The 20th UK-Japan Annual Scientific Workshop
on Research into Environmental Endocrine Disrupting Chemicals

Cambridge, UK

7th November – 9th November
2018

Department
for Environment
Food & Rural Affairs

環境省

The 20th UK-Japan Annual Scientific Workshop
on Research into Environmental Endocrine Disrupting Chemicals

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2018

Abstracts and Programme Information

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Programme

7th November 2018

14:00-17:00 Site visit – 'Anglian Water' Waste Water Treatment Plant – "A modern and advanced water recycling centre in the UK which also performs generation of renewable energy."

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Policy Morning
Co-chairs: Taisen Iguchi & Charles Tyler

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16:10-16:35 Kiyoshi Soyano, Nagasaki University – 'New survey of EEs contamination using egg envelope protein in the grey mullet'
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Co-chairs: Tohru Kobayashi & Chris Green

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09:30-10:00 Frances Orton, University of the West of Scotland – 'Biomarkers for reproductive health in captive and wild frogs'
10:00-10:10 Speaker Q&A
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Core Project: Molecular & Genomic Approaches for Understanding EDC Impacts
Co-chairs: Norihisa Tatarazako & Charles Tyler

10:30-10:50 John Colbourne, University of Birmingham – 'Roadmap for Precision Environmental Health'
10:50-11:20 Shinichi Miyagawa, Taisen Iguchi, Yokohama City University – 'Approaches for studying endocrine disruption and estrogen receptor subtypes in fish'
11:20-11:50 Anke Lange, Charles Tyler, University of Exeter – 'New developments in fish ecotoxicology models'
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Delegate List

Delegate Name	Affiliation	Country
Alice Baynes	Brunel University London	UK
Andrew Johnson	Centre for Ecology and Hydrology	UK
Anke Lange	University of Exeter	UK
Arthur Thornton	Atkins Global Ltd	UK
Callum Harris	Defra	UK
Camilla Alexander-White	Royal Society of Chemistry	UK
Charles Tyler	University of Exeter	UK
Christopher Green	Defra	UK
Christina Lye	Enviresearch Ltd	UK
Claire Massey	Environment Agency	UK
David Williams	Defra	UK
Dominique Butt	Natural Environment Research Council (NERC)	UK
Elen Strale	Defra	UK
Frances Orton	University of the West of Scotland	UK
Fumiaki Ogawa	Public Works Research Institute	Japan
Hiroaki Tanaka	Kyoto University	Japan
Hiroshi Yamamoto	National Institute for Environmental Studies	Japan
Holly Yates	Defra	UK
Ioanna Katsiadaki	Cefas	UK
Jeanette Rotchell	University of Hull	UK
John Colbourne	University of Birmingham	UK
Kay Williams	Defra	UK
Kiyoshi Soyano	Nagasaki University	Japan
Kunihiko Yamazaki	Ministry of the Environment	Japan
Liz Lawton	Defra	UK
Masaki Nagae	Nagasaki University	Japan
Masaru Ihara	Kyoto University	Japan
Nick Cartwright	Environment Agency	UK
Norihisa Tatarazako	Ehime University	Japan
Renato AM Silvano	Universidade Federal do Rio Grande do Sul	Brazil
Rieko Nakamura	Ministry of the Environment	Japan
Shinichi Miyagawa	Tokyo University of Science	Japan
Steve Dungey	Environment Agency	UK
Steve Morris	Defra	UK
Susan Jobling	Brunel University London	UK
Suzane Qassim	Natural England	UK
Taisen Iguchi	Yokohama City University	Japan
Takuma Kato		Japan
Tetsuro Okamura	IDEA Consultants, Inc.	Japan
Tohru Kobayashi	University of Shizuoka	Japan
Yuki Tateishi	Kyushu University	Japan
Yukio Kawashima	JAPAN NUS Co., Ltd.	Japan
Yuta Onishi	IDEA Consultants, Inc.	Japan

Presentation Abstracts

Core Project: Environmental Exposure of Emerging Contaminants

Speaker: Andrew Johnson, Centre for Ecology and Hydrology

Title: 'Chemical prioritization and using long-term macroinvertebrate monitoring data to assess river health'

Assessing the risk to aquatic wildlife from the many 1000's of chemicals discharged in wastewater is hard. We are all aware of the rising consumption of pharmaceuticals and personal care products and these have raised many questions. In the laboratory we can now see a range of responses to these different chemicals and micro pollutants in unprecedented detail. But it is hard to decide what the real impacts might be. It is hard for regulators to judge risks from chronic effects and ambiguous end-points and so inevitably they must take the precautionary point of view. We can try to assess relative risk by comparing toxicity data with monitoring information. But most chemicals are not monitored routinely, so we can only offer relative risk assessments on those which have been well studied.

A radically different approach is to compare wildlife population trends with respect to chemical exposure. The best dataset we have available in UK waters are for macroinvertebrates. Thus, it is possible to follow the fortunes of macroinvertebrates in river water which have high wastewater exposure. This may go some way to answer the original question as to how damaging are the 1000s' of chemicals discharged in wastewater to wildlife? Our preliminary studies at Swindon, Aylesbury, Basingstoke and Chesham would suggest macroinvertebrate populations have become more diverse and contain more sensitive species than they did 20 years ago. This implies today's chemical wastewater mix is not impeding many macroinvertebrate taxa.

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Speaker: Hiroaki Tanaka *et al*, Kyoto University

(Masaru Ihara[1], Norihide Nakada[1], Han Zhang[1], Fumiaki Ogawa[2], Seiya Hanamoto[2], Andrew Johnson[3], Monika Jürgens[3]. [(1)]Kyoto University, [(2)]Public Works Research Institute, [(3)]Centre for Ecology and Hydrology)

Title: 'Prediction and management of emerging contaminants in the water environment'

The sorption of pharmaceuticals in river water to river sediment was studied by scientists from PWRI. Azithromycin (AZM), an adsorptive antibiotic, was selected as a model compound. Sediment samples (n=9) were collected from the Katsura River near Kyoto, Japan, the Tama River near Tokyo, in Japan and the Thames River in the UK. The sorption tests of azithromycin in water were conducted for the nine sediments using $CaCl_2$ solution (5mM) in accordance with OECD test guideline No. 106. The concentration of AZM in the solution, C_w was varied from 0.5 to 4.0 μg/L to create sorption isotherms. Sorption intensity constants,1/*n* of Freundlich isotherm (Eq. 1) were in the range of 0.7 to 1.0, indicating the sorption isotherms were mostly linear. Therefore, partition coefficients (K_d), which was estimated as C_s/C_w, mostly do not relate with the AZM concentration of water, C_w. K_d showed significant correlation with cation exchange capacity (CEC) of the sediments, indicating that K_d of AZM could be predicted by a sediment property, CEC. On the other hand, no significant correlation was observed between K_d and organic carbon content of the sediments, which would be attributed that sorption mechanism of AZM was not hydrophobic but electrostatic interaction.

$C_s = K_f {C_w}^{1/n}$ Eq.1

where *Cs*: AZM concentration of sediment, *Cw*: AZM concentration of water, K_f and *n*: constants

In cooperation with colleagues at CEH, the KU scientists conducted field surveys of biological activity of G protein-coupled receptors (GPCRs)-acting pharmaceuticals in effluents from municipal sewage treatment plans (STPs) in the UK from 2014 to 2016 using the *in vitro* TGFα shedding assay. The results indicated that compounds were present in the treated sewage with antagonistic activities against angiotensin (AT1), dopamine (D2), adrenergic (β1), acetylcholine (M1) and histamine (H1) receptors in UK. Now KU scientists have been trying to prioritize GPCR-acting pharmaceuticals responsible for the observed AT1, H1, D2, M1 and β1 receptors activity in the treated sewage by looking at the pharmaceutical consumption data (e.g., NHS online database in the UK). In addition, they are now evaluating the activity (i.e., potency) of the individual chemicals by the *in vitro* TGFα shedding assay, because activity data are also required to prioritize GPCR-acting pharmaceuticals for environmental monitoring and toxicity testing in future study.

So far, KU measured the biological activity of antidepressants in treated sewage in Japan using the fluorescence-based *in vitro* assay (namely called antidepressant assay here), which detects the monoamine transporters inhibition (antagonism). In this assay, antagonism for human serotonin transporter (SERT) gene was measured. Now,

KU has started to investigate the antagonism for fish SERT (e.g., medaka and zebrafish) and compare with that for human SERT. As to estrogenic activity in the sewage and treated sewage against estrogen receptor (ER), we know that fish ER shows different response with human ER. Similarly, antidepressants activity in sewage might be different between human and fish SERT.

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Speaker: Dominique Butt, Natural Environment Research Council (NERC)

Title: 'NERC Research Programme on Emerging Risks of Chemicals in the Environment'

The Natural Environment Research Council (NERC) is part of UK Research and Innovation (UKRI), a new body which works in partnership with universities, research organizations, businesses, charities and government to create the best possible environment for research and innovation to flourish. NERC invests public money in world-leading science, designed to help us sustain and benefit from our natural resources, predict and respond to natural hazards and understand environmental change. We work closely with policymakers and industry to make sure our knowledge can support sustainable economic growth and wellbeing in the UK and around the world.

NERC has invested £6m in the Emerging Risks of Chemicals in the Environment programme which aims to conduct research to predict how the environment and its functioning will respond to chemical exposure. The anticipated high level outcome is a transformation in the way chemical risk assessment is considered; to move towards an ecosystems approach with greater ecological relevance. The programme will address three interlinked research questions:

1. What are the impacts of chemicals on populations, ecosystems and ecosystem services?
2. What are the risks from chemical mixtures?
3. How important are chemical stressors in relation to other stressors?

The following projects have been funded under the programme:

- Does the discharge of chemicals to the environment harm wildlife populations? Professor Andrew Johnson, CEH (link)
- Classic and temporal mixture synergism in terrestrial ecosystems: Prevalence, mechanisms and impacts. Dr. David Spurgeon, CEH (link)
- A novel framework for predicting emerging chemical stressor impacts in complex ecosystems. Professor Guy Woodward, Imperial College London (link)

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Speaker: Arthur John Thornton, Atkins Global

Title: 'Investigation and Management of Contaminants of Emerging Concern in the United Kingdom'

Chemical Investigations Programme: A collaborative research programme between UK environmental regulators and the UK Water Industry. It is the largest programme of its type in the world (c£200m). The most recent studies include over 60,000 samples, one million chemical determinations and the demonstration of innovative treatment processes and Feasibility and Pilot Trials and five catchment studies. Responsibilities. Now in a scoping phase for the 2019-25 programme including the risk assessment of micro-plastics and antimicrobial resistant organisms. The presentation will set out the approach to programme design, proficiency testing and will highlight emerging substances of concern including human hormones and endocrine disruptors.

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Core Project: Research on EDC Impacts on Aquatic Wildlife and Predictive Approaches for Risk Assessment

Speaker: Steve Dungey, Environment Agency

Title: ‘Updates in the identification of EDCs in the EU and the ways testing has developed’

An update will be provided summarizing which “substances of very high concern” have been identified due to environmental endocrine disruption under the REACH Regulation in the past 12 months, developments in EU testing approaches and chemicals subject to further ecotoxicity testing to address suspicions about endocrine disruption.

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Speaker: Jeanette Rotchell, University of Hull

Title: 'Sullied Sediments: Sediment Assessment and Clean Up Pilots'

Many of the inland waterways in Europe are under threat due to the introduction of Watch List chemicals that are not currently regulated under the European Water Framework Directive. These chemicals are introduced into our waterways as a result of our day-to-day activities and through industry and many have been shown to be harmful to the aquatic environment. Regardless of the source, they accumulate in the sediments in our rivers and canals.

Water regulators and managing authorities do not always know the levels, locations or impacts of these emerging pollutants. Nor do they have the tools to assess sediments confidently and make informed decisions about their management. An interdisciplinary partnership of scientific experts, regulators and water managers, led by the University of Hull (UK), is developing and testing new tools and methods that will help them to overcome these challenges.

Through the implementation of these new tools and methods, the 'Sullied Sediments' project aims to empower water sector partners across the North Sea Region to make better decisions with regard to the management, removal and disposal of sediments, thereby reducing economic costs to private and public sector organisations, and the impact of these pollutants on the environment (link).

We also working to reduce the amount of unwanted chemicals entering the water system by raising awareness about what we, as consumers, are releasing into the environment through the use of common drugs and household products. This includes the involvement of volunteers in a sediment sampling initiative across the region. The overall success of the 'Sullied Sediments' project will be based on its impact in the real world and this will also be discussed in the presentation.

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Speaker: Renato Azevedo Matias Silvano, Universidade Federal do Rio Grande do Sul (UFRGS)

Title: 'Linking fishers' knowledge, fish ecology and ecotoxicology to promote safe food from sustainable sources in tropical aquatic ecosystems'

This talk aims to provide a brief overview of the joint project being conducted by the presenter and collaborators from UK, Colombia and Peru. The main goal of this proposal is to address the relationships among four important topics related to the food security of people living from tropical freshwaters: fish contamination, fisheries (fish use by people), fish ecological roles and people's perception about fish ecology and contamination potential. I will focus on the Brazilian component of this project in the Tapajos River of the Brazilian Amazon, by describing the main environmental impacts, ecological features and small-scale fisheries. This river has been affected by heavy metals from gold mining at its medium and upper reaches, besides being threatened by proposals of dam building.

I shall outline our plans on how to relate fish contamination, fish consumption and fishers' knowledge in the Tapajos River, discussing potential fish species as indicators of contaminants, based on data gathered during this and previous research projects conducted in this region. The information gathered in the Tapajos River regarding fish contamination and fish use should be compared with similar assessments in Peru and Colombia.

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Speaker: Tohru Kobayashi, University of Shizuoka

Title: 'Testis-ova and sex ratio in the anuran *Pelophylax nigromaculata* complex and development of non-invasive methods for evaluation of ecological impacts'

Testis–ova occur with high frequency in wild populations of the Japanese pond frog *Pelophylax nigromaculata* from the Niigata area of northern Japan. We compared the dynamics of testis–ova in a wild population of Japanese pond frogs sampled from 2010–2017 with those from studies conducted between 1972 and 1973. Although the frequency of testis–ova differentiation in the puberty-stage of frogs was similar in the studies from the two periods, the number of testis–ova in mature frogs was reported in markedly higher numbers in the 1970s. Approximately 30% and 8% of the frogs from 1972–1973 had >11 and >100 testis–ova, respectively, compared with <10% and 0% of the frogs assessed over the last eight years. Frogs with testis–ova have also been observed in the Toyama and Gifu populations, with specimens recorded in the Toyama population with >11 testis–ova within the last five years. Additionally, newly metamorphosed frogs with >11 testis-ova were seen in Toyama area in 2018, while we did not observe these frogs in the Niigata area.

The sex ratio of frogs from the newly metamorphosed stage to puberty stage was approximately 1:1 in 1972-1973 and 1982-1984 in the Niigata area. In recent years (2015, 2017, and 2018), we examined the sex ratio of newly metamorphosed frogs derived from several egg clutches in the Niigata area, resulting that there are more female frogs than males. In particular, we examined the sex ratio of frogs in 2015 and 2017, indicating all female frogs after metamorphosis.

To evaluate ecological impacts, we tried to develop non-invasive methods for estimation of ecological impacts on wild amphibian and teleost populations. First, we examined whether external development of nuptial pad, which is an androgen-dependent male sexual characteristics, is indicative of ecological impacts, compared with histology and SEM analyses. Consequently, it was demonstrated that measurement of the relative area of the nuptial pad by portable microscope was correlated with the results of histological analysis and SEM analysis. To evaluate the development of the nuptial pads in terms of androgen production, we need further basal information on the hormonal regulation of nuptial pad development, in conjunction with field surveys. Second, we have tried to demonstrate whether the population of each species can be quantified using eDNA measurement from environmental water. The population size of the target species in amphibians may be estimated from the number of larvae in water, suggesting the number of egg sacs and number of mating pairs. To accomplish this, we have tried to develop a quantitative assay using q-PCR for medaka and several other anurans. I will discuss about the quantification of the number of target species using eDNA measurements by the q-PCR method.

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Speaker: Kiyoshi Soyano, Nagasaki University

Title: 'New survey of EEs contamination using egg envelope protein in the grey mullet'

The zona pellucida protein (ZP), which is constitutive protein of egg envelope, has potential as a new biomarker like vitellogenin (VTG) for evaluating the influence of environmental estrogen (EE) pollution. Therefore, we began to study the evaluation of influence of EEs using ZP. In our study, the gene expression of ZPB and ZPC5, which are one of ZP subtype, was high in ovary of the grey mullet, suggesting that *zpb* and *zpc5* have a great potential as a biomarkers for understanding the influence of EEs in wild fish. Thus we examined the expression of *zpb* and *zpc5* in testis or immature ovary of the grey mullet captured in various places in Kyushu, Japan. The expression of both genes indicated high levels in fish captured near the sewage treatment plant (STP). In addition, abnormal gonad with a large amount of connective tissue or without germ cells was observed in fish capture near STP. However, the relationship between STP and physiological abnormality has not been clarified.

We are also investigating the impact of pharmaceutical compounds on fish behavior by video analysis. The medaka was exposed to diphenhydramine that is anti-histamine regent (H1 receptor blocker), and the behavior was recorded in a video. The fish exposed diphenhydramine was restless and often attacked the glass surface of the aquarium. The detailed analysis of the behavior is in progress.

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Speaker: Masaki Nagae *et al*, Nagasaki University

(Masaki Nagae[1], Madoka Moriwaki[1], Kanako Yamashiro[1], Minako Kajima[1], Yuji Takao[1], Norihisa Tatarazako[2], Taisen Iguchi[3]. [(1)]Nagasaki University, [(2)]Ehime University, [(3)]Yokohama City University)

Title: 'Comparison of response sensitivity to steroid hormones between two species of medaka, Minami-medaka (*Oryzias latipes*) and Kitano-medaka (*Oryzias sakaizumii*)'

Medaka is one of the most useful animals in biological research, it has been used in many research areas of biology, developmental biology, reproductive biology, etc. Also in the research field of aquatic toxicology, this fish is useful test animals to evaluate various kinds of toxicity of chemicals. In some OECD test guidelines, medaka is listed as one of the recommended small teleost species.

Oryzias latipes is only major medaka species inhabited in Japan for a long time. However, in 2012, *Oryzias sakaizumii* has been recorded as a newly classified medaka species inhabited in Japan. Therefore, many scientists have been confusing by appearance of *O. sakaizumii*, because no one knows which medaka species is suitable for the test animal.

Aim of this study is to compare which medaka species is sensitive for detection of steroidogenic potency of chemicals. In addition, the existing PCR method for genetic sex determination of medaka was improved (new primer set applicable for both medaka species was designed).

1. Comparison of response sensitivity for E2
Adult male of both medaka species were treated with E2 (12.5, 25 and 50ppt) using flow-through system for 1 week at 25±1 °C. After exposure, liver VTG concentrations were measured by ELISA. Liver VTG concentrations increased in E2 dose-dependent manner, there was no significant difference in VTG concentration between two species at any E2 dose.

2. Comparison of response sensitivity for MT
Adult female of both medaka species were treated with 17a-methyltestosterone (MT, 33.3 and 100ppt) using flow-through system for 1 or 2 weeks at 25±1 °C. After exposure, a number of papillary processes (PPs) on anal fin were counted. PPs number on anal fin increased in MT dose-dependent manner. However, the number of PPs in *O. latipes* was significantly more than that of in *O. sakaizumii*. These results suggested that sensitivity for androgen in *O. latipes* was slightly higher than that in *O. sakaizumii*.

3. Improvement of the method for genetic sex determination
The existing PCR primers recorded in OECD TG234 is available for *O. latipes*, but not for *O. sakaizumii*. Therefore, the partial genomic DNA of DMY and DMRT1 of both medaka species were cloned and sequenced, the new PCR primers enable to determine genetic sex in both species is investigated now.

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Core Project: New Test Method Development Using Amphibians & Fish

Speaker: Ioanna Katsiadaki, Cefas

Title: 'Recent developments at OECD test guidelines from the UK'

The UK along with European colleagues have been trying for many years to standardise not only the technical aspects of international test guidelines but also the ethical aspects of testing when it involves animals protected under Animals (Scientific Procedures) Act animals. In collaboration with Switzerland our latest efforts (2017) resulted in a proposal to refine TG203 (the fish acute toxicity test), one of the most frequently conducted toxicity tests internationally which has not been updated since 1992. As well as modernising the protocol, we proposed to include the obligatory collection of data on clinical signs as part of the test guideline that could be used in the future to produce scientifically robust and international acceptable definition of humane endpoints that are predictive of death. The overall aim is the eventually move from mortality as an endpoint in TG203, similarly to the mammalian equivalent tests. The work is ongoing and a summary of where we are currently will be presented.

More recently, the UK expressed an interest in participating in the RADAR (Rapid Androgen Disruption Adverse Outcome Reporter) inter-laboratory validation work. This is of particular interest to the UK-Japan cooperation as the model combines an iconic Japanese fish (medaka) with a UK developed biomarker (spiggin). We are looking forward in conducting this transgenic fish test and evaluating the specificity and sensitivity of this assays for the detection of environmental (anti-) androgens.

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Speaker: Frances Orton, University of the West of Scotland

Title: ‘Biomarkers for reproductive health in captive and wild frogs’

Worldwide, many amphibian populations are declining at rapid rates. There is evidence that chemicals may be contributing to some of these declines including via effects on reproduction. Biomarkers of chemical effects on frogs are dominated by analysis of gonadal histopathology, primarily the identification of intersex. Alternative biomarkers of reproductive health are needed as intersex has not been linked to functional outcomes in amphibians. In addition, it may not be a very reliable indicator of reproductive health in amphibians, since ‘background’ levels are found in many species. I will present findings on alternative biomarkers of reproductive health in 4 species: *Xenopus tropicalis* (laboratory), *Bufo bufo* (wild, UK), *Rana temporaria* (wild, UK) and *Engystomops pustulosus* (wild, Trinidad).

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Core Project: Molecular & Genomic Approaches for Understanding EDC Impacts

Speaker: John Colbourne, University of Birmingham

Title: 'Roadmap for Precision Environmental Health'

Building on the sequencing of the human genome in 2001, the healthcare revolution known as 'precision health' has allowed the rapid discovery of a more complete spectrum of disease pathways, asking not whether a defect of a 'known candidate gene' contributes to elevated death rates, but rather what known and unknown genes conspire within pathways to impact health (doi.10.17226/13284). This data-rich, untargeted multi-omics approach at uncovering the origins of disease now arms medicine with new predictors (i.e., potential biomarkers) for over 10,000 monogenetic human diseases and over 8,000 new potential drug targets valued at more than $50 billion (doi.10.1038/nrd2199). However, it is well known that phenotypes, including disease phenotypes, are the result of gene by environment (G x E) interactions. As such, precision health is unlikely to reach its ultimate goals until environmental factors are considered.

Thus, there is the need for similar data-rich approaches for a 'precision environmental health' revolution, which allows the rapid discovery of a more complete spectrum of environmental health hazards and toxicity pathways that are directly integrated into the known and rapidly growing databases of genetic and regulatory pathways associated with disease. In other words, there is a timely opportunity for the environmental health sciences research community to fill a knowledge gap to prevent exposure-related harm, building upon the already huge investments in the discovery of gene-related disease pathways, most of which have unknown associations with environmental conditions including exposure to toxicants.

A roadmap is drawn to identify five utilities of scientific data, mapped to interventions that affect the likelihood of more effective evidence-based regulation to achieve environmental health.

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Speaker: Shinichi Miyagawa, Taisen Iguchi *et al,* Yokohama City University

(Saki Tohyama[1], Anke Lange[2], Charles Tyler[2]. [(1)]Yokohama City University, [(2)] University of Exeter)

Title: 'Approaches for studying endocrine disruption and estrogen receptor subtypes in fish'

A wide range of chemicals are now known to mimic hormones based on their structure or via their interference with hormone biosynthesis and/or degradation. Exposure to these so-called endocrine disrupting chemicals (EDCs) can cause disruptions in development, alter sexual differentiation and function, and impact adversely on reproduction in various animal species. A primary focus of Core Project 2 is to develop methods and systems to help better inform on EDCs, especially those acting through nuclear receptor pathways, through the application of molecular/genomic tools.

Estrogens play fundamental roles in regulating reproductive activities and they act through estrogen receptor (ESR) in all vertebrates. Most vertebrates have two ESR subtypes (ESR1 and ESR2), whereas teleost fish have at least three (Esr1, Esr2a and Esr2b). Intricate functionalization has been suggested among the Esr subtypes, but to date, distinct roles of Esr have been characterized in only a limited number of species. Study of loss-of-function in animal models is a powerful tool for application to understanding vertebrate reproductive biology. Recently, zebrafish Esr mutant study has been reported and a single Esr mutation is dispensable for normal reproduction in zebrafish.

Medaka is an excellent model for studying sex determination and differentiation during early gonadal development as genetic and intrinsic sexes can be identified. We previously found that esr1 KO medaka did not show any significant defects in gonadal development, sexual characteristics and reproductive activity as in the case for zebrafish. Esr2a KO female medaka, however, showed abnormal abdominal swelling with ovarian expansion and are infertile. We thus provide an evidence even within the teleost lineage, roles and functions of Esr are diverged. Our findings will also benefit interpretation of studies into the toxicological effects of estrogenic chemicals in fish and building adverse outcome pathways (AOPs).

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Speakers: Anke Lange, Charles Tyler, University of Exeter

Title: 'New developments in fish ecotoxicology models'

Over the duration of the UK-Japan partnership, core project two has developed and applied a wide range of molecular and genomic tools to advance mechanistic understanding on how EDCs and other emerging chemical contaminants mediate their effects in both sentinel and model fish species and to better understand population levels impacts. In recent years, this partnership has largely focussed on developing transgenic and knockout models (both, medaka and zebrafish) for use in establishing the mechanisms underlying observed adverse physiological effects for exposure to xenobiotics, especially environmental oestrogens. These models have been applied to study aspects of basic biology (e.g. role of ESR1 on sexual development and reproduction), test for chemical effects and chemical potencies, and screen environmental samples for hormone activity.

In the first part of this presentation, we will introduce the viviparous teleost Redtail splitfin (*Xenotoca eiseni*), a live bearing freshwater fish, as a potential novel model for investigating the effects of maternally derived micropollutants on development in early life stages. We first established a basic understanding on the reproductive physiology of the Redtail splitfin, documenting the ontogeny of both gonad and embryo development via histopathology. In males, the testis was comprised of two lobes merged at the anterior end and spermatogenesis started around four weeks after birth. Spermatozeugmata first appeared at between four and eight weeks. Females had a hollow structured ovary that was divided into two compartments by a highly folded septum and oogenesis was initiated between two and four weeks after the young were born. Females reach full sexual maturity at around twelve weeks, at a body length of around 3cm. Fertilisation and gestation took place in the ovary and two weeks after fertilisation, embryos hatched within the ovarian lumen. At this time the yolk reserve had been depleted and the fish larvae developed trophotaeniae (hindgut extensions) through which nutrients were absorbed from their mother. Gestation took around six weeks. To investigate the potential for maternal transfer of endocrine disrupting chemicals in the Redtail splitfin, we exposed females to 17α-ethinyloestradiol (EE2), including at environmentally relevant concentrations, and assessed effects on sex partitioning and early life development, the findings for which we will present at the meeting.

The second part of this presentation will illustrate various transgenic zebrafish models developed for application to studies on basic biology (e.g. investigations into the roles of oestrogens in brain development and function) and for studies into the effects of exposure to a wide range of toxicants, including agrochemicals and pharmaceuticals of environmental health concern and nanomaterials. The transgenic fish we will describe include those for detecting responses to oestrogens, aromatase inhibitors, and oxidative stress and for assessing neural activity in the brain. These transgenic fish are being applied to identify target tissues and chemical exposure effects, including for chemical mixtures, via imaging techniques (e.g. fluorescence, confocal and light sheet microscopy). Responses in some models are captured in real time using light sheet microscopy. The transgenic models provide valuable tools for studies into adverse outcome pathways of chemicals and as much of this work is being

conducted in embryo-larval stages prior to exogenous feeds, the work is also supporting the use of non-regulated animals for chemical research and testing.

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Presentation Slides

To be populated for hard copies.

Maps and Plans

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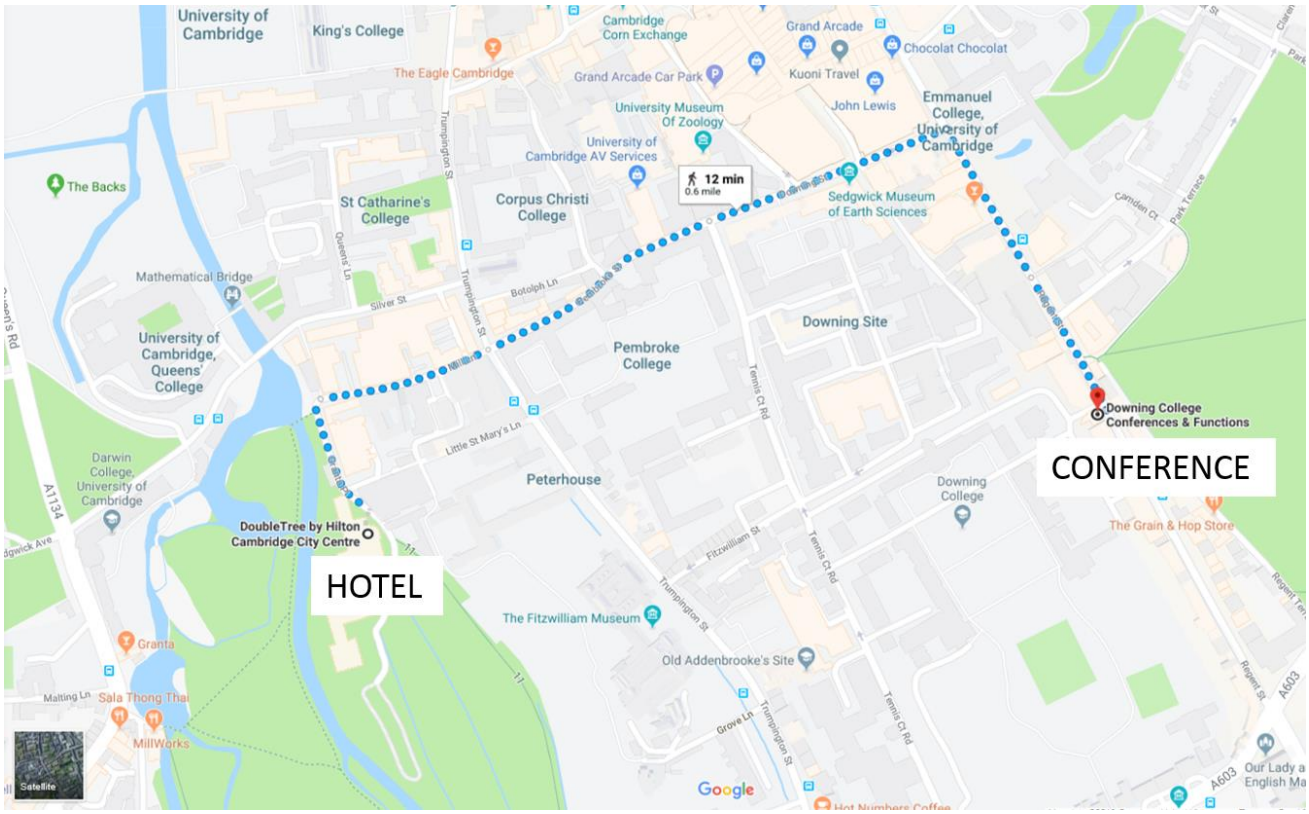
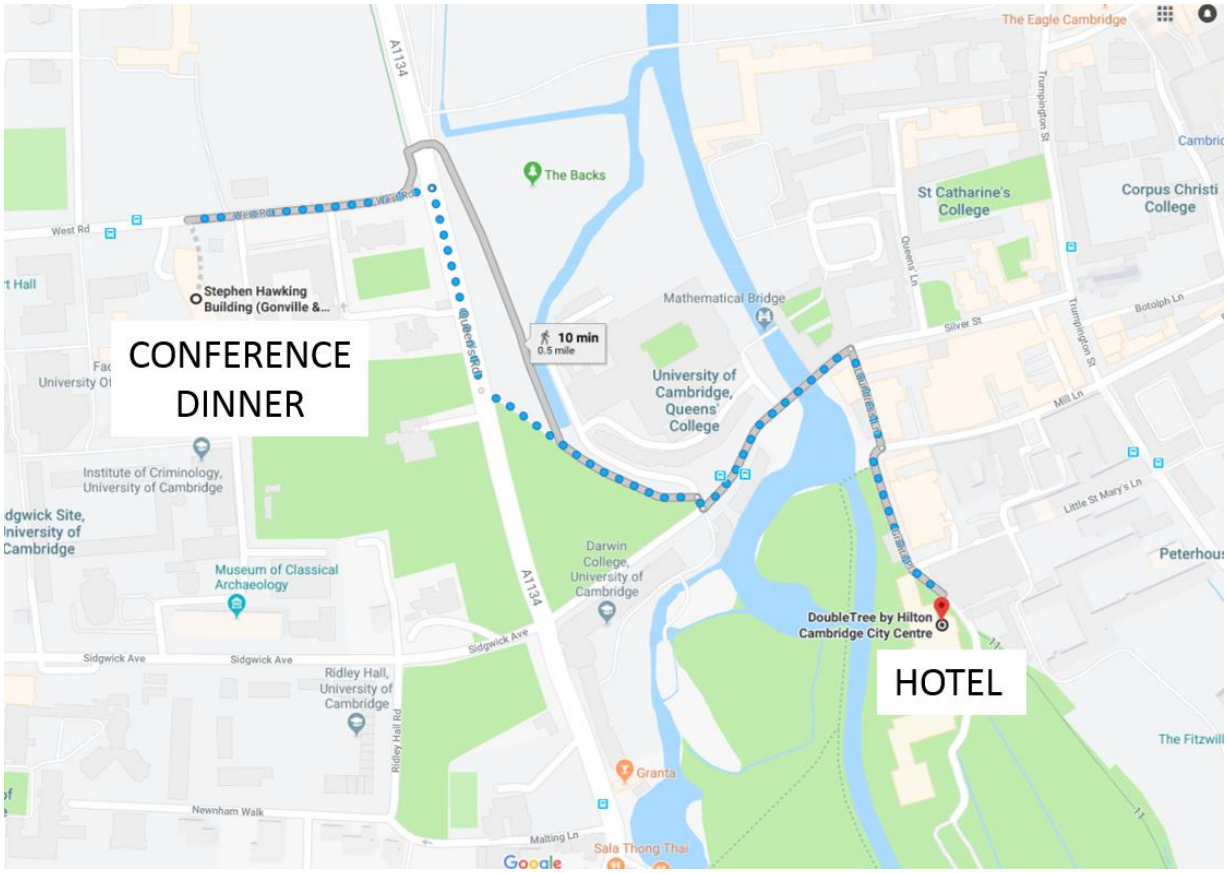
Phone: 01223 259988

Conference Centre: Howard Building, Downing College
Address: Downing College, Cambridge, CB2 1DQ
Phone: 01223 334860

Conference Dinner: Stephen Hawking Building, Gonville & Caius College
Address: Trinity Street, Cambridge, CB2 1TA
Phone: 01223 332400

Visit: Anglian Water Services, Cambridge Water Recycling Centre
Address: Cowley Road, Milton, Cambridge, CB4 0AP
Phone: 0345 791 9155

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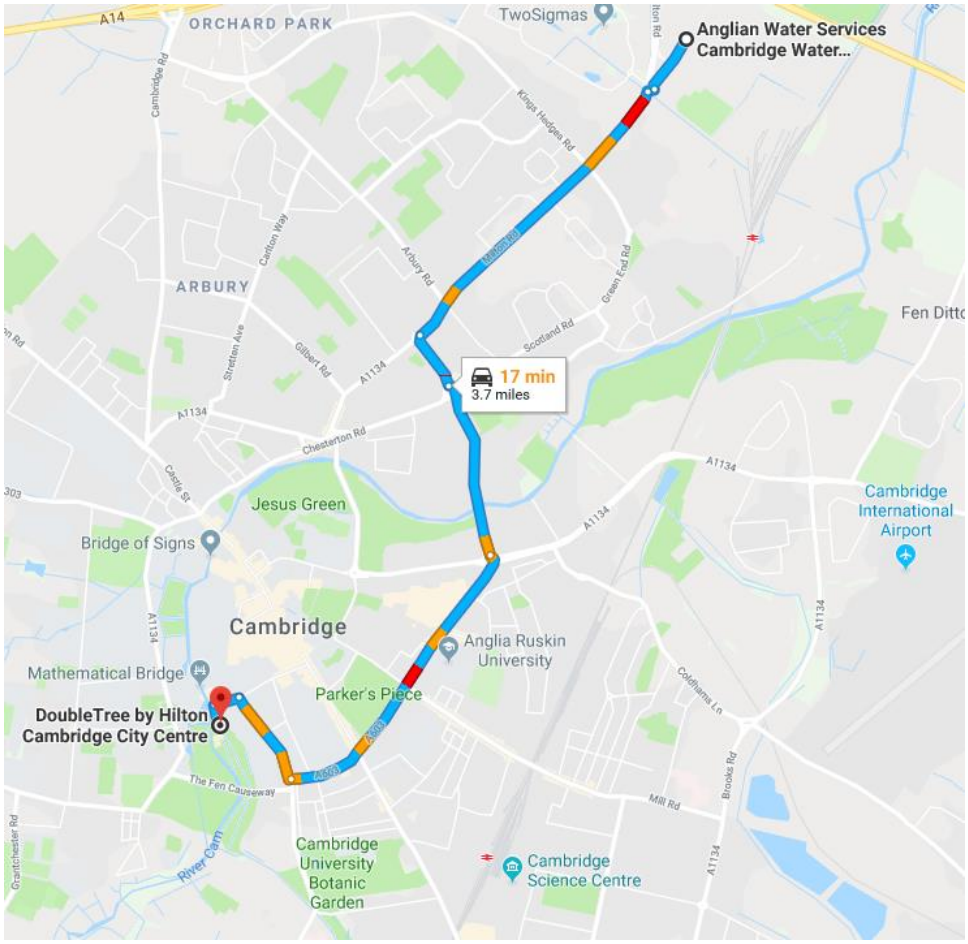
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