

10th International

2026

The 10th Joint Symposium of the Hokkaido-Tohoku Regional Chapter and the
Chugoku-Shikoku Regional Chapter, Japan Society for Environmental Chemistry

CHEMICAL HAZARD

SYMPOSIUM



Theme animal Steller sea lion

Photo: Kei Nomiya

PROGRAM AND ABSTRACTS

プログラム・講演要旨集

SYMPOSIUM THEME

**Creation of a New Academic Research Field
Based on a Decade of Networking**

22-23 OCT

2026

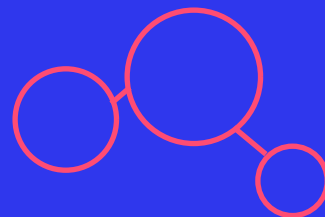
Faculty of Veterinary Medicine Auditorium, Hokkaido University

Sapporo, Japan | On-site + Zoom hybrid

WELCOME

開催にあたって

A DECADE OF NETWORKING. NEW FIELDS AHEAD.



01 / ご挨拶

第10回国際ケミカルハザードシンポジウムにご参加いただき、誠にありがとうございます。北海道大学の会場とオンラインで、国内外の皆様をお迎えできることをうれしく思います。

本シンポジウムは、2017年度に北海道大学と愛媛大学を中心として始まり、皆様のご協力のもと、第10回を迎えました。学生や若手研究者が研究成果を発表し、専門や立場の異なる参加者と率直に議論できる場を大切にしてきました。これまで支えてくださった皆様に、心より御礼申し上げます。

今回のテーマは「10年のネットワークを基盤とした新たな学術領域の創出」です。環境化学、毒性学、医学、獣医学などの分野を越え、産学官の多様な経験や技術を持ち寄ることで、これから取り組むべき研究課題を考えたいと思います。ヒト、動物、環境を一体として捉えるOne Healthの視点を共有し、これまで築いてきた交流を、新しい研究へと発展させる機会にしたいと考えています。

ご自身の専門から少し離れた発表にも、ぜひ耳を傾けてみてください。素朴な疑問や、まだ形になっていない着想を交わすことが、次の共同研究のきっかけになるかもしれません。この2日間で、若い研究者の挑戦を後押しし、次の10年につながる出会いと議論の場になることを願っています。

02 / WELCOME MESSAGE

Welcome to the 10th International Chemical Hazard Symposium. It is a pleasure to welcome colleagues from Japan and abroad, both to Hokkaido University and online.

The symposium began in fiscal year 2017, with Hokkaido University and Ehime University at its core. It has given students and early-career researchers opportunities to present their work and discuss it across disciplines. We are grateful to everyone whose support has sustained these exchanges and brought us to our tenth meeting.

Our theme is “Creation of a New Academic Research Field Based on a Decade of Networking.” We will build on these connections to explore research questions that bring together academia, industry and government. A One Health perspective, linking human, animal and environmental health, offers a shared basis for this dialogue.

We encourage you to ask questions beyond your own specialty and to share ideas that are still taking shape. We hope these two days will open new conversations, encourage the next generation of researchers and lead to collaborations that continue after the symposium. Thank you for joining us.

ONE HEALTH

HUMAN / ANIMAL / ENVIRONMENT

WITH OUR THANKS

池中 良徳

Yoshinori Ikenaka

北海道大学
One Healthリサーチセンター

One Health Research Center,
Hokkaido University

長谷川 菜々子

Nanako Hasegawa

北海道大学
Hokkaido University

野見山 桂

Kei Nomiyama

愛媛大学
Ehime University

渡邊 可菜実

Kanami Watanabe

北海道大学
Hokkaido University

22

DAY 01 / THURSDAY, OCT 22, 2026

PROGRAM

MORNING

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TIME / ID PRESENTATION / PRESENTER / CHAIR

09:20 Opening remarks (Prof. Mayumi Ishizuka)

English Session: One Health Session-1 ~Environmental Exposure, Wildlife Monitoring and Comparative Toxicology~

09:30 SS1-1 p.6	Infectious Bioaerosols and Airborne Particles (Fomites): Factors Affecting Microbial Viability in the Air Jun Noda / 酪農学園大学 Chair: Kei Nomiyama / English / 20 min
09:50 P1-1 p.7	Trace Metal Contamination in Settled Dust from the Erongo Region, Namibia. Ilana RAUTENBACH / 北海道大学 Chair: Kei Nomiyama / English / 12 min
10:02 P1-2 p.8	猛禽類における複数組織の鉛濃度・鉛同位体比からみた鉛曝露履歴 Lead Exposure History in Raptors Based on Lead Concentrations and Isotope Ratios in Multiple Tissues Mizuki MARUYAMA (D3) / 北海道大学 Chair: Kei Nomiyama / English / 12 min
10:14 P1-3 p.9	タンチョウにおけるボリコナゾールの代謝特性およびCYP酵素の関与 Voriconazole Metabolism and CYP Isoforms in Red-Crowned Cranes Mizuki TAKATORI (M1) / 愛媛大学 Chair: Yared Beyene / English / 12 min
10:26 P1-4 p.10	OECD TG 319Bを用いたBCF予測精度の向上：コイの肝代謝を考慮したモデル評価 Improving BCF Prediction Accuracy Using OECD TG 319B: Model Evaluation Incorporating Hepatic Metabolism in Common Carp Yuzuki MINEMURA (M1) / 愛媛大学 Chair: Yared Beyene / English / 12 min
10:38 P1-5 p.11	メキシコ産モレレットワニ（ <i>Crocodylus moreletii</i> ）におけるPOPs曝露と健康影響のオミクス解析 POPs Contamination and Health Effects in Wild Morelet's Crocodiles (<i>Crocodylus moreletii</i>) from Mexico: An Omics-Based Assessment Ryo NAKANISHI (M2) / 愛媛大学 Chair: Yared Beyene / English / 12 min
10:50-11:05	Break
11:05 P1-6 p.12	HFPO-TeA曝露ゼブラフィッシュ胚における甲状腺ホルモン攪乱及び循環器毒性影響評価 Evaluation of Thyroid Hormone Disruption and Cardiovascular Toxicity in HFPO-TeA-Exposed Zebrafish Embryos Mei YAMAMOTO (M2) / 愛媛大学 Chair: Kanami Watanabe / English / 12 min
11:17 P1-7 p.13	母牛と子牛の血清およびその飼育農場用水におけるレガシーおよび前駆体PFASの共存：インド農業システムにおけるフィールド調査 Co-Occurrence of Legacy and Precursor PFAS in Paired Bovine Mother-Calf Serum and Corresponding Farm Water: A Field Study in the Indian Agricultural System Snigdha (D2) / 北海道大学 Chair: Kanami Watanabe / English / 12 min
11:29 P1-8 p.14	ネオニコチノイド曝露によるマウス脳の領域特異的な神経活動およびモノアミン作動性変化 Neonicotinoid Exposure Induces Region-Specific Neuronal Activation and Monoaminergic Alterations in Mouse Brain Natamon JIANPRAPHAT / 北海道大学 Chair: Kanami Watanabe / English / 12 min
11:41	1st day Group Photo
11:51	Lunch Break

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DAY 01 / THURSDAY, OCT 22, 2026

PROGRAM

AFTERNOON

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TIME / ID PRESENTATION / PRESENTER / CHAIR

English Session: One Health Session-2 ~Health Effects, Animal Health and Environmental Epidemiology~

13:30

K1

p.15

リソソーム損傷が引き起こすパーキンソン病の病態と治療薬開発
Lysosomal Damage in Parkinson's Disease: Pathogenesis and Therapeutic Development
Kensuke Ikenaka / 大阪大学 | Chair: Yoshinori Ikenaka / English / 50 min

14:20-14:30

Break

14:30

SS1-2

p.16

暑熱ストレスと牛の健康
Heat stress and health in cattle
Takashi Tanida / 北海道大学 | Chair: Akifumi Eguchi / English / 20 min

14:50

SS1-3

-

環境省PFAS総合研究のプロジェクト概要と環境疫学からの知見
Project Overview of the Comprehensive Study on PFAS by Ministry of the Environment and Findings from Environmental Epidemiology
Yu Ait Bamai / 北海道大学 | Chair: Akifumi Eguchi / English / 20 min

15:10

P1-9

p.17

デクスメドミジンによるアストロサイト形態変化の解析
Analysis of dexmedetomidine-induced astrocyte morphological changes
Shu WATANUKI (D4) / 北海道大学 | Chair: Ilana Rautenbach / English / 12 min

15:22

P1-10

p.18

シヌクレイノパチーにおけるスベルミン- α シヌクレイン相互作用と脳領域特異的脆弱性
Spermine- α -synuclein interactions and regional vulnerability in synucleinopathies
Nan WANG / 大阪大学 | Chair: Ilana Rautenbach / English / 12 min

15:34-15:48

Break

Japanese Session: One Health Session-3 ~Ecotoxicology and Developmental Studies~

15:48

SS1-4

p.19

ネオニコチノイド系殺虫剤が海産甲殻類アミの繁殖と行動に及ぼす影響
Effects of Neonicotinoid Insecticides on Reproduction and Behavior in the Marine Mysid Crustaceans, Americamysis bahia
Masaya Uchida / 有明高専 | Chair: Tetsushi Hirano / 日本語 / 20 min

Industrial Session-1 ~Cutting-edge Analytical Technology~

16:08

C1-1

p.20

医学者向けの探索メタボロミクス ~LC/MSは代謝物の夢を見るか?~
Nontargeted Metabolomics for Medical Researchers ~Does LC/MS Dream of Metabolites?~
Akio Hayashi (Agilent) | Chair: Yoshinori Ikenaka / 日本語 / 15 min

16:23

C1-2

p.21

トラップドイオンモビリティ質量分析計を用いた次世代PFAS分析
Next level PFAS detection and annotation using Trapped Ion Mobility Spectrometry (TIMS)
Minoru Hirata (Bruker) | Chair: Yoshinori Ikenaka / 日本語 / 15 min

16:38

1st day Closing remarks (Prof. Kei Nomiyama)

17:30~

Banquet

17演題

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DAY 02 / FRIDAY, OCT 23, 2026

PROGRAM

MORNING

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TIME / ID PRESENTATION / PRESENTER / CHAIR

09:20 Opening remarks by Prof. Yoshinori Ikenaka

Japanese Session: One Health Session-4 ~Emerging Technologies for New Academic Fields~

09:30 固体ナノポア1分子計測による非標的異常検知 - One Healthのための早期警戒基盤
K2 Target-Agnostic Anomaly Detection by Solid-State Nanopore Single-Molecule Sensing: An Early-Warning Platform
 p.22 for One Health
[Sotaro UEMURA / 東京大学](#) | Chair: Yoshinori Ikenaka / 日本語 / 50 min

10:20-10:30 Break

10:30 ソリッドステートナノポアを用いた単分子識別技術
SS2-1 Solid-State Nanopore Technology for Single-Molecule Identification
 p.23 [Itaru YANAGI / 東京大学](#) | Chair: Yoshinori Ikenaka / 日本語 / 20 min

Japanese Session: One Health Session-5 ~Chemical Mixture Neurotoxicity and Wildlife Monitoring~

10:50 化学物質の複合曝露が引き起こす神経毒性の新規評価法の開発：AOP-based approachによる相乗作用の同定
SS2-2 A Novel AOP-Based Approach for Assessing Neurotoxicity of Chemical Mixtures: Identification of Synergistic Effects
 p.24 [Tetsushi HIRANO / 富山大学](#) | Chair: Tomoya Koike / 日本語 / 20 min

11:10 化学物質混合曝露における注目すべきグループ・成分の探索
SS2-3 Prioritizing Groups and Components in Chemical Mixtures
 p.25 [Akifumi EGUCHI / 千葉大学](#) | Chair: Tomoya Koike / 日本語 / 20 min

11:30-11:45 Break

11:45 無卵殻培養（ex-ovo）ニワトリ胚を用いたTCEPの発生毒性評価と作用機序解析
SS2-4 Developmental Toxicity and Mechanistic Analysis of TCEP Using a Shell-less (ex-ovo) Chicken Embryo Model
 p.26 [Kazuki KANDA / 愛媛大学](#) | Chair: Mizuki Maruyama / 日本語 / 20 min

12:05 PFDA曝露によるミナミメダカの繁殖阻害とその潜在的メカニズムの解明
P2-1 Elucidation of reproductive inhibition induced by PFDA exposure in Japanese medaka (*Oryzias latipes*) and its
 p.27 potential mechanisms
[Kenta MATSUBARA \(M2\) / 愛媛大学](#) | Chair: Mizuki Maruyama / 日本語 / 12 min

12:17 ネオニコチノイド系殺虫剤曝露と新奇環境負荷後のマウス脳におけるLC/TQ/MSを用いたモノアミン濃度変動の解析
P2-2 Analysis of Changes in Monoamine Concentrations in the Mouse Brain Following Neonicotinoid Insecticide
 p.28 Exposure and Novel Environment Stress by Using LC/TQ/MS
[Tomoya KOIKE \(D2\) / 北海道大学](#) | Chair: Mizuki Maruyama / 日本語 / 12 min

12:29 肝臓試料を対象とした前駆体PFASの分析法開発および野生鳥類のPFAS蓄積評価
P2-3 Development of analytical method for PFAS precursors in liver samples and assessment of PFAS accumulation in
 p.29 wild birds
[Hiromi SEIMIYA \(M2\) / 愛媛大学](#) | Chair: Mizuki Maruyama / 日本語 / 12 min

12:41 2nd day Group Photo

12:47 Lunch Break

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DAY 02 / FRIDAY, OCT 23, 2026

PROGRAM

AFTERNOON

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TIME / ID PRESENTATION / PRESENTER / CHAIR

Industrial Session-2 ~Cutting-edge Analytical Technology~

13:35 C2-1 p.30	LC-MSの最新ソリューション情報 The Latest LC-MS Solutions 服部 考成 (島津製作所) Chair: Kei Nomiyama / 日本語 / 15 min
13:50 C2-2 p.31	LC-MSを用いた化合物検出/同定手法の進展 Recent Advances in LC-MS Techniques for Compound Detection and Identification 建田 潮 (Sciex) Chair: Kei Nomiyama / 日本語 / 15 min
14:05 C2-3 p.32	PFAS分析を支える試薬・標準品・前処理技術の最新動向 Latest Trends in Reagents, Standards, and Pretreatment Technologies Supporting PFAS Analysis 森 太紀 (関東化学) Chair: Yoshinori Ikenaka / 日本語 / 15 min
14:20 C2-4 p.33	固相抽出法を用いた試料前処理の最新情報 The latest advances in sample preparation using solid-phase extraction 太田 茂徳 (GL Science) Chair: Yoshinori Ikenaka / 日本語 / 15 min

14:35-14:50

Break

Japanese Session: One Health Session-6 ~Comparative Toxicology, Xenobiotic Metabolism and Evolution~

14:50 SS2-5 p.34	鳥類グルクロン酸抱合酵素 (UGT) の機能とin silico予測 Function of avian UDP-glucuronosyltransferase and prediction in silico approach Yusuke Kawai / 帯広畜産大学 Chair: Hisato Iwata / 日本語 / 20 min
15:10 P2-4 p.35	ヘム鉄・非ヘム鉄摂取に対する生体メタロームの変動と代謝応答の解析 Changes in the Metallome and Metabolic Responses to Dietary Heme and Non-Heme Iron Nanako HASEGAWA / 北海道大学 Chair: Hisato Iwata / 日本語 / 12 min
15:22 P2-5 p.36	グルクロン酸抱合酵素 (UGT) の鯨類における遺伝子発現および代謝活性解析 Gene Expression and Metabolic Activity Analysis of UDP-Glucuronosyltransferases (UGTs) in Cetaceans Nami IWATA (B6) / 北海道大学 Chair: Hisato Iwata / 日本語 / 12 min
15:34 P2-6 -	カイウサギにおけるノミ・ダニ駆除薬フィプロニル高感受性の薬物代謝・薬物動態学的解析 Pharmacokinetic and drug metabolism analysis of the high sensitivity of domestic rabbits to the flea and tick control drug fipronil Ayano YOSHIMOTO (D2) / 北海道大学 Chair: Hisato Iwata / 日本語 / 12 min
15:46 P2-7 p.37	鳥類におけるグレリン関連遺伝子の比較進化的解析 Comparative evolutionary analysis of ghrelin-related genes in birds Taku OTSU (B6) / 北海道大学 Chair: Yusuke Kawai / 日本語 / 12 min
15:58 P2-8 p.38	シマハイエナの腐肉食性に着目した、苦味受容体T2Rの腐肉由来化学物質に対する応答解析 Analysis of Bitter Taste Receptor T2R Responses to Carrion-Derived Compounds with a Focus on the Scavenging Ecology of the Striped Hyena Shiori MIYAMOTO (D3) / 北海道大学 Chair: Yusuke Kawai / 日本語 / 12 min
16:10 P2-9 p.39	シリアンハムスターにおける自発的エタノール摂取量の個体差と腸内細菌叢の関連 Individual Differences in Voluntary Ethanol Intake and Their Association with the Gut Microbiota in Syrian Hamsters Toko IKEGAMI (B6) / 北海道大学 Chair: Yusuke Kawai / 日本語 / 12 min
16:22 P2-10 p.40	アジアゾウにおけるピレン曝露の検出とUGTおよびSULTを介した抱合代謝能の評価 Detection of Pyrene Exposure and Evaluation of UGT- and SULT-Mediated Conjugation Activities in Asian Elephants Kanami WATANABE / 北海道大学 Chair: Yusuke Kawai / 日本語 / 12 min

16:34

2nd day Closing remarks (Prof. Hisato IWATA)

20演題

p. = Abstract page; - = Abstract not included

SS1-1

感染性バイオエアロゾルと空気中粒子（媒介物）：微生物の空気中生存性に影響する因子

Infectious Bioaerosols and Airborne Particles (“Fomites”): Factors Affecting Microbial Viability in Air

Authors: Jun Noda¹, Tomoko Ando¹, Teruya Maki², Xiangrui Kong³, Christian Salvador³, Epameinondas Tsiligiannis³, Mattias Hallquist³, Kozo Morimoto⁴, and Satoshi Mitarai⁴

Affiliations: ¹Rakuno Gakuen Univ.; ²Kindai Univ.; ³Göteborg Univ.;

⁴Research Inst. of Tuberculosis

Bioaerosol research related to airborne infection has received increasing attention following the COVID-19 pandemic. An important remaining question is how environmental factors influence the survival and persistence of infectious microorganisms after they become airborne. During atmospheric transport, bioaerosols are exposed to multiple stresses, including desiccation, ultraviolet (UV) radiation, oxidation, and interactions with surrounding airborne particles. We therefore hypothesized that particulate matter may act as an airborne “fomite,” modifying the microenvironment surrounding microorganisms and thereby influencing their viability.

Using a controlled simulation chamber, we investigated the survival of bacterial bioaerosols under different environmental conditions in the presence of soot particles as a model of combustion-derived particulate matter. The presence of soot was associated with marked changes in microbial survival under UV stress, although the

magnitude of the effect depended on particle concentration and exposure conditions. Chemical characterization further indicated that UV irradiation altered the composition of the soot, suggesting that particle-associated effects on microbial viability may involve not only physical shielding but also chemical interactions.

These findings indicate that the fate of infectious bioaerosols in air cannot be explained solely by conventional environmental factors such as temperature, humidity, and UV radiation. Interactions with coexisting airborne particles may also influence microbial persistence and should therefore be considered when evaluating the environmental behavior and potential transmission of infectious bioaerosols. Further studies are needed to clarify the underlying physical and chemical mechanisms and to determine their relevance under real-world atmospheric conditions.

P1-1

Trace Metal Contamination in Settled Dust from the Erongo Region, Namibia.

Authors: Ilana Rautenbach¹, Yared Beyene Yohannes¹, Collins Nimako¹, Nelly Banda⁵, John Yabe², Rio Doya¹, Yoshinori Ikenaka^{1,3}, Mayumi Ishizuka¹, Shouta M.M. Nakayama^{1,4}

Affiliations: ¹Hokkaido Univ.; ²Univ. of Namibia; ³North-West Univ.; ⁴Univ. of Zambia; ⁵Univ. of Lusaka

Background: Environmental dust in Namibia reflects both natural and human activities and is fundamental to characterising trace contamination. Major dust sources such as the Namib Desert and ephemeral river valleys enrich regional dust transport, compounded by mining, urbanisation, traffic and deteriorating infrastructure. Indoor settled dust is an important but understudied exposure medium given its greater particle retention in enclosed spaces, yet information remains limited in the Erongo Region.

Method: This study investigated trace metal concentrations in settled indoor and roadside dust (<100 µm) from residential areas in three Erongo towns (Arandis, Swakopmund, and Walvis Bay). Samples were primarily analysed using ICP-MS; however, mercury was measured using a Direct Mercury Analyser and iron was cross-validated by AAS. Multivariate analysis was utilised to understand

contamination patterns and variability within the dataset.

Result: Indoor dust demonstrated significantly higher concentrations than roadside dust for most metals, particularly zinc, copper, and nickel. Uranium and vanadium were elevated in roadside dust and strongly associated with Arandis, the town closest to the region's uranium and base-metal mining operations. Multivariate analysis distinguished a geogenic / mining signature (uranium, vanadium and to a lesser extent chromium and cobalt) in roadside dust from a more anthropogenic signature (lead, zinc, copper and cadmium) in indoor dust. Further pollution index assessment reaffirmed considerable to very high indoor contamination of anthropogenic metals. Carcinogenic risk for arsenic was established during health risk screening and exceeded the USEPA acceptable threshold for nearly all samples and was especially high in children in Walvis Bay.

P1-2

猛禽類における複数組織の鉛濃度・鉛同位体比からみた鉛曝露履歴

Lead Exposure History in Raptors Based on Lead Concentrations and Isotope Ratios in Multiple Tissues

Authors: Mizuki Maruyama¹, Hikaru Adachi², Yukiko Watanabe², Keisuke Saito², Taku Otsu¹, Takeshi Kuritani¹, Yoshinori Ikenaka^{1,3}, Mayumi Ishizuka¹, Shouta Nakayama^{1,4}

Affiliations: ¹Hokkaido Univ.; ²Institute for Raptor Biomedicine Japan; ³North-West Univ.; ⁴Univ. of Zambia

Background: Lead (Pb) exposure remains a conservation concern for White-tailed eagles (*Haliaeetus albicilla*) and Steller's sea eagles (*Haliaeetus pelagicus*). Pb concentrations in soft tissues mainly reflect recent exposure, whereas bones can retain Pb over longer periods. Pb isotope ratios can help estimate exposure sources. Combining these measurements may therefore provide information on Pb exposure history. This study examined Pb exposure history in eagles from Hokkaido.

Method: Pb concentrations were measured by ICP-MS in liver and kidney samples from 61 White-tailed and Steller's sea eagles collected in Hokkaido between 2022 and 2026. Birds with >2 ppm Pb (w.w.) were classified as poisoned and those with 0.2–2 ppm as highly exposed. Pb concentrations and isotope ratios were further analyzed in available soft tissues and bones from selected poisoned and highly exposed birds using ICP-MS and multi-collector ICP-MS.

Result: Three eagles were classified as poisoned and 5 as highly exposed. One poisoned and 4 highly exposed birds died in train collisions, suggesting Pb exposure associated with feeding on deer carcasses. Pb-poisoned birds tended to have higher soft-tissue Pb concentrations relative to bone than highly exposed birds, suggesting a longer interval since exposure in the latter. Soft-tissue Pb isotope ratios were similar to those of shot pellets or fishing sinkers in 3 birds and to those of rifle bullets or shotgun slugs in the others. The ratios differed between soft tissues and bones in 4 birds, suggesting exposure to different Pb sources at different times, whereas 1 poisoned bird showed similar ratios across all tissues examined, consistent with exposure to a single source. These findings indicate multiple Pb exposure events in some birds and demonstrate that combining Pb concentrations and isotope ratios across tissues can provide information on Pb exposure history.

P1-3

タンチョウにおけるボリコナゾールの代謝特性およびCYP酵素の関与

Voriconazole Metabolism and CYP Isoforms in Red-Crowned Cranes

Mizuki Takatori¹, Rumi Tanoue¹, Akira Kubota², Kei Nomiya¹,
Tomoo Yoshino³, Hiroko Iima³

¹Ehime University, ²Obihiro University of Agriculture and Veterinary Medicine,

³Kushiro City Zoo

Background: Avian aspergillosis is a fungal disease primarily affecting the respiratory system, with increased risk in immunocompromised individuals. Voriconazole (VRCZ), an azole antifungal agent, is a first-line treatment due to its potent antifungal activity against *Aspergillus* species; however, its narrow therapeutic window and substantial interspecies variability in metabolism pose challenges for maintaining therapeutic plasma concentrations. The red-crowned crane (*Grus japonensis*), classified as Near Threatened (NT) in the Japanese Red List, cannot be subjected to in vivo pharmacokinetic studies for ethical reasons. This study aimed to evaluate VRCZ metabolism and identify the enzymes involved using hepatic microsomes from an accidentally deceased individual.

Materials and Methods: Hepatic microsomes were incubated with VRCZ in the presence of an NADPH-regenerating system. Metabolites were comprehensively screened using LC-QToF-MS and structurally characterized. VRCZ and two identified metabolites were quantified by LC-MS/MS. The disappearance of VRCZ and the formation rates of the metabolites were calculated, and kinetic parameters (K_m , V_{max}) were estimated using the Michaelis–Menten model. Intrinsic clearance (CL_{int} , in vitro) was calculated as the V_{max}/K_m ratio. Enzyme involvement was evaluated using ketoconazole (a CYP3A4 inhibitor) and omeprazole (CYP2C19 inhibitor).

Results and Discussion: Several metabolites were tentatively identified, with VRCZ N-oxide and a hydroxylated form showing the largest peak areas; these two metabolites were therefore selected for quantitative analysis. The N-oxide metabolite exhibited higher V_{max} (85 pmol/min/mg) and K_m (13 μM), indicating a low-affinity, high-capacity pathway. The hydroxylated metabolite showed lower V_{max} (3.2 pmol/min/mg) and K_m (1.4 μM), suggesting a high-affinity, low-capacity pathway. CL_{int} was higher for the N-oxide pathway, suggesting that N-oxide formation is the predominant metabolic pathway in red-crowned crane hepatic microsomes. The scaled in vivo hepatic clearance (CL_H) was intermediate among reported avian species and comparable to in vivo values reported for the red-tailed hawk. Ketoconazole strongly inhibited VRCZ metabolism (IC_{50} : 4.8 μM), with particularly potent inhibition of hydroxylation (IC_{50} : 1.5 μM) and weaker inhibition of N-oxide formation (IC_{50} : 32 μM), indicating predominant CYP3A involvement in hydroxylation and partial contribution to N-oxide formation. Omeprazole also reduced VRCZ clearance (IC_{50} : 96 μM), suggesting that CYP2C enzymes may contribute to VRCZ metabolism. Taken together, the inhibitor experiments suggest that VRCZ hydroxylation is predominantly mediated by CYP3A, whereas N-oxide formation likely involves both CYP3A and CYP2C subfamily enzymes.

P1-4

OECD TG 319Bに基づく生物濃縮係数予測の精度向上：コイの肝代謝を組み込んだモデル評価

Improving BCF Prediction Using OECD TG 319B: Evaluation of Models Incorporating Hepatic Metabolism in Common Carp

Yuzuki Minemura¹, Rumi Tanoue¹, Midori Iida², Kei Nakayama¹

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Background: The Bioconcentration Factor (BCF) is widely used to assess the bioconcentration potential of chemicals; however, *in vivo* testing is time-consuming, costly, and animal-intensive. New Approach Methodologies (NAMs) integrating *in vitro* metabolism with *in silico* models have therefore attracted increasing attention. Although OECD TG 319B provides a framework for metabolism-based BCF prediction, its applicability to common carp (*Cyprinus carpio*), the model species under Japan's Chemical Substances Control Law (CSCL), remains unclear. This study evaluated the impact of incorporating metabolism measured using common carp liver S9 fractions into B-compass and Hendriks BCF models.

Methods: Fourteen chemicals, including UV stabilizers and pesticides (log Dow: 3–7), were evaluated for their *in vitro* hepatic metabolic clearance and BCF prediction. Each compound (300 nM) was incubated with liver S9 fractions at 25°C and quantified by LC–MS/MS. Intrinsic clearance ($CL_{int, in vitro}$) was calculated for compounds showing

significant time-dependent decreases ($p < 0.05$). The metabolic parameters were incorporated into the BCF prediction models to evaluate the contribution of hepatic metabolism to chemical elimination and prediction accuracy.

Results: Incorporating of hepatic metabolism into the B-compass model increased the coefficient of determination (R^2) from 0.29 to 0.33, and decreased the root mean squared logarithmic error (RMSLE) from 3.3 to 2.1. Similarly, in the Hendriks model, R^2 increased from 0.32 to 0.40, while RMSLE decreased from 3.1 to 1.8. The improvement was particularly evident for highly hydrophobic compounds ($\log D_{ow} > 5$), including UV-329, UV-328, triclosan, triclocarban, and carbosulfan in which hepatic metabolism accounted for more than 80% of total chemical elimination.

Conclusions: Hepatic metabolism can substantially influence BCF prediction, particularly for highly hydrophobic chemicals. Incorporating species-specific *in vitro* metabolic clearance can improve BCF prediction in common carp.

P1-5

メキシコ産モレレットワニ (*Crocodylus moreletii*) におけるPOPs曝露と健康影響のオミクス解析

POPs Contamination and Health Effects in Wild Morelet's Crocodiles (*Crocodylus moreletii*) from Mexico: An Omics-Based Assessment

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Background: Environmental pollutants, such as Persistent Organic Pollutants (POPs) and pesticides affect ecosystems through endocrine disruption and neurotoxicity, and crocodilians, as apex predators in aquatic environments, are no exception. In Mexico's Yucatán Peninsula, where DDT was used in the past, there are concerns about the impact on the Morelet's crocodile, but data on their contamination status is insufficient. However, due to legal and ethical constraints, it is essential to conduct research using non-invasive samples. Therefore, in this study, we investigated the actual contamination levels of POPs using blood plasma from Morelet's crocodiles inhabiting the Yucatán Peninsula in Mexico. Furthermore, we investigated the effects of these environmental pollutants on the crocodiles' metabolome.

Method: We measured the concentration of POPs (DDTs, PCBs) in blood plasma from 59 samples. We also performed non-target metabolomics. OPLS-DA was then used to evaluate the effects of DDE on metabolites, using 10 samples with high DDE concentrations and 10 with low DDE concentrations.

Result: The analysis revealed that *p,p'*-DDE, a metabolite of DDT, was frequently detected at high concentrations, confirming the persistence of POPs contamination. OPLS-DA using a total of 78 annotated metabolites demonstrated a clear separation between the groups. Among the metabolites contributing to this separation, 14 metabolites including creatine increased, while 6 metabolites including glucose decreased. These findings suggest that DDE may inhibit normal mitochondrial energy production and induce an antioxidant response against oxidative stress.

P1-6

HFPO-TeA曝露ゼブラフッシュ胚における甲状腺ホルモン攪乱及び循環器毒性影響評価

Evaluation of Thyroid Hormone Disruption and Cardiovascular Toxicity in HFPO-TeA-Exposed Zebrafish Embryos

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Background: Perfluorooctanoic acid (PFOA), a type of per- and polyfluoroalkyl substance (PFAS), was listed for regulation under the Stockholm Convention in 2019, and HFPO homologues (HFPO-DA, HFPO-TA, and HFPO-TeA) have been developed as alternative substances. In our previous studies, we investigated the effects of exposure to PFOA and HFPO homologues on thyroid hormone (TH) homeostasis in zebrafish (*Danio rerio*). The results demonstrated that HFPO-TeA, in particular, increased thyroxine (T4) levels at lower concentrations than PFOA, suggesting its potential to disrupt TH homeostasis. However, the mechanisms underlying HFPO-TeA-induced TH disruption remains unclear.

Method: Zebrafish embryos were exposed to HFPO-TeA, and neurotransmitter (NT) levels were measured using LC-MS/MS. Developmental toxicity was also evaluated. In addition, agonistic and antagonistic of HFPO-TeA toward TR β were

assessed using the TR-CALUX assay. Mitochondrial toxicity was evaluated using an OXPHOS activity assay.

Result: NT analysis revealed no significant changes in GABA levels. In addition, neither PFOA nor HFPO-TeA exhibited agonistic or antagonistic activity toward TR β . These results suggest that the observed changes in T4 levels are unlikely to be mediated by GABA signaling or direct effects on TR β . In the developmental toxicity test, HFPO-TeA exposure increased heart rate and decreased trunk blood flow, indicating potential cardiovascular toxicity. Because these cardiovascular effects suggested a possible involvement of mitochondrial dysfunction, mitochondrial toxicity was further evaluated using an OXPHOS activity assay. The assay showed that HFPO-TeA inhibited Complex II activity in the electron transport chain. These findings suggest that mitochondrial dysfunction may contribute to the cardiovascular toxicity induced by HFPO-TeA.

P1-7

母牛と子牛の血清およびその飼育農場用水におけるレガシーおよび前駆体 PFASの共存：インド農業システムにおけるフィールド調査

Co-Occurrence of Legacy and Precursor PFAS in Paired Bovine Mother-Calf Serum and Corresponding Farm Water: A Field Study in the Indian Agricultural System

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Background: Per- and polyfluoroalkyl substances (PFAS) are highly persistent contaminants that enter agricultural food chains through contaminated water, soil and feed. Livestock that drink from or graze near industrial and sewage-affected sources can accumulate PFAS in blood and edible tissues, creating a dietary route of human exposure. The European Union has set maximum levels for the sum of PFOS, PFOA, PFHxS and PFNA in foods of animal origin (e.g., 1.3 µg/kg in bovine meat and 1.7 µg/kg in eggs). Comparable guidance and baseline data are lacking in India, despite its very large livestock population. Moreover, compound-specific PFAS transfer from bovine lactating adults to their calves remains uncharacterized in paired field samples linked to their drinking water source.

Method: In this study, we quantified 37 legacy, precursor and emerging PFAS in serum from cattle and water buffaloes (n= 95), comprising lactating adults, heifers and paired calves on lactation. We also analysed (n=24) drinking-water samples collected from the same farms where the animals reside in Faridabad and Gurugram district, of Delhi NCR, India. Serum was extracted using an Oasis

WAX 30 mg cartridge. Water was extracted using Bond Elut PFAS WAX 150 mg following EPA Method 1633. Quantitative and comprehensive analyses of all samples were performed using liquid chromatography tandem mass spectrometry (LC/MS/MS) on Shimadzu LCMS-8050 (recoveries 75–125%; MDLs [0.05- 2 ng/mL] in serum and [1.2~10 ng/L] in water).

Results: Short-chain PFAS dominated farm water, while long-chain compounds dominated serum, with apparent water-to-serum transfer factors rising with chain length, particularly for sulfonates. Within dam–calf pairs, calves carried higher levels of most long-chain PFAS than their mothers and had a distinct serum profile. With age, the pair moved in opposite directions: PFAS levels rose with calf age (1–6 months) but declined with increasing maternal age (5–9 years), suggesting that mothers eliminate PFAS to their calves through lactation (milk) as an elimination pathway. These findings establish a paired dam–calf PFAS field concentration data identifying bovine calves as a sensitive exposure group. This poses a threat to human food safety through livestock products.

P1-8

ネオニコチノイド曝露によるマウス脳の領域特異的な神経活動およびモノアミン作動性変化

Neonicotinoid Exposure Induces Region-Specific Neuronal Activation and Monoaminergic Alterations in Mouse Brain

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Background: Recent research provide insight into the effect by which neonicotinoid insecticides (NNIs) might influence cognitive processes, motor coordination, learning, or memory. *Arc* gene is closely related to neuronal signaling and synaptic function. Using *Arc*TRAP (*Arc*Cre^{ER/+}R26^{AI14/+}) mice allows us to label activated cell population expressing *Arc* in response to NNI exposure during specific window period. Therefore, this study aims to identify the brain regions that show altered *Arc* promotor activation as well as neurotransmitter changes after exposure to NNI.

Method: *Arc*TRAP (*Arc*Cre^{ER/+}R26^{AI14/+}) double heterozygous mice were administered with clothianidin (CLO) at 50 mg/kg (NOAEL). Then, tamoxifen (4-OHT) was administered to induce recombination of the activity-dependent recombinase CreER^{T2}, permanently labeling the *Arc*-expressing neurons induced by CLO. C57BL/6 mice were also exposed to CLO at NOAEL to 5x NOAEL. Different

brain regions were then harvested for monoamine quantification using LC/TQ/MS.

Result: Following a 1 h exposure to CLO, a significant increase in *Arc*TRAP-positive cells/mm² were observed in the granular layer of hippocampal dentate gyrus, basolateral amygdala, and caudate putamen. These findings indicated that acute CLO exposure induces region-specific alterations in *Arc*-dependent neuronal activity. In addition, dopamine, 3-MT, and serotonin levels were also altered in the cerebral cortex, hippocampus, and striatum consistent with brain regions with increased *Arc*TRAP labeling. This suggests a link between acute CLO-induced neuronal activation and monoaminergic dysregulation, implying that NNI exposure activates circuits associated with anxiety-related behavior and locomotion. Overall, this study provides spatial evidence of NN-induced alterations in neural circuit activity that underlie neurotoxicant-associated behavioral dysfunction in mammals.

リソソーム損傷が引き起こすパーキンソン病の病態と治療薬開発

Lysosomal Damage in Parkinson's Disease: Pathogenesis and Therapeutic Development

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Lysosomal dysfunction has emerged as a central pathogenic mechanism in Parkinson's disease (PD) and related synucleinopathies. We previously demonstrated that phosphatidylinositol-3,4,5-trisphosphate (PIP3) directly promotes α -synuclein aggregation, identifying the lysosomal lumen as a potential site of pathological protein assembly. We subsequently showed that lysosomal membrane damage facilitates the propagation of α -synuclein pathology by allowing internalized fibrils to access cytosolic α -synuclein and initiate templated aggregation.

More recent findings from our laboratory suggest that lysosomal damage itself can recruit endogenous α -synuclein to damaged lysosomes and promote its aggregation. Together, these observations support a model in which lysosomal membrane injury, altered

lipid composition, and impaired clearance form a self-amplifying cascade driving both the initiation and propagation of α -synuclein pathology. An important emerging question is how environmental chemical exposure contributes to this process. In collaboration with Prof. Yoshinori Ikenaka, we are investigating whether environmental pollutants and chemical hazards induce lysosomal stress and thereby increase vulnerability to α -synuclein aggregation. These mechanistic insights also provide therapeutic opportunities. We are developing small molecules that inhibit the PIP3- α -synuclein interaction, as well as Rubicon-targeting antisense oligonucleotides to enhance autophagic-lysosomal clearance. This lecture will discuss lysosomal damage as a potential nexus linking environmental exposure, α -synuclein aggregation, propagation, and therapeutic development in PD.

SS1-2

暑熱ストレスと牛の健康

Heat stress and health in cattle

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Dairy products are nutrient-dense foods and as the human population grows, those consumptions are expected to increase in the future. Thus, milk is in high global demand, leading to the increasement of number of dairy cows kept in farms in the world. In 2024, a total of 291 million dairy cattle were kept in the world although a total of 258 million dairy cattle were kept in 2010. However, recently, heat stress caused by high temperature and humidity negatively affects animal welfare and milk productivity of dairy cows due to global warming. Low milk productivity leads to the increasement of number of cows to produce enough amount of milk, which causes the increasement of total greenhouse gas emissions from cows.

The temperature humidity index (THI) is an indicator for estimating how degree of heat stress would be affected. The optimal THI for dairy cows is below 65. However, when the temperature is over 25°C, THI

usually become over 70. In this condition, cows are under heat stress. In this condition, cows are under heat stress. Under heat stress conditions, the amount of milk produced by a cow decreased and the milk composition, such as protein, fat, solid non-fat, casein, and lactose, is also altered. Furthermore, the fertility is reduced due to the reproductive problem in cows under heat stress, leading to the low milk productivity in cows. Heat stress detrimentally affects on immune resilience and, thus, cow health. Leaky gut also induced by heat stress, which leads to the increasement of mycotoxin exposure in dairy cows.

In this presentation, I would like to introduce the situation of dairy industry and a problem caused by heat stress in dairy cows due to global warming. I hope that the present topic will serve as an opportunity for audience to think about what we can do to solve this problem related to animal welfare, food security and environmental problems.

P1-9

デクスメトミジンによるアストロサイト形態変化の特徴解析

Analysis of dexmedetomidine-induced astrocyte morphological changes

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Background: Dexmedetomidine, an α_2 -adrenoceptor (α_2 -AR) agonist, is widely used as a sedative. However, dexmedetomidine also has affinity for imidazoline receptors. Both imidazoline I1 receptors (I1R) and I2 receptors (I2R) are expressed in multiple cell types in the brain, including astrocytes. Astrocytes are characterized by their complex morphology with numerous branches, which play essential roles in regulating central nervous system function as components of the blood brain barrier and tripartite synapses. Although astrocytic branch morphology is known to be altered by various drugs such as morphine, the effects of dexmedetomidine on astrocytic branches remain unclear. Therefore, this study investigated the effects of dexmedetomidine on the morphology of hippocampal astrocytic branches and the receptors involved.

Method: Male mice received intraperitoneal injections of dexmedetomidine, α_2 -AR antagonist atipamezole, I1R antagonist efroxan, I2R antagonist

idazoxan, or I1R agonist moxonidine. After administration, hippocampal slices were obtained and immunostained for GFAP, an astrocyte-specific intermediate filament protein. GFAP-positive structures were traced to analyse astrocyte morphology.

Result: Dexmedetomidine significantly reduced multiple morphological parameters of astrocytes, including branch length and the number of branches. These effects were significantly suppressed by I1R antagonist efroxan but not by atipamezole or idazoxan. Furthermore, I1R agonist moxonidine also decreased morphological parameters which were significantly suppressed by efroxan. These results indicate that I1R activation promotes astrocytic branch retraction. We are considering these astrocyte morphological change regulates waste clearance in the brain. We will introduce our approach to evaluating brain waste clearance.

P1-10

シヌクレイノパチーにおけるスpermin- α -シヌクレイン相互作用と脳領域特異的脆弱性

Spermine- α -synuclein interactions and regional vulnerability in synucleinopathies

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The pathological accumulation of α -synuclein (α Syn) aggregates is a hallmark of synucleinopathies, including Parkinson's disease (PD) and multiple system atrophy (MSA). Although altered polyamine metabolism has been implicated in these diseases, the regional distribution of polyamines in the human brain and their contribution to α Syn aggregation remain unclear. Using mass spectrometry imaging combined with immunohistochemistry, we found that spermine shows a distinct physiological enrichment pattern in the human brain, and that phosphorylated α Syn pathology preferentially occurs in spermine-enriched regions. LC/MS/MS further

revealed disease-associated alterations in individual polyamine species, particularly spermine. In cells, exogenous spermine enhanced pSyn accumulation only under proteostasis-impaired conditions. In vitro, polyamines interacted weakly with the α Syn C-terminal region and altered its conformation, but showed only modest effects on aggregation under physiologically relevant ionic conditions. These findings suggest that spermine alone is insufficient to drive α Syn pathology, but may provide a permissive regional environment that facilitates aggregation in the presence of additional disease-related stressors.

SS1-4

ネオニコチノイド系殺虫剤が海産甲殻類アミの繁殖と行動に及ぼす影響

Effects of Neonicotinoid Insecticides on Reproduction and Behavior in the Marine Mysid Crustaceans, *Americamysis bahia*

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Neonicotinoid insecticides, such as imidacloprid (IMI), are widely used globally because of their high safety for mammalian health. However, the environmental leakage of these insecticides raises concerns regarding their impact on non-target organisms, including marine organisms. Therefore, this study evaluated the chronic effects of IMI on the reproduction and behavior of the marine crustacean *Americamysis bahia*. A 21-day reproduction test was conducted using control, solvent control, and IMI exposure groups ranging from 2.5 to 20 µg/L. At the end of the exposure period, behavioral analysis was performed. The ovaries and brood pouches of the females were examined microscopically. In a reversibility test, mysids exposed to IMI for 1–3 weeks were transferred to seawater without IMI. The results showed a concentration-dependent decrease in neonates, until reaching a complete absence in the

10 and 20 µg/L groups. Although ovarian development was observed even at higher concentrations, the eggs in the brood pouches were either unfertilized or absent. Behavioral analysis revealed a significant decrease in both total movement distance and average swimming speed, accompanied by an increase in the wall-staying rate at higher concentrations. Furthermore, the reversibility test demonstrated complete recovery of reproductive abilities at 7 days after the mysids were transferred to clean seawater, indicating that the adverse effects of IMI on mysid reproduction were reversible. These findings suggest that IMI inhibits reproduction by disrupting reproductive behavior rather than inhibiting oogenesis. Further studies should investigate sex-specific behaviors, male spermatogenesis, and impacts on the next generation to further elucidate these mechanisms.

C1-1

医学者向けの探索メタボロミクス
～LC/MSは代謝物の夢を見るか？～

Nontargeted Metabolomics for Medical Researchers ～Does LC/MS Dream of Metabolites?～

Authors: Akio Hayashi

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Abstract: Recent advances in LC/MS have rendered the technology increasingly accessible to researchers without specialized training in analytical chemistry, thereby facilitating broader investigation of metabolic processes. This session presents a nontargeted metabolomics analysis designed to

comprehensively detect metabolites in datasets related to inflammatory bowel disease. The technologies employed to generate these findings are also introduced, and the implications of artificial intelligence for the field are discussed.

C1-2

トラップドイオンモビリティー質量分析計を用いた次世代PFAS分析

Next level PFAS detection and annotation using Trapped Ion Mobility Spectrometry (TIMS)

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講演要旨: 2016年にリリースされたトラップ型イオンモビリティー質量分析計「timsTOF」は、プロテオミクスやメタボロミクス分野を中心に幅広く活用され、多くの研究現場で導入が進んでいます。イオンモビリティー分離は、オミクス解析にとどまらず、医薬品、機能性食品、さらには環境汚染物質分析においても優れた分離能力を発揮し、新たな知見の獲得に貢献しています。

近年、環境汚染物質として世界的な関心を集めるPFAS (Per- and Polyfluoroalkyl Substances) には、多数の構造異性体が存在し、その多くは依然として未同定のままとなっています。このような複雑な化合物群の解析において、イオンモビリティー分離は有効なアプローチとして期待されています。

本講演では、timsTOFの特長を活かしたPFAS分析事例を紹介するとともに、イオンモビリティー技術がPFASの検出・同定にどのように貢献するのかについてご紹介します。

Abstract: The "timsTOF," Trapped Ion Mobility Spectrometry released in 2016, is widely utilized—particularly in the fields of proteomics and metabolomics—and is being adopted in numerous research settings. Beyond omics analysis, ion mobility separation demonstrates exceptional separation capabilities in areas such as pharmaceuticals, functional foods, and the analysis of environmental pollutants, thereby contributing to the acquisition of new insights.

In recent years, PFAS (per- and polyfluoroalkyl substances) have garnered global attention as environmental pollutants; however, they comprise numerous structural isomers, many of which remain unidentified. Ion mobility separation holds promise as an effective approach for analyzing such complex groups of compounds.

This presentation will introduce examples of PFAS analysis leveraging the unique features of the timsTOF and explain how ion mobility technology contributes to the detection and identification of PFAS.

固体ナノポア1分子計測による非標的異常検知
— One Healthのための早期警戒基盤

Target-Agnostic Anomaly Detection by Solid-State Nanopore Single-Molecule Sensing: An Early-Warning Platform for One Health

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Background: One Health threats, such as emerging pathogens, new pollutants and diseases in livestock and wildlife, often first appear as subtle, unanticipated changes in molecular composition. Conventional methods (immunoassays, PCR, LC-MS/MS) are highly sensitive but target-specific: they ask only whether a predefined molecule is present, and cannot respond to what has not been anticipated. We propose a complementary paradigm: measuring the molecular population of a sample one molecule at a time and detecting deviations from a normal baseline without specifying a target.

Method: Each molecule passing through a solid-state nanopore (a nanometer-scale pore in a thin SiN membrane) produces a transient ionic-current blockade. Thousands of label-free events are recorded, and the distribution of their waveform features is treated as a single-molecule fingerprint of the sample. The fingerprint is enriched by the Voltage Matrix method (measurement at multiple voltages)

and a 16-channel parallel nanopore array, then classified by machine learning with cross-individual validation.

Result: In a mouse model of colitis-associated colorectal cancer (AOM/DSS), serum fingerprints discriminated tumor-induced from control animals as early as one month after induction, and we are now testing whether cancer can be separated from chronic inflammation, which single biomarkers cannot reliably do. In environmental analysis, we detected PFAS with solid-state nanopores for the first time to our knowledge: ~1-nm pores stabilized in CaCl₂ yielded blockade events from a 26-component PFAS mixture, with event frequency scaling with concentration; coupling with fluoruous solid-phase extraction is under way to reach regulatory levels. I will discuss how this target-agnostic approach can serve as an early-warning platform linking animal, environmental and human health, and the challenges toward field deployment.

SS2-1

固体ナノポアを用いた単分子識別技術

Solid-State Nanopore Technology for Single-Molecule Identification

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Abstract: Long-read DNA sequencing with high throughput at low cost is strongly anticipated especially for the advancement of personalized medicine. To meet this requirement, DNA sequencing with nanopores has been attracting a lot of attention. Nanopore technology can be categorized broadly into two types according to the constituent materials of the nanopore. One is “biological”, i.e., nanopores that are formed with biological molecules. The other is “solid-state”, i.e., nanopores that are formed with semiconductor-related materials (“solid-state nanopores”). Solid-state nanopores have advantages in terms of robustness and possible large-scale integration. However, many issues must be resolved to realize DNA sequencing with solid-state nanopores. In particular, from the standpoint of device fabrication and DNA manipulation, we believe that there are three key issues to be resolved: (1) stable fabrication of a small nanopore with a diameter on the same order as that of DNA, (2) fabricating an ultrathin membrane, (3) decreasing the DNA

translocation speed through the nanopore. In this study, we address these three issues.

Regarding the stable nanopore fabrication, we developed a technique called Multilevel Pulse-Voltage Injection (MPVI). The nanopore-fabrication mechanism of MPVI is based on the dielectric breakdown phenomena of a membrane induced by a high electrical-field stress. This technique enables the reliable fabrication of nanopores with diameters of 1–3 nm with sub-nm precision. For the fabrication of ultrathin membranes, we developed a poly-Si sacrificial-layer process that enables the fabrication of Si_3N_4 membranes with a thickness of approximately 2.6 nm. To slow DNA translocation through the nanopore, we successfully demonstrated direct control of DNA motion within the nanopore using a piezoelectric actuator. By combining these techniques, we successfully obtained, for the first time using a solid-state nanopore, signals reflecting the base sequence of a DNA strand.

SS2-2

化学物質の複合曝露が引き起こす神経毒性の新規評価法の開発：AOP-based approachによる相乗作用の同定

A Novel AOP-Based Approach for Assessing Neurotoxicity of Chemical Mixtures: Identification of Synergistic Effects

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In modern life, humans are exposed daily to hundreds of chemicals, some of which are known to contribute to the onset of various diseases. However, conventional toxicity assessments are generally conducted on a chemical-by-chemical basis and therefore fail to evaluate the risks associated with low-level exposure to multiple chemicals. In this study, we aimed to systematically evaluate the synergistic neurotoxicity of multiple pesticides based on an Adverse Outcome Pathway (AOP)-based approach.

Using LUHMES cells, a dopaminergic neuronal cell model, we established an *in vitro* assay platform to enable high-throughput assessment of chemical-induced alterations in common key events associated with neurotoxicity, including mitochondrial membrane potential, oxidative stress, and lysosomal activity. Based on the results of screening 12 pesticides from five major pesticide classes widely used in Japan, we identified groups of chemicals exhibiting similar biological effects. The

dose-response relationships of neurite length following single and combined exposures were used to calculate Combination Index (CI) values and evaluate additive and synergistic interactions.

Organophosphate, phenylpyrazole, pyrethroid, and diamide pesticides exhibited similar concentration-dependent effects, including decreased mitochondrial membrane potential, increased oxidative stress, and reduced lysosomal activity. Notably, combined exposure to pyrethroids with either organophosphate or phenylpyrazole pesticides resulted in strong synergistic effects, as indicated by low CI values. These findings demonstrate that combined exposure to pesticides at concentrations that produce little or no detectable effects individually can induce neurotoxicity and synergistically increase the potential risk to the central nervous system. Our study provides the first systematic identification of pesticide combinations that exhibit synergistic neurotoxicity based on shared cellular pathways.

SS2-3

化学物質混合曝露における注目すべきグループ・成分の探索

Prioritizing Groups and Components in Chemical Mixtures

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Humans are simultaneously exposed to a wide range of environmental chemicals, including emerging substances such as novel PFAS, pesticides, plasticizers, and VOCs whose health effects remain poorly characterized. As comprehensive (e.g., non-target) analyses expand the range of measurable chemicals, statistical methods that can evaluate the health effects of combined exposure to chemical mixtures are increasingly needed.

Although several mixture analysis methods have been developed (Bobb et al., *Environ Health*, 2018; Keil et al., *Environ Health Perspect*, 2020), none is tailored to the high-dimensional data generated by comprehensive analyses, and approaches for incorporating compound information such as chemical structure have yet to be established.

Against this background, we developed Sparse Group Lasso Weighted Quantile Sum regression (SGL-WQS), which combines WQS regression with SGL regularization. Given predefined chemical groups (e.g., by chemical structure), it explores

relevant groups and chemicals within them while simultaneously estimating the direction (positive or negative) of their effects (Kawashima et al., *Environ Int*, 2026).

At present, SGL-WQS supports only continuous and binary outcomes. We believe it will need to be extended to multinomial and ordinal outcomes, count data, and longitudinal study designs. Application to omics data, beyond chemical exposure data, is another important potential use. This presentation will therefore also discuss prospects for these future extensions.

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SS2-4

無卵殻培養（**ex ovo**）ニワトリ胚を用いた
TCEPの発生毒性評価と作用機序解析

Developmental Toxicity and Mechanistic Analysis of TCEP Using a Shell-less (**ex ovo**) Chicken Embryo Model

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Background: Tris(2-chloroethyl) phosphate (TCEP) is an organophosphorus flame retardant detected in the tissues and eggs of wild birds. However, its developmental toxicity in avian embryos and underlying mechanisms remain unclear. This study investigated TCEP-induced developmental toxicity and its mechanisms using a shell-less (**ex ovo**) chicken embryo model enabling continuous in situ observation.

Methods: Chicken embryos at Hamburger-Hamilton stage (HH) 1 were exposed to TCEP at 0, 50, 250, or 500 nmol/g egg. Survival, embryonic growth, heart rate, and extraembryonic vascular development were assessed on incubation days 3–9. The cardiac transcriptome was analyzed by RNA sequencing on day 5, and angiogenesis-related genes in vitelline membranes were examined by RT-qPCR. Genes involved in epithelial-mesenchymal transition (EMT) and mesoderm differentiation were also examined in HH4 blastoderms.

Results: TCEP exposure reduced survival and delayed embryonic growth, with decreases in body length, body weight, and skeletal length. Heart rate was reduced, and extraembryonic blood vessel formation was suppressed even at 50 nmol/g egg, indicating cardiovascular toxicity. Cardiac transcriptome analysis showed decreased expression of NKX2-5 and cardiac conduction-related genes. TCEP also downregulated genes involved in FGF and VEGF signaling in vitelline membranes. In HH4 blastoderms, the expression levels of FGF4, SNAI2, TBXT, and TBX6 were reduced, suggesting inhibition of EMT and mesoderm differentiation during gastrulation. These findings suggest that disruption of early EMT and mesoderm differentiation contributes to impaired cardiac and vascular development and developmental delay. The shell-less chicken embryo model is useful for linking phenotypic toxicity with molecular mechanisms.

P2-1

PFDA曝露によるミナミメダカに対する繁殖影響とその潜在的阻害メカニズムの解明

Reproductive Effects of PFDA Exposure on Medaka and Its Potential Inhibitory Mechanisms

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Per- and polyfluoroalkyl substances (PFAS) are widely used in industrial applications owing to their thermal and chemical stability and superior water and oil repellency. However, certain PFAS, including perfluorooctane sulfonate (PFOS, C8), perfluorooctanoic acid (PFOA, C8), and long-chain perfluorocarboxylic acids (LC-PFCAs, C9–C21), are regulated under the Stockholm Convention on Persistent Organic Pollutants because of their persistence, bioaccumulation, and toxicity. In contrast to PFOS and PFOA, there is limited information on the potential effects of LC-PFCAs on fish reproductive health. In this study, we examined the reproductive effects of perfluorodecanoic acid (PFDA, C10) on medaka (*Oryzias latipes*) exposed to 0.74, 2.2, 6.7, and 20 μ M for 21 days. Transcriptome analyses of the livers and gonads were also performed. The reproduction assay revealed no effect on the total number of eggs spawned during the 21-day exposure period; however, significant differences were observed in the number of fertilized eggs (20 μ M) and the fertilization rate (6.7 and 20 μ M). After the 21-d exposure period, although no

effect on the gonadosomatic index (GSI) was detected in either sex, a concentration-dependent increase in the hepatosomatic index (HSI) was noted at concentrations ≥ 2.2 μ M in both males and females. Transcriptome analyses demonstrated that various pathways, such as oxidative phosphorylation, tyrosine metabolism (involved in the production of thyroid hormones), and sphingolipid metabolism (involved in the acquisition of fertilizing capacity and sperm motility), were affected in the testicles exposed to 20 μ M PFDA. In contrast, pathways related to energy metabolism, lipid metabolism, and amino acid metabolism were affected in the ovary, suggesting that these pathways are involved in the suppression of yolk accumulation, steroid hormone synthesis, and oocyte maturation. Hepatic mRNA-seq analysis documented that pathways related to fatty acid degradation were affected in both sexes in the 2.2, 6.7 and 20 μ M groups, suggesting that these changes reduced fatty acid β -oxidation and contributed to hepatomegaly. These findings elucidate the reproductive effects and potential inhibitory mechanisms of PFDA for the first time.

P2-2

ネオニコチノイド系殺虫剤曝露と新奇環境負荷後のマウス脳におけるLC/TQ/MSを用いたモノアミン濃度変動の解析

Analysis of Changes in Monoamine Concentrations in the Mouse Brain Following Neonicotinoid Insecticide Exposure and Novel Environment Stress by Using LC/TQ/MS

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Background: Neonicotinoids (NNs) can induce mammalian neurotoxicity. However, their interactions with environmental stress remain unclear. This study investigated how combined exposure to acetamiprid (ACE) and novel environment affects brain serotonin (5-HT) changes in mice to unmask this latent neurotoxicity.

Method: C57BL/6JJcl mice were administered a single oral dose of ACE (130 mg/kg) or a vehicle, with or without a subsequent Elevated Zero Maze (EZM) test (four groups). Immediately following the test, brain tissues from seven regions were collected. We quantitatively profiled 5-HT concentrations using liquid chromatography triple quadrupole mass spectrometry (LC/TQ/MS; Agilent 6495) following primary amine-selective derivatization (Py-Tag for Histamine).

Result: Behaviorally, the ACE-exposed mice subjected to the EZM exhibited a profound reduction

in total moving distance, indicative of severe defensive freezing behavior. Neurochemically, while ACE exposure alone significantly increased 5-HT levels across all brain regions, the superimposition of EZM stress amplified 5-HT concentrations specifically in the brain stem, thalamus, and striatum.

Discussion: These findings suggest that ACE-induced direct stimulation of nicotinic receptors primes the serotonergic system. We hypothesize that subsequent excitatory input from stress-related circuits overwhelms local inhibitory controls, triggering a robust 5-HT release. This excessive 5-HT influx into the thalamus may disrupt sensory gating, which then propagates to the striatum to induce freezing behavior. Consequently, this study highlights that the combination of chemical exposure and environmental stress can unmask the latent neurotoxicity of NNs.

P2-3

肝臓試料を対象とした前駆体PFASの分析法開発
および野生鳥類のPFAS蓄積評価

Development of an analytical method for PFAS precursors in liver and assessment of PFAS accumulation in wild birds

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Background: While PFAS are widely used and highly persistent, information on diverse PFAS, including LC-PFCAs and precursor compounds, remains limited. Our previous study analyzed 33 PFAS compounds in six bird species, including aquatic and terrestrial birds (Seimiya et al., Environmental Chemistry Symposium, 2026). In this study, we developed an analytical method for 42 PFAS compounds, comprising the 33 previously targeted PFAS plus five FTCAs, three FTUCAs, and FOSA, and applied it to grey heron liver samples.

Methods: Recovery tests for FTCAs and FTUCAs were conducted using our previously established method. For FOSA, SPE eluates were collected separately as methanol and 1% ammonia in methanol fractions, and recovery was evaluated in the methanol fraction.

Results and Discussion: FTCAs and FTUCAs showed good recovery (70–120%). FOSA was

successfully analyzed using the methanol fraction, although further cleanup optimization is required due to substantial matrix interference. Of the 42 PFAS compounds, 18 were detected in grey heron liver. Among the newly added compounds, 7:3FTCA (7.59–19.3 ng/g), 5:3FTCA (N.D.–0.57 ng/g), and FOSA (0.18–3.22 ng/g) were detected. Among the seven bird species, aquatic species (grey heron, osprey, black-tailed gull, and herring gull) tended to have higher total PFAS concentrations than terrestrial species. PCA indicated two distinct groups among the aquatic birds based on PFAS composition: grey heron and osprey, and black-tailed gull and herring gull. These composition differences may reflect variation in foraging environments and prey organisms.

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C2-1

LC-MSの最新ソリューション情報

The Latest LC-MS Solutions

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Background: LC-MS is an analytical technology that combines the high separation capability of liquid chromatography with the high sensitivity and selectivity of mass spectrometry. In recent years, the need to rapidly and accurately identify and quantify trace components in complex samples has increased across a wide range of fields, including pharmaceutical development, biopharmaceuticals, clinical diagnostics, and food and environmental analysis. To meet these demands, LC-MS has become an essential platform technology that enables applications ranging from the structural analysis of unknown compounds to simultaneous multi-component and quantitative analyses. At the same time, further advances are required in sensitivity, throughput, ease of operation, and data analysis efficiency. Since Shimadzu Corporation entered the field of mass spectrometry with the introduction of the LKB-9000 magnetic-sector GC-MS system in 1970, the company has conducted research and developed products in a wide range of

MS-related fields, including GC-MS, MALDI, LC-MS, and MS imaging. Shimadzu has accumulated more than 50 years of experience in mass spectrometry. This presentation will introduce the latest technological trends and solutions in LC-MS, including the LCMS™-TQ RX Series of triple quadrupole mass spectrometers; Peakintelligence™, a deep-learning-based peak-picking algorithm; PERFORMANCE CONCIERGE™, a self-diagnostic function for mass spectrometers; and “Ready to Use Method” method packages. It will also discuss how these solutions can be used to improve the efficiency and accuracy of analytical workflows and create new analytical value.



Fig. 1 LCMS-8060RX

C2-2

LC-MSを用いた化合物検出/同定手法の進展

Recent Advances in LC-MS Techniques for Compound Detection and Identification

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Growing concerns regarding pharmaceuticals, pesticides, PFAS, and other emerging contaminants have increased the demand for analytical technologies capable of sensitive detection and confident identification of both known and unknown compounds. High-resolution LC-MS/MS has become an essential tool for environmental screening, enabling target, suspect, and non-target analyses within a single workflow.

ZT Scan DIA 3.0 utilizes continuous narrow isolation windows of approximately 1 Da to acquire comprehensive MS/MS data while maintaining high precursor selectivity. Compared with conventional DIA approaches, this strategy reduces spectral interference from co-eluting compounds and generates cleaner fragmentation spectra from complex environmental samples. The resulting datasets also support retrospective analysis.

The Analytics workspace in SCIEX OS software integrates accurate mass measurements, isotope patterns, retention time information, and MS/MS spectral matching to facilitate compound

identification. Spectral library searching enables rapid confirmation of known compounds, while computational structure elucidation tools can predict molecular formulas and structural candidates for compounds not represented in spectral libraries. This combined strategy enhances the characterization of unknown contaminants and transformation products.

Electron Activated Dissociation (EAD) provides complementary structural information to conventional Collision-Induced Dissociation (CID). EAD generates structure-specific fragment ions that improve the differentiation of isomers, enabling more confident compound identification. By combining ZT Scan DIA 3.0, SCIEX OS Analytics, spectral library searching, computational structure elucidation, and EAD fragmentation, a comprehensive workflow can be established for environmental contaminant screening and identification.

This presentation will highlight the principles of these technologies and present a variety of application examples across different analytical fields.

C2-3

PFAS分析を支える試薬・標準品・前処理技術の最新動向

Latest Trends in Reagents, Standards, and Pretreatment Technologies Supporting PFAS Analysis

関東化学株式会社 札幌営業所 森太紀

KANTO CHEMICAL CO., INC.
Sapporo Sales Office Taiki Mori

近年、有機フッ素化合物（PFAS）は環境中に広く存在する難分解性化学物質として社会的関心が高まっている。特にペルフルオロオクタンスルホン酸（PFOS）およびペルフルオロオクタン酸（PFOA）については、水道水や公共用水域、地下水等における監視体制が強化され、分析ニーズが急速に拡大している。また、ペルフルオロヘキサンスルホン酸（PFHxS）をはじめとする関連物質についても調査対象が拡大しており、今後はより高感度かつ高精度な分析体制の構築が求められている。

PFAS分析では、極微量レベルでの定量が求められるため、分析装置の性能のみならず、試料前処理から測定に至るまでの全工程においてバックグラウンド汚染の低減と品質管理が重要となる。特に、固相抽出工程で使用する抽出溶媒やLC/MS用移動相溶媒にPFAS由来の汚染が存在すると、測定精度に大きな影響を与える可能性がある。

本発表では、LC/MS分析に用いられる高純度溶媒について、PFAS試験適合性に加え、金属保証およびパーティクル保証を付与した「-Plus-」シリーズを紹介する。これらの製品は、イオンサプレッションの抑制やカラム寿命の延長、分析再現性の向上など、PFAS分析の信頼性向上とランニングコスト低減の両立を目的として開発された。また、PFASブランクを厳格に管理した前処理用溶媒として、PFAS試験用メタノールおよびPFAS試験用蒸留水を取り上げる。これらの製品は、水質基準および要検討項目に設定されているPFAS成分について極めて低いレベルで管理されており、高感度分析におけるバックグラウンド低減に貢献する。

PFAS規制や分析対象物質は、今後も拡大・高度化していくことが予想される。分析精度の向上、品質保証および作業効率化を支援する最新の製品ならびに技術動向を紹介し、PFAS分析の信頼性向上に向けた取り組みについて報告する。

C2-4

固相抽出法を用いた試料前処理の最新情報

The latest advances in sample preparation using solid-phase extraction.

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Affiliations: ¹GL Sciences Inc.

Background: As analytical instruments have become increasingly sensitive in recent years, sample preparation techniques are now expected to meet a wider range of requirements beyond simple sample concentration, including simplicity, speed, and the removal of interfering matrix components. Accordingly, there is growing demand for simplified cleanup methods in solid-phase extraction (SPE). Approaches such as direct sample loading onto SPE columns and QuEChERS methods, in which sorbents are dispersed directly into samples, have attracted considerable attention. In this presentation, we discuss the analysis of pesticide residues using an SPE column packed with a mixture of different sorbents and demonstrate how the addition of magnesium sulfate can further improve cleanup efficiency.

Method: The performance of the method was evaluated by conducting a matrix-spike recovery test for 169 pesticides using GC-MS. Sample extraction was performed according to the QuEChERS (EN) method. For cleanup, an InertSep AL-N/VRA-PR column (400 mg/1,600 mg, 6 mL; GL Sciences Inc.)

was used. This column is designed for rapid pesticide residue analysis and contains three cleanup sorbents (C18, SAX, and PSA) layered beneath the AL-N sorbent. A standard pesticide solution was added to the acetonitrile extract obtained using the QuEChERS (EN) method, and the resulting solution was passed through the SPE column. The purified extracts were subsequently analyzed by GC-MS.

Results: Pesticide recoveries were evaluated by GC-MS. Pesticides were spiked into spinach and barley extracts at a concentration of 0.1 µg/mL and purified using the InertSep AL-N/VRA-PR column (n = 3). Recoveries within the acceptable range of 70–120% were obtained for 86 of the 169 pesticides in spinach and 99 of the 169 pesticides in barley. In an additional experiment, an SPE column with 1 g of anhydrous magnesium sulfate packed above the sorbent layer was prepared and evaluated under the same conditions. With the addition of magnesium sulfate, the number of pesticides with recoveries within the 70–120% range increased to 162 in spinach and 155 in barley.

SS2-5

鳥類グルクロン酸抱合酵素（UGT）の機能と*in silico*予測

Function of avian UDP-glucuronosyltransferase and prediction in silico approach

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Background: Vertebrates possess a defense system that excrete foreign chemicals entering the body. UDP-glucuronosyltransferase (UGT) is involved in this defense system by transferring glucuronic acid from UDP-glucuronic acid to exogenous chemicals. In birds, despite the great need to elucidate foreign substance metabolic capacity—from the perspective of treatment of rare species to the prediction of susceptibility to environmental pollutants—many aspects of UGT expression regulation and substrate affinity remain unclear. Therefore, in this study, to predict the functions of the avian UGT1 and UGT2 families, we used chickens as a model avian species and investigated AhR-mediated induction of each UGT isoform, their affinity for acetaminophen (APAP), and the construction of predictive models through *in silico* analysis.

Method:

We estimated the basal expression levels of UGT isoforms in chicken liver by obtaining and reanalyzing

RNA-seq data from public databases. Additionally, we orally administered sudan III to chickens for 3 days and collected their livers and small intestines on the 4th day. Then, the expression level changes of each UGT were measured using real-time qPCR. We also expressed each UGT isozyme using a yeast heterologous expression system and measured their glucuronidation activity toward APAP. Furthermore, we constructed a *in silico* model to predict substrate affinity for avian UGTs.

Result: Reanalysis of the RNA-seq data revealed that UGT1E1, UGT1E3, UGT1E5, and UGT2-1 showed high basal expression levels. Meanwhile, it was demonstrated that UGT1E8 is induced by sudan III in male livers. In APAP metabolism, high conjugation activity was observed for UGT1E11 and UGT2-1. Furthermore, we constructed an *in silico* analytical model consistent with the APAP conjugation activity of each UGT.

P2-4

ヘム鉄または非ヘム鉄の継続摂食が鉄吸収・組織分配およびメタロームに及ぼす影響

Dietary Intake of Heme or Non-Heme Iron and Its Effects on Iron Absorption, Distribution, and the Whole-body Metallomes

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Background: Iron is an essential trace element for living organisms. However, the limited bioavailability of dietary iron can often lead to iron deficiency in both wild and domesticated animals. Dietary iron is broadly classified into heme iron, which is abundant in animal-derived foods, and non-heme iron, which is the predominant form in plant-derived foods. Although heme iron is generally more efficiently absorbed through the intestinal mucosa than non-heme iron, the effects of dietary iron form on subsequent tissue iron distribution and trace element homeostasis remain poorly understood. Here, we investigated the effects of long-term dietary intake of heme and non-heme iron on iron absorption, tissue distribution, and the whole-body metallome.

Methods: Male C57BL/6J mice were fed diets containing heme or non-heme iron at 5, 50, or 200 mg Fe/kg for 6 weeks. One week before the end of the feeding period, mice received a single oral dose of either ⁵⁷Fe-labeled FeSO₄ or ⁵⁷Fe-protoporphyrin IX. Trace element concentrations were determined by ICP-MS (Agilent 7850), and iron speciation was

analyzed by X-ray absorption near-edge structure (XANES) spectroscopy. The hepatic metabolome and lipidome were also analyzed using LC-QTOF/MS (Agilent 6546).

Results: Dietary iron form significantly affected four elements in erythrocytes, ten elements in the liver, and splenic Fe concentration ($p < 0.05$), whereas dietary iron concentration affected one, eleven, and two elements in these tissues, respectively. Metabolomic and lipidomic analyses of the liver showed no significant differences among the dietary groups. XANES analysis indicated a higher proportion of Fe(III) species in the liver and spleen of the non-heme iron group than in the heme iron group at 200 mg Fe/kg. In addition, ⁵⁷Fe tracer analysis showed greater hepatic distribution of newly absorbed iron in the non-heme iron group. These findings indicate that dietary iron form influences iron speciation and the tissue distribution of newly absorbed iron and is accompanied by changes in the tissue metallome.

P2-5

グルクロン酸抱合酵素（UGT）の鯨類における
遺伝子発現および代謝活性解析

Gene Expression and Metabolic Activity Analysis of UDP-Glucuronosyltransferases (UGTs) in Cetaceans

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Background: Cetaceans are apex predators in marine ecosystems, and there are concerns about the toxic effects resulting from the accumulation of persistent organic pollutants and other substances. While it has been suggested that cetaceans may have reduced or absent XMEs, such as UDP-glucuronosyltransferase (UGT), their specific expression profiles remain unknown. In this study, we conducted a comprehensive evaluation of cetacean glucuronidation capacity by performing interspecies comparisons of UGT gene coding regions and sequences using genomic data, quantifying gene expression levels via RNA-sequencing and conducting metabolic activity assays on fresh cetacean liver samples.

Method: For inter-species genomic comparisons, we obtained data on UGT genes from mammals, including cetaceans, from NCBI and performed

phylogenetic and copy number analyses. In in vitro assays, we used fresh cetacean liver samples to conduct UGT metabolic activity assays using 1-OH-pyrene and β -estradiol as substrates, and measured and analyzed the conjugated metabolites by LC-MS/MS. In addition, we performed RNA sequencing on several of the liver samples to quantify gene expression levels.

Result: Cetaceans exhibited a reduction or absence of the UGT1A/2B subfamily, including UGT1A6, and were characterized by the presence of UGT1A1 and UGT2Es. Furthermore, UGT1A1-like and UGT2Es genes accounted for a large proportion of total gene expression, and metabolic activity assays suggested that cetaceans tend to have glucuronidation activity as low as that of cats. It is possible that UGT2Es play some metabolic role in cetaceans.

P2-7

鳥類におけるグレリン関連遺伝子の比較進化学的解析

Comparative evolutionary analysis of ghrelin-related genes in birds

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Background: The Ghrelin-system is widely conserved across vertebrates, and plays a role in energy homeostasis. It consists of four genes, namely ghrelin-coding gene (GHRL), MBOAT4, GHSR and LEAP2. Although the system is regulated by all four components, research about regulation of the ghrelin system has historically focused mainly on ghrelin. Previous reports showed the loss of GHRL in a few avian lineages, thus control mechanism of the ghrelin system may differ among avian lineages. However, no one has conducted a comprehensive analysis of the four ghrelin-related genes in birds. The purpose of this study is to uncover interspecific differences of the avian ghrelin system by organising the information of ghrelin-related genes and comparing evolutionary constraints within birds or with mammals.

Method: We analysed GHRL, MBOAT4, GHSR, and LEAP2 in 101 species representing 29 avian orders and conducted selective constraints analyses among dietary categories. In addition to birds, we also conducted the same analyses in 79 mammalian

species representing 24 orders for comparison with birds.

Result: As a result, loss of GHRL and/or MBOAT4 were found in 12 avian orders. In contrast, coding integrity of GHSR and LEAP2 were conserved in all species examined in this study. In selective constraints analysis, the constraints of GHRL and MBOAT4 were relaxed in birds when compared to mammals. However, avian GHSR and LEAP2 demonstrated similar strong purifying pressure as mammals. Further, we detected differences in selective constraints on GHSR and LEAP2 among avian dietary categories. These findings suggest the possibility that the relaxed selective constraint of GHRL and MBOAT4 in avian lineages may have led to gene loss in several avian orders. In contrast, GHSR and LEAP2 were conserved and may have some roles related to food intake. Ultimately, to reveal interspecific differences of the avian ghrelin system, further physiological study about LEAP2 and GHSR would be essential.

P2-8

シマハイエナの腐肉食性に着目した、苦味受容体T2Rの腐肉由来化学物質に対する応答解析

Analysis of Bitter Taste Receptor T2R Responses to Carrion-Derived Compounds with a Focus on the Scavenging Ecology of the Striped Hyena

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Background: Bitter taste is thought to serve as a defense mechanism that influences the ingestion of potentially harmful chemicals. Animals generally tend to avoid foods they perceive as bitter. Thus, when assessing chemical hazards, it is important to consider not only toxicity after ingestion but also the potential effects of taste on exposure. Carnivores possess functional bitter taste receptors (T2Rs), which may help them detect harmful compounds in food. Carrion decomposition produces compounds that humans perceive as bitter. Yet some hyenids readily consume carrion, raising the possibility that their T2Rs respond differently to carrion-derived compounds than those of other carnivores. In this study, we characterized T2R responses to carrion-derived compounds and discussed their potential relationship to scavenging.

Method: T2R genes from the striped hyena (*Hyaena hyaena*) and, for comparison, the domestic cat (*Felis*

catus), both belonging to the suborder Feliformia, were artificially synthesized and cloned into expression vectors. The resulting constructs were transfected into HEK293T cells, and their responses to 22 bitter-related compounds were evaluated using a luminescence-based assay.

Result: Five striped hyena T2Rs responded to carrion-derived compounds. Consistent with previous reports, cat T2R2 showed significant responses to Acesulfame K and Ofloxacin, and striped hyena T2R2 exhibited similar responses to these compounds. Furthermore, striped hyena T2R41 responded significantly to nine chemicals, whereas dog and human T2R41 have been reported to exhibit high ligand selectivity. These results indicate that the striped hyena retains functional bitter taste perception and may have an expanded bitter ligand spectrum.

P2-9

シリアンハムスターにおける自発的エタノール摂取量の個体差と腸内細菌叢の関連

Individual Differences in Voluntary Ethanol Intake and Their Association with the Gut Microbiota in Syrian Hamsters

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Background: Ethanol is a widely consumed neuroactive substance, yet ethanol intake varies considerably among individuals. Recent studies have suggested that the gut microbiota may contribute to alcohol-related behaviors through the microbiota–gut–brain axis, which connects the gut and brain through microbial metabolites and neural pathways. Syrian hamsters (*Mesocricetus auratus*) voluntarily consume relatively high concentrations of ethanol and show marked individual variation in ethanol intake, making them a useful model for studying voluntary drinking behavior. However, little is known about whether the gut microbiota before ethanol exposure is associated with later voluntary ethanol intake. In this study, we therefore examined the association between pre-existing gut microbiota composition and individual differences in voluntary ethanol intake.

Method: Twenty 6-week-old male Syrian hamsters were used. After a 5-day baseline period with two water bottles, 16 animals were given free choice between 15% ethanol and water for 5 days, while 4 controls received water only. Animals were classified as High (n=5), Middle (n=6), or Low (n=5) based on

mean ethanol intake. Daily fecal samples were analyzed by 16S rRNA gene V3–V4 amplicon sequencing. PERMANOVA, LEfSe, and Spearman correlation were used for microbiota analysis. Striatal *Drd1* and *Drd2* mRNA expression was measured by RT-qPCR after the drinking period.

Result: Mean ethanol intake ranged from 10.78 to 22.50 g/kg/day. Baseline gut microbiota composition tended to differ among groups by PERMANOVA. Baseline abundances of *Barnesiella*, *Bacteroides*, *Victivallis*, and *Parabacteroides* were positively associated with later ethanol intake. *Drd1* and *Drd2* expression did not differ among groups, whereas the striatal *Drd1/Drd2* ratio was lower in the High group than in the Low group.

Discussion: These findings suggest that pre-existing gut microbiota differences are associated with individual variation in voluntary ethanol intake. The lower striatal *Drd1/Drd2* ratio in High animals may reflect an intake-related difference in dopamine receptor expression balance, although its biological significance remains unclear. Together, these results support a potential role of the gut microbiota in voluntary ethanol intake in Syrian hamsters.

P2-10

アジアゾウにおけるピレン曝露の検出とUGTおよびSULTを介した抱合代謝能の評価

Detection of Pyrene Exposure and Evaluation of UGT- and SULT-Mediated Conjugation Activities in Asian Elephants

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Background: Understanding xenobiotic metabolism in elephants is essential for interpreting chemical exposure biomarkers. Our previous findings of CYP2E and CYP4A loss highlight the need to characterize elephant xenobiotic metabolism across both phase I and phase II pathways. Pyrene provides a useful model for investigating sequential oxidation and conjugation, with its hydroxylated metabolite, 1-hydroxypyrene (1-OHP), undergoing glucuronidation and sulfation. This study aimed to characterize these conjugation pathways in elephant liver and establish a mechanistic basis for interpreting pyrene metabolites in biological samples.

Methods: We performed in vitro metabolic assays using 1-OHP as a substrate with elephant liver microsomal and cytosolic fractions to evaluate glucuronidation and sulfation, respectively. UGT and SULT gene repertoires and hepatic transcript expression were also examined. We collected blood and urine samples from captive elephants to assess environmental exposure and characterize pyrene metabolites.

Results: Glucuronidation activity toward 1-OHP was greater than sulfation activity under the experimental conditions examined. Sulfation was detectable but appeared to represent a less prominent conjugation pathway. Molecular analyses indicated more identified UGT genes and higher UGT transcript abundance than SULTs, consistent with the observed predominance of glucuronidation. The relative abundance of free and conjugated pyrene metabolites in blood and urine, and the greater contribution of glucuronidation, were also reflected in the other results.

Conclusions: These findings suggest that glucuronidation is the predominant hepatic conjugation pathway for 1-OHP in elephants under the conditions tested. Blood and urine metabolite profiles reflect this predominance of 1-OHP glucuronide, making it a biomarker of pyrene exposure. Further measurements of pyrene depletion and 1-OHP formation will help characterize phase I metabolic capacity and provide a more integrated understanding of pyrene oxidation and subsequent conjugation in elephants.

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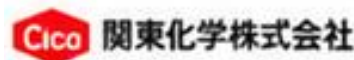
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