THE IMPACT OF EMERGING CONTAMINANTS TO THE ENVIRONMENT AND HUMAN HEALTH

Emerging contaminants are consistently and indirectly manufactured for global use and have now been found in all corners of the earth - in the water, soil, air, and biota. Agilent spoke to two of its research partners across the globe to understand the risk of these contaminants in each region.

Introduction:

Over the last century, the world has industrialized and urbanized at an extremely quick rate. While industrial and urban development has led to many benefits in society, the potential impact to our environment, and subsequently our human health, is slowly emerging. Advances in scientific research and technology in recent years have led to the widespread detection of 'emerging contaminants', which are chemical substances or compounds that are potentially threatening to environmental or human health, introduced by human activities in most cases such as perfluorinated compounds, water disinfection byproducts, gasoline additives, pharmaceuticals, man-made nanomaterials, microplastics and UV-filters.[1]

Many emerging contaminants have been in the environment for long periods of time but concerns over their presence and potential impact have often only recently been raised. Currently, little is known about many of these contaminants and most remain unregulated.[2,3] To understand more about this problem, Agilent interviewed two of its research partners from across the United States and Europe who shared their research and insights into this significant environmental challenge:

Exploring the increasing prevalence of emerging contaminants in US waters

We start with Professor Edward Kolodziej, whose research group at the University of Washington is working to characterize emerging contaminant fate and transport, and focuses in particular on understanding chemicals with adverse effects on fish that are present in urban storm waters and roadway runoff. Recently, they have analysed the chemical composition of roadway runoff, by applying high resolution mass spectrometry techniques to identify and characterize unknown chemicals in storm water sample types.

Tarun: Why is studying unknown or unregulated chemicals in the environment important?

Professor Edward Kolodziej: Unknown or unregulated chemicals are important because some of these contaminants induce adverse effects on biological organisms and humans. Particularly for sublethal impacts on growth, immune function, reproduction, neurodevelopment, endocrine signalling, etc, there still seems to be many not yet explained effects that arise when aquatic organisms are exposed to complex mixtures of human chemicals. We need to better understand these exposures and their implications to improve our management of environmental quality. Knowing what these chemicals are in these mixtures seems a good first step.

Professor Edward Kolodziej, Associate Professor of Civil & Environmental Engineering at the University of Washington, Division of Sciences and Mathematics, University of Washington-Tacoma, and Principal Investigator, Center for Urban Waters, Tacoma, WA

Dr. Leon Barron, Reader in Analytical & Environmental Sciences, Imperial College London

Tarun: Can you tell us about the research you and your team uncovered relating to the deaths of the coho salmon population in Puget Sound's urban streams? What was the cause of death for this species?

Professor Edward Kolodziej: For decades there have existed unexplained observations of water quality linked acute mortality in adult coho salmon after rainstorms in the Western US. When I arrived at the University of Washington in 2014, I was able to start collaborating with an excellent team of researchers to try and understand what was in the water when those salmon were dying.

Over time, our data and collaborative efforts led us closer and closer to roadway runoff and tire-rubber derived chemical contaminants as being important to this fish mortality phenomena. Once our ecotoxicology collaborators demonstrated acute mortality of juvenile coho salmon after exposure to a tire rubber leachate, we used an effects directed analysis approach to fractionate those samples chemically. This effort allowed our team to be the first to identify a highly toxic transformation product of 6PPD, which is an antioxidant chemical widely used in tires.[4] 6PPD reacts with ozone, as intended to protect the tire rubber, to produce a transformation product which then leaches into water. Unfortunately, coho salmon are especially sensitive to this transformation product, it's really lethal to them, indicating the importance of screening for bioactive transformation products in environmental systems, especially for sensitive or ecologically important species.

Tarun: How did these contaminants seep into the water and where did they originally come from?

Professor Edward Kolodziej: Bits of rubber and the chemicals in them don't disappear when they get out into the environment. These rubber particles leach chemicals into rainwater, and those get into our rivers and streams. Our efforts helped close the mass balance on a common antioxidant used in tire rubbers; we just started paying attention to a product that wasn't previously characterized in the environmental literature because the coho salmon were telling us it was important.

Tarun: What kind of research methods/instrumentation did you use to carry out your experiment?

Professor Edward Kolodziej: We use an Agilent 1290 UPLC and a 6530 QTOF-HRMS for primary sample characterization. The unbiased approach to sample screening and characterization was great; the instrument was telling us what was actually there instead of what we thought might be there. Thus, it provided the key analytical breakthrough for us to unlock the problem and figure out what was in that roadway runoff and tire rubber leachate that was important to watch.

Tarun: Is there anything we can do to mitigate the use of these emerging contaminants?

Professor Edward Kolodziej: We need to consider improving our ability to screen and simplify the chemical ingredients of our common consumer products. There is a significant mass of unknown chemicals hitting the environment everywhere, and we are trying to get a handle on what that means for biology so we can sort out the bad actors from the good. Building from a product sustainability and environmental safety perspective would seem like a good start, these are fundamental principles of green chemistry that need to be applied more widely.

River chemical monitoring at scale using new 3D-printing technology developed at Imperial with Agilent

Focusing on rapidly increasing chemicals in rivers of the United Kingdom

Dr. Leon Barron has an interest in how chemicals are being used, what happens to them after use, and whether they would pose a risk to our environment. Looking mainly at pharmaceuticals, pesticides, illicit drug, and personal care products, the scope of his team is steadily increasing to include more types of chemical classes including mobile chemicals which also display persistence and toxicity.

Tarun: As the leader of the Emerging Chemical Contaminants (ECC) team within the Environmental Research Group at Imperial College London, which areas of emerging contaminant research do you focus on specifically, and why?

Dr. Leon Barron: 'Emerging' chemical compounds have been monitored over the past 20 years and in many cases have been ubiquitously detected in the environment. Advances in knowledge have been largely underpinned by more widespread availability of better analytical technologies, such as high-resolution accurate mass spectrometry. However, often very little is known about their risks. One major focus of ours is whether such contaminants impact low-trophic level aquatic invertebrate behaviour. We are also involved in wastewater-based epidemiology which has been shown to be an excellent way to understand not just lifestyle and activity in a community,[5] but also health, as evidenced by national wastewater monitoring programmes for SARS-CoV-2,[6,7] and also potentially imminent threats to public safety.[8]

Tarun: Could you tell us more about the objectives of the ECC team at Imperial College London with regards to environmental research, and why your work is important for environmental, aquatic animal and human health?

Dr. Leon Barron: The aim of the ECC team is to understand, manage and mitigate chemical contamination in the environment, with a focus on compounds that we know very little about regarding their sources, distribution, fate and effects. Our work sits in several cross-cutting areas including:

a. Analytical methods: We develop new approaches for trace chemical monitoring, usually involving advanced separation science, mass spectrometry and chemometrics for large numbers of compounds (typically >200 for quantitative work, as well as high resolution untargeted analysis and suspect screening for >1000 compounds).

b. Wastewater-based epidemiology (WBE): We identify and monitor exposure, manufacture and/or consumption of chemicals at population scales in near real-time at wastewater treatment plants (WWTPs).

c. Environmental toxicology and risk assessment: The focus here is on occurrence, fate, toxicity and risks of emerging and new chemical contaminants in surface water, soil, sediment and predominantly low trophic-level aquatic and terrestrial invertebrates. This also includes application of 'omics-based' approaches such as lipidomics and metabolomics to help understand molecular-level initiating events and effects in these species.

Across these objectives, we are currently pursuing several funded research projects, often in close collaboration with industry, which really strengthens the value and impact of our work including translating it into practice. We are very keen to understand and prioritise wildlife health in our work, not just in terms of monitoring, but also predictively by developing new approaches to understand and predict which compounds may be an issue now or in the future, as well as management options to potentially minimise exposure.

Tarun: Could you share more about your group's recent research in screening 'emerging' contaminants along the estuarine River Colne in Essex, UK?

Dr. Leon Barron: This work[9] followed on from a previous invertebrate monitoring campaign in rural Suffolk, UK, in the summer of 2018, where we detected large numbers of emerging contaminants in a benthic amphipod, Gammarus pulex.[10] We wanted to see what the contrast was when moving to an urban area and to extend this to more species and also to more sample types. We took several samples of river water, sediment and four different invertebrate species including G. pulex, Peringia ulvae (a mollusc), Hediste diversicolor (a polychaete) and Crangon crangon (an amphipod). These invertebrates often play critical roles in river health, by cleaning detritus and acting as a food source for predators. Therefore, any significant disruption to their behaviour or health could potentially be quite an issue.

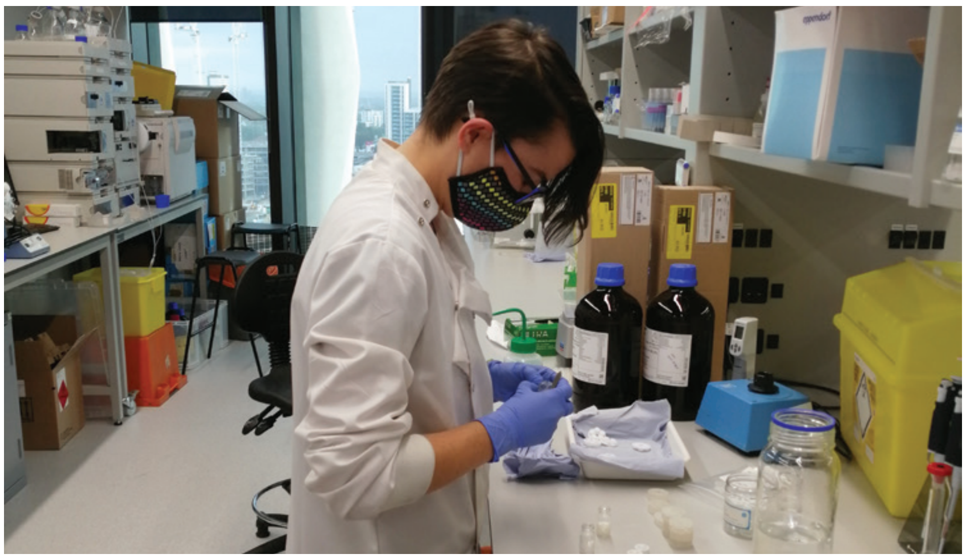
Following analysis, 33 compounds were detected in the macroinvertebrates sampled, 39 compounds detected in sediment and 59 compounds detected in surface water samples. The most impacted site was next to a wastewater treatment plant discharge point. Over half of the drugs detected in invertebrates were psychoactive substances including pharmaceuticals and illicit drugs. For the latter, cocaine and its metabolite benzoylecgonine were the most frequently detected and highest average concentration compounds which was the same in samples from Suffolk.

Previous work in our laboratory showed that, despite this, the risk posed by cocaine and benzoylecgonine is likely low in comparison to other psychoactive compounds. In addition to drugs, the banned neonicotinoid pesticide imidacloprid was also found in G. pulex and P. ulvae at 8 ± 3 ng/g and 24 ± 8 ng/g, respectively. Given that no usage was reported around this time, the conclusion was that occurrence was possibly based either on persistence of these compounds in soils which may leach into surface water or veterinary pesticide use, such as flea/tick medication. Interestingly, higher concentrations of emerging contaminants were found in infaunal species than epibenthic organisms showing that the main route of exposure for these organisms was likely through the higher measured concentrations in sediment.

Tarun: How do you think further research in this area could be enhanced?

Dr. Leon Barron: The important thing for us now is to broaden the chemical coverage to include more types of compound, to try to characterise more of the exposome. Aside from 'omics' approaches to understand internal biological processes in invertebrates under chemical stress,[11,12] next for us is to develop and apply more efficient suspect screening workflows using high resolution mass spectrometry to detect (bio) transformation products and refining analytical methods to minimise extraction steps.

Although challenging to do just yet for semi-solid samples, we are already performing this type of work for wastewater and river water. Advances in instrumental sensitivity now allow us to perform direct quantitative analysis of hundreds of compounds in under five minutes at low ng/L concentration levels, and at scale, with just a few millilitres of sample.[13]



The new Environmental Research Group facility based at Imperial's White City Campus

A team led by researchers at the University of Washington Tacoma, UW and Washington State University Puyallup have discovered a chemical that kills coho salmon in urban streams before the fish can spawn. Shown here Zhenyu Tian, a research scientist at the Center for Urban Waters at UW Tacoma, holds a sampling pole, which is used to collect creek water for future tests. ***Credit:*** *Mark Stone/University of Washington*

Tarun: Are you utilizing the Agilent Measurement Suite (AMS) at Imperial College London to conduct your research? If so, what kind of methods/instrumentation are you using, and how has this impacted your research?

Dr. Leon Barron: We've recently engaged with the Agilent Measurement Suite (AMS) and it has been an excellent resource for academic researchers, especially to try out new instrumentation and access the wealth of experience Agilent has in analytical method development and complex data analysis.

We currently have a Biotechnology and Biological Sciences Research Council (BBSRC)-funded project in partnership with Agilent looking at development of 3D-printed passive samplers. We recently engaged with the AMS to perform machine learning-assisted high-resolution suspect screening on sampler extracts from the River Thames in Central London, which was published on the cover of RSC Analytical Methods.[14] Victorian sewers in London have been coping with a rapidly expanding population and combined sewer overflows occur on a regular basis here making understanding any impacts of chemical pollution from wastewater an important issue. In this work, 65 unique targeted compounds were detected in passive sampler extracts of river water using an Agilent 1290 Infinity II LC system coupled to a 6546 LC/Q-TOF mass spectrometer.

Over the past number of years, we have been working hard to develop retention time prediction models to refine mass spectral suspect shortlists.[15,16,17,18] Even with high resolution measurements, several candidates often exist and the ability to differentiate them often lies in the chromatography. For use in passive sampler extract suspect screening, we developed a machine-learning based tool for retention time prediction for a gradient separation on an Agilent Zorbax Eclipse Plus C_{18}, 2.1 × 100 mm, 1.8 µm column. This enabled *in silico* tentative identification of 59 additional compounds in the same extracts. In general, we shortlist suspects first based on mass spectral database matching, followed by machine learning-assisted retention time prediction and the latter usually reduces these shortlists by roughly two thirds, which makes things more manageable. This serves therefore as an excellent way to prioritise the identification and confirmation of new and emerging contaminants more rapidly. Whilst we now do this routinely ourselves, the focus now is to make this an integrated application and it is excellent to have the AMS now on our doorstep at the Imperial White City campus to extend our collaborations.

Tarun: How do you think New Approach Methods (NAMs) – such as those involving machine learning – will continue to shape the future of chemical risk assessments in environmental research?

Dr. Leon Barron: There is an increased interest and demand for development and integration of machine learning-type tools within environmental risk assessment (ERA) as a perceived 'quick-win' & prioritization mechanism for such a vast array of chemicals, their metabolites and transformation products.[19] However, regulatory bodies such as the European Chemicals Agency have suggested that *in silico* approaches cannot yet replace classical toxicology, as more underpinning science and assurance is required. I think this is probably a fair appraisal for now given the current lack of skills, research and knowledge of the benefits and pitfalls of machine learning in ecotoxicology even though they have several benefits.

It is a disruptive technology and holds much potential, especially to help prioritise laboratory-based experimental ERA. It could also be used to rapidly assess management strategies for hazardous substances, for example, through *in silico* prediction of removal during wastewater treatment, as well as compound stability, (bio)transformation and distribution in the environment. This could also be integrated earlier in the product design pipeline to potentially risk assess new candidate chemicals. We have recently successfully used it to help estimate toxic or effect pressure from several emerging contaminants detected in real water samples as part of persistent, bioaccumulative and toxicity (PBT) assessments.

Certainly, there are issues to overcome, including approaches to determine suitable model inputs, interrogability for deeper mechanistic understanding of how models work, predictive accuracy for new compounds and generalisability across compartments, locations and/or species. However, at least for now, it is a very worthwhile option to explore and it is vital to properly validate it first and down the line perhaps use it in combination with more accepted laboratory-based approaches.

Conclusion:

Monitoring and mitigating our exposure to emerging contaminants is essential for the future of human and wildlife health. While researchers have already uncovered consequences of some of these contaminants to our environment, there are a plethora of unknown chemicals we are exposed to which we have almost no information on still. Further information and a paradigm shift in our thinking about lifecycle assessment of chemical introduction into manufacture and the environment is required. Robust monitoring & stringent regulation to lessen the use of these contaminants in society are essential. As technology continues to progress, scientists are in a better position to highlight the true nature of these contaminants and bring about real change.

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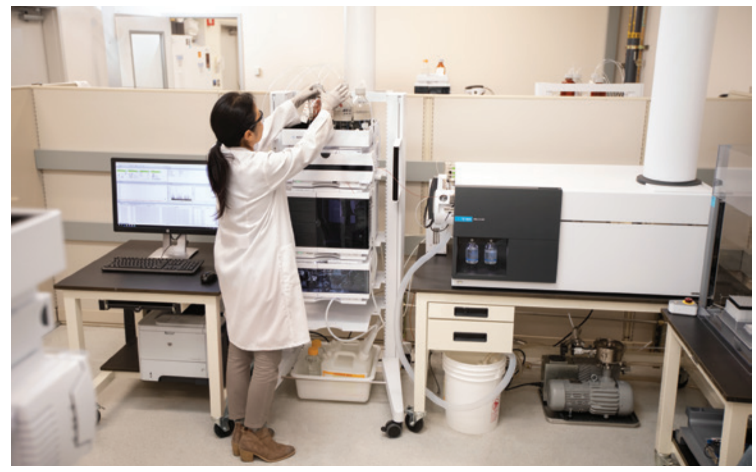

Agilent 1290 Infinity II LC system coupled with a 6546 Q-TOF Mass Spectrometer

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Author Contact Details

Tarun Anumol, PhD, Director, Global Environment & Food Applied Markets, Agilent Technologies. • Email: tarun.anumol@agilent.com • **Web: www.agilent.co**