assay. Although this system enabled continuous measurements over a 3-day period and captured more detailed changes in developmental toxicity than conventional manual measurements, it also raised several issues. The measurement system revealed the edge effect of the culture plate and experimental errors in each run. Therefore, a protocol to reduce these problems was investigated in detail and reconstructed in this study. This includes optimization of the pre-culture schedule, reduction of evaporation of culture medium, and concentration of luminescent substrates. In the Wnt signal disruption test, we prepared Wnt signal reporter cells and examined for the signal disruption assay. In addition, we tested Wnt ligands to FGF/SRF signal reporter cells. In particular, the latter showed that intracellular signaling network links may be responsible for the high probability of teratogen detection in this assay, suggesting that the number of signaling pathways in the future battery assay could be reduced.

Timeline: March 1, 2023 –

Topics:
Y. Okubo and J. Fukuda, Evaluation of developmental toxicity analyzing by dynamics of signal disruption, Seikagaku Mini Review, 95, 2, 1-6, 2023, Doi: 10.14952/SEIKAGAKU.2023.95

Presentation Award, K. Mizota, Y. Okubo, M. Shibata, R. Ohara, Y. Nakajima, J. Fukuda: Developmental toxicity testing of chemicals based on long-term signal disruption using human iPS reporter cells, 61st Japanese Society for Artificial Organs, Nov. 9-11, Tokyo

Publications:
Invited talk. Y. Okubo: Developmental toxicity detection via dynamics of FGF-SRF signal disruption in human iPSC-based assay. 13TH Global Summit on Regulatory Science (GSRS23) In-Person Annual conference. Sep. 27, 2023.

Y. Okubo, K. Mizota, M. Shibata, R. Ohara, S. Kitajima, Y. Hirabayashi, Y. Nakajima, J. Fukuda: Developmental toxicity test using human iPS cells based on signal disruptions induced by chemical substances. 12th World Congress on Alternatives and Animal Use in the Life Sciences (WC12), Niagara Falls, Canada. (Aug. 30, 2023). Oral presentation.

K. Mizota, Y. Okubo, M. Shibata, R. Ohara, S. Kitajima, Y. Hirabayashi, Y. Nakajima, J. Fukuda: Developmental toxicity testing of chemicals based on long-term signal disruption using human iPS reporter cells. 12th World Congress on Alternatives and Animal Use in the Life Sciences (WC12), Niagara Falls, Canada. (Aug. 30, 2023). Poster presentation.

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: Physiologically based pharmacokinetic (PBPK) modelling can be used to evaluate internal exposure in humans without any reference to experimental data. The input parameters for PBPK models (i.e., fraction absorbed × intestinal availability, absorption rate constants, volumes of the systemic circulation, and hepatic intrinsic clearances) were estimated for a panel of ~350 chemicals using a light gradient boosting machine learning algorithm (LightGBM) based on within 30 *silico*-calculated chemical descriptors. The parameters for the human and rat PBPK models for a diverse range of compounds were successfully estimated using chemical descriptors. Significant inverse relationships between the hepatic/plasma concentrations of selected lipophilic food chemicals using forward dosimetry and reported rat hepatic lowest-observed-effect level (LOEL) values were observed. The output values from rat pharmacokinetic models based on *in silico* liver-to-plasma partition coefficient values derived from the primary Poulin and Theil model can be used to estimate toxicokinetics or internal exposure to substances. This approach to pharmacokinetic modeling has the potential for application in computational toxicology and in the clinical setting for assessing the potential risk of general chemicals.

Timeline: From March 1, 2023 to February 29, 2024

Publications:

1) Adachi K, Nakano H, Sato T, Shimizu M, Yamazaki H. Liver and Plasma Concentrations of Food Chemicals after Virtual Oral Doses Extrapolated Using in Silico Estimated Input Pharmacokinetic Parameters to Confirm Reported Liver Toxicity in Rats. *Biol Pharm Bull*, **46**, 1133-1140 (2023).
2) Adachi K, Ohyama K, Tanaka Y, Nakano H, Sato T, Murayama N, Shimizu M, Saito Y, Yamazaki H. Plasma and Hepatic Exposures of Celecoxib and Diclofenac Prescribed Alone in Patients with Cytochrome *P450 2C9*3* Modeled after Virtual Oral Administrations and Likely Associated with Adverse Drug Events Reported in a Japanese Database. *Biol Pharm Bull*, **46**, 856-863 (2023).
3) Adachi K, Utsumi M, Sato T, Nakano H, Shimizu M, Yamazaki H. Modeled Rat Hepatic and Plasma Concentrations of Chemicals after Virtual Administrations Using Two Sets of in Silico Liver-to-Plasma Partition Coefficients. *Biol Pharm Bull*, **46**, 1316-1323 (2023).
4) Adachi K, Ohyama K, Tanaka Y, Sato T, Murayama N, Shimizu M, Saito Y, Yamazaki H. High hepatic and plasma exposures of atorvastatin in subjects harboring impaired cytochrome *P450 3A4*16* modeled after virtual administrations and possibly associated with statin intolerance found in the Japanese adverse drug event report database. *Drug Metab Pharmacokinet*, **49**, 100486 (2023).
5) Shimizu M, Uehara S, Ohyama K, Nishimura H, Tanaka Y, Saito Y, Suemizu H, Yoshida S, Yamazaki H. Pharmacokinetic Models Scaled-up from Humanized-liver Mouse Data Can Account for Drug Monitoring Results of Atomoxetine and Its 4-Hydroxylated and N-Demethylated Metabolites in Pediatric Patients Genotyped for Cytochrome *P450 2D6*. *Drug Metab Dispos*, **52**, 35-43 (2024).

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:

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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:

In the current fiscal year, we first established the passive dosing method. Ant adsorption from the Ant-adsorbed O-ring to MP was more than twice as high as that of the conventional method, which involved dissolving Ant in a solvent and adsorbing it onto MP. Consequently, with the Ant-adsorbed MP created using this method, in vitro tests simulating the gastrointestinal environment became possible.

Next, we investigated the cause of the vector effect of MP using simulated intestinal in vitro tests. As a result, the concentration and proportion of Ant eluted from MP in the intestinal washout fluid were approximately three times higher in the High_conc group compared to the control group. In simulated intestinal fluid containing taurocholic acid, the concentration of Ant eluted from MP was about 10 times higher than in the control. This mechanism is attributed to the surface-active properties of taurocholic acid, a major component of bile, which led to significant adsorption and desorption of Ant on MP. Therefore, the vector effect of MP on Ant is explained by the enhanced desorption due to the surface activity of bile in the gastrointestinal tract, resulting in increased absorption of Ant.

In the Phe+PE-MP co-exposure test, the aqueous-phase Phe concentration in the Phe+MP group was approximately 30% lower than in the Phe group, while there was no difference in Phe concentrations between the fish body Phe group and the Phe+MP group. Analyzing the

results, the elimination rate constants (k2) were 0.133 and 0.136, showing no significant difference between the two groups. The bioconcentration factor (BCF) was calculated to 2823, and the uptake clearance (k1) was determined to be 375. We estimated the internal concentration using a one-compartment model using these values and the aqueous-phase Phe concentration in the Phe+MP group (average = 0.102 mg/L). As a result, the Phe taken up from the aqueous phase in the fish body in the Phe+MP group contributed only about two-thirds of the internal concentration, demonstrating the vector effect. Considering that Phe adsorbed to MP at a concentration of 2.63 mg/g MP, the desorption (vector effect) of Phe adsorbed to MP is possible. The results of the 5.3 PaHs exposure experiment also suggest a vector effect for Phe.

In the preliminary experiment of the one-day co-exposure of Japanese medaka (Himedaka) with PaHs+PE-MP, none of the PaHs showed good dissolution in water due to the absence of surfactant additives. However, we performed calculations. In the Acy, Ace, Phe, and Ant MP co-exposure groups, an increase in the internal concentration of each PaH was observed, indicating a strong vector effect for these PaHs. However, Flu, Flt, Pyr, BaA, Chr, BbF, BkF, BaP, BghiP, Da, and IP were not detected in the fish body, likely due to their high log Kow, making desorption from MP difficult and resulting in no vector effect.

In future work, we will verify the vector effect using Japanese medaka exposed to MP and different PaHs or CBs with significantly different log Kow values for 10 days. Simultaneously, we will conduct in vitro desorption tests simulating the intestinal tract to investigate the vector effect further.

Timeline:

March 1, 2023- Feb28, 2024

Topics:

Publications:

1. Takai, Y., Tominaga, A., Honda, M., Qiu, X., Shimasaki, Y., Kang, I.J., Oshima, Y., 2023. Combined effect of anthracene and polyethylene microplastics on swimming speed and cytochrome P4501A monooxygenase expression of Java medaka (*Oryzias javanicus*). Ecotoxicology. https://doi.org/10.1007/s10646-023-02700-4
2. Takai, Y., Tominaga, A., Uchida, Y., Honda, M., Qiu, X., Shimasaki, Y., Oshima, Y., 2023. Size effect of polystyrene microplastics on the accumulation of anthracene for Java medaka (*Oryzias javanicus*). Chemosphere 338, 139543. https://doi.org/10.1016/j.chemosphere.2023.139543

3. Takai et al., Concentration effect of polystyrene microplastics on the accumulation of anthracene for Java medaka (*Oryzias javanicus*). Chemosphere (2024.4，投稿予定)
4. Md Al-Emran et al., No vector effects of polystyrene microplastics (PS-MP) on the accumulation chlorobenzenes (CBs) in Japanese Medaka (*Oryzias latipes*). Chemosphere (2024.4，投稿予定)
5. Md Al-Emran et al., Effect of polyethylene microplastics concentration on the accumulation of co-exposed anthracene for Japanese medaka, *Oryzias latipes.* Chemosphere. (2024.5，投稿予定)

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:

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Collaborators:

Jun-ichi Takeshita (National Institute of Advanced Industrial Science and Technology (AIST))
Tomomichi Suzuki (Tokyo University of Science)

Summary of Research:

Repeated-dose toxicity tests are important for chemical safety evaluation. 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, using chemical information and in vitro assays, as an alternative to rat repeated-dose toxicity tests. This year, we evaluated a two-step read-across method using molecular descriptors and in vitro test results for hepatotoxicity and hematotoxicity. Initially, 96 substances were selected as test substances, and 17 or 74 neighbor substances were chosen based on chemical descriptor-based Euclid distances between substances. Among them, the substances that matched the results of toxicity-related in vitro tests were used to predict the toxicity of the test substances for each endpoint. Finally, the prediction accuracy was verified with in vivo test results. The results demonstrated that some substances' toxicity was accurately predicted using in vitro tests, while the selection based on in vitro test results had a negative impact on the toxicity predictions of some substances. Next year, we will analyze the results to identify useful tests and endpoints and validate them through case studies. In addition, we will explore the utility of comprehensive gene expression data for read-across.

Timeline:

March 1, 2023 - February 29, 2024

Topics:

None

Publications:

1. S Kaito, M Iwata, T Hosaka, R Shizu, J Takeshita, K Yoshinari: Development of a read-across method for predicting drug-induced liver injury. 50th Annual Meeting of the Japanese Society of Toxicology, June 19th-21st, 2023, Yokohama.
2. Y Harakawa, J Takeshita, R Shizu, T Hosaka, K Yoshinari: Development of a read-across method for evaluating repeated-dose toxicity using molecular descriptors and in vitro test data. id.
3. K Yoshinari: Development of an objective read-across method for the evaluation of systemic toxicity of chemical substances. id.
4. K Yoshinari, Y Shimizu, T Sasaki, T Hosaka, R Shizu, J Takeshita: The association between cytochrome P450 inhibition and hepatotoxicity of chemical compounds. The 15th International Symposium on Cytochrome P450 - Biodiversity and Biotechnology, July 27th-30th, 2023, Copenhagen, The Netherlands.
5. S Kaito, M Iwata, T Hosaka, R Shizu, K Yoshinari: Prediction of drug-induced liver injury using read-across method, 69th Pharmaceutical Society of Japan Tokai Branch Meeting, July 8, 2023, Nagoya.
6. S Utsumi, T Suzuki, J Takeshita, S Itaka: Repeated-dose Toxicity Evaluation based on Similarity indices using molecular descriptors, 46th Chemo-informatics Workshop, November 22, 2023, Tokyo.

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:

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Summary of Research:

The purpose of this research is quantitative elucidation of the formation mechanism of microplastics under natural environments and development of a method for the preparation of “reference” microplastics for risk assessment. We conducted precise analyses of plastics degraded in the natural environment by outdoor exposure tests, and we found that photo-oxidation reactions induce crystallization in the weathering layer at a depth of 50-250 μm from the surface, which leads to slow crack growth inside the material owing to volume shrinkage. Kinetic analysis using artificially degraded materials by accelerated tests revealed that microplastics of about 0.2 mm in diameter are slowly formed over several years by similar mechanisms. We compared the artificially accelerated test and outdoor exposure test, which generally gave different results, we found that hydrolysis in the presence of water is important for PET. A method of analysis for airborne microplastics was also developed. Based on the microplastic formation mechanism, a method for preparing reference microplastics was developed, and the “reference” microplastics of low-density polyethylene in a size of 10, 30, and 100 μm were provided for risk assessments.

Timeline:

March 1, 2022-.

Topics:

July 14, 2023: Cite visiting at Kanazawa University
Aug 25, 2023: Oral presentation at 2023 LRI Research Report Meeting

Publications: (Only PI)

An oral presentation is scheduled in Sep. 2024; Y. Hiejima, “Formation of parallel cracks driven by chemicrystallization and subsequent fragmentation into microplastics”, 11th Conference of the Modification, Degradation, Stabilization of Polymers Society (MoDeSt2024)
To be submitted to Polymer Degradation and Stability; Y. Hiejima *et al.*, “Parallel crack formation in thermal degradation of isotactic polypropylene and subsequent spontaneous fragmentation into microplastics”

Title of Research:

23-1-03
Development of an alternative method for teratogenicity using zebrafish

Principal Investigator:

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Junichi Tasaki (Senior Scientist, Safety Science Research, Kao Corporation)
Shujie Liu (Scientist, Safety Science Research, Kao Corporation)

Summary of Research:

We performed whole genome sequencing of 15 zebrafish strains including AB, TU, RW and WIK and determined genetic tree. We used these strains for developmental toxicity tests and found that there were no strain difference of susceptibility to chemicals. In addition to teratogenicity, which is evaluated by appearance, gene expression changes by RNA-Seq were also examined as a hazard identification. We confirmed that the differences by strains were smaller than the differences by exposure concentration. These results suggest that it is not necessary to use specific strain when conducting developmental toxicity tests using zebrafish and that any strain can be used in the tests. We also found a correlation between changes in gene expression and teratogenicity that could explain the characteristics of teratogenicity. This suggests the possibility of using gene expression changes by RNA-Seq as a useful strategy to identify the AOP of developmental toxicity.

Timeline:

March 1, 2023 -

Topics:

1. "Development of an alternative method for teratogenicity using zebrafish" at the 2023 LRI Research report meeting of the Japan Chemical Industry Association. August 25, 2023.

Publications:

1. Kenichiro Sadamitsu, Fabien Velilla, Minori Shinya, Makoto Kashima, Yukiko Imai, Toshihiro Kawasaki, Kenta Watai, Miho Hosaka, Hiromi Hirata and Noriyoshi Sakai. Establishment of a zebrafish inbred strain, M-AB, capable of regular breeding and genetic manipulation. Scientific Reports. (Under revision)
2. Hiromi Hirata. Investigation of strains in toxicity testing of chemicals using zebrafish. Japan Pharmaceutical Manufacturers Association "Current status of alternative methods for reproductive and developmental toxicity" Symposium. Nihonbashi life science building, Tokyo. February 8, 2024. (Invited symposium speaker)
3. Kota Ujibe, Makoto Kashima, Rintaro Shimada, Masanari Okamoto, Seiji Wada, Hiroki Matsuda, Akira Sakamoto and Hiromi Hirata. Zebrafish lacking Werner syndrome gene wrn cause early nutritional deficiency. The 9th Zebrafish and Medaka-based Drug Discovery Meeting. MEXT research exchange center, Tsukuba. November 6, 2023. (Oral presentation)
4. Kenichiro Sadamitsu, Minori Shinya, Makoto Kashima, Noriyoshi Sakai and Hiromi Hirata. Establishment and whole genome analysis of zebrafish inbred strain M-AB," The 96th Annual Meeting of the Japanese Biochemical Society. Fukuoka International Conference Hall, Fukuoka. November 1, 2023. (Poster presentation)

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

Principal Investigator:
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Collaborators:
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Gomez Christopher (Professor), Graduate School of Maritime Sciences, Kobe University
Akira Ijiri (Associate Professor), Graduate School of Maritime Sciences, Kobe University
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Summary of Research:
We investigated microplastic residues in the sea surface water of Osaka Bay area, in order to set the microplastic exposure conditions. As a results, plastic particles accounted for only 22.4% of particles. Microplastic pollution values in the Osaka Bay (range abundance of 7.6 to 61.4 particles/L) is consistent with previous reports in other semi-enclosed bays. Fragments appeared in the size less than 100 μm, fibers had much smaller sizes in the range 10–30 μm, films appeared in the size range 34–409 μm, and beads appeared in the size around 30 μm. Atmospheric transport plays a critical role in the deposition and accumulation of microplastics in marine environments. Therefore, we aimed to investigate the abundance of microplastics in rainwater outfalls from five prefectures (Hokkaido, Akita, Fukuoka, Hiroshima, and Hyogo) across Japan The overall microplastics concentrations ranged from 379 to 1,790 particles/L.

Fish are thought to employ color vision to detect microplastics, prompting our investigation into microplastic ingestion patterns in three marine fish species, *Chrysiptera cyanea, Hypoatherina tsurugae,* and *Plotosus japonicus*, and three freshwater fish species, *Rhodeus ocellatus*, *Pseudorasbora parva*, and *Misgurnus anguillicaudatus*. Notably, *C. cyanea*, *P. japonicus*, and *R. ocellatus* exhibited color preferences in microplastic ingestion, with *C. cyanea* favoring red, *P. japonicus* preferring blue and gray, and *R. ocellatus* favoring red and yellow.

Finally, using *D. magna* (crustacean) and zebrafish (fish), we investigated whether the time required to expel microplastics varies when ingestion occurs through a food-chain. As a results, we found that microplastics excretion occurs within 48 h and 24 h for direct ingestion and ingestion through food chain (via *D. magna*), respectively.

Timeline:
March 1, 2023

Topics:
2023 年度 日化協 LRI 研究報告会にて発表「閉鎖性海域 大阪湾をモデルケースにした MP の生態リスク評価」
セタックジャパン ミニシンポジウムにて発表「Abundance of microplastics in a semi-enclosed Osaka Bay area -Ecotoxicological risk assessment of microplastics-」

Publications:
第 26 回日本水環境学会シンポジウムにて発表「閉鎖性海域 大阪湾をモデルケースにした MP の生態リスク評価」
令和 5 年度 海洋プラスチックごみ学術シンポジウムにて発表「「食う−食われる」過程を介した マイクロプラスチックの排出時間の変化」
Horie Y, Mitsunaga K, Yamaji K, Hirokawa S, Uaciquete D, Ríos JM, Yap CK, Okamura H.　Variability in microplastic color preference and intake among selected marine and freshwater fish and crustaceans,Discover Ocean, 1(5), 2024.
Horie Y, Uaciquete D, Mitsunaga K, Akkajit P, Ríos JM, Naija A. Food chain-mediated variation in excretion times of microplastics: Unraveling the interactions with plasticizers. Regional Studies in Marine Science. 69, 103343, 2024.

Title of Research:

XX-XX-XX

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), Research Institute of Science for Safety and Sustainability (RISS))

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), Yuichi Iwasaki (AIST, RISS), 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 2023 -

Topics:

JCIA-LRI Annual Workshop “Assessing sources, emissions and environmental risk of microplastics in support of effective risk reduction strategies”, August 2023

Publications:

Ono K, Naito W, Ogura I, Xue M, Kato E, Uesaka M, Tsunemi K (2023). Estimation of microplastic emission and transfer into Tokyo Bay, Japan, using material flow analysis. *Marine Pollution Bulletin.* 194. 115440. 10.1016/j.marpolbul.2023.115440

Iwasaki Y, Takeshita K.M, Ueda K, Naito W. Estimating species sensitivity distributions for microplastics by quantitively considering particle characteristics using a recently created ecotoxicity database. *Micropl.&Nanopl.* 3, 21 (2023). https://doi.org/10.1186/s43591-023-00070-6

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:

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Summary of Research:

In Japan, risk assessments of chemical are carried out based on the Chemical Substances Control Law. The risk assessments of difficult-to-test substances such as UVCB, which is a complex mixture of multiple substances, and cationic surfactants whose toxicity varies depending on water quality, have begun or is about to begin. Metals were once thought to be difficult-to-assess material because their toxicity varies depending on water quality. After the arise of the concept of "bioavailability", it was accepted that the varying toxicity of metals could be predicted with considerable accuracy, and now the ecological risk assessments are carried out with the concept. The toxicities of cationic surfactant also vary depending on the water quality; however, their characteristics of the toxicity variation depending on the water quality are similar to those with metals, and hence there is a possibility that we can predict such the varying toxicities by the bioavailability concept as well. The aim of this research is to quantitatively understand the toxicities of cationic surfactants, and to develop a model that predicts the toxicities to support the ecological assessments for such substances.

This year, we discussed: [1] effects of surfactants on water quality (hardness, ion concentration, etc.), [2] effects of surfactants on the available amount of cationic metals, [3] roles of DOC (humate) on the bioavailability of surfactants and [4] roles of algae of which toxicities will be investigated on the bioavailability of surfactant was investigated.

We found that [1] surfactants do not significantly change hardness or cation concentrations, [2] although further investigations remain, surfactants may not alter the bioavailability of cationic metals except a specific metal, [3] DOC alters the bioavailability of surfactants, thus the addition of DOC decreases the toxicity of the surfactants and [4] it seems to be unlikely that the presence of algae alter the bioavailability of surfactants.

Construction of a model for predicting the amount of bioavailability of the surfactants has also begun, and a preliminary model was developed in the limited water quality. The model will be developed next year to predict the toxicity of surfactants in various water qualities.

Timeline:

March, 1, 2023 -

Topics:

Oral presentation at the 2023 LRI research report workshop by JCIA (Tokyo)

Publications:

Title of Research:

23-6-03

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

Principal Investigator:

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Summary of Research:

The aim of this research is to finalize the AOPwiki entry for AOP475 proposed to the OECD. During this term, we participated in AOP coaching and incorporated intracellular Ca^{2+} overload, dendritic spine abnormality, and decreased neuronal network function into the Key Event (KE). We are currently gathering literature to link KE1 to KE4 and examining papers to connect KE4 to KE6. Experiments utilizing frozen rat neurons for primary culture and live rats were carried out to demonstrate the relationship between risk compounds of Impairment of Learning and Memory (AO) and Loss of Drebrin (KE3). Nine compounds were administered to cultured neurons, fixed, and immunocytochemically stained for drebrin and MAP2. Neuronal cell death was observed after 1 and 7 days of treatment with Compound A, but not at 1 hour. However, the number of high-intensity drebrin clusters was significantly reduced even at 1 hr. After a single oral dose of Compound B and Compound C to pregnant rats on gestation day 15, changes in drebrin expression were detected in the hippocampus and neocortex of the offspring (6 weeks). Changes in localization and expression of drebrin are useful indicators for risk assessment of compounds.

Timeline:

March 1, 2023 –

Topics:

Oral presentation at LRI research report meeting (25th Aug. 2023)

Publications:

[Original paper] Lin Waka, Shiomoto Shusaku, Yamada Saki, Watanabe Hikaru, Kawashima Yudai, Eguchi Yuichi, Muramatsu Koichi, Sekino Yuko “Dendritic spine formation and synapse maturation in transcription factor-induced human iPSC-derived neurons.” iScience. 26(4):106285, 2023, and another publication

[Conference presentation] Yuko Sekino, Izuo Tsutsui, Tomoaki Shirao, Shihori Tanabe “Impairment of learning and memory via loss of drebrin from dendritic spines of neurons.” The 97th Annual Meeting of the Japanese Pharmacological Society, Kobe, December 2023, and five other publications.

【Lecture】Yuko Sekino, "Actin cytoskeleton in synapses controls memory," The 46th Annual Meeting of the Japanese Neuroscience Society, Luncheon Seminar, Sendai, August 1, 2023.

LRI Annual Report 2023

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