NIHR
Health Protection Research Unit in
Chemical and Radiation Threats and Hazards

2020/21 Annual Report

MRC Centre for Environment & Health
Medical Research Council
Imperial College London

NIHR | Health Protection Research Unit in Environmental Exposures and Health at Imperial College London

NIHR | Health Protection Research Unit in Chemical and Radiation Threats and Hazards at Imperial College London

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1. OVERVIEW

Mission and vision

The mission of the National Institute for Health Research (NIHR) Health Protection Research Unit in Chemical and Radiation Threats and Hazards (HPRU-CRTH) is to undertake the highest quality research on the health effects of exposures to hazardous chemicals and radiation to improve their assessment, management and control. Our aim is to gain new knowledge on the distribution, determinants, mechanisms and pathways linking these exposures to health effects and to advance our understanding of how the everyday and exceptional contact we have with chemicals and radiation leads to ill health, in order to strengthen the scientific evidence underpinning public health practice and policy.

Our vision is to establish the HPRU-CRTH as a world-class resource for knowledge, expertise and training on today's most serious chemical and radiation threats and hazards, producing evidence to ensure effective protection of the population and to mitigate health inequalities from these exposures.

Strategy and objectives

The HPRU brings together leading expertise in epidemiology, biology, chemistry, toxicology, statistics and 'omics' technologies. Our multidisciplinary approach will identify markers of exposure and risk based on mechanistic understanding of disease pathogenesis, integrate these into epidemiological studies and investigate novel approaches to risk assessment, mitigation and health protection. Our research programme is organised into four complementary Themes focusing on exposures to priority hazards, including ionising radiation, electromagnetic fields, ultra-violet light, re-developed brownfield sites, neurotoxins and other high-toxicity chemical agents and drinking water contaminants. Priorities were selected to represent areas of scientific uncertainties and policy concern.

Our strategy will be informed by, and remain responsive to, the priority research needs and evidence gaps in the UK identified in partnership with Public Health England / UK Health Security Agency[1] (PHE/UKHSA), other public health agencies, our International Scientific Advisory Board (ISAB), Public and Community Oversight Group (PCOG) and local communities. Particular attention will be given to engaging with the diverse range of communities affected by the hazards under study, training the next generation of research leaders in environment and health and to the translation of scientific findings into policy or commercial exploitation.

Progress/achievements in the first year

This report will present how, during its first year, the HPRU-CRTH successfully set up operations and started to deliver on its research programme, producing evidence supporting the protection of public health from chemical and radiation hazards.

Leadership, governance and management arrangements

We established the governance structures of the HPRU-CRTH, including the HPRU's Steering Committee, joint Academic Career Development and Public and Community Involvement Engagement and Participation (PCIEP) Committees in partnership with the HPRU in Environmental Exposures and Health (HPRU-EEH) and Medical Research Council Centre for Environment and Health (MRC-CEH) (details of the leadership, governance and management arrangements can be found in Annex 1). These Committees meet regularly to oversee and coordinate the operations of the HPRU. The PCIEP, Academic Career Development and Knowledge Mobilisation strategies were developed in the first year and are being implemented (see below Sections 3, 4 and 5).

In addition, we established two independent external advisory groups, again jointly with the HPRU-EEH and MRC-CEH: the International Scientific Advisory Board (ISAB) and the Public and Community Oversight Group (PCOG). The ISAB is composed of experts in the main research areas of these units who advise on strategic direction and implementation of their research programmes. The PCOG is

[1] Public Health England will transition to a new agency, the UK Health Security Agency, from October 1, 2021

composed of members of the public, industry, local government, non-governmental organisations (NGOs) and community and patient groups representing 20 organisations. Its role is to advise on HPRU-CRTH activities ensuring they remain responsive to the interests and concerns of the broad community of stakeholders of our research (see Section 4 and Annex 3).

Substantial progress has also been made in building on the research networks between the partners. To foster the development of multidisciplinary, multi-partner research teams, we held three rounds of theme meetings identifying multiple opportunities for collaboration across themes and projects.

Implementation of the research strategy

The challenges posed by the COVID-19 pandemic have affected some projects, delaying recruitment and some early milestones; however, we have succeeded in keeping these impacts to a minimum.

- All but two of the projects (Theme II, Project 4 and Theme IV, Project 4) were underway by the end of Year 1 and nearly all the first-year milestones were completed on or ahead of time;
- All but one of the PhD students were recruited with all the appointed students and projects starting by October 2021.

Most short-term objectives listed in our application were achieved in Year 1 or are on track for being fully achieved within the first half of Year 2. Below is a summary of the changes to the research strategy included in our application (with further details given below):

- Following review of the potential policy impacts, two closely related projects in Themes II and III (Theme II, Project 3 and Theme III, Project 3) were re-focussed from studying the health effects associated with closed landfill sites to analysing the effects of residual chemical exposures from brownfield sites;
- Two additional projects leveraging external funds were associated with the HPRU-CRTH (Theme III:Project 5 and Theme IV:Project 5) addressing high priority areas for public health protection, namely the health effects of pesticide traces in food and detection of chemical threat agents in wastewater.
- In addition, we were successful in the award of an extra PhD student by PHE/UKHSA to support work on Theme II, Project 1, studying biomarkers of radiation exposure in blood samples of nuclear medicine technologists.

The research programme of the HPRU-CRTH has started to produce new insights into some of the priority chemical and radiation exposures with health impact in the UK. Below is a selection of key outputs of the HPRU-CRTH during 2020-2021:

- Results of analyses of childhood cancer near nuclear installations were presented to the Committee on Medical Aspects of Radiation in the Environment (COMARE) to inform radiation protection policy.
- We have identified a two-phase response to the effects of UV on nitric oxide (NO) production in skin cells suggesting that the vasodilatory effect may operate over a longer time-scale than previously thought, extending beyond the time of direct exposure. We are now investigating the effects of sunlight on NO production directly.
- Work on detection of the production of ‘homemade’ chemical threat agents through analyses of municipal wastewater is providing new capability to identify imminent chemical threats to the public. A new database of the chemical composition of several high-priority homemade explosives is now used by the UK Intelligence Community (UKIC).

Significant challenges faced during 2020-2021

The disruption caused by the COVID-19 pandemic resulted in delays in student and staff recruitment, in data access for non-priority COVID-19 projects, and reduced capacity in some laboratories. In addition, some staff working on the HPRU-CRTH, both at Imperial College London (ICL) and at PHE, were co-opted to support COVID-19 projects or response. The workplans for the projects affected have been adapted and these delays will be recovered in the coming years with negligible impact on our medium- to long-term deliverables. Conversely, CRTH-HPRU researchers have made important contributions to the COVID-19 response, notably through the REal-time Assessment of Community Transmission (*REACT*) programme.

2. RESEARCH THEMES

Theme I – Adverse outcome pathways and exposure-response relationships for ionising and non-ionising radiation

Theme Leads – Ken Raj (PHE/UKHSA) and Mireille Toledano (ICL)

Theme I Overview

Human exposures to ionising and non-ionising radiations are ubiquitous. They include natural and man-made sources of ionising radiation, radiofrequency electromagnetic fields (RF-EMF) from telecommunications, and ultra-violet (UV) and visible light exposures (both indoors and outdoors). There is greater awareness in society of the increasing exposure to EMFs, and growing concern that they may be a potential cause of ill-health. Research projects in this Theme address the potential health effects - good or bad - of the most prevalent electromagnetic waves that humans are exposed to, including both ionising and non-ionising radiations, the latter including sunlight, mobile phone and radio waves.

Our strategy is to gain improved understanding of these exposures and their effects on health through both epidemiological and mechanistic investigations. Research topics include quantifying cancer risk of populations living near nuclear installations, effects of ionising radiation exposure on circulatory disease and age-related pathologies, health impact of mobile telecommunications usage and assessing the risks and benefits of UV and light exposures on health. We will undertake studies to quantify population and age- and sex-related variation in response.

Objectives

- *Short term:* To update estimates of cancer risks among children living near nuclear installations; to establish human cell models to investigate effects of individual variation, age-at-exposure and cell type, and epigenetic ageing, from ionising radiation exposure; to establish cell-based assays to determine UV and light impacts on cardiometabolic risk using surrogate assays.
- *Medium term:* To obtain quantitative data on the impacts of age-at-exposure and inter-individual variation on IR responses related to circulatory disease and ageing; to delineate time trends in risk of childhood cancers near nuclear sites, and, if indicated, investigate causes of any persistent risk including effects of population mixing; to improve quantification of possible health impact of mobile telecommunications usage; to improve the evidence base for advice on 'damaging' vs 'healthy' levels of UV/sunlight exposure.
- *Long term:* To describe non-mutational mechanisms of ionising radiation action and identify biomarkers, to understand population variation in response to ionising radiation; to provide clear evidence on the harms, or lack thereof, due to RF-EMF exposure; to provide evidence to refine policy in relation to UV and light exposure.

Project 1 – Nuclear installations and childhood cancer

Project Lead – Bethan Davies (ICL)
Research Team – Paul Elliott, Fred Piel, Mireille Toledano and Daniela Fecht (ICL)

Summary and project aims

As part of ongoing surveillance of nuclear installations in Great Britain, the Committee on Medical Aspects of Radiation in the Environment (COMARE) requested that the UK Small Area Health Statistics Unit (SAHSU) undertake a re-evaluation of childhood cancer near nuclear installations following previous reports of an excess cancer risk near these sites. Data on incident cases of cancer from 1995-2016 from PHE/UKHSA and the Office for National Statistics (ONS) historical data, Welsh Cancer Intelligence & Surveillance Unit (WCISU) and Scottish Information Services Division (ISD) are being used to explore patterns in childhood cancer (<15 years) in the vicinity of nuclear installations, by comparing sex-specific annual incidence and the overall age distribution of childhood cancers to reference levels, and conduct specific analyses by country and cancer type (leukaemia and/or non-Hodgkin's lymphoma, Central Nervous System tumours and solid tumours).

Challenges to be addressed

The results will provide evidence concerning the safety and use of nuclear power in the UK and contribute to radiation protection policy. Outputs will be achieved through a combination of publications in peer-reviewed scientific literature, and presentations at national and international conferences, and improved evidence-based advice to the public. Intended audience includes the research community, the public and international regulatory bodies involved in radiation protection, facilitated by HPRU-CRTH staff involvement in COMARE, the United Nations Scientific Committee on the Effects of Atomic Radiation and the International Commission on Radiological Protection.

Year 1 progress and outputs

The analyses were presented to COMARE in March 2020 and are being written up for publication.

Future direction and objectives

This work is part of a periodic re-evaluation of possible health effects associated with nuclear installations (possible environmental risks; for biomarkers of occupational exposures to IR in the nuclear industry, see Theme III:Project 2 below). Following publication and dissemination of the findings, this project will be complete, although the HPRU and SAHSU remain ready to undertake additional work if and when required.

Project 2: Ionising radiation adverse outcome pathways for circulatory diseases and ageing

Project Leads – Ken Raj and Sylwia Kabacik (PHE/UKHSA)
Research Team – Donna Lowe and Christopher Whiteman (PHE/UKHSA)

Summary and project aims

Safe ionising radiation exposure limits are currently based on epidemiological estimates of the risk of cancer. Much has been learned in recent years about the biological effects of ionising radiation and it is becoming clear that a more comprehensive approach to radiation protection is necessary. First, not only is the risk of cancer increased by ionising radiation, but the risk of developing cardiovascular disease is also increased, especially in medical settings such as radiotherapy involving the chest area (see Theme II:Project 1). Second, cellular and tissue responses to ionising radiation are individual-dependent. While this may be due to genetic differences, it could also be due to other characteristics such as age and sex. The ultimate (long-term) aim of this project is to move health protection from the generic towards the individual, with risk assessment based on genetics, age and target cells/tissues for radiation, with a particular focus on circulatory diseases. We will establish systems to investigate the effect of age at exposure, degree of inter-individual variation and qualitative and quantitative differences between normal cell types in response to ionising radiation, focussing on the impact of radiation on cellular epigenetics with particular emphasis on ageing.

Challenges to be addressed

The main cellular target of ionising radiation with regard to cardiovascular disease is the coronary artery endothelial cell. We have previously demonstrated that upon irradiation, the apical surface of these cells becomes adhesive to monocytes, and their cell-cell junctions are weakened and become porous. This, we ascertained, was due to the irradiated cells becoming senescent. It is important to note that senescent cells accumulate with age, independently of radiation exposure. This raises the question of how is the risk of radiation to the coronary artery affected by the age of the irradiated tissue? We will address this by measuring the biological changes associated with senescence (apical surface adhesiveness and cell-cell junction function) of coronary artery endothelial cells derived from human donors of different ages. Senescence is but one of several ageing phenotypes and our investigations into non-senescent ageing show ageing to also occur at the epigenetic level. Having established assays for these two types of ageing in coronary artery endothelial cells, we will test their age-dependent responses to irradiation.

Year 1 progress and outputs

To carry-out the experiment described above we successfully sourced, obtained, immortalised and banked human coronary artery endothelial cells from donors of different ages. These cells and their non-immortalised counterparts are ready for use in the next phase of the study.

Future direction and objectives

We will use these cells to investigate the influence of age (senescence or non-senescence type) on cellular response to ionising radiation with respect to endothelial cell adhesiveness and permeability.

In parallel, we are testing the feasibility of using endothelial cells aged *in vitro*, with their ages measured using epigenetic age clocks, to generate a panel of quantified aged cell populations. If feasible, this will complement the original strategy, extending the biological age range afforded by donor tissues which is currently chronological age between 17 and 60 years. This approach can also address the question of inter-individual differences that may confound interpretation of the results obtained with donor tissues.

Project 3 – Health risks associated with mobile phones and police radios

Project Leads – Paul Elliott and Mireille Toledano (ICL)
Research Team – He Gao, Steven Shen and Joel Heller (ICL); Simon Mann (PHE/UKHSA),

Summary and project aims

Epidemiological studies on the safety of exposure to RF-EMF emitted by modern mobile communication devices have found no convincing evidence of adverse health effects, however, given their relatively recent introduction and almost ubiquitous use it is essential to continue investigation of any potential long-term effects. The aim of this project is to investigate long-term health effects of RF-EMF exposure in some of the largest worldwide study cohorts of mobile communications users.

Challenges to be addressed

We will analyse health outcome data in relation to mobile phone use in the international *COSMOS* study of over 300,000 mobile phone users (100,000 in the UK); we will continue follow up of the *Airwave* study of the British police forces; we will investigate the use of mobile phones and other wireless technologies and health, in the *SCAMP* cohort of ~6,000 adolescents across Greater London.

Year 1 progress and outputs

- *COSMOS* – NHS Digital application for UK health outcome data is approved and data will be available to the research team in UK. The Data Sharing Agreement (DSA) for the cancer analysis is subject to amendment to include a sub-licensing section. The DSA for headache analysis with Dutch collaborators is in place and data transfer is being arranged.
- *Airwave* – c. 5,800 participants were followed up through two COVID-19 related *REACT* studies. Home Office shared a baseline verification record form for the application for access to call data records which we have completed and are awaiting approval.
- *SCAMP* – Manuscript entitled "Salivary sex steroids as markers of puberty in boys during late childhood and adolescence" was drafted. Another paper entitled "Urinary gonadotropins as markers of puberty in boys during late childhood and adolescence" is under preparation. NHS Digital application is under review by the Independent Group Advising on the Release of Data.

Future direction and objectives

- *COSMOS* – Undertake analyses of cancer risk and mobile phone use in the international *COSMOS* study and initiate analyses of incident cardiovascular disease using well characterised information on mobile phone use prior to disease onset.
- *Airwave* – continue follow up of the *Airwave* study of the British police forces with a view to further quantifying cancer risk in relation to police radio use.
- *SCAMP* – Investigate in the *SCAMP* study the longitudinal association between RF-EMF from use of mobile phones and other wireless technologies, and cognitive development during adolescence.

Project 4 – Potential effects of ultraviolet light on cardio-metabolic risk

Project Leads – Gareth Hazell and Ken Raj (PHE/UKHSA)
Research Team – Marina Khazova (PHE/UKHSA)

Summary and project aims

Current health advice on sun exposure is heavily influenced by concerns about development of melanomas following UV light exposures. An increasing number of scientific reports purport to show positive effects of sunlight on health. Importantly, these are independent of vitamin D production resulting from ultraviolet B (UVB) exposure. The current advice to avoid sun exposure may therefore be overlooking other aspects with potentially greater positive impact on human health (beyond that of vitamin D production). There are encouraging reports on the benefits of light at wavelengths ranging from ultraviolet A (UVA) to near infrared on blood pressure, obesity, type 2 diabetes, glucose intolerance and insulin resistance. It is important to note that the prevalence of these health conditions in the population is greater than that of melanomas by several orders of magnitude. While it is always necessary to avoid active harm, it is also necessary to consider the passive harm that can be inflicted by safety guidelines that are not comprehensively informed by evidence and new developments.

Challenges to be addressed

We have previously tested and observed that the exposure of skin cells to UVA results in a significant increase in nitric oxide (NO). This is particularly significant because this natural molecule is the most potent vasodilator identified to date. NO was generated by all the different skin cell types that were tested – keratinocytes, fibroblasts and microvascular endothelial cells. Importantly, production of NO by endothelial cells is responsible for the relaxation of smooth muscle cells that envelop the endothelium. This would lead to blood vessel dilation and hence reduction of blood pressure. In this project we will ascertain the dynamics and mechanisms of NO production from acute exposure to UVA.

Year 1 progress and outputs

We have newly observed that exposure of skin cells to UVA, or UVA filtered to remove the short wavelengths leaving the 365nm peak, induces the instant production of NO (through breakdown of nitrite). Importantly, we showed that the initial decline in NO production post-exposure is followed by a gradual and steady recovery, and plateaus for the next 2 days. This second “dark reaction” peak of NO production is mediated enzymatically and is distinct from the first and immediate mechanism of NO production. These results suggest that exposure to sunlight may generate beneficial NO production not only during the period of exposure but after exposure as well.

Future direction and objectives

Having characterised the dynamics of NO production through controlled laboratory experiments, we are now establishing conditions to test these findings with actual sunlight. This entails establishing the logistics to allow experiments outside the laboratory with dosimetry monitoring of sunlight (March 2022).

Theme II – Biomarkers of exposure, effect and susceptibility to chemical and radiation exposures

Theme Leads – Marc Chadeau-Hyam (ICL) and Christophe Badie (PHE/UKHSA)

Theme II Overview

The overarching aim of this Theme is to adopt an exposome-based approach to the characterisation of the effects of exposures to chemicals and radiation. This approach will offer new insights into the internal and molecular response to such exposures and will allow us to: i) identify and validate biomarkers of exposure, early and long-term effects (such as toxicity and carcinogenesis) and susceptibility, ii) identify potential mechanisms involved, and iii) improve our understanding of causal pathways to evaluate their subsequent effects on health and ageing. These studies will use existing large and well-established population, patient and occupational cohort studies, as well as de novo collected biosamples, *in vitro* cell lines and blood samples exposed *ex vivo*. We will apply state-of-the-art 'omics' technologies and develop new statistical approaches to mine these large datasets for early

effect biomarkers of chemical and radiation exposures. Further mechanistic investigations will be carried out using causal modelling approaches, as well as cell line experiments.

Objectives

- *Short term:* Recruit one PhD student per project to investigate exposure and effect molecular biomarkers associated with IR, chemical exposures and mixtures, emissions from brownfield sites and early life exposures to a range of chemicals.
- *Medium term:* Characterise a range of exposure and early effect biomarkers for the various exposures above. We will further develop and validate gene expression assays for rapid assessment of exposure in radiation incidents. We will extend the omic/biomarker studies to: i) non-ionising radiation using unique collections of biological samples, omic analyses and police radio and mobile phone use already obtained in the *Airwave* (police cohort) and *SCAMP* (adolescents) studies respectively, ii) to other chemicals (e.g. halogenated compounds).
- *Long term:* Investigate the role of identified biomarkers in biochemical and mechanistic pathways to better understand potential health consequences and validate these hypotheses through linkage to data from other themes and already existing larger (mainly cohort-based) data.

Project 1 – Exposure and risk markers in medical uses of IR

Project Leads – Christophe Badie (PHE/UKHSA); Bijan Modarai and Samantha Terry (KCL)
Research Team – Liz Ainsbury, Grainne O'Brien, Lourdes Cruz-Garcia, Jayne Moquet, Mingzhu Sun, Stephen Barnard and Irene MButu-Austin (PHE/ UKHSA); Tian Yeong (KCL)

Summary and project aims

This project will study *in vitro*, and *in vivo* biomarkers of ionising radiation relevant to healthcare (e.g. CT scans, radiotherapy and man-made radioactive isotopes). The aim is to develop and validate novel biomarkers of radiation exposure, effect and susceptibility, notably exploiting existing cytogenetics techniques and developing markers such as expression of splice variants, microRNAs, RNA modification and mitochondrial DNA mutations. These and other markers will be applied to samples obtained from practitioners delivering X-ray guided endovascular procedures and their patients. This will contribute to a better understanding of the radiation doses delivered to specific tissues such as bone marrow and blood following radioactive isotopes injected for nuclear medicine purposes. We will evaluate DNA damage and activated pathways associated with low dose exposures from an intracellular radiation source *in vivo*. We aim to assess multiple existing and new sensitive biomarkers for reliable estimation of the low doses delivered internally *in vitro* and *in vivo* by radionuclides, respectively to blood and bone marrow, to develop calibration curves to compare external X-ray doses and internal exposure.

Ultimately, this knowledge will be used to develop new radiation treatment protocols with lower toxicity, allowing individual differences in sensitivity to be considered, to better protect those at higher risk by regularly monitoring them, to decrease risk and intervene to treat cancer earlier.

This project has two parts running in parallel, each associated with a PhD student:

- The first sub-project aims to identify biomarkers of deleterious biological effects in patients after medical radiation exposure of endovascular aortic repair (EVAR) and subsequent follow-up investigations. Work on this project will complement the pilot programme which has been established to explore the wider epidemiological aspects of vascular operator radiation risk and will complement the ongoing work under Theme III:Project 2.
- The second sub-project aims to determine if short and long-lived biomarkers of radiation exposure are present in blood samples of nuclear medicine technologists working in Positron Emission Tomography (PET) imaging centres. These biomarkers include looking at DNA damage (γH2AX) and chromosome mutations (e.g. dicentrics) as well as gene expression, which are known to be linked to high radiation dose and future health risks. The presence of these biomarkers in technologists' blood samples will be correlated with the low-level radiation they have been exposed to over the years.

Challenges to be addressed

- EVAR is increasingly used in preference to open repair. The former requires x-ray guidance for placement of stent grafts and could potentially last several hours for the more complex procedures. Although EVAR has lower risk of immediate post-operative morbidity and mortality, recent studies have demonstrated acute DNA damage in the circulating lymphocytes of patients after EVAR. Moreover, patients who underwent EVAR have been shown to have increased risk of abdominal cancer in the long-term.
- In nuclear medicine, radioactivity is used to image and treat a variety of diseases, from diagnosing cardiovascular disease to treatment of arthritis and cancer. Nuclear medicine technologists, who create the radioactive compounds and/or administer the radioactivity to patients, are exposed to low level radiation often across several years. Although the links between high levels of radiation and future health risks such as cancer are well-defined, any such link between chronic exposure to low radiation levels and future health issues is not clear.

Year 1 progress and outputs

- A HPRU-funded PhD student (Tian Yeong) has been recruited to work on the project on biological effects of low dose medical radiation exposure in the context of vascular surgery patients and operators (project will start in October 2021).
- A PHE/UKHSA co-funded PhD student (Irene MButu-Austin) has been recruited to work on the project on biomarkers of radiation exposure in of nuclear medicine technologists to start in October 2021.

Future direction and objectives

- Following work carried out previously, the aim will be to clearly establish details of doses received by both vascular patients and operators.
- Once appropriate procedures for exposure characterisation are in place, the aim will be to establish cytogenetic and genetic biomarker responses in vascular operators and to investigate the relationship between the observed biomarkers and dose.
- In parallel, we will seek to establish an experimental programme for investigation of biomarker responses in nuclear medicine technologists. The ultimate objective will be to establish a wider basis for data collection as part of PHE/UKHSA's ongoing research programme, including through molecular epidemiology (linking with Theme III:Project 1), to inform risks associated with medical uses of ionising radiation and the appropriate radiation protection legislation and guidance.

Project 2 – Pathways and biomarkers of mixtures of chemical exposures

Project Leads – Marc Chadeau-Hyam and Paul Elliott (ICL)
Research Team – Paolo Vineis, Ian Mudway, Sonia Dagnino, Dragana Vuckovic, Leon Barron and Sibo Cheng (ICL); Christophe Badie (PHE/UKHSA)

Summary and project aims

This project aims to develop statistical approaches to analyse mixtures of chemical exposures and their biological response and ultimately to identify biomarkers of these complex exposures. The project comprises a strong methodological component focusing on the development and application of novel statistical models and machine learning algorithms accommodating multiple and interacting compounds. The project will be based on the re-analysis of existing data as proof-of-principle and the resulting approach will be made available for the analysis of new data to identify validated biomarkers of exposure mixtures.

Challenges to be addressed

The main objectives are two-fold: i) to identify from existing data the biological imprint of chemical mixtures using exposure to chlorination by-products as an exemplar ii) to investigate the molecular signatures associated with these complex exposures. Adopting a xenobiotic approach, we will use clustering algorithms to define sets of exposures and/or markers of co-occurring exposures. Resulting cluster memberships will be used as a proxy to define multiple discrete exposure profiles. Their interpretation will rely on regression models or classification approaches quantifying the factors (and/or individual exposures) driving the cluster membership. To explore the internal response to these

exposures we will perform (multi) omic profiling using penalised regression and Partial Least square models, and visualise complex correlation patterns across omic signals and/or exposures via network inference.

Year 1 progress and outputs

A HPRU-funded PhD student has been recruited (Sibo (Lucas) Cheng) to work on this project piloting statistical and machine learning approaches for the identification of reproducible and quantitative biomarkers of exposure to disinfection by-products (DBP). This work will re-process and re-analyse the data from the *PISCINA* study, in which high resolution MS metabolomics data are available in 120 volunteers before and after a swim in a chlorinated pool.

Future direction and objectives

Multi-omic block regression models as well as correlation networks will be constructed using (targeted) metabolomic, transcriptomic and proteomic data measured in the same individuals to investigate the multi-omic signatures of exposure to DBP. These integrative analyses will be conducted using *PISCINA* and MCC (Spanish multicase-control study on cancer) study data, and will provide further molecular information on the acute response to the exposure to DBPs through different routes.

We will adapt existing machine learning-based algorithms to the annotation of xenobiotics in metabolomics profiles, using the *PISCINA* dataset as a training set, which will aid the annotation of the metabolomic signatures associated with DBPs and give better understanding of their biological roles. Such models have been applied and tested for the annotation of features analysed in water samples by Leon Barron's group (Theme IV:Project 3). In collaboration with Leon Barron's team, we aim to adapt and expand these models to the annotation of metabolomic signatures in human biosamples using the *PISCINA* dataset,.

With over 3.000 metabolic profiles acquired from other studies including experimental studies (*Oxford Street, TAPAS*) and cohort studies (*EPIC, NOWAC*), the metabolic/multi-omic signatures obtained from this project will be used to estimate the levels of exposure to DBPs in these other studies. As they were conducted in different locations in Europe, the findings may provide a description of the geographic distribution of DBP exposures in Europe. By linking to individual health outcomes, these biomarkers will also be used to investigate the potential health effects of DBP exposures.

Project 3 – Biomarkers of potential chemical exposures in populations living in new housing developments built on brownfield sites

Project Leads – Ian Mudway and Bethan Davies (ICL)
Research Team – Leon Barron, Stephanie Wright and Holly Walder (ICL); Tim Marczylo (PHE/UKHSA)

Summary and project aims

The urgent need for affordable and social housing in the UK has driven the exploitation of ex-brownfield sites but there is limited data on the likely residual exposures of populations to contaminants at these locations. In this project we aim to address this knowledge gap by monitoring a broad range of pollutants at selected brownfield sites, focusing on ex-gasworks sites where housing development is planned or ongoing.

The project will start with the identification of the locations of ex-gasworks sites throughout Great Britain and selection of sites for monitoring of emissions of volatile and semi-volatile organic compounds (VOC and SVOC). We plan to develop low-cost passive samplers to allow monitoring at scale. We also plan to conduct residential, ambient, and personal exposure assessments of populations living in residential developments built on ex-gasworks before expanding consideration to other types of brownfield sites. We will explore the feasibility of examining internal biomarkers of exposure and response in vulnerable populations living at these locations, based on the results of the monitoring campaigns.

Challenges to be addressed

There is significant public and policy concern about emissions from ex-brownfield sites and about the effectiveness of remediation measures but limited actual data on the exposures to VOC, SVOC and

other chemical agents during and after re-development. These issues are compounded by the fact that many of the developments are in densely populated and deprived inner-city areas already suffering from poor air quality. We will work closely with the local communities, local public health departments and where possible the developers themselves to address these concerns.

Year 1 progress and outputs

A PhD student was recruited to support the monitoring work in March 2020 (Holly Walder). We have now identified the locations of 4,195 former gas work sites in England and are extending this work to Wales and Scotland. Sites are currently being classified by current land use.

Through engagement with local communities around former gasworks sites, local government environmental departments and directors of public health we are currently planning a trial sampling campaign at the ex-gasworks sites in Southall, Ealing using novel passive samplers compared against current industry standard monitoring. An untargeted analysis of VOC and SVOC will be performed on the samples collected during this deployment. This activity is planned for the final quarter of 2021.

Future direction and objectives

Based on the results obtained above, we will expand the monitoring to a larger number of sites reflecting different stages of site decontamination and building works. This surveillance work will be complemented by location based and personal monitoring of populations living at varying distance from these locations using novel passive absorption badges to capture exposures to VOC and SVOC, with a focus on vulnerable groups: pregnant mothers, children, and aged individuals with chronic disease.

Project 4 – Biomarkers of early-life exposures and neurodevelopmental outcomes

Project Lead – Mireille Toledano (ICL)
Research Team – Ruth Parsons (ICL) and Ovnair Sepai (PHE/UKHSA)

Summary and project aims

This project will analyse questionnaire and biomarker data collected from women in the Breast Milk, Environment, and Early-life Development (*BEED*) study and follow up neurodevelopmental outcomes in their children. The *BEED* study was set up around 3 municipal waste incinerators (MWIs) across England and includes ~600 women and approximately ~1500 breast milk samples. The number of MWIs in the UK has increased due to EU restrictions on landfill, but there is ongoing public concern about the possible health effects of MWIs and resistance to their construction. *BEED* has gathered personal exposure profiles using biomarkers in breast milk, including persistent organic pollutants such as polychlorinated dibenzodioxins, polychlorinated dibenzofurans, dioxin-like polychlorinated biphenyls (PCBs), heavy metals, and brominated flame retardants. The breast milk metabolome has also been analysed using liquid chromatography-mass spectrometry (LC-MS).

Challenges to be addressed

The main objectives are: i) to analyse a large range of breast milk biomarker data to determine background population levels of these environmental chemicals; ii) to investigate personal exposure levels to these environmental chemicals in relation to both distance and particulate matter exposures from an MWI; iii) to investigate personal exposure levels to these environmental chemicals in relation to pregnancy, lifestyle and dietary questionnaire data; iv) to undertake further heavy metal analysis and GCMS metabolomic analysis on remaining samples of breast milk. However, the latter will require specialist analytical techniques and approaches to be adequately tailored to the complexities involved in analysing breast milk.

Year 1 progress and outputs

A PhD student was recruited to work on the project (Ruth Parsons) and is due to start October 2021. The student has already enrolled in the College's Transition to Zero Pollution PhD cohort.

Future direction and objectives

Based on the work above, we will consider running a follow up mailing of mothers and children (consent

already in place) to ascertain birth outcomes and early life neurodevelopmental outcomes in relation to personal exposure profiles. This may not be adequately powered statistically but will provide proof of concept/pilot work for further research at the individual level on mothers' exposures to environmental contaminants at the population level and potential early life impacts on their offspring.

Theme III – *In vitro* testing and integration with epidemiological data

Theme Leads – David Phillips (KCL) and Richard Haylock (PHE/UKHSA)

Theme III Overview

This Theme exploits our collective expertise in mechanisms of toxicity and carcinogenesis of environmental toxicants, their bioavailability, biological consequences of DNA damage and health effects of exposure to ionising radiation and toxic chemicals. It addresses questions on the origins of mutations in human tumours and the role of the gut microbiome in toxicity of environmental pollutants. It uses state-of-the-art *in vitro* mammalian and bacterial cell systems and high throughput analyses to apply insights and biomarkers (including those identified in Theme II), to epidemiological studies of health effects of exposure to ionising radiation and toxic chemicals.

Objectives

- *Short term:* To establish two PhD studentships (Projects 3, 4); to collect cohort samples from people occupationally exposed to radiation and from residents living near brownfield sites.
- *Medium term:* To establish the capacity of 3D human tissue organoids and gut bacteria to metabolically activate toxic chemicals and define their utilities as in vitro test systems.
- *Long term:* To define with whole genome sequencing the mutational signatures of environmental carcinogens and compare them with signatures in human tumours; to broaden our understanding of the toxic effects of food-borne pollutants and suggest new avenues for their detoxification via microbiome modulation; to identify biomarkers of occupational radiation exposure and quantify health effects of living on or near brownfield sites.

Project 1 – Mutagenesis and toxicology in 3D cell systems

Project Leads – David Phillips (KCL) and Christophe Badie (PHE/UKHSA)
Research Team – Rebekah Beck (KCL); Grainne O'Brien (PHE/UKHSA)

Summary and project aims

Human tissue organoids are grown in specialised 3D culture conditions maintaining the stem cell population and much of the tissue/cell architecture found *in vivo*. They represent a step change in the ability of *in vitro* systems to reproduce normal *in vivo* conditions, providing an important bridge between them. This project aims to use normal human tissue organoids to explore the mutagenesis and toxicology of environmental toxicants and ionising radiation *in vitro* in normal human tissues (e.g. gastric, intestine, liver, pancreas, kidney). An additional pilot project aims to identify a radiation exposure signature *in vivo* in humans, by studying genome-wide radiation-induced DNA methylation changes in radiotherapy patients developing therapy-related Acute Myeloid Leukaemia (AML); these patients are rare and difficult to identify.

Challenges to be addressed

The origins of ~50% of cancer cases remain unknown. Whole genome sequencing has revealed ~49 different patterns of mutation (mutational signatures) in human tumours. Some mutational signatures in human cancers can be replicated experimentally, strengthening epidemiological observations of possible causation by environmental agents. Experimental *in vitro* systems can shed new light on the origins of mutations in human cancer and the cellular processes that give rise to them following exposure to environmental agents, thus contributing to their overall risk assessment.

Year 1 progress and outputs

- Normal tissue organoids have been established in culture, derived from human gastric tissue, pancreas, kidney and colon. They have been karyotyped. A review on organoids for toxicology and genetic toxicology has been published[2].
- Gastric organoids have been exposed to a library of current chemotherapeutic agents and the mutation profiles are being determined by duplex sequencing; gastric, pancreas, kidney and colon organoids have been exposed to environmental mutagens and prepared for DNA sequencing to determine tissue-specific responses.

Future direction and objectives

- Determination of xenobiotic metabolic capability of organoids.
- Analysis of mutational signatures generated in organoids by chemotherapeutic agents and environmental mutagens to shed light on the origins of mutations in human tumours.
- Identify patients who have developed AML related to a previous radiotherapy (only) treatment for a solid tumour such as breast cancer.
- Obtain DNA from blood samples, extract DNA, analyse samples for DNA methylation analyses, apply specific bioinformatics pipelines to generate data.

Project 2 – Occupational exposure to ionising radiation

Project Leads – Richard Haylock and Liz Ainsbury (PHE/UKHSA)
Research Team – Catherine Smith, Les Scott, Jayne Moquet, Mingzhu Sun, Stephen Barnard and Wei Zhang (PHE/UKHSA)

Summary and project aims

PHE/UKHSA has over many years conducted epidemiological analyses of the health of the UK radiation workforce based on the National Registry for Radiation Workers (NRRW). For a few individuals in the NRRW cohort biological samples have been collected in the context of a study of the ex-BNFL workforce. This project will scope the feasibility of wider prospective sample collection with a view to identifying and integrating novel prognostic biomarker information to improve health risk estimation in support of UK radiological protection practices.

Challenges to be addressed

Due to the complexities of molecular epidemiology approaches, the suitability of biological samples collected from radiation workers for cytogenetic and genetic biomarker analysis in support of radiation risk assessment and health protection needs to be further evaluated. In particular, it is necessary to identify suitable biomarkers of radiation effect, and to demonstrate that use of such biomarker information together with individual data (e.g. age, sex, smoking status) can be used within epidemiological studies to better inform population and individual risk assessment, before longer term prospective programmes can be established.

Year 1 progress and outputs

- Agreement was received from the Nuclear Decommissioning Authority (NDA) for a proof-of-concept molecular epidemiology study using the retained samples from former Sellafield workers; biological and ethical approval to carry out this work is also now in place.
- The samples for the study above have been selected and are being transferred from Newcastle Biobank; cytogenetic and genetic analysis of the first set of samples is expected to begin in August 2021.
- The NDA also approved in principle the linkage of the biomarker and epidemiological data, so everything is now in place to proceed with this work as planned. In addition, this work will be carried out in partnership with the existing and newly established medical occupational dose assessment work under Theme II:Project 1.

[2] Caipa Garcia AL, Arlt VM, Phillips DH. Organoids for toxicology and genetic toxicology: applications with drugs and prospects for environmental carcinogenesis. Mutagenesis. 2021. https://doi.org/10.1093/mutage/geab023.

Future direction and objectives

The forthcoming milestones are:

- Initial genetic and cytogenetic analysis of 5 existing sample sets and concurrent testing in *ex vivo* exposed blood samples from healthy volunteers.
- Initial assessment of the use of biomarker data in the NRRW cohort to improve risk estimation.
- State of the art statistical approaches including those based on pathway identification and analysis for the transcriptional markers, will be used for targeted selection of biomarkers for analysis of the samples from the Sellafield workers with different exposure conditions.

Project 3 – Exposures and health effects near brownfield sites

Project Leads – Daniela Fecht (ICL) and Robie Kamanyire (PHE/UKHSA)
Research Team – Ian Mudway, Bethan Davies and Fred Piel (ICL)

Summary and project aims

The aim of this project is to study the exposure and health impacts on residents living on and near remediated brownfield sites, specifically near re-developed gasworks. This project is carried out in parallel with the closely related project in Theme II:Project 3, which is analysing biomarkers of exposures in populations living on or near these sites. We will conduct a systematic review of ex-gasworks sites and potential health effects. We will use spatial analysis methods to conduct a detailed exposure assessment and apply spatio-temporal epidemiological methods to quantify any adverse health effects in the population living in proximity of these sites.

Challenges to be addressed

To respond to the growing housing need and protect the countryside from development, ex-gasworks sites are increasingly being targeted for housing redevelopment. Depending on their previous use, ex-gasworks sites might pose potential risks to the health of residents in housing developments either in, on or in the vicinity of, redeveloped sites. To date, little research has been conducted on the link between brownfield sites and health and the evidence which exists has indicated a potential health concern. The current studies reflect PHE/UKHSA priorities and the growing public concern with housing developments on ex-gasworks sites.

Year 1 progress and outputs

- We were not successful in recruiting a PhD student in the second round of advertisements for the HPRU and are re-advertising the PhD project in September 2021, with an anticipated start date of January 2022, to conduct a detailed exposure assessment of residents on and near redeveloped gasworks. A Research Associate in Environmental Epidemiology is currently being recruited.
- A list of potential data sources for the identification of brownfield sites in England, differentiated by previous use, has been compiled in collaboration with PHE/UKHSA and Environment Agency.
- The literature scoping review has been completed.

Future direction and objectives

- Scoping of definitions for brownfield sites to be included in the epidemiological study, with a focus on ex-gasworks sites currently under development, is in progress.
- A systematic review of the potential health impacts of residence in proximity to ex-gasworks sites will start once the Research Associate has been recruited, to be completed by March 2022.

Project 4 – Gut microbiome mediation of toxicity of environmental pollutants

Project Leads – Anne Willis (UC) and Kiran Patil (UC)
Research Team – Anna Lindell (UC)

Summary and project aims

The overall goal of this project is to discover reciprocal interactions between commonly encountered environmental pollutants such as pesticides and human gut bacteria, to quantify the capabilities of gut

bacteria to detoxify pollutants and identify the biochemical mechanisms involved. Using a recently established *in vitro* microbiomics platform, a screen of ~40 bacterial species against ~100 pollutants will be carried out. Pollutants and degradation products will be tracked with a high- throughput LC-MS set-up. Bacterial genetic libraries and biochemical assays will be used to identify the responsible enzymes with the goal of developing a genomics-based method to predict detoxication potential of new species/strains. Additional food-borne toxic compounds will be included in the screen to synergise with Theme III:Project 1 and Theme III:Project 5.

Challenges to be addressed

Exposure to toxic compounds such as pesticides through food is harmful not only to human cells but also to the commensal microbiota. While the effect on the microbiota could increase the risk of, for example, metabolic diseases, certain bacteria might aid in detoxifying these compounds. This project will use an *in vitro* microbiomics platform to investigate the potential of gut bacteria to detoxify commonly found environmental pollutants and discover underlying biochemical mechanisms.

Year 1 progress and outputs

An HPRU part-funded PhD student (Anna Lindell) was appointed and started in October 2020. A library of pollutants is being arrayed for screening and the method for LC-MS quantification is being established. Currently, methods have been established for 55 compounds.

Future direction and objectives

- Pilot screen with 20 bacterial strains is in progress. Delays due to COVID-related disruptions. Expected completion by Oct. 2021.
- Analytical method for ~100 pollutants >50% completed. Expected completion as planned by December 2021.
- Final report on interaction between ~100 compounds against representative gut bacterial panel: Expected to be completed as planned by March 2022.

Project 5 – Pesticide toxicity

Summary and project aims

This project combines three additional ongoing studies by groups associated with the HPRU-CRTH funded from external sources, that have a common aim of improving our understanding of the bio-availability and effects of exposures to pesticides and related pollutants through the respiratory and intestinal routes.

P5A – Respiratory bioavailability.

Project Lead – Ian Mudway (ICL)
Research Team – Ben Forbes and Zachary Enlo-Scott (KCL)

Challenges to be addressed

This sub-project (funded via a BBSRC CASE studentship with Syngenta) examines the bioavailability and the associated toxicity of a range of fungicides at respiratory barriers, reflective of the upper and lower airways.

Year 1 progress and outputs

- A review of xenobiotic metabolism in the lung including the impact on metabolism of pesticides was published in March 2021[3].
- Bio-availability work has been performed on a range of lung epithelial barrier models, with results compared against *in silico* predictions. Parallel toxicological studies have also been completed with NMR-based metabolomics, assessment of cellular oxidative stress and induction of inflammatory pathways.

[3] Enlo-Scott Z, Bäckström E, Mudway I, Forbes B. Drug metabolism in the lungs: opportunities for optimising inhaled medicines. Expert Opinion on Drug Metabolism and Toxicology. 2021;17(5):611-25. https://doi.org/10.1080/17425255.2021.1908262.

Future direction and objectives
The experimental results outlined in Project 5A are currently being written up as part of Zachary Enlo-Scott's PhD thesis, which is due to be submitted in November 2021. Thereafter we envisage additional papers on assessment of bioavailability and local irritancy.

P5B – Unravelling system-wide biomolecular interactions of food-borne pollutants

Project Lead – Kiran Patil and Anne Willis (UC)
Research Team – Mike Chapman, James Thaventhiran, Ritwick Sarwarkar, Mathew Van de Pette (UC)

Challenges to be addressed
This sub-project (funded via a grant from MRC), aims to examine how environmental pollutants impact cell physiology and suggest potential targets towards mitigation, by large-scale profiling of the effects of hundreds of common food-borne pollutants (pesticides, herbicides, and veterinary medicines) using a multi-scale bioassay approach.

Year 1 Progress and outputs
The delivery of a library of pesticides for bioassay screening, originally expected by June 2021, has been delayed to ~ Oct. 2021. Two recruitment rounds for a post-doctoral researcher to work on the project have been unsuccessful. The milestones for this project will therefore need to be postponed by circa 6 months.

Future direction and objectives
Following the post-doctoral recruitment, we will accelerate the screening by focussing on selected bioassays and move to molecular target identification using proteomics. The goal will be to identify targets of pesticides and use these to gain insights into the adverse outcome pathways.

P5C – Identifying the effects of pesticides on intestinal permeability and gut-bacterial dysbiosis.

Project Lead – Michael Antoniou (KCL)
Research Team – David Phillips, Scarlett Ferguson, Halh Al-Serori and Robin Mesnage (KCL)

Challenges to be addressed
This sub-project (funded via a grant from the Sustainable Food Alliance), aims to establish the genotoxic potential of pesticide formulations and determine the effects of pesticides on intestinal permeability using an *in vitro* cell culture model system of the human gut.

Year 1 Progress and outputs
The PhD student recruited for this project (Scarlet Fergusson, started Oct 2020) has successfully tested the effects on cellular viability of active ingredients and the respective formulations of the 8 most frequently used herbicides in the UK.

The possible activation of mechanisms characteristic of carcinogens (genotoxicity, oxidative stress) in a human cell line is currently being investigated. Publicly available data from ToxCast are also being analysed using the Integrated Chemical Environment web resource to obtain more information on the toxicity of major pesticides. We used the ToxTracker® panel of six validated green fluorescent protein (GFP)-based mouse embryonic stem reporter cell lines, to evaluate whether glyphosate, 2,4-D and dicamba, either alone or in combination, can activate specific cellular signalling pathways predictive of their carcinogenicity.

Future direction and objectives
To gain insights about glyphosate effects on the human gut microbiome in a controlled laboratory setting, we are using a simulator of the gut microbiome (SHIIME) to evaluate the effects of glyphosate and Roundup on the gut microbiota of a healthy 3-year-old child. We are measuring the metabolic activity and composition of the microbiome and will use metabolomics to evaluate the effects on metabolite profiles.

Theme IV – Neurotoxins & high toxicity agents (HTA)

Theme Leads – Nora Bourbia (PHE/UKHSA) and Tom Welton (ICL)

Theme IV Overview

This theme aims to increase our understanding of significant chemical hazards to human health, ranging from household pollutants to toxic industrial chemicals and chemical warfare agents (CWA). In brief, the cycle of environmental toxic agents starts from: i) its presence in the environment, ii) its direct contact with humans, iii) its absorption by the body followed by the physiological process of its toxicity, and iv) its clearance from the body. The research in this Theme focuses on elucidating the mechanisms of action, presence in the environment, impact at the cellular level, defining decontamination protocols and developing novel routes to decomposition/disruption. A key aspect is the impact of such chemicals on humans, and how to decontaminate or alleviate exposure.

Objectives

- *Short term*: Recruit PhD students to Projects 2, 3 and 4 to conduct literature reviews and identification of simulants/development of analytical methods.
- *Medium term*: Determine the cellular uptake of neurotoxins and impact of household mould; develop analytical methods for highly toxic agents (HTA)/CWA simulant quantitation; investigate solvation and decomposition of CWA simulants; survey HTAs in UK drinking/waste waters.
- *Long term*: Human decontamination studies; urine analysis for quantification of HTA exposure; testing *in-silico* novel ionic liquids (ILs) with simulants and CWAs.

Project 1 – Novel screens for environmental neurotoxins

Project Lead – Nora Bourbia (PHE/UKHSA)
Research Team – Leon Barron (ICL)

Summary and project aims

This project aims to investigate the effect of environmental agents on neurological functions, with a focus on mycotoxins, and how these may impact on the establishment and development of brain disorders. We will start by developing a screen for environmental neurotoxins, which will enable us to investigate the effects of environmental neurotoxins found in other projects of the HPRU-CRTH or HPRU-EEH, e.g. following the results obtained from Theme IV:Project 3 we could screen the most relevant toxic agents found in water to assess whether they are neurotoxic.

Challenges to be addressed

Most brain disorders are multifactorial diseases/disorders with an interplay between genetic predisposition and environmental factors. For instance, toxic agents commonly used as pesticides and herbicides, like Rotenone and Paraquat respectively, are linked to the development of Parkinson's disease. The challenge is that we might be chronically exposed to environmental agents inducing or precipitating brain disorders and diseases. Therefore, these studies aim to screen and investigate environmental agents to assess their neurotoxic abilities when chronically exposed at low dose.

Year 1 progress and outputs

Despite having received the electrophysiology equipment for use in the development of the screens for environmental neurotoxins, the COVID-19 pandemic has delayed the installation/training required.

The investigation of the effect of the mycotoxin Ochratoxin A (OTA), on neuronal cells exposed for two days has shown that: i) at a 50% lethal dose, OTA induces the neurons to enter into a "survival mode" despite DNA damage, and ii) at a sublethal dose, the neurons already detect a threat by showing survival-related gene expression changes to a lesser extent than with the lethal dose.

Future direction and objectives

We plan to pursue the exciting results obtained in neuronal cells exposed to sublethal doses of OTA to investigate the survival mechanism of neurons exposed to neurotoxins and whether a chronic low dose

of mycotoxin induces long-term change to the neurons that can lead to cell death or pathology. The aim is to have a manuscript on this topic ready for publication by the end of the Year 2.

Project 2 – Identification and validation of novel simulants of CWAs and toxic industrial chemicals

Project Leads – Sam Collins and Tim Marczylo (PHE/UKHSA)
Research Team – Charlotte Hague and Tom James (PHE/UKHSA); Tom Welton (ICL)

Summary and project aims

Human decontamination is critical following release of toxic chemicals and simulants are often used to assess decontamination procedure efficacy. We will identify/evaluate novel simulants that address the spectrum of physicochemical properties of toxic chemicals, including novel and priority threats, e.g. Novichok. To date, simulants are typically hydrophobic liquids at ambient conditions, however, there are concerns around the use of highly toxic powdered substances which consequently may require different decontamination approaches. These studies will: i) identify potential simulants for novel and emerging chemical threats through a systematic review, ii) develop quantitative analytical methods for the identified simulants, iii) conduct proof-of-principle human decontamination studies with new candidate simulants, and iv) conduct studies to examine optimal decontamination strategies for toxic powders including studying the 'wash-in effect' with liquid and powder simulants.

Challenges to be addressed

UK emergency response procedures for decontamination of the public following a chemical incident have been developed using a limited range of simulants. This work will determine whether current best practice is optimal for chemicals possessing diverse physicochemical characteristics including whether use of water for decontamination can increase dermal absorption of some or all chemicals via the 'wash-in effect'.

Year 1 progress and outputs

An HPRU-funded part-time PhD student (Tom James) was appointed and started in October 2020.
Manuscript proposing appropriate novel chemical simulants submitted for publication in IJERPH[4].
Review of 'wash-in' effect is almost complete and ready for submission to journal.
Selection of 5 simulants for further method development: Rosmarinic acid, benzyl salicylate, octocrylene, diethyltoluamide, avobenzone.
Method development (LC-HRMS) has begun for development of quantitative methods for the 5 selected simulants.

Future directions and objectives

Submission of methodological paper developed specifically for analysing 5 new simulants identified for human volunteer studies.
Ethical application to enable application of these 5 simulants to volunteer's skin to assess efficacy of current national decontamination processes.
Develop analytical methods to quantify these 5 simulants and their metabolites in urine and use these methods to investigate efficacy of national decontamination processes and whether a 'wash-in' effect is observed.

[4] Collins S, James T, Carter H, Symons C, Southworth F, Foxall K, et al. Mass casualty decontamination for chemical incidents: Research outcomes and future priorities. International Journal of Environmental Research and Public Health. 2021;18(6):1-19. https://doi.org/10.3390/ijerph18063079.

Project 3 – Detection of HTAs in water

Project Lead – Leon Barron (ICL),
Research Team – Paolo Vineis, Marc Chadeau-Hyam and Davide Ciccarelli (ICL); Tim Marczylo (PHE/UKHSA)

Summary and project aims

The aim of this project is to develop and apply new analytical methods to determine human exposure to HTAs in drinking water, including ground water, bottled water and municipal treated drinking water. Initial work will focus on the development of comprehensive sample preparation and instrumental methods, capable of covering a large chemical space for small HTA molecules. The performances achieved with these developments will be systematically assessed against current mainstream methods. Even more challenging is the identification of HTAs that are not present in mass spectral libraries. The second main objective will be the development of a data processing workflow capable of: i) tracing all the masses generated by the instrument, ii) filtering them to isolate the ones that are related only to the sample, iii) apply data research tools for the screening of specific categories of substances, and iv) compile libraries by merging available spectra with new ones generated during the analysis.

Challenges to be addressed

This project will progressively build detailed knowledge of the chemical HTA composition from drinking water and potential for public health exposure, by compiling a library that will ultimately provide the ability to trace all components and spot the emergence of known and new HTA substances.

Year 1 progress and outputs

The project started in May 2021 with a new PhD student (Davide Ciccarelli). A detailed method development plan has been completed and all consumables and instrumentation purchased and installed. Over 70 representative HTA substances have been purchased for method development, performance evaluation and quantification in water samples. The features of various non-targeted analysis software for data processing have been thoroughly evaluated and are now ready for use.

Future direction and objectives:

An extensive literature review is underway covering non-target and suspect screening for HTAs, dissolved organic matter, and other classes of compounds. This is progressing well and expected to be submitted as planned to an international peer-reviewed journal.
Following installation and training on new liquid chromatography equipment (LC-QTOF-MS) in July 2021, method development for HTA screening will begin shortly. The first objective is to examine sample preparation approaches using solid phase extraction (SPE). The second objective is to assess the method performance using both SPE and direct injection LC-QTOF-MS for a selection of >70 HTAs. The third objective is to apply the method to a set of drinking water samples to identify known and unknown chemicals/HTAs.

Project 4 – Clearance of neurotoxins and other highly toxic chemicals from the environment

Project Leads – Tom Welton (ICL) and Patricia Hunt (ICL/Victoria University of Wellington)
Research Team – Charles Romain ad Gavin Smith (ICL); Samuel Collins (PHE/UKHSA)

Summary and project aims

In this project we will understand how ionic liquid (IL)-solute interactions can be manipulated to create solutions for the detection and destruction of CWAs. We will combine computational and experimental techniques to: i) identify the mechanism of solvation and decomposition of CWA in known ILs, ii) investigate solvation and decomposition of common CWA simulants, evaluate simulant fidelity against real CWA chemical structures, solvation and degradation processes, and identify better potential simulants, and their key chemical features, iii) design and test *in silico* novel ILs with simulants and CWA to improve containment and facilitate analytical processing; iv) design and test *in silico* novel ILs with simulants and CWA to enhance decomposition rates and promote generation of nontoxic degradation products.

Challenges to be addressed

Detection of and protection from CWAs have become more urgent with recent events. Agents can be highly volatile and non-persistent (e.g. sarin) or have lower volatility and only partial water solubility creating more persistent hazards (e.g. mustard gases). New systems that can be used to safely clear CWAs from the environment are urgently required. ILs are efficient in detecting and absorbing gases and liquids present in low concentrations. Selected ILs have also the ability to suppress solute vapour production. ILs have also been shown to accelerate the rates of chemical reactions, so providing the possibility to enhance decomposition of CWA but our understanding of these mechanisms is lacking.

Year 1 progress and outputs

PhD researcher successfully recruited (Gavin Smith; started August 2021).

Future direction and objectives

After initial training the PhD researcher will combine theoretical and experimental approaches to understand the mechanisms for the improved solubility, suppression of vapour pressure and reactivity of highly toxic agents in ILs, including:

- Literature review and introduction to computational techniques.
- Introduction to experimental techniques IL synthesis and analytical characterisation.
- 2-month placement in New Zealand to learn advanced computational techniques.
- Computational mechanistic study of VX in ILs to determine the decomposition process and compare to the aqueous phase data already obtained.
- Experimental study of existing VX mimics reactivity in ILs and comparison to water, to provide a benchmark for the computational work, kinetics (temperature, concentration dependencies, reaction rates).

Project 5 – Identification of illegal threat manufacturing activity via wastewater markers (*ThreatMARK*)

Project Leads – Keng Tiong Ng and Leon Barron (ICL)
Research Team – Tom B (Centre for the Protection of National Infrastructure), Peter Thompson (National Physics Laboratory)

Summary and project aims

This is a project associated with the HPRU-CRTH but funded from external sources. The aim of this work is to develop new capabilities for identifying and monitoring markers of explosive and chemical threats to monitor clandestine synthesis activity via wastewater analysis. As a result of time constraints, the focus lies on improvised explosives (e.g., sugar nitrates, nitro-based organics and inorganics). The project integrates the latest advances in high-resolution analysis and *in silico* AI-assisted technologies to rapidly achieve this. The outcome of this research will be to preliminarily set reliable baseline thresholds for explosive threat markers in city wastewater and subsequently, extend these capabilities towards the localisation of threat production activity.

Challenges to be addressed

A variety of legal and legitimate industrial, agricultural, pharmaceutical, veterinary chemicals and household products can be transformed for illegal purposes, such as explosives and chemicals manufacturing, including homemade explosives (HMEs), improvised explosive devices (IEDs) and nerve agents. This has become a major concern to governments and society around the world. Therefore, early identification of the illicit manufacture of threat agents is critical for protection of public safety. Success of this research will serve as an early warning monitoring tool and another new source of intelligence for law enforcements to intercept illegal manufacturing activity.

Year 1 progress and outputs

Initial work on this project has led to: i) impurity profiling of 35 homemade explosives (e.g., sugar nitrates, nitro-based organics, inorganics and peroxides) and 38 precursors known for their use in homemade explosives; ii) shortlisting of potential markers in seven sugar nitrates for later wastewater analysis; iii) invited to give oral presentation at a webinar and IC Academic Research symposium,

organised by the National Geospatial-Intelligence Agency and Director of National Intelligence.

Future direction and objectives

Future work will focus on: i) shortlist markers of other homemade explosives, their occurrence screening in wastewater and identity prediction; ii) publication of a review article of homemade explosives analytical methodologies.

3. TRAINING PROGRAMME

Our aim is to create a high-quality training and career development programme for PhD students and early career researchers (ECRs) in the HPRU-CRTH, combining multidisciplinary training in fundamental toxicological, epidemiological, and quantitative environmental sciences, to form the next generation of researchers with the skills needed to understand and tackle the complex human health risks and threats from environmental exposures to chemicals and radiation in the UK.

To realise this aim we created a joint academic career development (JACD) programme in partnership with the HPRU-EEH and the MRC-CEH, building on over 10 years of experience running a highly successful training programme in the MRC-CEH. The creation of a joint programme aimed to leverage synergies and complementary expertise and training opportunities available amongst the different partners while preserving the specificities of each core unit (for details on the JACD see Annex 2)

We established a JACD Committee including representatives of all the partners involved in the three units as well as representatives of the students and ECRs, to develop the training strategy and oversee the training activities. Our Joint Training Strategy for the HPRU-CRTH and HPRU-EEH (see Annex 2) outlines the approach for providing a wide range of innovative training opportunities, covering technical as well as soft skills, with a strong focus on career development and networking opportunities, including through activities organised by the NIHR Academy.

Our recruitment procedures are based on identifying excellent PhD candidates through consistent and inclusive processes encouraging diversity and equality. During the first year of the HPRU-CRTH we ran three PhD recruitment rounds, jointly with the MRC-CEH and the HPRU-EEH:
applications from 158 candidates, through three competitive rounds of recruitment. Overall, offers were made to 16 candidates

- We received at total of 158 applications over three competitive rounds of recruitment and made offers to 16 candidates (10% success rate);
- We successfully recruited 9 of the 10 PhD students included in the HPRU-CRTH application (5.5 funded from the HPRU award and 4.5 match-funded by the partners);
- Five of these students have already started and four will start in October 2021;
- Three additional PhD students working in externally-funded projects associated with this HPRU complete the cohort.

We hosted several activities through the JACD to welcome and support our new students and ECRs:

- We held three online induction sessions, with representatives from all partner institutions, where students were given an overview of the academic career development activities and resources;
- A buddy scheme was set up pairing each new PhD student with a current student to provide a point of contact for informal help and support.

The COVID pandemic and the change to working remotely created additional challenges in maintaining contact with the students and ECRs. Additional resources were specifically created to continue to engage with and support these groups throughout this period:

- We created a Training Portal in MS-Teams, providing a single point of access to a collection of remote training resources and information on news, events and career opportunities available across all partners in the three units and those provided by the NIHR Academy;
- We set up one-to-one online sessions with the JACD Leads to provide additional confidential support for any problems the students and ECRs may have been facing.

We put in place a number of measures to ensure that all the students in this cohort receive tailored

training reflecting both academic and health protection research perspectives:

- Each of our HPRU-CRTH PhD students are jointly supervised by researchers from the universities and from PHE;
- We will organise reciprocal exchanges for the HPRU-CRTH PhD students between the universities and PHE/UKHSA;
- We gave honorary ICL affiliations to all our PhD students primarily based in other institutions, to give them access to the wide range of career development opportunities hosted at ICL;
- We promoted participation in NIHR Academy activities with one of our HPRU students attending the NIHR academy training camp in early 2021;
- We explored opportunities for our PhD students and ECRs to shadow senior members of our HPRU involved in several national committees (e.g. COT, COMEAP, COMARE) and hope to be able to offer these in the near future.

To promote capacity building and multi-disciplinary career development opportunities, we held a range of activities jointly with the HPRU-EEH and the MRC-CEH, involving a cohort of approximately 79 PhD students and 76 ECRs:

- To build capacity in emerging research areas and methods, we launched a series of workshops, led by ECRs, combining theory and practical sessions. A first workshop on “Machine learning methods in Environment and Health Research” was run in December 2020 and again in January 2021 due to the high demand. A second workshop in “Data Visualisation Principles and Methods” is being planned in early 2022.
- Our annual training event, held on March 29-30 2021, offered opportunities to our HPRU PhD students and ECRs to present their research projects as posters or flash oral presentations and to co-chair sessions with some of their peers. The event took place online and was attended by close to 100 participants in each of the two days. The keynote presentation focused on climate change, an area of development and collaboration for our HPRU. Members of HPRUs in Environmental Change and Health, in Modelling and Health Economics, and in Respiratory Infections were invited to attend.

Our additional objectives for the future are threefold:

i) Further increase engagement and involvement of PhD students and ECRs in all our activities.
ii) Continue to promote interactions tailored towards career development with other HPRUs. We started with selected HPRUs (see above) and aim to expand this in the coming year, building on activities of the NIHR Academy.
iii) Further advertise and support participation in NIHR Academy training and career development activities, such as SPARC, to provide training in core skills required for CRTH research.

4. PUBLIC AND COMMUNITY INVOLVEMENT, ENGAGEMENT AND PARTICIPATION

Our aim for the PCIEP programme is to engage with and involve the public in a scientific dialogue to ensure that the impact of the research in the HPRU on chemicals and radiation in the environment extends beyond academic and policy domains and is responsive to the concerns of the public. To this end, we will embed PCIEP activities across all the Themes of this HPRU to ensure the public voice can impact on our research strategies, projects, and functions and to make our research accountable, transparent, and relevant to the communities concerned.

We have established a set of governance structures in the first year of the HPRU-CRTH to support and oversee the implementation of the PCIEP Programme (for more details see Annex 3):

- We appointed a dedicated PCIEP Coordinator, Ms Antoinette Amuzu to manage these activities;
- We created a Joint PCIEP Committee in April 2020, in partnership with the HPRU-EEH and the MRC-CEH, including representatives of all the partners, that is responsible for overseeing the coordination of PCIEP activities across the three units;
- We established a Joint Public and Community Oversight Group (PCOG) in October 2020. The PCOG is composed of members of the public, industries, local government, community and patient groups, academics and third sector organisations. The PCOG is a strategic resource whose role

is to advise on the development and implementation of the PCIEP strategy and activities from the perspective of the public and the wider community of stakeholders of our research.
- Two PCIEP representatives have been identified for each research Theme, one from PHE/UKHSA and the other from the partner universities, who are responsible for dissemination of PCIEP activities and reporting on progress.

Another key outcome in the first year has been the development of the joint PCIEP strategy for the HPRUs (see Annex 3). The strategy sets out the vision and commitment to make public involvement engagement and participation a core value of the HPRUs integrated across all its work programmes. This PCIEP Strategy was developed in consultation with the PCOG and was informed by the NIHR INVOLVE National Standards for Public Involvement in Research and the Going the Extra Mile Policy Review.

Learning & Support

We identified a programme of training courses to build PCIEP capacity and expertise in line with our objectives and offered these to all HPRU members as well as to the PCOG. The HPRUs PCIEP Coordinators are active members of the National HPRU Public Patient Involvement and Engagement Network.

Inclusive Opportunities

We are identifying ways to expand the diversity and inclusion in our PCIEP activities by developing links with various networks and online platforms to engage with difficult to reach groups, to include the use of the VOICE Digital Platform and The Young Persons' Advisory Network (YPAN).

Communication

During our first year, we engaged with a variety of audiences to communicate and to involve the public in our research through a variety of media, including interviews in newspapers, webinars, and workshops. We created a twitter account (@HPRU_CRTH) to disseminate our outreach activities, promote our PCIEP opportunities and establish a social media presence. We also have a dedicated section on PCIEP on the HPRU-CRTH website and will continue to develop our content there.

Capturing and reporting on impact

Post-event feedback forms are used to capture the impact of our activities on the public and researchers. In December 2020 we developed a PCIEP reporting tool to facilitate the accurate recording and reporting of all our events and activities and to help researchers demonstrate their impact on the research. A list of PCIEP activities over the first year can be found in Annex 3).

5. KNOWLEDGE MOBILISATION

Effective knowledge management and knowledge mobilisation (KM) brings together communities, scientists, public health practitioners, decision makers and other stakeholders with an interest in one or more topics, to catalyse change. Done well, KM maximizes the impact of health protection research to facilitate effect policy change and improved services for patients and the public.

KM will be an important component of the work undertaken under the HPRU CRTH, not least due to the diverse, multidisciplinary nature of the research programme and the intended outputs focused on strengthening the evidence to underpin development and implementation of legislation and guidance related to promotion and protection of the health of the UK population. In common with the Training and PCIEP work of this HPRU, and due to the clear mutual interests and potential for joint working and outputs, KM is being managed across the three Environmental HPRUs (two at Imperial College and the third, on in Environmental Change and Health at London School of Hygiene and Tropical Medicine), is also embedded into the training for students and early career researchers within each of the Themes, and is a key performance indicator for the success of the HPRU.

The primary development in KM activities during 2020-2021 was the appointment of Knowledge Mobilisation Manager (KMM) Dr Kerry Broom in April 2021, split across the three Environmental HPRUs. The KM Strategy (see Annex 4), currently under development, outlines our approach to promoting the use of the knowledge generated by the HPRU both internally and externally. The key objectives are to establish a cross cutting culture which focuses on translation of research evidence into policy, and to promote understanding of importance of high-quality scientific research to underpin effective policy making.

The first draft of the KM strategy was submitted to NIHR in May 2021. Following feedback from NIHR and further discussion and development of the strategy in partnership will all Themes and Projects, a second draft to include objectives and timeframes was being developed during August-September 2021. Once completed, the second version of the strategy will be published on the HPRU websites. In the meantime, a short section on Knowledge Mobilisation has been published on the HPRU website, which explains the strategy at a high level. In addition, presentations on KM have been given at each of the Theme meetings, at the Joint Training Meeting for PhD students and early career researchers which took place in March 2021, and at the Joint Training Committee and Joint Patient and Public Involvement and Engagement Committee Meetings (which the KMM attends).

Formal training on KM for the wider HPRU participants will take place at the September Annual Meeting; a session via MS Teams has already taken place for PhD students in July and slides are available on the Teams Training Portal.

A Science Converge Session was held at PHE/UKHSA CRCE during July to highlight the work of the three environmental HPRUs hosted by CRCE Lead Scientists. Presentations included overviews of the HPRUs and their research. Further, the KMM highlighted the work of the three environmental HPRUs at the PHE/UKHSA Environmental Public Health Practice Network in June. Clear areas of interest for PHE/UKHSA were identified for health effects of brownfield sites in Themes II and III and wastewater monitoring in Theme IV. There may also be potential interest with per- and polyfluoroalkyl substances (PFAS) exposure in Theme II.

KM within the HPRU is currently at an early stage. However, in the coming year a number of tasks have been identified to help further establish the programme. Firstly, primary stakeholder identification has taken place and initial discussions with the Theme Leads is taking place during the latter half of 2021. These discussions will identify existing and potential stakeholders, including industry and public liaison activities. These discussions will also consider opportunities for interaction with other HPRUs. There are existing connections with the Environmental Exposures and Health HPRU particularly around Themes II, III and IV.

Stakeholder mapping will continue to be conducted throughout the HPRU's lifetime. By identifying end-users and other stakeholders at the project and theme level, the HPRU-CRTH can map and reach out to facilitate knowledge transfer and identify areas for new work where there are evidence gaps.

In addition, attendance of the KMM as an observer at the expert Committee on Medical Aspects of Radiation in the Environment (COMARE) is planned, to examine the current work programme, meet members and assessors and opportunity to highlight the work of the three environmental HPRUs. There is representation from HPRU staff on this committee already. Work from Theme I focussed on childhood cancer and nuclear installations feeds directly into the work programme of COMARE, and COMARE also retains a watching brief on electromagnetic field exposures such as those from mobile phones in young people in Theme II.

Ultimately, participation in the pan-HPRU Knowledge Mobilisation network is helping to develop a knowledge mobilisation framework for health protection incorporating learning across KM.

6. COLLABORATION WITH OTHER HPRUs

From the start of the HPRU-CRTH we established a close partnership with the HPRU-EEH: i) we set up common governance and management structures, ii) we established a joint Executive Group and Steering Committee, iii) the Academic Career Development and a PCIEP Committees are jointly led

and include representatives from all the partners in from both HPRUs, and iv) the two external advisory groups, the PCOG and the ISAB, also provide recommendations on the work of both HPRUs.

The joint Academic and Career Development and a PCIEP programmes are delivered in a fully integrated way across both HPRUs in order to maximise the use of the available resources and provide a broad range of opportunities for our students ECRs and more senior researchers. It is our objective to extend the collaboration in these areas to other related HPRUs and to this end we invited the NIHR HPRUs in Respiratory Infections, in Modelling and Health Economics and in Environmental Change and Health (at the London School of Hygiene and Tropical Medicine) to take part in our Inaugural Annual Training Day.

Together with the other HPRUs hosted at Imperial College (HPRU in Respiratory Infections, HPRU in Healthcare Associated Infections and Antimicrobial Resistance, HPRU in Modelling and Health Economics and HPRU-EEH) we have been involved in the planning of joint public engagement and outreach events for the next Imperial Festival.

The Knowledge Mobilisation Manager has actively participated in the Pan-HPRU Knowledge Mobilisation Network. This network has had regular meetings where best practise is discussed and experiences shared. The appointed Knowledge Mobilisation Manager works across this HPRU, the HPRU-EEH and the HPRU in Environmental Change and Health facilitating efficient knowledge management, transfer and exchange.

Research collaborations

- Analysis of fungi and their associated mycotoxins in wastewater – we are discussing with the HPRU-EEH a possible collaboration for a pilot study to determine if fungi and/or their mycotoxins can be detected in wastewater as part of Theme IV.
- Modelling of SARS-CoV-2 transmission - We are collaborating with the HPRU in Respiratory Infections and the HPRU in Modelling and Health Economics in the analysis of data on household transmission of SARS-CoV-2, drawing on our expertise in statistical modelling including of longitudinal data.

Future collaborations

- We will expand the collaborations with the other HPRUs based at Imperial College on our JACD and PCIEP programmes;
- Together with the HPRU-EEH we will actively seek to establish collaborations with other relevant HPRUs, notably, Environmental Change and Health and some of the cross-cutting HPRUs such as Genomics and Data and will explore the option to host a cross-HPRU meeting in the next year.