ISTA21

International Symposium
on Toxicity Assessment

-Venue-
the Recent Hotel, Fukuoka, Japan
-Period-
August 25-30, 2024

〈 Program & Abstract Book 〉

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International Symposium on Toxicity Assessment

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ISTA 21 Organizing Committee

Chair:

Toshihiro HORIGUCHI, Head of Ecosystem Impact Research Section, Health and Environmental Risk Division, National Institute for Environmental Studies (NIES)

Secretary-General:

Ik-Joon KANG (Kyushu University, Associate Professor)

Members:

Masato HONDA (Kanazawa University, Assistant Professor)
Mayumi ISHIZUKA (Hokkaido University, Professor)
Tomohiko ISOBE (NIES, Chief Senior Researcher)
Hisato IWATA (Ehime University, Professor)
Hiroyuki KINTSU (NIES, Researcher)
Keita KODAMA (NIES, Chief Senior Researcher)
Gen KUME (Kagoshima University, Associate Professor)
Fumihiro MORISHITA (Hiroshima University, Assistant Professor)
Shigeharu NAKACHI (Kumamoto Gakuen University, Professor)
Kei NAKAYAMA (Ehime University, Lecturer)
Hideo OKAMURA (Kobe University, Professor)
Yuji OSHIMA (Kyushu University, Specially-appointed Professor)
Yohei SHIMASAKI (Kyushu University, Associate Professor)
Michio SUZUKI (University of Tokyo, Professor)
Yuji TAKAO (Nagasaki University, Professor)
Hiroshi YAMAMOTO (NIES, Director) (alphabetical order)

Co-organizer:

National Institute for Environmental Studies (NIES)

International Co-Chairs for ISTA

Ruth SOFIELD (Professor, Western Washington University, U.S.A.)
Markus HECKER (Professor, University of Saskatchewan, Canada)

ISTA 21 Organizing Committee

Scientific Program:
Masato HONDA, Keita KODAMA, Hiroyuki KINTSU, Hisato IWATA

Keynote Speakers:
Toshihiro HORIGUCHI, Ik-Joon KANG

Student Program:
Fumihiro MORISHITA, Mayumi ISHIZUKA, Michio SUZUKI, Hiroshi YAMAMOTO

Social Activities:
Shigeharu NAKACHI, Toshihiro HORIGUCHI, Ik-Joon KANG, Gen KUME, Yuji TAKAO

Logistics:
Ik-Joon KANG

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Tomohiko ISOBE, Yuji OSHIMA, Masato HONDA, Hideo OKAMURA, Yohei SHIMASAKI

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Keishiro TOKUDOME

Program & Abstract Book:
Sari OGIHARA, Hiroyuki KINTSU, Keita KODAMA

Special Issue (*Environmental Science and Pollution Research*):
Kei NAKAYAMA

Registration:
Kayo KAWAMURA, Yosuke MASUDA

Message from the ISTA 21 Chair

A very warm welcome to Fukuoka, Japan and the 21st International Symposium on Toxicity Assessment (ISTA 21).

Since the 13th International Symposium on Toxicity Assessment (ISTA 13) was held in Toyama in August 2007, ISTA conference is held as ISTA 21 in Japan for the first time in 17 years.

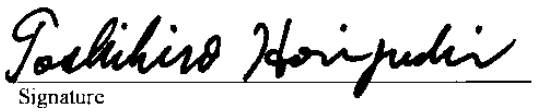
In the ISTA 21, in addition to the General Sessions, focusing on assessment of toxicities of various environmental attributes (not only chemical but also physical) on both aquatic and terrestrial organisms at different levels of organization (i.e., gene-, cellular-, organ-, individual-, population- and community-levels), a Special Session "Toxicity assessment and its environmental/ecological relevance" is planned. In this Special Session, we would like to focus on a knowledge gap between effects observed in the laboratory and those in the environment. Meanwhile, when we provide policy makers with information on conservation or protection of the environment, the estimation or assessment of adverse effects emanating from certain development activities or pollutants/contaminants is needed at population or community levels; however, most studies on adverse effects on organisms have been conducted at individual or sub-individual levels. For assessment of population-level effects, it is necessary to consider the combined effects of the environmental factor of concern (i.e., a pollutant/contaminant) with other abiotic and biotic factors together with the life-history traits and sensitivities of the species examined. For assessment of community-level effects, however, few methodologies have been proposed so far. In this special session, we would like to discuss these unresolved and challenging issues.

The reason why we selected Fukuoka as the venue of ISTA 21 is that we strongly wish to visit Minamata with ISTA 21 participants. Therefore, a special tour to Minamata, where Minamata disease had befallen its residents since the 1950s, is planned. We will visit the Hyakken drain outlet, where the factory of Chisso Corporation, the company responsible for this tragedy, had released methyl mercury-contaminated wastewater into the bay over several decades. We will also visit other historical sites/areas related to Minamata disease as well as the Minamata Disease Municipal Museum. A fetal Minamata disease patient, Ms. Shinobu Sakamoto, will be joining us as a storyteller. We will also enjoy the natural environs (forest and the sea) around Minamata, as well as drawing lessons from historical records of this tragedy.

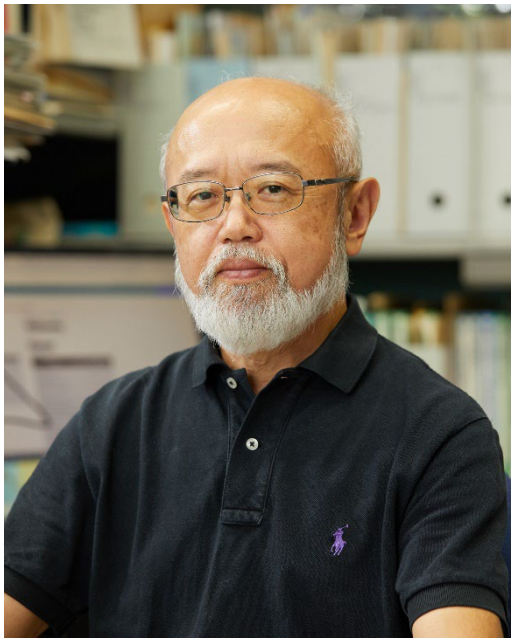
We hope all participants will get fruitful results from scientific sessions and social programs of ISTA 21. We also hope all ISTA 21 participants will enjoy the tradition and culture, interaction with people, and the environment, as well as various kinds of food in Japan!

Message from the ISTA 21 Chair

Sincerely,


Signature

Toshihiro Horiguchi
ISTA 21 Conference Chair



Message from the ISTA 21 Secretary-General

Welcome to ISTA 21! It is our pleasure to host the 21st International Symposium on Toxicity Assessment to be held in Fukuoka, on August 25-30, 2024. My first participation in the ISTA was in 2007, ISTA13 in Toyama. The ISTA13 was very special for me, because the ISTA gave me a great opportunity to get to know each other with Dr. Doris Au and many influential researchers. Therefore, it is a great honor and a privilege to serve you as the ISTA 21 Secretary-General with all members of ISTA21 organizing committee.

Fukuoka is the biggest city in Kyushu Island with its food culture and excellent transportation accessibility. From ancient times, Fukuoka city's proximity to the continent has made it an important gateway for cultural influences from China and Korea. You can feel a city known for its historic ambiance in many places. I sincerely hope that you actively participate and enjoy the ISTA21. We look forward to your wonderful experience in Fukuoka.

Sincerely,

Ik-Joon Kang
ISTA 21 Conference Secretary-General

History of ISTA

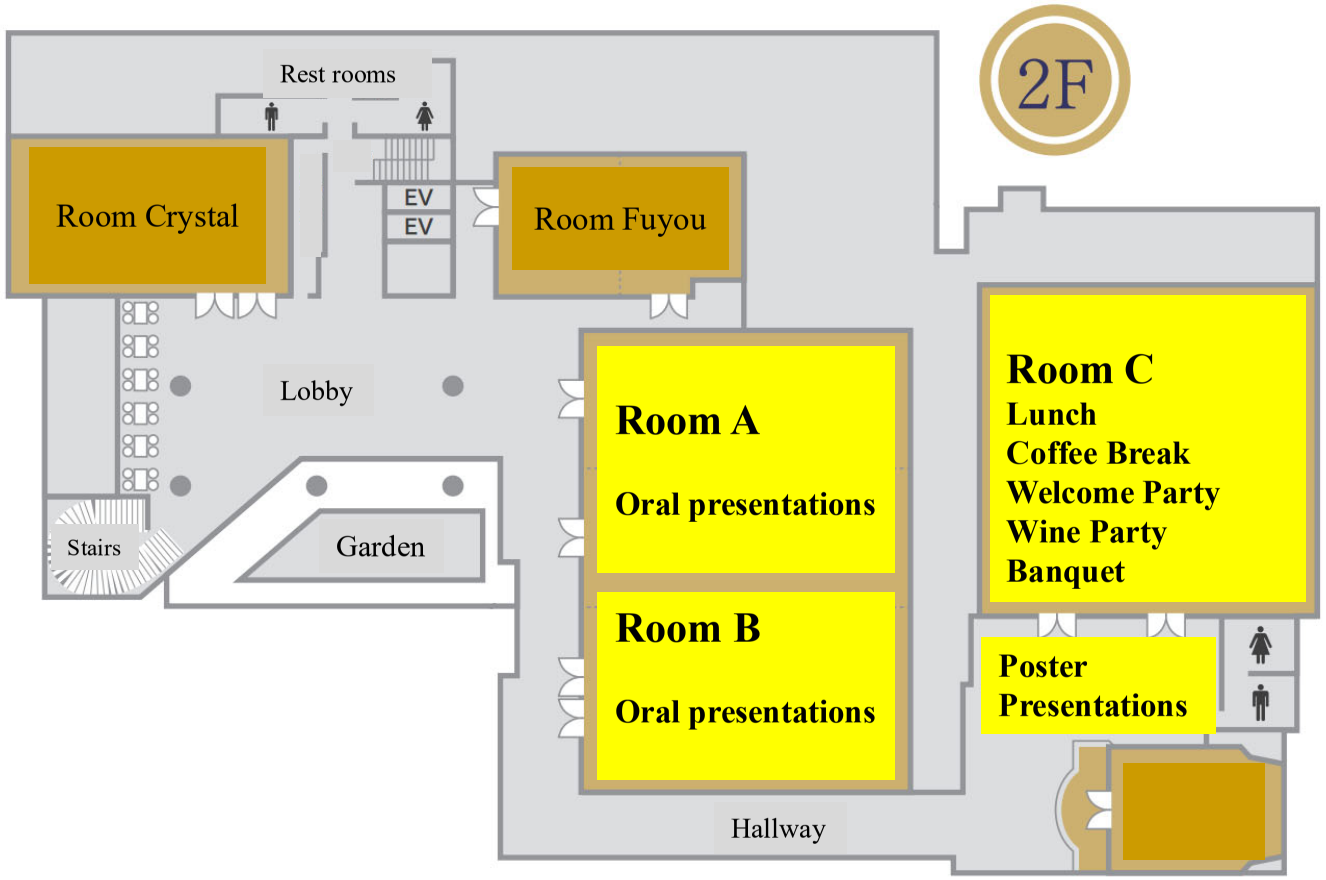
The ISTA symposium series has met regularly since 1983 when it was held for the first time in Burlington, Ontario, Canada. It provides a platform for scientists from around the world to discuss the latest developments in understanding levels of toxicity and mechanisms of action for chemicals of global concern, to better inform regulatory toxicology focused on public health and environmental safety. Current advances in the fields of toxicity assessment, biomarkers and bioindicators, environmental chemistry and green chemistry, exposomics, new approach methods, systems toxicology, risk assessment, and remediation and mitigation methodologies and technologies will be covered.

Previous ISTA symposia have been held in 20 cities in 13 different countries:

Burlington, Canada (1983)
Banff, Canada (1985)
Valencia, Spain (1987)
Las Vegas, USA (1989)
Kurashiki, Japan (1991)
Berlin, Germany (1993)
Santiago, Chile (1995)
Perth, Australia (1997)
Pretoria, South Africa (1999)
Québec City, Canada (2001)
Vilnius, Lithuania (2003)
Skiathos Island, Greece (2005)
Toyama, Japan (2007)
Metz, France (2009)
Hong Kong, China (2011)
Cape Town, South Africa (2013)
Bellingham, USA (2015)
Limeira, Brazil (2017)
Thessaloniki, Greece (2019)
Saskatoon, Canada (2022)

ISTA is a unique opportunity to bring together researchers, managers and practitioners, regulators, non-governmental organizations, and policymakers to exchange ideas, identify research priorities, and determine resource needs, to support better management of ecosystem health and minimize risks to public health. Early career researchers and students are strongly encouraged to participate in ISTA 21. These meetings have been shown to provide an excellent opportunity for networking and career advancement.

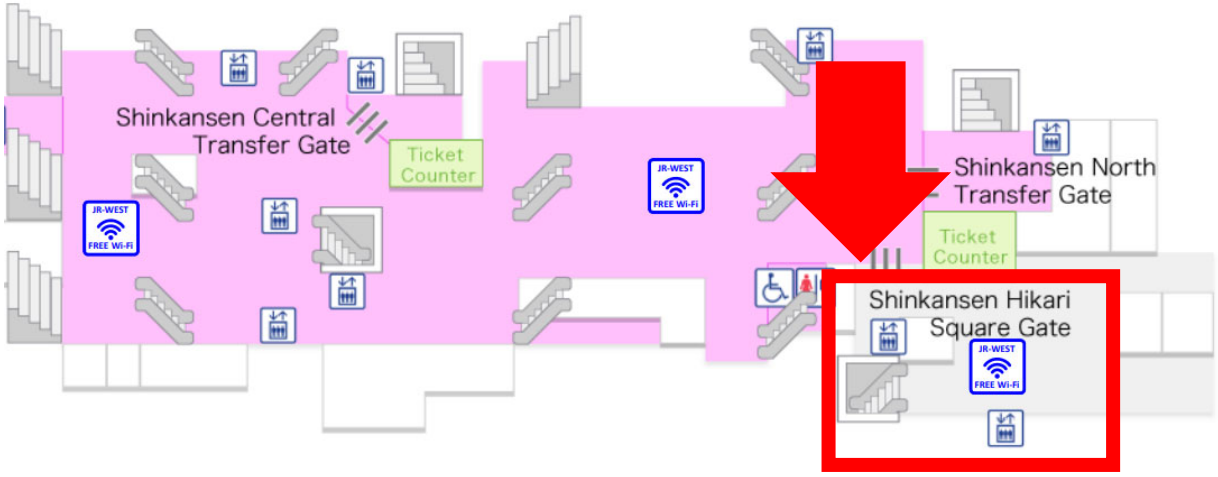
Map of the Venue

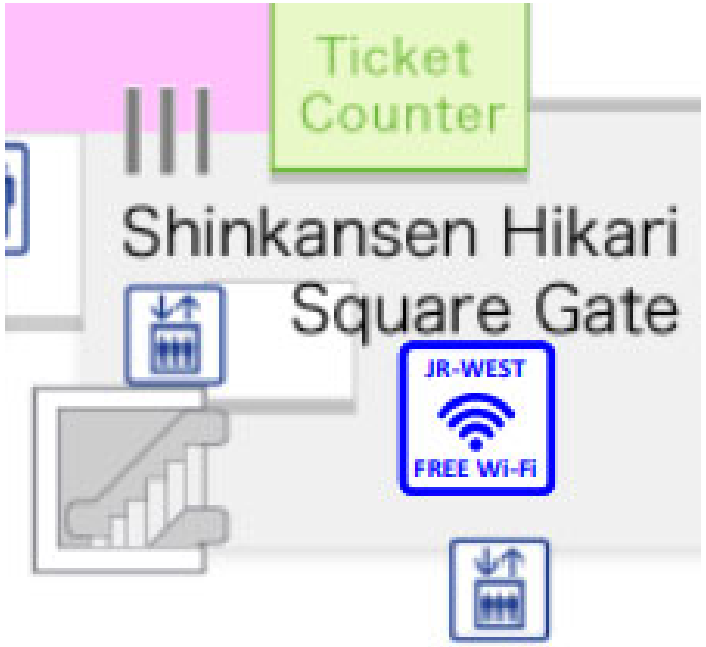


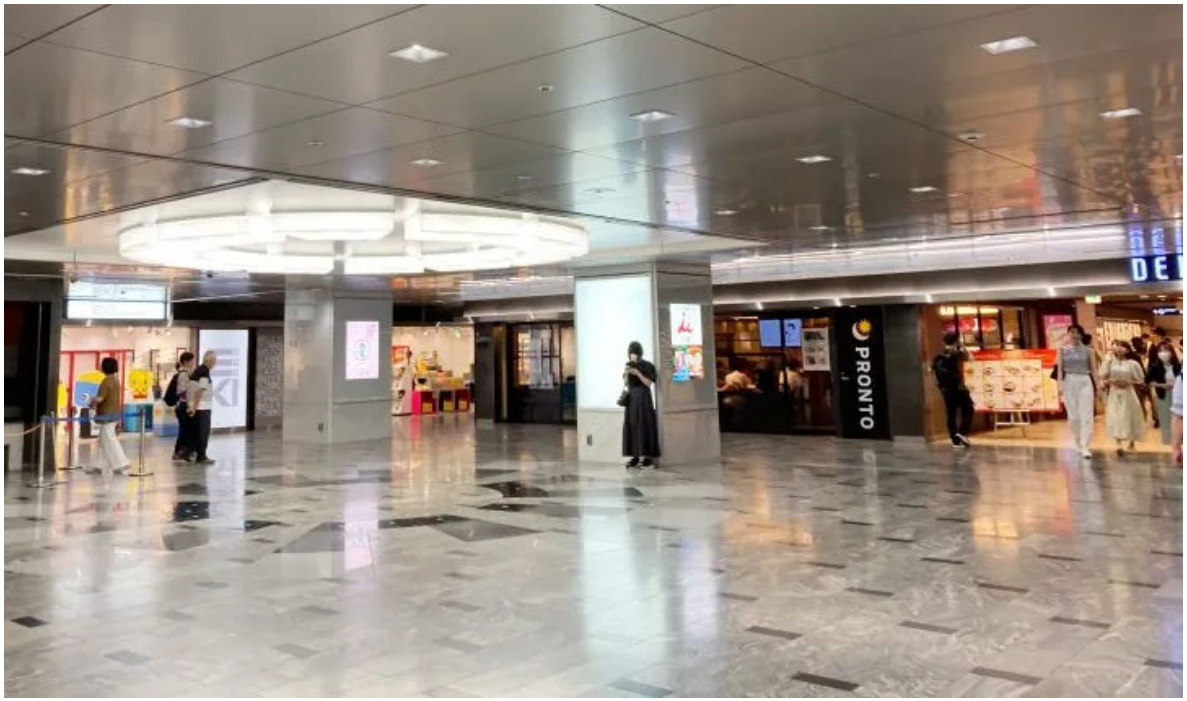
Map of the JR Hakata Station

When you arrive at the JR Hakata station, you can find the stairs and the escalator in front of the Chikushi Gate on the first floor, JR Hakata station. Please come up to the second floor.

Map of the JR Hakata Station







When you come up to the second floor, and you can find the meeting place, the Shinkansen Hikari Square. Please gather up the meeting place by 8:15 a.m. of August 28

Program summary

25-Aug	Hall A	Hall B	Hall C	Remarks
17:00-18:00				Reception
18:00-18:30			Opening ceremony	
18:30-19:30			Plenary lecture: **Dr. Markus Hecker**	
19:30-21:00				Welcome reception

26-Aug	Hall A	Hall B	Hall C	Remarks
9:10-10:00	Chairperson: Kodama, Horiguchi			
	Special Session “20. Toxicity assessment and its environmental/ecological relevance (1)”			
Break				
10:20-12:00	Chairperson: Kume, Horiguchi			
	Special Session “20. Toxicity assessment and its environmental/ecological relevance (2)”			
11:00-12:00	Chairperson: Horiguchi			
	Special Session lecture: **Dr. Akio Sohma** "			
Lunch break				

13:20-14:40	Chairperson: Okamura, Nakachi	Chairperson: Kintsu, Suzuki		
	General session "11. Environmental risk of antifouling biocides etc. (1)"	General session "1. Accumulation of and effects by heavy metals or radioisotopes in aquatic organisms"		
Break				
15:00-16:00	Chairperson: Kojima, Honda	Chairperson: Iwata, Isobe		
	General session "11. Environmental risk of antifouling biocides etc. (2)"	General session "17. New approach methodologies (NAMs) in toxicology and environmental toxicology (1)"		
Break				
16:20-18:00			General sessions (Poster)	

27-Aug	Hall A	Hall B	Hall C	Remarks
9:00-10:40	Chairperson: Takao, Isobe	Chairperson: Nakayama, Shimasaki		
	General session "2. Analytical methods and dynamic analysis of environmental chemicals"	General session "14. Hematotoxicity and immunotoxicity"		
Break				
11:00-12:00	Chairperson: Kodama			
	Keynote lecture (1): **Dr. Nathaniel Scholz**			
Lunch break				
13:20-14:20	Chairperson: Honda			
	Keynote lecture (2): **Dr. Ruth Sofield**			

Break				
14:40-16:20	Chairperson: Iwata, Suzuki	Chairperson: Maekawa, Morishita		
	General session "17. New approach methodologies (NAMs) in toxicology and environmental toxicology (2)"	General sessions "16. Neurotoxic chemicals and their effects on humans and wildlife"		
Break				
16:30-18:00			General sessions (Poster)	
18:00-21:00				Wine party

28-Aug	
8:00-18:00	Excursion (Special tour to Minamata)

29-Aug	Hall A	Hall B	Hall C	Remarks
9:00-10:20	Chairperson: Watanabe, Shimasaki	Chairperson: Ishizuka, Maekawa		
	General session "3. Bioassays and testing methods (1)"	General session "12. Environmental toxicology of terrestrial animals/ecosystems (1)"		
Break				
10:40-11:40	Chairperson: Kang			
	Keynote lecture (3): **Dr. Xuchun Qiu**			
Lunch break				
13:20-14:20	Chairperson: Iwata			

	Keynote lecture (4): **Dr. Cinta Porte**			
Break				
14:40-16:20	Chairperson: Oshima, Nakayama	Chairperson: Ishizuka, Iwata		
	General session "8. Effect of micro and nano plastics on aquatic animals at experimental and real condition (1)"	General session "12. Environmental toxicology of terrestrial animals/ecosystems (2)"		
Break				
16:30-18:00			General sessions (Poster)	
18:00-21:00				Banquet

30-Aug	Hall A	Hall B	Hall C	Remarks
9:00-10:00	Chairperson: Oshima, Shimasaki	Chairperson: Kang, Nakayama		
	General session "8. Effect of micro and nano plastics on aquatic animals at experimental and real condition (2)"	General session "19. Signals from aquatic organisms, the behavioral effects as a toxicity assessment indicator (1)"		
Break				
10:20-11:40	Chairperson: Yamamoto, Oshima	Chairperson: Kang, Shimasaki		
	General session "3. Bioassays and testing methods (2)"	General session "19. Signals from aquatic organisms, the behavioral effects as a toxicity assessment indicator (2)"		
11:40-12:00	Wrap-up			
12:00-12:30	Closing ceremony			

Program schedule

(S): The persons who are applying to Best Student Poster or Platform Awards. **(Y)**: The persons who are applying to Best Young Scientist Poster or Platform Awards.

25-Aug	Hall A	Hall B	Hall C	Remarks
17:00-18:00				Reception
18:00-18:30			Opening ceremony	
18:30-19:30			Plenary lecture: **Dr. Markus Hecker** "Unraveling the toxicity of 6PPD-quinone across fishes of commercial, cultural, and ecological importance"	
19:30-21:00				Welcome reception

26-Aug	Hall A	Hall B	Hall C	Remarks
	Special Session “20. Toxicity assessment and its environmental/ecological relevance (1)”			
	Chairperson: Kodama, Horiguchi			
9:10-9:20	**Toshihiro Horiguchi** "Explanation of aim"			
9:20-9:40	O-1. **(S) Alexis Trujillo** "Impairment of post translational histone modifications in osteoblast subpopulations after parental benzo[a]pyrene exposure"			

9:40- 10:00	O-2. **(S) Yuan-Ru Wu** "Toxicity of rare earth elements on medaka fish and associated ecological risk assessment"			
Break				
	Special Session “20. Toxicity assessment and its environmental/ecological relevance (2)”			
	Chairperson: Kume, Horiguchi			
10:20- 10:40	O-3. **Toshihiro Horiguchi** "Reproductive abnormalities observed in a rock shell population in the vicinity of the Fukushima Daiichi Nuclear Power Plant, Japan"			
10:40- 11:00	O-4. **Keita Kodama** "Long-term changes in the megabenthic community structure of Tokyo Bay in relation to shifts in environmental conditions"			
	Chairperson: Horiguchi			
11:00- 12:00	Special Session lecture: **Dr. Akio Sohma** "An assessment model of toxic chemicals integrating benthic-pelagic lower ecosystem and the lower-higher trophic food web"			
Lunch break				
	General session "11. Environmental risk of antifouling biocides etc. (1)"	General session "1. Accumulation of and effects by heavy metals or radioisotopes in aquatic organisms"		
	Chairperson: Okamura, Nakachi	Chairperson: Kintsu, Suzuki		

13:20-13:40	O-5. **(S) Hirohisa Mieno** "Recent trend of antifouling coating technology and related regulations"	O-12. **(S) Haksoo Jeong** "Single and combined effects of increased temperature and methylmercury on different stages of the marine rotifer *Brachionus plicatilis*"		
13:40-14:00	O-6. **Ryuji Kojima** "A bioassay for evaluating the efficacy of antifouling paints on algae using their CIELAB color coordinates with a flow-through system"	O-13. **(Y) Eunjin Byeon** "Toxicity and speciation of inorganic arsenics and their adverse effects on in vivo endpoints and oxidative stress in the marine medaka *Oryzias melastigma*"		
14:00-14:20	O-7. **(S) Jaehee Kim** "Detrimental effects of hull cleaning wastewater on oxidative status, life cycle parameters, and population growth of the monogonont rotifer *Brachionus manjavacas*"	O-14. **(Y) Izabela Jośko** "Game-changing transformations: Rethinking metal nanoparticle toxicity in water"		
14:20-14:40	O-8. **(S) Somyeong Lee** "Hull-cleaning wastewater poses serious acute and chronic toxicity to a marine mysid—a multigenerational study"			
Break				
	General session "11. Environmental risk of antifouling biocides etc. (2)"	General session "17. New approach methodologies (NAMs) in toxicology and environmental toxicology (1)"		
	Chairperson: Kojima, Honda	Chairperson: Iwata, Isobe		
15:00-15:20	O-9. **(S) Mi Zhou** "Antifouling and marine paint – related microparticles containing heavy metals in surface water in Osaka Bay, Japan"	O-15. **(S) Junyi Lin** "Unraveling the mechanism and species sensitivity differences in 6PPD-quinone toxicity by using whole transcriptome analysis in four fish species"		

15:20-15:40	O-10. **(Y) Lianguo Chen** "A new mechanism of reproductive endocrine disruption based on isothiazolinone"	O-16. **(S) Hannah Mahoney** "Unveiling the molecular effects of replacement and legacy PFASs: Transcriptomic analysis of zebrafish embryos reveals surprising similarities and potencies"		
15:40-16:00	O-11. **Toshimitsu Onduka** "Monitoring and ecological risk assessment of antibiotics and herbicides in the Seto Inland Sea, Japan"	O-17. **(S) Phillip Ankley** "Xenometabolome of early-life stage salmonids exposed to 6PPD-quinone"		
Break				
16:20-18:00			General sessions (Poster)	

27-Aug	Hall A	Hall B	Hall C	Remarks
	General session "2. Analytical methods and dynamic analysis of environmental chemicals"	General session "14. Hematotoxicity and immunotoxicity"		
	Chairperson: Takao, Isobe	Chairperson: Nakayama, Shimasaki		
9:00-9:20	O-18. **(S) Miho Nomura** "Residue analysis of non-phthalate plasticizers in seawater and sediments at Osaka Bay, Japan"	O-28. **Frauke Seemann** "Marine medaka (*Oryzias melastigma*) as a developmental immunotoxicity model for PFAS exposure "		
9:20-9:40	O-19. **Maciej Tankiewicz** "Organic pollutant impact from poultry farm on soil"	O-29. **(S) Jonathan Hoang** "Immunotoxicity of cryo-milled tire tread in Japanese medaka (*Oryzias latipes*)"		
9:40-10:00	O-20. **(S) Remi Labeille** "Benzo[a]pyrene as an epigenetic toxicant"	O-30. **Kei Nakayama** "Chronic exposure to tire particles induces anemia in common carp"		

10:00- 10:20	O-21. **(Y) Agnieszka Fiszka Borzyszkowska** "Antibiotic resistance in the soils of the intensive farming coop and fields nearby"			
10:20- 10:40	O-22. **(S) Yangqing Liu** "Kinetic analysis of microplastics and their impacts on Japanese medaka (*Oryzias latipes*)"			
Break				
11:00- 12:00	Chairperson: Kodama			
	Keynote lecture (1): **Dr. Nathaniel Scholz** "Coastal development, toxic urban runoff, and Pacific salmonid conservation in western North America"			
Lunch break				
13:20- 14:20	Chairperson: Honda			
	Keynote lecture (2): **Dr. Ruth Sofield** "Contaminants of emerging concern in the Salish Sea, USA"			
Break				
	General session "17. New approach methodologies (NAMs) in toxicology and environmental toxicology (2)"	General sessions "16. Neurotoxic chemicals and their effects on humans and wildlife"		
	Chairperson: Iwata, Suzuki	Chairperson: Maekawa, Morishita		
14:40- 15:00	O-23. **(Y) Kyoshiro Hiki** "Application of AmpliSeq to environmental RNA may enable non-invasive assessments of various activities in fish"	O-31. **(Y) Eiki Kimura** "Dioxin target neurons in the mammalian brain"		

15:00-15:20	O-24. **Steve Wiseman** "Towards development of an in vitro assay of oocyte maturation inhibition to predict reproductive capacity of fishes"	O-32. **Seico Benner** "Large-scale behavioral data-driven approach for neurotoxicity risk assessment using animals"		
15:20-15:40	O-25. **(S) Keito Takahashi** "Inferring the health outcomes of PFAS using a network biology approach"	O-33. **Tomohiro Ito** "Analysis of effects of nanoplastic particles on human neurons"		
15:40-16:00	O-26. **Daniela M. Pampanin** "Automated analysis of histological lesions in whole slide images of fish liver"			
16:00-16:20	O-27. **Akira Kubota** "Developmental effects of estrogenic endocrine-disrupting chemicals and the predictability of in vitro and in silico analyses using zebrafish (*Danio rerio*)"			
Break				
16:30-18:00			General sessions (Poster)	
18:00-21:00				Wine party

28-Aug	
8:00-18:00	Excursion (Special tour to Minamata)

29-Aug	Hall A	Hall B	Hall C	Remarks
	General session "3. Bioassays and testing methods (1)"	General session "12. Environmental toxicology of terrestrial animals/ecosystems (1)"		

	Chairperson: Watanabe, Shimasaki	Chairperson: Ishizuka, Maekawa		
9:00-9:20	O-34. **(S) Jed Mamish** "Investigation of cytotoxicity of lipofectamine in rainbow trout fish cell lines"	O-43. **(S) Woo-Seon Song** "Evolutionary decline of AHR2 and compensatory role of AHR1: Implications for environmental toxicology in mammals"		
9:20-9:40	O-35. **(Y) Yusuke Oda** "Mixture effects of dialkylesters of phthalic acids (PAEs) on aquatic organisms based on predicted environmental exposure profiles"	O-44. **(S) Kanami Watanabe** "Evaluation of heavy metal and pesticide residues in fecal samples of african savanna elephants in Lower Zambezi National Park, Zambia"		
9:40-10:00	O-36. **Gisela Umbuzeiro** "Comparison of the eco/genotoxicity of natural anthraquinone dyes"	O-45. **Patryk Oleszczuk** "Biochar: The key to eco-friendly biosolids management and a thriving circular economy"		
10:00-10:20	O-37. **Haruna Watanabe** "How much known chemical substances in receiving water can explain the measured toxicity to alga, daphnid and fish?"			
Break				
10:40-11:40	Chairperson: Kang			
	Keynote lecture (3): **Dr. Xuchun Qiu** "Effects of pharmaceutical contaminants on fish behaviors: Underlying mechanisms and ecological consequences"			
Lunch break				
	Chairperson: Iwata			

13:20-14:20	Keynote lecture (4): **Dr. Cinta Porte** "New approach methodologies for studying plastic related toxicity: A lipidomic perspective"			
Break				
	General session "8. Effect of micro and nano plastics on aquatic animals at experimental and real condition (1)"	General session "12. Environmental toxicology of terrestrial animals/ecosystems (2)"		
	Chairperson: Oshima, Nakayama	Chairperson: Ishizuka, Iwata		
14:40-15:00	O-38. **(Y) Jin-Sol Lee** "Synergistic toxic mechanisms of microplastics and triclosan via multixenobiotic resistance (MXR) inhibition-mediated autophagy in the freshwater water flea *Daphnia magna*"	O-46. **Sébastien Lemiere** "Integrated assessment of the toxicological, ecotoxicological and ecological effects of all phytopharmaceutical treatments used during a whole potato crop season"		
15:00-15:20	O-39. **Margit Heinlaan** "Long-term effects of plastic additives on aquatic and terrestrial biota"	O-47. **(S) Dongha Kim** "Molecular and ecological linkages to dioxin sensitivity of mammalian aryl hydrocarbon receptor 1"		
15:20-15:40	O-40. **(S) Nik Nurhidayu Nik Mut** "Biofouling alleviates acute and chronic toxicity of polylactic acid microplastics to *Daphnia magna*"	O-48. **(Y) Dani Sukkar** "Imidacloprid and amitraz synergistically alter immune responses in Apis mellifera hemocytes with fungal PAMPs exposure"		
15:40-16:00	O-41. **(S) Radwa Saad** "Uptake and depuration kinetics of small microplastics in juveniles of sea squirts (*Ciona intestinalis*)"			

16:00-16:20	O-42. **(S) Md Al-Emran** "Vector effects of polyethylene microplastics on the accumulation of phenanthrene in Japanese medaka, *Oryzias latipes*"			
Break				
16:30-18:00			General sessions (Poster)	
18:00-21:00				Banquet

30-Aug	Hall A	Hall B	Hall C	Remarks
	General session "8. Effect of micro and nano plastics on aquatic animals at experimental and real condition (2)"	General session "19. Signals from aquatic organisms, the behavioral effects as a toxicity assessment indicator (1)"		
	Chairperson: Oshima, Shimasaki	Chairperson: Kang, Nakayama		
9:00-9:20	O-49. **(S) Yasuharu Katte** "Kinetics of microplastics with different polymer types in *Grandidierella japonica*"	O-56. **(S) Fu-Mei Chan** "Reproductive toxicity of benzophenone-related derivatives used in food contact materials on medaka"		
9:20-9:40	O-50. **(S) Sana Mikami** "Predicting the combined effects of environmental contaminants adsorbed on beached plastic pellets using network science"	O-57. **(Y) Kahina Mehennaoui** "Assessing behavioral responses and toxicokinetic profiles of multicomponent nanoparticles in *Daphnia magna*"		
9:40-10:00	O-51. **(S) SeokHyun Lee** "Impact of polystyrene ingestion on the gut microbiota and metabolic processes of wharf roaches (*Ligia sp.*)"	O-58. **(S) Abrianna Elke Chairil** "First application of one-class support vector machine algorithms for detecting abnormal behavior of marine medaka *Oryzias javanicus* exposed to the harmful alga *Karenia mikimotoi*"		

Break				
	General session "3. Bioassays and testing methods (2)"	General session "19. Signals from aquatic organisms, the behavioral effects as a toxicity assessment indicator (2)"		
	Chairperson: Yamamoto, Oshima	Chairperson: Kang, Shimasaki		
10:20-10:40	O-52. **Mariliis Sihtmäe** "Toxicity of silver–chitosan nanocomposites to aquatic microcrustaceans *Daphnia magna* and *Thamnocephalus platyurus* and naturally luminescent bacteria *Vibrio fischeri*"	O-59. **(Y) Yuki Takai** "Peek-A-Boo test: Assessing the behavioral impact of diazepam and chlorpyrifos on medaka fish (*Oryzias latipes*)"		
10:40-11:00	O-53. **Kaja Kasemet**s "Novel synergistic antimicrobial silver-chitosan nanocomposites: Design, efficiency and safety"	O-60. **(S) Yuya Hojo** "Examination for effects of OxyPAHs in Japanese medaka embryo triggered by oxidative stress "		
11:00-11:20	O-54. **Seiichi Uno** "Comparison of sensitivities between *Oryzias Javanicus* and *Oryzias latipes* embryos exposed to sediments collected in Tokyo Bay"	O-61. **Ik-Joon Kang "**Effect of chlorpyrifos on alarm reaction of Japanese medaka (*Oryzias latipes*) induced by alarm substances"		
11:20-11:40	O-55. **(S) Gunay Karimova** "Chronic toxicity and multifaceted detrimental effects of diflubenzuron on *Daphnia magna*"	O-62. **Hyung Chul Kim** "Investigation of the cause of the mass mortality of sardines in Jinhae Bay of Korea, 2022"		
11:40-12:00	Wrap-up			
12:00-12:30	Closing ceremony			

Poster presentations

26th August	
P-1. Kenji Yoshino	A trophic dilution of Hg in the macrobenthos community from Minamata Bay
P-2. **(Y)** Hiroyuki Kintsu	Accumulation of radioactive strontium derived from the Fukushima nuclear accident in vertebral bones of marine fishes
P-3. Zin'ichi Karube	Radiostrontium (Sr-90) activity in shells of mussels from the coastal area of Fukushima Prefecture, Japan
P-4. **(Y)** Kyoshiro Hiki	Effects of tire-derived chemical 6PPD-quinone on mitochondrial function in salmonid fish species
P-5. **(S)** Zhaoya Li	Effect of antifouling agents dichlofluanid and chlorothalonil on growth and photosynthetic activity of marine diatom *Thalassiosira pseudonana*
P-6. Chon Rae Cho	A study on the distribution characteristics of organotin compounds in sediments around Ulsan Bay, Korea
P-7. **(S)** Islem Boukara	Assessment of the effects of short-, medium-, and long-chain chlorinated paraffins on the differentiation of human iPS cells to definitive endoderm
P-8. **(Y)** Živilė Jurgelėnė	Graphene oxide nanoparticles for water purification: Mitigating heavy metal contamination and toxicity in aquatic ecosystems
P-9. Danguolė Montvydienė	The impact of war on the aquatic ecosystems of Ukraine: Problems and solutions
P-10. **(S)** Hao Chen	New approach methodology for hazard assessment of short-chain chlorinated paraffins in *ex-ovo* chicken embryos
P-11. Hisato Iwata	New approach methodologies to predict transactivation potencies of environmental contaminants mediated by Baikal seal estrogen receptor α and β
P-12. Christina Emmanouil	The sewage sludge directive in EU and its imminent amendment
P-13. **(S)** Emily K. C. Kennedy	A method for non-lethal detection of endocrine disruption in fishes
P-14. Pei-Jen Chen	Assessing bioavailability and toxicity of contaminated sediment with medaka fish
P-15. Keita Kodama	Decadal monitoring of the megabenthic community in the coastal areas of Fukushima Prefecture, Japan, after the Great East Japan Earthquake
P-16. Emiko Kokushi	Examinations for potential of dietary accumulations for chemicals by common carp (*Cyprinus carpio* L.) and relationship between biomagnification factors and octanol water partition coefficient
P-17. Noriaki Namba	Evaluation of the toxicity to algae of leaches from rare-earth elements (REE)-rich mud from the seabed around Minami-Tori-Shima
P-18. **(S)** Dylan Mack	Comparative analysis of spine and otolith gene expression and mineralization changes due to ancestral BaP exposure
P-19. Michio Suzuki	Cd binding protein from the midgut of the scallop, *Mizuhopecten yessoensis*
P-20. **(S)** Yuto Namikawa	Iron distribution and iron-binding protein in the digestive diverticula of *Turbo sazae*
P-21. **(Y)** Yugo Kato	A histidine-rich protein detected from lower jaws of green lugworm accumulating zinc

27th August	
P-22. (S) Deejay Suen-yui Mak	An investigation into chloroxylenol, a popular antimicrobial ingredient in hygiene and disinfection products, in water environments of Hong Kong
P-23. Maciej Tankiewicz	Pollutant penetration from poultry farm into groundwater
P-24. Yoshikatsu Takazawa	Long-range transport of POPs from East Asia
P-25. **(Y)** Agnieszka Fiszka Borzyszkowska	Screening analyses of micropollutants originating from intensive poultry farming in core soils
P-26. Natsuki Ono	Changes in *Takifugu rubripes* liver gene expression after oral administration of tetrodotoxin
P-27. **(Y)** Midori Iida	Network-based approach to exploring mechanisms of action for PFAS
P-28. Hyeon Seo Cho	Distribution and bioaccumulation of perfluoroalkyl substances in freshwater ecosystem of Korea
P-29. Masato Honda	Estimation of physiological effects of perfluorooctane sulfonate in *Takifugu rubripes*
P-30. Fumihiro Morishita	Disrupted seasonal expression of neuropeptide precursor genes in the brain of *Reishia clavigera* with abnormal reproduction near Fukushima Daiichi Nuclear Power Plant
P-31. Moonho Son	*Alexandrium catenella* (Group I) causes higher and faster toxicity than *A. pacificum* (Group IV) in *Mytilus eduis*
P-32. **(S)** Shoma Inoue	Growth interactions between the harmful dinoflagellates *Heterocapsa circularisquama* and the diatom *Skeletonema dohrnii*
P-33. **(S)** Zhangbin Liu	Growth interactions between the dinoflagellate *Karenia selliformis* and the diatom *Chaetoceros diadema*
P-34. **(S)** Han Bao	Effects of *Karenia selliformis* exposure on the sea anemone *Actinia equina*
P-35. Toshihiro Horiguchi	Accumulation and excretion of ^{131}I and ^{134}Cs in the rock shell, *Reishia clavigera*, exposed in a simulating manner of a nuclear power plant accident: toward the dose estimation
P-36. Frauke Seemann	Sperm epigenome as an indicator of modified offspring brain development
P-37. Tomohiko Isobe	Intervention trial for pharmacokinetic study of chemicals in daily use products
P-38. Jurate Zaltauskaite	Veterinary antibiotics as an environmental threat for forage crops: Tetracycline (TC) and ciprofloxacin (CIP) impact on oilseed rape (*Brassica napus*)
P-39. Daylan Pritchard	Evaluating the actions of nitrate, nitrite and ammonia using salmonid epithelial cell lines

29th August	
P-40. Jae-Seong Lee	Synergistic adverse effects of microfibers and freshwater acidification on host-microbiota interactions in the water flea *Daphnia magna*
P-41. Yuji Oshima	No vector effect of polystyrene microplastics on the accumulation of chlorobenzenes in Japanese medaka (*Oryzias latipes*)
P-42. **(S)** Elizabeth Everett	Microplastics as a disturbance to food web dynamics in Texas Gulf Coastal Bays
P-43. Kei Nakayama	Investigating the effects of virgin or recycled polypropylene microplastics on fish growth
P-44. Te-Hao Chen	Microplastic ingestion in the anemonefish *Amphiprion ocellaris*: Influence of concentration, odor, and light
P-45. **(S)** Kasumi Komatsu	The combined effect of organophosphate pesticide chlorpyrifos and microplastics on medaka behavior
P-46. **(S)** Miharu Tokunaga	Adsorption of polycyclic aromatic hydrocarbons on microplastics and their desorption in simulated intestinal fluids
P-47. **(S)** Daniel Christian Wijaya	Uptake and depuration kinetics of aged polyethylene microplastics in Java medaka (*Oryzias javanicus*) through quantification of actual plastic concentration using μFT-IR
P-48. Seiichi Uno	Evaluations of effects in marine amphipod, *Ptilohyale barbicornis*, absorbed microplastics through feeding
P-49. Seiichi Uno	Inductions of chondrodysplasia in fish larvae hatched from embryos exposed oxygenated polycyclic aromatic hydrocarbons
P-50. **(S)** Woo-Seon Song	Molecular toxicological characterization of the novel AHR2 in cats: Comparative insights with AHR1
P-51. Jaïro Falla-Angel	Stress reduction with co-culture of *Miscanthus x giganteus* and *Pelargonium x hortorum* in a Pb-contained soil to improve biomass production
P-52. **(Y)** Daishi Inoue	Avoidance behavior of fish to anesthetic agent MS-222 in euthanasia treatment
P-53. **(Y)** Yuki Takai	Behavioral diversity and sample size optimization in medaka fish behavioral tests
P-54. Jurate Zaltauskaite	Effects of elevated temperature and decreased water pH on tetracycline toxicity to *Lemna minor*
P-55. Gen Kume	Effect of global warming on population dynamics of a critically endangered Ryukyu-ayu *Plecoglossus altivelis ryukyuensis* in Amami-Oshima Island, southern Japan

Important Notes about Program

The symposium scientific program will take place at the Halls A, B and C in the Fukuoka Recent Hotel. Social events will take place at the Hall C. Excursion will take place at the locations indicated on the schedule.

Attendees presenting posters can put them up during registration period on Monday morning (August 26). Posters will remain up for the duration of the conference for viewing and judging (student and young scientists (under 40 years old) posters only). Judging will take place during afternoon coffee breaks; please ensure you are at your poster during this time. Posters can be taken down during morning coffee break on Friday (August 30).

Platform presentations are to be 15 minutes in length, followed by a 5-minute question and discussion period. A computer with Windows 10 and Microsoft Office 2019 will be available in each presentation room. MS PowerPoint (either .ppt or .pptx) presentations on a USB stick are to be submitted to the ISTA 21 staffs to copy the presentation file into the mobile PC equipped with the Halls A or B in the Fukuoka Recent Hotel by the morning of the day when the platform presentation is to be made. We will recommend every presenter to check his/her presentation slide operation before the platform session.

Plenary lecture

Unraveling the toxicity of 6PPD-quinone across fishes of commercial, cultural, and ecological importance

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Stormwater runoff from urban landscapes has recently been linked to mass mortalities of coho salmon in the U.S., also dubbed urban runoff mortality syndrome (UMRS). The chemical responsible has been identified as N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-Q), a transformation product of the rubber tire antioxidant 6PPD. This presentation summarizes a series of studies that assessed the acute toxicity of 6PPD-quinone across eight other fishes of commercial, cultural, and ecological importance in North America, and to characterize the specific mechanisms that drive toxicity using acute and sub-chronic experiments with select fishes. Acute toxicity differed greatly among species: No mortality occurred for Arctic char (AC), brown trout, bull trout, westslope cutthroat trout, and white sturgeon even at the highest measured concentrations (>13 µg/L), while lake (LT), brook, and rainbow (RBT) trout were sensitivity to 6PPD-Q exposure with LC50 values between 0.33 and 0.59 µg/L. Furthermore, sub-chronic exposures of RBT and LT demonstrated teratogenic effects in both larval RBT and LT. Experiments with primary cultures of RBT gill cells suggest that the mechanism of toxicity may be related to uncoupling the mitochondrial electron transport chain. Comparative cardiac ultrasound, electrocardiography and blood gas analysis revealed significant decreases in hemoglobin oxygenation and sympathetic stimulation in sensitive RBT but not insensitive AC, further supporting this hypothesis. Whole transcriptome analysis in RBT identified several molecular toxicity pathways that may explain the apical effects described above. Further research supporting development of a comprehensive toxicity pathway model supporting risk assessment of 6PPD-quinone across fishes is underway.

Keywords: acute toxicity; hazard assessment; mechanism of action; rubber tire particles; species sensitivity; stormwater; sub-chronic toxicity; toxicity pathway

Keynote lecture (1)

Coastal development, toxic urban runoff, and Pacific salmonid conservation in western North America

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Development transforms natural landscapes to pavement and other impervious surfaces. Reductions in rainfall infiltration and corresponding increases in stormwater runoff drive biological decline, a phenomenon termed the urban stream syndrome. Although the physical and biological facets of the syndrome have been studied for decades, the water quality dimension, particularly the contributions of toxic chemicals, remains poorly understood. This knowledge gap is undermining ongoing investments in river restoration, particularly in watersheds where in-stream flows are dominated by non-point source pollution inputs. This presentation will explore the influence of motor vehicles and the transportation grid on riverine and estuarine habitats that support threatened Pacific salmon and steelhead (*Oncorhynchus* spp.) populations in the western U.S. Emerging contaminants are common within urban drainages but rarely addressed in stream restoration, despite major societal investments (i.e., billions $USD) to improve salmon habitats. Intensive research on urban stream restoration effectiveness in the early 2000's eventually led to the discovery of a novel chemical in vehicle tires, 6PPD-quinone, as the underlying cause of widespread spawner mortality phenomenon among coho salmon (*O. kisutch*). This single but ubiquitous chemical - among hundreds or thousands in runoff – is sufficient to reduce wild salmon abundances at the population scale. The novel, tire-derived compound also poses a substantial threat to conventional restoration practices by creating ecological traps (nuisance habitats) upstream of migration barrier removal projects. Current research priorities to guide salmonid conservation and recovery will be discussed, including mechanisms of 6PPD-q toxicity, interactions with other chemicals in complex stormwater mixtures, sublethal impacts, interactions with temperature stress (in the context of climate change), and cumulative risks at the landscape and watershed scales.

Keywords: *Oncorhynchus*, non-point source pollution, 6PPD-quinone, PAHs, mixture toxicity, urban ecology, habitat restoration, endangered species, climate change

Keynote lecture (2)

Contaminants of emerging concern in the Salish Sea, USA

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The Salish Sea is an inland sea that includes the marine waterways and watersheds of both British Columbia, Canada and Washington, United States. In Washington (WA), the Salish Sea encompasses the Strait of Juan de Fuca, Puget Sound, and a portion of the Strait of Georgia and is home to iconic species such as Orcas and salmon. Several monitoring campaigns have document Contaminants of Emerging Concern (CECs) in the WA Salish Sea. These CECs include pharmaceuticals, antibiotics, phthalates, industrial chemicals, and PFAS. An additional concern are the chemicals released from microplastics, which are largely uncharacterized. Extensive work has been done to identify which of these CECs should be further investigated for management efforts. The work has included lab-based assays to assess the toxicity of chemicals leached from microplastics; risk-based approaches which rely on traditional measures of toxicity and New Approach Methodologies; and on-going field-based work. This presentation will include a synthesis of this work as it applies to CECs in the Washington Salish Sea.

Keywords: Contaminants of Emerging Concern, Exposure Activity Ratios, estrogenic chemicals, microplastics, toxicity benchmarks.

Keynote lecture (3)

Effects of pharmaceutical contaminants on fish behaviors: Underlying mechanisms and ecological consequences

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The rising levels and frequencies at which pharmaceuticals are being detected in aquatic ecosystems elicit notable concern, particularly in light of their potential adverse impact on non-target species such as fish (1-3). Based on standard endpoints used in risk assessments (excluding effects on behavior), current levels of most pharmaceutical contaminants in the natural environment (ranging from ng/L to low ug/L) appear to have a low probability of affecting fish (3, 4). However, accumulating evidence suggests that exposure to pharmaceuticals can disrupt a range of behaviors, including those associated with activity, anxiety, aggression, and reproduction (*4-6*). These behavioral changes could potentially disrupt natural predator-prey interactions and reproductive behaviors, emphasizing the need for a comprehensive assessment of those pollutants to understand their impacts on individual fitness and population stability (*5-8*). Nevertheless, there are significant knowledge gaps in understanding both the underlying mechanisms and ecological consequences of pharmaceutical-induced behavioral changes (*7*, *8*). In this presentation, I will share our recent research findings on the impacts of several typical neuroactive pharmaceuticals (e.g., Diazepam, Amitriptyline, and Thimerosal) on the behaviors of zebrafish and Japanese medaka, focusing on the mechanisms and potential consequences. In addition, some recommendations for better assessing the effects of pharmaceutical contaminants on fish behaviors will also be discussed, aiming to prompt an in-depth discussion on this topic.

References:

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Keywords: behavioral abnormality: ecological consequences; emerging pollutants; fish; pharmaceuticals; mechanisms.

Keynote lecture (4)

New approach methodologies for studying plastic related toxicity: A lipidomic perspective

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The exponential increase in plastic production and use, combined with inadequate waste management and plastics' high resistance to degradation, has resulted in widespread pollution, posing serious threats to ecosystems and human health. Investigating the toxic effects of plastics on the environment and living organisms is a major challenge, as toxicity is linked not only to visible pollution from debris but also to micro- and nano-plastics (MNPs) and the leaching of hazardous chemicals from plastic materials. In recent years, global research efforts have focused on developing and applying New Approach Methodologies (NAMs), ranging from simple cell cultures to 3D and organ-on-a-chip models, which do not require the use of living organisms and provide information on chemical hazards by elucidating the mechanisms of toxicity at the subcellular level. Besides, there is growing interest in the developing models that predict realistic exposure scenarios using repeated or chronic exposures at lower doses, yielding more environmentally relevant results.

By combining –omic technologies, such as lipidomics, with traditional toxicological data (cell viability, gene expression, and enzymatic activities), we aim to characterize the complex biological responses of fish liver cells (PLHC-1, ZFL) to single plastic additives, their mixtures, MNPs, and newly developed bioplastics, as well as their toxic effects after photodegradation and composting. Fish cell monolayers have been successfully applied, revealing specific lipid fingerprints, such as the accumulation of neutral lipids, membrane lipid reorganization, and depletion of lipid molecules containing highly unsaturated fatty acids after exposure to plastic additives and MNPs.

The use of fish liver spheroids (PLHC-1, ZFL), which exhibit a more mature liver phenotype from a lipidomic perspective compared to cell monolayers, will hopefully allow the detection of more realistic responses to plastic exposure in the near future, and likely increase the sensitivity of traditional in-vivo approaches.

Keywords: spheroids; fish cells; liver; toxicity; bioplastics; lipidomics

Special Session lecture

An assessment model of toxic chemicals integrating benthic-pelagic lower ecosystem and the lower-higher torphic food web

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In toxicology, the development of research and accumulation of knowledge at the levels of individual organisms, cells, and genes are remarkable. However, when considering future guidelines for environmental management, it is essential to evaluate impacts at the population and community levels. For example, it is important to unify perspectives on the changes in the lower ecosystem spanning sediment-water, changes in the food web from lower trophic levels to higher trophic levels, and the behavior of harmful chemicals associated with measures such as controlling inflow loads, improving hypoxia, and mitigating climate change.

In this context, we are attempting to develop a prediction and evaluation system for harmful chemicals in Tokyo Bay, consisting of (1) an ecosystem model representing the dynamics of the lower ecosystem connecting sediment and water, (2) the relationship between sedimentary organic matter and chemicals, and (3) an analysis of the bioaccumulation of chemicals through the food web from lower to higher trophic levels. In (1), we analyze the dynamics of sedimentary organic matter, phytoplankton, zooplankton, benthic animals, and microorganisms. These metabolisms are formulated by nutrients, water temperature, light intensity, etc., and can analyze the ecosystem's response to inflow load reduction and climate change. In (2), we analyze the relationship between the quality of sedimentary organic matter and the adsorption of chemicals. In (3), we estimate the food web among major biological groups using stable isotope ratios and analyze the amount of harmful substances accumulated in organisms through this food web.

By integrating (1), (2), and (3), this system aims to clarify the response of water quality, lower and higher organisms, and the bioaccumulation of harmful chemicals to environmental policies. On the day of the presentation, we will introduce an overview of this system and some of the results.

Keywords: ecosystem model; food web; bioaccumulation; population dynamics; benthic-pelagic coupling, Tokyo Bay, TBT

Impairment of post translational histone modifications in osteoblast subpopulations after parental benzo[a]pyrene exposure

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Environmental stressors, such as Benzo[a]pyrene (BaP) have been repeatedly associated with developmental bone defects in offspring and are considered a risk factor for later life-stage osteoporosis in fish. Impaired osteoblast differentiation is marked by deregulated gene expression, which is likely induced through changes in the miRNA expression and in the gene-specific methylation pattern. Notwithstanding, further research is required to explain the inheritance mechanisms of this inherited bone phenotype. Epigenetic mechanisms influence osteogenic differentiation and bone related metabolic processes. More specifically, chemical modifications along the histone 3 (H3) and 4 (H4) tails are crucial for Bmp and Wnt/β-catenin signaling pathways during osteoblast differentiation. Histone modifications in parentally BaP-exposed offspring may play a dominant role in the epigenetically inherited bone phenotype. To understand the role of histone modification changes in osteoblasts, immunohistochemistry experiments have been conducted with selected histone modification antibodies for H3 tail methylation and H4 tail acetylation to assess their contribution to an osteoblast subpopulation-specific histone profile upon parental BaP exposure. F1 adults from a transgenic twist:dsred/col10:gfp medaka (*Oryzias latipes*) strain were assessed to yield novel data on the histone code of osteoblasts and allow quantification of parental environmental pollutant exposure's interference with chromatin structure regulation. Ancestral BaP exposure tended to deregulate the histone tail modifications resulting in a changed chromatin structure. Understanding the relationship between epigenetic modification and bone health will improve assessment of ecological risk and public health impact of BaP pollution and further address the hypothesis that BaP-induced histone modifications are inherited over generations and involved in bone formation in an osteoblast subpopulation specific manner.

O-2

Toxicity of rare earth elements on medaka fish and associated ecological risk assessment

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Rare earth elements (REEs), comprising the lanthanide series along with scandium (Sc) and yttrium (Y), are a group of 17 elements widely used in various industries such as electronics, energy, medicine, and agriculture. REEs are considered as emerging contaminants, as increasing global demands for REEs have led to their releases into the environment, resulting in pollution and bioaccumulation in the aquatic ecosystem around the world. In this study, we have used medaka (*Oryzias latipes*) larvae as a model organism to screen the lethal and sublethal toxic effects of 13 REEs. The 96-hour lethal concentration 50 (96 h-LC_{50}) of tested REEs showed that Yttrium (LC_{50} = 1.40 μM) and lanthanum (La, LC_{50} = 1.55 μM) were the most lethal toxic elements, gadolinium (Gd, LC_{50} = 2.15 μM) and dysprosium (Dy, LC_{50} = 2.20 μM) were followed. Seven-day sublethal exposure with medaka larvae showed impacts on swimming behaviors by La (50 nM) and Y (200 nM). As well, La and Dy dose-dependently decreased body length and weight of treated larvae after 7 day-sublethal exposure, whereas La and Y exhibited higher bioconcentration factors (BCFs). The species sensitivity distribution (SSD) and assessment factor (AF) approaches were used to assess the predicted no-effect concentrations (PNECs) of tested REEs. Risk quotients (RQs) were calculated through the ratio of measured environmental concentration (MEC) to PNEC to determine ecological risks of Y, La, Gd, and Dy on the aquatic environment.

Reproductive abnormalities observed in a rock shell population in the vicinity of the Fukushima Daiichi Nuclear Power Plant, Japan

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In 2012, after the accident at the Fukushima Daiichi Nuclear Power Plant (FDNPP) that followed the Tohoku earthquake and tsunami in March 2011, no rock shell (*Reishia clavigera*; Gastropoda) specimens were found near the plant from Hirono to Futaba Beach (a distance of approximately 30 km). In July 2016, however, rock shells were again found to inhabit the area. From April 2017 to June 2023, we collected rock shell specimens monthly at a site near the FDNPP (Ottozawa) and at a reference site ~ 120 km south of the FDNPP (Hiraiso). We examined the gonads of the specimens histologically to evaluate their reproductive cycle and sexual maturation. The gonads of the rock shells collected at Ottozawa, ~ 1 km south of the FDNPP, exhibited a certain reproductive abnormality, consecutive sexual maturation (CSM) during the 6 years from April 2017 to June 2023, whereas sexual maturation of the gonads of specimens collected at Hiraiso was observed only in summer. The CSM of the gonads of the specimens collected at Ottozawa might not represent a temporary phenomenon but rather a site-specific phenotype, possibly caused by specific environmental factors near the FDNPP. Meanwhile, the CSM of the gonads has been observed only in rock shell populations inhabiting the sites within 3 km south of the FDNPP: more severely affected in the specimens collected at Ottozawa (1 km south of FDNPP) than those at Koirino (3 km south of FDNPP). No or less recovery was observed from the CSM in rock shells collected at Ottozawa, even if they were kept at the laboratory of National Institute for Environmental Studies, namely, under different circumstances in the vicinity of FDNPP, for a year. It is suggested that unknown factor(s) derived from the FDNPP might have affected the regulatory system of physiology in the rock shell, resulting in the expression of CSM. Investigation on the causal factor(s) for CSM is currently ongoing with various analytical methods.

Long-term changes in the megabenthic community structure of Tokyo Bay in relation to shifts in environmental conditions

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Tokyo Bay is a heavily eutrophicated area in Japan that has experienced substantial changes in both its environment and biota due to human activities in metropolitan areas, as well as changes in meteorological and oceanographic conditions. To assess the effects of these factors on the ecosystem, monitoring surveys to investigate changes in the biocoenosis are essential. We have conducted fisheries-independent trawl surveys at 20 sampling sites covering the entire bay from 1977 to 1995 and from 2002 to 2023. Fishes, crustaceans, mollusks, and echinoderms were identified to the species level, and changes in their abundance and biomass were examined. We found substantial changes in the community structure during the sampling period. The total abundance and biomass exhibited an increasing trend until 1987, followed by a substantial decline from the late 1980s to the 1990s due to a decrease in small to medium-sized fish and crustacean species. Meanwhile, a marked increase in the number of large fish, including elasmobranchs, mollusks, and echinoids, was observed in the 2000s. These shifts in the megabenthic community structure were correlated with an increase in water temperature and a decrease in nutrient concentrations and copepod densities. Cumulative evidence suggests that a remarkable shift in the megabenthic community structure occurred between the 1970s and the 2020s, which was possibly associated with variations in the environmental conditions in Tokyo Bay.

Recent trend of antifouling coating technology and related regulations

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Antifouling coatings play an important role for maintaining the ship performance during the life span of ship by function of preventing the marine organisms attaching to the ship's underwater hull. To achieve the reduction of CO2 emission from the maritime sector, numerical ship performance parameters related with CO2 emission such as EEDI, EEXI, CII, target value and rating by each parameter are prescribed by the amended treaty of MARPOL Annex VI. Regulation for use of antifouling coating systems is also in progress. Cybutryne is the first alternative biocide which is banned by amended AFS treaty of 2023 after tri-butyl tin compounds were banned in 2008. Another new trend of the related treaty is the countermeasure for cross-border transfer of marine organisms by ships. Some local treaties have been already entered into force in United States (California), Australia and New Zealand. In this point of view, antifouling coatings is also playing great contribution for the past, present and future. Therefore, it should weigh in the balance with enough scientific evidence, both environmental adverse effects and contribution to ship performance, preventing the movement of marine organisms by using of antifouling coatings. In this presentation, recent trend of developments of antifouling coatings is introduced. For example, use of new active substance technology combined with self-polishing technology is introduced. Another new technology is silicone-based technology which is combined with surface active polymer and small amount of active agent. Use of information technology solution package is one of the most effective ways for optimal use of the antifouling coatings. Performance analysis visualize actual ship performance. Operational profile analysis is available for optimal antifouling coating selection for each vessel. Performance simulation considered both hull fouling and roughness conditions is also available for selection of adequate antifouling coating.

A bioassay for evaluating the efficacy of antifouling paints on algae using their CIELAB color coordinates with a flow-through system

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Biofouling on ships increases fuel consumption and can lead to the spread of non-indigenous species, impacting local ecosystems. Anti-fouling (AF) paints play an important role in the prevention of biofouling on ships. In this regard, the authors have already conducted efficacy tests of AF paints using mussels and barnacles. The authors focused on using algae, *Ectocarpus* sp., as the test organism. A laboratory bioassay was designed and its applicability for testing the efficacy of AF paints was investigated. Five types of AF coatings with cuprous oxide (0, 5, 10, 20 and 40 wt.%) were prepared as test paints. The test plates were coated on one side with the test paint. The plates were aged using a dynamic rotating device under controlled condition with light shielding prior to the bioassay. An algal suspension was filtered through a nitro cellulose membrane filter under vacuum filtration. The membrane filter with algae was cultured in PESI medium in a still water condition. After culturing the algae, the surface of the membrane with algae was affixed to the test and control plates. The laboratory bioassay was conducted under a controlled condition with a flow-through system using boiled seawater as the test medium. The efficacy of AF paints was evaluated by the CIELAB coordinates (L*, a*, b*) of algae after the bioassay. The value of L* increased with increasing Cu_2O content. A linear regression analysis was performed on the relationships between values of L* and survival rate of the algae. Results showed that the value of L* decreased with an increase in survival rate. Further, the assessment of the estimated paint performance from the bioassay was confirmed using correlation patterns between the value of CIELAB coordinates and the survival rate of the algae. The newly designed bioassay using brown algae under a flow-through system was successfully conducted for screening AF paints. This method proves to be reliable for evaluating current and future AF paints.

Detrimental effects of hull cleaning wastewater on oxidative status, life cycle parameters, and population growth of the monogonont rotifer Brachionus manjavacas

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While wastewater discharged from in-water cleaning process of ship hulls on rotifer consistently released into aquatic ecosystem, its detrimental effects on non-target animals are largely unclear. In this study, we provide evidence on detrimental effects of hull cleaning wastewater in the monogonont rotifer *Brachionus manjavacas* by analyzing biochemical and physiological parameters in its oxidative status, survival, lifespan, growth, fecundity, and population. The wastewater contained high concentrations of metals (Zn and Cu) and metal–based antifoulants (CuPT and ZnPT). Significant oxidative stress was observed in response to two wastewater samples [1) raw wastewater (RW) and 2) mechanical filtrated in the cleaning system (MF)]. Higher detrimental effects in survival, lifespan, fecundity, and population growth for 10 days were measured in the RW-exposed rotifers than those results analyzed in the MF-exposed rotifers. Two growth parameters, lorica length and width were also significantly modulated by both wastewater samples. These results indicate that even filtered hull cleaning wastewater would have deleterious effects on the maintenance of the rotifer population when they exposed constantly.

Hull-cleaning wastewater poses serious acute and chronic toxicity to a marine mysid—A multigenerational study

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We conducted a comprehensive assessment involving acute effects on 96-hour survival and biochemical parameters, as well as chronic effects on growth and reproduction spanning three generations of the marine mysid *Neomysis awatschensis* exposed to filtered wastewater to evaluate the potential impact of ship hull-cleaning wastewater on crustaceans. The analyzed wastewater exhibited elevated concentrations of metals, specifically zinc (Zn) and copper (Cu) and metal-based antifoulants, i.e., Cu pyrithoine (CuPT) and Zn pyrithoine (ZnPT). The results revealed dose-dependent reductions in survival rates, accompanied by a notable increase in oxidative stress, in response to the sublethal values of two wastewater samples: 1) mechanically filtered using the cleaning system (MF) and 2) additionally filtered in the laboratory (LF) for 96 h. Mysids exposed to MF displayed higher mortality than those exposed to LF. Furthermore, mysids subjected to continuous exposure of 0.001% LF across three generations exhibited significant inhibition of the feeding rate, more pronounced growth retardation along with an extended intermolt duration, and a diminished rate of reproduction compared to the control. A noteworthy inhibition of the feeding rate and growth was observed in the first generation exposed only to the LF sample. However, although the reproduction rate was not significantly affected. Collectively, these findings underscore the potential harm posed by sublethal concentrations of wastewater to the health of mysid populations under consistent exposure.

Antifouling and marine paint – related microparticles containing heavy metals in surface water in Osaka Bay, Japan

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Based on the assumption that particles containing high concentrations of copper originate from ship antifouling paint, we analyzed the heavy metals and polymer types of the anthropogenic microparticles (MPs) floating in sea-surface microlayer (S-SML) and bulk water (1m under the sea surface) in 8 sites of Osaka Bay for the years 2021–2023. The metal types in MPs were analyzed by X-Ray Fluorescence Spectrometer (XRF) and the abundance of MPs containing heavy metals (Metal MPs) was assessed. 7 types of metal MPs were found and the corresponding metal component was Cu, Cu-Zn, Zn, Ti, Sn, Ba and Fe-Mn-Ni (Fe-Mn or Fe-Ni). The abundance of Cu, Zn, Ti and Sn MPs was positively correlated with microplastics with various polymers from the same sampling site, found in a previous study ($p<0.05$). The polymer type for 97.8% of Cu and Cu-Zn MPs (41 samples) and 56.6% of Zn MPs (23 samples) was acrylic resins which are widely used as binders in contemporary antifouling paints for ships; as such these Cu, Cu-Zn and Zn MPs may show the occurrence of antifouling paint particles (APPs) that fall off from hulls. Then the calibration curve between fluorescent X-ray intensity for Cu and Zn and the metal concentrations in standard APPs measured by atomic absorption spectrometry was made for each particle size, and Cu and Zn concentrations (mg/kg) in MPs were quantified by the standard curves. It was found that the Cu concentrations in Cu MPs ranged from 511–53,600 mg/kg and the Zn concentrations in Zn MPs ranged from 95.2–13,300 mg/kg, respectively. Copper concentrations in some MPs of this study exceeded 1%, higher than the ones reported in literature in the North Atlantic Ocean. This study was the first to infer some Cu MPs in Osaka Bay as APPs according to their high Cu concentration and polymer types, and the burden of all metal MPs in Osaka Bay was also discussed.

O-10

A new mechanism of reproductive endocrine disruption based on isothiazolinone

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The unintended exposure of humans and animals to isothiazolinones has led to increasing concern regarding their health hazards. Isothiazolinones were previously found to disrupt reproductive endocrine homeostasis. However, the long-term reproductive toxicity and underlying mechanism remain unclear. In this study, life-cycle exposure of marine medaka to dichlorocthylisothiazolinone (DCOIT), a representative isothiazolinone for antifouling purpose, significantly stimulated the gonadotropin releasing hormone receptor (GnRHR)-mediated synthesis of follicle stimulating hormone and luteinizing hormone in the brain. Chem-Seq and proteome analyses revealed disturbances in G protein-coupled receptor, MAPK, and Ca^{2+} signaling cascades by DCOIT. The G protein αi subunit was identified as the binding target of DCOIT. Gαi bound by DCOIT had enhanced affinity for the mitochondrial calcium uniporter, consequently changing Ca^{2+} subcellular compartmentalization. Stimulation of Ca^{2+} release from the endoplasmic reticulum and blockage of Ca^{2+} uptake into the mitochondria resulted in a considerably higher cytoplasmic Ca^{2+} concentration, which then activated the phosphorylation of MEK and ERK to dysregulate hormone synthesis. Overall, by comprehensively integrating *in vivo*, *ex vivo*, *in silico*, and *in vitro* evidence, this study proposes a new mode of endocrine disrupting toxicity based on isothiazolinones, which is expected to aid the risk assessment of chemical library and favor the mechanism driven design of safer alternatives.

Monitoring and ecological risk assessment of antibiotics and herbicides in the Seto Inland Sea, Japan

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Antibiotics and herbicides are being increasingly used globally. Although many studies have reported the environmental fate and ecological risks of antibiotics and herbicides from a land viewpoint, few have assessed them in coastal waters. This study monitored antibiotics and herbicides in Japan's Seto Inland Sea to examine their dynamics, and their ecological risks were evaluated by comparing their concentrations in environmental water with previously reported predicted no-effect concentrations (PNECs). Surface water samples were collected from several points in the Seto Inland Sea from 2019 to 2022. Three herbicides and 12 antibiotics were selected as analytes, and samples pretreated via solid-phase extraction were analyzed using liquid chromatography-tandem mass spectrometry.

In total, 266 seawater samples were analyzed. The antibiotic sulfamethoxazole exhibited the highest detection rate (71%), followed by herbicides (bromacil and diuron) and other antibiotics (clarithromycin, metribuzin, azithromycin, erythromycin, roxithromycin, and spiramycin). No other analytes were detected in any sample. Measurements of antibiotic and herbicide concentrations in the environmental water showed that those of Hiroshima Bay were generally higher in the inner part of the bay than at the bay mouth. Likewise, they were higher inside the weir built at the river mouth inflowing Fukuyama Port, indicating their land origin. A comparison of the antibiotic and herbicide concentrations with previously reported PNECs showed that diuron, sulfamethoxazole, azithromycin, clarithromycin, and erythromycin existed at concentrations higher than the PNECs. For antibiotics, fewer than 10 samples exceeded the PNECs, and it was assumed that the ecological risk would be higher near sewage treatment plants and in limited estuarine areas. However, more than half of the diuron samples exceeded the PNEC, indicating concerns regarding the ecological risk over a wider coastal area.

O-12

Single and combined effects of increased temperature and methylmercury on different stages of the marine rotifer Brachionus plicatilis

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Rapid, anthropogenic activity–induced global warming is a severe problem that not only raises water temperatures but also shifts aquatic environments by increasing the bioavailability of heavy metals (HMs), with potentially complicated effects on aquatic organisms, including small aquatic invertebrates. For this paper, we investigated the combined effects of temperature (23 and 28 °C) and methylmercury (MeHg) by measuring physiological changes, bioaccumulation, oxidative stress, antioxidants, and the mitogen-activated protein kinase signaling pathway in the marine rotifer *Brachionus plicatilis*. High temperature and MeHg adversely affected the survival rate, lifespan, and population of rotifers, and bioaccumulation, oxidative stress, and biochemical reactions depended on the developmental stage, with neonates showing higher susceptibility than adults. These findings demonstrate that increased temperature enhances potentially toxic effects from MeHg, and susceptibility differs with the developmental stage. This study provides a comprehensive understanding of the combined effects of elevated temperature and MeHg on rotifers.

Toxicity and speciation of inorganic arsenics and their adverse effects on in vivo *endpoints and oxidative stress in the marine medaka* Oryzias melastigma

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Here, we investigate the effects of acute and chronic exposure to arsenate (AsV) and arsenite (AsIII) in the marine medaka *Oryzias melastigma*. *In vivo* effects, biotransformation, and oxidative stress were studied in marine medaka exposed to the two inorganic arsenics for 4 or 28 days. An investigation of embryonic development revealed no effect on *in vivo* parameters, but the hatching rate increased in the group exposed to AsIII. Exposure to AsIII also caused the greatest accumulation of arsenic in medaka. For acute exposure, the ratio of AsV to AsIII was higher than that of chronic exposure, indicating that bioaccumulation of inorganic arsenic can induce oxidative stress. The largest increase in oxidative stress was observed following acute exposure to AsIII, but no significant degree of oxidative stress was induced by chronic exposure. During acute exposure to AsV, the increase in the enzymatic activity of glutathione-S-transferase (GST) was twice as high compared with exposure to AsIII, suggesting that GST plays an important role in the initial detoxification process. In addition, an RNA-seq–based ingenuity pathway analysis revealed that acute exposure to AsIII may be related to cell cycle progression. A network analysis using differentially expressed genes also revealed a potential link between the generation of inflammatory cytokines and oxidative stress due to arsenic exposure.

Game-changing transformations: Rethinking metal nanoparticle toxicity in water

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The increasing production and use of nanoproducts has led to the environmental release of engineered nanoparticles (ENPs). Once in the environment, ENPs undergo various physical, chemical, and biological transformations that affect their properties, fate, and toxicity. This study investigated the chemical and biological transformations of two commonly used ENPs, nano-CuO and nano-ZnO, and evaluated the toxicity of the transformed ENPs (trans-ENPs). The chemical transformation involved sulfidation, while biological modification entailed the formation of a protein corona (BSA) on the ENP surface. As transformed ENPs may undergo continuous modification, sulfidized ENPs were also coated with a protein corona. The trans-ENPs were physicochemically characterized using TEM, DLS, BET, XRD, XRF, XPS, FTIR, and ICP-OES. The acute toxicity of pristine (p-) and trans-ENPs was assessed using *Daphnia magna* and Lepidium sativum as test organisms. The p-ENPs exhibited higher toxicity to both organisms than the trans-ENPs. However, the phytotoxicity of BSA@ZnS was greater than that of ZnO, ZnS, and BSA@ZnO, based on EC50 values. In contrast, the toxicity to *D. magna* followed the trend: ZnO≈BSA@ZnS < ZnS < BSA@ZnO. Transforming nano-CuO significantly reduced its toxicity to crustaceans and plants in the order: BSA@CuS < BSA@CuO < CuS < CuO. While the bioactivity of ENPs is attributed to the released metal ions, the ENPs themselves, or both, no clear relationship was found between the concentrations of Zn^{2+} and Cu^{2+} in the test media and the observed endpoints. This suggests that other mechanisms, such as the diverse bioaccumulation of ENPs with different biological identities due to the presence of a biocorona, may be responsible for the toxicity. This study provides valuable insights into the transformations, behavior, and toxicity of ENPs, which can inform risk assessments related to nanopollution.

Unraveling the mechanism and species sensitivity differences in 6PPD-quinone toxicity by using whole transcriptome analysis in four fish species

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N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone(6PPD-quinone), has recently been identified as a cause of acute mortality in coho salmon exposed to urban runoff. 6PPD-quinone poses significant concern in aquatic environment as it exhibits a wide distribution of species sensitivities among fishes and the underlying mechanism of toxicity remains unknown. Therefore, there is urgent need for assessment of hazards and risk associated with 6PPD-quione; however, current methods in chemical hazard assessment heavily rely on costly, time-consuming, and ethically questionable live animal testing. This study applied an alternative approach that employs next generation whole transcriptome analysis to investigate the toxicity of 6PPD-quinone in early-life stages of rainbow trout (*Oncorhynchus mykiss*), lake trout (*Salvelinus namaycush*), Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*). An advanced dose-response modeling approach to derive transcriptomics points of departure (tPODs) was successfully applied to both rainbow trout (Acute LC_{50}: 1 µg/L) and lake trout (Acute LC_{50}: 0.50 µg/L). Data for Atlantic salmon and brown trout are still in the process of analysis. We found that tPODs effectively estimated previously reported apical benchmark concentrations (BMC) from acute and sub-chronic tests with adult and early life stages. Lists of differential express genes were used for pathway analysis, which indicated that rainbow trout and lake trout shared several pathways that were indicative of certain apical outcomes. In contrast, Atlantic salmon exhibited a greater number of unique pathways when compared to both rainbow trout and lake trout, which suggests that potential compensatory mechanism may dominate responses in insensitive species. Our results highlight the potential of using transcriptomics for chemical hazard assessment and provide a deeper understanding of species-specific responses to 6PPD-quinone.

Unveiling the molecular effects of replacement and legacy PFASs: Transcriptomic analysis of zebrafish embryos reveals surprising similarities and potencies

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The prevalence of per- and poly-fluoroalkyl substances (PFASs) in the environment has prompted restrictions on legacy PFASs due to their recognized toxic effects. Consequently, alternative "replacement" PFASs have been introduced and are prevalent in environmental matrices. Few studies have investigated the molecular effects of both legacy and replacement PFASs under sub-lethal exposures. This study aimed to address this by utilizing transcriptomic sequencing to compare the molecular impacts of exposure to the legacy perfluorooctane sulphonic acid (PFOS) and two of its replacements, perfluoroethylcyclohexane sulphonate (PFECHS) and perfluorobutane sulphamide (FBSA). Using zebrafish embryos exposed to concentrations 0.001 – 5 mg/L for 72 hours, the research assessed sub-lethal apical effects (mortality, morphology, and growth), identified differentially expressed genes (DEGs) and enriched pathways, and determined transcriptomic points of departure (tPoDs) for each compound. Results indicated that PFOS exhibited the highest relative potency, followed by PFECHS and then FBSA. While similarities were observed among the top DEGs across all compounds, over-representation analysis revealed slight differences. Notably, PFOS demonstrated the lowest tPoD identified to date (0.0025 mg/L). This study also investigated the feasibility of applying network-based analyses to quantitatively link individual and population adverse outcomes with molecular dysregulation and concluded that adverse outcome pathway-based network analysis is likely an accessible and efficient method for identifying the main mechanisms of complex chemicals. These findings raise concerns regarding the safety of emerging replacement PFASs and challenge assumptions about PFAS toxicity solely resulting from their accumulative potential. As replacement PFASs proliferate in the environment, this study underscores the need for heightened scrutiny of their effects and questions current regulatory thresholds.

Xenometabolome of early-life stage salmonids exposed to 6PPD-quinone

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N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-Q) is a ubiquitous and acutely toxic transformation product (TP) derived from the rubber tire antioxidant 6PPD. While not all salmonids are sensitive to acute lethality, sensitivity to 6PPD-Q can vary by several orders of magnitude among fish species. The main driver(s) of species sensitivity differences is a pressing question, with one area of interest examining whether differences in their ability to biotransform and detoxify 6PPD-Q could be a driving factor. This study utilized high-resolution mass spectrometry (HRMS) to assess biotransformation and metabolome-wide effects of 6PPD-Q on early-life staged salmonids, including two sensitive species, rainbow trout (*Oncorhynchus mykiss*) and lake trout (*Salvelinus namaycush*), and one tolerant species, brown trout (*Salmo trutta*). We detected three Phase I TPs and seven Phase II TPs and revealed that brown trout had the greatest ability to detoxify 6PPD-Q. TP-OH1, a phase I TP, was detected in both rainbow and lake trout, but not in brown trout, with more research needed to understand potential TP-OH1 mediated toxicity. Several endogenous metabolites were found to be dysregulated in rainbow and lake trout, indicative of mitochondrial dysfunction and altered metabolism. Our results indicate a difference in the biotransformation capability of 6PPD-Q among salmonid fish species and subsequent unique metabolome responses.

Residue analysis of non-phthalate plasticizers in seawater and sediments at Osaka Bay, Japan

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Due to endocrine-disrupting effects of phthalate esters on humans, non-phthalate plasticizers (NPPs) are used as alternative plasticizers to phthalate esters, and their usage have been increasing. There is, however, limited knowledge on their environmental residues worldwide, and they have not been reported in Japanese aquatic environments. In this study, a method to analyze NPPs in seawater using solid-phase extraction was developed, and the residual concentrations of five NPPs (Diisobutyl adipate (DIBA), Acetyl tributyl citrate (ATBC), Di-(2-ethylhexyl) adiate (DEHA), Di-(2-ethylhexyl) sebacate (DEHS) and Trioctyl trimellitate (TOTM)), and two phthalate esters (Dicyclohexyl phthalate (DCHP) and Diethylhexyl phthalate (DEHP)) in seawater and sediment collected from Osaka Bay were quantified. The recovery of the target substances in seawater was more than 68 % using an Oasis Max column and acetone as eluting solvent. The recovery of the target substances in freeze-dried sediment was more than 63 % using ethyl acetate and dichloromethane as extracting solvent. In seawater NPPs were not detected, and only DEHP was detected at 22-515 ng/L from two sites out of 12 sites. On the other hand, ATBC and TOTM in sediments from about half of the 14 sites ranged from 36-69 ng/g and 47-131 ng/g, respectively, while DEHA and DEHS at only one site were detected at 83 ng/g and 181 ng/g, respectively. Higher concentrations of ATBC, DEHS and TOTM were observed at close to factories, suggesting the NPPs may be present in effluent from the factories. The residual concentrations of TOTM were correlated with the ignition loss of sediments. DEHP concentrations in sediments were ranging in 53-1161 ng/g at all sampling sites, with a trend of decreasing concentrations from coastal areas to center of the bay. This is the first report on the environmental occurrence of NPPs in the coastal area of Japan.

Organic pollutant impact from poultry farm on soil

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Intensive rearing aims to maximize profit or efficiency while minimizing time and costs. Unfortunately, intensive animal husbandry may lead to adverse effects on the environment and therefore on human health, especially since it concerns living organisms, which can only intensify the negative effects. The aim of the study was to identify organic contaminants in soil samples collected at a farm and from nearby agricultural fields fertilized with manure. The obtained results showed the presence of various substances, including drugs and antibiotics, which are closely correlated with their use in intensive poultry farming. Moreover, polycyclic aromatic hydrocarbons and pesticides were also detected. Of particular note was the presence of the pesticides o,p'-DDE and p,p'-DDE, the use of which was banned in 1972. In addition, the ecotoxicity assessment of the collected samples was also performed using the Phytotoxkit and Ostracodtoxkit F bioassyas. This way, more complete information was obtained about the condition of the soil and the possible impact of farms on the surrounding area.

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Benzo[a]pyrene as an epigenetic toxicant

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Benzo(a)pyrene (BaP), an environmental toxicant, is associated with transgenerational inheritance of adverse phenotypes at background concentrations. Previous research point towards disruption of the inheritable epigenetic profile as the underlying mechanism of the changed phenotype in the offspring of Japanese medaka (*Oryzias latipes*) and other fish models. Currently it the extent of epigenetic alteration in the parental genome is unknown. To elucidate the interactions of BaP and its metabolite, benzo(a)pyrene diol epoxy (BPDE), with chromatin structure and chromatin modulating enzymes, transposase-accessible chromatin sequencing (ATACSeq) and *in silico* computational simulations have been conducted. It hypothesized that parental BaP exposure is modifying the epigenetic profile of mature oocytes in a site-specific manner targeting phenotypically relevant genomic loci. Genes with significantly modified epigenomic marks are expected to be related to bone and brain developmental pathways associated with the inherited phenotype. The exploration of BPDE involvement in chromatin alterations was investigated through computational docking of different methyltransferases (polycomb repressive complex 2, mixed lineage leukemia complex, DNA methyltransferase), which were differently transcribed in ancestrally exposed fish. The docking results confirmed the hypothesis that epigenetic methyltransferase's function may be dysregulated by BPDE. Results showed a conserved BPDE binding pocket in the active site of methyltransferases at the SET domain, suggesting BPDE may competitively inhibit these enzymes. Quantum/molecular mechanics computational modeling will assess the kinetics and energetic aspects of the BPDE interaction within the epigenetic methylation reactions. This research enhances the understanding of phenotypical ramifications from background BaP exposure, refining risk assessment for human and wildlife health by using *in silico* and *in vivo* methods.

Antibiotic resistance in the soils of the intensive farming coop and fields nearby

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Poultry production by industrial farms has a negative impact on people and the environment, in particular, by emissions of greenhouse gases and pollution of soil, surface water, and air, causing epidemiological threats and loss of biodiversity. Environmental conditions in the immediate vicinity of areas with intensive poultry breeding are constantly under pressure to emit different compounds, such as pesticide residues, various pathogens, pharmaceuticals (antibiotics and hormones), and metals. Among them, antibiotics are of high interest because of the possibility of the formation of antibiotic-resistant strains of pathogens, and consequently, are able to rapidly spread resistance genes (Gržinić et al. 2023). Therefore, we examined the impact of pharmaceuticals emitted by intensive farming on soil microbiology.

In the present study, microbiological tests were performed on soil collected around an intensive poultry farm and crop fields fertilized with manure. Apart from slight microbiological contamination with fecal flora (*E. coli*, coliform groups, and *Clostridium* spp.), no increased critical microbiological parameters were found in the tested samples. Only a few coliform strains that were naturally resistant to 3rd generation cephalosporins of the *Enterobacter* genus were found. The selected pharmaceuticals were quantitatively measured, confirming the presence of e.g. sulfacarbamide, sulfamerazine, sulfadiazine, and metoclopramide. Based on this, the detailed pharmaceutical effects on anti-biological resistance will be discussed.

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*Kinetic analysis of microplastics and their impacts on Japanese medaka (*Oryzias latipes*)*

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Microplastics (MPs) and its impacts on aquatic organisms have drawn worldwide attention. However, the knowledge of the kinetics of MPs in fish still needs to be updated. We conducted exposure and depuration tests of spherical fluorescent-labeled polyethylene (PE) and polystyrene (PS) MPs in 10^6 particles/L (PS 2μm, PS 20μm, PE 20μm) and 10^4 particles/L (PE 200μm) concentrations using Japanese medaka (*Oryzias latipes*). The distribution and concentration of MPs in medaka were determined in-situ after tissue clearance. During the 14-day exposure, MPs were mainly detected in the intestines of fish. The bioconcentration factor (BCF; L/kg) for MPs in medaka was estimated as 74.4 (200μm PE), 25.7 (20μm PE), 16.8 (20μm PS), and 139.9 (2μm PS). MPs were eliminated from the fish body within five days of depuration, but 2μm PSMPs could still be detected in the intestine at the 10-day depuration phase. These suggested 2μm MPs may pose higher risks to aquatic species due to their relatively higher BCF and the potential for long-term persistence in the body. A second experiment was performed to verify the residual of 2μm MP in the fish body and its distribution. We conducted exposure and depuration tests of 2μm spherical fluorescent-labeled PS under 10^6 particles/L concentration in Japanese medaka. The distribution and concentration of MPs in medaka were determined in-situ after tissue clearance and in histological analysis. During the exposure period (4-day exposure, 8-day exposure, 18-day exposure), MPs were mainly accumulated in the intestine. After seven days of depuration, $10.1*10^3 \pm 4.9*10^3$, $17.4*10^3 \pm 11.4*10^3$, $128*10^3 \pm 46.4*10^3$ particles/g of 2μm MPs were detected in the fish intestine. Our results showed a certain amount of 2μm MPs residue in fish after depuration and could be found in the cavity of the medaka's intestinal tract near the intestine wall. Additionally, we found a positive correlation between exposure time and number of residual MP in fish.

Application of AmpliSeq to environmental RNA may enable non-invasive assessments of various activities in fish

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Toxicity testing using aquatic animals is a common practice for chemical risk assessments. While analyzing tissues or blood samples from animals exposed to chemicals can provide valuable insights into the mechanisms of toxicity, these methods cause pain or death to the animals. Increasing concerns over animal welfare and ethical issues have necessitated the development of New Approach Methodologies (NAMs), which aim to reduce animal use, replace it with alternative methods, and refine testing procedures to minimize distress.

Environmental RNA (eRNA) is RNA released by organisms into their surrounding environment such as water and soil. Unlike DNA, RNA is produced only for actively transcribed genes, making it a useful tool for understanding the real-time status of the organisms. Recent studies have demonstrated that various types of messenger RNA, including those related to environmental stresses, can be retrieved from water surrounding macroorganisms such as fish and water fleas. In addition, these studies have revealed that eRNA profiles change in response to environmental stresses such as chemical exposure and temperature increases.

Despite the potential of eRNA to assess toxic effects in aquatic organisms, eRNA collected from water contains significant amounts of RNA of nontarget organisms (e.g., bacteria and fungi), hampering the efficient sequencing of target organisms. To address this challenge, we developed a set of 1687 genes for Japanese medaka (*Oryzias latipes*) and applied targeted RNA sequencing (AmpliSeq) using the developed primer set to eRNA derived from *O. latipes*. Our results showed that AmpliSeq could detect 1286 genes in eRNA collected from laboratory and > 700 genes in eRNA collected from field waterways. One million reads by AmpliSeq provided sufficient sequencing depth and reached a plateau in the number of detected genes, indicating high accuracy and reliability of AmpliSeq for eRNA analysis even in the presence of abundant non-target RNA.

Towards development of an in vitro *assay of oocyte maturation inhibition to predict reproductive capacity of fishes*

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In fish, oogenesis is a dynamic process by which fertilizable oocytes develop from germ cells. Oogenesis can be divided into a growth phase and a maturation phase. Many endocrine disrupting chemicals impair reproduction by disrupting vitellogenesis that occurs during the growth phase. From this research, several AOPs have been developed for decreased fecundity due to disruption of vitellogenesis, including AOP 30 (direct estrogen receptor antagonism), and AOP 25 (depression of E2 synthesis through inhibition of aromatase). Our lab has been investigating inhibition of the second phase of oogenesis, oocyte maturation, as a mechanism of decreased fecundity. Based on a previously developed *in vitro* assay of oocyte maturation in zebrafish, we have developed an *in vitro* assay to assess oocyte maturation in Japanese medaka. We quantify inhibition of oocyte maturation using *in vitro* exposures of isolated immature oocytes to candidate chemicals, or by assessments of oocyte maturation following *in vivo* exposure of sexually mature female fish to candidate chemicals. In one study with Japanese medaka, *in vitro* exposure of stage IX oocytes to the brominated flame retardant, TBCO, caused a 40% reduction in oocyte maturation, which was approximately equal to the decrease in fecundity of females exposed to dietary TBCO. Lack of changes in plasma E2 or expression of vitellogenin suggest that impairment of oocyte maturation likely caused the decreased fecundity. In another study, fecundity of Japanese medaka exposed as embryos to maternally deposited TBCO was significantly decreased and corresponded with a decrease in oocyte maturation. These studies, and others, will be presented as evidence that assays of oocyte maturation might be useful to predict impaired fecundity.

Inferring the health outcomes of PFAS using a network biology approach

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Per- and polyfluoroalkyl substances (PFAS) are widely used in various products due to their waterproof and oil-repellent properties. While certain PFAS, such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), have been linked to immune system alterations and cancer, leading to international regulatory measures, the health risks associated with the majority of commercially used PFAS remain unclear. This study employs a network biology framework to predict target proteins of PFAS by leveraging structural similarity and investigates the relationships between diseases and PFAS target proteins within the human protein interaction network. We found that proteins associated with PFOA and PFOS form PFAS modules that are network-proximal to disease modules, suggesting a higher likelihood of these PFAS influencing disease. Using network proximity, we confirmed known associations such as breast cancer and hypertension for PFOA and PFOS, and identified Alzheimer's disease as a novel high-risk prediction. Furthermore, we suggest that the emerging PFAS compound perfluoro (2-methyl-3-oxahexanoic) acid (HFPO-DA) may potentially cause various diseases, including lung cancer, ovarian cysts, and multiple sclerosis. The predictive approach developed in this study offers a novel method for assessing the health impacts of PFAS and highlights the need for further research on emerging PFAS compounds.

Automated analysis of histological lesions in whole slide images of fish liver

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Tissue changes in aquatic organisms are important to consider during environmental monitoring programs to understand individual health, and possibly ecosystem health. However, the traditional estimation by a trained histopathologist is often costly, time consuming, and prone to operator bias. Digital pathology consists in computer-based analyses with artificial intelligence and high-throughput methodologies. These approaches, which have already been adopted in biomedical applications, have not been implemented in environmental studies yet.

In this proof-of-concept research, fish liver samples, collected during an environmental monitoring study in the North Sea, were analysed. A selection of histological lesions (steatosis, melanomacrophage aggregates, haemocytes infiltration, and granulocytoma) was estimated by a trained histopathologist (traditional approach) and using a neural network model for image recognition implemented in the open-source software, QuPath (automated approach) in whole slide image (WSI) scans. A subset of WSI was used to train a pixel classifier (random forest), which was then used to automatically detect and quantify the lesions. Lesion coverage data were compared to traditional manual scoring results using various statistical tools. Obtained results were compared to manually assigned severity scores. Assuming the traditional estimation by a trained histopathologist as reference, the classifier model correctly identified 91% for steatosis, 95% for melanomacrophage aggregates, 79% for haemocytes infiltration, and 95% for granulocytoma. To improve the model prediction, data driven decision boundary between classes are also proposed. No differences between species were observed while applying the automated approach, confirming the possibilities to utilise the same lesion classifier in various fish species. These results provide the first successful base for automated lesion detection and quantification methodologies for use in aquatic organisms.

Developmental effects of estrogenic endocrine-disrupting chemicals and the predictability of in vitro *and* in silico *analyses using zebrafish (*Danio rerio*)*

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The present study aimed to evaluate the developmental effects of estrogenic endocrine-disrupting chemicals (E-EDCs) with the predictability of *in vitro* and *in silico* analyses, as well as to give insight into their potential mechanism of action, using zebrafish. In this concept, embryos were exposed to three distinct E-EDCs, including diethylstilbestrol (DES), zearalenone (ZEN), and genistein (GEN) at different concentrations. The same set of E-EDCs was used for an *in vitro* luciferase reporter gene assay in which expression plasmids of zebrafish estrogen receptors (zfERs) were transfected into COS-1 cells. We also performed *in silico* simulations to predict interactions between E-EDCs and ligand binding domains of zfERs. Concentration-dependent increases in the expression of the ER target gene *CYP19A1b in vivo* and in the luciferase activity *in vitro* were observed for all tested E-EDCs, with distinct potency based on the EC_{50}. Positive correlations were exhibited for EC_{50} from *in vivo CYP19A1b* induction and interaction energy of E-EDCs to each of the zfERs from *in silico* docking simulation. Furthermore, a variety of developmental toxicities was displayed by all tested E-EDCs, with differing incidence and severity among chemicals. An interactive effect was observed with the combined exposure to DES and ZEN, where DES-induced developmental toxicities were alleviated by co-treatment with low concentrations of ZEN and vice versa. On the other hand, no effect was exhibited by DES or ZEN on toxicities mediated by GEN and vice versa. Developmental toxicities elicited by DES and ZEN, but not GEN, were improved by co-exposure to an ER antagonist fulvestrant. Altogether, the *in vitro* zfER transactivation assay and the *in silico* simulations of interactions between ligands and zfERs may help predict the *in vivo* estrogenic potency of untested chemicals. The possibility of *in silico* simulations to predict the *in vivo* potency of developmental toxicity warrants further investigation.

*Marine medaka (*Oryzias melastigma*) as a developmental immunotoxicity model for PFAS exposure*

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Production and use of legacy per- and polyfluoroalkyl substances (PFAS) is heavily regulated due to their immunotoxicy. Currently it is unclear if replacement PFAS pose a similar risk. To evaluate potential immunotoxic effects of PFAS during critical windows of immune system development, marine medaka (*Oryzias melastigma*) were exposed to PFAS levels equivalent to prenatal/postnatal human blood concentrations. Legacy PFAS (PFOS, PFOA) and replacements (PFHxS, PFBS, PFHxA, GenX) were assessed for immunotoxic impacts using a bacterial challenge and transcriptomics. Exposure windows encompassed lymphocyte progenitor cell migration and thymus colonization (7-11 days post fertilization (dpf)) and the establishment of immune competence in the thymus (12-19 days post hatching (dph)). PFHxA exposure at 7-11dpf reduced immune competence, while exposure during 12-19dph revealed diminished immune competence in response to PFHxS, PFBS, PFOA, and GenX. The transcriptome indicated changes in expression profiles of PFAS-exposed larvae (12-19 dph). Similar numbers of genes were differentially regulated for PFOA (919) and its replacement GenX (964). Comparatively, fewer genes were differently expressed after exposure to PFOS (492) and its replacement PFHxS (666). However, the shortest PFOS replacement, PFBS, resulted in the highest deregulation with 1697 differentially expressed genes. Modified genes were found to be associated with Gene Ontologies (GOs) related to immune function including thymus development, B-cell and T-cell differentiation, and myeloid and lymphoid progenitors. The data demonstrate variability in mechanisms of PFAS-induced immunotoxicity. Thus, specific critical windows may be more susceptible to certain PFAS which is important to consider for risk assessment.

*Immunotoxicity of cryo-milled tire tread in Japanese medaka (*Oryzias latipes*)*

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Tire particles are a substantial source of microplastic (MP) pollution, yet are greatly under-researched compared to other microplastics. Tire particles have been found to account for 28.3% of all microplastic pollution in the ocean. Tire particles and their leachates caused a decrease in embryonic heart rates, hatching success, and body length in fathead minnow. In addition, increased morphological deformities in embryos such as diminished eye and body pigmentation were observed post-exposure. 6PPD-quinone, a chemical used in the production of tire particles was found to be lethal to brook trout and in rainbow trout. The objective of this research is to examine the immunotoxicity of a low (0.16547 μg/mL) and high (1.6545 μg/mL) concentration of cryo-milled tire tread (CMTT) using Japanese medaka (*O. latipes*). Japanese medaka 0 days post hatching were collected and exposed to CMTT solution for 7 days. Post exposure, the medaka were processed and assessed for CMTT gut content, immune organ pathology, and innate immune gene expression. Gut contents revealed a dose-dependent retention of CMTT with a significant increase in the high concentrations. Histology revealed a significant increase in thymus size in the high concentrations. On the molecular level, Lysozyme C expression was significantly reduced in the highest concentration. The data indicates a possible immune compromise related to CMTT ingestion in fish larvae. This information aims to fill gaps within current tire particle research, as well as shed light on the environmental impact of tire particle waste and the associated health risks.

Chronic exposure to tire particles induces anemia in common carp

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This study investigated the chronic toxicity of tire dust on common carp to contribute to the ecological risk assessment of tire dust.

Cryo-Milled Tire Tread (CMTT) samples were obtained from the USTMA. UV irradiated CMTT was prepared by irradiating with ultraviolet light for 8 weeks. In this study, three independent exposure tests were conducted. In Test 1, three groups of carp were fed diets containing 0, 2.5, and 10% CMTT for 4 weeks, respectively. In Tests 2 and 3, four groups of carp were fed diets containing 0, 0.625, 1.25, and 2.5% CMTT for 2 weeks, and in Test 3, 0, 1.25, 2.5, and 5% UV-irradiated CMTT, respectively. In all tests, the standard body length and weight of carp were measured and blood samples were collected at the end of the exposure period. The blood was used to count the total number of blood cells and measure hemoglobin (Hb) concentration and hematocrit (Ht) values. In addition, in Test 1, spleen tissue sections stained with Berlin blue were observed.

There were no deaths in any of the tests during the test period. In Test 1, there were no significant differences in body length, weight, or growth rate between treatment groups. This result suggests that chronic exposure to CMTT does not affect the growth of carp. On the other hand, Hb concentration and Ht values were significantly lower in both concentration groups compared to the control group. In the spleen, many hemosiderin deposits were observed, confirming that red blood cells were damaged. In Test 2, Hb concentration was significantly lower only in the 2.5% group compared to the control group. The results of Tests 1 and 2 revealed that CMTT administration causes anemia in carp, and its LOEL and NOEL were 0.75 and 0.375 mg/g-b.w./day, respectively. In the exposure test with UV irradiated CMTT in Test 3, no significant changes in Hb concentration were observed in any treatment group. From the above, it was inferred that chemicals derived from tire additives are the main cause of anemia.

Dioxin target neurons in the mammalian brain

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Epidemiological and experimental studies have shown that exposure to dioxin impairs cognitive and neurobehavioral functions in humans and laboratory animals. The aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, exists in diverse species, including mammals, birds and fish. The AhR binds to dioxin and translocates from the cytoplasm into the nucleus, where it induces the expression of multiple target genes, leading to various toxic effects. Therefore, accurate identification of AhR-expressing neurons in the brain is indispensable to clarify molecular mechanisms of dioxin neurotoxicity. To detect AhR in the mouse brain, we performed immunohistochemistry, a powerful technique for the analysis of molecular dynamics at the single-cell level *in vivo*. Specific and distinctive expression of AhR in brain tissue sections was captured by a confocal microscopy. AhR dynamics in individual cells was quantitatively analyzed with high-resolution images. Gene expression was measured by quantitative PCR using adult mice orally exposed to 2,3,7,8- tetrachlorodibenzo-*p*-dioxin (TCDD). AhR was expressed in nearly all neurons of the locus coeruleus (LC) in wild-type, not Ahr-null, mice at 5-, 7- and 14- days old. No distinct change in AhR dynamics of LC neurons was found during development; in contrast, TCDD exposure significantly increased nuclear translocation of AhR in adults. Expression of the three target genes (i.e., Cyp1a1, Cyp1b1 and AhRR) in the brains of the TCDD-exposed mice was obviously upregulated, indicating disruption of AhR-regulated signaling pathways. Collectively, the new method we established provides experimental evidence showing AhR expression in brain neurons from early life stage and the dioxin-induced change of AhR dynamics *in vivo*. As the LC is commonly found in vertebrates, our results suggest the need for studies focusing on AhR agonists in the environment upon the brain development and function of humans and wildlife.

Large-scale behavioral data-driven approach for neurotoxicity risk assessment using animals

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Behavioral analysis using animal models remains indispensable for assessing the mental health risks associated with chemical exposures, as *in vitro* and *in silico* methods cannot fully elucidate the complexities of brain function. However, the majority of conventional animal behavior analysis methods have persisted unchanged for over half a century, and there are growing concerns regarding their reliability and efficiency. To address this challenge, we have been developing risk assessment methodologies driven by cutting-edge digital technology for large-scale animal behavior data collection. Specifically, we will introduce the Automated Home-Cage Monitoring (AHCM) approach, which integrates RFID (Radio Frequency Identification) and various behavioral sensors. Traditional methods involve direct experimenter contact with the animal, which is known to compromise the experiment reliability and efficiency. In contrast, the AHCM approach achieves unmanned, automated data collection, enabling the analysis of a diverse repertoire of animal behaviors in a low-stress environment, continuously throughout the year. The resulting extensive datasets can reveal brain and behavior abnormalities overlooked by traditional methods. We will present the technical specifics, significance, and applications of the our proposed large-scale behavioral data collection technology (AHCM approach), highlighting its potential in revolutionizing neurotoxicity risk assessment in the future.

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Analysis of effects of nanoplastic particles on human neurons

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In recent years, environmental pollution by micro- and nano-sized plastic particles has become a social issue. Regarding toxic effects, many studies have investigated the effects on aquatic organisms and wildlife. Moreover, in humans, microplastic particles have been detected in various organs including placenta. Therefore, there are concerns about the impact of microplastics and nanoplastics on developing children as well as adults. Until now, there have been reports about transfer of plastic particles into the brain, and concerns about their effects on the brain, but there are still few reports on the detailed effects. In this study, we used LUHMES cells, human mesencephalic neuronal cells, as a model for detecting developmental neurotoxicity, and exposed to fluorescently labeled polystyrene (PS) particles with average particle diameters of either 50 nm or 500 nm. The uptake of PS particles with each size into LUHMES cells was confirmed by both fluorescent microscopic and flow cytometry analyses. In addition, LUHMES cells internalized PS particles via clathrin-mediated and macropinocytotic pathways. Uptake of PS particles by the neurons was low compared to other cells in brain such as microglia, and the exposure to PS particles for 24 hr did not exhibit severe acute cytotoxicity even at high concentrations at least in our experimental condition. These results suggest that neurons might not be quite sensitive to nanoplastic particles, but accumulate certain amount of nanoplastic particles. We need to examine further detailed analysis including the functions of neuron and the effects of long-term exposure.

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Investigation of cytotoxicity of lipofectamine in rainbow trout fish cell lines

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Transfection, the process of introducing nucleic acids into eukaryotic cells, is a vital technique used in molecular biology research. Various forms of transfection exist, including biological, chemical, and physical approaches, each with distinct advantages and considerations. Cationic lipid-mediated transfection, such as the use of transfection reagents, is widely used due to its efficiency and versatility. This technique is based on the neutralization of cationic lipids to negatively charged nucleic acids followed by the formation of lipoplexes, allowing for endocytosis to occur at the negatively charged cell membrane. This study focuses on evaluating the cytotoxic effects of transfection reagents Lipofectamine 3000 and Lipofectamine RNAiMAX in two established rainbow trout cell lines: a gill epithelial cell line (RTgill-W1), and a gut epithelial cell line (RTgutGC). While Lipofectamine dosage and toxicity has been studied extensively in mammalian cells, its potential toxicity in non-mammalian cell lines, particularly fish cell lines, remains understudied. Understanding these mechanisms may provide a potential alternative to antibiotic use in aquaculture, which contributes greatly to antimicrobial resistance in fish. Scratch-wound assays and cell viability assays are employed to determine the amount of viable cells remaining upon exposure to varying concentrations of Lipofectamine. The results indicate that both transfection reagents result in cytotoxic effects within both cell lines, especially upon increasing the concentration. Further research can look at the potential risk of other transfection reagents in fish cell lines, and the mechanism to which these cytotoxic effects occur within rainbow trout cell lines.

Mixture effects of dialkylesters of phthalic acids (PAEs) on aquatic organisms based on predicted environmental exposure profiles

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Dialkylesters of phthalic acids (PAEs) are primarily used as plasticizers in industrial applications and includes structurally diverse congeners, differing in the number of carbon atoms in their alkyl chains and whether those chains are linear or branched. Despite their similar usage, PAEs have a wide range of physico-chemical properties, leading to varying mixing ratios of components in different aquatic environments. However, there are no studies quantitatively evaluating the aquatic toxicity of the multi-component PAEs at environmentally relevant exposure profiles. In this study, we predicted exposure profiles of six PAEs (alkyl side chains of C1 to C6) in rivers across Japan and performed shortterm chronic ecotoxicity tests under those predicted profiles on three aquatic organisms (alga, daphnid, and fish). We used the spatially resolved multimedia environmental fate model G-CIEMS with estimated emission information for individual PAEs to determine three representative exposure profiles based on mean predicted PAE concentrations in the followings: (1) top 10 river segments where dialkyl isobutyl phthalate (DIBP) is predominant; (2) top 5 % river segments with the highest relative toxic potential derived from potency of each PAE; (3) top 10 river segments where dialkyl methyl phthalate (DMP) is predominant. When these profiles were converted into relative contributions as toxic unit (TU, 50% effect is assumed at TU = 1), it was found that dialkyl butyl phthalate (DBP) or DIBP accounted for more than 90% of the total TU for all PAEs. The effects of PAE mixtures with each exposure profile, in the range of total TUs from 0.5 to 2, were compared from the prediction of concentration addition (CA) model. Our results suggest the mixture toxicity of PAEs can be adequately predicted using the CA model, and therefore that the ecological risk of PAE mixtures in different aquatic environments can be quantified using monitoring data and relative potency for individual PAEs.

Comparison of the eco/genotoxicity of natural anthraquinone dyes

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Anthraquinones are used as colorants for textiles and other applications. There is a growing interest in the replacement of synthetic dyes with dyes from natural origins. Dyeing processes can generate high volumes of water and dyes can reach the aquatic environment depending on the type of wastewater treatment. This indicates a need for their hazard evaluation to aquatic organisms. In this work we evaluated four biocolorants, three from the fungi *Cortinarius sanguineus* (dermorubin, dermocybin and emodin) and one from the plant madder (alizarin). Dermorubin was not toxic to algae (chronic, *Raphidocelis subcapitata*), crustacea (acute *Daphnia similis* and *Parhyale hawaiensis*, chronic *Ceriodaphnia dubia*) and fish (embryo test, *Danio rerio*). It was not mutagenic in the Ames test. Dermocybin was toxic with a lowest IC_{10} of 0.13 mg/L (*C. dubia*) but not mutagenic in the Ames test. Emodin and alizarin were the most toxic dyes with lowest LC_{50} observed for *D. rerio* (0.025 mg/L and 0.040 mg/L, respectively). When comparing the present results with results in the literature regarding the aquatic toxicity of anthraquinone dyes either from synthetic or natural origins, the latter two dyes are by far the most toxic ones. Additionally, both were mutagenic in Ames test and had their mutagenicity confirmed after 96h exposure to *P. hawaiensis* measured by the increase of micronuclei in hemocytes. It is anticipated that these results can guide future risk assessments of the presence of such dyes in the aquatic environment and, also enable smart decisions prior to their approval to be marketed.

How much known chemical substances in receiving water can explain the measured toxicity to alga, daphnid and fish?

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Current environmental monitoring and risk assessment programs for selected individual chemical substances possibly overlook the effects of new substances without toxicity data and chemical mixtures in the environment. To address this issue, effect-based methods, which use bioassays to assess the adverse effects of environmental water samples including chemical mixtures, is expected to be implemented in the monitoring program. When the effect is detected in the samples, effect-directed analysis (EDA) is conducted to identify suspect toxicants, by the combination of bioassays and comprehensive chemical analysis on fractionated samples. However, there are limited EDA studies in Japan using *in vivo* assay addressing sub-chronic toxicity. To develop the EDA methods for environmental monitoring and chemical risk assessment in Japan, we conducted three short-term sub-chronic toxicity tests using alga, daphnid, and fish and multiple-component chemical analysis using Automated Identification and Quantification System (AIQS) with GC/MS and LC-QTof/MS for organic compounds (pesticide, PPCPs), and ICP-MS for metals. The chemicals with hazard quotient (HQ = detected concentration/toxicity values) above 0.1 included pesticides (e.g. herbicide bromacil, insecticide fenitrothion, carbofuran, diflubenzuron), industrial chemicals (e.g. anilines), and metals (Ni and Zn). Although the toxicity of metals was overestimated in many samples, the predicted toxicity of detected organic toxicants could not explain all the measured toxicity in several samples, indicating the presence of unmeasured toxicants. Expansion of chemical substances to be measured and/or non-target screening of the toxic fractionations will be needed to identify the other unknown toxicants.

Synergistic toxic mechanisms of microplastics and triclosan via multixenobiotic resistance (MXR) inhibition-mediated autophagy in the freshwater water flea Daphnia magna

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Since a mixed state of environmental contaminants, including microplastics (MPs), heavy metals, pharmaceuticals, and personal care products (PPCPs), exists in aquatic ecosystems, it is necessary to evaluate not only the adverse effects of exposure to a single stressor but to combined stressors. In this study, we exposed the freshwater water flea *Daphnia magna* to 2 μm MPs and triclosan (TCS), one of PPCPs, for 48 h to investigate the synergistic toxic consequences of simultaneous exposure to both pollutants. We measured *in vivo* endpoints, antioxidant responses, multixenobiotic resistance (MXR) activity, and autophagy-related protein expression via the PI3K/Akt/mTOR and MAPK signaling pathways. While MPs single exposure did not show toxic effects in water fleas, simultaneous exposure to TCS and MPs was associated with significantly greater deleterious effects in the form of increased mortality and alterations in antioxidant enzymatic activities compared with water fleas exposed to TCS alone. In addition, MXR inhibition was confirmed by measurement of the expression of P-glycoproteins and multidrug-resistance proteins in MPs- exposed groups, which led to the accumulation of TCS. Overall, these results suggest that simultaneous exposure to MPs and TCS resulted in higher TCS accumulation via MXR inhibition, leading to synergistic toxic effects such as autophagy in *D. magna*.

Long-term effects of plastic additives on aquatic and terrestrial biota

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Plastic is a chemically diverse material, a blend of polymer(s) and additives. Additives have the potential to migrate and induce toxicity throughout the life-cycle of the plastic product. However, compared to microplastics, the risks related to plastic additives are poorly studied. Addressing this knowledge gap is a prerequisite for the sustainability of plastics. Phthalic acid esters (phthalates) are representatives of plasticizers, one of the largest functional additives groups. Formerly dominant but now largely restricted low molecular weight phthalates are being increasingly replaced with alternatives - high molecular weight (high) phthalates and non-phthalate plasticizers. As a result of this large-scale substitution, alternative plasticizers, with largely unknown environmental impacts, are already considered emerging contaminants. We investigated the long-term impact of high phthalate di(2-propylheptyl)phthalate (DPHP) on aquatic (ostracod *Heterocypris incongruens*, water flea *Daphnia magna*) and terrestrial arthropods (mealworm *Tenebrio molitor*, woodlice *Porcellio scaber*). DPHP toxicity potential was compared with that of the restricted di(2-ethylhexyl)phthalate (DEHP). Toxicity exposures were performed either via phthalate-spiked food (*T. molitor*) or soil/sediment (other organisms) over the range of 5-1000 mg phthalate/kg medium.

Ostracods' and mealworms' growth was stimulated in the presence of phthalates. For *D. magna*, survival, body size and time to first brood were affected by both DPHP and DEHP. For *P. scaber*, acetylcholinesterase, glutathione S-transferase (GST) and electron transfer system activities were altered at 100 mg DEHP/kg. DPHP increased GST activity at 1000 mg/kg. No clear immune response was observed in either case, except for a decrease of total haemocyte count at 50 mg DPHP/kg. In conclusion, across the organisms, the scale of long-term toxicity induced by the emerging plasticizer DPHP was comparable to that of the restricted DEHP.

Biofouling alleviates acute and chronic toxicity of polylactic acid microplastics to Daphnia magna

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Microplastics (MPs) that end up in aquatic environments are naturally subjected to a biofouling process, which occurs at an accelerated rate for biodegradable types. In this study, we investigated the toxicity of pristine and biofouled polylactic acid (PLA) MPs on *Daphnia magna* across different life stages. Chronic toxicity experiments were conducted using juvenile *D. magna* (4 days old) exposed to environmentally relevant concentrations (2.5 mg/L and 5.0 mg/L) of PLA MPs over 17 days. Observation of gut damage and the absence of significant sublethal effects across all treatments suggest that gut damage could be the primary contributing factor to mortality induced by pristine PLA MPs. Interestingly, biofouling appeared to alleviate toxicity, possibly through the provision of food from the biofilm formed on the MPs, which may have contributed to the higher reproduction rate observed in *D. magna* exposed to biofouled PLA MPs. To further elucidate toxicity mechanisms, acute toxicity tests were conducted on both juvenile and adult *D. magna* (10 days old) using higher concentrations (10 mg/L and 40 mg/L) of PLA MPs. Only adult *D. magna* exposed to pristine PLA MPs at the highest concentration exhibited mortality, suggesting a potential age-dependent capability of digesting PLA MPs within a specific size range (25-53 μm). Subsequent evaluation of sublethal effects, including oxidative stress, gene expression, food uptake, bioconcentration of MPs, and gut damage, provided insights into the physiological responses of *D. magna* to acute exposure across different life stages. Overall, our findings highlight the complex interplay between biofouling, MP characteristics, and *D. magna* life stage in determining toxicity, emphasizing the importance of considering multiple factors in assessing the ecological impacts of MPs in aquatic environments.

*Uptake and depuration kinetics of small microplastics in juveniles of sea squirts (*Ciona intestinalis*)*

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Microplastics (MPs) have been detected in shallow water of many marine habitats, which are inhibited by sea squirt filter feeders; known to uptake suspended particles from water column. Juvenile Sea squirt was chosen as a model organism to study the kinetics of MPs for their bodies transparency, wide distribution and small body size. High concentration (10^5 per liter) of fluorescent polystyrene microplastics particles (PSMPs) were used for the exposure of 21 juvenile sea squirts for five days, followed by an extended depuration phase. PSMPs were found in both intestine and stomach after 24 hours (16.7 ± 14.4 and 2.3 ± 2.08 particles. individual^{-1}, respectively), which significantly increased after 48 hours reaching (45.3 ± 10.6 and 21.67 ± 22.55 particles. individual^{-1}, respectively). Sea squirts egested (53.3 ± 36.23 particles. individual^{-1}) after 24 hours of exposure, while (536 ± 161.5 particles. individual^{-1}) were observed in fecal matter after 4 hours of starting the experiment, representing approximately 0.5 % of the initial concentration of PSMPs in water, this percentage was nearly doubled after 120 hours of exposure to reach 0.89 % of PSMPs of the initial concentration in the exposure water. After 24 hours of rinsing sea squirt and transferring them to MPs- free artificial sea water, most of particles were depleted from guts of all individual, however a complete elimination was reached after 48 hours of depuration. PSMPs were found adhered to the gelatinous tunics from day one of exposure and even after several rinses and depuration period. Juvenile sea squirts rapidly ingested and egested PSMPs, without obvious threatening effects on survival rates. Further experiments are planned to determine the potential of resuspension of PSMPs deposited in feces into water column and the possibility to recontribute to diet intake.

Vector effects of polyethylene microplastics on the accumulation of phenanthrene in Japanese medaka, Oryzias latipes

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Microplastics (MPs) and polycyclic aromatic hydrocarbons (PAHs) coexist in the environment. PAHs are hydrophobic and can be absorbed by MPs. Phenanthrene (Phe), a PAH, is a priority pollutant in water bodies, according to USEPA. Thus, the vector effects of polyethylene microplastics (PE-MP) on Phe accumulation are assumed. We carried a co-exposure test to clarify the possibility of PE-MP being a vector for Phe in fish. We exposed 35 medaka in each group to Phe (0.2 mg/L) only or Phe (0.2 mg/L)+PE-MP (6 mg/L) for 10 days and allowed to depurate for 4 days. We also set up a control group (free of Phe and MPs). Water and fish samples were collected on day 1, 3, 6, 9, and 4, 7, 10, 10(+8h), 11, 12, and 14, respectively. Fish samples were dissected, only muscle portion was homogenized and extracted, and Phe was measured. Phe in water samples was measured in three different phases: total phase (with MPs), water phase (without MPs), and MP phase (Phe absorbed by MPs). The HPLC system measured both fish and water samples. The pharmacokinetics of Phe were calculated according to OECD guidelines TG305, and one compartment model estimated the vector effect. Phe was detected in the MP phase, suggesting absorption of Phe by MPs. No Phe and MPs were detected in the water and fish of control group. The one-compartment model revealed that a significant portion of Phe was accumulated in fish contributed by MPs (vector effect). This result showed that MPs absorbed Phe, which were desorbed in the fish intestine and subsequently accumulated in fish body. Our study reveals that PE-MP can be a potential carrier of Phe and act as a vector for enhanced bioaccumulation compared to single exposure (without vector). Studies on different kinds of polymers, sizes, shapes, concentrations of MPs, and various concentrations of Phe and conditions can be carried out for further clarification.

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Evolutionary decline of AHR2 and compensatory role of AHR1: Implications for environmental toxicology in mammals

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The aryl hydrocarbon receptor (AHR) plays an important role in mediating a variety of physiological processes, including immune regulation through endogenous ligands as well as mediating toxic responses such as carcinogenesis triggered by dioxin-like compounds. Vertebrates typically express multiple isoforms of AHR, each performing a variety of physiological functions. However, mammals universally conserve AHR1, while some species conserve AHR2. The function of AHR2 in these species remains largely unexplored, representing a critical gap in our understanding of environmental toxicology. The aim of this study is to investigate the biological properties of mammalian AHR genes and elucidate their environmental toxicological implications. We investigated the ligand responsiveness and tissue-specific expression levels of AHR2 to understand its molecular properties. AHR2 from cats and seals has demonstrated the ability to regulate transcriptional activity by binding to agonists such as TCDD and FICZ. However, the expression level of AHR2 was found to be extremely low in tissues such as cats and cattle. These results suggest that mammalian AHR2 retains its ligand transactivation capacity but is undergoing degeneration. We collected data on 220 mammalian species with AHR1 and confirmed the conservation of AHR2 in 63 species through phylogenetic tree analysis. This analysis revealed clear differences in AHR2 conservation at the specific order or family level. We also investigated whether various ecological and biological factors may contribute to AHR2 conservation. Our results indicate that factors such as body weight and diet type may influence the preservation of AHR2. Understanding the evolutionary decline of AHR2 and the functional compensation of AHR1 to environmental pollutants may provide insights into environmental toxicology.

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Evaluation of heavy metal and pesticide residues in fecal samples of African savanna elephants in Lower Zambezi National Park, Zambia

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Heavy metal and pesticide contamination are major environmental issues with severe ecological impacts. Elephants, due to their varied diets and extensive migration patterns, are highly susceptible to accumulating these toxic substances. This study examines the presence and concentration of heavy metals and pesticides in fecal samples from African savanna elephants in Zambia's Lower Zambezi National Park. Human activities, including mining and tourism, exacerbate environmental pollution in the park, necessitating this study.

Samples were collected in two phases: November 2022 from the east side and September 2023 from the west side of the park. The 2022 collection included 30 plant, 59 soil, and 33 elephant fecal samples; the 2023 collection comprised 150 soil, 120 plant, and 75 elephant fecal samples. Heavy metals were analyzed using Inductively Coupled Plasma-Mass Spectrometry, and 368 pesticides were identified and quantified using Liquid Chromatography (LC-MS/MS and Q-TOF/MS) and Gas Chromatography-Mass Spectrometry. Significant variations were found between the 2022 and 2023 samples, particularly in Selenium, Cobalt, and Nickel levels. High concentrations were detected in one of the 2023 elephant fecal samples, indicating a potential area with elevated metal presence. The increased metal levels in 2023 are likely due to higher human activity, especially tourism, on the west side of the park. Seasonal environmental changes also contributed to pollutant accumulation. Trace amounts of various pesticides were detected, highlighting their pervasive presence in the environment despite generally low concentrations.

The findings indicate significant heavy metal accumulation which necessitates environmental monitoring and protective measures to mitigate human impact on the park's ecosystem. Effective environmental management, including regular monitoring, is essential for maintaining and ensuring the health of the environment and its wildlife, particularly elephants.

Biochar: The key to eco-friendly biosolids management and a thriving circular economy

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The surge in industrialization and urbanization has led to an increase in sewage sludge (biosolid) from wastewater treatment plants. While sewage sludge is rich in phosphorus, nitrogen, microelements, and organic matter—making it a valuable soil amendment—its contamination with toxic compounds, trace metals, and pathogens limits its use in agriculture and soil reclamation. This work explores strategies to harness the benefits of sewage sludge while mitigating its drawbacks, particularly regarding organic pollutants. Converting sewage sludge to biochar offers a promising solution to minimize the negative impacts of its agricultural use. Biochar production can reduce trace metal leaching, enhance soil enzymatic activity, and decrease the bioaccumulation of trace metals and polycyclic aromatic hydrocarbons (PAHs). The high sorption capacity of biochar allows it to bind and immobilize trace metals and organic contaminants, reducing their long-term bioavailability. Another approach is to mix biochar with sewage sludge to decrease PAH bioavailability and the overall toxicity of the sewage sludge. Amending soil with a biochar-sewage sludge blend can both minimize the environmental impact of contaminants introduced by the sewage sludge and enhance the beneficial effects on biota through the additional nutrients provided by the biochar.

Integrated assessment of the toxicological, ecotoxicological and ecological effects of all phytopharmaceutical treatments used during a whole potato crop season

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Two laboratories of the University of Lille and JUNIA, have partnered with the Hauts-de-France Agriculture Regional Chamber to study the effects of the use of phytopharmaceutical products (PP) during a whole potato crop season. Multidisciplinary researches were carried out successively at three scales (fields, experimental plots and lab), in order to establish an inventory of agricultural practices and to study the ecological, ecotoxicological and toxicological effects of the possible PP multi-residual contamination on soil functioning, the crop quality and different plant and animal models.

The study first phase involved conventional or organic (C or O) potato culture plots, all located in the French North region in two distinct contexts (large-scale or peri-urban fields). On each plot was carried out an analysis of cultural practices and uses, a soil recognition and sampling, a crop sanitary state characterization, a measurement of PP multi-residual contaminations, a study of plot biodiversity and an assessment of the impregnation of lichens transferred around plots. The second phase concerned potato crops (C or O) on experimental plots under better controlled conditions (soils, technical routes, PP natures and doses) and a same follow-up was conducted. Finally, the last phase used the soils and part of the harvested tubers of these experimental plots to conduct lab-exposures, to evaluate the ecotoxicological and toxicological responses of four biological models, namely earthworm, clover, cabbage and mouse. A nutrigenomic approach was implemented in mice, and the genotoxic effects studied in plants and worms, co-exposed or not.

After a brief recall of the results obtained in the first phases, we will discuss in more detail of the toxicological and ecotoxicological part results (nutrigenomic and biomarker data for exposed biological models). They highlight the complementarity of our different test-organisms, and the interest of realistic exposures in microcosms.

We will conclude on the interest of our integrated assessment, inspired by the “One Health” approach favoured and supported by the French authorities, which consider possible effects and interactions between PP residues for a whole crop season.

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Molecular and ecological linkages to dioxin sensitivity of mammalian aryl hydrocarbon receptor 1

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Aryl hydrocarbon receptor 1 (AHR1) regulates toxicity caused by dioxin-like chemicals (DLCs). Our previous avian studies have shown that key residues (324 and 380) in the ligand binding domain (LBD) influence DLC sensitivity and are affected by ecological factors. However, the determinants of mammalian AHR1 genotype and ligand sensitivity remain unclear. This study aims to elucidate the molecular characteristics of mammalian AHR1 and their association with ecological factors related to species-specific dioxin sensitivity. We identified six amino acid types (IA, VA, LA, IS, VS, IV) at residues 325 and 380 of the human sequence reference across 220 mammalian AHR1 sequences. Type IA was the most common (65%), followed by VA (22%) and LA (11%). *In vitro* reporter gene assays showed that the IS type was the most sensitive to TCDD, with IA and LA showing intermediate sensitivity and VA being generally insensitive. This indicates that these residues significantly impact TCDD sensitivity. IA types exhibit varying sensitivities, suggesting the involvement of additional factors. We further investigated how amino acid conservation is related to ecological factors, discovering an association between AHR1 genotypes and the environment. The IA type was particularly closely associated with marine habitats, while the VA type was more commonly linked to herbivores; however, the connections with other factors were not clear. Additionally, even with different ecological factors, families with phylogenetically close relationships tended to preserve the same type. Furthermore, we identified various structures within the transcriptional activation domain (TAD) of mammalian AHR1, which may potentially influence differences in TCDD sensitivity. This study enhances our understanding of the molecular and ecological factors shaping the DLC sensitivity of mammalian AHR1, providing insights for future ecotoxicological assessments.

Imidacloprid and amitraz synergistically alter immune responses in Apis mellifera *hemocytes with fungal PAMPs exposure*

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The European honeybee (*Apis mellifera*) is an important global pollinator and the source of honey production having both ecological and economic importance. However, the immune-competence of honeybees is greatly affected by pesticide exposure rendering them more susceptible to diseases. Honeybee hemocytes were exposed to different concentrations of imidacloprid and amitraz pesticides in combinations with variable immune stimulation conditions to mimic fungal interaction. Parameters measured include phagocytosis, viability, and immune-gene expression in different pathways. The results showed that there is a synergistic effect of imidacloprid and amitraz on honeybee hemocytes reducing phagocytosis while intriguingly increasing viability. Genes of the Toll pathway were also dysregulated by pesticides and zymosan A exposures indicating a competitive action between pesticides and zymosan on the immune system. The interaction between risk factors such as pesticides and pathogens is complex requiring more studies of different parameters to be fully comprehended, and further considerations are needed in pesticide assessments before application.

Kinetics of microplastics with different polymer types in Grandidierella japonica

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Marine environment contamination by microplastics (MPs; <5000 µm) has become a growing concern as marine animals can ingest MPs, causing potential ecosystem damage. The accumulation and impact of MP ingestion by benthic invertebrates are particularly concerning, yet their kinetics remain poorly understood. This study investigates the internal kinetics of MPs using the amphipod *Grandidierella japonica*, a benthic invertebrate model animal for toxicity assessment. *G. japonica* were exposed to 10 µm polystyrene particles (PSMP) and 30 µm aged and fragmented polyethylene microplastics (afPEMP) to simulate environmental conditions. Subsequently, the MP kinetics were analyzed.

10 µm PSMP Exposure Experiment: *G. japonica* (2-4 mm) were placed in 10 mL glass vials with seawater (30‰) and 2 mg/L PSMP (n=80) and exposed for 6 days. Following exposure, amphipods were transferred to clean seawater for an 8-day depuration period. Sampling was conducted at multiple intervals up to 14 days. Samples were digested with hydrogen peroxide (30%), filtered through 5 µm PTFE filters, imaged with a fluorescence microscope, and PSMPs were counted using ImageJ.

30 µm afPEMP Exposure Experiment: *G. japonica* were placed in 10 mL glass vials with seawater and 10 mg/L afPEMP (n=50) and exposed for 3 days. Amphipods were then transferred to clean seawater for an 8-day depuration period. Sampling was conducted at multiple intervals up to 216 hours. Samples were digested with hydrogen peroxide (30%), filtered through 5 µm PTFE filters, imaged with µFT-IR, and afPEMPs were measured using ImageJ.

In the PSMP experiment, MP concentration in *G. japonica* peaked at 24 hours with a bioaccumulation of $3x10^5$ compared to the water. After transferring to clean seawater, 80% of PSMPs were excreted within 96 hours but 5-10% remained even after 14 days, indicating residual retention. Detailed results of the afPEMP experiment will be discussed.

Predicting the combined effects of environmental contaminants adsorbed on beached plastic pellets using network science

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There is concern about the effects of chemical mixtures on humans and wildlife. However, methods to predict the effects on biological systems of chemical mixtures is lacking. In this study, we propose a network-based method to predict relationships between various chemical mixtures, and genes and diseases.

The prediction is based on the chemical-disease association networks obtained from the Kyoto Encyclopedia of Genes and Genomes and Toxicant and Disease database and chemical-gene association networks retrieved from the CTD database. Using network propagation methods, prediction scores for chemicals−genes and,−diseases associations were calculated from these networks and similarity networks.

First, we focused on the relationship between each chemical isomer and genes and diseases. We found that PCB153 is related to genes, such as TRHR and diseases such as acne. DDE is associated genes, such as FOXC2 and diseases, such as melanoma, a type of skin cancer. Second, we investigated the relationships between chemical families and genes and diseases, by calculating the sum of prediction score for each isomer. We found that PCBs are likely associated genes, such as NR1I3. DDTs are associated genes, such as FOXC2. We also found that PCBs are likely associated with acne. DDTs are associated with melanoma. Finally, we analyzed diseases related to chemical mixture including PCBs, DDTs, PAHs, chlordanes and HCHs which adsorbed on beached range pellets. Assuming that chemicals were adsorbed in equal amounts onto the pellets and that these chemicals had the same toxic equivalents, it was expected that they could cause melanoma.

In this study, we predicted diseases and genes associated with chemicals and chemical mixtures. However, there are problems. First, chemical concentrations are not taken in the prediction. Second, the toxic strength of the chemicals is not taken. In the future, resolving these issues will enable more realistic and accurate predictions.

*Impact of polystyrene ingestion on the gut microbiota and metabolic processes of wharf roaches (*Ligia *sp.)*

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Microplastics (MPs) pollution in aquatic environments is a significant global concern. Marine organisms inhabiting the tidal line such as wharf roach (*Ligia* sp.), may ingest MPs, affecting their overall health. In our previous study, we sampled wharf roaches from the coasts of Japan and found that they tend to ingest polystyrene (PS), presumably expanded PS (EPS), commonly used in fisheries activity. We are following up on this study by evaluating the effect of PS and EPS on the gut microbiome and gene expression of wharf roaches.

First, we confirmed the ingestion of EPS to the wharf roach. Then, wharf roaches from an EPS-contaminated port in Fukuoka were fed an EPS diet for 10 days. Metagenomic analysis showed significant changes in gut microbiota composition, notably enhancing methanogenic archaea *Methanospirillum* in the EPS-feed group, though no significant changes in microbiome diversity indices were observed.

In the following experiment, we fed wharf roaches collected from control site, tidal zone in Noto peninsula where few EPS were observed) with diets containing 2-μm fluorescent PS beads (0, 0.1, 1 mg EPS/g-diet) and analyzed changes in their gut microbiota using 16S and 18S rRNA analysis, no methanogenic archaea was detected in control and exposure group. Metabolomic analysis using GC-MS/MS suggested that the concentration of ingested polystyrene affected metabolic pathways.

Our research has revealed that the ingestion of EPS significantly impacts the gut microbiota and metabolic processes in wharf roaches, a finding that underscores the potential health implications of EPS pollution for organisms in tidal zones. Furthermore, our results indicate a potential increase in methane gas production due to MP ingestion. These findings emphasize the urgent need for further research to explore the broader ecological impacts of MPs, including bioaccumulation and effects on other bioindicator species.

Toxicity of silver–chitosan nanocomposites to aquatic microcrustaceans Daphnia magna *and* Thamnocephalus platyurus *and naturally luminescent bacteria* Vibrio fischeri

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For the successful commercialization of new materials data on their environmental safety are obligatory. Silver and chitosan have antimicrobial properties, making them a promising combination in biomedicine (e.g., wound dressings and antimicrobial surfaces). In this study, the toxicity of different silver–chitosan nanocomposites (nAgCSs) was evaluated using environmentally relevant test organisms: microcrustaceans *Daphnia magna* and *Thamnocephalus platyurus*, and naturally luminescent bacteria *Vibrio fischeri*. Three nAgCSs with different weight ratios of Ag to CS were studied. Citrate-coated silver nanoparticles (nAg-Cit), AgNO3 (ionic control), and low molecular weight chitosan (LMW CS) were evaluated in parallel. The primary size of nAgCSs was ~50 nm. In deionized water, the average hydrodynamic sizes were ≤100 nm and the ζ-potentials positive (16–26 mV). The nAgCSs proved highly toxic to aquatic crustaceans: the 48-h EC_{50} value for *D. magna* was ~0.06 mg Ag/L, and the 24-h LC_{50} value for *T. platyurus* was ~0.2 mg Ag/L. The toxic effect correlated with the shedding of Ag ions (~1%) from nAgCSs. Upon exposure of *V. fischeri* to nAgCSs for 30 minutes, bacterial luminescence was inhibited at 3-25 mg Ag/L. However, the inhibitory effect on bacterial growth upon 1 or 24 h exposure was observed at higher concentrations of nAgCSs, 10-50 mg Ag/L. LMW CS inhibited bacterial luminescence upon 30-min exposure at 6 mg/L, but bacterial growth was inhibited at a much higher concentration (MBC >100 mg/L). The multi-trophic test battery, where *D. magna* was the most sensitive test organism, ranked the nAgCSs as 'extremely toxic' [$L(E)C_{50} \leq$ 0.1 mg/L]. Chitosan was toxic to crustaceans at ~12 mg/L, and bacterial growth was not inhibited up to 100 mg/L and ranked accordingly as 'harmful' [$L(E)C_{50}$ 10-100 mg/L]. Thus, silver-chitosan nanocomposites may pose a hazard to aquatic organisms and must be handled accordingly.

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Novel synergistic antimicrobial silver-chitosan nanocomposites: Design, efficiency and safety

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The continuous surge in healthcare-associated infections and antimicrobial resistance (AMR) urgently calls for new antimicrobial solutions. Nanotechnologies utilizing Ag, ZnO, and CuO nanoparticles (NPs) are increasingly used in various biomedical applications, such as wound dressings, implants, and antimicrobial surfaces, to reduce microbial infections. Indeed, due to the multiple simultaneous modes of action, metal-based NPs are unlikely to induce AMR. Moreover, combining these NPs with a biocompatible polymer, e.g., chitosan with antimicrobial but immuno-modulating properties, may yield synergistically acting nanomaterials with enhanced antimicrobial efficacy.

In the current study, nanosilver was combined with chitosan: silver-chitosan nanocomposites (nAgCSs) with varying Ag:CS weight ratios (1:0.3, 1:1 and 1:3) were synthesized, characterized and evaluated for their antimicrobial activity against critical pathogenic bacteria *Escherichia coli* MG1655, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC6538, and fungi *Candida albicans* ATCC10231. The nAgCSs were also assessed for biocompatibility using human macrophage THP-1 cells *in vitro*.

The nAgCSs had primary and hydrodynamic sizes of ~50 and ~120 nm, respectively, with a ζ-potential of ~+25 mV. Shedding of Ag-ions ranged from 2–4%. According to our results, the nAgCSs were more effective against bacteria than fungi, with 24-hour minimum bactericidal and fungicidal concentrations (MBC and MFC) ranging from 0.2–0.75 mg Ag/L for bacteria and 10–33 mg Ag/L for fungi. The most significant antimicrobial activity was observed at an Ag:CS ratio of 1:3, presumably due to nAgCS absorption on the microbial surface, as confirmed by flow cytometry and confocal microscopy. The antimicrobial activity of nAgCss was not solely attributable to the shedding of Ag-ions, indicating to the synergistic effect of chitosan and silver.

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Comparison of sensitivities between Oryzias javanicus *and* Oryzias latipes *embryos exposed to sediments collected in Tokyo Bay*

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Chemicals discharged by human activities move to aquatic environment with the various events on earth, and a part of them achieves to coastal sediments. Sediments are also important habitat and spawning places for a lot of benthic aquatic organisms. Chemicals in sediments are possibly a certain of risk to the organisms, and we need to monitor the current situations of actual impacts caused there. However, generally, it's difficult to observe the conditions in and on coastal sediments, and irreversible impacts might be silently progressing. In previous study, we tried to evaluate the toxicities of sediments collected in the coastal area of Tokyo Bay, Japan, using Japanese medaka (*Oryzias latipes*) embryos, lived in freshwater, put on sediments only with the pore water and found the slight toxicities. However, the risk evaluations of seawater area are supposed to be evaluated with the data obtained from the marine organisms. Java medaka (*Oryzias javanicus*) is in estuarine areas of East to Southeast Asia, and the breeding conditions, except for salinity, are almost similar with those for Japanese medaka. Therefore, Java medaka is a good test organism for the risk evaluations in sea water area. In this study, we evaluated toxicities in sediments collected from Tokyo Bay using the Java medaka embryos, and compared the results obtained by Japanese medaka embryos. At last, the embryos of Java medaka were examined whether that test marine organisms for the evaluation of sediment toxicities.

The most serious effects in Java medaka embryos as causing 37.5 % mortality in a site, while those of Japanese medaka embryos were only 6.7%. Additionally, we found both 20% of the combined ratios with mortality and unhatching rate in other two sites, although those of Japanese medaka were 3.3% and 0%, respectively. Those suggests that Java medaka embryos are sensitive and useful to evaluate toxicities in coastal sediments.

Chronic toxicity and multifaceted detrimental effects of diflubenzuron on Daphnia magna

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The global demand for pesticides has surged in recent decades, driven by the need to enhance agricultural productivity and control pest populations. Among these pesticides, diflubenzuron (DFB), a benzoylurea insecticide, has gained prominence due to its effective inhibition of chitin synthesis in arthropods, which is crucial for the formation of exoskeletons. Consequently, DFB has been extensively used in both agricultural and aquacultural practices. However, the extensive use of pesticides like DFB has raised significant concerns regarding their potential toxicity to non-target aquatic organisms. Despite diflubenzuron's low solubility in water, it can cause chronic toxicity and death in *Daphnia magna*, an important freshwater organism, even at very low concentrations. This study aimed to assess the long-term toxic effects of DFB, an insect growth regulator, on this species. Chronic toxicity assessment was conducted over a 21-day period, utilizing a range of environmentally relevant exposure concentrations of diflubenzuron (DFB) (1, 3, 5, 8, and 10 ng/L-1). Chronic exposure to DFB elicited significant dose-dependent impairments in key life history parameters of *Daphnia magna*, including survival, growth, and reproductive capacity. A notable observation was a concentration-dependent increase in the production of male offspring, concomitant with a statistically significant decrease ($p < 0.05$) in the proportion of normal, non-deformed offspring. Furthermore, chronic exposure to DFB resulted in reduced reproductive output, delayed maturation, and the manifestation of developmental abnormalities in offspring, collectively underscoring the detrimental impact of this insect growth regulator on *Daphnia magna* population dynamics and overall fitness. This research elucidates the multidimensional toxicity profile of DFB, encompassing not only acute mortality but also sublethal physiological impairments and chronic disruptions in key life history traits of *Daphnia magna*. By integrating chitin analysis results with the observed physiological and life history impairments, this study provides a comprehensive understanding of DFB's multidimensional toxicity profile. The findings underscore the critical necessity for comprehensive and rigorous ecological risk assessments of pesticides, particularly insect growth regulators like DFB, to ensure the long-term conservation and sustainability of aquatic ecosystems and the protection of non-target organisms. Additionally, research on the adsorption effects and toxicity testing of DFB with biodegradable microplastics is still ongoing.

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Reproductive toxicity of benzophenone-related derivatives used in food contact materials on medaka

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Benzophenones (BPs) are a group of organic aromatic ketones with similar structures, commonly added into sunscreen as chemical UV filters to absorb UVA or UVB radiation. BPs also have a very wide range of applications in the industry, being as UV filters in plastics or daily care products to prevent them from sun-degradation. As well, BPs are key components of printing inks (acting as a photo-initiator) used in food contact materials (FCMs). Due to high usages of BPs and their potential of environmental persistence, residues of these chemicals are frequently detected in various environmental matrices and organisms. BPs have attracted people's attention because BP-3 was reported to increase coral bleaching rate, which poses a threat to marine ecosystem. However, there're less studies on ecotoxicity of other BPs, especially those used in FCMs. Therefore, first aim of this study is to systematically analyze *in vivo* toxic effects of 10 BPs used in FCMs on Japanese medaka (*Oryzias latipes*) fish. The 96 hr-acute mortality test with medaka larvae showed that DEAB has the highest acute toxicity (LC_{50}=0.43 μM) among tested BPs. With the 7-day sub-lethal exposure, only 4PBP and DEAB dose dependently increased larval mobility at tested concentrations (5-50 nM). Additionally, DEAB (1.00 μM) had high impact on reproduction of medaka adults, including decreases of egg numbers and fertilization rate. Furthermore, the vtg1 and vtg2 expression in the liver of female and male medaka adults were significantly decreased by DEAB at 0.10 and 1.00 μM, which supports the phenomenon of reduced egg production. The toxic molecular mechanism regarding DEAB-altered reproductive toxicity in medaka fish will be further investigated.

Assessing behavioral responses and toxicokinetic profiles of multicomponent nanoparticles in Daphnia magna

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Multicomponent nanoparticles (MCNMs) are emerging nanomaterials developed by combining individual nanoparticles (NPs) to enhance their properties. Despite their prevalence, there is a limited knowledge about the behaviour, fate, and potential effects of MCNMs on aquatic organisms. Zinc oxide (ZnO) is a widely used NM, particularly in cosmetics and sunscreens. When coupled with manganese (Mn) NPs, ZnO is anticipated to exhibit increased stability and potentially reduced toxicity. To explore this hypothesis, a 72h experiment was conducted, consisting of 24h of exposure of *Daphnia magna* to the EC_5 of ZnO NPs and ZnO:Mn NPs followed by 48h of depuration. Toxicokinetic profiles of Zn and Mn were assessed. Additionally, behavioural responses corresponding to mobility and velocity of *D. magna* were assessed every 24h.

Organisms exposed to ZnO NPs and ZnO:Mn NPs exhibited a rapid uptake of Zn and Mn within 1h of exposure. Interestingly, at the end of the experiment, there were no indications of Zn elimination in either exposure condition. However, organisms exposed to ZnO:Mn NPs displayed a slow but significant elimination of Mn. Furthermore, the mobility and velocity of exposed organisms was significantly reduced compared to the control group at 48h and 72h, indicating delayed effects of ZnO and ZnO:Mn NPs. The application of a light stimuli had significant effects only on non-exposed organisms highlighting the impact of ZnO and ZnO:Mn on *D. magna* phototaxis. The current study gives a better understanding of the exposure kinetics of ZnO and ZnO:Mn NPs and their potential toxicity on *D. magna*, as they may have deleterious effects on population dynamics which may alter the functioning of aquatic ecosystems.

First application of one-class support vector machine algorithms for detecting abnormal behavior of marine medaka Oryzias javanicus *exposed to the harmful alga* Karenia mikimotoi

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It is empirically known that fish exposed to harmful algal blooms (HABs) exhibit abnormal behavior. This might serve as a method for early detection of HABs. There has been no report of the detection of behavioral abnormalities of fish exposed to harmful algae using machine learning. In this study, the behavior of *Oryzias javanicus* (Java medaka) exposed in a stepwise manner to the HAB species *Karenia mikimotoi* at densities of 0 cells mL^{-1} (control), 1×10^3 cells mL^{-1} (non-lethal), and 5×10^3 cells mL^{-1} (sub-lethal) was recorded for 30 minutes at each cell density using two digital cameras connected to a software that tracked behavioral metrics of fish. Recording was conducted in three trials using three Java medaka per trial. The level of anomaly in the behavior of Java medaka was then analyzed using One-Class Support Vector Machines (OC-SVM) to determine whether the behavioral changes could be considered abnormal. This was conducted by taking a midpoint coordinate from each Java medaka group's swimming activity and measuring the average distance and swimming speed of each medaka from the midpoint as parameters of observation. The results revealed abnormal swimming behavior evidenced by an increase of swimming speed, a decrease of shoaling behavior, and a greater depth of swimming in Java medaka exposed to the sub-lethal *K. mikimotoi* density. The medaka exposed to *K. mikimotoi* also displayed physical deformities of their gills that were thought to have caused their abnormal behavior. This supposition was confirmed by further analysis using OC-SVM because the behavior of groups exposed to non-lethal and sub-lethal densities of *K. mikimotoi* were considered abnormal compared to that of the control groups. The results of this study show the possibility of using this system for early real-time detection of HABs as well as other environmental pollutants.

*Peek-A-Boo test: Assessing the behavioral impact ofdiazepam and chlorpyrifos on medaka fish (*Oryzias latipes*)*

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Organism behavior is crucial for survival, and recent studies have raised concerns about "ecological death", where non-lethal chemical exposure can lead to behavioral changes resulting in population declines (Scott and Sloman, 2004). We believe that a simple but effective behavioral test is required to assess the actual effect of pollutants on aquatic organisms. In 2024, we devised a simple behavioral test, named the Peek-A-Boo test, to assess the effect of anxiolytics on the behavior of a model fish (medaka, *Oryzias latipes*), where we investigated the response of medaka to an image of a predator fish (donko fish, *Odontobutis obscura*). To evaluate the potential of the Peek-A-Boo test as a fish behavioral assessment, we conducted a diazepam (benzodiazepine) exposure test and a chlorpyrifos (organophosphate pesticide) exposure test. The diazepam exposure test revealed that the time taken for test medaka exposed to diazepam (0.8, 4, 20, or 100 μg/L) to approach the image was shorter by a factor of 0.22 to 0.65, and the time spent in the area close to the image was longer by a factor of 1.8 to 2.7 than in the solvent control group for all diazepam exposure groups ($P < 0.05$). On the other hand, the chlorpyrifos exposure test indicated that the time taken for test medaka exposed to chlorpyrifos (5 μg/L) to approach the image tended to be longer than the solvent control group. Quantification of swimming trajectories using Shannon's information entropy at 1 minute after image presentation observed a trend of reduced information entropy (monotonous trajectories) in the exposure groups (1.25, 5, 20 μg/L) (P value, 0.101–0.158), suggesting that exposed fish continued to exhibit wary behavior towards the image longer than the solvent control group. Hence, we confirmed that the Peek-A-Boo test can be used for pollutant toxicity assessment on medaka behavior, particularly for evaluating anti-predator behavior.

Examination for effects of OxyPAHs in Japanese medaka embryo triggered by oxidative stress

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Generations of oxygenated polycycle aromatic hydrocarbons (OxyPAHs) are derived from the combustion of organic matters or the emissions from vehicles and industries. They are one of a group of highly toxic pollutants contained in diesel exhaust particles (DEP), which have attracted attention as the air pollutants. OxyPAHs have a structure in which one or more oxygen molecules are doubly bonded on the benzene rings of polycyclic aromatic hydrocarbons (PAHs). It's concerned the effects of OxyPAHs to aquatic organisms because they have been widely detected from the aquatic environment. Their effects and toxicological mechanisms of OxyPAHs in fish are poorly under, despite extensive examinations for the effects in mammals.

In the previous study, we confirmed that OxyPAHs could cause some effects in Japanese medaka embryos and exhibit some malformations such as the abnormalities of bone formation, cardiotoxicity, and anemia in the hatched larvae. The effects possibly cause the excess oxidative stress (OS) and disrupting ascorbic acid (AA) in the embryos by examining the variations of some biomarkers.

In the present study, we generated AA-deficiency embryos and exposed them to OxyPAHs, because of elucidating the contributions of OS causing embryo toxicities, while also exploring their action mechanisms. If some symptoms on hatched larvae increased as AA-deficiency during embryo development, those could be considered the effect by OS. As a result, OxyPAHs induced remarkably serious effects in the embryos reduced up to over 1/20 of AA from normal individuals and exacerbated depending on the exposure time. Furthermore, exposure with high concentrations of OxyPAHs resulted the excess OS, which was 1.4 times compared with the group of solvent control. On the other hand, because curvature of spine was induced in up to approximately 20% individuals of hatched larvae even in the groups of solvent control and lower exposure, OS might not relate to this symptom.

*Effect of chlorpyrifos on alarm reaction of Japanese medaka (*Oryzias latipes*) induced by alarm substances*

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It was known that fish exhibit several alarm substances (AS)-mediated anti-predator behaviors for their population viability. Previous studies demonstrated that most anti-predator behavior in fish would be impaired by disrupting the nervous system such as AChE activity. Organophosphorus pesticide chlorpyrifos (CPF) is widely detected in aquatic environments and might exert its toxicity to non-target aquatic organisms. Therefore, we aimed to elucidate the effects of CPF on alarm substance-meditated anti-predator response of medaka fish (*Oryzias latipes*). Each of the 8 fish (male:female = 4:4) was exposed to CPF for 4 days as follows: No-exposure + Water group, No-exposure + AS group, CPF-exposure + Water group, and CPF-exposure + AS group. During the behavior test, individual fish were placed in the observation chamber. One mL of AS was delivered in the No-exposure + AS group and CPF-exposure + AS group, and 1 mL of ultra-pure water was added in the No-exposure + Water group and CPF-exposure + Water group. Fish behaviors were recorded for 3 min before and 15 min after the stimulus delivery. The AChE activity in the brain samples were measured according to the colorimetric method. As a result, No-exposure + AS and CPF exposure + Water groups showed a significant decrease in swimming speed and increase in freezing duration ($p < 0.05$). In the CPF-exposure + AS group, consistent longer freezing duration was significantly found ($p < 0.01$, $p < 0.05$). In addition, the AChE activity in the CPF-exposure + Water group dramatically decreased compared to the No-exposure + AS group ($p < 0.05$). Our study demonstrated that CPF impaired the anti-predator behavior in medaka fish induced by alarm substances in a time-dependent manner. Besides, we suggested that medaka exposed to CPF became more sensitive to weak stimuli.

Investigation of the cause of the mass mortality of sardines in Jinhae Bay of Korea, 2022

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The cause of the mass mortality of sardines in Jinhae Bay of Korea in 2022 was comprehensively investigated by various fields such as pathology, marine physics, marine environment, and fisheries resources to identify the cause.

Most of the dead bodies found at the sites were sardines, and most of the waters where the death occurred were semi-closed coasts.

A number of open-mouthed individuals were observed as external characteristics of death, and no pathogens were found that could cause death due to disease. The water quality in the areas where death occurred was good, and organic matter in the sediments was diagnosed as contaminated, but there was no temporary inflow of pollutants that could cause death. However, in some dead zones, hypoxia water masses were formed from the bottom layer to a depth of 4 m.

From a marine physical point of view, as a result of backtracking of floating dead bodies using a marine numerical model, the dead body occurred inside the bay, and the possibility of inflow from outside the bay was low. In addition, it was confirmed that no anchovy gendered fish vessels operated inside the bay where the dead body was found during the closest period to mass death.

From a fishery resource perspective, it is estimated that the large inflow of sardines into Jinhae Bay is due to an increase in the inflow of individuals spawned from the eastern coast of the South Sea and the eastern sea of Jeju of Korea. The mass death of sardines in Jinhae Bay of Korea in 2022, which was examined through overseas cases and expert discussions along with the results of the comprehensive investigation, was identified to be the main cause of the lack of oxygen, and more careful observation and investigation are required in case the same case occurs in the future.

A trophic dilution of Hg in the macrobenthos community from Minamata Bay

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Mercury is a toxic heavy metal. The content of Hg in organisms generally increase through food chains, leading to high bioaccumulation in consumers of higher trophic levels like fish, a main exposure source of Hg to humans. While bioconcentration from ambient water by primary producers is particularly high and therefore important process that determine the overall degree of bioaccumulation in the ecosystem, bioaccumulation of Hg in consumers is considered to be mainly derived from their diets rather than from ambient water. In shallow coastal ecosystems, macrobenthos are important consumers that become important diets for not only demersal fish but also bentho-pelagic fish. Macrobenthos are fueled by both phytoplankton and microphytobenthos, which are main primary producers in shallow coastal waters. Macrobenthos therefore work as a conduit of Hg from primary producers to fish, and the information on the Hg burden and trophic level of each species becomes a key for understanding Hg bioaccumulation in shallow coastal ecosystems. In this study, we measured total Hg contents (THg), together with carbon and nitrogen isotope values (δ13C and δ15N) for macrobenthos and fish collected from Minamata Bay. Macrobenthos was collected by Eckman grab or sledge from September 2022 to September 2023. Fish was collected by fishing in December 2022. δ13C of macrobenthos ranged from ca. -19‰ to -13‰. Their THg did not significantly increase with δ13C, but significantly decreased with δ15N, indicating that trophic dilution occurred. Although THg of macrobenthos tended to differ among seasons, the slope of trophic dilution did not significantly differ among seasons. In contrast, fish THg clearly increased with δ15N. Many studies show that Hg almost always biomagnifies through trophic levels. To the best of our knowledge, our results show a rare example that Hg do not necessarily increase with trophic relationships.

Accumulation of radioactive strontium derived from the Fukushima nuclear accident in vertebral bones of marine fishes

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The research on the accumulation of Sr-90 derived from an accident at the Fukushima Dai-ichi Nuclear Power Plant (FDNPP) in various marine fishes has been limited. In this study, to analyze the spatial distribution of Sr-90 in fish and identify differences in Sr-90 radioactivity concentrations among fish species, we measured vertebral Sr-90 radioactivity concentrations of various fishes collected off the coast of Fukushima Prefecture in 2014. The spatial distribution of radioactivity concentrations tended to be high in the central area at shallow water depths as well as throughout the southern sites. The spatial distribution of vertebral Sr-90 reflects that of Sr-90 in seawater predicted from previous modeling and measuring results. Among the species we examined, the highest radioactivity concentrations and detection frequencies of Sr-90 were observed in rays such as *Okamejei kenojei* and *Hemitrygon akajei*, an elasmobranch. Soles, Cynoglossidae, had relatively high Sr-90 concentrations. Focusing on *Okamejei kenojei*, Sr-90 concentrations of the individuals collected from 2013 to 2015 were further measured. Sr-90 were still detected in 2015 and the weight of Sr-90-detected individual tended to increase as time passed, indicating that Sr-90 in vertebrae may remain until fish dies. Measurement of stable Ca concentrations and Sr in ashed vertebrae revealed that approximately 35% for most species, whereas stable Sr concentrations in ashed vertebrae ranged from 0.1% to 0.3% and were species-specific. Rays and soles were higher in the ratio of concentrations of stable Sr to Ca in ashed vertebrae than other species. Thus, there could be differences in the Sr-90 accumulation among marine fish species, which may be important factor in determining Sr-90 radioactivity in fish.

Radiostrontium (Sr-90) activity in shells of mussels from the coastal area of Fukushima Prefecture, Japan

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In the coastal area of Fukushima Prefecture, Japan, the radiostrontium (Sr-90) activity in seawater temporarily increased because of the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident in 2011. Still, the activity has recovered to the levels before the accident by 2015 (Karube et al. 2016). As of 2024, treated water, which may contain not only tritium but also radiostrontium, has been released from the FDNPP into the ocean, suggesting that the release of radiostrontium has occurred again. Therefore, it is necessary to continue radiostrontium monitoring in this coastal area. In this study, we investigated the space and temporal variation of Sr-90 activity in shells, which can uptake and accumulate strontium from seawater in the coastal area of Fukushima Prefecture. We also examined the availability of shells for radiostrontium monitoring in seawater.

We analyzed Sr-90 activity in the shells of the mussel (*Septifer virgatus*, shell length: 38-48 mm) collected from the coasts of Minamisoma City, Namie Town, Tomioka Town, Hirono Town, and Iwaki City in Fukushima Prefecture from 2013 to 2022 using the solid phase extraction method (Tazoe et al. 2016).

The Sr-90 activity of shells in 2013 showed the highest value (50 Bq/kg) in Tomioka Town, the nearest and south side of the FDNPP. In 2022, Sr-90 activities of shells in Hirono Town, Tomioka Town, and Iwaki City were lower than the minimum detectable activity (2.7-7.9 Bq/kg). The radiostrontium released from the FDNPP has been dispersed in a southerly direction from the FDNPP by coastal currents and has recovered to levels before the FDNPP accident by 2015 (Karube et al. 2016). Therefore, our results suggest that the Sr-90 activity of shells of mussels reflects the spatial and temporal variation of the activity in seawater, and shells are available for radiostrontium monitoring in seawater.

Karube et al. (2016) Environ Sci Pollut Res 23: 17095–17104

Tazoe et al. (2016) Talanta 152:219–227

Effects of tire-derived chemical 6PPD-quinone on mitochondrial function in salmonid fish species

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N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-Q), a transformation product of the tire antioxidant 6PPD, has raised significant global concerns due to its high acute toxicity towards salmonid fish species. The parent chemical, 6PPD, belongs to the class of substituted para-phenylenediamines (PPDs), is used in many commercial and industrial products, including tire rubber. Due to the ubiquitous occurrence of 6PPD and its rapid transformation, 6PPD-Q has been detected globally in stormwater, road dust, atmospheric particles, and human urine.

The mechanisms of acute lethality by 6PPD-Q to some salmonid species remain unclear. Uncoupling of the electron transport chain has been observed in rainbow trout gill cells exposed to 6PPD-Q, but not in rainbow trout liver cell line (Mahoney et al., 2022, ES&T Letters). The uncoupling effect is a possible molecular initiating event for 6PPD-Q acute lethality and may explain species sensitivity differences, however, the uncoupling by 6PPD-Q has never been examined for salmonid species other than rainbow trout.

To address this gap, we examined the effects of 6PPD-Q on oxygen consumption rate (OCR) in mitochondria isolated from the liver and brain of sensitive (white-spotted char, *Salvelinus leucomaenis pluvius*) (Hiki and Yamamoto, 2022, ES&T Letters) and tolerant salmonid species (kokanee salmon, *O. nerka*), both native species in Japan. For these species, 6PPD-Q exposure did not increase OCR in mitochondria isolated from liver and brain, whereas CCCP, a well-known uncoupler, showed dose-dependent increases in OCR. The reason for the inconsistencies between this study and the previous study (Mahoney et al., 2022, ES&T Letters) is unclear but might be due to the difference in target organs, which should be investigated further.

Effects of antifouling agents dichlofluanid and chlorothalonil on growth and photosynthetic activity of marine diatom, Thalassiosira pseudonana

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Alternative antifouling agents have been adopted following the prohibition of organotin compounds. However, these have also been detected in environmental samples from coastal areas (e.g., dichlofluanid 13.44 μg L^{-1} and chlorothalonil 3.17 μg L^{-1} in Turkey; Korkmaz et al., 2023), and it is necessary to evaluate their impacts on primary production. This study tested the toxicity effects of two common antifouling agents, dichlofluanid and chlorothalonil, on the growth and photosynthetic activity of marine planktonic diatom species *Thalassiosira pseudonana*. *T. pseudonana* was exposed to varying concentrations of dichlofluanid (0, 1, 5, 10, 50, 100, and 200 μg L^{-1}) and chlorothalonil (0, 1, 5, 10, 50, and 100 μg L^{-1}) using DMSO as a solubilizing agent (0.01% in each test solution). The fluorescence intensity and photosynthetic activities were measured every 24 hours during the 72-hour experimental period using an in-vivo fluorometer (Model 10-AU, Turner Design) and an Aqua Pen fluorometer (PSI, Photon Systems Instruments). The results indicated decreasing F/Fm ratio and PIabs in higher doses (200 μg L^{-1} for dichlofluanid; 100 μg L^{-1} for chlorothalonil), implying a disruption in the photosynthetic system attributable to intrinsic damage to PSII components. EC_{50} values for dichlofluanid (127 μg L^{-1}) and chlorothalonil (24.89 μg L^{-1}) were calculated post-exposure based on their growth rate and fast chlorophyll fluorescence induction (OJIP transient) analysis. Lower dose ranges for dichlofluanid (1 μg L^{-1} to 50 μg L^{-1}) and chlorothalonil (1 μg L^{-1} to 10 μg L^{-1}) had no significant effect on *T. pseudonana* proliferation or photosynthesis activities. The individual risks of dichlofluanid and chlorothalonil might be negligible within environmentally detected concentrations. However, low concentrations of various antifoulants may coexist in environments such as ports or inland seas. This requires further investigation into the combined toxicity of antifoulant mixtures.

A study on the distribution characteristics of organotin compounds in sediments around Ulsan Bay, Korea

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Ulsan bay is 8.5 km long and 3.2 km wide, and various ports and industrial facilities such as a big ship buildings and some chemical complex are surrounding the bay. The objective of this study is to evaluate the concentration distribution of butyltin compounds such as TBT (tributyltin), DBT (dibutyltin) and MBT (monobutytin) in sediments around the bay. Organotin compounds (OTs) have been used widely in antifouling paints to prevent adherence of sedentary organisms to ship hull and other structural surfaces immersed in seawater from the 1960s. In Korea, the national restriction on the use of TBT-based antifouling paints was introduced in 2000 for small boats, and they were totally banned in 2003.

The sediments were collected in total 42 sites from the Taehwa river (T1-T7; 7 sites), Gosa stream (G1-G5; 5 sites), Ulsan bay (U1-U18; 18 sites), Onsan bay (O1-O8; 8 sites), and Oehwang river (W1-W4; 4 sites) from April 25-29, 2022.

The TBT were detected in 41 sites of 42 sites, the range of concentrations were 1.06 to 398.62 (average 14.30±61.22) ng Sn/g dry wt., the DBT were detected in 20 sites of the 42 sites, the range of concentrations were 17.80 to 21.38 (average 19.55±1.01) ng Sn/g dry wt., the MBT were detected in 40 sites of 42 sites, the range of concentrations were 3.11 to 742.90 (average 24.56±115.10) ng Sn/g dry wt.. The range of total BTs concentrations were 3.12 to 1162.90 (average 47.80±177.20) ng Sn/g dry wt.

The highest mean concentration(256.15±453.71) ng Sn/g dry wt. was detected in the Gosa stream area where chemical plants are located in the upstream. The second higher mean concentration(22.89±21.51) ng Sn/g dry wt. was shown in the Ulsan bay area where higher ship activity such as a big shipyards and big harbor are located around there. However, the lowest mean concentration(8.67±7.27) ng Sn/g dry wt. was shown in the Onsan bay area located near the Onsan chemical plants complex.

Assessment of the effects of short-, medium-, and long-chain chlorinated paraffins on the differentiation of human iPS cells to definitive endoderm

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Chlorinated paraffins (CPs) are mixtures of linear chloroalkanes classified into short-, medium-, and long-chain chlorinated paraffins (i.e., C10-13-SCCPs, C14-17-MCCPs, and C18-30-LCCPs) based on the length of their carbon chains. Although the transfer of CPs to human fetuses and embryos has been documented, their risk to human development is poorly characterized. To elucidate the effects of CPs on human definitive endoderm (DE), a 4-day *in vitro* induction method from induced pluripotent stem cells (h-iPSCs) was developed under chemically defined conditions on a 96-well plate platform, and its efficiency was tested by qPCR analysis. Differentiating h-iPSCs were exposed to SCCPs (63% Cl), MCCPs (57% Cl), and LCCPs (49% Cl) at 1, 10, 100, and 500 µg/L for 4 days, followed by a WST-8 assay. The qPCR measurement of the mRNA expression levels of the DE-specific genes SOX17, FOXA2, GATA4, and CXCR4 revealed induction folds of $2.8x10^7$, $3.7\ x10^2$, $1.5x10^5$, and $3.9x10^3$, respectively. In contrast, the mRNA expression of the pluripotency marker gene OCT4 was significantly repressed compared to undifferentiated h-iPSCs, highlighting the efficiency of the differentiation method. Exposure to CPs revealed a dose-dependent decrease in cell viability at the end of the 4-day induction period for SCCPs and MCCPs, with statistical significance appearing at the 10 µg/L level, whereas LCCPs showed no toxic effects over the entire exposure range. Compared to our previous results on the weak, nonmonotonic cytotoxic potency of CPs on undifferentiated h-iPSCs, the current results suggest that SCCPs and MCCPs at environmentally relevant concentrations have a developmental toxicity potential on human DE.

Graphene oxide nanoparticles for water purification: mitigating heavy metal contamination and toxicity in aquatic ecosystems

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Restoring environmental integrity in water bodies contaminated with toxicants due to military activities presents a significant challenge. This study explores the potential of graphene oxide (GO) nanoparticles, known for their exceptional sorption capabilities, as a remedial agent for the removal of heavy metals (Cr, Cu, Ni, and Zn) from water contaminated during military conflicts. The research focuses on assessing the toxicity effects of GO in conjunction with a heavy metal mixture on algae *Desmodesmus communis*, crustaceans *Daphnia magna* neonates and fish *Salmo trutta* embryos and larvae.

The metal mixture's concentration was set to the maximum allowable limits in EU inland waters and increased up to 10-40 times to simulate realistic postwar pollution scenarios. Test organisms were exposed to three different concentrations of GO, the metal mixture (MIX), and a combination of GO and the metal mixture (GO+MIX) over a short-term period. Results demonstrated that GO's capacity to sorb metals from the GO+MIX combination exceeded 90%, significantly reducing metal bioavailability and subsequent toxicity. Co-exposure to GO+MIX mitigated the toxic effects commonly associated with heavy metal exposure, as evidenced by changes in physiological parameters of the test organisms. These findings underscore the dual role of GO in not only removing heavy metals from aquatic environments but also diminishing their bioavailability and toxicity to aquatic life. Nevertheless, the environmental presence of GO necessitates careful evaluation of its potential mechanical effects (shadowing, digestive blockage, damaged protective barriers), particularly on sensitive developmental stages of aquatic organisms.

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The impact of war on the aquatic ecosystems of Ukraine: Problems and solutions

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The ongoing conflict in Ukraine has precipitated an environmental crisis, significantly affecting the country's aquatic ecosystems. The contamination of water sources by chemicals from munitions, the improper disposal of human and animal remains, and the substantial mortality among aquatic species, including fish, have resulted in significant ecosystem degradation, as evidenced by reduced biodiversity, habitat destruction, and disruptions to essential ecosystem services. To address these challenges, our study plans to collect water samples from war-affected aquatic ecosystems.

The hydrochemical analysis and microbiological characteristics of the aquatic ecosystems will be analyzed and the toxicity of the contaminants will be assessed using model organisms, including *Danio rerio*, *Daphnia magna*, *Lepidium sativum*, and *Desmodesmus communis*, etc. Following this, the risks associated with these contaminants will be evaluated, with experiments conducted with nanoparticles in order to mitigate chemical pollution. Furthermore, we intend to establish a consortium of microorganisms to purify water bodies of organic materials and develop a comprehensive strategy for ecosystem restoration.

This research integrates economic, environmental, and social factors to propose sustainable solutions for the restoration of aquatic bioresources. Our methodology includes rapid and cost-effective assessments of aquatic ecosystem health, identification of risks, restoration of bioresources, and future protection of water bodies for fisheries and aquaculture purposes. The introduction of algorithms for the swift qualitative and quantitative assessment of damage to freshwater ecosystems caused by military operations will enhance current conditions and establish a resilient management framework for aquatic ecosystems in conflict-affected regions.

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New approach methodology for hazard assessment of short-chain chlorinated paraffins in ex ovo *chicken embryos*

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Short-chain chlorinated paraffins (SCCPs) are found in various environmental matrices and biota, including avian eggs. The presence of SCCPs in avian eggs raises concerns about their potential impacts on embryonic development. Traditional *in ovo* studies are limited by the eggshell, hindering direct observation of early-stage embryos. However, recent advances in shell-less (*ex ovo*) incubation systems, utilizing artificial transparent vessels, have enabled real-time monitoring of chick embryos from early stages. This study investigated the developmental toxicity of SCCPs in early-stage chicken embryos using such an *ex ovo* system. Results demonstrated that exposure to SCCPs at 20 μg/g egg wet weight resulted in lethality during early embryogenesis. Furthermore, exposure to SCCPs was associated with a significant reduction in various growth parameters, including body length, head and bill length, and eye diameter, indicating developmental delays by day 9. SCCPs exposure was found to alter the expression of genes involved in TH transport (SLC16A10), signaling (THRA), and metabolism (DIO3) in the developing embryos. Notably, the downregulation of SLC16A10 and THRA suggests impaired TH uptake and signaling, respectively, potentially contributing to the observed developmental delays. Additionally, the upregulation of DIO3, a TH-inactivating enzyme, may lead to decreased thyroxine (T4) levels, further exacerbating the disruption of TH homeostasis. Interestingly, SCCPs exposure also affected the expression of TH-related genes (TYR, SLC16A2, DIO2, TTR, SLCO1C1, TSHB, CGA, TSHR, TPO and TG) in the developing brain and eyes. Further research is warranted to elucidate the specific molecular mechanisms underlying the observed effects in the brain and eyes. This study highlights the utility of our *ex ovo* incubation system as a new approach methodology for investigating the developmental toxicity of environmental contaminants like SCCPs.

New approach methodologies to predict transactivation potencies of environmental contaminants mediated by Baikal seal estrogen receptor α and β

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High levels of environmental contaminants including dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), and their metabolites (OH-PCBs) have been detected in Baikal seals (*Pusa sibirica*). These chemicals have shown toxic effects via estrogen receptor (ER) signaling pathways in model and non-model species. However, study on marine mammal ER remains limited. Thus, we aimed to evaluate the transactivation potencies of BPs and OH-PCBs in *in vitro* Baikal seal ERs (bsERα and bsERβ) reporter gene assays. We then performed *in silico* docking simulations for these chemicals and bsERs and examined whether computational models built using the *in silico* data account for the *in vitro* activities.

Transactivation potencies of 11 BPs, 9 OH-PCBs, DDT, and DDE were measured by using the in vitro reporter gene assay system where bsERα or bsERβ were transiently expressed in COS-1 cells. *In silico* homology modelling based on human ERα and ERβ templates and docking simulation were carried out using the Molecular Operating Environment (MOE) software. The docking simulation parameters (interaction energy and Protein-Ligand Interaction Fingerprint (PLIF) indices) and molecular descriptors including MOE-VSA and MACCS were used to build Quantitative Structure-Activity Relationship (QSAR) models which explain the ligand-specific *in vitro* transactivation of bsERs.

Out of the 22 tested chemicals, 21 and 18 chemicals induced luciferase activities for bsERα and bsERβ, respectively. From their dose-responsive curves, 19 and 15 chemicals showed EC_{50} values for bsERα and bsERβ, respectively. We successfully generated QSAR models with Partial Least Square (PLS) algorithm. The EC_{50} values calculated from the PLS regression models for both bsERs were highly correlated with those observed from *in vitro* reporter assays. The PLS Discriminant Analysis (PLSDA) classification models also clearly separated the active and non/low-active chemicals with a high performance accuracy. Some molecular descriptors and docking simulation indices concertedly contributed to the ligand-specific activity for both bsERα and bsERβ. In conclusion, by combining chemical descriptors and *in silico* ligand-receptor docking simulations, we were able to predict the *in vitro* bsER-mediated transactivation potencies of these chemicals.

The sewage sludge directive in EU and its imminent amendment

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Wastewater Treatment Plants (WWTP) are facilities where municipal wastewater is treated as to become harmless for public health and safe for environmental release. A typical WWTP employs the activated sludge process; the initial step involves the removal of large debris through screening and grit removal, which is followed by physical separation to minimize suspended solids. The activated sludge process is characterized by an aerobic suspended growth treatment system where microorganisms utilize the organic content in wastewater as their energy source for survival and replication. Tertiary treatment further enhances the quality of the effluent by eliminating residual contaminants, and finally disinfection of the effluent eradicates pathogens. One of the waste by-products of this procedure is sewage sludge that is mainly produced during the secondary treatment in the secondary precipitation clarifier vessel. This waste that is defined in 91/271/EEC as "(the) residual sludge, whether treated or untreated, from urban waste water treatment plants" and needs to undergo extensive treatment before leaving the WWTP. Sludge that has been properly treated so that water content has been reduced and pathogens have been eliminated is preferentially called "biosolids" (BS). The term "biosolids" emphasizes the beneficial aspects of this material which can become a fertilizer, a backfilling component or an incineration substrate. In the first case (use in agriculture) the Directive 86/278/EEC has been a seminal regulation that lays down the requisites for sludge use in crop fertilization, for almost 30 years now. The Directive sets limit values for seven metals (Cd, Cu, Ni, Pb, Zn, Hg, and Cr) in soil and in the BS. It is also dictates time period between the application of BS and livestock grazing or crop harvesting. The present review highlights the additional limitations and precautions that each Member State has set, besides the Directive and the rationale behind them, in the light of the following

-the additional limitations set on the amended Waste Landfill Directive

-the imminent amendment and adaptation of the Sludge Directive

-the priorities of the New European Green Deal

-the serious lack of incorporation of ecotoxicity indices in the limitations set in the original Directive and the need for relevant propositions

A method for non-lethal detection of endocrine disruption in fishes

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The introduction of endocrine disrupting compounds (EDCs) to aquatic environments following anthropogenic activity poses a significant threat to the well-being of fish populations. The adverse effects of such disruptions often target steroidogenic pathways and thus the evaluation of circulating steroid hormone concentrations provides a crucial means of understanding these impacts. While conventional blood sampling is commonly employed for such purposes, it presents challenges, especially with small fish species like the fathead minnow, commonly utilized in studying EDCs. These fish often lack sufficient blood volume for analysis, necessitating the pooling of multiple samples, increasing animal sacrifice and diminishing statistical power. The goal of this project was thus to develop a method for the quantification of steroid hormones excreted into fish holding tank water using liquid chromatography tandem mass spectrometry (LC-MS/MS) as a means of assessing the effects of EDCs. Preliminary results have indicated that 17β-estradiol, progesterone, testosterone and 11-ketotestosterone are detectable using 500 mL of water collected from a tank containing a single male or female minnow at concentrations ranging from 0.08-6 ng/L. Moving forward, this method will be tested for its ability to detect endocrine disruption in fish following exposure to known EDCs. This step will include a comparison of blood and tank water hormone concentrations as well as transcriptomic analyses to anchor responses to known biological pathways. Once fully validated this method will be highly useful in the non-lethal screening of EDCs, and thus, the assessment of fish health and improving fish welfare.

Assessing bioavailability and toxicity of contaminated sediment with medaka fish

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Sediment pollution by heavy metals has been an important environmental issue for decades. With the rapid development of high-tech industries, sediment contamination by technology-critical elements (TCEs) has raised the attention. The sediment of the aquatic environments often acts as the sink of contaminants, likely becoming a source of pollutants if the polluted sediment is disturbed or changes its chemical condition. The sediment pollutants could be released into the water column. In this study, we used Copper (Cu), a commonly detected sediment contaminant as a surrogate; the bioavailability and toxicity of Cu from different Cu-contaminated environmental sediments are assessed using a whole sediment exposure strategy with embryos of medaka fish (*Oryzias latipes*) as a model organism. Our results demonstrated that sediment with lower pH values, cation exchange capacity, or organic matter tended to release Cu ions in the pore and overlaying water, thus causing higher copper bioavailability (e.g., copper accumulation in embryos) and toxicity (e.g., embryo mortality). Either dissolved Cu in overlaying water and pore water or Chelex 100 extractable Cu showed a good correlation with Cu bioaccumulation and embryo mortality. Our results demonstrated that the established embryo exposure system can successfully assess the copper bioavailability and toxicity from sediment exposures. We will use the established sediment exposure system to assess the bioavailability and toxicity of TCE-contaminated sediment.

Decadal monitoring of the megabenthic community in the coastal areas of Fukushima Prefecture, Japan, after the Great East Japan Earthquake

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The Great East Japan Earthquake and the subsequent major tsunami on March 11, 2011, had significant impacts on the regional coastal ecosystem. These impacts included land subsidence, alteration of substrates, and changes in the vegetation and community structure of marine organisms. Additionally, the disastrous accidents at the Fukushima Daiichi Nuclear Power Plant (1F) caused leaks of radioactive cooling water into the sea, raising serious concerns about adverse effects on marine organisms. Investigating the status of the megabenthic community along with environmental changes is essential to evaluate the effects of natural and anthropogenic disasters on marine organisms. In this study, we conducted fisheries-independent trawl surveys to reveal the community structure of megabenthic species (fishes, crustaceans, mollusks, and echinoderms) following the disastrous events in the coastal areas of Fukushima Prefecture, Japan. The trawl survey data were analyzed for the summer and winter from 2013 to 2023 at three latitudinal transects along the coast: off Soma (north), off 1F (central), and off Iwaki (south). Each transect consisted of three sites at different depths (10, 20, and 30 m). We collected megabenthic species and investigated their spatio-temporal changes throughout the survey period. The total abundance declined from 2013 to 2015, primarily due to a decrease in crustaceans and echinoderms. In particular, low larval density was evident for decapod shrimps in 2020, although adult males and females had reached sexual maturity, implying recruitment failure during their early life stages. The total biomass fluctuated from 2013 to 2020 and exhibited a decreasing trend from 2021, which was attributed to declines in most of the dominant species such as flatfish, puffers, and elasmobranchs. Factors affecting changes in the megabenthic community will be discussed in relation to environmental conditions.

*Examinations for potential of dietary accumulations for chemicals by common carp (*Cyprinus carpio *L.) and relationship between biomagnification factors and Octanol water Partition coefficient*

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Various chemicals are released into environment with human activities. Generally, hydrophobic substances adsorb on the suspended solids in the water column, eventually settle on bottom sediments, and are absorbed by the aquatic organisms possibly through magnification. In general, intakes of chemicals into the organisms occurs through two distinctly different pathways; that is, trans-gill uptake, chemicals dissolved in water are absorbed with the gill respiration; and dietary uptake, chemicals in food are absorbed with feeding. Most of previous studies for accumulations of chemicals have often showed bioconcentration factors (BCFs), obtained in the exposures expected the absorbing chemicals dissolved in test water. On the other hand, information for their accumulation with dietary exposures have been limited. A correlation between logKow of individual chemicals and their BCFs or biomagnification factors (BMFs) is assumed. However, currently empirical evidences to confirm the correlation only for BMFs are insufficient, and, therefore, a greater number of examinations for their relationship are required. The purposes in this study were to estimate BMFs in carp (*Cyprinus carpio* L.) orally exposed to several of structurally similar substances spiked in the feed, and to elucidate the relationship between BMFs and logKow. The substances with logKow within 3.5 to 5, or greater than 5 were selected in the oral exposure tests. Their target substances were included polyaromatic hydrocarbons (PAHs), terphenyls, and pesticides. As a result, substances with high logKow values did not always exhibit high BMFs. Meanwhile, a few substances such as phenanthrene showed slightly higher BMFs, despite being below 1. Additionally, the clear correlation between BMFs and logKow were not found, except for a part of chemical groups, in the present study. These results suggest that it is possibly difficult to predict BMFs only from logKow of individual chemicals.

Evaluation of the toxicity to algae of leaches from rare-earth elements (REE)-rich mud from the seabed around Minami-Tori-Shima

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In 2013, extremely rare-earth elements (REE)-rich mud, with concentrations reaching 7,000 ppm—the highest grade in the world—was discovered in the Japanese exclusive economic zone (EEZ) around Minami-Tori-Shima. Consequently, industry-academia-government collaboration is now underway to develop practical uses for REE. However, the full extent of the environmental impact of deep-sea mining focused on REE-rich mud remains unclear. The International Seabed Authority (ISA) states that the environmental impact assessment should address not only areas directly affected by mining but also the winder region impacted by the discharged plume and material released by the transportation of minerals to the ocean surface.

Our aim in this study is to assess the toxicity to algae of leaches from REE-rich mud collected from the seabed around Minami-Tori-Shima, focusing on the ocean surface area. To assess the toxicity of many mud samples, we used high-throughput method employing 96-well plates as an alternative to the general growth inhibition test using 300 mL flasks. Additionally, we used the fresh water unicellular alga *Raphidocelis subcapitata* as the test species, considering that the sensitivity to metals in freshwater algae is generally higher than that in the marine species and the impact after being brought ashore.

Several leaches from the muds collected during sampling program conducted by KM21-06 and KM21-03 cruise greatly promoted the growth of *R. subcapitata*, indicating that the leaches from REE-rich mud might induce algal blooms. Although inductively coupled plasma-mass spectrometry (ICP-MS) analysis of the leaches revealed high concentrations of molybdenum and vanadium, the factors promoting growth are not yet clear. To demonstrate the effect of the metals in the leaches, inhibition tests using mixed-metal solution with the same composition as the leaches, in addition to assessing the toxicity of each metal detected in the leaches, should be conducted in the future.

Comparative analysis of spine and otolith gene expression and mineralization changes due to ancestral BaP exposure

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Parental benzo[a]pyrene (BaP) exposure is associated with developmental bone defects, increased risk factors for late-onset osteoporosis, and possible hearing loss in the offspring. While previous research indicates that BaP significantly impacts vertebral bone, little is known about the impacts of ancestral BaP exposure on calcified structures such as otoliths. Using Japanese medaka (*Oryzias latipes*), this study aims to describe the transgenerational effects of BaP exposure on both otoliths and spines. F1 generation male and female adult medaka (6 months-old) were used for mineral quantity assessment of eight elements (Na, Mg, P, Ca, Mn, Zn, Sr, Ba) using laser ablation-inductively coupled plasma-mass spectroscopy. Using linear mixed models, differences based on sex, treatment, and tissue type were analyzed. In general, female medaka showed increased levels of Na, Mg, P and Mn compared to male fish in the otolith. The same four minerals were significantly reduced in parentally BaPexposed female offspring. No differences were observed in the spine mineral composition between the sexes or the treatment groups. This is the first report of a sex difference in otolith mineral deposition from a controlled lab experiment. Further, the data demonstrate that ancestral BaP exposure reduces the mineral deposition in otoliths of female fish. Additional assessment is needed to better characterize how reduced otolith mineral amounts may affect vestibular and auditory senses. Changes in expression of key mineralization genes between the treatment groups will be analyzed using qualitative Real Time-PCR for seven genes (Osx, twist1b, Col10a1b, Col2a1a, otol1, sparc, cnmd) to determine statistically significant differently expressed genes. This research advances the knowledge of transgenerational impacts of environmental pollutants on mineralized tissues and argues towards a refined environmental risk assessment for BaP.

Cd binding protein from the midgut of the scallop, Mizuhopecten yessoensis

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Scallops (*Mizuhopecten yessoensis*) accumulate heavy metals such as cadmium (Cd), zinc (Zn), iron (Fe), etc. In this study, we separated and purified the Cd binding proteins of scallops. We dissected scallops by tissue and measured Cd concentrations. In order to estimate the size of the Cd binding molecules, HPLC-ICP-MS gel filtration was carried out. Fraction including Cd binding proteins was isolated by HPLC and analyzed by LC-MS/ MS. Cd binding protein was identified as novel protein (myCRP), which has Cysteine-rich domain. Gene expression levels in scallop tissues were determined by quantitative PCR. myCRP was found to be expressed specifically in the midgut glands of scallops. Further functional analysis was performed using recombinant myCRP (rmyCRP) expressed in *E. coli*. The ITC measurement revealed that 9 Cd ions bind to 1 molecule of rmyCRP. Quantitative analysis using antibody showed that *M. yessoensis* contained 132.2 nmol/g of myCRP. We found that Cd binding to myCRP accounted for 93.7% of all Cd bound to myCRP. The findings provide new insights into the mechanisms by which invertebrates defend against Cd.

Iron distribution and iron-binding protein in the digestive diverticula of **Turbo sazae**

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Molluscan species accumulate heavy metals in the digestive diverticula. However, molecular mechanism of metal accumulation in the gastropods remains unclear. This study investigated heavy metal accumulation in *Turbo sazae* through elemental quantification, elemental mapping, and identification of metal-binding organic molecules.

Elemental analysis of *T. sazae* was conducted using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), revealing that the digestive glands contain approximately 1% iron (Fe). To investigate the distribution of Fe within the digestive glands, elemental mapping was performed using Particle Induced X-ray Emission (PIXE, PASTA, QST) for light elements and Synchrotron Radiation X-ray Fluorescence (SR-XRF, BL37XU, SPring-8) for heavy elements. The analysis indicated that Fe is localized in the colored granular tissue of the digestive glands. PIXE analysis showed that the Fe distribution does not align with the distribution of phosphorus (P), sulfur (S), chlorine (Cl), and bromine (Br). Additionally, SR-XRF measurements revealed that other heavy metals, such as cadmium (Cd) and zinc (Zn), are also concentrated in the Fe-rich areas.

To further study the Fe-binding organic molecules in the digestive glands of *T. sazae*, the extracts from the digestive glands were applied to High-Performance Liquid Chromatography coupled with Inductively Coupled Plasma Mass Spectrometry (HPLC-ICP-MS). The purified fraction was analyzed by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) to determine the amino acid sequences of Fe-binding proteins. Two isoforms of ferritin, which bind Fe, were identified.

T. sazae feeds on seaweed by scraping rocks on the seabed with their radula. The seabed contains insoluble iron compounds such as $Fe(OH)_3$, while seawater has low concentrations of soluble Fe ions. The findings of this study suggest that *T. sazae* probably plays important roles in the Fe cycle in seawater.

A histidine-rich protein detected from lower jaws of green lugworm accumulating zinc

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Biominerals are minerals produced by living organisms and are regulated by organic matter. Among biominerals, the lower jaws are subjected to very high forces during predation, so the organism concentrates metallic elements such as calcium, which is found in human teeth, to strengthen the lower jaws. The green lugworm (*Nereis aibuhitensis*) has zinc (Zn)-accumulated lower jaws. Biominerals using transition metals are extremely rare. In this study, we focused on the chemical form of zinc in the lower jaws and organic molecules that contributes to Zn accumulation.

Elemental imaging using micro-PIXE (Particle Induced X-ray Emission) on the cross section of lower jaws from the green lugworm revealed the distribution of Zn inside the apical portion of the lower jaw and the presence of halogens complementary to zinc. To analyze the chemical form of the zinc, the lower jaw was ground and subjected to XRD (X-ray Diffraction) and XAFS (X-ray Absorption Fine Structure). No specific peaks were detected in the XRD pattern of the lower jaw powder, indicating that Zn is present in a noncrystalline form. Comparison of XAFS spectra of the Zn-K absorption edge with several standards suggested that Zn is bound to an imidazole group. HPLC-ICP-MS (High-performance liquid chromatography-inductively coupled plasma mass spectrometry) of lower jaws extraction suggested the presence of organic molecules binding to Zn. To identify the organic molecules that contributes to Zn accumulation, lower jaws were divided into Zn-rich apical and Zn-poor root sides and treated with acetic acid and urea solution. The extracts from the lower jaws were subjected to SDS-PAGE (Poly-Acrylamide Gel Electrophoresis), and a histidine-rich protein Nai11527 was identified in a band specific to the apical side. The imidazole group in the histidine of Nai11527 may contribute to zinc accumulation by binding to Zn in the lower jaw of the green lugworm.

An investigation into chloroxylenol, a popular antimicrobial ingredient in hygiene and disinfection products, in water environments of Hong Kong

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Chloroxylenol, a halogenated phenolic compound, is an antimicrobial ingredient. After it was first reported to be effective in inactivating SARS-CoV-2 virus that causes Coronavirus Disease-2019, the demand for popular brands (e.g., Dettol and Walch) of antibacterial hand sanitizers and household hygiene disinfectants has surged. As Hong Kong is a city with a high population density and a massive use of disinfection products, a significant amount of chloroxylenol may discharge to water environments through drainage, posing toxicological threat to humans and aquatic animal. However, its occurrence in the water environments both in Hong Kong and during the pandemic has not been investigated.

Surface water and wastewater samples were collected with an automated water sampler which were sent to the automatic solid phase extraction system for extraction and liquid chromatography-tandem mass spectrometry for concentration measurements after optimization of parameters. Temporal variations (diurnal, weekly and seasonal) and ecological risk of chloroxylenol were investigated.

In a two-year period, the concentrations of chloroxylenol in Tuen Mun River and Yuen Long Creek ranged from 2.12 to 42.95 μg/L, with the highest concentration detected in Yuen Long Creek. These concentrations were higher than previous studies (in ng/L), suggesting the massive use of chloroxylenol in Hong Kong. Furthermore, more than half of the environmental samples had concentrations higher than 4.20 μg/L (i.e., a concentration causing chronic effects in freshwater fish, involving genotoxicity and histopathology).

As chloroxylenol is known to be stable in water, potential ecotoxicity and health risks induced by chronic exposure cannot be ignored because of its massive use in Hong Kong. The reported concentrations of μg/L indicated the potential risk of aquatic toxicity, showing the importance of public awareness of the environmental consequences from overuse of chloroxylenol-based disinfectants.

Pollutant penetration from poultry farm into groundwater

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Intensive poultry farming helps to meet the ever-increasing demand for animal protein. On the other hand, it can be a source of environmental pollution, thus affecting the health of local residents, farm workers, and consumers. In particular, the use of animal manure for soil fertilization represents a route for the spread of these substances in the environment, including aquatic compartments, due to their persistence, mobility (low sorption resulting in a tendency to remain in aqueous phase) or interaction with solid particles (high sorption causing accumulation in sediments and/or sludge). The objective of this study was to identify pollutants present in groundwater samples, resulting from intensive poultry production. In particular, the possibilities of contaminants penetrating through the soil into groundwater were examined. Additionally, microbiological tests were likewise performed. Apart from limited presence of *Pseudomonas* spp. strains and slight microbiological contamination with fecal flora *(E. coli* and coliform groups), no increased microbiological parameters were found in the tested samples. The analysis of the occurrence of drug-resistant strains did not reveal the presence of Enterobacterales strains resistant to cephalosporins or carbapenems nor of *Enterococcus* spp. resistant to glycopeptides.

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Long-range transport of POPs from East Asia

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We determined the concentrations of airborne organochlorine compounds, including hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane and its metabolites (DDTs), chlordane-related compounds (CHLs), and hexachlorocyclohexanes (HCHs) at Cape Hedo, Japan during November 2007 to April 2008. In general, these compounds are called persistent organic pollutants (POPs). Of the POPs measured in this study, HCB and HCHs were predominant, followed by CHLs, DDTs, and heptachlor. The sum of POPs concentrations ranged from 82 to 164 pg/m^3 in this period. On the other hand, continuous air mercury analysis has been conducted by the Ministry of the Environment, Japan, using Tekran ambient air monitor system, which provides us with mercury concentration data in every 2 hrs at Cape Hedo. Therefore, we compared data between POPs and gaseous elemental mercury (GEM), and calculated correlation coefficients. As a result, the correlation coefficient between the sum of measured POPs and GEM showed 0.51 with a significant difference ($p < 0.05$).

Screening analyses of micropollutants originating from intensive poultry farming in core soils

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Agricultural activities are an important source of organic pollution, especially in animal farming, which is related to the use of a variety of chemicals, including plant protection products, veterinary antibiotics, and fastidious odors, etc. Xenobiotic contamination affects various environmental compartments, including air, water, and living organisms (M. Krupka et al., 2024). Contamination by persistent, bioaccumulative, and mobile compounds in the environment is considered a global environmental problem. Their concentration in the environment depends on the physicochemical properties of soils, the dose of manure fertilized, weather conditions (with the large impact of rainfall), and their susceptibility to degradation. Due to complex factors, the pathway of the environmental fate of contamination is not entirely known; specifically, there is a lack of knowledge about their penetration into the core soil (Gržinić et al., 2023). Thus, research on the xenobiotic presence of core soils is of high importance because of the risk of micropollutant emissions into groundwater.

The aim of this study was to identify chemical contamination in core soil samples originating from intensive poultry farming, focusing on antibiotics, pesticides, and other xenobiotics. These results explain the pathway of the migration of micropollutants in soil and allow us to study the risk of leaking them into groundwater.

G. Gržinić et al., STOTEN 858, 2023, 160014

M. Krupka et al., Agriculture 2024, 14, 87

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Changes in Takifugu rubripes *liver gene expression after oral administration of tetrodotoxin*

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Tetrodotoxin (TTX) is a natural neurotoxin produced by marine bacteria. *Takifugu rubripes*, a commercially important pufferfish species, is known to accumulate TTX particularly in the liver and matured ovaries through the food chain in wild life. However, the biological responses to the TTX accumulation in pufferfish are not well understood. We investigated the responses of non-toxic *T. rubripes* to TTX exposure by administering TTX-containing food (1,125 μg TTX/kg b.w.) and TTX-free food to two groups of juvenile *T. rubripes* (5 fish each, 7-month-old) using oral gavage. Pufferfish livers were collected for mRNA-seq, proteome analysis, and TTX measurement 72 hours post-administration. There were no observed deaths during the exposure period. Total RNA was extracted from pufferfish livers and subjected to mRNA-seq analysis. mRNA-seq results showed 12,727 expressed genes; 29 genes were differentially expressed (FDR<0.05, 28 upregulated; 1 downregulated) in the TTX administrated group compared with the control group. The differentially expressed genes included tumor necrosis factor (TNF) super family member 12 and semaphorin-3F-like, which are involved in apoptosis, suggesting that TTX accumulation induced apoptosis in hepatocytes. On the other hand, no significant changes were observed in the expression levels of Na+ channel-related gene and pufferfish saxitoxin and tetrodotoxin binding protein (PSTBP), which are known to be involved in TTX resistance and transport. We will analyze the relationship between TTX concentration and each gene expression level for further clarifications.

Network-based approach to exploring mechanisms of action for PFAS

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Per- and poly-fluoroalkyl substances (PFAS) are widely used synthetic chemicals. They raise global concerns due to their environmental persistence and links to adverse health outcomes like cancer. However, the targets and action mechanisms of most PFAS remain unclear. Recent findings in network medicine have shown that drugs closely located to each other on the human protein-protein interaction network tend to share chemical, biological, functional, and clinical similarities. Building on this insight, we hypothesized that PFAS closely located to drugs on the human protein-protein interaction network may exhibit similar effects through similar mechanisms of action (MOA).

To test our hypothesis, we analyzed the proximity between 900 drugs and 45 PFAS on the human protein-protein interaction network. We found that perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are closely located to estradiol, an estrogen receptor agonist. Estrogen receptor antagonist activity is one well-known effect of PFOA. Furthermore, antineoplastic agents, such as doxorubicin and arsenic trioxide, are in close proximity to PFOA and PFOS, both of which are known carcinogens. These results suggest that PFAS and drugs in close proximity on the protein-protein interaction network may share some of the same MOAs, although the PFAS and drugs have different effects and outcomes.

These findings provide new insights in MOA of PFAS where toxicity information is limited.

Distribution and bioaccumulation of perfluoroalkyl substances in freshwater ecosystem of Korea

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In this study, we determined the presence of nineteen perfluoroalkyl substances (PFASs) including 17 legacies and 2 alternative substances (GenX and F-53B) in the water, and sediment samples from major rivers of Korea in 2023, namely Nakdong, Namhan, Yeongsan and Geum river. In addition, the PFAS accumulation of 23 freshwater species and its trophic levels (TL) in Yeoju food chain from Namhan river were investigated using bioaccumulation factor (BAF) and biomagnification factor (BMF) values.

In the water samples, legacy PFASs concentrations (ng/L) were in a range of 4.20 to 114.60. Besides, the concentrations (ng/L) of GenX and F-53B were ranged from 0.19~7.48 (mean = 1.21) and ND~0.28 (mean = 0.18), respectively. In the sediment samples, PFASs concentrations (ng/g dw) were detected in low concentrations ranging from 0.26~0.99 (mean = 0.51). In addition, GenX and F-53B concentrations were detected in all sampling sites ranging from ND~0.07 (mean = 0.03) and ND~0.10 (mean = 0.02), respectively. Almost high PFASs concentrations were found in the midstream of Nakdong river located near several possible discharge sources.

The PFAS concentration (ng/g ww) in food chain samples were in the range of 2.24~31.92 (mean = 12.57). Among these species, PFAS concentrations in the predatory carp, and barbel steed species (TL = 3) were higher than those in other species. Besides, the high frequency detection of alternative PFASs were detected. Most BAF values of PFASs were over than the bioaccumulative level (>2000 L/Kg), especially PFOS and PFUnDA, while BAF values of PFAS alternatives was lower than those.

Regarding BMF in predators by feeding zooplankton, the high BMF values of PFOS were found in the predatory carp (7.31), barbel steed (5.56), and *pungtungia herzi* (5.05), while the BMF values of PFOA in all food chain samples were low (approximately 1). Low BMF values of GenX (mean = 1.22) and F-53B (mean = 0.77) were detected in most samples.

Estimation of physiological effects of perfluorooctane sulfonate in Takifugu rubripes

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Perfluorooctane sulfonate (PFOS) is well known fluorinated environmental chemical, and concerned about the physiological effects on organisms. Interestingly, PFOS preferentially accumulates in not only fatty tissue, but also blood of wildlife and humans. Although earlier studies have suggested that this was due to binding of PFOS to a plasma protein, definite characterization of the protein in in vivo exposure studies was not conducted thus far. In this study, we tried to identify binding protein in the blood, estimate tissue distribution and related physiological effects of PFOS in puffer fish. For the studies, PFOS was administered intraperitoneally to tiger puffer fish, *Takifugu rubripes*. After administration, the highest concentration of PFOS was found in the plasma, followed by the mucus, liver, and other tissues of fish. A gradual upward trend in PFOS concentration was observed in the mucus and liver whereas there was no change in the plasma. These results may suggest that mucus is one of the elimination pathways of PFOS in tiger puffer fish. In the results of analysis on plasma protein, the PFOS-binding protein was identified as an apolipoprotein A-I, which has important role on cholesterol metabolism. From these results, we tried to estimate physiological effects of PFOS in fish.

Disrupted seasonal expression of neuropeptide precursor genes in the brain of Reishia clavigera *with abnormal reproduction near Fukushima Daiichi Nuclear Power Plant*

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A marine snail, *Reishia clavigera*, living within 3-Km south to the FDNPP exhibits consecutive gonadal maturation (CSM) that maintains gonadal maturation all the year around, despite of the fact that the normal mating season of this snail is early summer. Given the crucial role of neuropeptides in reproductive regulation, we hypothesized that unknown environmental factor disrupted the neuropeptide system, leading to the CSM. To verify this hypothesis, we conducted RNA-seq analysis on CNS of the normal and CSM snails in mating season and non-mating season, respectively, and succeeded to constitute more than 60,000 of transcript models including more than 90 kinds of neuropeptide precursors, computationally, without the genome sequence data. Interestingly, expression levels of neuropeptide precursor genes in CSM snails were concomitantly lower than those in normal snails in non-mating season, while those in CSM snails was comparable to the normal one in mating season, suggesting that expression of neuropeptide precursor has seasonal change. When the expression levels of 25 kinds of neuropeptide precursors were determined by quantitative PCR every two-months, expression levels of some precursors such as FLRFamide and whitnin in CNS of male snail were peaked in August and October, while those of female snail were peaked in December. The seasonal changes of a set of neuropeptide precursors seemed negatively correlated to the maturation of gonads. Notably, CSM snails exhibited reduced peak levels of neuropeptide precursors, yet maintained higher gonadal maturation than normal snails. These raise the possibility that CSM snails maintain gonadal maturation during the non-mating season due to insufficient levels of neuropeptides that typically suppress this process. Our data provide a molecular basis for monitoring environmental factors that influence gonadal maturation in this marine snail by quantifying the expression of specific neuropeptide precursor genes.

Alexandrium catenella *(Group I) causes higher and faster toxicity than* A. pacificum *(Group IV) in* Mytilus eduis

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Consumption of poisoned shellfish poses serious health risks, including the potential for severe health problems and even death. In Korea, *Alexandrium catenella* (Group I) and *A. pacificum* (Group IV) are known to cause paralytic shellfish poisoning (PSP), with an increasing prevalence observed in a broader geographical area. Despite these findings, the association between toxic dinoflagellates and shellfish poisoning remains unclear. This study aimed to investigate the toxicity, detoxification, and compositional changes in PSP within *Mytilus edulis* exposed to *A. catenella* and *A. pacificum*-induced PSP. High-performance liquid chromatography with post-column oxidation was employed to analyze PSP toxicity in poisoned *M. edulis*. Results revealed that PSP in *M. edulis* increased with the cell density of *A. catenella* and *A. pacificum*. Interestingly, *A. catenella* exhibited a faster peak cell density than *A. pacificum*, leading to a higher level of toxicity. In detoxification experiments, PSP in *M. edulis* rapidly decreased within 24 hours in filtered seawater. However, continuous detection persisted without reaching below the detection limit until the experiment's conclusion. Furthermore, carbamate composition (GTX1+4) emerged as the primary toxic component in poisoned *M. edulis*, diverging from the composition in vegetative cells. This finding suggests that GTX1+4 can swiftly poison shellfish when toxic dinoflagellates are present in the marine environment. Despite this, complete detoxification of poisoned shellfish is a time-consuming process, and continuous exposure to poisonous dinoflagellates may lead to a rapid increase in toxicity due to biotransformation. These results contribute valuable insights into the mechanisms of shellfish toxicity and detoxification following PSP induced by toxic dinoflagellates, aiding in the identification of potential health risks associated with shellfish consumption in affected regions.

Growth interactions between the harmful dinoflagellates Heterocapsa circularisquama *and the diatom* Skeletonema dohrnii

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Harmful dinoflagellate *Heterocapsa circularisquama* has caused significant fisheries damage since it was first identified in Uranouchi Bay, Kochi Prefecture, in 1988. This species has spread along the coast of western Japan, causing mass mortality of bivalves, such as cultured Akoya oysters and Pacific oysters. While it has been suggested that the growth of *H. circularisquama* is inhibited by diatoms, there are few reports on these competitive relationships. Therefore, we investigated the involvement of allelopathy (actions through extracellular exudates) and cell-to-cell contact in the competitive relationship between *H. circularisquama* and the diatom *Skeletonema dohrnii* under bialgal culture conditions. First, we conducted bi-algal culture experiments with combinations of various initial cell densities, which resulted in higher density species suppressing the growth of lower density species. The growth of *H. circularisquama* was strongly inhibited by *S. dohrnii* at all initial cell density combinations, whereas the growth of *S. dohrnii* was slightly suppressed by *H. circularisquama*. In addition, we found that *H. circularisquama* with *S. dohrnii* were more likely to form round shape cell. The culture filtrates of *S. dohrnii* did not suppress the growth of *H. circularisquama*, indicating no allelopathic effect. However, the growth of *H. circularisquama* in bi-algal cultures under contact conditions was significantly lower than the mono-algal and noncontact bi-algal cultures. These results suggest that cell-to-cell contact with *S. dohrnii* strongly inhibits the growth of the growth of *H. circularisquama*.

Growth interactions between the dinoflagellate Karenia selliformis *and the diatom* Chaetoceros diadema

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A large-scale red tide by *Karenia selliformis* along the western coast of Hokkaido, Japan emerged in the autumn of 2021, severely affecting local fisheries. Field monitoring studies have suspected that the bloom formation of *K. selliformis* was linked to competitive interactions with other algal species, especially diatoms. Therefore, the present study investigated the growth competitive relationship between *K. selliformis* and a species of diatoms, *Chaetoceros diadema*, isolated from the eastern coastal region of Hokkaido. We conducted a bi-algal culture experiment with varying initial cell densities (200 cells ml^{-1} or 2000 cells ml^{-1}) for both species under the following conditions: 15°C temperature, 150 μmol m^{-2} s^{-1} light intensity, and a 14h:10h light-dark cycle. The results showed the pivotal role of initial cell concentration in determination of competitive outcomes, with higher algal concentrations exerting inhibitory effects on lower concentrations. *K. selliformis* consistently outcompeted *C. diadema* at the same initial concentration, indicating competitive advantages in *K. selliformis*. *K. selliformis* filtrate also demonstrated potent growth inhibition of *C. diadema* whereas *C. diadema* filtrate did not inhibit the growth of *K. selliformis.* In a follow up non-contact experiment using a 6-well plate with cell culture insert (pore size 3 μm), we observed that the suppressive effect of *K. selliformis* on *C. diadema*, or vice versa, was significantly weaker compared with co-cultures under the contact conditions. These findings indicate that the allelopathic effect of *K. selliformis* on *C. diadema* observed in culture filtrate and the cell contact enhanced the growth suppression effect. These growth interactions may be involved with the HAB formation of *K. selliformis*.

Effects of Karenia selliformis *exposure on the sea anemone* Actinia equina

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The dinoflagellate *Karenia selliformis* is a harmful algal bloom (HAB) species that caused massive red tide exceeding 1,000 cells/ml in Pacific coast of Hokkaido in autumn, 2021, leading to mass mortality of marine organisms including fish, bivalves, and sea anemones and so on. However, there is very inadequate knowledge on the susceptibility of each organism to *K. selliformis*. In this study, *Actinia equina*, a species of sea anemone inhabiting rocky shores from Hokkaido to Okinawa in Japan was selected to investigate the effects of *K. selliformis* on sea anemone. *A. equina* (1.9 ± 0.8 g) were assigned to the exposure and control groups (4 individuals each). The exposure group was exposed to *K. selliformis* at about 3000 cells/ml as initial cell density for 72 hours after which they were moved to clean artificial sea water. The conditions of *A. equina* in the exposure and control groups were observed and recorded during and after the exposure. Body weights of *A. equina* were recorded on day 0, 3, 4. Behavior of *K. selliformis* was recorded for the first 2 hours of exposure and observations of appearance changes were taken at days 2, 3, 4, 5, 6, 7, 14. *A. equina* were continuously observed for another 15 days to further observe their survivability. As a result, tentacles of *A. equina* in the exposure group were retracted into their bodies and the defensive behaviors against *K. selliformis* continued until the end of exposure. In the control group, the body weights of *A. equina* after 72 hours ranged from 78% to 203% of their initial weights at the start of the experiment. In contrast, the body weights of the exposure group significantly decreased, ranging from 25% to 54% of their initial weights. In addition, two out of four individuals in the exposure group died within one month and all individuals in the control group survived. These results revealed that exposure to bloom level of *K. selliformis* causes behavioral abnormality, growth inhibition and death in *A. equina*.

Accumulation and excretion of ^{131}I and ^{134}Cs in the rock shell, **Reishia clavigera**, *exposed in a simulating manner of a nuclear power plant accident: toward the dose estimation*

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In 2012, after the accident at the Fukushima Daiichi Nuclear Power Plant (FDNPP) that followed the Tohoku earthquake and tsunami in March 2011, the number of intertidal species decreased significantly with decreasing distance from the power plant and no rock shell (*Reishia clavigera*; Gastropoda) specimens were found near the plant from Hirono to Futaba Beach (a distance of approximately 30 km). The collection of rock shell specimens at many other sites hit by the tsunami suggests that the absence of rock shells around the plant in 2012 might have been caused by the nuclear accident in 2011. To verify this working hypothesis, we have conducted laboratory exposure experiments of radionuclides (i.e., ^{137}Cs and ^{90}Sr), using the rock shells, for several times. Here, we will report results on exposure experiments of ^{131}I and ^{134}Cs, using the rock shells, conducted in a simulating manner of a nuclear power plant accident. Average concentrations (± standard deviations) of ^{131}I and ^{134}Cs in seawater in exposure groups during the experiments were 2.58 ± 0.68 MBq/L and 2.88 ± 0.33 MBq/L, respectively. Average concentrations of ^{131}I and ^{134}Cs in whole bodies of the rock shells in exposure groups at the end of exposure were 124,213.72 Bq/g (= 124.21 MBq/kg) and 5,530.13 Bq/g (= 5.53 MBq/kg), respectively. Thus, concentration factors of ^{131}I and ^{134}Cs in the rock shell were estimated to be 48 and 2, respectively. Interestingly, marked concentrations of ^{131}I ((144.34 Bq/g (in average) and 2431.80 Bq/g (in average)) were observed in outside of shell and operculum, respectively, compared with those (174.05 Bq/g (in average)) in whole bodies of the rock shells in exposure groups even after 3 months of the experiments. We also tried to directly estimate doses of the rock shells exposed to ^{134}Cs, using grass rod dosimeter (1.5mm φ and 12mm in length): absorbed dose in the rock shell was estimated to be 217.05 mGy at 30 days of exposure.

Sperm epigenome as an indicator of modified offspring brain development

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DNA methylation has the potential to serve as molecular marker for intergenerational inheritance of modified offspring development extending the developmental onset of health and disease (DOHaD) hypothesis towards the pre-conceptional parental environment. The ubiquitous environmental pollutant Benzo[a]pyrene (BaP) reduces DNA methyltransferase activity in vertebrate models and affects offspring methylation profiles. Sperm harbors a repertoire of methylation markers that are associated with different physiological functions, especially neurobehavioral activity. Sperm-borne differential DNA methylation in genes that regulate the development and function of the CNS in offspring has been demonstrated in fish and mammals. Comparing sperm bisulfite sequencing data from adult male and gene expression, acetylcholinesterase activity and swimming behavior in larval offspring upon parental BaP exposure for 21 days (1µg/L) revealed modified methylation in pathways associated with neuronal development and function (netrin signaling; synaptogenesis pathway). Rho guanine nucleotide exchange factor 7, neuroligin 2 and adenylate cyclase 8 were found hypomethylated in the offspring sperm and exhibited reduced gene expression in the larvae, and thus, are possibly responsible for an increased anxiety behavior identified in larval and adult offspring. The data presented here provide a potential connection between parental BaP exposure, neurodevelopmental impacts, and the risk of behavioral and psychiatric disorders in vertebrates over generations.

Intervention trial for pharmacokinetic study of chemicals in daily use products

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To investigate the pharmacokinetics of the chemicals in the daily use products, we conducted an intervention trial with controlled diet and use of personal care products. This approach allows us to obtain pharmacokinetic parameters without administering chemicals to the participants. Study participants were requested to consume foods and personal care products that we provided for 5 days, and blood and urine were collected before, during and after the intervention period. Biological half-lives were calculated using temporal variation of excretion rates of some chemicals. Some chemicals such as parabens, triclosan, neonicotinoids showed decreasing trends in urinary excretion rates during the intervention period. Elimination half-lives were estimated for chemicals showed decreasing trends, and results were in the same range of the previous reports. This study design may provide the essential information on pharmacokinetics of chemicals that can be utilized for the risk assessment.

*Veterinary antibiotics as an environmental threat for forage crops: tetracycline (TC) and ciprofloxacin (CIP) impact on oilseed rape (*Brassica napus*)*

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Antibiotics have received growing attention in recent years as emerging aquatic and terrestrial contaminants. The use of animal manure and slurry for soil fertilization in agriculture represents a major route for the spread of veterinary antibiotics in the environment. Due to their broad spectrum of activity, tetracyclines and fluoroquinolones are widely used to treat various bacterial infections in the veterinary raising concern about their wide distribution in the environment. Therefore, the residues of veterinary antibiotics in soil are a worldwide problem of increasing concern. However, the knowledge about their phytotoxicity and the mode of action is still limited. The study was aimed to analyze tetracycline (TC) and ciprofloxacin (CIP) toxicity to oilseed rape (*Brassica napus*) with the focus on morphological, biochemical, and physiological effects. Plants were grown in the soil amended with 1 – 500 mg kg^{-1} of TC and CIP, representing the range from environmentally relevant to high antibiotic concentration. Environmentally relevant TC and CIP concentrations had no significant adverse effect on most parameters. CIP was found to be more toxic than TC, especially when applied at high concentrations. The highest CIP concentrations induced complete *B. napus* growth retardation caused by oxidative burst, impaired photosynthetic performance and collapse of antioxidative protection.

Evaluating the actions of nitrate, nitrite and ammonia using salmonid epithelial cell lines

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An ecotoxicological problem of current concern is evaluating the impact on fish, of ammonia (NH_3), nitrite (NO_2^-) and nitrate (NO_3^-), which are reactive nitrogen compounds (Nr's) whose deposition into the aquatic environment is being increased by human activities. Fish cell lines are increasingly being used to study aquatic toxicants especially to elucidate mechanisms of toxicity and toxic potencies but to the best of our knowledge, they have yet to be applied to Nr's. In addition to being ecotoxicants, Nr's are involved in cellular metabolism and signalling. These roles have been intensively investigated for NH_3 and NO_2^- in mammalian cells but less is known about their involvement in other vertebrates. Few alternative methods (i.e., not using animals) have been approved for ecotoxicological purposes, and RTgill-W1 has recently been approved as the only cell line as animal alternatives for use of effluent testing and chemical registration by the Organization for Economic Co-operation and Development as OECD test 249. As well, the International Standards Organization has approved the use of RTgill-W1 as a standard for water quality testing, ISO 21115:2019. Therefore, RTgill-W1 along with another gill cell line, ASG-10 derived from Atlantic salmon gills, and two other rainbow trout epithelial cell lines, RTgutGC and RTolf-UFV1, were exposed in L15 medium with fetal bovine serum to $NaNO_3$, $NaNO_2$, and NH_4Cl. The cells were assessed for their ability to maintain basic epithelial cell functions: energy metabolism with Alamar Blue (AB), adherence and wound healing. The toxic ranking of these three Nr's *in vitro* at the cellular level is overall similar as those observed *in vivo* with fish. Knowing the tolerance range of salmonid cells for NH_3, NO_2^- and NO_3^- should better allow the use of diverse fish cell lines as tools to explore the physiological roles and toxic mechanisms of these compounds in epithelial membranes of commercially important fish such as rainbow trout and Atlantic salmon.

Synergistic adverse effects of microfibers and freshwater acidification on host-microbiota interactions in the water flea Daphnia magna

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Microfibers are the most common type of microplastics in freshwater environments. Anthropogenic climate stressors, such as freshwater acidification (FA), can interact with plastic pollution to disrupt freshwater ecosystems. However, the underlying mechanisms responsible for the interactive effects of microfibers and FA on aquatic organisms remain poorly understood. In this study, we investigated individual *Daphnia magna*–microbiota interactions affected by interactions between microfibers and FA (MFA). We found that the accumulated amount of microfibers in pH-treatment groups was significantly higher than in the control groups, resulting in negative consequences on reproduction, growth, and sex ratio. We also observed that MFA interactions induced immunity- and reproduction-related biological processes. In particular, the abundance of pathogenic bacteria increased only in MFA groups, indicating that MFA interactions can cause intestinal damage. Our integrated analysis of microbiomes and host transcriptomes revealed that synergistic adverse effects of MFAs are closely related to changes in microbial communities, suggesting that *D. magna* fitness and the microbial community are causally linked. These finding may help elucidate the toxicity mechanisms governing the responses of *D. magna* to microfibers and acidification interactions, and to host-microbiome-environment interactions.

*No vector effect of polystyrene microplastics on the accumulation of chlorobenzenes in Japanese Medaka (*Oryzias latipes*)*

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The coexistence of microplastics and hydrophobic organic chemicals is widespread in the environment. Microplastics can act as vectors for transmitting organic pollutants to organisms. This study investigated whether polystyrene microplastics (PSMP) impact fish bioaccumulation of chlorobenzenes (CBs). We co-exposed adult medaka fish to CBs and two sizes of PSMP. As a result, there was no increase in the concentration of CBs in the medaka co-exposed to MPs or PSMP sizes. This result might have been due to the strong sorption of CBs to PSMP and little or no desorption of CBs in the gut. Our study revealed no vector effects of PSMP on the bioaccumulation of CBs in medaka. Our results suggest that MPs in the environment accumulate CBs, thereby reducing the concentrations of CBs in the aqueous phase and inhibiting the accumulation of CBs in organisms. Further studies with different chemicals and concentrations of MPs should be performed to investigate the vector effect further. This study was supported by the Long Range Initiative (LRI, 22-5-03), Grants-in-Aid for Scientific Research (JSPS KAKENHI Grant Numbers JP21H05058, JP22H03760), and by the Environmental Research and Technology Development Fund of the Ministry of the Environment of Japan (JPMEERF20231001).

Microplastics as a disturbance to food web dynamics in Texas Gulf coastal bays

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Studies have improved our understanding of how aquatic organisms' uptake microplastics (MPs), but there is a lack of information on their transfer through the food chain. To address this gap, a study combining field observations and lab experiments on ingestion is needed. This research will focus on the impact of MPs in the marine food web of Matagorda Bay in the Gulf of Mexico. By studying the abundance, composition, and distribution of MPs in this ecosystem and conducting feeding experiments on a two-level trophic chain from copepod to jellyfish, critical information on microplastic retention and its effects on fecundity, behavior, and growth will be provided. Copepods will be sampled *in situ* and examined for the microplastic body burden. MPs obtained from the field will be used in the lab-feeding experiments with copepod nauplii and jellyfish ephyra. It is expected that the consumption of MP contaminated copepods will negatively impact jellyfish development, with reduced pulse-perminute rates and survival indicating physiological stress. Additionally, MP-fed copepods are likely to experience reduced growth, reproductive output, or impaired physiological functions. Initial findings revealed MP concentrations ranging from 1,591 to 15,275 MPs per liter across the 7 sampled bays. The three highest MP/L concentrations were observed in bays influenced by freshwater input. The initial analysis demonstrated that adult copepods exhibit a higher MP body burden compared to nauplii stages. Adult copepods average 4.0 MPs per individual, while nauplii average 1.7 MPs per individual. Additionally, the copepods from sites with freshwater input recorded the highest MP body burden. This data will inform future policies to reduce microplastic pollution in the marine environment and protect aquatic resources.

Investigating the effects of virgin or recycled polypropylene microplastics on fish growth

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This study investigated the effects of virgin and recycled polypropylene (PP) microplastics (MPs) on fish growth, focusing on potential differences in impact and threshold levels based on contaminated chemicals and particle size.

In vitro bioassays were conducted to measure the cytotoxicity and agonist activity of some receptors in extracts of virgin and recycled PP pellets. The pellets were then cryogenically ground and sieved through 300 µm and 106 µm meshes to obtain MPs with two different size distributions (<106 µm, 106–300 µm). Fish exposure tests were conducted by adding MP into the diet and feeding the fish for four weeks. During the experiment, the total weight of fish in each tank was measured weekly and the feeding amount was recalculated. In Test 1 and Test 2, 10% of each size of recycled or virgin MP was added to the diet, respectively. In Test 3, <106 µm virgin PP was added at 2.1 or 4.5% to the diet. In each test, the MP-contaminated diets were fed at 3% of fish body weight per day. At the end of the experiment, the body length and weight of each individual were measured, and blood samples were collected. Total blood cell count, hemoglobin concentration, and hematocrit values were measured.

The extracts from recycled PP showed relatively high agonist activity for receptors such as AhR and PXR, although cytotoxicity was low. In contrast, no biological activity was detected in virgin PP. When fish were exposed to these MPs, both virgin and recycled PP at 10% in the diet for the <106 µm size significantly reduced specific growth rate. For 106–300 µm MP exposure, these effects were weaker for virgin PP and not observed for recycled PP. Further exposure to lower concentrations of <106 µm virgin PP revealed a LOEL and NOEL for fish growth inhibition of 1.4 and 0.62 mg/g-b.w./day, respectively. No hematological effects were observed in any of the tests.

Microplastic ingestion in the anemonefish Amphiprion ocellaris*: Influence of concentration, odor, and light*

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This study investigated the ingestion of microplastics by false clown anemonefish *Amphiprion ocellaris*, focusing on their behavioral responses under various conditions. Utilizing polyethylene terephthalate (PET) microplastics, we assessed the effects of food presence, microplastic concentration, and environmental factors such as odor and light on the anemonefish's behavior and microplastic ingestion. Results indicated that anemonefish ingested more microplastics when food was present, with a plateau in ingestion at higher concentrations. Behavior and ingestion were significantly influenced by light conditions, with brighter environments leading to higher ingestion rates, while biofilm odor had no significant effect. This research highlights the complex interplay between environmental factors and microplastic ingestion, emphasizing the need for further exploration in this field.

The combined effect of organophosphate pesticide chlorpyrifos and microplastics on medaka behavior

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Microplastic (MP) pollution in aquatic ecosystems is a worldwide problem. At the same time, exposure to organophosphorus pesticide chlorpyrifos (Cpf) are known to inhibit AChE activity in the medaka (*Oryzias latipes*) brain, affecting medaka swimming and social behavior (Qiu et al., 2017; Khalil et al., 2017). We evaluated the combined effect of Cpf and polystyrene MP (2-µm diameter) on the predator avoidance behavior of medaka by using the Peek-A-Boo test (Takai et al., 2023) with six treatment groups: 1.25 µg/L Cpf (LCpf group), 5.0 µg/L Cpf (HCpf group), 40 µg/L fluorescent MPs (MP group), 1.25 µg/L Cpf with 40 µg/L MP (LCpf+MP group), and 5.0 µg/L Cpf with 40 µg/L MP (HCpf+MP group). After 96 hrs of exposure, one individual from each group was placed in a 19 cm × 19 cm tank for 10 minutes of behavior recording. A photo of the predator fish (donko, *Odontobutis obscura*) was revealed to the test medaka (n=19) after five minutes of recording. The area within 6 cm × 6 cm around the predator photo was defined as the "donko area." The time taken to enter this area for the first time after the photo display was measured as an indicator of predator avoidance behavior. After the behavior test, medaka brain, gastrointestinal (GI) tract, and muscle were dissected to measure brain AChE activity, MP weight in the GI tract, and Cpf concentration in the muscle, respectively. Statistical analysis was performed using the Steel-Dwass test. AChE activity was expressed as µmol/min/mg-protein. AChE activity of the LCpf group (0.053) and HCpf group (0.022) were significantly lower than the control group (0.075; $p<0.05$). HCpf group AChE activity was also significantly lower than the LCpf group. However, AChE activity of the HCpf+MP group (0.044) was significantly higher than the HCpf group (0.022), indicating that MP suppressed the chlorpyrifos AChE activity inhibition. We will discuss these results in relation to the Cpf body concentration and Peek-A-Boo test results.

Adsorption of polycyclic aromatic hydrocarbons on microplastics and their desorption in simulated intestinal fluids

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Microplastics (MPs, < 5 mm in size) are emerging pollutants which co-exists with contaminants such as polycyclic aromatic hydrocarbons (PAHs) in aquatic environments. MPs tend to be hydrophobic and may absorb hydrophobic chemicals. Previous studies have discussed the acceleration of pollutant accumulation via MP (vector effect) using anthracene (Ant) (Qiu et al, 2020; Takai et al, 2023a,b). The vector effect might be attributed to the desorption of Ant from MP when ingested by fish. To understand the mechanism of the vector effect, we performed an adsorption test of PAHs to MPs under solvent and solvent-free conditions along with an in vitro desorption test using simulated intestinal fluid (SIF) containing taurocholic acid, which is a primary component of bile fluids. Spherical polystyrene MP (10 μm, PS-MP), naphthalene (Nap), Ant, and chrysene (Chr) were used in this study. We utilized the passive dosing (PD) method in the adsorption test; PS-MPs were incubated with PAHs-adsorbed silicone O-ring and the adsorption rate of PAHs was evaluated. Our results observed that the adsorption rate was measured from highest to lowest for Chr, Ant, and Nap, respectively, indicating that higher PAH hydrophobicity leads to higher adsorption rates. Ant-adsorbed-PS-MPs from the PD method were incubated in SIF (500 mM phosphate buffer pH 8.0 with 5% sodium taurocholate) for one day and desorption of Ant was evaluated. As a result, the amount of Ant desorbed from the PS-MPs in SIF was 10-fold higher than that of the control (SIF without sodium taurocholate). This might be due to the surfactant action of taurocholic acid enhancing desorption from PS-MPs. Our results suggest the vector effect mechanism here involves the bile surfactant action, which promotes desorption in the digestive tract. Further investigation of desorption conditions is needed to confirm this vector effect contribution.

*Uptake and depuration kinetics of aged polyethylene microplastics in Java medaka (*Oryzias javanicus*) through quantification of actual plastic concentration using µFT-IR*

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Microplastic (MP) pollution is a significant global challenge. Marine species in estuarine habitats, such as the Javanese medaka (*Oryzias javanicus*), are at high risk of ingesting these particles, negatively affecting their survival and health. Our previous study detailed polystyrene and polyethylene microbeads' uptake and depuration kinetics in Japanese medaka (Liu et al., 2021). However, bead-shaped microplastics are not commonly found in the environment. This study aims to elucidate the uptake and depuration kinetics of aged fragment polyethylene microplastic (afPEMP), one of the most prevalent forms of MPs in the environment, using Java medaka.

We conducted a meticulous experiment, exposing adult Java medaka (n=50) to 30 µm afPEMP at a concentration of 0.01 mg/L in 10 liters of 3.5% artificial seawater over a period of seven days, with water changes every two days. We collected water samples at the start, before, and after each water change, and at the end of the exposure period for quantification of afPEMP particles using fluorescence microscopy. Post-exposure, the Java medaka were transferred into clean artificial seawater for a three-day depuration period with daily water changes. During this period, we sampled medaka intestines and body tissues at intervals (0h, 3h, 12h, 24h, 48h, 72h; n=5) for residual afPEMP quantification.

Samples were immersed in hydrogen peroxide to dissolve over seven days, then filtered through 5 µm PTFE filters. The filtered contents were imaged using micro-Fourier Transform Infrared (µFT-IR, Nicolet iN10, ThermoFisher) spectroscopy, and the amount of afPEMP was quantitatively assessed using ImageJ software. We will present the detailed results and further discuss afPEMP accumulation in Java medaka. This study enhances our understanding of MPs and their impact on marine life and thus adds to future conservation and remediation strategies with emphasis on the adaptability of Java medaka.

Evaluations of effects in marine amphipod, Ptilohyale barbicornis, *absorbed microplastics through feeding*

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Plastics has been essential materials in our life due to cheap and facilities, and used all over the world. However, 4.8–12.7 million tons of used plastics were introduced in the ocean's environments and an estimated over their 5 trillion pieces are floating in the world's oceans. Larger plastic debris can become small fragments by photodegradation and sea wave, and possibly settled on marine sediments. Several toxic effects of those microplastics with small size to marine organisms have been reported. *Ptilohyale barbicornis*, ovoviviparous marine amphipods, widely distributes on sediments in Japanese coastal areas. Because this amphipod feed the carrions and detritus settled on sediments, and is also a prey species for upper animals in the food chain, it's an important species to maintain the biodiversity in coastal benthic environment. The microplastics remained in high concentrations in sediments could have adverse effects, and their risk information including toxicities induced by their dietary exposures is not enough yet. In the present study, we prepared the feeds spiked 1-25% of microplastics (g/g), had continuously exposed those to *P. barbicornis* within 48 hours of birth for 14 days, and evaluated the exposure effects. We obtained each two sizes of new and recycle plastics with below 106 and 106-300 μm, respectively, and estimated the individual effects.

Although new plastics with 106-300 μm reduced about 20% growth compared with control group, there were not clear concentration dependent. However, recycle plastics significantly reduced the growth rate, and the effects showed depending on concentrations in feeds within range of 1 to 25%. Additionally, new plastics with below 106 μm significantly reduced the growth rates within 0.2-25%, and, similarly, recycle plastics with same size, 1-25%. Those plastics couldn't kill amphipod during exposure periods for 2 weeks. As a result, we concluded the microplastics might slightly affect to amphipods in the coastal areas.

Inductions of chondrodysplasia in fish larvae hatched from embryos exposed oxygenated polycyclic aromatic hydrocarbons

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Oxygenated polycyclic aromatic hydrocarbons (oxyPAHs) are a group that contains one or a few oxygens bonded on benzene rings with double bonds. They are discharged from automobile exhaust gas directly to atmosphere, and generated by the photoreactions of polycyclic aromatic hydrocarbons (PAHs), while they are as metabolites of PAHs in the invertebrates. OxyPAHs widely distribute in the field, and sometimes detected even in aquatic environment, but information of their toxicities to aquatic organisms has been limited. In our previous study, we showed their toxicities in embryos exposed to oxyPAHs (Kawano et al., 2017), such as embryonic lethality and abnormalities including tuber heart, pericardial edema, altered axial curvature, and incomplete development of the cephalic region and palate. As a result, oxyPAHs possibly affect the skeletal development in fish embryos. In the present study, we focused the abnormality especially for chondrogenesis, and examine the effects to cartilage developments in the hatched larvae from Japanese medaka embryos exposed to individual oxyPAHs.

We exposed acenaphthenequinone (ANQ), 1,2-benzanthraquinone (BAQ), 1,4-naphthoquinone (NAQ), and 9,10-phenanthrenequinone (PHQ) to Japanese medaka embryos just before hatching, and stained the cartilage of the larvae. Their exposures resulted some chondrodysplasia in the larvae, and, especially, several individuals lacking Meckel cartilage, should form the underjaw, and cartilage in the caudal fin were observed. Additionally, basihyal, ceratohyal, palatoquadrate, and ceratobranchial were sometimes malformed in larvae. Those shortages were caused in embryos exposed to target oxyPAHs as ANQ, BAQ, NAQ, and PHQ. Those affected individuals were remarkably decreased hydroxy proline in collagen, which is main component in cartilage. Therefore, the lacks of cartilages in larvae were suggested deeply to relate decreasing of hydroxy proline caused by exposure of oxyPAHs.

Molecular toxicological characterization of the novel AHR2 in cats: comparative insights with AHR1

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The aryl hydrocarbon receptor (AHR) plays an important role in mediating a variety of physiological processes, including immune regulation through endogenous ligands as well as mediating toxic responses such as carcinogenesis triggered by dioxin-like compounds. In mammals, AHR1 is universally conserved, whereas AHR2 is conserved only in certain species, such as cats. The functional significance of AHR2 in species such as cats remains largely unexplored, representing a critical gap in our understanding of environmental toxicology. The purpose of this study is to confirm the functional and toxicological characteristics of cat AHR1/2. First, we aligned the sequences of cat AHR1 and AHR2, observing a 43% similarity, and confirmed that AHR2 lacked a Q-rich region in the transactivation domain, unlike AHR1. These findings suggest that the responsiveness and functionality of cat AHR1 and AHR2 to environmental pollutants may differ. Next step, we examined the tissue-specific expression levels and ligand-binding potential of AHR2 to understand its molecular characteristics. In both cat liver tissue and kidney cell lines, AHR1 was predominantly expressed at more than 99%. Also, both cat AHR1 and AHR2 can bind to DLCs and endogenous ligands to regulate transcriptional activity. However, AHR1 exhibited more sensitivity to PCDDs, while AHR2 showed slightly higher sensitivity to PCDFs. Additionally, it was observed that changing the key amino acid at 326 from isoleucine to valine in AHR2 resulted in decreased sensitivity to TCDD. These findings indicate that ligand sensitivity varies depending on the AHR isoform and that residue 326 in AHR2 can regulate ligand sensitivity. These results suggest that while AHR2 in cats retains ligand-binding functionality, its functional significance may be declining due to its low expression levels within tissues. This study may contribute to enhancing understanding of toxicology by elucidating the molecular properties of mammalian AHR1/2.

Stress reduction with co-culture of Miscanthus x giganteus *and* Pelargonium x hortorum *in a pb-contaminated soil to improve biomass production*

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The industrial past of most regions in Lorraine increased the number of polluted sites. In this study, two plants: *Miscanthus x giganteus* and *Pelargonium x hortorum* were used to clean up soil mainly contaminated by lead (Pb). At the end of the experiment, Pb concentrations were measured in the soil and plants to know if plants chosen can induce a toxicity decrease. Furthermore, phytohormones such as auxins, abscissid acid, jasmonates and salicylates were also measured to evaluate the defense mechanisms of the selected plants in front of Pb concentrations. Results obtained showed that both plants are able to develop despite contamination by Pb and also reduce Pb concentrations in soil. Concerning the molecules synthetized by the plants under stress conditions, only salicin was found in *MxG* roots and aerial parts, especially for plants grown in individual culture. These results may be linked to Pb contamination and *PxH* which increases competition for nutrients. But Results showed also that co-culture was beneficial to the development of *MxG*. Indeed, Pb accumulation by *PxH* could reduce the stress induced on young rhizomes and their implantation could be facilitated.

Avoidance behavior of fish to anesthetic agent MS-222 in euthanasia treatment

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In fish ecotoxicity testing, euthanasia of surviving fish is required after the end of exposure to relieve of pain. An overdose of anesthetic is a simple method of euthanasia, and lethal via sedation is considered desirable. However, the information of fish euthanasia is insufficient. In this study, behavioral responses to the anesthetic agent MS-222 were confirmed in medaka (*Oryzias latipes*), zebrafish (*Danio rerio*), carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*), which are fish species recommended in ecotoxicity testing. As a results, avoidance behavior was observed in several fish species.

The number of avoidance behavior (hyperactive) increased more than 600 mg/L in medaka (juvenile and adult stage) and 400 mg/L in zebrafish. On the other hand, no change in the number of avoidance behaviors were observed in carp and rainbow trout. Tracking analysis was conducted using behavioral videos of medaka (adult stage), and total swimming distance during the 10 sec after the start of immersion were calculated. As a results, the total swimming distance increased more than 600 mg/L, consistent with the effect concentrations in the behavior test. These results revealed that several fish species show avoidance behavior when MS-222 concentration exceed a fixed value. Furthermore, it is suggested that euthanasia treatment with excessively high concentrations of MS-222 is unsuitable from an animal welfare perspective because it caused avoidance behavior and was not via sedation.

Behavioral diversity and sample size optimization in medaka fish behavioral tests

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Even without direct lethality from environmental pollutants, behavioral changes can lead to "ecological death," reducing individual or population numbers (Scott and Sloman, 2004). Behavioral tests using aquatic organisms, primarily fish, have recently gained attention as evaluation methods for waterborne pollutants. However, behavioral test results are often seen as less reproducible, due to reflections of individual behavioral characteristics. While previous studies, including ours, have increased the number of observed individuals to address this, no statistically optimal sample size has been determined. Our study aimed to statistically verify the optimal sample size for behavioral tests in medaka (*Oryzias latipes*) by considering individual behavioral diversity. We conducted behavioral tests on 121 medaka fish (68 males, 53 females), namely the Open Field test, Peek-A-Boo test (Takai et al., 2023), Sociability test (Takai et al., 2022), and Color Preference test, using a glass tank (19 cm × 19 cm × 19 cm). In the Peek-A-Boo test, the median time, the time taken for test fish to approach the predator fish picture was 102 s (mean 100.5 ± 59.7 s, relative standard deviation 59.4%). Random sampling indicated that observing 22 or more test fish resulted in a mean and median change rate within 10%, with a relative standard deviation around 60%; observing 22 or more individuals in Peek-A-Boo tests yields reproducible results reflecting individual behavioral characteristics. This study concludes that while behavioral tests, especially the Peek-A-Boo test, can provide reproducible results with 22 or more test fish, this number is not ideal from an animal welfare perspective. Future behavioral tests should aim to develop versatile methods that accurately assess behavioral effects with fewer organisms, balancing the utility of behavioral indicators for toxicity evaluation with animal welfare considerations.

P-54

Effects of elevated temperature and decreased water pH on tetracycline toxicity to Lemna minor

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Vytautas Magnus University, Lithuania

Because of persistence in the environment and wide use for human and animal applications antibiotics are recognized as an emerging class of environmental contaminants. Effluents are the major pathway of antibiotic entrance into aquatic environment. Therefore, the occurrence of antibiotics in surface waters is of high concern, while no data are available on antibiotic toxicity changes to aquatic organisms under global climate change. The aim of the study was to evaluate the influence of elevated temperature and decreased water pH on tetracycline toxicity to *Lemna minor*. *L. minor* were exposed to tetracycline (TC, 1-500 µg L-1) for 7 days and response was assessed though the effects on the growth rate, biomass, secondary metabolites and antioxidant enzymes. TC reduced *L. minor* growth rate, frond area, affected biomass production and altered the activity of antioxidant enzymes leading to induced lipid peroxidation. Elevated temperature and reduced pH impaired exacerbated TC effect on *L. minor* physiological and biochemical parameters.

Effect of global warming on population dynamics of a critically endangered Ryukyu-ayu Plecoglossus altivelis ryukyuensis *in Amami-Oshima Island, southern Japan*

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The Ryukyu-ayu *Plecoglossus altivelis ryukyuensis* belongs to the family Plecoglossidae of the order Osmeriformes and is a subspecies of the ayu *Plecoglossus altivelis altivelis*, which is distributed widely from Japan and the Korean Peninsula to northern Vietnam. It has been speculated that the ancestor of *P. altivelis ryukyuensis* was isolated from populations inhabiting the mainland of Japan in the Middle Pleistocene with the formation of the Ryukyu Islands (a chain of islands extending from southwestern Kyushu to northern Taiwan) and then evolved separately for a million years. Amami-Oshima Island in the Ryukyu Islands was registered as Japan's latest world natural heritage site in July 2021. The Island is the only area where wild populations of *P. altivelis ryukyuensis* are found. The subspecies was designated as critically endangered by the Japanese Ministry of the Environment and the Kagoshima Prefecture, and fishing them has been completely banned since 2004. Although administrative supports contribute to the conservation of the Ryukyu-ayu, the subspecies is continually facing the risk of extinction. Since 1992, we have conducted monitoring surveys in their main river habitats of the Ryukyu-ayu every year. The number of up-migrating juveniles greatly differed among years, which was inversely correlated with water temperature in the coastal area in winter. In this presentation, we explain life history and population dynamics of amphidromus *P. altivelis ryukyuensis* and then introduce a significant adverse effect of global warming on their recruitment.

The Best Student Poster or Platform Presentation Awards (Students only)

The Best Young Scientist Poster or Platform Presentation Awards (Young Scientists (Under 40 years old only)

These awards will be judged/evaluated in terms of the following five viewpoints: Scientific significance, originality, unique idea, ripple effects/social impact, and his/her presentation (i.e., a clear explanation and appropriate responses to questions). Four recipients (in total) will be awarded at the closing ceremony on August 30. Certificates and prizes are to be provided with recipients by the ISTA 21 Organizing Committee.

Dr. Doris Au Student Excellence Award

The Dr. Doris Au Student Excellence Award has been created since the ISTA 20 held in Saskatoon, Canada in August 2020. The Dr. Doris Au Student Excellence Award will be given to two students at ISTA 21 for their research excellence and impact. This award will be judged/evaluated in terms of the following three viewpoints: Applicability to toxicity testing, scientific significance, and ripple effects/social impact. Two recipients will be awarded at the closing ceremony on August 30. Certificates and prizes are to be provided with recipients by the ISTA 21 Organizing Committee. Moreover, recipients of this award will present a five-minute “lightning” talk during the closing ceremony.

Funding / Support / Sponsorship

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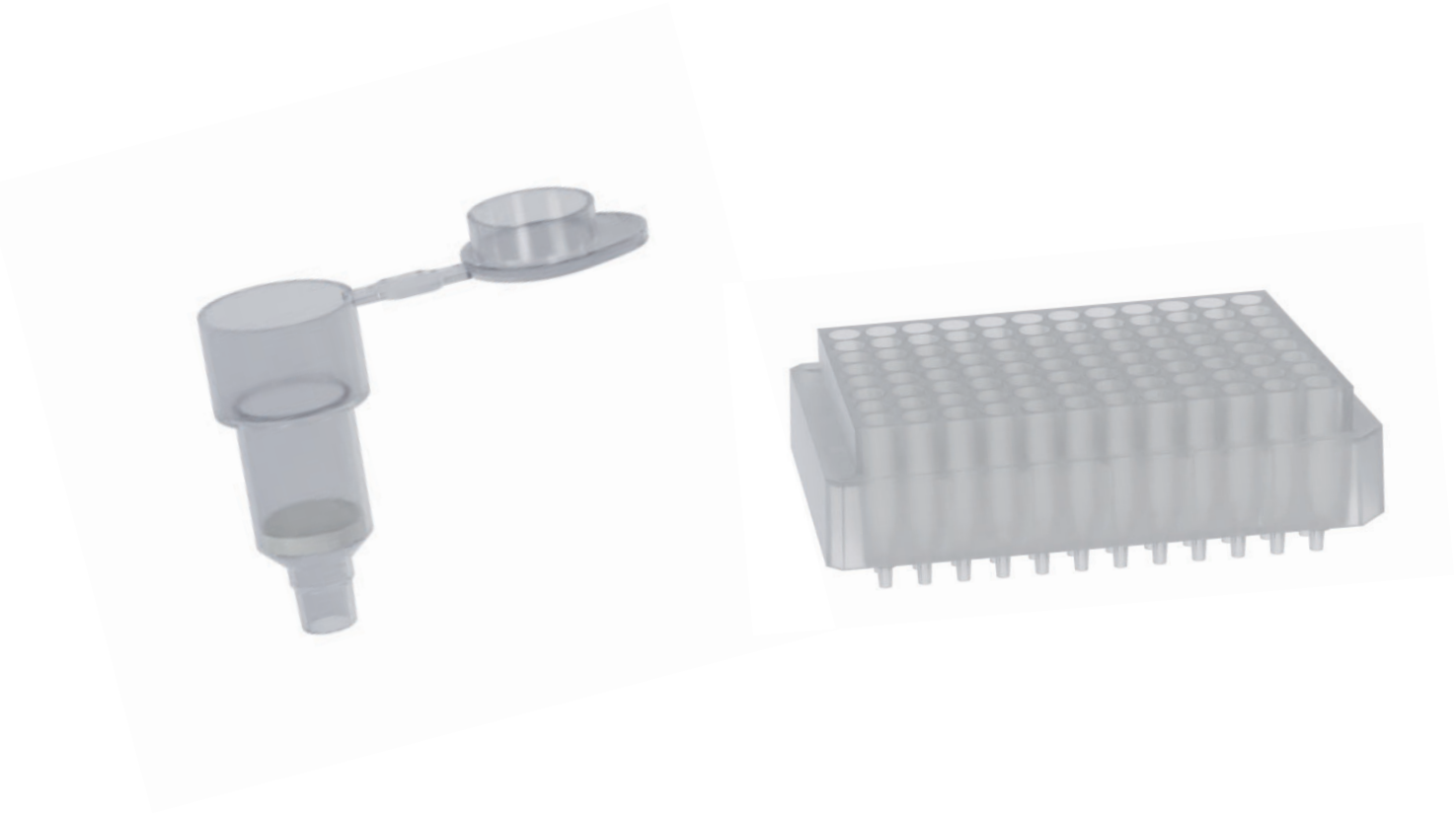

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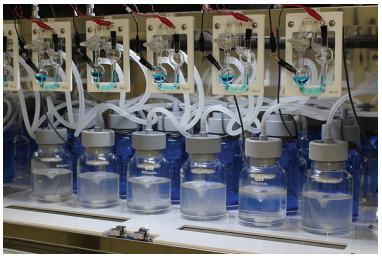
Application

- Japan's Chemical Substance Control Law (CSCL)
- Japan's Industrial Safety and Health Act



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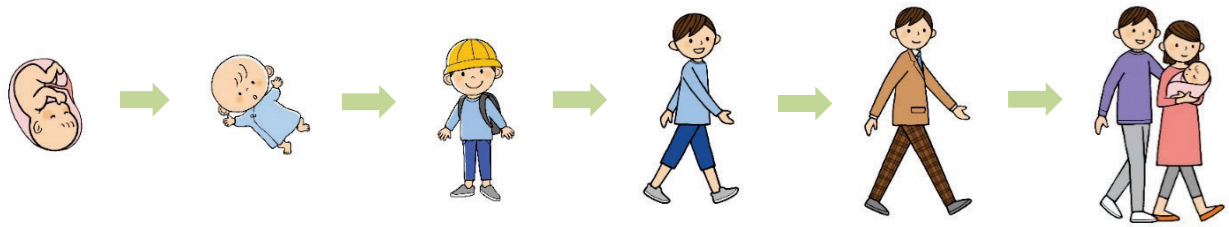
Japan Environmental and Children's Study, "JECS"

What are being studied in JECS?

National Institute for Environmental Studies (NIES) has been conducting the study called "Japan Environment and Children's Study (JECS)". JECS is a large-scale cohort study involving 100,000 mother-child dyads living throughout Japan. It started in 2011. JECS investigate children from foetus, examining how chemical substances in the environment and their lifestyles are related to the development and diseases of children. The study is attracting worldwide attention!

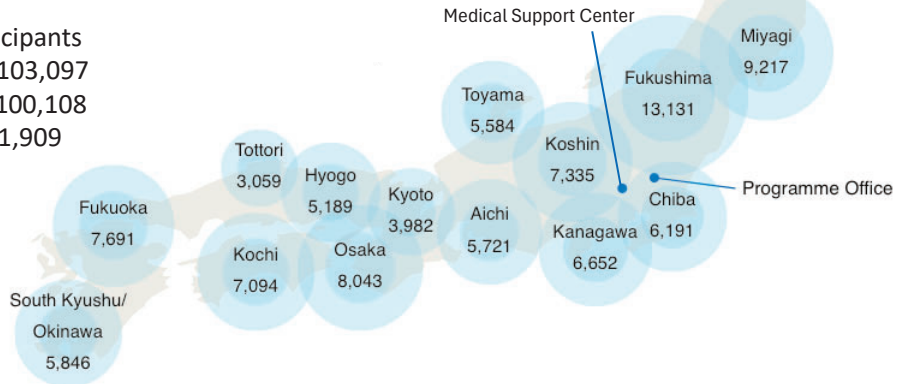
We follow children from foetus up until they become adults.



Ongoing in 15 regions throughout Japan!

As the Programme Office, NIES has been conducting the study in cooperation with 15 Regional Centres throughout Japan. The Medical Support Centre situated in the National Center for Child Health and Development provides medical expertise.

JECS Participants
Mothers:103,097
Children:100,108
Fathers:51,909



452 peer reviewed publications! (May,2024)

To date, 452 papers have been published using data from JECS.
Studies on the relationship between chemical exposure (metals, PFAS, bisphenols, neonicotinoid pesticides) and health outcomes are included.

List of papers

Japan Environment and Children's Study Programme Office
National Institute for Environmental Studies,
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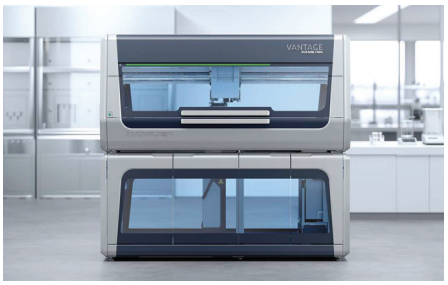

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