The British Toxicology Society awards bursaries to individuals in non-profit making educational or research organisations to support their attendance at EUROTOX and IUTOX meetings in addition to bursaries for domestic **bts** meetings, such as the annual Congress. Priority is given to students and Early Career Toxicologists. For more information on how **bts** bursaries are awarded and the eligibility criteria visit: http://www.thebts.org/Meetings/Bursaries.aspx

## SYMPOSIUM 1: USE OF NEW APPROACH METHODOLOGIES IN REGULATORY SCIENCE

Chairs: Dr. Carol Courage (Boots) and Dr. Camilla Alexander-White (Royal Society of Chemistry)

This symposium aimed to discuss the issues currently faced in the regulation of substances, from the side of the regulators and those seeking approval. Whilst the speakers were from differing sides, all focused on the requirement of clear benchmarks in the use of new approach methodologies, specifically in determining their correlation with in vivo effects.

Dr. David Bell (European Chemical Hazards Agency) started the session with his talk entitled "Using New Approach Methods in Regulatory Sciences". He highlighted the difficulty that a regulatory body faces when the science of the new approaches still requires refinement. Currently new and alternative methods (NAMs) are well used in the screening and/or prioritisation of compounds but less so in regulatory approval. He talked of work with the Commission, to update the REACH Annexes and so provide a basis for accepting NAMs.

Dr. Richard Currie (Syngenta) continued the session with an interesting talk entitled "New Approach Methodologies and Adverse Outcomes Pathways". Starting with the description of an "adverse outcome pathway (AOP)", his talk followed the process of using one to create a model for a particular adverse outcome. He stressed the interplay between his work (at Syngenta) and that of the EPA in determining suitable standards for regulatory safety that successfully avoided a 90 day rat study.

The symposium continued with Dr. Hans Ketelslegers (Concawe) talking on "Using New Approach Methodologies in Grouping in the Cat App Project". He explained the difficulties encountered when dealing with complex- and multi-constituent substances, as exemplified by UVCBs (unknown or variable composition, complex reaction products and biological materials). He showed work on petroleum substances (a prototypical example of UVCBs in their very nature) which are used in cosmetics. This work, as part of the Cat-App consortium (a multi-year consortium initiated by Concawe), used high-content screening data to group classes of UVCBs such that read-across can be performed under regulatory programmes. He included the difficulties faced when considering differing exposures (oral vs dermal testing).

Dr. Andreas Schepky (Beiersdorf) talked on "Cosmetics Europe's non-animal strategy: toxicokinetics for systemic toxicity assessment". Here, he presented animal-free approaches for the testing of cosmetic ingredients; namely in silico skin penetration models to predict exposure. Five mechanistic models could accurately predict bioavailability of 25 chemicals. However, the amounts of these chemicals in the epidermis and dermis was less well predicted. He did mention that, when using these models, scientists should be aware that diverse industrial representation is not the same as chemical diversity and so care should be taken in their application.

The final speaker was Dr. Elena Fioravanzo (ToxNavigation Ltd.), who gave a talk entitled “New approach Methodologies in Read-Across: a workflow based on chemoinformatics”. She talked on her experiences using read-across (RA) and the resultant workflow that attempts to reduce uncertainties in RA by identifying biologically meaningful and relevant analogues. Quantitative uncertainties were shown using a skin sensitization case study.

**Ben Alexander-Dann**, University of Cambridge

## BARNES PRIZE LECTURE

It was a great experience attending the 2019 BTS annual congress as a bursary recipient. The congress featured exciting, highly stimulating, and educational lectures. The lecture by Prof Andy Smith, the 2019 Barnes Prize Award winner, titled “Toxicology concepts from applied and fundamental studies of iron and haem biology” was particularly relevant to me as it discussed the convergence of haem metabolism and toxicity.

In his lecture, Prof Smith talk through the biochemistry of haem and its role in respiration, steroid metabolism and cell signalling. He further highlighted the many crucial interaction between haem biology and chemical toxicity including drugs, especially how a defect in haem metabolism can be a susceptibility factor to drug toxicity. Prof Smith particularly cited disorders in haem metabolism such as acute intermittent porphyria (AIP), an inheritable mutation in HMBS (hydroxymethylbilane synthase) gene, can be considered a predisposing factor to adverse drug effects. He also discussed how uroporphyria, a multifactorial haem metabolic disorder, could be viewed as an iron-based disorder potentiated by chemicals such TCDD, HCB and drugs.

Prof Smith went further to discuss the role of haem oxygenase, an enzyme in haem metabolism, in protection against cytotoxicity. Referencing the work of Takeda 2015, he pointed out that continuous de-novo biosynthesis of haem and its rapid turnover to bilirubin are necessary for cytoprotection against drug or chemically induced cell damage. In general, Prof Smith lecture advanced my understanding of haem and iron biology and how this could be crucial in determining susceptibility to toxicants.

Overall, the 2019 BTS congress was a great experience and help broadened my toxicological knowledge considerably. The congress brings experts in the field toxicology and addressed topics of toxicology in the 21st century and I returned to the lab better informed on the various topics discussed at the congress. The congress also provided excellent networking opportunities among academics and researchers with diverse research interest and allowed me to present and receive feedback on the research conducted in our lab which was presented during the poster session. I look forward to attending future congresses.



**Oluwaseun Akinyele**, University of Aberdeen

## ASCEPT LECTURE: RENAL TRANSPLANTATION: TRANSPORTERS, GENETICS AND IMMUNOSUPPRESSANT NEPHROTOXICITY

This year's BTS annual Congress included a lecture delivered by Dr Benedetta Sallustio, a long-standing member of the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT). The prestigious lecture was provided as part of the biennial lecture exchange agreement between ASCEPT and the BTS. The theme was 'Renal transplantation: transporters, genetics and immunosuppressant nephrotoxicity', a very engaging and clinically relevant topic.

Dr Sallustio highlighted the current issues related to the complex management of immunosuppression in renal transplant recipients, whose therapeutic regimen encompasses calcineurin inhibitors (CNI) combined with prednisolone. The high inter-individual variability observed in CNI pharmacokinetics and toxicity implications requires patients to undergo strict therapeutic drug monitoring (TDM) to achieve optimal therapy, however graft failure due to either rejection or nephrotoxicity is a significant tangible problem. Currently TDM is based on whole blood CNI concentrations which, Dr Sallustio explained, does not correlate with CNI concentration in the allograft tissue, and therefore offers a poor predictive value.

Dr Sallustio defined several pharmacogenomic aspects that could thus improve clinical management and prediction of CNI-associated nephrotoxicity. In particular, genetic polymorphisms in the recipient's CYP3A5 gene have been shown to alter systemic responses to CNI, and their characterisation could therefore be useful in individualising immunosuppressive therapeutic approaches. The speaker presented exciting innovative data in relation to the distribution of the CNI's cyclosporine and tacrolimus to their target sites, demonstrating differential concentrations within the allograft sites and other target sites. These findings suggest that donor's genetic polymorphisms are also a major contributor and play a critical role in CNI uptake and consequential nephrotoxicity. Consequently, in combination with optimised analytical methodologies, this highlights the need for a more integrative TDM and genotyping approach for the management of renal transplant patients, which could not only positively impact clinical outcomes, but could help elucidate unexplored toxicological mechanisms of CNI-associated toxicity.

From my perspective, this lecture truly reflected the translational aspects of toxicology, drug exposure, and clinical responses. I now feel further inspired and enthused around the value of the type of studies I do, albeit in the cardiotoxicity rather than nephrotoxicity area, and the impact this could have upon the clinical setting.

**Carol De Santis**, Newcastle University

**SHORT ORAL COMMUNICATION**

I found that the BTS Annual Congress-2019 an ideal platform for scientific communication and knowledge transfer as it is an international forum, consisting of researchers, academics and industry representatives who are experts in the field of Toxicology. Since my research is mainly about mitochondrial dysfunction and liver toxicity, this congress provided me with an opportunity to network with people working in this field and other related fields. Moreover, I presented part of my PhD research about Primary Biliary Cholangitis as a poster presentation and received useful feedback from scientists and PhD students who attended the conference.

It was rewarding experience for me to attend the different congress symposia about Occupational, Pharmaceutical, Regulatory, Environmental and Molecular Toxicology. The RSC/BTS selected short oral communications session was one of the best sessions that I attended. It was judged by Dr Sarah Judge (Newcastle University) and Dr Amy Wilson (AstraZeneca). It consisted of four short oral talks presented by 4 young researchers;

- 'Protein targets associated with post-marketing adverse events of drugs'

Miss Ines Smit (University of Cambridge) presented her research around Pharmacovigilance, specifically the correlation between the in vitro activities of different drugs and chemicals against protein targets extracted from the ChEMBL database and the post-marketing adverse events reported from the Food and Drug Administration Adverse Event Reporting System (FAERS).

- 'Investigating the toxicity of modified therapeutic IVT mRNA'

Mr Thomas Murloney (MRC Toxicology Unit, University of Cambridge) presented his work about toxicity screening of In Vitro transcribed mRNA that are considered as proposed potential therapeutic agents (Phase I/II clinical trials) for treating some hereditary diseases. He showed that ribonucleotide modifications may decrease immunogenicity however, that it also has effects on mRNA processing and translation.

- 'Absence of metabolism-dependant toxicity of paracetamol in primary hepatocyte culture'

Miss Lucia Livoti (University of Liverpool) indicated that paracetamol has a metabolism-independent toxicity pathway in isolated human and rodents' hepatocytes. This differs from the well-known cytochrome P450-dependentNAPQI mechanism of toxicity that leads to the depletion of the anti-oxidant reduced Glutathione (GSH).

- 'Long-Fibre Carbon Nanotubes induce Pleural Mesothelioma Recapitulating Human Disease: a role of Epigenetic mechanisms in disease development'

Dr Joaquin Cabeza (MRC Toxicology Unit, University of Cambridge) showed in his talk that prolonged exposure to Long-Fibre Carbon Nanotubes (Long-CNT) may trigger pleural carcinogenesis (Mesothelioma), which is similar in mechanism to that seen after asbestos exposure. He also revealed that long-CNT exposure is associated with epigenetic changes which play crucial roles in the progression of pleural inflammation to malignant mesothelioma.

**Tarek Mamdouh Abdelghany**, Newcastle University

**SYMPOSIUM 7**

Dr Roel Schins presented a talk entitled 'Usefulness of advanced *in vitro* models of the GI tract to study the genotoxicity of particulates'. While there is much knowledge on the exposure of the lungs to particulates by inhalation, this is not the same for the gastrointestinal tract (GI). Particles such as engineered nanoparticles may reach the GI tract in one of two ways. Either particles may be excreted into the digestive tract after being cleared from the lungs by mucociliary clearance, or particles may be ingested if they are used in drug delivery, food additives, or food packaging. In the case of inhalation, it is well known that factors such as size, shape, solubility, surface charge, aggregation, and agglomeration of particles influence toxicokinetics, toxicodynamics, and *in vitro* toxicity outcomes. Therefore, *in vitro* testing of particles should take these factors into account. Endpoints of interest for nanoparticles are inflammation, oxidative stress, and genotoxicity. *In vitro* models of the GI tract may be co-cultures of macrophages, epithelial cells, and dendritic cells. In addition, M-cells and Goblet cells can be used which produce mucous. The GI tract is characterised by unique processes and conditions including metabolism; the presence of enzymes, mucous, and microbes; a food matrix; and contraction. The "digestion" of particles may change their properties such as reducing reactive oxygen species and DNA-damaging potential. *In vitro* systems to evaluate toxicity of engineered nanoparticles should take into account the rapid turnover of GI epithelial cells (5-day renewal time), as well as potential secondary genotoxicity. The latter refers to genotoxicity caused by persistent inflammation as opposed to particles interfering in the nucleus or cytosol (primary genotoxicity).

Dr Elisa Passina's talk asked "Can human *in silico* drug trials replace the need for animal studies?" Her talk focused on the prediction of drug-induced cardiotoxicity in humans using computer models. Such models are now a mature technology with a high level of knowledge following many years of development. The computer models are based on biophysical variables from human hearts, and can therefore more easily translate to human effects. The models consider different scales: ion currents (subcellular level), the action potential (cellular level), or the whole heart (organ level). The cellular models can simulate the electrocardiogram (ECG), abnormalities in which are strongly related to arrhythmias such as Torsade de Pointes (TdP). In the population, there is inter-subject variability in the shape and duration of the action potential. This variation is reflected in the ECG simulation by varying parameters in the model. By statistical sampling of the parameters, many different models are generated which are then calibrated with human data. The population of models can be used to do an *in silico* drug trial in a matter of hours on a normal desktop computer. Retrospective drug trials for drugs with known TdP risk showed good performance. Passina is working with industry and regulators to bring the technology into practice, which would contribute to reducing the use of animals. Future work includes organ-level models, which are still computationally expensive, as well as personalising models to specific patients.

Professor Thomas Hartung presented on "Alternatives in 21st century toxicology". Considering the disadvantages of animal testing, including costs, lack of translation and lack of reproducibility, there are many reasons to move to alternative methods. One such approach is read-across, which is based on chemical and biological similarity, but which faces a major hurdle in data accessibility. Hartung's group has used natural language processing to extracted data on over 10,000 chemicals from ECHA registration documents. They used this data to build a network of chemicals based on chemical similarity. Classifying skin sensitization potential by

the nearest neighbour in this network showed performance comparable to the local lymph node assay. Combining read-across and QSAR methods and using databases with millions of structures are promising methods for the replacement of animal testing. For example, such methods may be used to find chemicals with certain desired properties but lower predicted or known toxicity. However, there is not enough data for many endpoints such as chronic endpoints and carcinogenicity. In addition to computational methods, 3D cellular co-cultures have been developed to represent the complexity of human organs, such as an organo-typic mini-brain system using induced pluripotent stem cells. The neurons in the mini-brain can fire spontaneously and may be useful in studying developmental toxicity as well as gene-environment interactions.

Dr Gerry Kenna's talk was titled "Can we use *in vitro* data to predict human hazard? – A comparison of three evidence streams for troglitazone and rosiglitazone." The occurrence of post-marketing safety events shows that there is a need for improving the prediction of drug safety. Kenna presented a case study on rosiglitazone and troglitazone, evaluating preclinical data for their safety. While troglitazone was withdrawn due to drug-induced liver injury (DILI), rosiglitazone is classified as having less DILI concern.

To investigate whether animal studies were able to predict human liver outcomes for these two drugs, a protocol-driven systematic review of scientific literature was designed. The animal studies reviewed showed limited evidence of liver toxicity for troglitazone and rosiglitazone. Results from the high-throughput screen ToxCast were analysed and normalised with the Cmax values for the drugs' respective therapeutic doses. Troglitazone showed 7-fold more off-target effects in cell cycle processes, gene expression, and transcription factor activity in the *in vitro* screen. The WHO pharmacovigilance database contains more reports of adverse events for troglitazone than rosiglitazone, while rosiglitazone is more widely used. Given clinical trials have limited power to detect rare events, reproducible workflows are needed to evaluate preclinical and *in vitro* data relevant to drug safety.

**Ines Smit**, University of Cambridge

**SYMPOSIUM 10**

Chaired by Dr Jill McKay and Prof T Gant, a well-attended tenth symposium discussed the health impacts of pollution with two talks and three short oral presentations.

The first speaker, Dr Nelly Saenen from Hasselt University in Belgium, presented the importance of understanding air pollution, and the effect it has on early development and later-life consequences. Air pollution is a complex mixture of toxicants and varied sizes of particles and is one of the top 5 causes of human disease. Current levels of pollution are at lower levels than European standards and have been decreasing. This talk focussed on multiple methods of detecting carbon black concentrations within fibroblast cells, urine, cord blood and placental samples at different stages of development and later life. Results generated have shown that children living near busy roads have up to 9% higher carbon black concentrations and that carbon black is able to travel to the foetal side of the placenta. Data also highlighted that higher exposure to PM 2.5 is related to DNA damage, telomere shrinkage and oxidative stress. In relation to human health, this could lead to an increased risk of atherosclerosis and other disease later in life. The environment is therefore equally important to lifestyle, and socioeconomic status as risk factors, but most importantly, pollution can have an effect before birth, and is evidence to support the need for improved air quality.

The second talk, 'Mutational Signatures of environmental Mutagens and Chemotherapeutic Agents' was given by Prof David Phillips from King's College London. This talk discussed a recently published data set which looked at whole genome sequencing, showing thousands of genetic mutations once exposed to certain mutagens. Overall, there were 79 difference mutagenic agents assessed, including aldehydes, alkylating agents, chemotherapies, PAHs, nitrosamines, reactive oxygen species (ROS) and simulated sun light. Human induced pluripotent stem cells were exposed to the IC50 of each agent, and then allowed to recover for 7 days. Whole genome sequencing and computational analysis of samples then generated multiple (41) mutagen signatures. Data showed a mix of expected results – such as signatures generated from UV exposure, tobacco smoke and benzopyrene, however, some results were less expected, such as those generated from alkylating agents; which may be due to the cells used in this work. In summary, similar compounds gave similar signatures, but this data also saw differences between signatures of similar compounds, and some dissimilar chemicals gave very similar signatures, therefore showing that caution is required when interpreting mutagen signatures.

The first short oral presentation was delivered by Dr Ruth Morse from the University of West England, who described the vaping habits of e-cigarette users, and the toxic effect of nicotine on lung cell cultures. A549 and U937 cells were used both as mono- and co-cultures and exposed to tobacco smoke and e-liquids containing nicotine. Results showed that the presence of macrophages caused significantly different toxicological outcomes to monocultures, tobacco particulate reduces A549 cell viability and evidence of inflammation after exposure to e-cigarette vapours. Furthermore, observations of tobacco cigarette and vape users showed that vapers use e-cigarettes for longer, and more often than tobacco cigarette users, showing that exposure levels may be different in vape users, and must be considered when testing for toxicity.

The second presentation by Ms Lucy Swithenbank from the University of Swansea, was entitled 'Measuring tumour response to chemotherapy using blood-based biomarkers'. This project focussed on oesophageal cancer, which has a low prognosis due to late and difficult diagnosis, and chemotherapy is also not often successful. For these reasons, a blood-based biomarker to

assess the severity of the cancer and response to chemotherapy would be beneficial. The pig-A mutation, where a lack of GPI-anchors are seen on human erythrocytes, may provide a much quicker and efficient method of assessing response to chemotherapy. 8 cancer patients showed higher levels of pig-A mutations from analysis of 10 µL of blood, using fluorescent labels (CD235a, CD59 and CD55). Healthy, wild-type cells show high frequencies of GPI anchors, and cancer patients show a lower frequency. Results from the test can be received in as little as 2 hours. Significant changes in mutation frequency are seen after 6,9,12 and 15 weeks of chemotherapy treatment. The hypothesis going forward is that an increase in mutant frequency could correspond to a higher success rate of chemotherapy treatment.

The final oral communication was given by Mr Camilo de Lelis Medeiros-De-Morais from the University of Central Lancashire, who spoke briefly about the 'Differentiation of meningioma brain tissue using Raman hyperspectral imaging'. Raman spectroscopy can detect biological markers of interest, such as proteins, DNA or carbohydrates with high specificity and little interactions with cell culture reagents. Focussing on meningioma, the most common form of brain tumour, Raman spectroscopy was used to differentiate between grade I and grade II meningioma, with a high accuracy of 99%, showing Raman spectroscopy to be a valuable and accurate tool in clinical settings, with the ability to differentiate between meningioma tumours.

**Sarah Mitchell**, Swansea University

**CEP COURSE**

The continuing education programme (CEP) discussed new approaches to genotoxicity testing, with the reduction of animal use a key focal point. Dr Laura Brierley opened the session focusing on *in silico* techniques such as quantitative/ qualitative structural activity relationships (QSAR) to predict genotoxic endpoints. QSAR is a key aspect of early chemical evaluation in drug discovery and regulatory processes, using a mathematical model to accurately predict the biological activity and chemical reactivity of novel compounds where experimental data is limited. Dr Brierley emphasized the importance of expert user judgement and interpretation of the chemical theory behind QSAR structural alerts, rather than taking the alert at "face value", e.g. does the target substance fall within the models' applicability domain. Furthermore, read-across can be used to complement QSAR predictions, increasing reliability and negating the need for further testing.

The succeeding talk from Dr Ann Doherty gave an outline of the *in vitro* test battery for agrochemical and pharmaceutical genotoxicity testing, and an insight into recent advances. Typically, drug development, and chemical and food safety guidelines recommend conducting the Ames test to assess for mutagenic potential in bacteria, accompanied by testing for clastogenicity and aneugenicity in mammalian cells. A novel *in vitro* Pig-A assay has been developed to measure mutagenicity in mouse lymphoma L5178Y cells. The X-linked Pig-A gene encodes a protein required for early stage synthesis of glycosyl phosphatidylinositol (GPI) anchors. Inactivation of the Pig-A gene leads to a defect in the first step of GPI-anchor biosynthesis, decreasing the cellular expression of GPI-anchored markers, which can be measured using flow cytometry.

Next, Mr Jonathan Howe gave an overview of the current and recent advances in *in vivo* genotoxicity testing. The majority of OECD test guidelines have been updated in recent years, including the Mammalian erythrocyte micronucleus test, alkaline comet assay, Mammalian bone marrow chromosomal aberration test, and the transgenic rodent somatic and germ cell gene mutation assay. Furthermore, the Organisation for economic co-operation and development (OECD), and the International guidelines for harmonisation (ICH) S2 guidelines recommend competent laboratories dose a group of positive control animals and create a bank of samples, negating the need for a positive control group per study.

Finally, Mr Thomas Holmes gave a cross-industry view on genetic toxicity testing, looking at cross-sector requirements within Europe, and data interpretation from regulators. Most recently, some data requirements have evolved, for example the guidance for crop metabolites states the *in vitro* micronucleus assay should now be used to measure aneugenicity in place of the chromosome aberration assay, and the European food safety authority (EFSA) has stated a preference for the more sensitive mouse lymphoma assay, which can theoretically detect clastogens. For compounds that give positive results, but are not necessarily genotoxic, emphasis should be placed on understanding the mechanism of action (MOA) to comply with the International programme on chemical safety framework. There are various assays that can be used to determine the MOA, such as the FISH assay to determine aneugenicity or clastogenicity, and new reporter gene assays measuring oxidative stress. Photogenotoxicity testing requirements were described, EFSA require testing for active substances when an ultraviolet/ visible molar extinction coefficient (MEC) is greater than 1000L/mol/cm. ICH guidelines recommend the *in vitro* photoxicity test (OECD 432) or ROS assay for a MEC >1000 and significant distribution to the skin.

The CEP gave a comprehensive overview of current and new methods to evaluate genotoxicity across the European chemicals agency, EFSA, and ICH. New developments with *in silico* prediction software, *in vitro* methods, and more efficient *in vivo* testing will help to reduce the number of animals needed for novel and untested chemicals.

**Nathan Goldsmith**, Public Health England