

Chemical Hazard Symposium

(Joint Seminar on Environmental Chemistry and Toxicology)



Widens the knowledge and construction of research network!

August 2nd, 2017, 9:30~18:20

AM: Lecture Hall

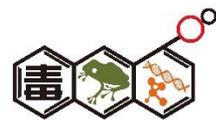
PM: 2nd Lecture Room



北海道大学
大学院獣医学研究科・獣医学部
Graduate School of Veterinary Medicine, Hokkaido University



Hokkaido University
Leading Graduate School
Veterinary Science for One Health



研究拠点形成事業

Participant: Hokkaido University, Ehime University, Kyoto University, Rakuno Gakuen University, Obihiro University of Agriculture and Veterinary Medicine, Chiba University

共催:(一社)日本環境化学会北海道東北地区部会
愛媛大学化学汚染・沿岸環境研究拠点(LaMer)



Chemical Hazard Symposium 2 August 2017

Time	Venue	Presenter	Chair and time keeper	
9:55	Lecture Hall	Opening remark (Prof. Ishizuka)		
10:00		Dr. Rialet	Dr. Ikenaka	
10:20		Dr. Noda	Dr. Mizukawa	
10:40		Dr. Alex		
11:00-11:10		Break		15 min presentation 5 min Q&A
11:10		Dr. Aoshima	Dr. J.Yamazaki	
11:30		Dr. Ichii		
11:50		Dr. Eguchi	Dr. Nomiya	
12:10		Dr. Fujimori		
12:30-13:30		Lunch		
13:30	Tanoue (Ehime)	Tanaka (Hokkaido)		
14:10	Kondo (Hokkaido)	Mukai (Kyoto)		
14:35	Kojima (Kyoto)	Morita (Obihiro)		
15:00-15:10	Break			
15:10	Takaguchi (Ehime)	Yokoe (Rakunou)		
15:35	Yokoe (Rakunou)	Kondo (Hokkaido)		
16:00	Ito (Obihiro)	Kojima (Kyoto)		
16:25-16:35	Break			
16:35	Mukai (Kyoto)	Takaguchi (Ehime)		
17:00	Morita (Obihiro)	Tanaka (Hokkaido)		
17:25	Kraisiri (Som) (Hokkaido)	Ito (Obihiro)		
17:50	Tanaka (Hokkaido)	Tanoue (Ehime)		
18:15	Closing remark (Dr. Nomiya)			
19:00	Banquet			

Participating University

Hokkaido University
 Ehime University
 Kyoto University
 Obihiro University of Agriculture and Veterinary Medicine
 Rakuno Gakuen University
 Chiba University

言語: 基本英語、ただし学部生に関してはスライドのみ英語でもOK
 要旨: 7月上旬に要旨を提出してもらう(A4、1枚、基本英語)
 午前は北大教員による発表、午後は4大学の学生による研究報告会
 午前: 15分発表+5分質疑応答
 午後: 15分発表+10分質疑応答、ディスカッション

HIV antiretrovirals in the aquatic environment of South Africa

Authors: Rialet Pieters¹, Elisca Gerber¹, Danél Fouché¹, Jurie Potgieter¹, Lindri van der Merwe¹, Suranie Horn¹, Tash Vogt¹, Hindrik Bouwman¹

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South Africa is the largest user of antiretroviral drugs (ARVs) in the world (WHO, 2017) because of the high prevalence of the human immunodeficiency virus (HIV) and the consequent acquired immunodeficiency syndrome (AIDS). Not all of the consumed pharmaceuticals are degraded in the human body and find their way to wastewater treatment plants (WWTPs) in slightly modified and even unmetabolised form (Debska *et al.*, 2004). Unfortunately, sewage treatment methods are not highly effective in removing pharmaceuticals which leads to contamination of surface water. Some South African communities have inadequate or failing sanitation facilities contributing to further pharmaceutical contamination of environmental water (Madikizela *et al.*, 2017).

The presence of HIV treatment ARVs in the freshwater environment of South Africa has been confirmed by Wood *et al.*, (2015), ranging between 26.5 and 430 ng/L. And Schoeman *et al.* (2015) reported wastewater effluent concentrations for nevirapine (350 ng/L) and efavirenz (7 100 ng/L) at a WWTP in Gauteng, the country's most populated and geographically smallest province.

At therapeutic levels the ARV medication has many side effects (Meintjies *et al.*, 2014). There is growing evidence of ARVs causing unintentional interference with the patient's endocrine system: ritonavir binds to and inhibits oestrogen receptors (Xiang *et al.*, 2014); efavirenz may help cause enlarged breasts in males (Sikora *et al.*, 2010); and Svärd *et al.* (2014) provided evidence that some of the adverse health effects of ARVs might be caused through nuclear receptor activation including the oestrogen and glucocorticoid receptors.

The question arises: Would non-target aquatic organisms experience endocrine disruptive effects at environmental levels? Here we report on base-line studies involving *in vitro* reporter gene assays to determine endocrine disrupting effects elicited by the active ingredient of six ARVs (stavudine, lamivudine, efavirenz, lopinavir, ritonavir, and nevirapine) commonly used in South Africa. The concentrations range was 0.8, 8, 80, 800, 8 000, and 80 000 ng/mL.

(Anti-)androgen activity (binding to the androgen receptor, AR) was tested with the MDA-kb2 reporter gene assay (Wilson *et al.*, 2002) and activation of the aryl hydrocarbon receptor (AhR) was investigated with the H4IIE-*luc* cells (Aarts *et al.*, 1993). A second mechanism of action—hormone production—was tested with the H295R adrenocortical carcinoma cells: the ability of the target compound to up- or down-regulate oestrogen and androgen levels was investigated (OECD, 2011). A non-hormonal effect was also investigated: the influence of the ARV active ingredients on the viability of human intestinal cells (HuTu). Each of the respective tests were run with the necessary controls and repetitions.

The ARV active ingredients did not activate the AR in the MDA-kb2 cells. However, there was evidence of AR inhibition, and although none was statistically significant, some were practically significant. In some cases, the inhibition was traditionally dose-dependant, eg., efavirenz, and in others, like stavudine, the dose-dependance was U-shaped. There was no activation of the AhR, which agrees with literature. All of the ARV active ingredients influenced the levels of oestradiol and testosterone levels. The exposure concentrations mostly decreased the levels of the respective hormones but not always to the same extent. The target compounds did not inhibit the viability of the human intestinal cells statistically significantly at the concentrations (1, 2, 4, 6, 8 & 10 mg/mL) and exposure periods (12 h, 24 h, & 26 h) investigated. However, there was a tendency for efavirenz to decrease viability at longer exposure periods.

This information was needed to be able to now compare with responses caused by the mixtures found in environmental water.

Aarts *et al.*, 1993. *Organohalogen Compounds* 13:361–364.

Debska *et al.*, 2004. *Critical Reviews in Analytical Chemistry* 34:51–67.

Madikizela *et al.*, 2017. *Journal of Environmental Management* 193:211–220.

Meintjes *et al.*, 2014. *Southern African Journal of HIV Medicine* 15(4):121–143.

OECD <http://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oced/ocedtg456-2011-508.pdf>

Date of access: 28 Feb. 2017.

Schoeman *et al.*, 2015. *Journal of Chromatography and Separation Techniques* 6:272.

doi:10.4172/2157-7064.1000272.

Sikora *et al.*, 2010. *HIV Medicine* 11(9):603–607.

Svärd *et al.*, 2014. *British Journal of Pharmacology* 171:480–497.

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Wilson V, *et al.*, 2002. *Toxicological Sciences* 66:69–81.

Wood *et al.*, 2015. *Environmental Pollution* 199:235–243.

Xiang J *et al.*, 2014. *Experimental Cell Research* 327:318–330.

BIOAEROSOLS AND CHEMICAL SUBSTANCES - COEXISTENCE AND POSSIBLE INTERACTIONS-

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The aerosols with biological components known as bioaerosols can transport a long distance to cause possible health effects to human, livestock, and ecosystem. There are many unknown aspects of bioaerosols to evade degradation in the atmosphere thus the level of possible adverse health effects can vary. An airborne transmission of pathogens such as influenza, tuberculosis, Legionella is a typical example of bioaerosols to cause adverse health effects in a different environment; however, a role of co-existing aerosols is not well understood. Here we present some of the laboratory measurements of the viability of bioaerosols together with different environmental aerosol dust. As a model of airborne bacteria, *Escherichia coli* was used to assess the dust affecting a viability reduction rate of the bacteria in a Teflon reaction chamber. The viability reduction rates of the bacteria were measured with culture method. The bacteria were nebulized into the chamber and mixed with airborne dust materials 1) Phosphate Buffer Solution (PBS) as a control, 2) desert sand from Mongolia, and 3) sludge from the coastal area of Japan. The result of comparisons with PBS control aerosols indicated that the coexistence of DH5 α with desert dust from Mongolia significantly decreased the viability; however, with the sludge dust from Japan significantly increased the viability of the airborne bacteria ($p < 0.05$). The results indicated that the different types of airborne environmental dust influenced the viability of airborne bacteria. The chemical and physical characteristics of environmental aerosol are considered to act as important factors attributing the viability of the bioaerosols. Further understanding of the dust characteristics as co-existing aerosols in the atmosphere may contribute to a health risk assessment of bioaerosols.

ASSESSMENT OF HEAVY METAL POLLUTION IN SOUTHERN NIGERIA: ECOLOGICAL AND HUMAN HEALTH IMPLICATIONS

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Introduction

Heavy metal pollution in aquatic and terrestrial ecosystems is a worldwide environmental problem that has received increasing attention owing to its toxicity, abundance, persistence and subsequent bioaccumulation in biota. In order to assess heavy metal pollution of environmental matrices and selected fauna of southern Nigeria, road side soils and earthworms from high traffic areas (HTAs) of Benin City and Benin River ecosystem were studied in two phases with emphasis on ecological and human health implications. Phase 1 was on the assessment of levels, distribution and ecological risk of heavy metals in road side soils and earthworms from urban high traffic areas while Phase 2 was assessment of heavy metal levels in surface water and selected fauna of Benin River, both in Southern Nigeria. The aim of these studies was to assess the ecological and human health risk of metal pollution of Southern Nigeria.

PHASE 1

Methods

Soil and earthworm samples were collected from three high traffic areas in Benin City and one pristine area away from the city. Digestion of samples was according to AOAC, (2000) and analysis for heavy metals was done using atomic absorption spectrophotometer (Buck Scientific, model VGP 210). Assessment of pollution status of soils was conducted using pollution indices (Hakanson, 1980, Sutherland, 2000, Zhang and Liu 2002).

Results and Discussion

Figure 1 shows box and whisker plot for spatial distribution of heavy metals in HTAs of Benin City.

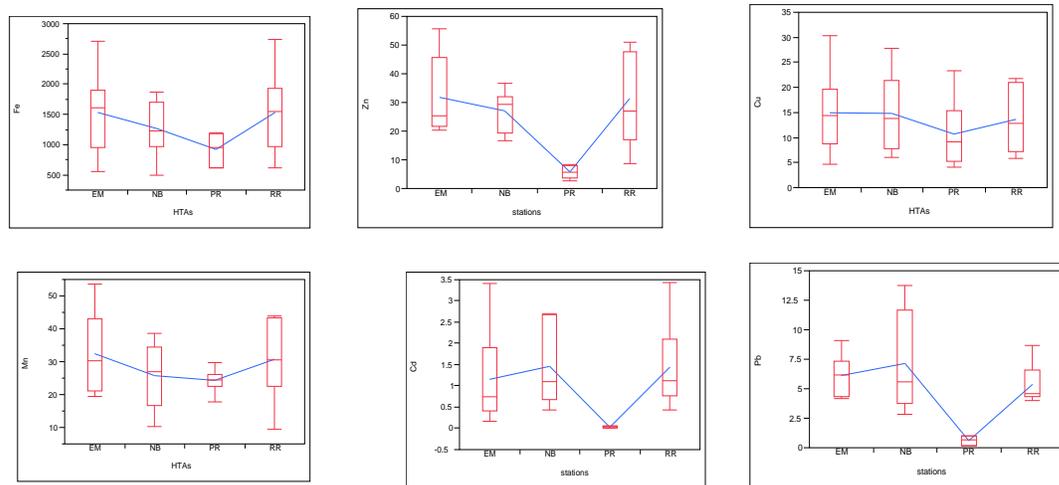


Figure 1: Box and whisker plot for spatial distribution of heavy metals in HTAs of Benin City. EM: Ekiosa Market; NB: New Benin; PR: Pristine; RR: Ring Road. Boxes enclose the 25th and 75th percentiles within which the medians are indicated by a horizontal line. The bars at the ends of the tails mark the extremes.

Contamination Factor (CF) showed very high contamination for Pb and Cd in all HTAs. Enrichment Factor (EF) indicated significant anthropogenic enrichment for Pb as EF for Pb in these locations were $5 \leq EF < 20$. There was an extremely high enrichment for Cd in all the study areas as EF was > 40 . This study also showed that all HTAs studied were polluted by heavy metals as pollution load index, PLI was > 1 . The soils of New Benin, Ring Road and Ekiosa areas showed elevated levels of Zn, Pb and Cd while the Pristine area was low. PCA (figure 2) showed a clear separation of pristine area from sampled HTAs.

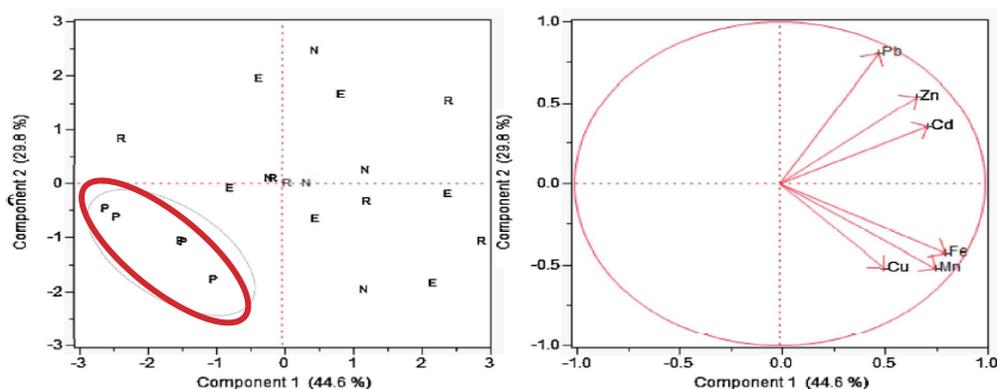


Fig. 2. Distribution patterns heavy metals in the HTAs and pristine area characterized by PCA. P: Pristine area, N: New Benin, E: Ekiosa Market, R: Ring Road.

Bioaccumulation factor (BAF) for earthworms were less than 1 except for Zn at New Benin high traffic area. Potential ecological risk indices (PERI) showed a very high ecological risk for heavy metal pollution across all HTAs.

PHASE 2

Methods

Water, fish (*Brycinus longipinnis*) and shrimp (*Macrobrachium macrobrachion*) samples were collected from three sampling stations of the Benin River with industrial installations and tank farms belonging to petrochemical companies. Digestion of samples was according to AOAC, (2000). Analysis of heavy metal concentrations was carried out using atomic absorption spectrophotometer (Buck Scientific, model VGP 210).

Results and Discussion

Results showed that Fe (0.14mg/l), Cu (0.02mg/l), and Zn (0.01mg/l) were below the recommended safe limits of 0.3, 0.05, 0.01 and 3.0mg/l respectively set by WHO for drinking water while Pb (0.18mg/l) was above the recommended limit of 0.05mg/l. This could be as a result of the anthropogenic activities of industries and petrochemical installations situated at the banks of the river. Cd concentration was exactly at the 0.01mg/l level of WHO safe limit. Mean levels (mg/kg) of Fe (114.78) and Cu (60.83) in shrimp were above FAO/WHO standards of 5 and 0.4mg/kg in food respectively. Similarly, mean levels of Fe (66.13mg/kg) and Cu (7.05) mg/kg in fish were above the FAO/WHO limits (Table 1). EDI for heavy metals in water from dermal exposure were below the reference doses. The hazard quotient (HQ) for water showed that Zn had the lowest value while Pb had the highest (>1) for risk through ingestion. HI was >1 indicating possible health risk from ingestion of water from the Benin river. Target Hazard Quotient (THQ) for Pb was highest in fish with the value of 2.9300 indicating potential health risk through consumption. TTHQs in shrimps and fish were >1 which portends non carcinogenic health risks

through consumption. These species of fish and shrimp have been among the most common and important sources of protein for Koko community that inhabit the area where samples were collected.

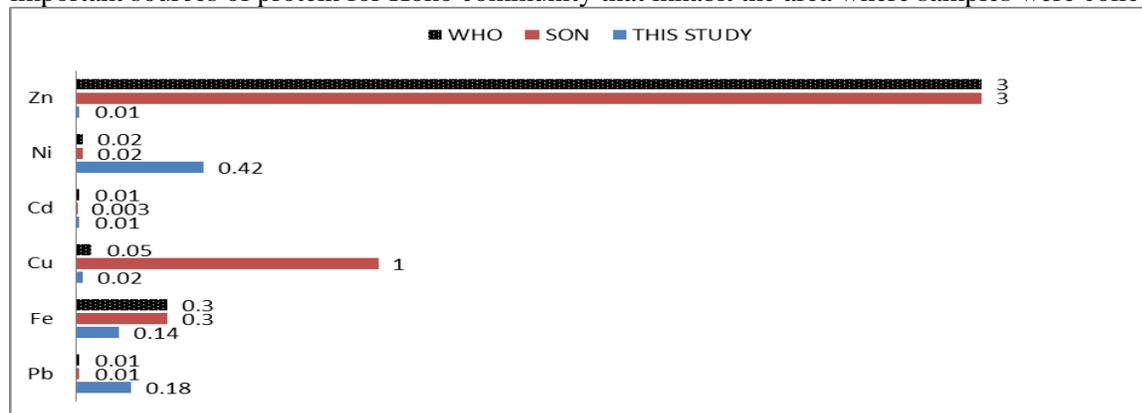


Figure 3 Mean metal levels in water in this study compared with SON and WHO standards

Table 1: Mean levels of heavy metals in shrimp and fish (mg/kg) compared with FAO/WHO Standards

Metal	SHRIMP	FISH	WHO/FAO 2011
Fe	114.78	66.13	5 (Foods)
Cu	60.83	7.05	0.4 (Foods)
Cd	0.93	0.98	2 (Cephalopods)
Ni	33.03	53.57	NA
Pb	22.39	36.04	0.3 (fish)
Co	18.01	31.14	NA

Conclusion

These studies have clearly shown that soils of high traffic areas (HTAs) of New Benin, Ring Road and Ekiosa area showed high levels of Pb and Cd and with EFs >1.5 which indicated anthropogenic enrichment. Also, surface water and fauna of Benin River was polluted by heavy metals especially Pb from industrial activities around the river. Pb showed high levels in shrimp and fish which were above FAO/WHO 2011 standards of 0.3mg/kg for fish. A regular monitoring of the environmental matrices and fauna of Southern Nigeria for heavy metal contamination is pertinent. Eco-friendly policies must be adopted as a matter of urgency. A stricter enforcement of already existing environmental laws is advocated.

References

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A Look at the Relationship between Epigenetics and Environment

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Epigenetics is a mechanism to regulate gene expression without changing DNA sequence. Epigenetic modifications such as DNA methylation and histone modifications activate or repress gene expression depending on the context by responding to various stimuli in the body. It is also possible to react environmental changes and can alter people's life.

If a pregnant woman is in a severe famine, she cannot take lots of food and give enough nutrition to her baby. You can guess her baby would have some effects from this situation but they are not always the same. Fetuses who could not take enough amount of nutrition in the late stage of pregnancy indicated lower weight and lower obesity rate than ordinary people even if they could take a lot of food freely after birth. Only a short period of nutritional deficiency before birth affected their long life. On the other hand, fetuses who could not have good nutrition only in the early stage of pregnancy showed higher obesity rate than common people. Interestingly, in this case, the effect can be seen in the baby's children, which means that it can be transmitted to the mother's grandchildren. Recent research identified epigenetics is highly involved in these phenomena. Epigenetic alteration caused by environmental change in mother's body affected whole life of the baby and even the baby's baby.

Lamarckism has not been accepted for a long time. It has been believed that acquired character cannot be inherited to the next generation because it is unable to affect DNA sequence in germ cells and wasn't proven experimentally. However, the story above implies that some environmental effects can be inherited beyond generations. Some papers succeeded to prove the similar effect in animal models. Based on these information, do you think Lamarckism can be accepted or still unaccepted? Here, I want to share some current information about the relationship between epigenetics and environment, and also want to discuss how environment affects epigenetic regulation and the possibility that environmental effects can be inherited to our descendants through epigenetic mechanisms.

Glomerular injury caused by indoxyl sulfate, a uremic toxin and aryl-hydrocarbon receptor ligand

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Indoxyl sulfate is a uremic toxin and a ligand of the aryl-hydrocarbon receptor (AhR), a transcriptional regulator. Elevated serum indoxyl sulfate levels may contribute to progressive kidney disease and associated vascular disease. We asked whether indoxyl sulfate injures renal glomeruli, especially focusing on podocytes *in vivo* and *in vitro*. Mice exposed to indoxyl sulfate for 8 weeks exhibited prominent tubulointerstitial lesions with vascular damage. Indoxyl sulfate-exposed mice with microalbuminuria showed ischemic changes, while more severely affected mice showed increased mesangial matrix, segmental solidification, and mesangiolysis. In normal mouse kidneys, AhR was predominantly localized to the podocyte nuclei. In mice exposed to indoxyl sulfate for 2 hours, isolated glomeruli manifested increased *Cyp1a1* expression, indicating AhR activation. After 8 weeks of indoxyl sulfate exposure, podocytes showed foot process effacement, cytoplasmic vacuoles, and a focal granular and wrinkled pattern of podocin and synaptopodin expression. Furthermore, vimentin and AhR expression in the glomerulus was increased in the indoxyl sulfate-exposed glomeruli compared to controls. Glomerular expression of characteristic podocyte mRNAs was decreased, including *Actn4*, *Cd2ap*, *Myh9*, *Nphs1*, *Nphs2*, *Podxl*, *Synpo*, and *Wt1*. *In vitro*, immortalized-mouse podocytes exhibited AhR nuclear translocation beginning 30 min after 1 mM indoxyl sulfate exposure, and there was increased phospho-Rac1/Cdc42 at 2 hours. After exposure to indoxyl sulfate for 24 hours, mouse podocytes exhibited a pro-inflammatory phenotype, perturbed actin cytoskeleton, decreased expression of podocyte-specific genes, and decreased cell viability. In immortalized human podocytes, indoxyl sulfate treatment caused cell injury, decreased mRNA expression of podocyte-specific proteins, as well as integrins, collagens, cytoskeletal proteins, and bone morphogenetic proteins, and increased cytokine and chemokine expression.

In conclusion, we propose that basal levels of AhR activity regulate podocyte function under normal conditions, and that increased activation of podocyte AhR by indoxyl sulfate contributes to progressive glomerular injury.

Exploration of lead exposure related biological pathways in Vietnamese acid-lead battery recycling workers

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Lead poisoning is now considered a public health risk, particularly in developing countries. Indeed, health problems from Pb exposure have broken out in many parts of the world, especially near lead mines, lead smelters, and used lead–acid battery recycling plants.

In a previous study, we surveyed Pb levels in the surface soil in Hung Yen, a ULAB recycling village in northern Vietnam, in 2011, 2013, and 2014 ². We noted high Pb levels along battery transportation routes in the smelter or in the battery collection site.² Blood Pb levels in residents of the village (median 34 µg/dL, range 14-122 µg/dL) were also significantly higher than in residents of control sites (median 3.3 µg/dL). ³ Importantly, levels in the former exceed the recommended levels for adults (< 25 µg/dL) as set by the U.S. Department of Health and Human Services ⁴, indicating that health effects from Pb exposure are a concern to these residents, even though toxicological problems, if any, have not been documented.

In this study, we analyzed the urine metabolome of residents in a used lead–acid battery recycling village to investigate the biological effects of Pb exposure. Random forest regression models were moderately predictive of serum Pb, as evaluated by 5-fold cross-validation ($R^2_{CV} = 0.541$, $RMSE_{CV} = 21.6$) and against an external test set ($R^2_{EXT} = 0.420$, $RMSE_{EXT} = 21.5$). In random forest discriminant models, areas under receiver operating characteristic curves, as measured by 5-fold cross-validation ($AUC_{CV} = 0.950$) and against an external test set ($AUC_{EXT} = 0.923$), indicate accurate classification of urine samples from the recycling village and from a reference site. Candidate biomarkers identified at false discovery rate < 0.05 were associated with small-molecule transport,

amino acid metabolism, neuronal function, and gluconeogenesis. Collectively, the results highlight the nephro- and neurotoxicity of Pb.

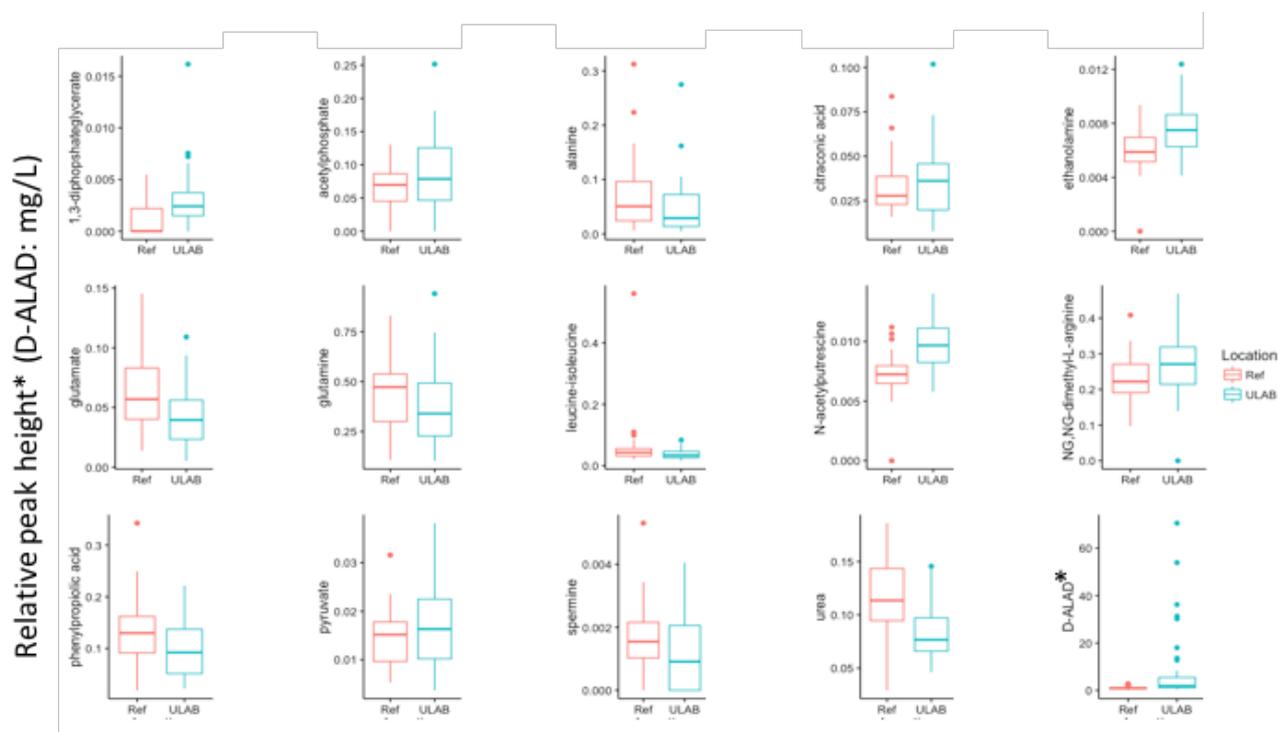


Figure 1. Urinary metabolites significant in both regression and discriminant models of Pb exposure.

*D-ALAD: δ -aminolevulinic acid

Heavy Metals and Organohalogen Compounds from Primitive E-Waste Recycling: Pollution, Distribution, Interplay, and Mechanism Implication

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Recent years, we have studied on local pollution issue by electronic waste (e-waste) recycling in several developing countries such as the Philippines, Vietnam, and Ghana. Primitive e-waste recycling activities including transporting, dismantling, and open burning are serious sources of heavy metals and organohalogen compounds (**Figure 1**).

In this symposium, representative results of this issue by our research group were reviewed. Main findings were summarized as following: (i) *Heavy metals*. Quantitative analyses and hazard assessment suggested high toxic risk of oral exposure to multi-element contaminated soil and dust from e-waste recycling sites around Metro Manila, the Pshilippines (**Figure 1a**). Workers and children exposed to surface matrices should protect themselves from specific toxic metals (especially, Pb and Cu) (Fujimori *et al.*, 2012). On-site X-ray measurement revealed pollution distribution of heavy metals in surface soil at an informal e-waste recycling workshop (Fujimori and Takigami, 2014).

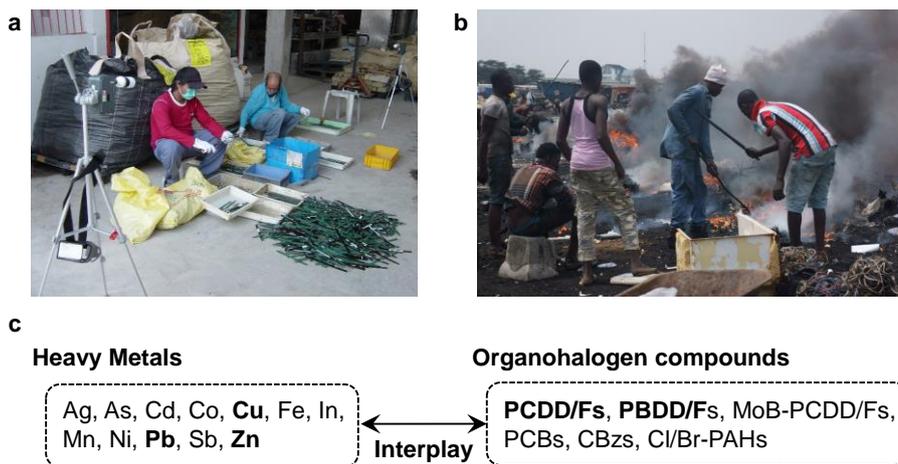


Figure 1 E-waste recycling activities as a source of heavy metals and organohalogen compounds. (a) E-waste dismantling (Philippines), (b) E-waste open burning (Ghana), (c) Interplay of heavy metals with organohalogen compounds in e-waste open burning soil.

(ii) *Organohalogen compounds*. Surface soil at e-waste open burning site in Ghana was a serious sink of heavy metals and chlorinated/brominated aromatic compounds (**Figure 1b**). Except for polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs), biphenyls (PCBs), and chlorobenzenes (CBzs), large part of organic chlorine was unidentified and anthropogenic chlorinated organic compounds generated by e-waste open burning (Nishimura *et al.*, 2014). Soil pollution of chlorinated and brominated polycyclic aromatic compounds (Cl/Br-PAHs) was ubiquitously found in e-waste open burning sites in Vietnam, the Philippines, and Ghana (Nishimura *et al.*, 2017).

(iii) *Interplay and implication*. Cu, Pb, and Zn contained in various e-wastes possibly influenced generation of many dioxin related compounds (DRCs) through e-waste open burning as shown in **Figure 1c** (Fujimori *et al.*, 2016). X-ray absorption spectroscopy revealed chlorides of Cu, Pb, and Zn as main chemical forms in residual soil in e-waste open burning site. Catalytic behavior of specific forms of heavy metals might play key role to generate DRCs (Fujimori and Takaoka, 2009; Fujimori *et al.*, 2009; Fujimori *et al.*, 2011; Fujimori *et al.*, 2013).

Comprehensive understanding of formation and health effect of hazardous compounds derived from e-waste recycling activities will be required in the future. Recently, we have progressed related studies including thermochemical formation mechanism of organohalogen compounds, bioaccessibility by using *in vitro* assay, assessment of unidentified organohalogen compounds, and quantification of insoluble/extractable halogens. These recent findings will be presented by Mr. Yusuke Kojima and Mr. Kota Mukai from our research group in this symposium.

Acknowledgements

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UPTAKE AND METABOLISM OF OPIOID ANALGESIC TRAMADOL IN FISH, AND A LINKAGE BETWEEN INTERNAL CONCENTRATIONS AND BEHAVIOURAL EFFECTS

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Recent species-extrapolation approaches to predict the potential effects of pharmaceuticals present in the environment on wild fish are based on the assumption that pharmacokinetics and metabolism in humans and fish are comparable. To test this hypothesis, we exposed fathead minnows to the opiate pro-drug tramadol and examined uptake from the water into the blood and brain, and metabolism of the drug into its main metabolites. We further examined the linkage between plasma drug concentrations and the effects on fish exploratory behaviour quantified by performing the novel tank diving test. As a result, we found that plasma concentrations could be predicted reasonably accurately based on the lipophilicity of the drug, once the pH of the water was taken into account. The concentrations of the drug and its main metabolites were higher in the brain than in the plasma, and the observed brain/plasma concentration ratios were within the range of values reported in mammalian species. This fish species was able to metabolise the pro-drug tramadol into the highly active metabolite *O*-desmethyl tramadol (T-M1) and the inactive metabolite *N*-desmethyl tramadol (T-M2) in a similar manner to mammals. However, we found that concentration ratios of T-M1 to tramadol were lower in the fish than values in most humans administered the drug (Fig. 1). Statistically significant behavioural effects (e.g., decreased swimming speed in the upper two-third of the novel tank) were observed in fish exposed to 100 µg L⁻¹. In this group, plasma concentrations of tramadol and T-M1 were approximately half and one-tenth of the effective plasma concentrations reported in humans, respectively. However, this effect was not concentration-dependent and not consistent among time points. Our pharmacokinetic and pharmacodynamics data of tramadol in fish help bridge the gap between widely available mammalian pharmacological data and potential effects on aquatic organisms, and highlight the importance of understanding drug uptake and metabolism in fish to enable the full implementation of predictive toxicology approaches.

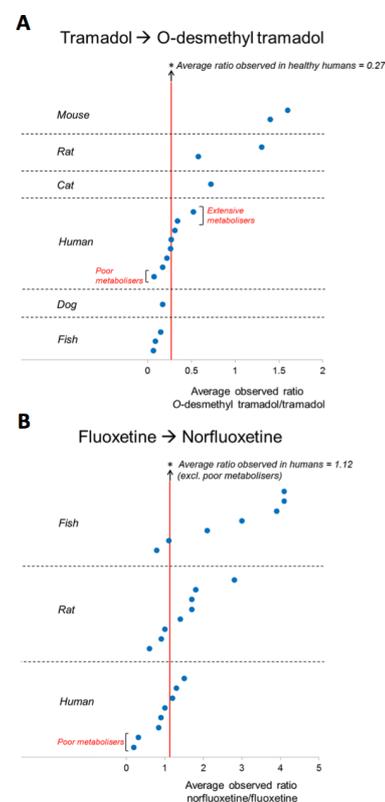


Fig. 1. Inter-species differences in metabolism. (A) *O*-desmethyl tramadol/tramadol concentration ratios in plasma of mouse, rat, cat, human, dog, and fish. (B) Norfluoxetine (*N*-desmethyl fluoxetine)/fluoxetine concentration ratios in plasma of fish, rat, and human.

Evolution and enzymatic activity of Phase II drug metabolism enzymes in Carnivores

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There have been many reports that Carnivore species are highly exposed to numerous environmental chemicals, such as PCBs (poly-chlorinated biphenyls), DDT (Dichloro-diphenyl trichloroethane), and other POPs (Persistent Organic Pollutants). Almost all Carnivores are top predators of their respective food chains, and therefore they are highly affected to the biomagnification of environmental chemicals, and they are considered a significant global threat for these species. Therefore, xenobiotic metabolism as a defense system for these chemicals should be investigated in these animals. Xenobiotic compounds such as drugs and environmental pollutants are metabolized by phase I enzymes, conjugated by phase II enzymes, and eliminated in urine or bile through phase III transporters. Phase I enzymes include primarily the cytochrome P450 (CYP), whereas phase II conjugating enzymes include many enzymes such as UDP-glucuronosyltransferase (UGT), sulfotransferase (SULT). Some xenobiotics are metabolically activated by CYP and the resulting intermediates cause health dysfunction. Therefore, the Phase II reaction is essential factors for the xenobiotics detoxification. This study was performed to elucidate the species differences in two major Phase II drug metabolism enzymes, UGTs and SULTs. To reach our aim of the study, we conducted two analyses. Firstly, we analyzed the *in vitro* enzymatic activity using the typical substrates for UGTs (lorazepam, oxazepam, temazepam: UGT2Bs) and SULTs (estradiol: SULT1E1) in liver samples from Steller sea lions, Northern fur seals, Caspian seals, Harbor seals, cats, and rats. Secondly, using the genome database, we conducted the genetic comparative analysis for the gene sequences and the coding locus in carnivore species. In this seminar, we would like to show the species differences in UGTs and SULTs in the carnivores, and current progress and future plans of our research for drug metabolism enzymes in wildlife mammalians.

The Thermochemical Formation of Brominated Aromatic Compounds from Tetrabromobisphenol A at E-waste Open Burning Sites

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Introduction

Brominated aromatic compounds (ArBr-compounds) including polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs) are produced by the open burning of electronic waste (e-waste) in developing countries. The ArBr-compounds are thought to be produced from brominated flame retardants (BFRs). E-waste contained a lot of plastics including much BFRs and a variety of metals especially copper (Cu) and iron. Fujimori *et al.* showed that the toxic equivalent concentrations (TEQs) of PBDD/Fs were higher than those of polychlorinated dibenzo-p-dioxins and dibenzofurans, and that the concentration of Cu had strong positive correlations with the total concentration of PBDFs in soil sampled from sites in Agbogbloshie, Accra, in Ghana, where e-waste is burned openly¹.

Another study showed heating CuBr₂ and AC matrix for 1 hour at 300°C, 400°C, and 500°C created PBDD/Fs. Furthermore, the presence of CuBr₂ and oxygen accelerated the decomposition of carbonaceous structure.²⁾

Previously, we investigated the model sample which is composed of the same weight of CuBr₂ and AC when it received thermal load so that proved copper bromine (I) (CuBr) has a catalytic effect in the creation of ArBr-compounds.³⁾ In this study, we measured the *in situ* X-ray absorption fine structure (XAFS) of Cu and bromine (Br) on heating samples. To investigate the thermochemical mechanisms by which ArBr-compounds are produced in the open burning of e-waste, we studied the combustion of samples consisting of Cu, the BFR tetrabromobisphenol A (TBBPA), and activated carbon (AC).

Materials and methods

The model samples consisted of Cu, TBBPA, and AC. The ratio of Cu to Br was fixed at 1:2 (mol ratio), and the overall ratio of the three was 1:5:3 (by weight). The sample was measured using *in situ* Cu and while being heated. The XAFS measurement was performed using Quick XAFS on the Super Photon ring-8 GeV (SPring-8). The *in situ* cell used in the experiments was T-shaped, and the X-rays passed through Kapton polyimide film. Each XAFS measurement was taken for 2 minutes and the measurement interval was set to 1 minute so that one measurement cycle was 3 minutes. A temperature-control unit

(SY2121-3R; CHINO) was used to heat the cells in two stages: first from room temperature to 140°C over 12 minutes (at about 10°C/min) and then from 140°C to 503°C over 6 hours and 3 minutes (at about 1°C/min). This was done because the speciation of the model mixture changed at temperatures from 200°C to 500°C, and this enabled the XAFS to be observed in detail. The samples were heated in synthetic air (4:1 N₂/O₂) flowing at 50 mL/min. The XAFS data were processed by linear combination fitting (LCF) with the XAFS of standard substances using Athena software.⁴⁾ LCF was performed for three different ranges.

Results and discussion

Fig.1 represents spectra of standards (Cu, CuBr, and CuO) and three spectra of sample of 172°C, 300°C, and 399°C. The shapes of spectra of sample have dramatically changed as they were heated. It showed each spectra at 172°C, 300°C, and 399°C have similarities that of Cu, CuBr, and CuO, respectively.

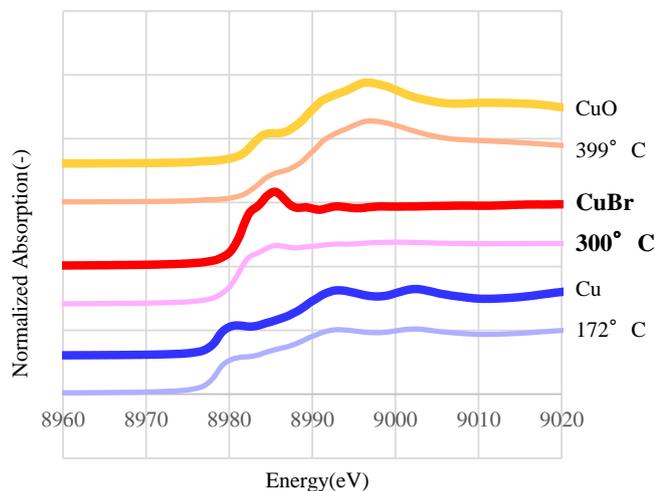


Figure.1 Spectrum of sample (narrow line) and standards (bold line)

Fig.2 indicates the results of LCF, meaning changes of copper chemical forming ratio from 150°C ~

400°C. This quantitative result agreed with the qualitative result of Fig.1. It was thought the Cu combined with HBr or Br₂, which was decomposed or created from TBBPA, especially at 250~300°C.⁵⁾ In addition, the oxidation of Cu started at around 250°C, creating Cu₂O. Then, this oxidation progressed rapidly at 300~350°C. Finally, the Cu was changed almost completely into CuO at 350°C. The maximum ratio of CuBr was around 300°C, and oxidation of Cu almost completed at 350°C. These combined changes not only in Cu and Br but also in Cu and O were in

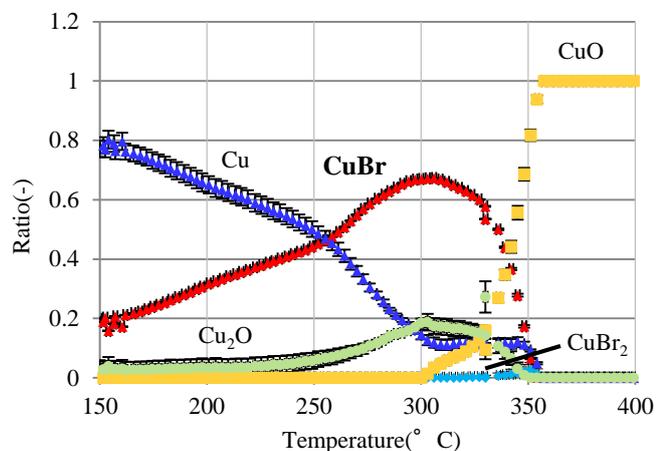


Figure.2 Average of LCF of Copper XANES

agreement with our prior study, implying that the ArBr-compounds were unintentionally created at around 200~300°C. These results imply that ArBr-compounds are likely to form unintentionally when BFRs and Cu are heated during the open burning of e-waste. Moreover, PBDD/Fs would be created at e-waste open burning. Future studies need to quantify PBDD/Fs and examine the behavior of other BFRs on heating.

Acknowledgements

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PCB-induced disruption of thyroid hormone homeostasis in dogs and cats

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Thyroid hormones (THs) such as 3,3',5-triiodo-L-thyronine (T₃) and L-thyroxine (T₄) are essential for the regulation of growth and development. Recent studies reported that polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs) affect the TH homeostasis. In our previous studies, concentrations of PCBs and OH-PCBs in pet cat serum showed significant negative correlation with THs. However, there are no *in vivo* evidences on the adverse effects of PCB exposure on thyroid hormone homeostasis of pet animals. The present study determined the serum TH levels in dog (*Canis lupus familiaris*) and cat (*Felis silvestris catus*) following PCB exposure, and examined the relationships with levels of PCBs and OH-PCBs. PCB and OH-PCB levels in dog serum were higher than those in cat serum after 24 hours exposure to the same dose of PCBs. THs levels in cat serum were not changed by PCBs exposure. In dog serum, free T₄ level increased and was positively correlated with the levels of T₄-like OH-PCBs including 4OH-CB107, 4OH-CB202 and 4OH-CB187. These results suggest that the increase of free T₄ in dog serum may be induced by competitive binding of T₄-like OH-PCBs to TH transport protein. Additionally, total T₄ and total T₃ in PCB-exposed dog serum decreased and were negatively correlated with PCB concentrations. It was reported that PCB exposure induced the decrease of total T₄ in serum due to induction of UDP-glucuronosyl transferase (UGT) and accumulation of T₄ in liver. These results imply that PCB exposure enhanced TH excretion in dog due to increased uptake of THs and induction of UGT in the liver.

Plasma Endotoxin Activity and Trace Elements Kinetics in *Macropus giganteus* infected-Lumpy Jaw Disease (LJD)

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Lumpy Jaw Disease (LJD)は飼育下のカンガルーでおきる下顎骨または上顎を含む進行性の肉芽腫性骨髄炎である。LJDは主に *Fusobacterium necrophorum* の口腔粘膜侵入による歯周炎を起点とし、隣接した骨に感染が波及して骨髄炎に発展する。LJD罹患カンガルーは *F. Necrophorum* から放出されたエンドトキシンにより全身性炎症を呈することから本疾病の重症度は血漿中エンドトキシン活性値と関連性があることがわかっている。牛の大腸菌性乳房炎罹患牛では血漿 Fe および Zn 濃度が低値を示しことや、ハムスターの急性炎症時には血中 Cu 濃度が増加するなどエンドトキシンに起因する全身性炎症は血中微量元素動態に影響を及ぼすことが知られている。そこで、LJD と血漿中微量元素濃度との関係に注目し、検証を行うことで炎症時の血漿中の微量元素動態について解析を進めた。LJD 罹患ハイロオオカンガルーの血漿中微量元素を Particle Induced X-ray Emission (PIXE)法により多元素同時定性定量でスクリーニングを行った。血漿エンドトキシン活性値が 0.22 EU/mL 以上で、顔面腫脹、体重の減少、流涎など LJD の臨床所見があり、X線検査で骨吸収が見られたカンガルーを ETX 検出群とし、それ以外のカンガルーを対照群として PIXE 法による測定を行った。その結果、27 元素が血中から検出され、ETX 検出群で Na、Si、S、Cl、K、Ca の 6 元素で有意に低値を示した。炎症時に血中濃度が変化すると報告のある Fe、Cu、Zn は ETX 検出群と対照群の間で有意な差は見られなかったが、これまでの報告と同様に ETX 検出群で Fe および Zn 濃度が減少し、Cu 濃度が増加する傾向が見られた。

De novo* sequencing and mRNA expression of cytochrome P450 1–3 genes in red-crowned crane, *Grus japonensis

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【目的】 タンチョウ (*Grus japonensis*) は環境省の絶滅危惧Ⅱ類に分類され、現在、保護増殖事業が行われている。その一環として傷病個体保護や、必要に応じて投薬による治療が行われる。タンチョウに対する薬剤の投与量は、知見の不足から、他の鳥類での報告を基に決定されている。しかし薬物感受性には種差があることが知られ、外挿を基にした投薬は副作用の危険性もある。したがって、タンチョウ傷病保護個体の獣医療を高度化するためには、種固有の異物代謝酵素システムと代謝能を明らかにすることが重要である。そこで本研究では、タンチョウを対象に薬物感受性種差の要因となりうる cytochrome P450 (CYP) 1-3 遺伝子の特徴や発現パターンを明らかにし、比較生物学的視点で考察した。

【方法】 本研究では交通事故や電線事故等により傷病または死亡し、釧路市動物園に搬入された野生のタンチョウ臓器を用いた。肝臓から全 RNA を抽出し、Illumina HiSeq 2500 により配列情報を得た。これを基に BLAST 検索で CYP1-3 ファミリー遺伝子と推定される配列を収集し、系統解析によって遺伝子の分類を行った。次いで各臓器（肝、腎、心、脳、肺、生殖巣）から全 RNA を抽出し逆転写反応により cDNA を得て、リアルタイム PCR 法で肝臓の各遺伝子発現量の定量と臓器間の発現比較を行った。

【結果】 次世代シーケンスおよび系統解析から、タンチョウ肝臓において優位に発現している CYP1-3 遺伝子として、CYP1A5、CYP2C23、CYP2C45、CYP2D49、CYP2G19、CYP2U1、CYP2AC1、CYP3A37、CYP3A80 の 9 分子種が検出された。肝臓の各分子種の発現量は、中央値で比較して CYP3A37 が最も多く、次いで CYP2AC1、CYP2C45 の順であった。雌雄、成長段階、飼育の有無による発現量の差は今回みられなかった。また臓器間の発現量比較では、CYP2C45 を除く分子種で肝臓の発現量が最も多く、次いで腎臓の発現が多い傾向がみられた。CYP2C45 は肝臓の発現は高いものの、肺での発現がより高かった。

【考察】 発現量解析では、タンチョウ肝臓で CYP3A37 の発現量が最も多く、異物代謝において重要な役割を担っている可能性が示唆された。他の鳥類で報告された発現量の多い CYP1-3 分子種とタンチョウの結果は一致せず、鳥類間で CYP 分子種の発現パターンは異なることが示唆された。タンチョウでは真菌感染が比較的頻繁にみられ、アゾール系抗真菌薬が臨床ではよく用いられている。アゾール系抗真菌薬はヒトにおいて CYP3A4 阻害による薬物相互作用が知られている。鳥類 CYP3A 分子種に対する阻害効

果の報告はないが、CYP3A37 が阻害を受ける可能性はあり、他の鳥類の臨床結果をタンチョウに外挿する場合、薬物相互作用を考慮する必要性が考えられた。

今後は、タンチョウの異物代謝能を明らかにするため、肝ミクロソーム画分を用いた薬剤の代謝活性や阻害効果の検証、ならびに異物代謝に重要と推察された CYP3A37 や CYP2C ファミリーの機能解析が必要であると考えられた。

Total Concentrations and Chemical Forms of Chlorine in Insoluble Fractions of Environmental Solid Samples

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Introduction

Organochlorine compounds (OCs) have drawn much attention because of their persistence, bioaccumulation, and toxicity. Most studies have been focusing on the individual OCs but it is challenging to determine all the individual OCs. This is why a mass balance approach by measuring total amounts of organically bound chlorine (Cl) is needed. In this study, we quantified the total Cl concentration in the original sample (TCI) and in insoluble fractions (TisCl). We used the same pretreating method and combustion-ion chromatography (combustion-IC) to various environmental solid samples; forest soil, paddy soil, electronic waste (E-waste) open burning soil, house dust, municipal solid waste incineration (MSWI) bottom ash, and MSWI fly ash by applying. To clarify the chemical forms of Cl measured as TisCl, we measured Cl 1s X-ray adsorption near-edge structure (XANES) and analysed.

Material and methods

Sample: Forest soil was collected from the A horizon (depth: 5–10 cm) at Mt. Yoshida, Japan, in August 2016. The vegetation in the area is dominated by deciduous trees, and soil from this area has previously been classified according to soil taxonomy¹. Paddy soil was collected from a rice paddy field in Aomori, Japan, in March 2003. This soil was an Andosol, and concentrations of ions were determined by NAA and ICP-MS after combustion². E-waste open burning soil was collected from the Agboghloshie market, Ghana, in 2013. Soil collected from the same point was studied in previous research³⁻⁵. The house dust sample came from the NIST standard reference

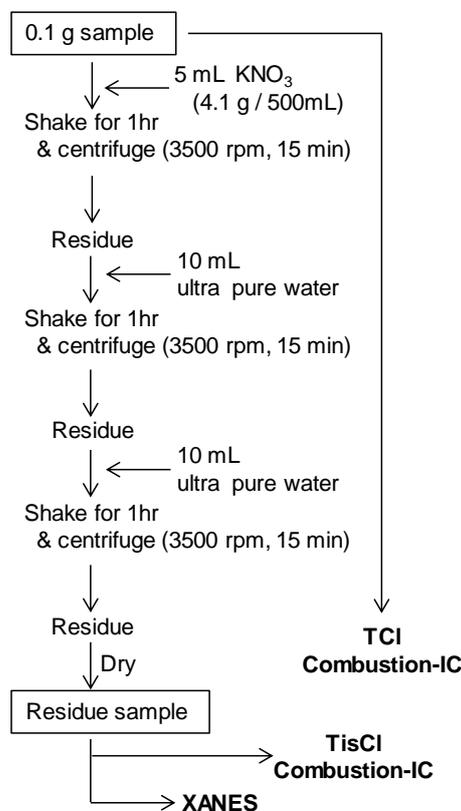


Figure 1 The scheme diagram showing the pretreatment and analysis methods for combustion-IC & XANES.

material 2585, and was taken from vacuum cleaner bags collected from homes, cleaning services, motels, and hotels in America in 1993–1994. The MSWI bottom ash sample was taken from the standard reference material, JSAC 0512, and the MSWI fly ash sample was taken from the standard reference material, JSAC 0511. These ash samples were collected from a waste (mainly wood waste) incineration plant in Japan.

Pretreatment for TisCl: Dried and sifted samples (0.1 g) were mixed with 5 mL of KNO₃ (4.1 g/500 mL) and shaken for 1 hour. After the suspension was centrifuged, the residue was washed into another bottle by 10 mL of ultra-pure water and shaken for 1 hour. The same process (wash into bottle, shake, and centrifuge) was repeated once and the residue was dried in desiccator (Figure 1).

Quantification of total concentrations: Total concentrations (TCI and TisCl) were measured by using combustion-IC. Five milligrams of dried sample was placed into a ceramic boat with 25 mg of WO₃ and introduced into a combustion unit (AQF-2100H, Mitsubishi Chemical Analytech). After halogens were decomposed and absorbed into H₂O₂, the solution was pumped into a suppressor-type ion chromatography (HIC-20ASP, Shimadzu).

Cl 1s XANES measurement and linear combination fitting (LCF) analysis: XANES spectra were measured between 2810 and 2860 eV using the total-fluorescence-yield (TFY) method and total-electron-yield (TEY) method at beamline BL-11B of the Photon Factory in Tsukuba, Japan. Each residue sample was applied to carbon tape and irradiated with soft X-rays. Our group have accumulated spectra data of more than ten standard compounds since 2006. We fit spectra of each residue sample as a linear combination of less than 4 chosen standard compounds spectra using analytical software (Athena, Demeter 0.9.25; Matt Newville, Univ of Chicago).

Results and discussion

The results in this study were summarized in Table 1. Considerable amounts of Cl were detected in all samples and the concentrations varied greatly between samples. TisCl was about 1/6–1/13 of TCI because water soluble Cl was removed.

Table 1 Concentrations of TCI, TisCl and the percentage of aromatic, aliphatic and inorganic Cl in residue sample.

	forest ^a	paddy ^b	e-ob ^c	house ^d	bottom ^e	fly ^f
<i>Concentrations (mgCl/kg)</i>						
TCI*	750 ± 21	1200 ± 21	3600 ± 360	10000 ± 380	6100 ± 270	110000 ± 2200
TisCl**	69 ± 0.83	120 ± 10	240 ± 97	940 ± 40	990 ± 68	830 ± 22
<i>Percentage of each Cl chemical form in TisCl (%)</i>						
Aromatic Cl	51	31	39	19	9.6	24
Aliphatic Cl	22	11	17	53	0	0
Inorganic Cl	28	58	45	28	90	76

^a Forest soil, ^b Paddy soil, ^c E-waste open burning soil, ^d House dust, ^e MSWI bottom ash, ^f MSWI fly ash
* concentrations in the original samples, ** Concentrations in the residue samples.

Total Cl concentration in insoluble fractions (TisCl): House dust, bottom ash, and fly ash showed relatively high concentrations of TisCl. Focusing on forest soil, TisCl was the same concentration level with the previous study on Swedish forest soil⁶ (35–76 mg/kg).

Linear combination fitting (LCF) analysis: Figure 2 shows the results of LCF analysis. From this analysis, the proportion of Cl bonded with inorganic elements (inorganic Cl), Cl bonded with aromatic carbon (aromatic Cl) and Cl bonded with aliphatic carbon (aliphatic Cl) was calculated (Table 1).

Concentrations of each forms of Cl in residue: We estimated the concentrations of aromatic, aliphatic and inorganic Cl in each residue by multiplying TisCl and the proportion (Figure 3). Bottom ash and fly ash contains large proportion of inorganic Cl in their residues. This is because of insoluble inorganic salts like Friedel's salt. Comparing forest soil, paddy soil and E-waste open burning soil, E-waste open burning soil showed the highest concentrations of both aromatic and aliphatic Cl in the residue. This indicates high level contamination from E-waste open burning. House dust showed a unique character; containing an extremely high concentration of aliphatic Cl (about 500 mg/kg) in the residue. This is because typical components of house dust are dominated by fabric fibers, which include chlorine polymer such as polyvinyl chloride (PVC) and polyvinylidene chloride. Some toxic chlorinated hydrocarbons such as polychlorinated *n*-alkanes and chlorinated paraffins may also contribute to the concentration of aliphatic Cl. In the residue of house dust, aromatic Cl concentration was also high. These aromatic Cl may originate from some chlorinated pesticides and chlorinated polycyclic aromatic hydrocarbon. These high concentrations of aromatic and aliphatic Cl indicate that we should pay more attention to house dust.

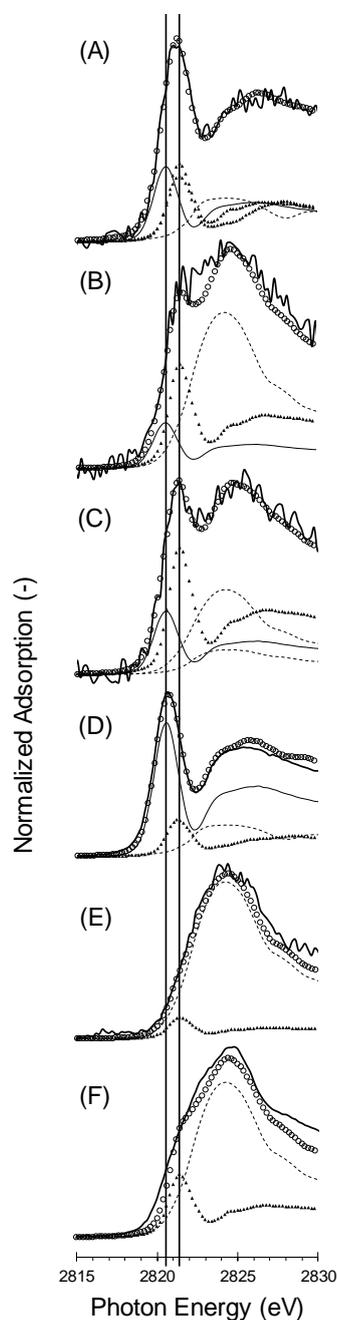


Figure 2 Cl K-edge XANES spectra
 Bold line: spectra of residue sample.
 Circle: calculated spectra by fitting.
 Triangle: weighted spectra of aromatic Cl standard compounds. Solid line: weighted spectra of aliphatic Cl standard compounds. Dotted line: inorganic Cl standard compounds.
 (A) Forest soil. (B) Paddy soil (C) E-waste open burning soil. (D) House dust. (E) MSWI bottom ash. (f) MSWI fly ash

Mass balance consideration: We compared the estimated total concentrations of aromatic Cl in residue with the individual concentrations in the original sample. Individual concentrations are from other studies or certified concentrations of standard materials. About house dust, concentrations of PCBs, DDE, DDD and DDT are certified. Cl concentration originates from these compounds was calculated as 0.27 mg/kg and this contributes 0.15% of total aromatic Cl concentration. About E-waste open burning soil, two studies report the concentrations of DL-PCBs, PCDDs, PCDFs and Cl-PAHs in the sample from the same point. Cl concentration originates from these compounds calculated as 0.52 mg/kg and this contributes 0.56% of total aromatic Cl concentration. About fly ash, concentrations of PCBs, PCDDs and PCDFs are certified. Cl concentration originates from these compounds was calculated as 0.7 mg/kg and this contributes 0.35% of total aromatic Cl concentration.

Conclusion and possibility of research collaborations

TisCl and EOCi can be powerful approaches to understand the transfer of OCs between environment, animals and humans. We can measure TisCl and EOCi and also have the method to estimate the total aromatic and aliphatic Cl in environmental solid samples. We are welcome to have many research collaborations.

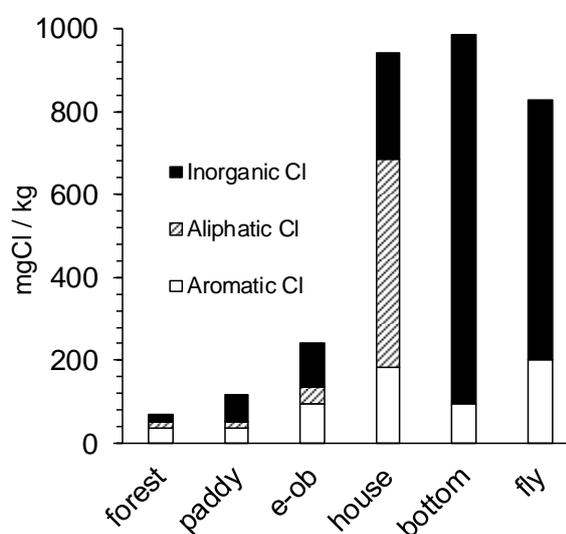


Figure 3 Estimated concentrations of aromatic, aliphatic and inorganic Cl in each residue sample. Forest: forest soil. Paddy: paddy soil. E-ob: E-waste open burning soil. House: house dust. Bottom: MSWI bottom ash. Fly: MSWI fly ash.

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Developmental effects of organophosphate flame retardants and their metabolites in zebrafish

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【目的】有機リン系難燃剤（OPFRs）は、使用規制が進む臭素系難燃剤の代替として世界中で使用が増加している。一方で、OPFRs は揮発性が高く室内空気汚染物質として問題視されてきた物質でもある。一部の OPFRs は発がん性を示すことが古くから報告されてきたが、最近では魚類胚や鳥類胚に対して発生毒性を示すことも報告されている。魚類胚を用いた研究では、OPFRs による発生毒性のメカニズムも検討されてきており、一部の OPFRs の毒性には芳香族炭化水素受容体（AHR）やレチノイン酸受容体（RAR）の関与も指摘されている。これに対し、OPFRs の代謝物は、*in vitro* 試験により変異原性を示すことや、レポーターアッセイ系において親化合物よりもエストロゲン活性が高いという報告があるものの、発達期に対する毒性については不明な点が多い。そこで本研究ではゼブラフィッシュ胚を用いて、OPFRs およびその代謝物の発生毒性とその分子機構を明らかにすることを試みた。

【方法】受精後 72 時間（72 hpf）のゼブラフィッシュ胚に 4 種類の OPFRs (triphenyl phosphate (TPP)、tris(1,3-dichloro-2-propyl)phosphate (TDCPP)、tricresyl phosphate (TCP)、tris(2-chloroethyl)phosphate (TCEP))および TPP、TDCPP の主要代謝物である 4-hydroxyphenyl diphenyl phosphate (HO-*p*-TPP)、bis(1,3-dichloro-2-propyl)phosphate (BDCPP)を 24 時間水性曝露した。曝露胚を 96 hpf で洗浄し、3% carboxymethyl cellulose で不動化させ倒立顕微鏡により体幹血流を評価した。さらに側面像を取得し心臓周囲面積と全長の計測を行った。また、72 hpf のゼブラフィッシュ胚に上記と同様に OPFRs およびその代謝物を水性曝露し、96 hpf で洗浄・採材した。採材した曝露胚から全 RNA を抽出し、cDNA 合成を行った後、標的遺伝子(*cytochrome P450 19A1b (CYP19a1b)*, *growth hormone (gh)*, *insulin-like growth factor 1 (igf-1)*)の mRNA 発現量をリアルタイム PCR 法で測定した。

【結果】OPFRs の親化合物および主要代謝物を用いた曝露実験の結果、TDCPP、BDCPP、TPP、OH-*p*-TPP、TCP は 96 hpf のゼブラフィッシュ胚に心臓周囲浮腫や全身血流の低下を誘発した。TDCPP および TPP 曝露により胚に体躯の矮小が認められたが、各代謝物では体躯のサイズに影響はみられなかった。また、TPP および HO-*p*-TPP 曝露は *CYP19A1b* 発現量を増加させ、HO-*p*-TPP の方で最大効力はより高値を示した。TDCPP 曝露は *gh* 発現量を顕

著に低下させ、TPP、TCP 曝露は *igf-1* 発現量を有意に低下させた。

【考察】OPFRs の親化合物だけでなく、一部の代謝物もゼブラフィッシュ胚に心血管毒性を誘発することが明らかとなった。一方で、TDCPP および TPP 曝露群でみられた稚魚の成長阻害は親化合物特異的であり、GH/IGF 系の遺伝子発現の低下に起因すると推察された。また、TPP よりも HO-*p*-TPP において *CYP19A1b* 誘導が顕著であったことから、TPP の代謝はエストロゲン様作用を増強すると考えられた。

今後は、エストロゲン様作用と心血管毒性の関連性について検討するとともに、心血管系発生への関与が報告されている遺伝子を対象に、OPFRs および代謝物の遺伝子発現に対する影響を明らかにすることが必要と考えられた。

SERUM BIOCHEMICAL RESPONSES IN CATS EXPOSED TO ORGANOHALOGEN COMPOUNDS

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The organohalogen compounds (OHCs) including polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are persistent organic pollutants. Even though the production of PCBs and some formulations of BDEs (octa-BDE, penta-BDE) was banned, they're remain ubiquitous environmental pollutants due to their resistance to degradation and bioaccumulated in human, livestock, and wildlife. Because of their chemical structures, they're highly toxic and can cause endocrine problems by blocking hormone functions, especially thyroid hormone axis. Currently, an increasing number of studies demonstrated that feline hyperthyroidism correlated PCB/PBDE levels in cat serum. However, the underlying mechanisms and effects of these compounds on cat thyroid hormones, or even on liver and kidney functions are not defined. The objective of this study was therefore to investigate the effects of PCBs and BDE209 on liver and kidney functions by evaluating serum biochemical including liver enzymes, albumin, total protein, blood urea nitrogen (BUN), creatinine, and lipids by using the collected time-series serum from cats (*Felis catus*) exposed to PCBs (single dose exposure; i.p.) and BDE209 (one-year oral administration). In PCBs exposure cats, the albumin and total protein levels were significantly decreased at one time by comparing with control cats. The significant negative correlation between 4'OH-CB70 and GPT (Glutamic Pyruvic Transaminase) ($R=-0.58$, $P<0.05$), and between 4'OH-CB79 and total bilirubin ($R=-0.68$, $P<0.05$) were also found in PCBs exposure cats. Meanwhile the significant decreasing albumin level and the increasing high-density lipoprotein (HDL) and triglyceride levels were found in BDE209 exposure cats at one time or another. In addition, the significant negative correlation between BDE209 and creatinine ($R=-0.51$, $P<0.05$), and between total PBDEs and total bilirubin ($R=-0.56$, $P<0.05$) were observed in BDE209 exposure cats. Although there are no evidences of liver and kidney cell damage, which was indicated by no significant difference of liver enzymes, BUN, and creatinine levels between control and exposure cats of both experiments. However, these results demonstrated that both PCBs and BDE209 exposures can

affect liver and kidney functions, as well as disturb lipid levels. These negative correlations revealed that some OH-PCBs and BDE209 might be key effectors for these biochemical responses. However, the further studies are needed to verify these finding.

Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics

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Increasing amounts of plastics are entering the oceans on account of increases in production and poor waste management. Plastics are fragmented into smaller pieces and ingested by a variety of marine organisms such as seabirds. This study focused on the chemical effects of ingested plastics, i.e., transfer and accumulation of the plastic-derived chemicals to biological tissue.

We analyzed polybrominated diphenyl ethers (PBDEs), one of the major flame retardant applied to plastics, in tissues and ingested plastics of four species of Procellariiformes, i.e., short-tailed shearwaters (*Puffinus tenuirostris*) from North Pacific Ocean, northern fulmar (*Fulmarus glacialis*) from North Atlantic Ocean, white-chinned petrel (*Procellaria aequinoctialis*) and shy albatross (*Thalassarche cauta*) from South African waters. In 5 of 30 short-tailed shearwaters, BDE209 was detected in both tissue and ingested plastics. BDE209 is not present in their natural prey, but is a main congener of deca-BDE technical product. It was suggested that the seabirds accumulated additive-derived BDE209 from plastics into their tissue. In the other 2 birds, similarly, transfer of octa-BDE or hexa-BDE technical products from plastic to tissue was suggested. Sporadic accumulation of BDE209 was also observed in tissues of northern fulmar, which could be derived from ingested plastics.

In addition, laboratory experiment was conducted to study leaching of additive PBDEs from plastics into seabirds' digestive fluid. The experiments suggested that stomach oil, which present in stomach of birds in the order Procellariiformes, facilitated leaching of PBDEs from plastic to digestive fluid.

The conclusion is that plastics are transported in the ocean retaining additives, and after ingestion by seabirds, chemicals in plastics can be rapidly extracted and exposed to the birds. Marine plastics also contain many other chemicals than PBDEs, such as UV-stabilizers, antioxidants, flame retardants, and byproducts of manufacturing. Bioaccumulation and toxicological risks of these chemicals should be studied in future.