LRI

Long-range Research Initiative

Annual Report 2024

2024

Japan Chemical Industry Association

Annual Report 2024

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:
The aim of this study was to develop an in vitro assay (DynaLux/c) that can predict human developmental toxicity, in order to solve the problems of current developmental toxicity testing methods, such as the low accuracy of predictions for humans due to interspecies differences. Focusing on the FGF signaling pathway, which plays an important role in the developmental process, we proposed a testing system that evaluates developmental toxicity by detecting the disruption of the FGF signaling pathway by chemical substances using human iPS cells. In this study, real-time luminescence measurement was introduced to overcome the problem of manual luminescence measurement in this assay, and it became possible to monitor signal disruption for longer periods of time. We demonstrated the ability of this approach to determine 19 known developmental/non-developmental toxicants with 100% accuracy. Reporter iPS cells for Wnt, BMP, and activin signals did not yield high luminescence signals. FGF reporter cells activated with Wnt ligands showed potential for detection of developmental toxicants. Time-lapse RNA-seq analysis may elucidate the link between signal disruption and developmental toxicity, but further studies will be needed.

Timeline:
March 1, 2024 – February 28, 2025

Topics:
Presentation at the 2024 JCIA LRI Research Report Meeting, “Establishment of Human iPSC Reporter-Based Developmental Toxicity Assay that Detects FGF Signal Disruption”

Publications:
Yusuke Okubo, Yoko Hirabayashi, Junji Fukuda, Advances in Genomic Toxicology: In vitro Developmental Toxicity Test based on Signal Network Disruption Dynamics, Current Opinion in Toxicology, 39, 100489 (2024) doi.org/10.1016/j.cotox.2024.100489

Kashu Mizota, Rintaro Ohara, Rieko Matsuura, Yoko Hirabayashi, Yoshihiro Nakajima, Yusuke Okubo, Junji Fukuda, Developmental Toxicity Assessment Using Human iPSCs by Automated Measurement of FGF Signaling Disruption, 58th Congress of the European Societies of Toxicology, Copenhagen, Denmark, poster, 2024.09.08-11

Okubo Yusuke, Mizota Kashu, Matsuura Rieko, Hirabayashi Yoko, Nakajima Yoshihiro, Fukuda Junji: in vitro developmental toxicity testing based on real-time monitoring for signal disruption. 58th Congress of the European Societies of Toxicology, Copenhagen, Denmark, poster, 2024.09.08-11

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), 3-2-1, 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 systemic circulation, and hepatic intrinsic clearances) were estimated for a panel of approximately 350 chemicals. These parameters of the human and rat PBPK models for a diverse range of compounds were successfully estimated using a light gradient boosting machine learning algorithm (LightGBM) based on < 30 *silico*-calculated chemical descriptors. This approach to pharmacokinetic modeling has the potential for application in computational toxicology and in clinical settings to assess the potential risk of drugs and general chemicals. Based on estimating interspecies toxicokinetics or internal exposures of lipophilic food components after oral doses in humans, this approach, which applies simple PBPK modeling with no reference to experimental pharmacokinetic data, has the potential to play a significant role in the extrapolation of reported liver toxicity levels in rats to humans.

Timeline: From March 1, 2024 to February 28, 2025

Publications:

1) Adachi K, Shimizu M, Shono F, Funatsu K, Yamazaki H. Octanol/water partition coefficients estimated using retention times in reverse-phase liquid chromatography and calculated *in silico* as one of the determinant factors for pharmacokinetic parameter estimations of general chemical substances. *J Toxicol Sci*, **49**, 127-137 (2024).

2) Adachi K, Sasaki T, Arai A, Shimizu M, Yamazaki H. Impact of variability of *in silico* and *in vitro* octanol/water partition coefficients of compounds on the input parameters and results of simplified human physiologically based pharmacokinetic models after virtual oral administrations. *J Toxicol Sci*, **49**, 459-466 (2024).

3) Adachi K, Ohyama K, Tanaka Y, Saito Y, Shimizu M, Yamazaki H. Modeled Hepatic/Plasma Exposures of Fluvastatin Prescribed Alone in Subjects with Impaired Cytochrome P450 2C9*3 as One of Possible Determinant Factors Likely Associated with Hepatic Toxicity Reported in a Japanese Adverse Event Database. *Biol Pharm Bull*, **47**, 635-640 (2024).

4) Adachi K, Ohyama K, Tanaka Y, Murayama N, Shimizu M, Saito Y, Yamazaki H. Modeled Hepatic/Plasma Exposures of Omeprazole Prescribed Alone in Cytochrome P450 2C19 Poor Metabolizers Are Likely Associated with Hepatic Toxicity Reported in a Japanese Adverse Event Database. *Biol Pharm Bull*, **47**, 1028-1032 (2024).

5) Adachi K, Hosoi M, Shimura Y, Shimizu M, Yamazaki H. Reported liver toxicity of food chemicals in rats extrapolated to humans using virtual human-to-rat hepatic concentration ratios generated by pharmacokinetic modeling with machine learning-derived parameters. *J Toxcol Sci*, in press.

Title of Research:

22-5-03

The development of a model to evaluate the vector impacts of microplastics and to predict their effects under actual environmental conditions using this model

Principal Investigator:

Yuji Oshima (Kyushu University, Faculty of Agriculture, Laboratory of Marine Environmental Science Professor)
744 Motooka, Nishi-ku, Fukuoka 8020345, Japan
(tel&fax)+81-092-802-4605

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:

To prove that the vector effect of MPs is caused by the adsorption and desorption of adsorbents by intestinal bile, we performed in vitro desorption tests using taurocholic acid for Ant, Phe, Nap, and CBs adsorbed MP. The results showed that Ant and Phe showed high adsorption and that taurocholic acid promoted desorption. However, Nap and CBs showed weak or no enhancement of desorption by taurocholic acid. The results of the in vitro desorption study were in agreement with the results of in vivo co-exposure studies of Ant, Phe, Nap, CBs and MP in medaka (Ant and Phe showed vector effects; Nap showed weak vector effects; CBs did not show any effects). The reason for this is considered to be the high-water solubility of Nap and the high Log Kow of CBs, which are difficult to elute from MP. The taurocholic acid concentration and its analogues were confirmed by LCMSMS analysis of medaka bile. Based on these results, the desorption test method using simulated intestinal fluid containing taurocholic acid as a substitute for bile is considered a useful approach for screening the elution of chemical substances adsorbed on MP. In the future, this screening method could be used to examine the elution of not only chemicals adsorbed on plastics, but also a wide variety of additives (plasticizers, antioxidants, stabilizers, etc.) to evaluate vector effects and their toxicity.

Timeline:

March 1, 2024- Feb28, 2025

Topics: "Construction of a Vector Effect Estimation Model for Microplastics and its Application to Prediction of Effects in the Real Environment - Elucidation of the Mechanism of Vector Effects" Yuji Oshima. LRI meeting. Aug23, 2024

Publications:

Al-Emran, M., Matsudera, M., Honda, M., Takai, Y., Lee, S., Uchida, Y., Qiu, X., Shimasaki, Y., Oshima, Y., 2024. Accumulation of chlorobenzenes in Japanese medaka (Oryzias latipes) co-exposed to 10- or 45-μm polystyrene microplastics. 環境毒性学会誌 27, 73–86.

Al-Emran Md., Takai Y., Shimasaki Y., Oshima Y. Vector effect of polyethylene microplastics on accumulation of phenanthrene in Japanese Medaka, *Oryzias latipes.* Chemosphere (Preparing manuscript for submission)

Tokunaga M., Takai Y., Komatsu K., Al-Emran Md., Shimasaki Y., Oshima Y. Adsorption of polycyclic aromatic hydrocarbons on microplastics and their desorption in simulated intestinal fluids. Chemosphere (Preparing manuscript for submission)

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 (National Institute of Advanced Industrial Science and Technology (AIST))
Tomomichi Suzuki (Tokyo University of Science)

Summary of Research:

Repeated-dose toxicity (RDT) tests are essential for assessing the safety of chemical substances. However, due to the complexity of RDT, developing alternative methods to animal testing remains challenging. In this study, we aimed to develop an objective read-across method utilizing information on chemical structures and toxicity-related in vitro tests. First, we prepared subgroups of chemical substances based on their partial structures and performed predictions for liver and blood toxicity using the results of in vitro tests. We found that toxicity could be accurately predicted in some subgroups (e.g., aromatic amines) with in vitro tests. Next, using model substances containing aniline- and phenol-like structures, we explored a method for grouping based on toxicity profiles across multiple toxicity endpoints. We found that predicted biological activity values are useful for grouping substances with similar toxicity. Additionally, using three or four molecular descriptors, we identified several subgroups of chemicals that contained only negative or positive substances for specific liver or blood toxicity endpoints. Finally, we found that for certain groups of chemicals, cytochrome P450 inhibition assay data are valuable for quantitatively predicting LOEL values for hepatotoxicity. In conclusion, our present results may assist in establishing a methodology for predicting the RDT of chemical substances through read-across, taking into account multiple toxicity endpoints and utilizing molecular descriptors and in vitro test data.

Timeline:

March 1, 2024 - February 28, 2025

Topics:

None

Publications:

1. J Takeshita, Y Goto, S Yamamoto, T Sasaki, K Yoshinari. Comprehensive analysis of the toxicity-related findings from repeated-dose subacute toxicity studies of industrial chemicals in male rats. *Crit Rev Toxicol,* 54:996-1010, 2024.
2. N Uchida, Y Harakawa, T Hosaka, R Shizu, J Takeshita, K Yoshinari: Quantitative correlation analysis between the liver toxicity LOEL and P450 inhibitory activity, 51st Annual Meeting of the Japanese Society of Toxicology (July 3-5, 2024, Fukuoka)
3. N Uchida, M Shibata, Y Harakawa, A Ooka, T Hosaka, R Shizu, J Takeshita, K Yoshinari: Development of a toxicity evaluation method for structurally similar compounds using RNA sequence data, 7th Symposium on Drug Toxicity Mechanisms (January 8-9, 2025, Shizuoka)
4. Y Harakawa, J Takeshita, A Ooka, T Hosaka, R Shizu, K Yoshinari: Development of a read-across method for repeated dose toxicity evaluation: Evaluation of the usefulness of in vitro test data related to toxicity mechanisms, Ibid.

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:
Yusuke Hiejima, Dr. (Sci.) (Professor, Graduate School of Natural Science and Technology, Kanazawa University)
Kakuma Campus, Kanazawa, 920-1192
(tel) +81-76-264-6256, (e-mail) hiejima@se.kanazawa-u.ac.jp

Collaborators:
Shinichi Kuroda (Gunma University), Hiroyuki Oku (Graduate School of Science and Engineering, Gunma University), Takashi Kuriyama (Yamagata University), Go Matsuba (Graduate School of Organic Materials and Systems Science, Yamagata University), Hitoshi Mizuguchi (Graduate School of Technology, Tokushima University), Hiroaki Kouzai (Department of Science and Engineering, Kanto Gakuin University)

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 X-ray microbeam analysis of plastics degraded in the natural environment by outdoor exposure tests. We found that the depth profile of the crystallinity was explained by the weathering layer model proposed by our group. We have analyzed PET bottles collected in the Arakawa riverbed. We found that degradation is not obvious even after outdoor exposure for 20 years, suggesting the PET bottle may not be important as the source of microplastics. We also analyzed airborne microplastics by using the pyrolytic GC/MS. We found that the size of rubber particles was significantly larger than those of PET particles. The kinetic analysis for microplastic formation was conducted by using the ultraviolet exposure tests. We found that the crack propagation speed during the ultraviolet exposure tests is significantly faster than that during the high-temperature exposure tests, suggesting the acceleration of microplastics formation by the ultraviolet irradiation. We prepared the spheric particles of polyethylene and polypropylene by using cryogenic milling technique, where the typical size was about 100 and 10 μm. We expect the “reference” microplastics can be obtained by applying the photochemical oxidation treatment.

Timeline:
March 1, 2023-

Topics:
July 7, 2024: Cite visiting at Kanazawa University
Aug. 23, 2024: Poster presentation at 2024 LRI Research Report Meeting

***Publications:* (only PI)**
An oral presentation “Formation of parallel cracks driven by chemicrystallization and subsequent fragmentation into microplastics” was given at 11th conference of the Modification, Degradation, Stabilization of Polymers Society (MoDeSt 2024) in Sept. 1-4, 2024.

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 Technician, Aoyama Gakuin University)
Kota Ujibe, MSc (Graduate Student, Aoyama Gakuin University)
Kenichiro Sadamitsu, MSc (Graduate Student, Aoyama Gakuin University)
Makoto Kashima (Lecturer, Toho University)
Junichi Tasaki (Kao Corporation)
Shujie Liu (Kao Corporation)

Summary of Research:

Various attempts have been made to use zebrafish as a novel approach to current developmental toxicity testing. However, a standardized test protocol has not yet been established, and concerns remain regarding reproducibility and reliability, such as trial variability, strain differences, and inter-laboratory inconsistencies. This study aims to develop an alternative method for teratogenicity assessment using zebrafish and to utilize it for identifying adverse outcome pathways (AOPs). Initially, the principal investigator established a developmental toxicity testing protocol using zebrafish and then addressed the issue of strain differences. By decoding the whole genome sequences of zebrafish strains, their genetic relationships were clarified, and the strain-specific differences in toxic responses were investigated. The results revealed no significant strain differences in teratogenicity based on malformation observations following exposure to six compounds or in gene expression responses analyzed through RNA-Seq across low to high concentrations of three compounds, even among genetically distinct strains. Based on these findings, it was concluded that teratogenicity assessmentusing zebrafish provides reproducible and reliable test results, irrespective of the strain utilized. One of the key advantages of zebrafish-based developmental toxicity testing is the simplicity of conducting AOP analysis. By leveraging the whole-organism RNA-Seq technique developed during the strain difference analysis, AOP analyses focusing on gene expression were conducted. Multiple universal toxicity response genes, whose expression consistently decreased across exposures to several compounds, were identified. Moreover, a detailed time-course analysis following valproic acid exposure revealed early gene expression changes, including the downregulation of genes related to erythrocyte function and reactive oxygen species (ROS) elimination. These findings led to the hypothesis that key events in valproic acid-induced developmental teratogenicity include early-stage anemia and oxidative stress, ultimately resulting in energy deficits and oxidative damage. These mechanisms affect specific tissue development, culminating in teratogenicity.

Timeline:

March 1, 2023 – February 28, 2025

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.
2. "Development of an alternative method for teratogenicity using zebrafish" at the 2024 LRI Research report meeting of the Japan Chemical Industry Association. August 23, 2024.

Publications:

1. Kenichiro Sadamitsu, Fabien Velilla, Minori Shinya, Makoto Kashima, Yukiko Imai, Toshihiro Kawasaki, Kenta Watai, Miho Hosaka, **Hiromi Hirata*** and Noriyoshi Sakai*. (2024)

Establishment of a zebrafish inbred strain, M-AB, capable of regular breeding and genetic manipulation. Sci. Rep. 14(1): 7455. (*Corresponding authors)
2. Kanako Mori, Yoshinobu Aoki, Fumito Mikashima, Kazushige Maki, Toshio Tanaka, Mai Hayashi, Wataru Sugimoto, Mizuho Ono, Saaya Umekita, Tatsuhiro Niino, Michio Fujiwara, Tomonori Ebata, **Hiromi Hirata** and Hajime Kojima. (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. Tox. Sci. 49(8): 337-348.
3. Kenichiro Sadamitsu, Kumiko Yanagi, Yuiko Hasegawa, Yoshiko Murakami, Sean E. Low, Daikun Ooshima, Yoichi Matsubara, Nobuhiko Okamoto, Tadashi Kaname* and **Hiromi Hirata***. (2024) A novel homozygous variant of the PIGK gene caused by paternal disomy in a patient with neurodevelopmental disorder, cerebellar atrophy, and seizures. J. Hum. Genet. 69: 553-563. (*Corresponding authors)
4. **Hiromi Hirata***, Tsuyoshi Tezuka and Kota Ujibe. (2024) Aging and Senescence Studies in Human and Zebrafish. Gerontology as an Interdisciplinary Science (Current Topics in Environmental Health and Preventive Medicine). Springer. Edited by Shiozawa, T., Hirata, H., Inoue, T., Kanikowska, D. and Takada, H. p3-22. (*Corresponding author)
5. Kenta Watai, Kenichiro Sadamitsu, Seiji Wada, Makoto Kashima and **Hiromi Hirata***. (2024) Zebrafish trpm7 mutants show reduced motility in free movement. Dev. Growth Differ. 66 (6): 349-356. (*Corresponding author)
6. Kenichiro Sadamitsu, Makoto Kashima, Seiji Wada, Akiko Ishioka, Satomi Nakayama, Ryoko Nakayama, Hitoshi Okamoto* and **Hiromi Hirata***. Establishment and genetic characterization of zebrafish RW line. Sci. Rep. Under Revision. (*Corresponding authors)

Title of Research:

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

Principal Investigator:

Yoshifumi Horie, PhD (Associate 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 (Associate Professor), Graduate School of Maritime Sciences, Kobe University
5-1-1　Fukaeminami-machi, Higashinada-ku, Kobe 658-0022, JAPAN

Summary of Research:

In the second year of the study, fine particles (including microplastics) collected from the surface layer of Osaka Bay were chronically exposed to medaka to assess their toxicity. First, an investigation was conducted to determine the extent of microplastic contamination in tap water across Japan. A total of 43 samples were collected from 23 prefectures, including households, parks, hotels, coastal facilities, universities, and hospitals. To clarify differences in residual concentrations, samples were taken from various locations and water sources; however, no significant differences in microplastic concentrations were observed. The detected microplastic concentrations ranged from 1 to 18 particles per liter, with an average of 6 ± 4 particles per liter. In total, 255 microplastic particles were detected across all 43 analyzed samples.

Next, in 2023, fine particles (including microplastics) collected from the surface layer of Osaka Bay were chronically exposed to medaka to evaluate their toxicity. Specifically, exposure experiments were conducted on both the F0 generation (from immediately after fertilization to 14 weeks of age) and the F1 generation (up to the second week after fertilization). Seven observational parameters were set: (1) embryonic development, (2) growth, (3) secondary sexual characteristics, (4) histological analysis of the gonads, (5) residual microplastics in the digestive tract, (6) reproduction, and (7) survival.

As a result, no statistically significant differences were observed between the exposed group and the control group in terms of embryonic development, growth, secondary sexual characteristics, histological analysis of the gonads, reproduction, and survival. Based on these findings, it was concluded that chronic exposure of medaka to fine particles (including microplastics) collected from the surface layer of Osaka Bay did not exhibit any detectable toxicity at present.

Timeline:

March 1, 2024

Topics:

2024 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」

Publications:

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 2024

Publications:

Iwasaki Y., Ueda K., Naito W. (2024) Converting effect concentrations obtained from laboratory toxicity tests into concentrations relevant to polydisperse microplastic particles present in the environment. *Jpn. J. Environ. Toxicol.* 27: 46-52

Ueda K., Iwasaki Y., Kameda Y., Naito W. (2024) Estimation of Microplastic Particle Concentration Considering Unmeasured Particle Size Range and Concentration Conversion. *Jornal of Japanese Society on Water Environment* 47: 105-112.

Ueda K., Kameda Y., Fujita E., Rachi S., Iwasaki Y., Tai R., and Naito W. (2025) Concentrations and characteristics of microplastic particles collected by neuston net or pump system in the surface layer of Tokyo Bay. *Regional Studies in Marine Science,* 84, 104108.

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 institute of science for safety and sustainability (RISS))
16-1 Onogawa, Tsukuba, Ibaraki, 305-8569, Japan
(tel)+81-80-7799-6536, (e-mail) Masashi-kamo@aist.go.jp

Collaborators:

Yuriko Ishikawa (AIST, RISS)
Hiroyuki Mano (AIST, RISS)
Tetsuro Okamura (IDEA Consultant, Inc. Institute of Environmental Ecology)
Atsushi Sawai (IDEA Consultant, Inc. Institute of Environmental Ecology)
Jun Yamamoto (IDEA Consultant, Inc. Institute of Environmental Ecology)

Summary of Research:

This study aims to provide information to support risk assessment of monoalkyl cation C16 (Hexadecyltrimethylammonium chloride: HTAC), a cationic surfactant designated as a priority assessment chemical substance under the Chemical Substances Control Law of Japan. It is known that the toxicity of HTAC changes depending on the water quality, and a more sophisticated risk assessment is possible by relating the water quality and the toxicity of HTAC. First, we examined water quality items that may affect the toxicity of HTAC and found that dissolved organic matter (DOC) has a significant effect, but other water qualities such as pH, hardness or salinity have almost no effect. To know the effect of DOC on the toxicity, we performed a growth inhibition test using *Raphidocelis subcapitata*. Using the test results for DOC=0 mg/L and DOC=5 mg/L, we constructed a model to predict the toxicity at various DOC concentrations. The prediction accuracy of the model was quite high, but it is necessary to verify next year whether it can properly predict the effects of DOC concentrations other than the two implemented this year. Finally, for exposure assessment, AIST-SHANEL Ver.3.0 was used to perform tertiary mesh and monthly concentration analysis of HTAC concentrations in 109 first-class water systems nationwide.

Timeline:

March, 1, 2023 -

Topics:

Oral presentation at the 2024 LRI research report workshop by JCIA (Tokyo)

Publications:

Yamamoto, J., Yamaguchi, N., Okamura, T., Sawai, A., Mano, H., Kamo, M. (2024) Analysis of Hexadecyltrimethylammonium Chloride using LC-MS/MS. July 3, 2024. 3rd Joint Conference on Environmental Chemicals

Ueda, K., Naito, W., Mano, H., Kamo, M. (accepted) Current status and issues of difficult-to-test substances such as cationic surfactants under the Chemical Substances Control Law of Japan. Japanese Journal of Environmental Toxicology (in Japanese).

Title of Research:

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

Principal Investigator:
Yuko Sekino, PhD (Project Professor, The University of Tokyo, Graduate School of Agricultural and Life Sciences, Department of Veterinary Pathophysiology and Animal Health) 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, The University of Tokyo, Graduate School of Agricultural and Life Sciences, Department of Veterinary Pathophysiology and Animal Health) 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, 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
Shihori Tanabe, PhD (Senior Researcher, Division of Risk Assessment, Center for Biological Safety and Research, 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 (Toyohashi University of Technology, Center for Diversity and Inclusion, Appointed to Dept. Applied Chemistry & Life Science) 1-1 Hibarigaoka, Tenpaku-cho, Toyohasi-city, Aichi, 441-8580, Japan (tel) +81-532-81-5106, (e-mail) syoshida@tut.jp

Summary of Research:

This project aims to finalize the AOPwiki entry for AOP 475, proposed to the OECD. During this term, we participated in AOP coaching three times from Dr. Rex FitzGerald, a member of the OECD's Extended Advisory Group on Molecular Screening and Toxicogenomics (EAGMST).

AOP 475 outlines a pathway involving loss of drebrin (KE_2078) and dendritic spine abnormalities (KE_2242) leading to learning and memory impairments, without neuronal death. To integrate new KEs, we propose several key event relationships (KERs), including: Increased Intracellular Calcium Overload leading to Loss of Drebrin (KER_3091), Loss of Drebrin leading to Dendritic Spine Abnormality (KER_3298), and Dendritic Spine Abnormality leading to Dysfunctional Synapses (KER_3301). Literature and experimental data are being reviewed to validate these links.

To explore the relationship between compounds affecting learning and memory (AO) and drebrin loss (KE3), we conducted experiments using primary rat hippocampal neurons. 7 compounds were tested, with Compound A reducing the number of drebrin clusters without inducing cell death after 1 hour of exposure. Compound B had no effect alone but enhanced the effect of Compound A when co-administered. These findings provide critical evidence supporting the linkage between KE3 (drebrin loss) and AO (memory impairment) in AOP 475.

Timeline:
March 1, 2024 –

Topics:

Publications:
[Original paper] Koganezawa N, Roppongi RT, Sekino Y, Tsutsui I, Higa A, Shirao T. “Easy and Reproducible Low-Density Primary Culture using Frozen Stock of Embryonic Hippocampal Neurons” J Vis Exp. Jan 27;(191). 2023, Video disclosed in Public at April 2024.and another publication
[Conference presentation]
[Lecture]

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. (Group leader, Research Institute of Science for Safety and Sustainability (RISS), 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 (Research Institute of Science for Safety and Sustainability (RISS), 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 preventing plastic pollution. Quality of recycled plastic left undefined, although it is acknowledged as very important. 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 and resource recycling in the supply chain. We assumed that the risks associated with the use of recycled plastics come from plastic chemicals such as plastic additives, contaminants, and by-products contained in plastics. A risk-based grading approach for recycled plastics has been developing by conducting a trial grading of recycled plastics based on a risk assessment framework for chemicals contained in plastics.

Timeline:

March 1, 2024-

Topics:

The presentation entitled “Study on Risk-Based Grading of Recycled Plastic and its Application for Enhancing Plastic Recycling” was held in JCIA LRI Annual Workshop 2024

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" Society for Risk Analysis Annual Meeting 2024, Austin, Texas, USA (2024/12), Poster 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 alternatives to animal testing for respiratory sensitization is currently underway at the OECD with the preparation of a Detailed Review Paper. It includes descriptions of our dendritic cell (DC) coculture system (DCsens) and the two-step DC/T coculture system (DC/Tsens). DCsens is a coculture system with the human bronchial epithelial cell line BEAS-2B and the human monocyte cell line CD14-ML cells using a Scaffold, while DC/Tsens is a coculture system in which T cells are added to the DC layer stimulated in DCsens. Their markers are OX40 ligand, an important costimulatory molecule for T-cell helper (Th) 2 differentiation, and IL-4, a potent inducer of Th2 differentiation and effector molecule for Th2 cells, respectively. DC/Tsens uses T-cell activation at key event (KE) 4 of the respiratory sensitization AOP as a marker, and KE4 is an important KE where signals from KE1 to KE3 converge, but no globally accepted test has yet been developed to use it. We have previously shown that it is possible to discriminate between respiratory and skin sensitization using primary human peripheral blood $CD4^+$ T cells in the DC/Tsens system. To improve the versatility of the system, a human allogeneic Th2 cell line was generated and applied to DC/Tsens. Using the Th2 cells, IL-4 production was detected by ELISA in culture supernatants 24 hours after the addition of T cells. DC/Tsens successfully discriminated between 13 representative respiratory and skin sensitizers with more than 80% predictability after correction for cell viability and T-cell activation using the WST-8 assay and IL-2 production, respectively. Although the addition of T cells in DC/Tsens is more complicated than DCsens, it is expected to increase the accuracy using T cells and could become the world's first evaluation method using KE4 as a marker aiming at the OECD test guideline.

Timeline: March 1, 2019-Feberary 28, 2022

Topics:

2024 Annual Meeting of The Japan Chemical Industry Association LRI. Poster presentation. “Development of a coculture system to assess respiratory sensitization using IL-4 production from human Th2 cells as a marker.” (2024.8.20)

Publications:

1. Yoshimoto, et al. Development of respiratory sensitization assessment methods. Special lecture. The 31st Annual Meeting of the Japanese Society of Immunotoxicology.（2024.9.19-20）Hyogo
2. Mizoguchi I, et al. Development of a two-step DC/T cell co-culture system to discriminate between respiratory and skin sensitizers. The 31st Annual Meeting of the Japanese Society of Immunotoxicology.（2024.9.19-20）Hyogo
3. Mizoguchi I, et al. Development of an in vitro respiratory sensitization assay using TNFSF4 (OX40L) expression as a marker in a three-dimensional coculture system consisting of immature DCs from the human monocyte-derived cell line CD14-ML. The 37th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments.（2024.11.29-12.1）Utsunomiya

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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