18th ANNUAL CEFIC-LRI WORKSHOP
AOPs and Genomics: How useful, how to address risk, and where next?
16-17 November 2016
Brussels

LRI Innovative Science Award 2016

The 18th LRI Workshop opened on the evening of 16 November at Le Plaza Hotel in Brussels with a poster session and gala dinner during which the 2016 LRI Innovative Science Award was presented.

The LRI Award dinner session was chaired by **Dr Nicolas Cudré-Mauroux,** Research and Innovation Group General Manager at Solvay, who first introduced the 2015 LRI Innovative Science Award winner: **Dr Alice Limonciel** from Innsbruck Medical University. She described the results of her project on "Linking stress response pathways and markers of nephrotoxicity into renal adverse outcome pathways" that investigated cellular responses to the acceleration of chronic kidney disease following chemical exposure. The project looked to identify the genes involved in cellular stress response pathways, quantify these responses in parallel with markers of cellular dysfunction and deliver a new generation of quantitative tools based on gene expression. These tools could be used to evaluate the hazard linked to chemical exposure for use in risk assessment strategies. The project built on a number of technological advances and increased knowledge of molecular pathways that have created a paradigm shift in toxicology with the monitoring of molecular mechanisms as a driving force. She was currently finalising the characterisation of gene signatures and modelling the stress responses. Implementation of quantitative Adverse Outcome Pathways (AOPs) was being undertaken with Effectopedia – an online encyclopaedia/wiki of AOPs - and then the model would be applied to nephrotoxins.

Dr Cudré-Mauroux then presented the 2016 LRI Innovative Science Award to **Dr Wibke Busch** of the Helmholtz Centre for Environmental Research in Germany. Dr Busch outlined her project concept: "Genome wide profiling of molecular responses related to toxicokinetic and toxicodynamic processes for the determination of key events as basis for quantitative AOPs". By applying state-of-the-art molecular, analytical, and data analyses methods, the project aims to develop and test an experimental design to investigate the time and concentration dependence of effect propagation by quantitatively linking toxicokinetics, toxicogenomics and phenotype observations. The results of the project should help establish a quantitative link between molecular effects and adverse outcome, identify key responses and key event sequences within AOPs, and provide an experimental approach for a systematic development of advanced predictive models, for example toxicokinetic-toxicodynamic models or quantitative AOPs in the future.

The dinner poster session featured presentations by 10 LRI projects covering a wide range of LRI topics plus a poster giving further details of the work of the 2015 LRI Innovative Science award. Delegates and dinner guests were able to talk to the poster presenters over networking drinks before the dinner and award ceremony. Brief details of all the posters are given in Appendix 1.

LRI key questions – Workshop day

The next morning (17 November) the delegates reconvened for the first main session of the workshop that took place in the impressive surroundings of theatre hall at Le Plaza Hotel. **Dr Stuart Marshall** of Unilever and Chair of the Cefic LRI Strategy Implementation Group welcomed delegates and reminded them of LRI's current four focus areas: intelligent testing; complex environments; acceptance of technologies; and, on the social science side, policy impact. He also reminded his audience of the seven key long-term questions that the research programme is seeking to address over the next ten years or so. The workshop would directly address the first six of these questions:

- Omics / 21st Century Toxicology: How to link information at the molecular level to health impacts and interpreting results for meaningful decision making?
- Predictive tools for health impact: What are pragmatic approaches for reducing complexity, whilst maintaining robust predictions of health effects?
- Combination effects: How to identify combination effects scenarios of concern?
- Eco-systems approach: Which new concepts enhance ecological relevance of risk assessment?
- Real life Exposure: Which predictive, validated exposure scenarios apply to assessing environmental stressors?
- Comparative assessment: How to interpret impact of health and environmental stressors?
- Risk/benefit approaches: Can we understand societal drivers for public acceptance of innovation?

The main morning plenary session was chaired by **Dr Bruno Hubesch**, LRI Programme Manager at Cefic, and covered a wide range of projects from the programme including environmental methodology, nanomaterials, dust and workers exposure, dermal absorption, eye irritation, epidemiology and epigenetics.

Dr Timo Hamers, of the Vrije University of Amsterdam described the results from LRI project ECO23: TIPTOP (Time Integrative Passive sampling combined with Toxicology Profiling) to assess mixture toxicity in surface waters. Sampling was mainly in the main Dutch river delta and found that generally the surface water and also samples from Waste Water Treatment Plants (WWTP) effluent streams were clean. Could this be due to successful regulatory policies such as REACH and the WFD (Water Framework Directive)? He noted that attempting to analyse many chemicals at concentrations lower than their limit of detection (LOD) was not cost-effective! In conclusion he proposed an alternative monitoring strategy based on determining the distribution of time-integrated concentrations in water to generate toxic pressure data. This had the potential to significantly reduce the cost of analysis for the sampling programme for the WFD. Setting the standards for the maximum acceptable toxic pressure would be required but he suggested that 5% could be acceptable.

Project ECO24 - Prediction of Non-Extractable Residues (NERs) using structural information ('structural alerts') was described by **Dr Ralph Kuhne** via a phone link from the Umwelt Forschung Zentrum in Leipzig. The project kept up the environmental theme by looking to establish a viable computer model to help determine the fate of xenobiotic NERs in soil and sediments. Modelling initially suffered from a lack of data and large variability due to experimental and environmental uncertainties when more data was available. However the methodology could identify NER potential increasing and decreasing with substructures and property changes and combine them in a non-linear manner for classification of substances. An artificial neural network

model was used to give a quantitative prediction but, again, this was only used to classify compounds although some rules for classification was emerging. In conclusion the project had achieved rough estimation of xenoNER formation from NER and CO2 and identified properties and substructures of substances relevant to NER formation and delivered a classification approach. A computer implementation of the project outcomes called ChemProp would be available soon for free from the UFZ website.

Science-based grouping of nanomaterials and establishing criteria for 'Safe by Design' applications (LRI Project N4) was presented by **Dr Hans Bouwmeester** of Wageningen University. No single property groups all nanomaterials so we need to refine grouping criteria and look at multi-perspective grouping. Optimisation of analytical methods for determining the solubility of nanoparticles in complex matrices is needed and larger datasets on nanomaterials are needed with consistent data and better data quality. However, decision trees can be used to refine descriptor selection and to set specific threshold values for structural features that relate to potential biological effects. These identified key nanoparticles descriptors can help in the design of new nanomaterials as they are the most relevant parameters for safety. He said that a predictive model (DF4nanoGrouping) had been developed to assist decision-making for the grouping and testing of nanomaterials.

Dr An Van Rompay of VITO moved the theme to in-vitro testing and toxicology with her presentation of LRI project AIMT6 - CON4EI: CONsortium for in-vitro eye irritation testing strategy. The aim was to see what the possibilities were to integrate the current range of in-vitro eye irritation tests for categorising substances. A number of similarities and differences were identified and likely combinations of tests proposed. Top down approaches represent many models and were more difficult to combine their assays. More work is required to fill in gaps for the significant drivers in terms of irritant substances here. A bottom up approach (using 3D models) was recommended as a first step strategy for identifying chemicals that do not require classification (No CAT). An also said that all results from the project will be available soon and will be published in a special edition of the ATLA (Alternatives to Lab Animals) journal. They will also be submitted for inclusion in OECD guidelines (TG 437).

After the morning coffee break, the theme moved to LRI exposure projects starting with B12 - DustEx: How relevant is the dust pathway for consumer exposure to SVOCs? The project was described by **Dr Natalie von Goetz** from ETH Zurich. Consumers spend a lot of time indoors and dust could be an important pathway for exposure to semi-volatile organic chemicals (SVOCs). A small scale field study was used with deuterium labelled substances which showed that dust is a more relevant pathways for substances with log K_{oa} greater than 10 and that the main transfer pathway to dust was via the air and direct transfer. In terms of the overall relevance of dust exposure, in general terms, ingestion of dust was much less than the direct exposure pathway, however it can be important and the relative exposure can now be calculated by using the DustEx model that will be web-based and should be available to all by the end of 2017 via the LRI toolbox.

The results of project B13 - Development of a mechanistic in-silico multi-scale framework to assess dermal absorption of chemicals - were presented by **Dr Rebecca Notman** from the University of Warwick. The aim of the project was to rationalise and predict chemical bioavailability of a broad range of substances through dermal exposure by developing a mechanistic in-silico multi-scale framework model combining macroscale and molecular models. Various pathways for substance exposure and penetration of the skin were modelled using a variety of techniques including molecular dynamics simulation. Key parameters were evaluated

for penetration data for water and ionic substances and structural changes to the lipid matrix of the skin for organic substances including phenol and ibuprofen were characterised. She noted the modelling highlighted the importance of hydrogen bonding networks for permeation in lipid bilayers and also the heterogeneity of the bilayers in the plane of the layer. She also said that no continuous aqueous pathway was seen due to 'pooling' of water. Modelling also showed that transient pore formation is normally highly unfavourable from an energetic point of view but additives, such as phenol and ibuprofen, dramatically reduced this energy barrier.

Project B16 - External validation of Tier-1 workers dermal exposure estimates in the ECETOC TRA (Targeted Risk Assessment) tool - was outlined by **Dr Wouter Fransman** from TNO. The tool calculates the risk of exposure from chemicals to workers, consumers and the environment but the study showed that dermal exposure was underestimated in 20% of cases and low exposures were overestimated while high exposures were underestimated. The model could be applied to solid-in-liquid products but dermal exposure measurement data is lacking over a large set of conditions. Wouter said that dermal sampling methods were not standardised yet, exposure limits are not established in all cases and exposure models are less sophisticated than other pathways. Improvement will require a collaborative effort. Although, in general, the knowledge was developed, there was not enough evidence for a validated quantitative risk assessment. This will help effective skin exposure management. More sophisticated Tier 2 models are required and more experimental and standardised field data is required.

Assessing causality is a key element of the LRI programme and project EMSG58 - Quality assessment of the epidemiological evidence of adverse effects to humans of endocrine active substances in the environment – was presented by **Prof. Carlo La Vecchia**, from the University of Milan and the Mario Negri Institute. The main aims of the project was to define a systematic evaluation scheme to assess the quality and reliability of epidemiological studies and to develop a methodology to evaluate health effect claims identified in epidemiological studies. The project discovered that the quality of epidemiological studies was variable and the results were heterogeneous. Only well conducted systematic reviews on specific (limited) topics can provide a clear evaluation of the epidemiologic evidence. Using the Epid-Tox framework both epidemiological and toxicological evidence suggests that industrial chemicals Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) interfere with intrauterine growth and result in decreased birth weight in humans and rodents. However, the effect in animals is only evident at extrapolated serum concentrations 100-1000 times higher than those observed in humans.

The final project presentation - LRI C3 Epigenetics: Normality in toxicologically relevant species, development of a framework to better understand the impact of epigenetics on (eco)toxicology – was presented by **Prof. Richard Meehan** of the University of Edinburgh. He described how transcriptomic and epigenetic landscapes can be rapidly perturbed in many diseases (such as cancer) and change in response to chemical exposure. Therefore a promising area for research is the combination of epigenomic and transcriptomic analysis for toxicity testing in particular the identification of characteristic early stage biomarkers. The project generated a huge amount of data and successfully generated the first comprehensive DNA modification maps (for 5hmC and 5mC – a stable marker in the genome) for two strains of rat liver and one strain of mouse liver. The global patterns showed that variance within a strain and gender is low and that epigenetic patterns reflect genetic makeup. He felt that epigenetics could contribute to define pathways and metrics of toxicology

The outcomes of the ICCA-LRI and the Japanese National Institute of Health Sciences (NIHS) workshop on Awaji Island, Japan held in June, was described by ECETOC General-Secretary **Dr Alan Poole**. The meeting addressed global challenges in applying new scientific methods to improve environmental and human health risk assessments. Prior to the workshop itself three educational courses were held covering ecological risk assessment, endocrine active substances and assessing persistent, bioaccumulative and toxic (PBT) substances and persistent organic pollutants (POPs). The workshop featured four concurrent sessions on decision making for PBT chemicals and POPs, emerging environmental issues, the challenges of read-across for decision-making, and mechanistic understanding in epidemiology. Alan highlighted the general conclusions of the workshop. To meet global challenges, global interaction and cooperation is required and a shift away from traditional toxicity testing towards new technologies for data collection, analysis, and interpretation is required – the LRI programme can act as an international catalyst here. Training programmes for developing countries are needed to ensure their scientists can also take advantage of these new technologies and to support the education of the next generation of environmental scientists, toxicologists and epidemiologists. A key issue is to balance global research needs with budget limits and regional priorities, as well as with country-specific concerns and regional policy terrains.

Genomics and AOPs

After lunch a panel discussion on "Genomics technology and AOP methodology: how useful, how to address risk, and where next?" was introduced by moderator: **Prof. Ian Kimber** of the University of Manchester. The session started with two short 'scene setting' presentations.

The first was from **Prof. Jos Kleinjans** of Maastricht University on genomics technology as alternative testing technologies. He described the FP7 carcinoGENOMICS project that aimed to develop in-vitro methods for assessing the carcinogenic potential of compounds and validated microarray-based technologies. Overall predictive accuracy increases when genomic technology was combined with classical methods. Microarray technology is at the end of its development lifecycle, he stated, and was being replaced by RNA sequencing technologies due to their improved accuracy for low abundance transcripts. He concluded that transcriptomics analysis has yielded promising results which can provide additional weight-of-evidence in chemical risk assessment and for developing predictive genomic profiles in-vitro, transcriptomics analysis will also work and a 'cross-omics' approach may not be necessary but could lead to deeper insights in toxic/carcinogenic mechanisms-of-action.

The second scene setting talk was on AOPs methodology from **Prof. Aldert Piersma** of the Institute for Risk Assessment Sciences (IRAS) at Utrecht University. Adverse outcome pathways (AOPs) describe the intermediate events or predictive relationships spanning various levels of biological organisation that link a molecular initiating event (exposure) and an adverse effect (toxicity). Developmental ontology brought together developmental biology, chemistry and toxicology to provide such a methodology. The final goal could provide an animal-free risk assessment methodology that combined molecule-cell-tissue-organ-organism physiologies to produce toxicity profiles through in-vitro testing only.

Panel discussion

Ian Kimber then introduced the six-man panel that would consider eight wide ranging questions on AOPs. The panel consisted of:

- Dr Rick Becker, American Chemistry Council
- Prof. Peter Kille, Cardiff University
- Prof. Jos Kleinjans, Maastricht University

- Prof. Aldert Piersma, Utrecht University (part)
- Dr Alan Poole, ECETOC
- Prof. Gilbert Schonfelder, Bf3R

How useful

Q1. Has the early promise (late 90s) of toxicogenomics been realised, and if not, why?

Ian Kimber noted that his first paper in this area had been published in 1999. **Peter Kille** thought we were "getting there". The ability to map genomics onto the AOPs resulted in a tool that is fit for purpose now. Recently high throughput toxicology experiments had shown how data can be used effectively in toxicology. But you needed the AOP framework to sense of it all. **Rick Becker** agreed saying that having AOPs helps to focus where data can be used. He thought the field had suffered from a tendency to "leap ahead" to prediction, when we needed to step back and get a better understanding. **Alan Poole** saw only one real success and that was in read across applications. The initial promise had not been fulfilled. In particular, the regulatory community really did not understand the technology and, therefore, do not have confidence in it.

Gilbert Schonfelder disagreed saying institutions have been using the technology and he thought regulators did understand not the technology, but the data does not have the gold standard compared to normal toxicological methods. He thought the technology had promise and was very Very important from an animal alternatives perspective. **Aldert Piersma** had been working for the past 40 years with 'omics'. He thought that science had been naïve initially looking for single gene effects, when the reality was much more complex. It was a shame that the technologies were still not at a stage when we can be confident that the technology could be used in a regulatory regime. There was always a mismatch between scientific and regulator progress, but it was paramount that we, as scientists, can convince ourselves that the basis is good before we try to sell anything to regulators. Essentially we must know what is happening at a mechanistic level, he stated, as that would help to generate confidence. **Jos Keinjans** noted that our understanding of the complexity of biology has radically changed since 1999 and this has raised the required bar. He firmly believed that the technologies would provide the basis for better understanding.

A question from the audience wondered if we were addressing the wrong question. The bottom line must be to reduce animal use in testing and we need systems that show absence of biological activity that will cover most of the chemicals that need to be tested!

Q2. To what extent do AOPs need to be described and quantified for use in chemical hazard assessment?

Gilbert Schonfelder thought we are at an early stage, only starting to understand at the cellular level. AOP is just wording, but it gives us a structure for understanding and brings learning together. Ian Kimber described AOPs as "a new lexicon for a debate". **Peter Kille** said that AOPs describe biochemistry and are phenomenally useful framework that allow a common structure. The quantitative aspect is prediction, and this is really important, but a 'Black box' concept is not good for use with mixtures as it is impossible to predict interactions.

Ian Kimber commented that it was "remarkable that AOP [research] was looking at causality related to outcomes, but it was very rare to find work on quantitative relationships." **Aldert Piersma** would like to see response relationships, more work on individual assays looking for order of magnitude differences. **Rick Becker** thought we needed to define the question more accurately: was there an adverse effect was one question, but no biological effect was a different

question. There was a need to understand the analytical systems, but also to understand the relationships between events was important.

Gilbert Schonfelder had been a medical doctor and he said that adverse effects in human, effectively meant disease, and the key was to understand how we make models to protect humans. AOPs gave a structure that helps to raise the right (toxicological) questions around adverse outcomes.

Q3. What are the challenges in using omics data to discriminate adaptive and adverse changes resulting from chemical exposure?
Alan Poole said there were many challenges in particular we must get the experience and knowledge to be able to have confidence that we can recognise an adaptive response from an adverse response. In this context **Jos Kleinjans** thought the design of the study is important and that an appropriate hypothesis for the adaptive case was required, **Aldert Piersma** said that better understanding of what is the threshold of adverse effects was important. An effe can now be observed on the genome level - even at single molecule dose - but high dose was required for an actual adverse outcome. Better understanding of tIming and dosing are some of the biggest challenges.

Gilbert Schonfelder wanted to know what is the adverse change that leads to disease? What is a 'normal' gene expression profile during the day? "We need simpler questions and establish a basis for comparison," he said. When we measure changes we need to know what the thresholds are. **Peter Kille** noted that the presentation from 2015 LRI award winner **Alice Limonciel** had addressed this issue: what you see in a normal situation, then an adaptive situation that is then pushed over the threshold for an adverse effect including an explanation of the mechanistic situation explained.

Q4. Omics is a rapidly developing field. Considering the current state of the science, which applications in hazard assessment: a) look most promising and b) pose the greatest challenges?
Rick Becker stated that we can use these technologies to understand biological activity of molecules that are not designed to be bioactive and provide an effective screening approach that can define a zone of safe exposure. **Gilbert Schonfelder** wondered what is required. He thought that we need to work together with pathology which was opening new fields using phenotypes.

For **Alan Poole** the most useful application was most in read-across. If we know the mode of action (MOA) we can understand the key events, he said. **Rick Becker** indicated that working in tandem with gene knockout technology to loose gene expression offered a good combination. **Peter Kille** thought the technology could be used for the "unknowns" where you did not know the chemistry and also for read-across on species groups. He said that the greatest challenge was to understand the threshold where the genetic level change leads to the physiological issue. **Gilbert Schonfelder** also indicated there was a need to better characterise the sensitivity of the technique. "We need to know the number of experiments that we will need to do," he concluded.

How to address risk
Q5. Can omics be used to screen for hazards/ MOAs and thereby help focus hazard assessment? Or better as supporting approach for making other tools more useful?
Ian Kimber speculated if we can develop risk assessments via the technology. **Peter Kille** saw it as a supporting technology able to identify the key events that would require the development of other technologies to quantify the event and elucidate the dose and the temporal questions. **Gilbert Schonfelder** agreed and said that the technology could identify the pathway, while **Jos**

Kleinjans said it could identify a limited amount of genes, but we need to get other biological methods right then we can more usefully use the omics he suggested. Overall he thought the technology had great potential.

In contrast **Alan Poole** said he knew of fine chemicals companies using omics to predict characteristics of new products. There was a lots of data out there in industry. For **Rick Becker** it all comes back to how are we are going to use this information. We could use pathways to screen chemicals through species comparison, but this was more challenging for chemicals that do not have high biological activity. **Gilbert Schonfelder** highlighted an issue of different phenotypes for standard cell lines, obviously it was important that we know what cell type is being used.

Where next

Q6. Is it too early for a pre-regulatory debate? If so, where should we be heading next?

In terms of AOPs **Peter Kille** reiterated that AOP was a great framework and the OECD wiki for AOPs should be populated by everyone. He was worried to hear at the awards dinner about Effectopedia – another one wiki. He felt there was no need to dilute the action. **Ian Kimber** was worried about the wiki approach: "Where is the quality control, the robustness?" he asked. **Gilbert Schonfelder** agreed but asked where the money to enable quality control is? **Rick Becker** said that we need to elaborate case studies for use of AOPs to do category formation studies, but he felt there was not enough understanding for hazard assessment studies as yet.

In terms of where next for genomics **Alan Poole** reported on a joint ECETOC/ OECD regulators. Regulators needed to build confidence in using omics. This would require consensus on a standardised framework for analysing results. In addition how can all the data be brought together in a weight of evidence approach? It all comes down to the level of confidence in using the methodology, compared to the high level of confidence in animal testing methodologies. **Peter Kille** was concerned that many of labs doing this work were not toxicology laboratories and lacked the necessary methodology rigour required to "do this right".

Gilbert Schonfelder said that regulators would be able to understand the data using a standardised and controlled system; this was the usual evolution of a new technology. **Jos Kleinjans** said that the European Commission always emphasised the need to sit together with regulators. The difference in the US is the the regulators follow the science and know better where the technology is going. **Gilbert Schonfelder** said that regulators in Europe also followed technology developments closely.

Q7. What research is needed to deal with the previous questions? Is there enough valid data to build case studies?

To conclude the panel session **Ian Kimber** asked each panelist to give him one example of a tangible thing we can do. **Rick Becker** reiterated the need to "test drive an AOP and learn from doing". This will tell us what we need to do more and/or better. **Alan Poole** suggested that we need develop some technology to support the OECD, while **Peter Kille** said that we needed to ensure that all data produced by the techniques is public: submitted and available for all. **Gilbert Schonfelder** said that we needed to identify gaps in our knowledge and also identify those people who can be involved in our programmes to make progress. Finally, **Jos Kleinjans** suggested that pesticide research on molecular pathology be pursued and we should continue this sort of discussion on a regular basis.

Dr Bruno Hubesch concluded the workshop. Commenting on the morning session he noted that there was a tendency in LRI projects to focus on regulatory aspects, while companies require screening level models to assess new products at before market entry or the regulatory aspect.

He reminded participants that the LRI website carries information on all the programme's projects and all the materials from the workshop will be available there too. The 19th annual Cefic-LRI workshop will take place in Brussels during November 2017.

Appendix - The 2016 LRI Workshop Poster Session

ECO31 - Identifying strategies that will provide greater confidence in estimating the degradation rates of organic chemicals in water, soil and sediment - Prof. Damian Helbling, Cornell University, USA

ECO32 - Aqueous biodegradation and desorption of poorly soluble substances – improving experimental tools and models - Prof. Andreas Schäffer, RWTH Aachen University, DE

ECO34 - A tiered testing strategy for rapid estimation of bioaccumulation by a combined modelling - in vitro testing approach - Prof. Kristin Schirmer, Eawag, CH

ECO35 - Interference of hepatotoxicity with endocrine activity in fish - Prof. Thomas Braunbeck, University of Heidelberg, DE

B17 - SHINE: Target and non-target screening of chemicals in the indoor environment for human exposure assessment - Dr Marja Lamoree, IVM University Amsterdam, NL

B18 - Carcinogen Dose-Response Database for Threshold of Toxicological Concern (CDRD-TTC) - Prof. Mark Cronin, Liverpool John Moores University, UK

AIMT5 - Building a prenatal developmental toxicity ontology, integrating existing biological, chemical, in silico models and in vitro methods and data, aiming at an alternative integrated AoP/MoA framework for mechanistic hazard and risk assessment in developmental toxicology - Prof. Aldert Piersma, RIVM, NL

ECO20.2 - Development of an alternative testing strategy for the fish early life-stage test for predicting chronic toxicity: Assay validation - Prof. Dries Knapen, University of Antwerp, BE

LRI AWARD 2015 - Establishment of thresholds of activation of stress responses pathways and ligand-activated receptors for chemical classification - Dr Alice Limonciel, Innsbruck Medical University, AT

ECO11.3 - Ring test OECD 306 - Dr Russell Davenport, University of Newcastle, UK

ECO39 - Review, ring-test and guidance for TKTD modelling - Dr Roman Ashauer, York University, UK