ANNUAL CONGRESS 2016

bts

THE BRITISH TOXICOLOGY SOCIETY

Manchester Conference Centre

CONFERENCE PROGRAMME

3rd-6th April 2016

Badge Colour Guide

This year the BTS have added colour coded stripes to delegate badges so that different groups are easily identifiable. These colours are:

Member Delegate

Non- Member Delegate

BTS Executive Committee Member

BTS Speciality Section Chair

Speaker

Exhibitor

Ambassador

Contents

The BTS is grateful to the following organisation for their donations

Covance Laboratories Ltd,

Charles River Laboratories

Unilever

AstraZeneca

Novartis Pharma

BTS Bursary Recipients for this meeting

Dr Philip Probert (Newcastle University)

Dr Jonathan Lea (University of Liverpool)

Dr Tim Allen (University of Cambridge)

Mr Alistair Leitch (Newcastle University)

Miss Amy Ball (University of Liverpool)

Mr Alex Cooper (Public Health England)

Miss Kimberley Rockley (Durham University)

Miss Nathalie De Bois Brillant Andreu (University of Liverpool)

Miss Fiona Mutter (University of Liverpool)

Miss Joanna Clarke (University of Liverpool)

Miss Kirsty Meldrum (Imperial College London)

Welcome…

I am pleased to welcome you to the 37th Annual Congress of the British Toxicology Society taking place here in Manchester, with a rich and varied scientific programme and outstanding speakers.

Following the Continuing Education Programme on Sunday 3rd April which will update us on "Carcinogenicity testing" the BTS President, Professor Heather Wallace, will open the main Congress. Dr Muireann Coen will be giving a "Hot Topic" lecture, "The microbiome in toxicology". The scientific sessions of the day will end with Early Career Oral Communications, a new slot for 2016 where post-doctoral researchers will present their work.

Our Plenary Lecture this year is being given on Monday April 4th by Professor Martin van den Berg (Utrecht University) exploring "why are we not using more replacement *in vitro* models for human risk assessment to disease uncertainties ". Dr Toby Athersuch (Imperial College) will present the Early Career Prize investigator lecture on the morning of Tuesday April 5th, followed later that day by another Congress highlight - the delivery of the Paton Prize Lecture by Professor Ted Lock (Liverpool John Moores University).

This year we have an exciting set of symposia covering "New Frontiers in Predictive approaches to Safety Assessment" to "New Technologies in Discovery Toxicology " to "Cancer Pathology ", "Novel Psychoactive Agents and Drugs of Abuse", " In Vitro Methods for Genotoxicity and Carcinogenicity", "Assessing the Safety of Naturals" and "Therapeutic Vaccines".

The newly invigorated Study Director/Study Monitor workshop on Tuesday April 5th provides a great forum to exchange ideas between scientists and there are numerous other opportunities to network and get to know each other during the Congress (e.g. Early Career and the BTS Speciality Sections). Please also make time to join an informal lunchtime session "Developing Government Scientists" on Monday April 4th.

This year we have an impressive number of poster presentations and oral communications. We are again pleased to offer several prizes this year: two supported by the Society, one by the In Vitro Toxicology Society and the fourth is the Toxicology Research Prize sponsored by the Royal Society of Chemistry. Prize winners will receive their certificates at the Congress dinner on Tuesday April 5th. We also have the return of our Trade Exhibition this year, organised by Executive Business Support Ltd (EBS), which I encourage you all to visit during your time in Manchester. All are welcome too at the two lunchtime workshops which are being hosted by Bioreliance and Cyprotex.

Last but by no means least the Congress will close on Wednesday April 6th with a Technology lecture "The application of novel genome engineering techniques in health and disease" which is being given by Dr Hamid Dolatshad (University of Oxford).

My thanks go to everyone who has been involved with the design and organisation of the Annual Congress but in particular to my colleagues on the BTS Scientific Subcommittee, Speciality Sections and the BTS Education, Training and Career Development Subcommittee and to Alice Higgins and Victoria Blincoe from EBS for their sterling work in support of our Congress. I would also like to express my thanks and that of the BTS to the Manchester Congress Centre and the Midland Hotel management for their help in preparing for this meeting. I hope you all enjoy the Congress, I look forward to meeting with as many of you as possible over the next few days and, as ever, we would greatly appreciate your feedback on the Congress and suggestions for future topics of interest.

We are going to be live tweeting the Congress this year – do follow us at @BritToxSoc and retweet us to your followers! Also feel free to add your own experiences on Twitter using #BTSCongress.

Dr Maria L Beaumont,
BTS Scientific Meetings Secretary

Congress Programme

SUNDAY – 3rd April 2016	
11:30	Registration desk open for CEP and Congress delegates
12:00 – 12:40	Lunch available for CEP delegates – **Pioneer Room**
All sessions in the Pioneer Theatre **Continuing Education Programme: An update on carcinogenicity testing: hazard identification and human risk assessment** **Chair: Professor Alan Boobis** (Imperial College, UK)	
12:40 – 13:00	Introduction – overview/scene setter – Chemical carcinogenicity: Past, present, future **Professor Alan Boobis** (Imperial College, UK)
13:00 – 13:45	Waiving Carcinogenicity studies: Carcinogenicity assessment documents (CAD) – Biologics/Pharmaceutical **Dr Tim MacLachlan** (Novartis, USA)
13:45 – 14:30	Hazard identification and risk assessment based on epidemiology and animal data, the example of perfluorinated compounds. **Dr Tony Fletcher** (Public Health England, UK)
14:30 – 15:00	*Refreshments – Pioneer Room*
15:00 – 15:45	Mode of Action/Human Relevance Framework for carcinogens **Dr Richard Currie** (Syngenta, UK)
15:45 – 16:10	Round-up session **Professor Alan Boobis** (Imperial College, UK)
All sessions in the Pioneer Theatre **Congress Programme**	
16:20 – 16:30	**Welcome by the BTS President Professor Heather Wallace** (University of Aberdeen, UK)
16:30 – 17:30	**Hot Topic Lecture -** The microbiome in toxicology **Dr Muireann Coen** (Imperial College, UK) *Chair: Professor Tim Gant (Public Health England Centre for Radiation, Chemical and Environmental Hazards, UK)*
17:30 – 18:30	**Early Career Oral Communications** *Chair: Dr Chris Powell (GlaxoSmithKline, UK)* Using *in vitro* toxicity assays to track soil contaminants – Dr Philip Probert Investigating the *in vitro* utility of keratin-18 forms as biomarkers of drug-induced liver injury (DILI) – Dr Jonathan Lea An MIE atlas – Dr Tim Allen Switching cells to galactose results in metabolic reprogramming and profound changes in cellular signalling, the mitochondrial proteome and ultrastructure – Gareth Miles
18:30 – Late	Wine reception and buffet supper – **Pioneer Room**
19:30 – 20:30	**Conference Room 5** Early Career Networking Quiz

MONDAY - 4th April 2016		
08:30	Registration opens	
09:00 – 10:00	**Pioneer Theatre** **Plenary Lecture:** **Why are we not using more replacement in vitro models for human risk assessment to disease uncertainties?** **Professor Martin van den Berg** (Utrecht University, Netherlands) *Chair: Professor Heather Wallace (University of Aberdeen, UK)*	
	Pioneer Theatre **Symposium 1:** **New Frontiers in Predictive Approaches to Safety Assessment** **(BTS Regulatory Toxicology Speciality Section and Royal Society of Chemistry)** *Chairs: Dr Henry Stemplewski (MHRA, UK)* *Professor Andrew Smith (MRC Toxicology Unit, UK)*	
10:00 – 10:30	Toxicology predictions: present issues, future challenges **Professor Alan Boobis** (Imperial College, UK)	
10:30 – 11:00	Modelling of the toxicity of small molecules as an aid in designing new drugs **Professor Hans Westerhoff** (University of Manchester, UK)	
11:00 – 11:30	*Refreshments – Pioneer Room*	
11:30 – 12:00	Systems biology in toxicology **Professor Steve Oliver** (University of Cambridge, UK)	
12:00 -12:30	Application of transgenic models in metabolism and toxicity **Dr Colin Henderson** (University of Dundee, UK)	
12:30 -14:00	*Lunch and Trade Exhibition in Pioneer Room*	
12:45 – 13:45	Lunchtime Workshop: **Conference Room 7** **Developing Government Scientists** **Professor Penny Bramwell** (Food Standards Agency, UK) *Chair: Dr Henry Stemplewski (MHRA, UK)*	
12:45 – 13:30	Cyprotex hosted Lunchtime Workshop: **Conference Room 5** **The Application of Imaging for Toxicity Detection and Mechanistic Understanding** 'Real-time *in vivo* bioluminescence imaging of Nrf2 activity reveals localised chemical stress responses associated with organ-specific drug toxicity' **Fiona Mutter, MRC centre for Drug Safety Science, Dept. of Molecular & Clinical Pharmacology** 'Development of 3D cardiac and hepatic microtissue models for the improved prediction of clinical cardio- and hepatotoxicity' – **Paul Walker, Cyprotex**	
	Pioneer Theatre **Symposium 2 - New Technologies in Discovery Toxicology: Case Studies in Optimising Drug/Chemical Design (BTS Discovery Toxicology Speciality Section)** *Chairs:* *Dr Pete Newham (AstraZeneca, UK)* *Professor Heather Wallace (University of Aberdeen, UK)*	**Cotton Theatre** **Symposium 3- Cancer Pathology** **(British Society of Toxicological Pathology)** *Chairs:* *Dr Catherine Ross (Covance Laboratories, UK)* *Dr Chris Powell (GlaxoSmithKline, UK)*
14:00 – 14:30	Improving toxicity predictions to design safer acidic molecules **Dr Nigel Swaine** (Pfizer, UK)	The two-year bioassay: pros/cons/provisos **Dr John Foster** (Independent Consultant, UK)

14:30 – 15:00	Applying data from exploratory omics experiments for early safety screening **Dr Hinrich Goehlmann** (Janssen Pharmaceutical Companies of J&J, Belgium)	Animal models of cancer **Dr Jennifer Morten** (Cancer Research, UK)
15:00 – 15:30	*Refreshments – Pioneer Room*	
15:30 – 16:00	Exploiting the promise of advanced 2D and 3D cell systems for selection of safe and efficacious drugs **Professor Magnus Ingelman-Sundberg** (Karolinska Institute, Sweden)	Translation of animal carcinogenicity data to humans **Dr Chris Powell** (GlaxoSmithKline, UK)
16:00 – 16:30	Combining in vitro and in vivo PK/PD (TK/TD) modelling data to guide patient-centric risk assessments **Dr Teresa Collins** (AstraZeneca, UK)	Genetic diagnosis and treatment of cancer: the answers in our genes **Professor Mark Caulfield** (Genomics England, UK)
16:30 -18:00	**BTS AGM – Pioneer Theatre**	
18.00 onwards	BTS Speciality Section Networking: *Information on Room Locations for each speciality section can be found on signage in the Pioneer Room or at the Registration desk*	

TUESDAY – 5th April 2016		
08:30	Registration opens	
08:40 – 09:25	**Pioneer Theatre** **Early Career Investigator Prize Lecture:** Investigating the Human Exposome – **Dr Toby Athersuch**, Imperial College London *Chair: Professor Tim Gant (Public Health England Centre for Radiation, Chemical and Environmental Hazards, UK)*	
	Pioneer Theatre **Symposium 4 - Novel Psychoactive Agents and Drugs of Abuse: Where are we now? (Human Toxicology Speciality Section)** *Chairs:* *Dr John Thompson (Cardiff University, UK)* *Dr David Wood (Guys and St Thomas' NHS Foundation Trust, UK)*	**Cotton Theatre** **Symposium 5 - In Vitro Methods for Genotoxicity and Carcinogenicity (In Vitro Toxicology Society):** *Chairs:* *Dr Ray Boughton (Delphic HSE Solutions Ltd, UK)* *Dr Jonathan Welch (Charles River Preclinical Services, UK)*
09:30 – 10:00	Novel Psychoactive agents – 'Legal highs': what's out there? How do we know? **Dr John Thompson** (Cardiff University, UK)	Industry considerations for in vitro modelling of genotoxicity and carcinogenicity **Dr Ann Doherty** (AstraZeneca, UK)
10:00 – 10:30	Legal highs – clinical effects **Dr David Wood** (Guy's and St Thomas' NHS Foundation Trust, UK)	Investigating the potential of nanofibre physico-chemical properties to elicit genotoxicity in vitro **Dr Martin Clift** (Swansea University, UK)
10:30 – 11:00	*Refreshments- Pioneer Room*	
11:00 – 11:30	Severe clinical toxicity caused by unlicenced weight loss agents including dinitrophenol **Professor Simon Thomas** (Newcastle University, UK)	An integrated "in vitro carcinogenicity predictive tool", utilising in vitro cell signalling and cell behaviour assessment coupled with in vitro genotoxicity data **Professor Gareth Jenkins** (Swansea University, UK)
11:30 – 12:00	Drugs of abuse: regulatory challenges **Dr Michael Evans-Brown** (European Monitoring Centre for Drugs and Drug Addiction, Portugal)	Adverse Outcome Pathways in genotoxicity & carcinogenicity **Dr Andrew Scott** (Unilever, UK)
12:00 – 13:30	*Lunch and Trade Exhibition – Pioneer Room*	
12:15 - 13:15	BioReliance supported workshop: **Conference Room 1** **ICH M7: Dealing with genotoxic impurities**	
13:30 – 14:30	**Pioneer Theatre** **Paton Prize Lecture** Biochemical mechanisms and biomarkers of toxicity to the kidney: advances over the last 40 years **Professor Ted Lock** (Liverpool John Moores University, UK) *Chair: Dr Ernie Harpur (Newcastle University, UK)*	
14:30 – 15:00	*Refreshments – Pioneer Room*	
15:00 – 17:00	**Pioneer Theatre** **Oral Communications** *Chair: Dr Tina Mehta (Dow Agrosciences, UK)* - MicroRNA-122 provides sensitive detection of paracetamol-induced acute liver injury and dysfunction – a large prospective study of 985 patients –	**Cotton Theatre** **Study Director/Study Monitor Workshop** *Chairs:* *Karen Laidlaw (Charles River Laboratories, UK)* *Stuart Purbrick (AstraZeneca)* The new and improved Morris Maze **Lisa Burdett** (Charles River, UK)

	Joanna Clarke, University of Liverpool - Neurotoxicity of novel psychoactive substances – **Peter Hanson, University of Newcastle** - Optimisation of air-liquid interface cultures of human bronchial epithelial cells and their use in the investigation of potential health effects of diesel exhaust particles and cerium dioxide nanoparticles – **Kirsty Meldrum, Imperial College London/Public Health England** - A novel role for the microRNA biogenesis apparatus in the repair of double-stranded breaks – **Ben Hawley, MRC Toxicology Unit** - Early studies assessing the cardioprotective properties of metformin during sunitinib-induced cardiotoxicity – **Refik Kuburas, Coventry University** - Phototoxicity Testing for Agrochemicals: A Proposed Human Risk Assessment Framework and Case Studies – **Fiona Macleod, Dow Agrosciences** - Toxicological evidence underpinning the assessment of the carcinogenicity of alcoholic beverages by the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment – **Britta Gadeberg, Public Health England** - Assessment of reactive metabolite-induced mitochondrial toxicity and generation of personalised *in vitro* models as a tool to determine susceptibility to idiosyncratic hepatotoxicity – **Amy Ball, University of Liverpool**	Safety Pharmacology end-points on Toxicology studies **Stuart Purbrick** (AstraZeneca, UK) The SD and SM Role, "out of the frying pan, into the fire" **Clare Horne** (GW Pharmaceuticals, UK) Pioneering better science through the 3Rs: an introduction to the NC3Rs **Fiona Sewell** (NC3Rs, UK)
17:00 – 18:30	**Poster Session – Leader Suite**	
19:00 – 19:30	**At the Midland Hotel - Pre-Dinner Drinks Reception**	
19:30 – Late	**At the Midland Hotel - Congress Dinner and Entertainment**	

WEDNESDAY – 6th April 2016		
08:30	Registration Opens	
08:40 – 09:25	**BTS Ambassadors Breakfast – Conference Room 1**	
	Pioneer Theatre **Symposium 6 - Assessing the Safety of Naturals** **(BTS Risk Assessment Speciality Section)** *Chairs:* *Dr Carol Courage (Unilever, UK)* *Dr Theresa Neely (Unilever, UK)*	**Cotton Theatre** **Symposium 7 - Therapeutic Cancer Vaccines** **(BTS Regulatory Toxicology Speciality Section)** *Chairs:* *Dr Henry Stemplewski (MHRA, UK)* *Professor Paul Baldrick (Covance Laboratories, UK)*
09:30 – 10:00	What are natural compounds – are they all safe? **Dr Anne Constable** (Nestle, Switzerland)	Therapeutic cancer vaccines: where are we now? **Professor Farzin Farzaneh** (Kings College London, UK)
10:00 – 10:30	Natural product characterization – separating the wheat from the chaff **Dr Paul Russell** (Unilever, UK)	Regulatory considerations for clinical and non-clinical development of therapeutic cancer vaccines **Dr Bridget Heelan** (Parexel, UK)
10:30 – 11:00	*Refreshments – Pioneer Room*	
11:00 – 11:30	Novel food approach to safety assessment **Dr Patrick O'Mahony** (Food Safety Authority of Ireland, Ireland)	Selecting pre-clinical models for cancer immunotherapy **Professor Michael Lisanti** (University of Manchester, UK)
11:30 – 12:00	History of safe use – a Unilever approach to assessing naturals **Dr Theresa Neely** (Unilever, UK)	Immuno-oncology: investigating cancer therapies powered by the immune system **Professor Hardev Pandha** (University of Surrey, UK)
12:10 – 13:00	**Pioneer Theatre** **Technology Lecture:** The application of novel genome engineering techniques in health and disease **Dr Hamid Dolatshad** (Nuffield Division of Clinical Laboratory Sciences, University of Oxford UK) *Chair: Dr Ernie Harpur (Newcastle University, UK)*	
13:00	*Lunch and End of Congress- Pioneer Room*	

Organisers and Chairs

Symposium 1: New Frontiers in Predictive Approaches to Safety Assessment - (BTS Regulatory Toxicology Speciality Section and Royal Society of Chemistry)
Dr Henry Stemplewski (MHRA, UK) and Professor Andrew Smith (MRC Toxicology Unit, UK)

Symposium 2: New Technologies in Discovery Toxicology: Case Studies in Optimising Drug/Chemical Design (BTS Discovery Toxicology Speciality Section)
Dr Pete Newham (AstraZeneca, UK) and Professor Heather Wallace (University of Aberdeen, UK)

Symposium 3: Cancer Pathology (British Society of Toxicological Pathology)
Dr Catherine Ross (Covance Laboratories, UK) and Dr Chris Powell (GlaxoSmithKline, UK)

Symposium 4: Novel Psychoactive Agents and Drugs of Abuse: Where are we now? (Human Toxicology Speciality Section)
Dr John Thompson (Cardiff University, UK) and David Wood (Guys and St Thomas' NHS Foundation Trust, UK)

Symposium 5 : In Vitro Methods for Genotoxicity and Carcinogenicity (In Vitro Toxicology Society)
Dr Ray Boughton (Delphic HSE Solutions Ltd, UK) and Dr Jonathan Welch (Charles River Preclinical Services, UK)

Symposium 6: Assessing the Safety of Naturals (BTS Risk Assessment Speciality Section)
Dr Carol Courage and Theresa Neely (Unilever, UK)

Symposium 7: Therapeutic Cancer Vaccines (BTS Regulatory Toxicology Speciality Section)
Dr Henry Stemplewski (MHRA, UK) and Professor Paul Baldrick (Covance Laboratories, UK)

Scientific Sub-Committee:

Chair – Tim Gant
Scientific Meetings Secretary – Maria Beaumont
Ordinary Members – Jonathan Tugwood, Marion MacFarlane, Richard Currie and Muireann Coen
Abstract Editor – Dominic Williams
Co-opted Members:
BSTP Representative – Catherine Ross
IVTS Representative – Ray Boughton
ITTP Co-ordinator – Andy Smith
Speciality Section Chairs – John Thompson, Henry Stemplewski, Carol Courage, Peter Newham
Treasurer – Guy Healing

Congress Secretariat:

Event Manager - Alice Higgins
Executive Business Support
City Wharf
Davidson Road
Lichfield
WS14 9DZ
meetings@thebts.com
01543 442158

Conference Info

The full address of the conference centre is:

Manchester Conference Centre
78 Sackville St,
Manchester
M1 3NJ

Registration Desk Opening Times
Sunday 3rd April – 11:30 -18:00
Monday 4th April – 08:30 – 18:00
Tuesday 5th April – 08:30 – 18:00
Wednesday 6th April – 08:30 – 13:00

Exhibition Opening Times
Monday 4th April – 11:00 – 18:30
Tuesday 5th April – 10:30 -18:30

BTS Exec - Meet and Greet
The BTS stand will be situated in the exhibition area and will be manned during refreshment and lunch breaks by members of the BTS Executive committee. Please do come along and feedback to the team your views on any aspect of BTS or areas that you believe BTS could be working better for you. This is a good opportunity to shape the future of your BTS so please come along and get involved.

Poster Sessions
There are 33 posters on display in the Leader Suite, next to the exhibition area throughout the conference.
There is a directed poster viewing on Tuesday from 17:00 – 18:00
Authors should make sure that they attend their posters during this time.
Each poster has been assigned a number which appears alongside it's abstract in this brochure.
Posters should be mounted by 10:00 on Monday 4th April and taken down by 13:00 on Wednesday 6th April. Any posters not taken down by this time will be removed. The Society cannot be held responsible for any lost or damaged posters.

Poster Prizes
The BTS is pleased to be able to provide two student prizes this year – one for best oral communication and one for poster presentation.
The Royal Society of Chemistry will award a Toxicology Research Poster Prize.
The IVTS will also be awarding a poster prize for the best poster presenting work in the *in vitro* field of toxicology.
All prize winners will be awarded their certificate and prizes at the Congress dinner.

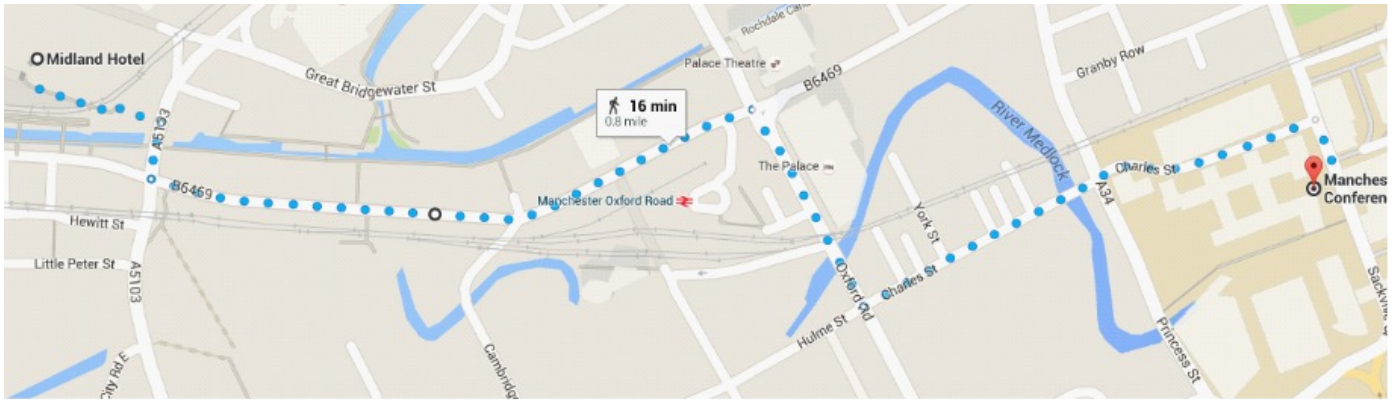
Social Timetable

Sunday – 18:30 – Welcome wine reception and buffet supper – Pioneer Room
Sunday – 19:30 – Early Career Networking Quiz – Conference Room 5
Monday – 18:00 – Wine reception and speciality networking sessions – Please see notices about which session is taking place in which room. The BTS Speciality sections are:

- *Discovery Toxicology*
- *Regulatory Toxicology*
- *Human Toxicology*
- *Risk Assessment*

Tuesday – 19:00 for dinner at 19:45 – Congress Dinner and Ceilidh – Midland Hotel

The Midland Hotel is just under a mile from the Conference Centre following the map below. Alternatively we recommend Manchester Cars who can be contacted on Tel: 0161 2283355



Meals and Refreshments

Full delegate registration includes lunches and refreshments for the duration of the Congress with the exception of Monday evening meal.
All meals, with the exception of the Congress Dinner, will be served in the Pioneer Room.

BTS Committee Meetings

The following meetings are scheduled during Congress:

Sunday	20:00 – 21:00	Scientific Sub Committee	Conference Room 4
Monday	18:30 – 20:15	Executive Committee	Conference Room 1
Tuesday	12:15 – 13:15	ETCD	Conference Room 7

BTS Annual General Meeting

The AGM will take place on Monday 4th April at 16:30 in the Pioneer Theatre. This is open to all BTS members. Please come along to see what is happening in the society and meet the incoming Executive Committee.

Facilities Information

Accommodation at Pendulum Hotel – Check in at the Pendulum Hotel is from 2pm at Hotel reception. This is open 24 hours a day.

Internet Access – Free wifi is available throughout the conference centre and hotel. Please see the registration desk if you have any issues logging in.

Parking - The hotel doesn't have its own car park but there is a multi-story car park on Charles Street at a cost of £10 per 24 hours.

Local Area - Maps of the local area are available from the registration desk or can be seen here: http://www.visitmanchester.com/what-to-do/maps/

Medical Services – For minor incidents and first aid, please contact the registration desk. In the event of an emergency please dial 999 and inform a member of staff on the registration desk.

Certificates and CPD

Certificates and CPD information for Congress and the CEP will be sent electronically after the meeting following receipt of an online feedback form.

Are you a Member of the BTS? -A Message from the General Secretary

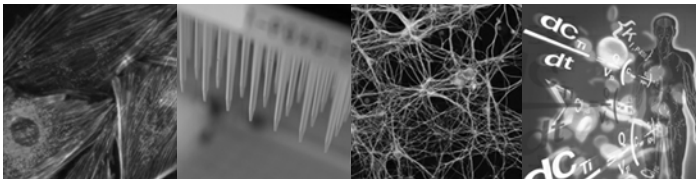
I hope you enjoy this Congress and the opportunity of networking. I wonder if you have considered joining the Society? We offer a wide range of benefits to those with an interest in toxicology and related disciplines including access to travel grants, awards and prizes, our on –line news "Toxfeed" and discounts on meeting registration and books and journal subscriptions. Please look at the website for more information (www.thebts.org).

If you are interested in finding out more about our Society please find any of our Executive Committee (EC) members (identified by their badges) and we will be happy to tell you more. We can also guide you through the simple membership application process and explain how you can benefit from an introductory offer applying to non-members attending this congress.

We look forward to welcoming you to our Society as a member.
Yours sincerely

Shirley Price
General Secretary.

cyprotex



In vitro Toxicology

Mechanistic Toxicity

Skin & Ocular Sensitisation, Irritation and Corrosion

Genotoxicity

Systemic Toxicity
Cardiotoxicity Hepatotoxicity
Neurotoxicity Nephrotoxicity

Endocrine Disruption

Regulatory Genotoxicity

Ames Test OECD TG 471
Salmonella typhimurium TA98
Salmonella typhimurium TA100
Salmonella typhimurium TA1535
Salmonella typhimurium TA1537
Escherichia coli WP2 uvrA pKM101
In vitro Chromosome Aberration OECD TG 473
In vitro Micronucleus Test OECD TG 487

In vitro ADME

Permeability and Transporters

Distribution and Protein Binding

Drug Metabolism

Drug-Drug Interactions

***In Silico* Prediction**
PBPK Modelling
QSAR Modelling

Physicochemical Profiling

Bioanalysis & PK

DDI Packages

In vitro drug metabolism (CYP and non-CYP) and transporters (efflux and uptake)

Scientific guidance and consultancy

Screening and regulatory services

Bioanalytical expertise

Novel Innovations

eCiphr®Cardio - High throughput MEA to monitor cardiac electrophysiology.

eCiphr®Neuro - High throughput MEA to monitor neuronal activity with 11 end points.

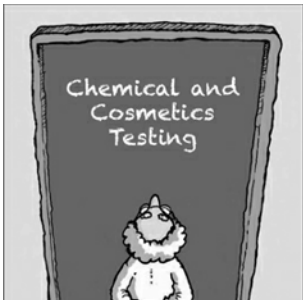
3D microtissues - Organ-specific spheroids which closely mimic native tissue and allow for extended repeat dosing.

CellCiphr® Premier - Comprehensive hepatotoxicity assessment using multiple cell types, time points and end points.

NEW from Cyprotex

To complement our ADME, DDI and Toxicology guides, we have recently introduced our Chemical and Cosmetics Testing guide.

Order your free copy at www.cyprotex.com/guides



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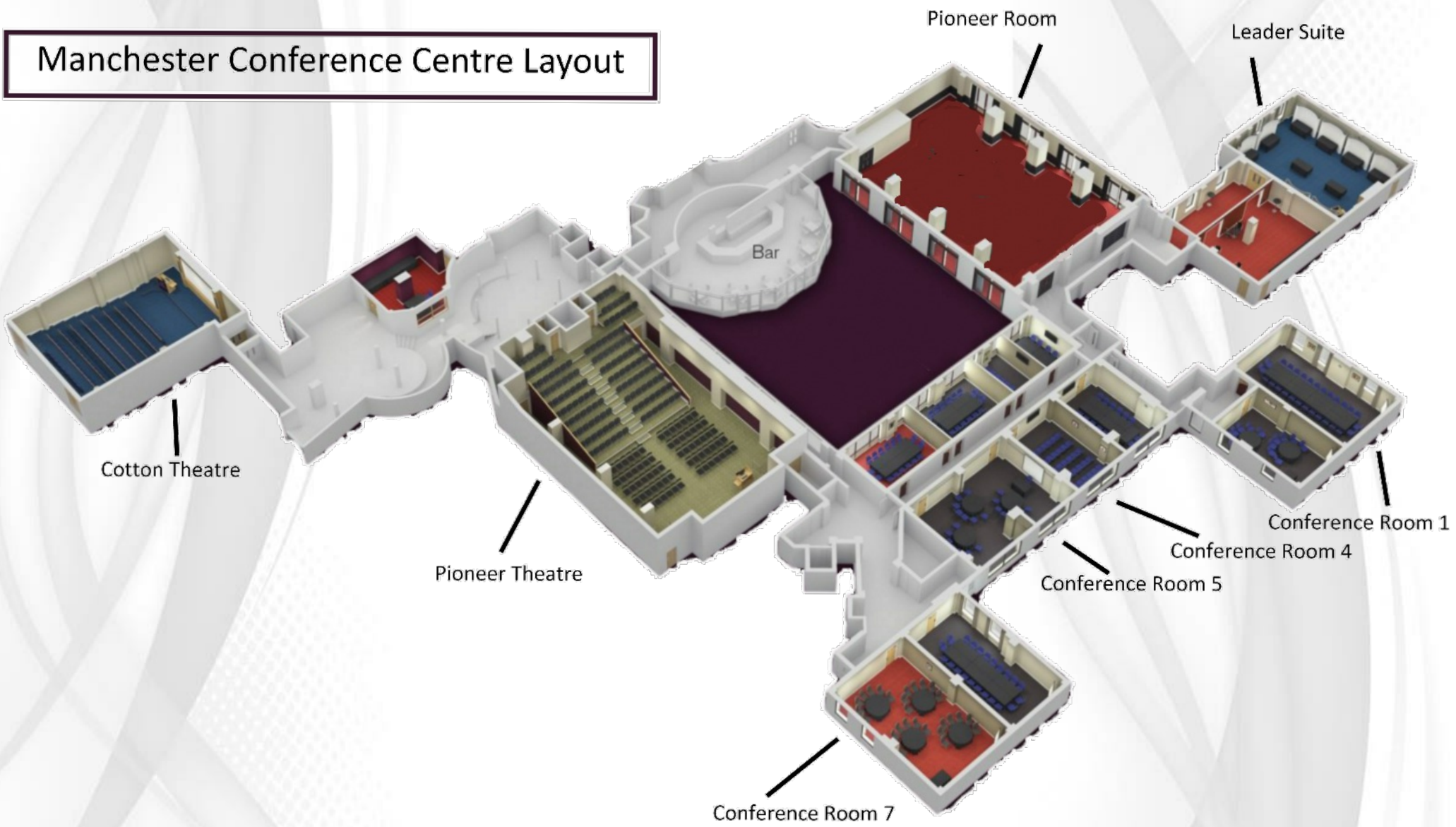
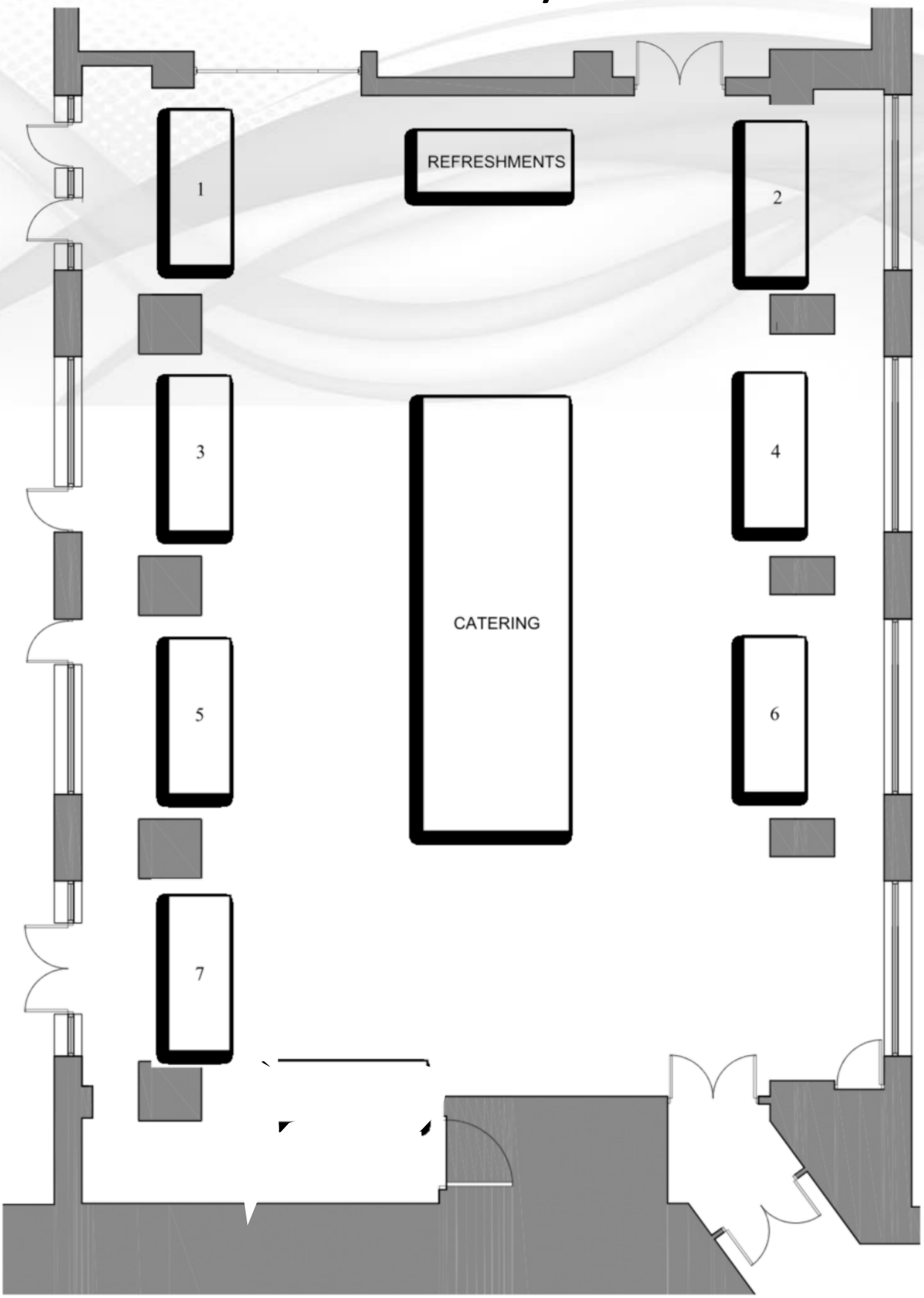
Manchester Conference Centre Layout



Exhibition Layout



BTS Annual Congress 2016
Manchester Conference Centre
Pioneer Room - 270m²
(Not to Scale)

BTS Congress 2016 - Trade Exhibition

The Trade Exhibition is taking place in the Pioneer Room which is where all refreshments will be served. Please take time to visit the trade stands and benefit from the technical and product advice that they have available. You can also visit the BTS Stand to meet the Executive Committee, find out the benefits of membership and talk about what the BTS can do for you.

The following companies will be exhibiting:

Company Name	Stand Number
British Toxicology Society	7
CXR Bio	4
Cyprotex	5
Kinesis	6
Micromatrices	1
Royal Society of Chemistry	2
Sciex	3

CXR Biosciences Ltd - CXR Biosciences provides preclinical services, specialising in investigative toxicology, exploratory & discovery toxicology, metabolism & PK, and dermal absorption. CXR's collaborative and advisory approach, toxicological expertise and extensive laboratory capabilities have helped customers resolve issues relating to the safety of compounds or selection of drug/chemical candidates. Our customers include leading pharmaceutical, agrochemical, chemical, consumer product and biotech companies.

Cyprotex - Cyprotex is listed on the AIM market of the London Stock Exchange (CRX). It has sites at Macclesfield and Alderley Park, both of which are near Manchester in the UK, and at Watertown, MA and Kalamazoo, MI in the US. The Company was established in 1999 and works with more than 1400 partners within the pharmaceutical and biotech industry, cosmetics and personal care industry and the chemical industry. Cyprotex acquired Apredica and the assets of Cellumen Inc. in August 2010 and the combined business provides support for a wide range of experimental and computational ADME-Tox and PK services. The acquisition of the assets and business of CeeTox in January 2014 has enabled Cyprotex to expand its range of services to target the personal care, cosmetics and chemical industries. In 2015, Cyprotex launched its new bioscience division to expand its capabilities into phenotypic and target based screening. The Company's core capabilities include high quality *in vitro* ADME services, mechanistic toxicology and high content toxicology screening services, including our proprietary CellCiphr® toxicity prediction technology, bioscience services, predictive modelling solutions including Cloe® PK, chemPK™ and chemTox, and a range of skin, ocular and endocrine disruption services. For more information, please visit www.cyprotex.com.

Kinesis is a leading international supplier and servicer of chromatography, liquid handling, sample storage and medicinal chemistry consumables and equipment. The company's products are used extensively in the Pharmaceutical, Environmental, Forensic, Life Sciences, Food, Biotechnology and Academic Markets.

Kinesis was established in 1997 in the UK. Today, the company has expanded its reach globally with subsidiary offices in Australia, Germany, Italy and USA. A network of international distributors ensures the Kinesis range of products is available worldwide.

Kinesis works with industry leading manufacturers, many on a global exclusive or preferred supplier basis. Key suppliers to the Kinesis Group include AccuStandard, ChromSword, Corning, Diba Industries, GL Sciences, Hamamatsu, Heraeus, HTL, IDEX Health & Science, Knauer, Lipomed, Micronic, Parker domnick hunter, SGE Analytical Science and Tosoh Bioscience.

The Kinesis UK facility houses state of the art processes for the pre-alignment of deuterium lamps. Approved by the world's two leading lamp manufacturers, Hamamatsu and Heraeus, these accredited processes have helped Kinesis become the world's largest 3rd party provider of pre-aligned deuterium lamps. Kinesis have recently launched a new Instrument Demonstration Suite to display and train customers on the systems they sell.

Pipette service and calibration suites at both the UK and German locations offer back to base or field-based pipette service and calibration. The UK facility is UKAS accredited.

MicroMatrices (micromatrices.com) provides molecular toxicology services to the chemical, agrochemical and pharmaceutical industries. Since 2011, our investigations have produced valuable information for our clients for their compound development planning, product safety stewardship, and mode of action studies.

MicroMatrices has core expertise in Pharmacology, Toxicology, Pathology and Histology networked to facilities of excellence across the UK. We possess a suite of analytical applications which we apply to FFPE, as well as fresh or frozen samples, and update frequently to incorporate recent findings and evolving technologies.

Our applications support Mode of Action understanding, and hypothesis generation, and have been used in the development of Threshold Based Risk Assessments and Human Relevance arguments. Services include, but are not restricted to: immunohistochemistry and in situ hybridisation (RNA scope), laser microdissection, proteomic and genomic profiling, image analysis and RT-PCR to produce integrated analyses for our clients.

Our current in house research effort is focussed on developing mass spectrometry-based solutions for localising, visualising and quantifying proteins and small molecules (environmental toxins, drugs) in tissues to facilitate understanding of mechanisms underlying toxicity and/or efficacy.

MicroMatrices is based in Dundee, Scotland, United Kingdom, a centre for world class life science research and innovation.

SCIEX helps to improve the world we live in by enabling scientists and laboratory analysts to find answers to the complex analytical challenges they face.

In Forensic Toxicology, accurate results delivered quickly mean everything. SCIEX offers a comprehensive portfolio of preconfigured LC/MS/MS methods, libraries and software to meet the most challenging applications. Our QTRAP® and TripleTOF® systems with enhanced sensitivity enable rapid screening, identification and quantitation of hundreds of compounds in a single analysis with confidence. SCIEX strives to exceed expectations and empower customers to find accurate answers.

For research use only.

Workshop supporter:

BioReliance, which is a business of Merck KGaA, Darmstadt, Germany, is a leading global contract services company in the area of product safety. We specialise in genetic toxicology screening and GLP assays, as well as transgenic mouse carcinogenicity testing. Whether requiring pre-clinical toxicology for small molecule development, hazard assessment for REACH registrations, or other regulatory, development, safety and risk assessment; BioReliance has all the experience and expertise needed to design and execute a toxicology testing program to meet your needs. Offering superlative scientific expertise, dedicated operations and specialised services, BioReliance is passionate about Toxicology and Focused on our Customer to Provide the Most Dependable Results.

Speaker Biographies

PATON PRIZE WINNER:

PROFESSOR E A LOCK - I trained at the MRC Toxicology Unit, then at Carshalton in Surrey, and obtained MIBiol (Biochemistry) and then a PhD in Biochemical Toxicology, under the supervision of Dr W N Aldridge. In 1974 I joined ICI Industrial Hygiene Research Laboratories working with Dr MS Rose in the Biochemical Mechanisms Unit. In 1980 I was appointed head of Biochemical Toxicology Section then I took sabbatical leave in 1985 to work at CIIT, Research Triangle Park, returning to CTL in 1986 as a senior scientist. I "retired" from Syngenta in 2003 as a Senior Syngenta Fellow. Then spent 2 years as a visiting Professor at Medical University of South Carolina working with Professor Rick Schnellmann. For the last 10 years I have been Professor of Industrial Toxicology at Liverpool John Moores University.

CEP and CONGRESS SPEAKERS:

PROFESSOR ALAN BOOBIS - Alan Boobis is Professor of Biochemical Pharmacology and Director of the Public Health England-supported Toxicology Unit at Imperial College London. He has been a member of Imperial College London (originally at the Royal Postgraduate Medical School, with which it merged) for almost 40 years. His main research interests include drug metabolism, mechanisms of toxicity, chemical carcinogenesis and risk assessment methodology. He has published around 240 original research papers. He is a member/chair of a number of national and international scientific advisory committees, including chair of the Committee on Toxicity. He was awarded an OBE in 2003.

PROFESSOR MARK CAULFIELD - Mark Caulfield graduated in Medicine in 1984 from the London Hospital Medical College and trained in Clinical Pharmacology at St Bartholomew's Hospital where he developed a research programme in molecular genetics of hypertension and clinical research. In 2009 he won the Lily Prize of the British Pharmacology Society. In 2000 he established the Barts and The London Genome Centre which now underpins over 40 programmes of research. Since 2008 he directs the Barts National Institute for Health Research Cardiovascular Biomedical Research Unit. Mark was appointed Director of the William Harvey Research Institute in 2002 and was elected a Fellow of the Academy of Medical Sciences in 2008. He led on fundraising towards the £25m William Harvey Heart Centre which created a translational clinical research centre. Mark served on the NICE Guideline Group for hypertension and leads the Joint UK Societies' Working Group and Consensus on Renal Denervation and was President of the British Hypertension Society (2009-2011). In 2013 he became an NIHR Senior Investigator and was appointed as the Chief Scientist for Genomics England (NHS 100K Sequencing Project) 2013-2017.

DR MARTIN J D CLIFT - Martin, a newly appointed lecturer (in Nanotoxicology) at Swansea University Medical School, gained his PhD from Edinburgh Napier University in 2009 followed by 7 years' post-doctoral research experience in Switzerland. Always within the field of Nanotoxicology, Martin predominantly focusses upon the nanoparticle-(mammalian)cell interaction, with a view to determining the mechanistic toxicological, immunological and genotoxic effects that nanoparticles, with varying physico-chemcial characteristics, may cause at the cellular level by using advanced, next-level *in vitro* systems. Martin is an editorial board member of *Particle and Fibre Toxicology*, and to date, has authored over 85 publications in the area of *in vitro* nanotoxicology.

DR MUIREANN COEN - Dr Coen is a lecturer in Metabonomics and Toxicology at Imperial College London. Her research interests include the application of metabonomics in pre-clinical and clinical toxicology and liver disease, with a particular focus on mechanistic toxicology and the study of adverse drug reactions and variable response phenotypes. This is achieved through application of advanced analytical technologies including NMR spectroscopy and mass spectrometry to characterise endogenous and xenobiotic metabolic phenotypes in tissues and biofluids. Dr Coen was awarded the BTS early career award in 2010 and the MRC ITTP fellowship in 2009.

TERESA COLLINS - In 2003 after graduating in Biochemistry from Sheffield, Teresa joined AstraZeneca and the Drug metabolism and Pharmacokinetics department. In 2009 she did a 6 month secondment to AZ Mölndal to further develop an interest in PK/PD modelling and simulation, and she completed an MSc in Modelling and Simulation with University of Manchester in 2013. She joined Drug Safety and Metabolism in 2012, and is part of team dedicated to the modelling of pre-clinical safety findings and using this information to make quantitative predictions of clinical findings.

ANNE CONSTABLE PhD - Senior Expert and Manager of chemical food safety evaluation for the Nestle Research Centre, Switzerland, providing global support and risk assessments of chemicals and ingredients for new product development, and for existing food products to Nestlé R&D communities and to corporate management. Anne started as medical microbiologist within UK Public Health Service, studied in Liverpool and Manchester, served time at the European Molecular Biology Laboratories in Heidelberg, joined Nestlé in 1992, and has been based in UK since 2004. Anne has contributed to several ILSI Europe activities and EU funded networks in chemical risk assessment and novel food safety.

RICHARD CURRIE - Richard Currie is a Principal Technical Expert at the Syngenta (Bracknell, UK) responsible for predictive and computational toxicology. He has interests in the development and application of SAR, mechanism of toxicity (including MOA/AOPs), systems biology models for toxicology. He is the lead toxicologist for some Crop Protection Chemistry research projects, the senior toxicologist for the insecticide research portfolio and is an internal consultant on investigative toxicology projects. Previously he lead research activities on mechanisms of non-genotoxic carcinogens, the role of epigenetics in toxicology, and the application of new systems approaches to developmental toxicity prediction.

ANN DOHERTY - Ann Doherty is currently Director of Genetic Toxicology Group at AstraZeneca. Ann gained her PhD in Genetic Toxicology on the *in vitro* micronucleus test in 1995 from Swansea University supervised by Dr Elisabeth Parry. Ann then completed a post-doctoral projects at Swansea University, University of Leicester and a research fellowship at University of Bristol. Ann is currently the former chair of the UK Industrial Genotoxicity Group (IGG) 2011-2015. Ann Doherty has strong links with the Genetic Toxicology Group at Swansea co-supervising two BBSRC funded PhD students. In January 2014 Ann was awarded an honorary Professorship at Swansea University.

HAMID DOLATSHAD - Hamid Dolatshad obtained his PhD at the University of Cambridge in the field of genetics and developmental biology. He has many years of experience in stem cell and molecular genetics which includes the use of CRISPR/Cas9 genome editing technology.

MICHAEL EVANS-BROWN - Mike is a Scientific Analyst at the EMCDDA working on the EU Early Warning System on New Psychoactive Substances. His main areas of work are on toxicovigilance, signal management, monitoring open source information, pharmacovigilance, risk communication, and risk assessment.

FARZIN FARZANEH - Following studies at Aberdeen and Sussex, and postdoc fellowships (Beit, EMBO and MRC), in UK and the Netherlands, Farzin joined King's College London as a lecturer in 1985. He founded the Molecular Genetics Unit in 1986 and was appointed the founding Head of the Department in 1993. In 1996 he was awarded a Personal Chair in Molecular Medicine. He co-founded the International Society for Cell and Gene Therapy of Cancer (ISCGT), serving as it's President in 2007/8. Farzin has published over 200 scientific papers and two edited books on the Functional Analysis of the Genome and Cancer Gene Therapy. He is a Qualified Person and has, since 2001, directed a GMP facility at King's, manufacturing products for clinical studies in regenerative medicine, gene therapy and immune therapy of cancer. He was awarded the Distinguished Scientist Award of the US Society for Experimental Biology & Medicine in 2016.
DR TONY FLETCHER - Tony Fletcher works as Senior Environmental Epidemiologist in the Climate Change Department, at Public Health England's Centre for Radiation Chemical and Environmental Hazards (CRCE). He is part time, also at the London School of Hygiene and Tropical Medicine (LSHTM), and Adjunct Research Professor at Boston University. He was President of International Society for Environmental Epidemiology, 2003-6. During 2006 to 2013, he was running a major research effort on the health effects of drinking water exposure to Perfluorooctanoic acid (PFOA or C8), in West Virginia and Ohio, USA. He was part of the "C8 Science Panel" established to assess the links between PFOA and disease.

PROFESSOR JOHN R FOSTER - John's first role in Toxicology was in 1978 at the British Industrial Biological Research Association (BIBRA), at Carshalton having successfully completed a PhD from University College, Cardiff. He left BIBRA in 1983 to join ICI Central Toxicology Laboratory in Cheshire, United Kingdom where he stayed until 2000. He worked for AstraZeneca Pharmaceuticals from 2001-2013 as a Senior Principal Pathologist and Deputy Director of Pathology. He became a member of the UK Royal College of Pathologists in 1988, was elected a Fellow in 1997, and was Chair of the Panel of Examiners for Toxicology from 2006-2012. He was President of the British Society of Toxicological Pathologists from 2002-2004, was appointed an Honorary Fellow of the British Society of Toxicology in 2008, and was made an Emeritus Professor in the Faculty of Health and Medical Sciences at the University of Surrey in 2010. He has published over 140 research papers, review articles and book chapters in toxicological pathology, and was the Editor in Chief of the journal, *Toxicologic Pathology,* from 2008-2013.

HINRICH W.H. GÖHLMANN - Hinrich Göhlmann did his PhD in molecular biology at the University of Heidelberg (Germany) and started his career at Janssen in November 1999 as a PostDoc focusing on establishing microarray technology at Janssen. He received the Hofmann award for leading the team that unravelled the mechanism of action of Janssen's anti-tuberculosis compound Sirturo (Bedaquiline) using next-generation sequencing technology. Since 2014 Hinrich is leading a team of scientists within Computational Sciences (Discovery Sciences) who are supporting the disease areas with the analysis of high dimensional, biological data sets generated from technologies such as high content imaging, next generation sequencing, microarrays or L1000.

BRIDGET HEELAN - Dr Heelan is Vice President (technical) at Parexel since January 2014. Her role involves advising on clinical development plans, reviewing/writing documents for regulatory submissions and providing responses to clinical questions from regulatory authorities.
Prior to this she worked as a senior clinical assessor at the Medicines and Healthcare Products Regulatory Agency, was chair of the Rheumatology Immunology Working Party and UK delegate for the Committee for Advanced Therapies at the EMA. Before joining pharmaceutical medicines she worked as a consultant immunologist in the NHS.

DR COLIN HENDERSON - Colin graduated in Biochemistry from the University of Edinburgh, where he also did his PhD in the Medical School. Following a Training Fellowship with the MRC Reproductive Biology Unit, Colin joined Roland Wolf's Molecular Pharmacology Unit, then relocating with the Unit to the University of Dundee. In the Medical School Colin developed a Transgenic programme, generating a number of knockout and reporter mouse models with applications in the fields of drug metabolism, toxicology and carcinogenesis. Currently a Senior Lecturer in the Division of Cancer, Colin continues to work closely with Roland Wolf in the development of transgenic mouse models.

MAGNUS INGELMAN-SUNDBERG - Magnus Ingelman-Sundberg, PhD; BSc.Med is Professor of Molecular Toxicology since 1996 and research group leader in Pharmacogenetics at the Department of Physiology and Pharmacology , Karolinska Institutet since 2006. He has more than 420 original papers, 24 500 citations (32 000 in Google Scholar) and an h-factor of 83 (ISI) or 97 (Google Scholar). He is a member of The Nobel Assembly at Karolinska Institutet since 2008. Recently categorized by Thomson Reuters as one of the World's Most Influential Scientific Minds (http://sciencewatch.com/sites/sw/files/sw-article/media/worlds-most-influential-scientific-minds-2015.pdf). His research focuses on genetics, epigenetics, polymorphism, regulation, function and toxicology of the hepatic ADME system with aims at understanding interindividual differences in drug response. Furthermore he develops novel hepatic in vitro systems for studying liver function and validation of drug targets.

GARETH JENKINS - I have spent 20 years studying DNA damage and DNA mutation and its relevance to cancer. I obtained a BSc in Biophysics from Kings College London in 1990, then an MSc in Biotechnology at University of West of England in 1991. I worked for 2 years at Cardiff University before starting a PhD in Professor Jim Parry's group at Swansea University. After completing this and carrying out a postdoc in the same lab, I moved to the new Medical School at Swansea University. Was promoted to Professor in 2010, joined the UK government's Committee on Mutagenicity in 2009.

PROFESSOR MICHAEL P. LISANTI, MD-PHD - Professor Lisanti serves as the Director of the Manchester Breast Cancer Now Research Unit and holds the Muriel Edith Rickman Chair of Breast Oncology within the Institute of Cancer Sciences. He is also Professor of Cancer Biology and the Director of the Manchester Centre for Cellular Metabolism (MCCM).An active research scientist for more than three decades with a broad background in cell biology and genetics, Professor Lisanti graduated with a degree in Chemistry (Magna Cum Laude) from New York University and obtained his MD-PhD at Cornell University Medical School in Cell Biology and Genetics. From 1992-96, he was a Skeggs Fellow at the Whitehead Institute for Biomedical Research at the Massachusetts Institute of Technology (MIT), followed by several distinguished appointments at the Albert Einstein College of Medicine and the Kimmel Cancer Center. Professor Lisanti joined the Breast Cancer Now Unit in 2012 as Professor of Cancer Biology. He has an H-index >130, with over 60,000 citations. Moreover, he has published >500 papers and is the former Editor-in-Chief of The American Journal of Pathology.

TIM MACLACHLAN - Tim MacLachlan, PhD, DABT, joined Novartis Institutes of Biomedical Research in 2010 and is currently the Global Head of Biologics Safety Assessment and Executive Director within the Department of Preclinical Safety. He is responsible for the oversight of the biologics portfolio including therapeutic proteins, monoclonal antibodies and gene and cell therapies. Prior to this Tim held roles of increasing responsibility in pharmaceutical preclinical safety assessment at Genzyme and Curagen. Tim is currently on the leadership committee and past-chair of "BIOsafe". Tim received his PhD from Thomas Jefferson University and performed his postdoctoral research at the University of Pennsylvania.

JEN MORTON - I am currently a joint leader of the pancreatic cancer preclinical team at the Beatson Institute. My research focuses on (a) using mouse models of pancreatic cancer to determine the importance of mutations found in human tumours, (b) profiling subsets of pancreatic cancer to better understand the disease and identify targets for therapy, and (c) running preclinical trials of targeted therapies in clinically relevant models. I also run the Glasgow Cancer Centre preclinical trials unit and am part of the wider Glasgow Pancreatic Cancer Research Group, which brings together scientists, oncologists, pathologists and surgeons in an effort to move pancreatic cancer research from bench to bedside.

DR THERESA NEELY - My career in toxicology started out as a study supervisor – progressing to Study Director and team leader, in reproductive toxicology at Huntingdon Life Sciences, Eye Research Centre in Suffolk.
I then joined Unilever within the Safety and Environmental Assurance Centre (SEAC) based at Colworth Science Park in Bedfordshire. My initial role was as a toxicological risk assessor for the Homecare and Personal care categories within Unilever dealing with products such as Persil and Dove.
I then moved over to the Food and Beverage side of the Unilever business to become the lead toxicological risk assessor for products such as Lipton tea, Magnum ice cream and Flora Proactive margarine.
Throughout my career in Unilever there has always been a business interest in naturals – both at the functional and emotive level. This has been the driver for SEAC to develop a safety risk assessment framework for naturals.

PROFESSOR STEPHEN G OLIVER - Steve Oliver is Professor of Systems Biology & Biochemistry and Director of the Cambridge Systems Biology Centre. His research employs a range of comprehensive, high-throughput analytical techniques and exploits genome-scale logical and stoichiometric models to investigate protein synthesis/secretion, metabolism, and networks involved in neurodegenerative diseases. Together with Ross King (Manchester), he has developed a high-throughput, intelligent drug-screening system, based on genetically engineered yeast and a Robot Scientist. This system is able to identify compounds that discriminate between equivalent pathogen and human proteins, and screen against general cytotoxicity. Steve is the 2016 recipient of the Microbiology Society's Marjory Stephenson Prize.

PATRICK O'MAHONY - Dr Pat O'Mahony has worked with the Food Safety Authority of Ireland since 2000 as their Chief Specialist in Food Technology. While his primary role with the Authority relates to the implementation of the novel food Regulation, he is also responsible for GM food, irradiated food, food allergens, food labelling and advertising as well as the technical aspects of food fraud investigations. The FSAI has delivered 35 novel food initial assessments to date and issued 60 substantial equivalence opinions. A plant molecular biologist by training, Dr O'Mahony has more than 15 years in the Irish and EU food regulatory arena.

PROFESSOR HARDEV PANDHA - Prof Hardev Pandha is a clinician scientist who trained in internal medicine and subsequently in medical oncology at Hammersmith Hospital and at the Royal Marsden Hospital, London. He completed his PhD in the CRUK labs at the Hammersmith. He was appointed Professor of Urological Oncology at the University of Surrey in 2007. He is the Director of the Surrey Cancer Research Institute. His research focus centres on the development and clinical evaluation of cancer immunotherapy, oncolytic viral therapy and targeted agents for the treatment of solid tumours.

CHRIS POWELL - Chris is Vice President of Safety Assessment at GSK, leading a group responsible for the translational safety of potential new medicines. He trained in biochemistry, pharmacology and histopathology at the University of Wales and Imperial College London and is a Fellow of the Royal College of Pathologists. In 1996, he was Reader in Pharmaceutical and Chemical Safety at St Bartholomew's Hospital Medical College in London: investigating mechanisms of hepatotoxicity and directing postgraduate training in toxicology. Prior to GSK, he worked for small Biotech start up company: Vanguard Medica. His interests are: mechanisms of toxicity, chemical carcinogenicity and the translational relevance of experimental safety studies. Chris has served on safety advisory committees for the WHO and for UK/EU Regulatory Authorities and is a member of the BTS Executive Committee.

DR PAUL RUSSELL - Paul Russell Ph.D. Chem MRSC, is a Science Leader at Unilever's Safety and Environmental Assurance Centre where he has a specific focus on the development of chemistry based approaches to aid the risk assessment of novel materials. Paul gained a BSc in Chemical and Pharmaceutical Science from the University of Sunderland and a PhD from King's College London in developing analytical fingerprinting techniques in support of natural product risk assessment. Paul has nearly 20 years industrial experience in analytical chemistry and is a technical specialist in LC-MS. He regularly presents at international conferences and is a committee member of the Toxicology SIG of the Royal Society of Chemistry.

DR ANDREW SCOTT - I joined Unilever in January 2001 after completing a BSc at Leeds University; a PhD and post-doc at the University of Wales Swansea; and a European Science Foundation fellowship at the Institut Gustave Roussy in Paris. I was awarded an honorary professorship by the College of Medicine, Swansea University in April 2013. I have experience in risk assessment and consumer safety, particularly in relation to Mutagenicity and Carcinogenicity, and a strong interest in the development and application of animal alternative approaches to safety assessment. My current role has a focus on the development of Experimental capabilities in Unilever, working with others to progress the science of toxicity pathways & mechanistic safety science, and helping to set in place the tools and novel thinking needed to bring increased mechanistic understanding to decisions on human and environmental safety.

DR NIGEL SWAIN - Nigel joined Pfizer in 2003 as a medicinal chemist where he has worked in Anti-Infectives, Allergy & Respiratory and for the past decade in the Pain therapeutic area. Following the Sandwich site closure in 2011 Nigel transferred to the Pfizer research site in Cambridge, UK continuing to support projects in the Pain research area with a particular focus on ion channel targets, most notably Nav1.7.

PROFESSOR SIMON THOMAS - Simon Thomas is Professor of Clinical Pharmacology and Therapeutics at Newcastle University and Director of the National Poisons Information Service (Newcastle) Unit and the UK Teratology Information Service. He is a co-opted member of the Advisory Council on Misuse of Drugs Technical Committee and is a past President of the European Association of Poisons Centres and Clinical Toxicologists and member of the Commission for Human Medicines. Prof Thomas trained at St Thomas's Hospital in London, the Freeman Hospital in Newcastle and at Newcastle University. His research interests include adverse drug reactions and clinical toxicology.

DR JOHN P THOMPSON - Dr John Thompson is Senior Lecturer in Clinical Pharmacology at Cardiff University where he is the lead for prescribing education for the undergraduate medical course. He is also Director of the National Poisons Information Service (Cardiff), one of four units responsible for delivering advice on the management of poisoned patients within the United Kingdom and which also provides services internationally. He is the national lead for the United Kingdom Poisons Information Database (UKPID), a comprehensive database capturing clinical data from telephone enquiries, used increasingly in monitoring the changing pattern of poisoning in the UK and for identifying clinical features of emerging poisons including novel psychoactive agents.

PROFESSOR DR. MARTIN VAN DEN BERG - Prof. Dr. Martin van den Berg is Professor in Toxicology, deputy director of the Institute of Risk Assessment Sciences(formerly RITOX) of the University of Utrecht, The Netherlands and head of the Toxicology and Pharmacology Division of IRAS. He is also appointed as an honorary professor in environmental toxicology at the University of Queensland (Brisbane) and a visiting/adjunct professor at the Royal Chulabohorn Research Institute in Bangkok. In autumn 2006 he received an honorary doctorate from the University of Umea, Sweden for his research on mixture toxicity of dioxinlike compounds. His current areas of research include: toxicokinetics, metabolism and reproductive and interactive effects of halogenated polyaromatics, interactions of xenobiotics and phytochemicals on steroid hormone syhthesis, metabolism and their relation with hormone dependent tumors, development of in vitro assays to detect endocrine disruptors. The results of his scientific work and that of his research group have been published in approx. 350 scientific articles, shortpapers and conference proceedings. He is a European registered toxicologist and connected to several national and international organizations which are involved with the toxicological risk assessment of environmental and food contaminants, and pesticides. Presently he is Associate Editor of Toxicological Sciences, Environmental Health Perspectives and Toxicology Reports.

HANS V. WESTERHOFF - EXPERTISE: systems biology, biochemistry, microbiology, mathematical biology, biotechnology
CAREER: PhD, University of Amsterdam, Postdoc University of Padova, Huygens Fellow (NWO), postdoc and visiting scientist at the National Institutes of Health (NIH), Bethesda. MD, USA, Pionier (NWO) and Staff Scientist at the Netherlands Cancer Institute, Professor of Mathematical Biochemistry, University of Amsterdam, Fellow of the Stellenbosch Institute for Advanced Study, South Africa (2001-2002), Director of the VUA Institute of Molecular Cell Biology, Director of the Manchester Centre for Integrative Systems Biology, Director of FEBS Advanced Lecture Course Systems Biology (2005-2011)
PRESENT ACADEMIC POSITIONS:
Professor of Microbial Physiology, VU University Amsterdam, Professor of Systems Biology, the University of Manchester, UK, Professor of Synthetic Systems Biology, the University of Amsterdam

DR DAVID WOOD - David is consultant physician and clinical toxicologist at Guy's and St Thomas' NHS Foundation Trust and King's Health Partners, London. He has a clinical, research and academic interest in the epidemiology of use of and acute harms related to classical recreational drugs and novel psychoactive substances (NPS). He has established a network of specialist European centres to monitor the acute harms associated with recreational drug/NPS use (the European Drug Emergencies Network (Euro-DEN)). He is a co-opted member of the UK Advisory Council on the Misuse of Drugs and expert advisor to the European Monitoring Centre for Drugs and Drug Addiction.

List of Posters

Below you will find a list of posters with the name of the lead author. For a list of all authors and to see the abstract, please see the pages later in this booklet.

Posters will be displayed in the Leader Suite for the duration of Congress

P001 - A Novel Methodology to Test Dry Dislodgeable Foliar Residue of Agrochemical Spray for *In Vitro* Dermal Absorption Using Human Skin

Manoj Aggarwal

P002 - Application of the comet assay to detect metal-induced DNA strand breaks in cultures of the marine sponge *Hymeniacidon Perleve*

Rachael U Akpiri

P003 - Deconvoluting target and chemistry related toxicology in the rat through the utilization of 'inactive' and 'active' paired enantiomers

Mark Anderton

P004 - Evaluation of a Chemical Genetic Approach to Determine GPCR Function

Simon Brooke

P005 - The utility of QSARs in predicting acute fish toxicity of pesticide metabolites: a retrospective validation approach

NatalieBurden

P006 - Adverse effects of anti-tuberculosis drugs on HepG2 cell bioenergetics

WayneCarter

P007 - Age is a factor of the extent of anti-cancer Sunitinib therapy induced cardiotoxicity: Studying haemodynamic parameters and cardiotoxicity specific microRNA expression involved

Samantha Cooper

P008 - Re-shaping acute toxicity testing of agrochemical formulations by combining the GHS ATE formula and in vitro approaches

Marco Corvaro

P009 - Toxicogenomics in Agrochemicals: Developing a Predictive Systems Toxicology Platform

Marco Corvaro

P010 - Disruption of the immunodominant T cell epitope of Green Fluorescent Protein (GFP): impact on immunogenicity

Rebecca Dearman

P011 - Subvisible protein aggregates result in T helper (Th) 1 skewing of immune responses

Rebecca Dearman

P012 - The combination of measuring oxygen consumption and extracellular acidification with the Glu/Gal assay improves the detection of mitochondrial toxicants.

Julie Eakins

P013 - Experience With In Vitro EPISKIN<trade> Corrosivity Testing in a CRO

David Esdaile

P014 - Updated guidance on risk assessment of chemical carcinogens by the UK Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

Britta Gadeberg

P015 - SMAC mimetics induce liver-specific NF-<Kappa>B activation in vivo and protect against hepatotoxicity

Giles Hayward

P016 - The international regulatory need for tests and information to develop an Integrated Approach to Testing and Assessment (IATA) of non-genotoxic carcinogens.

Miriam Jacobs

P017 - Can the *In vitro* Glu/Gal mitochondrial assay be used to predict rat *In vivo* toxicity outcome?Undefined

Stefan Kavanagh

P018 - PH dependent model of human hepatic metabolism

Ross Kelly

P019 - Assessment of the effect of mitochondrial DNA polymorphisms and mutations on drug sensitivity

Jasper Komen

P020 - Diesel exhaust particulate associated chemicals elicit inhibitory effects on human airway epithelial derived antiviral and Th1 type T-cell signals.

Martin Leonard

P021 - Could smokers' socio-demographic and housing factors affect and influence the choice between smoking cessation therapies?

Silvia Leone

P022 - Comparative Systems Toxicology Assessment of the Tobacco Heating System 2.2 and reference cigarettes (3R4F), on human organotypic respiratory tissue cultures.

Patrice Leroy

P023 - Designing novel mRNA-based therapeutic approaches for non-toxic delivery of genetic material

Luke Mulley

P024 - Are Inhaled Iron Oxides Human Lung Carcinogens?

Camilla Pease

P025 - High content analysis for prediction of human drug-induced liver injury across several pharmaceutical companies within the IMI MIP-DILI consortium

Mikael Persson

P026 - Measurement of Cyp2b1 protein induction in laser dissected FFPE liver samples by Nano-LC Mass Spectrometry

Simon Plummer

P027 - Global cross-company data-sharing on the housing of non-rodents during the recording of cardiovascular telemetry data on toxicology studies

Helen Prior

P028 - The potential of hLiMTs and HepaRG spheroids to improve the *in vitro* prediction of drug-induced liver injury (DILI) using a repeat dose high content screening (HCS) approach.

Stephanie Ravenscroft

P029 - Target organ profiles in toxicity studies supporting human dosing: does severity progress with longer duration of exposure?

Ruth Roberts

P030 - Drinking water fluoridation and PHE monitoring report

Stephen Robjohns

P031 - Molecular initiating events in toxicity pathways using organotypic human *In vitro* models

Paul Russell

P032 - Molecularly Imprinted Polymer Sensor as an alternative to binding assays

Julie Settipani

P033 - Metabolic reprogramming of HepG2 cells alters mitochondrial function and expression of Bcl-2 family proteins that regulate both autophagy and cell death

Kayleigh Frame

Delegate list

Jeppe	Agner	Lundbeck
Rachael Ununuma	Akpiri	University of Birmingham
Timothy	Allen	University of Cambridge
Toby	Athersuch	Imperial College London
Ian	Bailey	University of Surrey
Paul	Baldrick	Covance Laboratories Ltd
Amy	Ball	University of Liverpool
Amy	Ball	University of Liverpool
Susan	Barlow	None
Stine	Bartelt	Novo Nordisk A/S
Maria	Beaumont	GlaxoSmithKline
Michelle	Beharry	Medicines and Healthcare products Regulatory Agency
Michaela	Benton	Health and Safety Executive
Simon	Brooke	MRC Toxicology Unit
Paul	Brooker	Envigo
Werner	Brueller	Austrian Agency for Health and Food Safety AGES
Lily	Buckley	Food Standards Agency
Tilmann	Buerckstuemmer	Horizon Discovery Ltd
Natalie	Burden	NC3Rs
Lisa	Burdett	Charles River Laboratories Edinburgh Ltd
Kelvin	Cain	MRC Toxicology Unit
Laura	Caneva	European Medicines Agency
Desmond	Cave	BioReliance
Tanya	Chambers	MHRA
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Joanna	Clarke	University of Liverpool
Alex	Cooper	Public Health England
Ian	Copple	University of Liverpool
Carol	Courage	Unilever
Ana	Cravo	Fontem Ventures
Anna	Cronin	AstraZeneca
Richard	Currie	Syngenta Ltd.
Andy	Danks	Charles River Laboratories, Edinburgh
Theo	Dare	GlaxoSmithKline
Coleman	David	Envigo
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Clare Margaret	Horne	GW Pharmaceuticals
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Sean	O'Halloran	Covance Laboratories Ltd
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Patricia	Parris	AstraZeneca
Joel	Parry	GlaxoSmithKline
Nektaria	Pasiotis-Tsantila	Exponent International Ltd
Gary	Peart	Inutox Limited
Camilla	Pease	MKTox
Jacqui	Piner	GlaxoSmithKline
Simon	Plummer	MicroMatrices Associates
Shirley	Price	University of Surrey
Philip	Probert	Newcastle University
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Jean Pierre	Valentin	UCB BIOPHARMA SPRL
Corinne	van Dorp	Grünenthal GmbH
Paul	Walker	Cyprotex
Nayna	Walker	Syngenta Ltd
Heather	Wallace	University of Aberdeen
Ian	Waterson	Medicines and Healthcare products Regulatory Agency
Margaret	Whittaker	ToxServices LLC
Faith	Williams	Newcastle University
Steve	Williams	SWCL
Adam	Woolley	ForthTox Ltd
David	Woolley	ForthTox Ltd
Nazia	Yamin	SGS Ashby Ltd
Eric	Yau	Infineum UK Ltd
Mouna	Zachary	ToxServices LLC

Speaker Abstracts

S001 Toxicology predictions: present issues, future challenges

Author: A. Boobis
Affiliation: Imperial College London Centre for Pharmacology & Therapeutics, Department of Medicine

Abstract Body: Toxicology is undergoing profound changes, as we seek to increase the accuracy and predictive power of toxicity/safety assessment, to reduce attrition during product development, to develop informative biomarkers and to incorporate the rapid advances in biological knowledge, technology and computational sciences into public health protection. Studies on adverse outcome pathways (AOPs) (aka modes of action) have played a key role in determining the relevance of effects observed in experimental animals to humans, in interpreting dose-response relationships and interindividual variability and in the development of informative biomarkers. However, this is largely reactive – explaining the biological significance of adverse effects observed in experimental animals, i.e. a 'top-down' approach. In addition, throughput is low. As a new generation of non-animal methods is developed, where perturbations of biological pathways leading to adverse responses are identified and effects in vivo are predicted using physiologically-based models, AOPs have a key role to play in the design of such methods. The nature and magnitude of the effects studied should be relevant to potential adverse outcomes, and it should be possible to postulate a plausible adverse outcome pathway, i.e. a 'bottom-up' approach. However, for these approaches to fulfil their full potential, there is need for an agreed strategy for establishing their fitness-for-purpose. Progression to their use in quantitative risk assessment will require a more systems-based approach, whereby quantitative information on biological processes, PK and metabolism is integrated to provide a more accurate prediction of the adverse effects of a compound.

Notes:

S002 Mode of Action/Human Relevance Framework for Carcinogens

Author: R. Currie
Affiliation: Syngenta Jealotts Hill International Research Centre

Abstract Body: An increase in treatment-related tumours is a common finding of chronic cancer bioassays conducted at high doses of chemicals. Decades of investigative toxicology has demonstrated that some tumour types are produced by Modes of Action (MoA) that are not relevant for the assessment of carcinogenic risk to humans. A MoA is a biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data. A key event is a measurable change in biological state that is essential, but not necessarily sufficient, for the progression from a defined biological perturbation toward a specific adverse outcome. Therefore the MoA/HRF framework describes key cytological and biochemical events—that is, those that are both measurable and necessary to the adverse observed effect—in a logical framework that permits the evaluation of causality, excludes other potential MoAs and explicitly describes uncertainties and inconsistencies in the available data and only then determines whether those key events are plausible in humans. The MoA/HR Framework provides a logical structured test for whether a MoA is relevant to humans. The first step is to determine whether the MoA has been adequately established in rodents and other MoAs are excluded. Then an assessment of human relevance is performed by asking, for each key event, whether there is either a qualitative or quantitative difference between rodents and humans. If the answer to either of these questions is yes then the MoA is not plausible for human health risk assessment. The data required to answer the species difference question can come from a fundamental difference in the biology of rodents and humans, or it can be developed from experimental demonstrations that some key events do not occur in human systems. I will illustrate the practical use of the MoA/HRF with some examples of liver, thyroid and uterine tumours.

Notes:

S003 Waiving carcinogenicity studies: Carcinogenicity assessment documents (CAD)

Author: T. MacLachlan
Affiliation: Novartis Institutes of Biomedical Research

Abstract Body: Assessment of the potential for carcinogenicity has been at the core of the safety evaluation of pharmaceuticals since the beginning of the industry. Indeed, the induction of cancer by dyes in the late 19th century began to formalize the field of toxicology. Today, long-term studies in rodents are a standard part of the nonclinical package for small molecule drugs programs reaching the end of clinical development to assess the potential for generation of tumours. Such studies are run over the projected lifetime of a rat or in shorter-term mouse models that are predisposed to cancer. Designs of such studies are communicated to health authorities via a Carcinogenicity Assessment Document, or CAD. For large molecule "biopharmaceuticals", where the majority of agents are pharmacologically active only in non-human primate species, the approach has generally been to submit a request for a waiver from conducting such studies, via the CAD, based on a weight-of-evidence suggesting the potential or lack thereof for carcinogenesis. In rare cases, biopharmaceuticals that are cross reactive to rodents have been used in rodent studies aimed at answering specific questions relating to tumour generation or growth. Recently, regulators and industry have considered the possibility of employing a waiver approach for small molecules as well and are accumulating data that will determine if the principles in the ICH S1 guidance document should be modified to incorporate this possibility. The status of this effort and examples of CADs will be presented.

Notes:

S004 Metabolic phenotyping and elucidating the role of the microbiome in drug metabolism and toxicology

Author: M. Coen
Affiliation: Computational and Systems Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London

Abstract Body: Metabolic phenotyping involves the characterisation of the small molecule complement of complex biological samples using high-resolution analytical platforms such as NMR spectroscopy and mass spectrometry [1,2]. Data modelling and mining and the subsequent identification of panels of candidate biomarkers are typically approached with multivariate statistical tools. Metabolic phenotyping has been shown to provide a unique, systems-wide window into the biochemical status of an organism, through the generation of information-rich metabolic profiles that reflect both genetic and environmental influences and simultaneously capture both endogenous and xenobiotic metabolites. A major influence on the metabolic phenotype of the host is provided by the gut microflora which represent a major, but often forgotten, organ of metabolism, with potential to influence both the pharmacology and toxicology of ingested compounds [3,4]. An overview of important examples of such effects will be provided illustrating the development of the field and our understanding of the host-microbiome interactions in relation to drug metabolism and toxicity. In addition, recent applications of metabolic phenotyping in exploring inter-individual variability in drug metabolism and toxicology and the mediatory effect of the microbiome, together with novel statistical tools [5] that have been developed to maximize biomarker extraction will be discussed. The model hepatoxotoxin, galactosamine (galN), is associated with marked inter-animal variability in response, with clear responder phenotypes presenting with differential degrees of liver necrosis whereas non-responders exhibit no toxic response. Application of metabolic phenotyping to characterize biofluids (urine, faecal water, blood plasma) and liver from a range of pre-clinical studies of galN hepatotoxicity, showed differential urinary and faecal gut microbial co-metabolites and plasma bile acids in responders and non-responders suggesting a role for the gut microflora in determination of differential response [6,7]. In addition, differential hepatic metabolism of galN in responders and non-responders provided novel xenobiotic data on the fate of galN together with mechanistic insight.
The application of a pharmacometabonomic [8] approach will also be discussed in the context of both pre-clinical and clinical studies of the widely-used anti-tubercular drug, isoniazid and the nephrotoxic antibiotic, gentamicin. With respect to isoniazid, this approach enabled the discrimination of post-treatment outcome from pre-treatment urinary metabolic phenotypes and revealed an association between altered acetylation capacity, a gut bacterial co-metabolite and toxic outcome [9].

As will be shown, the gut microbiota clearly hold potential to have a profound influence on the outcome of drug testing and toxicology, and may represent an important confounding factor that needs to be taken into consideration in such work.

Notes:

S005 Translation of Animal Carcinogenicity Data to Humans.

Author: C. Powell
Affiliation: GSK

Abstract Body: 50-60% of chemicals & medicines tested for carcinogenicity in 2 yr studies in rats & mice, increase the incidence of tumours (Swirsky Gold 1977). Yet, only 118 of the many thousands of chemicals & medicines to which humans are exposed, have been recognised to cause cancer in humans (IARC). Why are these data so discrepant? First, many chemicals tested for carcinogenetic by the US Govt (NTP) were tested because there was cause for concern: due to positive results in genotoxicity tests, or a chemical structure relationship to other suspect carcinogens – therefore, they are not representative of all chemicals. Second, many chemicals which only cause tumours in rodents at a so called "maximally tolerated dose level", or at an exposure level >50 times greater those which humans experience are unlikely to be human relevant – adaptive & physiological control mechanisms will have been overwhelmed. Third, rodents and humans respond to chemical exposure in qualitatively and quantitatively different ways; i.e. distribution, metabolism, excretion, responses to DNA damage, impact on physiological control mechanisms e.g. hormonal systems etc. Fourth, rodents and humans have different spontaneous tumour profiles (more epithelial tumours in rodents, more mesenchymal tumours in humans) possibly related to species differences in tumour viruses, immune responses, genetic diversity and exposure to other environmental factors, e.g. sunlight. Fifth, human exposure has been restricted when chemicals are considered likely to cause cancer,

Are *all* chemicals which cause cancer in animals, human relevant? NO, e.g. phenobarbital and subcutaneously implanted biomaterials - IARC

Are positive results in animal carcinogenicity studies *all* irrelevant for humans? NO, all proven human carcinogens have tested positive in at least one animal carcinogenicity study.

1. Can we determine which of the chemicals that cause cancer in animal carcinogenicity studies are human relevant? NOT WITH CERTAINTY, e.g. pioglitazone induced bladder tumours in rodents that were, initially, thought *not* to be human relevant (but see Pio epidemiology). Chemicals that cause cancer in animals at exposure levels equivalent to those which humans experience, are ***the*** greatest cause for concern.
2. Are genotoxic chemicals more *human relevant* and *more potent* than non-genotoxic carcinogens? **NOT NECESSARILY,** e.g. B-raf kinase inhibitors appear to be non-genotoxic, yet they cause skin tumours in 10-25% humans within 6-8 weeks of the start of exposure.
3. Are data from carcinogenicity studies with transgenic mice more human relevant than data from other studies? **NOT YET KNOWN** – although there are fewer positives.

More understanding of the fundamental biology, triggering the rare events which lead to cancer in humans and rodents, is necessary to determine which animal carcinogens are human relevant.

Notes:

S006 Why are we not using more replacement in vitro models for human risk assessment to decrease uncertainties?

Author: M. V. Berg
Affiliation: Institute for Risk Assessment Sciences, Utrecht University, the Netherlands

Abstract Body: After decades of toxicological research the use of results from in vitro studies in human risk assessment remains disappointing. This is in spite of the large societal pressure to reduce the number of animals for toxicological research and risk assessment. Unfortunately, most in vitro studies are still aimed to only elucidate a mechanism of action, not in the least because regulatory authority show little interest to incorporate these in vitro data in the actual risk assessment process. Without doubt, this lack of use of in vitro results in the risk assessment process represents a significant waste of financial resources as well as the unnecessary use of experimental animals. During the last decades there has been a large improvement with respect to the use of in vitro human cell systems. Among others, this includes an increasing knowledge how to culture (human) primary cell systems, besides the more traditional use of immortal human tumour cells. With our increasing knowledge about paracrine and endocrine processes the use of co-culture systems has become increasingly more common. More and more, these multi-culture cell systems provide realistic opportunities to study effects of compounds for which intercellular communication is an important part of the mechanism or mode of action. The use of uncertainty or safety factors is clearly a necessity when results from animal experiments are used for human risk assessment. Species differences in toxicokinetics, noticeable bioavailability from the gastro-intestinal tract and metabolism are important reasons to apply these uncertainty or safety factors. When reviewing the use of in vitro data for human risk assessment, it may be argued that in vitro concentrations may very well resemble human systemic exposure levels e.g. in blood. Consequently, the use of in vitro concentration-effect relationships could provide us with estimations of human systemic concentration – effect relationships. For this comparison lower uncertainty factors can be used, because species differences in toxicokinetics are less relevant.
Nevertheless, data obtained from in vitro experiments may still have several aspects that cause deviation from real life systemic levels and associated effects in humans. One of these aspects is the amount of bioavailable molecules in in vitro systems as well as in e.g. human blood, which is significantly influenced by differential binding to e.g. blood and medium proteins. However, recent studies have shown that calculation of the free and protein bound fractions can adequately be calculated which depends among others on the physico-chemical properties of the compound.
In this presentation a range of examples from our institute will be given to show the usefulness of advanced human co-culture systems for which paracrine or endocrine interactions play an important role in the mechanism or mode of action of compound. In addition, examples will be given to illustrate the more efficient use of results from in vitro assays for human risk assessment.

Notes:

S007 Networking in toxicity: Mapping and Modelling detoxification capacity

Author: H. Westerhoff
Affiliation: Swammerdam Institute of Life Sciences, University of Amsterdam

Abstract Body: Molecules act in networks before they affect biological function. Systems Biology examines how and to what extent the networking determines that function. The effect of a medicinal drug is determined by its pharmacodynamics, by its direct effect on its molecular target and by the network effect of that target. We will discuss three issues. First we will review the development and implementation of a strategy of differential network-based drug targeting. This strategy puts molecule and network, action and toxicity in the single frame it may deserve. Then we shall discuss how we implement systems biology to pre-evaluate the performance of proposed biomarkers for glutathione-mediated detoxification. This leads us to propose multi-dimensional biomarking supported by systems pharmacology. Finally we shall suggest how revolutionary ICT may put the Humpty-Dumpty together again that we have all been taking apart so successfully, in a strategic activity towards the human *in silico*.

Notes:

S008 Systems and synthetic biology in toxicology

Author: S. Oliver
Affiliation: Cambridge Systems Biology Centre & Dept. Biochemistry University of Cambridge

Abstract Body: The use of the model eukaryote, the brewing and baking yeast, *Saccharomyces cerevisiae,* to determine the route of ingress and egress of drugs and other molecules into and out of the cell will be discussed. The engineering of yeast to mimic either parasites or their human hosts will be explained. It will be demonstrated how such synthetic biology constructs may be used to effect cheap and efficient drug screens that identify compounds that lack general cytotoxicity and also effectively discriminate between a target protein in a pathogen and its human equivalent. The use of a Robot Scientist to further increase the efficiency of such screens by using machine-learning approaches will also be explained. Finally, the prospects for using model-driven approaches to predict useful, and exclude toxic, drug combinations, as well as to anticipate likely resistance mechanisms, will be discussed.

Notes:

S009 Application of transgenic models in metabolism and toxicity

Author: C. Henderson
Affiliation: Division of Cancer, School of Medicine, University of Dundee

Abstract Body: Over the past two decades transgenic models have contributed significantly to our understanding of the role of drug metabolising enzymes (DME) and transporters in the disposition of xenobiotics. The ability to express foreign genes, or delete—conditionally or globally —or mutate specific endogenous genes in a living multicellular organism, has vastly added to our knowledge of drug-metabolizing enzymes and their transcription factors — how they are expressed and regulated, how they function, and how they interact. Much of this work has been carried out in the mouse, and increasingly in other species, including the rat; one issue that has become increasingly apparent with such rodent models is that of species specificity. Whilst animal models remain central to the study of drug metabolism, disposition and development, the often marked species differences in metabolic pathways – leading to profound differences in toxicity, efficacy and pharmacokinetics – can represent a major limitation in their use. It is well recognised not only that xenobiotic metabolism is around 10-fold faster in mice and rats than in humans, but also that the DMEs (and transporters) found in rodents are significantly different from those in man in terms of both expression and functionality. These factors seriously handicap the use of rodents in their ability to model human drug metabolism and disposition, and in recent years there have been a number of efforts to overcome such shortcomings by generating humanised mouse models where murine genes have been deleted and replaced by their appropriate human counterparts. In collaboration with Taconic, we have generated a mouse model where 33 functional mouse genes (the murine *Cyp2c*, *Cyp2d*, and *Cyp3a* gene clusters and the nuclear receptors Car and Pxr) have been replaced by six human genes CYP2C9, CYP2D6, CYP3A4/3A7, CAR, PXR), representing one of the most complex genetically humanised mouse models to date and involving the human cytochrome P450s that contribute ~80% to the Phase I metabolism of marketed drugs. In addition, the application of increasingly sophisticated reporter systems also allows us to determine where DME expression occurs, and in response to which type of chemicals, *in vivo* and in real time, for example with bioluminescent imaging. As part of an EU FP7 Innovative Medicines Initiative programme (MARCAR) we have investigated the role of oxidative stress in the mechanism of non-genotoxic hepatocarcinogenesis *in vivo*, utilizing viral 2A peptide sequences to allow the generation of multiple reporters from the same locus in novel haem oxygenase-1 reporter mouse models.

Notes:

S010 Improving toxicity predictions to design safer acidic molecules

Author: N. Swain
Affiliation: Pfizer

Abstract Body: Predicting toxicity of small molecules remains an area of significant interest and our analysis for acidic drug space highlights the challenge for this ion class. The talk describes how we identified gaps in our assessment of promiscuity for acidic compounds leading to changes in an off-target screening panel. An attrition based medicinal chemistry case study also outlines the use of zebrafish studies in a retrospective fashion to assess pre-clinical toxicity predictive power and refine hypothesis around improving safety in an acidic lead series. An improved physical chemical assessment for predicting safety of acidic molecules to aid future design in this space will also be presented.

Notes:

S011 Applying data from exploratory omics experiments for early safety screening

Author: H. Goehlmann
Affiliation: Janssen Pharmaceutical Companies of J&J, Belgium

Abstract body: During drug discovery and development, the early identification of adverse effects is expected to reduce costly late stage failures of candidate drugs. As risk/safety assessment takes place rather late during the development process and due to the limited predictivity of animal models to the human situation, modern unbiased high-dimensional biology read-outs are sought, such as molecular signatures predictive for in vivo response using high-throughput cell-based assays. The talk will highlight findings of an in-depth exploration of a single chemical core structure. Via transcriptional profiling we identified a subset of close analogs which commonly down-regulate multiple tubulin genes across cellular contexts, suggesting possible spindle poison effects. Confirmation via a qualified toxicity assay (in vitro micronucleus test) and the identification of a characteristic aggregate-formation phenotype via exploratory high content imaging validated the initial findings. SAR analysis triggered the synthesis of a new set of compounds and allowed us to extend the series showing the genotoxic effect. This proof-of-concept demonstrates the potential to flag toxicity issues by utilizing data from exploratory experiments which are typically generated for target evaluation purposes during early drug discovery and considers how this approach may be incorporated into drug development strategies.

Notes:

S012 Combining in vitro and in vivo PK/PD (TK/TD) modelling data to guide patient-centric risk assessments

Author: T. Collins
Affiliation: AstraZeneca

Abstract Body: Pharmacokinetic/pharmacodynamic (PK/PD) or toxicokinetic//toxicodynamic (TK/TD) modelling is concerned with the prediction of temporal timecourse of both drug concentration and biomarkers of interest. Systems pharmacology takes this a step further by inclusion of physiologically relevant turnover processes that govern the biomarkers of interest and provides a framework in which to characterise differences between species and models. An example is the model of suppression proposed by Friberg *et al.* (1, 2), which characterises the production rate of haematopoietic cells such as neutrophils. It is assumed that drugs impact immature cell counts in the early stages of the haematopoiesis, and these cells undergo a series of cell divisions and differentiation steps before being released into the circulation as mature cells. Rat pre-clinical blood cell count data is used to quantify the drug effect on the system, and the system properties in terms of turnover and feedback are also estimated. The estimate of drug effect in rat is corrected for plasma protein binding and potency differences between rat and human to give a predicted human drug effect, which is proportional to the concentration of drug present. This is used with the known system properties (baseline, turnover, feedback) in human to simulate the effects at therapeutic dose and different dose schedule. We can study the impact of Oncology drugs and combinations on circulating blood cell counts using this approach; however this assumes reasonably "healthy" haematopoiesis which includes a healthy blood cell count baseline and feedback. Additional factors need to be considered for patients with acute myeloid leukaemia (AML) where blasts are the predominant cell produced in the bone marrow. In this situation blood count recovery can be used as both a marker for safety and efficacy. Here we show how the model developed by Friberg *et al.* was modified for use in AML patients. The baseline cell production was reduced to reflect the reduced capacity to produce normal cell types, prophylactic transfusions were included and recovery rates were explored upon drug dosing. This resulted in an improved assessment of drug-induced toxicity in AML patients over the healthy predictions where the magnitude of effect is likely to be over predicted.

This model provided insight into a specific patient population (AML patients). This can be compared to clinical data from AML patients to confirm these assumptions. Future work is needed to incorporate the clinical prediction of efficacy into the model, which drives the recovery rate.

Notes:

S013 The Two-Year Rodent Bioassay: pros/cons/provisos

Author: J. Foster
Affiliation: Consultant Pathologist

Abstract Body: Throughout the last 50 years, the paradigm for carcinogenicity risk assessment has depended on lifetime bioassays in rodents. Since 1997, the International Conference on Harmonisation (ICH) S1B has permitted the use of a 2-year rodent bioassay (usually in the rat) and an alternative, genetically modified mouse, model to support cancer risk assessment of pharmaceuticals. Since its introduction, it has become apparent that many of the stated advantages of the 6-month Tg mouse bioassay have, in actual fact, not been realized, and the concern exists that an, albeit imperfect, 2-year mouse bioassay has been replaced by a similarly imperfect 6-month equivalent. This presentation will argue that model systems will always be imperfect representing as they are meant to, certain key aspects of the biological process under examination. However the two year rodent bioassay, using cancer as the end point, covers the majority of the biological changes that human cancer undergoes and, while they expensive and relatively long in duration, care needs to be taken not to embark on the introduction of new technologies which, while cheaper and quicker, provide an even less satisfactory conclusion in terms of human carcinogenic risk than the current assays where decades of experience, from many hundreds of assays provide confidence that the vagaries of the system are well understood and where confidence in the outcome is high. With the recent initiatives, from the OECD and the Institute of Peace and Conflict Studies, on ''mode of action,'' ''adverse outcome pathways,'' and ''human relevance framework'' the science of carcinogenic risk assessment is truly advancing and this coordinated, and integrated, approach to the science is being actively embraced by both the industry and regulatory community. The recent suggested revisions to the ICH S1 guidelines, utilizing carcinogenicity assessment documents, go some way to developing a science-based risk assessment that does not depend almost entirely on a single, imperfect, cancer-based end point in non-relevant animal species.

Notes:

S014 Adverse Outcome Pathways (AOPs) in Genotoxicity & Carcinogenicity

Author: A. Scott
Affiliation: Unilever

Abstract Body: The AOP concept is a multidisciplinary and mechanistically-based approach that has potential to transform safety risk assessment, negate the requirement to generate hazard data in animal models and reduce compound attrition. The concept links molecular initiating events and the interaction of a chemical at a biological target with the progression of an adaptive/adverse response across scales of biological organisation that lead to outcomes relevant for risk assessment. At the core of this framework is the use of *in vitro* approaches to investigate perturbations in the critical cellular processes that lead to adverse events (toxicity pathways), and a safety assessment approach that ensures human exposure is kept below the level expected to cause adverse effects. Using case study chemicals, a defined exposure scenario and a prototype pathway (p53) we have examined key elements of the AOP conceptual framework to determine the applicability of pathways based risk assessment of consumer product ingredients. Doses determined from *in vitro* assays including global gene expression and analysis of repair complex formation/resolution (measuring key components of the p53 pathway), were compared to the output from biokinetic modelling and the potential for *in vitro* to *in vivo* extrapolation explored. In addition, we have undertaken an evaluation of several emerging technologies (*e.g.* 3D-DIP-CHIP, an immunoaffinity, microarray-based analysis of genetic damage and its repair throughout the genome), to provide mechanistic information and refine our understanding of adversity versus adaptive response. We aim to demonstrate the practical applicability of using pathway-based risk assessment for consumer safety and highlight the challenges.

Notes:

S015 Biochemical mechanisms and biomarkers of toxicity to the kidney: advances over the last 40 years

Author: T. Lock
Affiliation: Liverpool John Moores University

Abstract Body: The lecture will focus on biochemical mechanisms of toxicity to the kidney which I have been involved with during my carrier in toxicology and will also highlight some of the advances in biomarkers of renal toxicity. Topics will include the renal toxicity of paraquat, the renal toxicity of a group of halogenated alkenes and alkanes, and the underlying mechanism for tissue selectivity and injury. Understanding the basis for the toxicity and carcinogenicity of gasoline to the male rat kidneys. Plus the relevance of these finding in experimental animals to humans. Discuss markers of renal injury in use at the start of my carrier and the recent step change in advances in renal biomarkers based on proteomic and genomic technology.

Notes:

S016 What are naturals – are they all safe? A food perspective.

Author: A. Constable
Affiliation: Nestle Research Centre

Abstract Body: There is a growing interest by both consumers and industry for the use of 'natural' foods and ingredients for technological effects and health reasons. There is however no consensus definition of 'natural'. There is widespread perception that artificial, or synthetic, equates to toxic, and that 'natural' or 'traditional' food ingredients are safe. Such foods and ingredients have rarely been subjected to standard toxicologically testing procedure, compared to regulated substances such as artificial food additives. Natural or traditional foods are considered safe when they are prepared and used in traditional ways (cultural practises) for the consuming population because of long-term human experience. Food crops produce not only nutrients but a vast array of non-nutrient secondary metabolites. Plant foods may contain inherent toxicants and anti-nutritional substances, but the consequences to the health of the consumer depend on how those foods are processed, prepared and the quantities consumed. The safety of these ingredients may change considerably if taken out of their cultural context, preparation and usage. This is particularly true for plants and plant derived ingredients possessing medicinal properties. Care must be taken not to automatically equate 'natural' or 'traditional' with safe. For a number of reasons it is not always straightforward to address the public health significance of naturally occurring chemicals
This presentation aims to provide an overview on the chemical diversity and toxicological profiles of substances occurring naturally in foods, using examples of staple foods, ingredients, and dietary supplements. Approaches to assess the safety of food plants and botanical ingredients will be discussed. Insights on how inherent toxins can be managed in order to ensure food safety will be provided.

Notes:

S017 Natural Product Characterisation – Separating the Wheat from the Chaff

Author: P. Russell
Affiliation: Unilever

Abstract Body: Consumer interest is growing in food and cosmetic products which contain botanicals as an ingredient with an established or perceived functional benefit. These materials are often intrinsically linked to traditional medicine cultures throughout the world. Due to their inherent chemical complexity, there are substantial challenges in safely commercialising truly efficacious plant derived materials capable of supporting strong functional claims. Chemical understanding of natural products is critical to enable effective safety risk assessment and both chromatographic and spectroscopic based analytical methods have a key role to play to inform on chemical composition. Traditional medicine based history of used is well documented for many natural products, but can only be used to support development programmes where both the indication and extract composition are the same as those historically used. This should be confirmed through raw material authentication and holistic fingerprinting approaches, the latter of which we define as a 'unique visual pattern representing the presence of known and/or unknown characteristic chemical components'. The reductive nature of pharmacopeia marker approaches does not provide sufficient insight into the composition of the complex material as a whole and the concept of a holistic analytical representation also retains respect for the synergistic philosophy of completeness that is embedded in many traditional therapies. Therefore, recently there has been increased recognition of the value that chromatographic chemical fingerprinting can bring to naturals research through its use as a quality control tool. However, the effectiveness of this approach is limited by the quality of the fingerprint obtained. The development of refined extracts are often desirable for efficacy, but increases the emphasis on the analytical chemist to understand the qualitative and quantitative chemical composition as history of safe use approaches become less applicable. Here, the use of robust analytical approaches coupled to chemometric data analysis [1] in support of the risk assessment of partially or heavily refined extracts will be discussed.

Notes:

S018 History of Safe Use – a Unilever approach to assessing naturals

Author: T. Neely
Affiliation: Unilever

Abstract Body: History of safe use is a well recognised assessment approach (Constable et al 2007). SEAC have adopted and developed this approach for the assessment of naturals in Unilever products (Neely et al 2011).A model of expert toxicological judgement that can be used to evaluate a natural has been developed by SEAC. The model compares the natural of interest (the substance which the business wants to incorporate into a new product) with a comparator (what people have historically already been exposed to). This approach can only be used if the natural of interest is to be used at similar or lower levels than the comparator. The model is composed of a number of criteria which evaluate the evidence of history of use and evidence of adverse effects. An important contributor to the evidence for a safe history of use is the chemical similarity that the natural of interest has with its comparator material. The model also assesses the evidence for concern associated with the natural. Again, a number of criteria are used to evaluated the evidence for concern of potential adverse effects of the natural.

Notes:

S019 Therapeutic Cancer Vaccines: where are we now?

Authors: F. Farzaneh
Affiliations: King's College London

Abstract Body: A broad range of tumour-associated antigens provide specific targets for immune therapy of cancer. These include antigens expressed by oncogenic viral vectors such as Human Papilloma Virus (HPV), as well as an array of mutated oncogenes, abnormally glycosylated proteins and a whole array of carcino-embryonic gene products that are expressed at elevated levels in a range of cancers. In addition to these common antigenic targets, recent data has demonstrated the presence of other, entirely patient and tumour specific mutations. Both the common and the private tumour associated antigens provide potential targets for the immune mediated eradication of cancer. Such immune therapy strategies are of particular relevance to the eradication of the residual cancer which when present after conventional forms of therapy, can cause the eventual relapse and recurrence, despite a successful initial response to therapy. The most promising of the new immune therapy based approaches include the use of broad-spectrum, largely antibody based, immune modulators (*e.g.* anti-CTLA4, anti-PD1/PDL1, *etc.).* The blockade of immune inhibitory feedback loops by these agents allow the stimulation and expansion of specific populations of tumour specific T cells. An alternative and highly promising new form of immune therapy is the generation of autologous and allogeneic T cells expressing antigen specific T cell receptors, including chimeric antigen receptors (CAR T-cells). Other strategies showing moderate clinical efficacy include the use of autologous dendritic cells that are pulsed with tumour-associated RNA, proteins and peptides, or with whole tumour lysate. Alternatively, autologous cancer cells can themselves be genetically modified to serve as whole cell vaccines. Yet other exciting new developments include the identification of new adjuvants and vaccination strategies for cancer specific induction of protective and therapeutic cellular immunity. The combination of such new vaccination strategies, combined with mass spectrometry and deep sequencing based identification of individualised cancer specific mutations, is holding out the promise of substantially more effective vaccination strategies. Given the rapid pace of these developments and the emerging clinical evidence of efficacy for both the broad spectrum immune modulators, and for the better induction of both adaptive and acquired cellular immunity against cancer associated antigens, the treatment of many cancers is now poised for dramatic improvements.

Notes:

S020 Regulatory considerations for clinical and non-clinical development of therapeutic cancer vaccines

Author: B. Heelan
Affiliation: Parexel

Abstract Body: Some of the challenges relating to proof of concept, immune monitoring, patient selection and clinical endpoints when developing therapeutic cancer vaccines will be discussed. While the expected main mechanism of action of cancer immunotherapy differs from standard chemotherapies, the need to show a clinically relevant benefit remains the ultimate goal of treatment. When (one of) the mechanism(s) of action can be shown, this aids in biomarker identification, proof of concept studies and dose finding. Some of the challenges in interpreting results from immune monitoring will be highlighted. The need for adequate proof of concept studies prior to initiating phase 3 trials will be discussed.

Notes:

S021 Common antibiotics target mitochondria and effectively eradicate cancer stem cells, across multiple tumour types

Author: M. Lisanti
Affiliation: University of Manchester

Abstract Body: Recently, we proposed a new strategy for the treatment of early cancerous lesions and advanced metastatic disease, via the selective targeting of cancer stem cells (CSCs), a.k.a., tumour-initiating cells (TICs). We searched for a global phenotypic characteristic that was highly conserved among cancer stem cells, across multiple tumour types, to provide a mutation-independent approach to cancer therapy. This would allow us to target cancer stem cells, effectively treating cancer as a single disease of "stemness", independently of the tumour tissue type. Using this approach, we identified a conserved phenotypic weak point - a strict dependence on mitochondrial biogenesis for the clonal expansion and survival of cancer stem cells. Interestingly, several classes of FDA-approved antibiotics inhibit mitochondrial biogenesis as a known "side-effect", which could be harnessed instead as a "therapeutic effect". Based on this analysis, we now show that 4-to-5 different classes of FDA-approved drugs can be used to eradicate cancer stem cells, in 12 different cancer cell lines, across 8 different tumour types (breast, DCIS, ovarian, prostate, lung, pancreatic, melanoma, and glioblastoma (brain)). These five classes of mitochondrially-targeted antibiotics include: the erythromycins, the tetracyclines, the glycylcyclines, an anti-parasitic drug, and chloramphenicol. Functional data are presented for one antibiotic in each drug class: azithromycin, doxycycline, tigecycline, pyrvinium pamoate, as well as chloramphenicol, as proof-of-concept. Importantly, many of these drugs are non-toxic for normal cells, likely reducing the side effects of anti-cancer therapy. Thus, we now propose to treat cancer like an infectious disease, by repurposing FDA-approved antibiotics for anti-cancer therapy, across multiple tumour types. These drug classes should also be considered for prevention studies, specifically focused on the prevention of tumour recurrence and distant metastasis. Finally, recent clinical trials with doxycycline and azithromycin (intended to target cancer-associated infections, but not cancer cells) have already shown positive therapeutic effects in cancer patients, although their ability to eradicate cancer stem cells was not yet appreciated.

Notes:

S022 Immuno-oncology: investigating cancer therapies powered by the immune system

Author: H. Pandha
Affiliation: University of Surrey

Abstract Body: The treatment of solid malignancies has evolved beyond traditional surgical, chemotherapy and radiotherapy approaches to more targeted approaches exploiting new insights in basic cancer biology. Although the era of small molecule inhibitors of cancer growth has resulted in astonishing progress in otherwise refractory cancers, these treatments offer long palliation rather than cure, and are associated with a plethora of new toxicities. Immunotherapy remains and intriguing and potentially curative treatment modality. The experience with recombinant cytokines and monoclonal antibodies has formed the basis of further strategies looking specifically to induce T cells to control tumour growth. Whilst prototype cellular vaccines were safe and feasible they were associated with low efficacy. We are now progressing at an astonishing pace along a number of parallel and interactive pathways addressing the tumour microenvironment as a whole. These include T cell activation through immune checkpoint inhibition, focal destruction of tumours to induce immunogenic cell kill, immunotherapy using oncolytic viruses and recent progress with engineered T cells. We are observing long term durable responses in patients with cancers thought to be refractory to immune manipulation. Finally, huge strides in dissecting the cancer genome is now allowing us to identify neo-epitopes which may break immune tolerance and thereby potentially identify patients most likely to respond to some of these treatments.

Notes:

S023 The application of novel genome engineering techniques in health and disease

Author: H. Dolatshad
Affiliation: Nuffield Division of Clinical Laboratory Sciences, University of Oxford

Abstract Body: Genome editing technologies have advanced significantly over the past few years, providing a fast and effective tool to precisely manipulate the genome at specific locations. The three commonly used genome editing technologies are Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)–associated Cas9 (CRISPR/Cas9) system. Unlike ZFNs and TALENs that use proteins to recognize specific sequences in the genomic regions of interest, the CRISPR/Cas9 system uses an RNA sequence complementary to the target genomic DNA as its recognition mechanism. The adaptability, simpler assembly, higher specificity and efficiency of the CRISPR/Cas9 system make it a valid alternative to ZFNs and TALENs. CRISPR/Cas9 is now the most commonly used genome editing method. The myelodysplastic syndromes (MDS) represent a heterogeneous group of myeloid malignancies. Recent studies have illuminated the molecular landscape of MDS. The most common mutations found in MDS occur in genes that are epigenetic modifiers (e.g. ASXL1) or regulators of RNA splicing (e.g. SF3B1). Approximately 78-89% of MDS patients harbour at least one known gene mutation. Although it is clear that the common gene mutations impact both the pathophysiology and prognosis in MDS, we do not fully understand their role in MDS disease initiation and progression. We have used the CRISPR/Cas9 system to correct the ASXL1 homozygous nonsense mutation present in the KBM5 myeloid leukaemia cell line, which lacks ASXL1 protein expression. We successfully managed to correct mutation in ASXL1 gene using three gRNAs yielding 2%, 0.46% and 1.4% single allele precise correction and biallelic precise correction in two of the three gRNAs at a yield of 1.63% and 1.13% among a total of 1027 PCR amplified sequencing analysis. CRISPR/Cas9-mediated ASXL1 homozygous correction resulted in protein re-expression with restored normal function, including down-regulation of polycomb repressive complex 2 target genes. Significantly reduced cell growth and increased myeloid differentiation, providing new insights into the role of ASXL1 in human myeloid cell differentiation. Mice xenografted with mutation-corrected KBM5 cells showed significantly longer survival than uncorrected xenografts. These results show that the sole correction of a driver mutation in leukaemia cells increases survival in vivo in mice.

Given its successful application in the KBM5 cell line, the CRISPR/Cas9 system has been used to correct recurrent gene mutations found in MDS haematopoietic stem and progenitor cells (HSPC). In order to improve the delivery efficiency in HSPCs we electroporate a complex composed of the recombinant Cas9 protein and gRNA with the DNA template containing a selection marker into MDS progenitor cells. The impact of mutation correction on cellular function is studied in Corrected cells.

This study provides proof-of-concept for driver gene mutation correction via CRISPR/Cas9 technology in human leukaemia cells and presents a strategy to illuminate the impact of oncogenic mutations on cellular function and survival. In addition, this technique provides a proof-of-concept for gene correction in primary adult HSCs derived from patients with a myeloid malignancy, paving the way for future gene therapy approaches.

Notes:

S024 Novel Food approach to safety assessment

Author: P. O'Mahony
Affiliation: Food Safety Authority of Ireland

Abstract Body: The EU novel food Regulation (EC) No 258/97, in force since May 15th 1997 has yielded almost 200 applications, with 89 authorised. In addition, more than 250 food products have been notified through the simplified procedure. Under the current Regulation, the initial safety assessment is carried out by an individual Member State selected by the applicant. Member States have 60 days in which to make comments about or raise reasoned objections to those applications and assessments. Where even one Member State raises a reasoned objection, the application goes to the European Food Safety Authority (EFSA) for additional assessment. In reality, only a handful of the 28 EU Member States carry out initial assessments or provide substantial equivalence opinions as part of the simplified procedure. Within these Member States the majority utilise a committee system whereby a specific committee of experts assess the safety of novel foods and report back to the Competent Authority which then represents that opinion at EU level. The Food Safety Authority of Ireland operates a more simplified system that generally involves one toxicologist, one nutrition expert and other experts as required. The experts assess the novel ingredients based on agreed parameters and submit their reports to FSAI. A final safety assessment is compiled by the FSAI and agreed with the experts and the applicant prior to submission to the EU Commission. Commission Recommendation 97/618/EC sets out how novel food application dossiers should be put together and the type of scientific and other information required. While the concept of "substantial equivalence" is cited frequently in this Recommendation, it does not have a significant role to play in modern day novel food applications, and is a mere remnant of the early days when GM foods were also regulated by this Regulation. The recommendation is not prescriptive, providing general headings to guide applicants on critical areas such as composition, nutritional value, toxicological considerations, intended uses and levels of undesirable substances. The type and extent of information provided in a dossier will depend on the novel food or ingredient itself and the intended targets. For example a single chemical or compound will be easier to assess in terms of absorption, distribution, metabolism and excretion (ADME), compared to a botanical or multi-ingredient complex which are made up of a large number of often unidentified chemicals, proteins and other compounds. Animal studies are desirable where there is little information on the prior consumption of an ingredient and human feeding trials add considerable weight to a products' safety profile. Undesirable substances are generally standardised in terms of heavy metals, microbiological contaminants, pesticide residues, environmental toxins like dioxins, PCBs etc.
A number of Member States carry out safety assessments on each novel food in parallel with the rapporteur Member State carrying out the formal initial assessment. Historically, about 80% of applications draw at least one reasoned objection which means EFSA then carries out an additional assessment. In essence this constitutes a triple assessment process for most applications, and a double assessment for a minority.

Notes:

S025 Animal Models of Cancer

Author: J. Morton
Affiliation: Cancer Research UK

Abstract Body: The complexity of cancer and the urgent need for improved options for patients mean that animal models of cancer are required to fully understand the disease and improve therapeutic options for patients. Although there can be differences between animal and human cancers there are also many similarities, and they are still the only way in which researchers can model the interactions between tumour cells and the complex microenvironment in which they exist. Animal models can be used to study the mechanisms of cancer development, progression and metastasis, and also how cancers may be detected earlier, particularly in those models where timing of initiation is well understood. Perhaps most importantly, they offer a system in which to test various novel anti-cancer therapies and investigate why some tumours respond to treatment while others are resistant. The value of animal models varies by species, and by similarity to humans. The most commonly used animal cancer models are mice and rats. Other animals, including hamsters, rabbits, dogs, cats, pigs, sheep and fish have been studied, however there are far more reagents available to support research in mice and rats, and their genomes have been far better annotated. There are several ways in which human cancer may be modelled in animals, for example, by exposure to chemical carcinogens or radiation, by genetic alteration throughout the animal, or by tissue-specific genetic alteration of the genes that cause tumours in those tissues in humans. In addition, there are some animal models that have a genetic predisposition to develop spontaneous tumours. Historically, xenograft models were used whereby long-established human tumour cell lines were grown subcutaneously in mice. Recent advances, however, mean that it is now possible to implant human tumour fragments directly from patients into mice, and approached like this allow the study of more personalised medicine. I will discuss some of these approaches and provide various examples based around our work on pancreatic cancer. Pancreatic cancer is an aggressive disease predicted to become the second commonest cause of cancer death in the western world by ~2025. It is characterised by its complex microenvironment, aggressive invasion and resistance to conventional chemotherapy, and as such is an excellent example of the need for the use of animal models in cancer research.

Notes:

S026 Genetic Diagnosis and Treatment of Cancer: The answer may be in the genes

Author: M. Caulfield
Affiliation: Genomics England

Abstract Body: The 100,000 Genomes project is using whole genome sequencing to bring diagnoses to patients with rare inherited disorders, identify drivers to cancer and response to therapy and drivers to antimicrobial resistance in pathogens. This will transform the capability and capacity of the NHS to apply genomic medicine for patient benefit. This programme will be carried out in England requiring the NHS and third party providers to create new leading edge infrastructure and operational plans for day to day NHS Practice. The cancer element of this programme sequences at least two genomes per person the germline genome and that of the cancer (the somatic genome). In the somatic genome there may be drivers to malignancy, to response, to relapse and to outcomes. Obtaining optimal tissue can be challenging and we have a series of pilot experiments that have led us to conclude the fresh or fresh frozen will be the optimal sample handling to obtain the highest quality genomes.To deliver this programme in partnership with NHS England we have created 13 NHS Genomic Medicine Centres across England to enable generation of clinical data and sample flows from NHS patients with broad consent for whole genome sequencing into our Genomics England Biorepository at the NIHR Biosample centre. We are creating one of the largest X Ten Next Generation Sequencing Centres in the World at Hinxton with our partner Illumina. The value of this programme will be the alignment of the highest fidelity and most comprehensive whole genome DNA sequence produced from patients to date with high fidelity clinical data stored in pseudonymised format within a multi-petabyte data infrastructure. This will allow ongoing refreshment from primary, secondary and tertiary NHS care to offer a picture of life-course health and disease progression for participants. To drive up diagnoses for patients we have created the Genomics England Clinical Interpretation Partnership where 2100 clinicians and scientists will work on pseudonymised builds to enhance value for patients. Alongside this significant enhancement of NHS capability for utilisation of next generation sequencing in clinical care we will train with 530 person years of Masters Training the next generation of clinicians and scientists to ensure that NHS capacity to harness Genomic Medicine is the most advanced in the world.

Notes:

S027 Novel Psychoactive Agents and Drugs of Abuse: Where are we now?

Author: J. Thompson
Affiliation: Cardiff University

Abstract Body: The last decade has seen a rise in the number of compounds available for recreational use. The pattern of use, and of adverse effects, is changing within the United Kingdom (UK) and overseas. In the UK many traditional recreational drugs are scheduled under the Misuse of Drugs act and possessing or supplying them can attract heavy penalties. By producing new chemical entities, often based upon traditional compounds, illicit drug manufacturers have sought to circumvent legislation and have marketed their products as 'legal highs' or 'bath salts.' These new psychoactive agents can have potent effects, and may produce clinical effects different from their 'parent' compounds. Once one new compound has been identified, and legal controls introduced, it may be replaced by another, similar but chemically distinct, compound. Keeping track of this constantly changing marketplace is difficult, as is identifying the clinical features associated with use. Tracking and identifying the chemical nature of these new psychoactive agents is challenging. Analytical systems for identifying drugs have included analysis of pooled urine samples from nightclubs and samples left in 'amnesty bins.' Schemes exist, for example the Welsh Emerging Drugs and Novel Substances Project (WEDINOS), whereby users may submit their own purchases for analysis and the results are made available publicly via a web site, together with harm reduction messages. These too can provide useful information regarding changing patterns of availability. Identifying the clinical features seen with new compound is also challenging. Often only the street name, rather than substance, will be known. The same 'brand' may contain different substances at different times. Similarly, little may be known about the human toxicity when a new product hits the streets, and users will be unaware of the expected dose – response relationship, which may result in accidental overdose. Enquiries to poisons information services are a source of data which can be used to detect trends in use, and to observe the effects of changes in legislation.

Due to the lag which has existed historically between identifying a specific compound and legislating for it, a new system for regulation has recently been introduced in the UK.

Notes:

S028 Legal highs – Clinical effects

Author: D. Wood
Affiliation: Guy's and St Thomas' NHS Foundation Trust

Abstract Body: There is increasing evidence of the use of novel psychoactive substances (NPS) (also known by users and others as "legal highs") across Europe and the world. Despite this evidence of increased use, currently there remains limited understanding and awareness of the potential unwanted clinical effects related to their use. Often there is no pre-clinical or animal testing of an NPS or data available from human clinical trials to be able to understand their unwanted clinical effects. Information on the acute harms and unwanted clinical effects related to the use of NPS can however be obtained from a variety of different sources. These sources of information include: i) user self-reports; ii) sub-population surveys; iii) information from calls / accesses of regional or national poisons information centres/services; iv) published case reports / series; and v) data from emergency department (ED) presentations. The latter of these can be collated through national or international networks of EDs [for example the European Drug Emergencies Network (Euro-DEN project run and co-ordinated by my group in London, UK, which has now been extended as the Euro-DEN Plus project] or combined with information from regional poisons information centres/services and analysis of biological samples from cases [for example the STRIDA project in Sweden]. Each of these different sources of information has their own limitations, however through combination of these different sources of information it is possible to describe the unwanted clinical effects of an individual or class of NPS and minimise the limitations of any one individual information source. In this presentation, I will use information from these information sources, in particular data from case reports/series, poisons information centres/services, the Euro-DEN/Euro-DEN Plus project and the STRIDA project to describe the unwanted clinical effects related to some of the more commonly used and encountered classes of NPS.

Notes:

S029 Severe clinical toxicity caused by unlicensed weight loss agents including dinitrophenol

Author: S. Thomas
Affiliation: Newcastle University

Abstract Body: There is substantial demand for medicines to enhance weight reduction. This is fuelled both by the potential health benefits of weight reduction and the desire to lose weight for societal reasons. As a result, the potential market for such products is very large and the pharmaceutical industry has invested considerable time and resource in product development. Unfortunately, although a number of medicines have been licensed, the majority of these have caused adverse reactions and in many cases the risks of these have outweighed potential benefits, resulting in restricted use of withdrawal from the market, with examples including fenfluramines, rimonabant and sibutramine. There is also a substantial market in complementary or herbal approaches to weight reduction but the benefits and risks of these products are much less clearly defined. Some pharmaceutical may be used inappropriately for weight reduction (e.g. emetics, laxatives, diuretics), while some apparently herbal products have caused adverse reactions as a result of contamination with pharmaceutical adulterants, an example being sibutramine found in traditional Chinese medicines. There is a wide range of herbal or borderlines substances currently available for purchase and marketed for weight reduction. Some are bulking agents, e.g.guar gum, methylcellulose, flax seeds or psyllium. Other herbal or natural products include Garcinia cambogia (Malabar tamarind), raspberry ketone, chitosan, konjac and Citrus aurantium (bitter orange). Benefits and risks associated with many of these are poorly defined. Of particular concern is use of the industrial chemical 2,4,dinitrophenol (DNP), because of the marked toxicity of this substance. DNP uncouples oxidative phosphorylation by dissipating the proton gradient required by ATP synthase for the formation of ATP from ATP. The consequences are an increased metabolic rate, increased glycolysis and lipolysis and a reduction in fat stores. This "fat burning" effect is sought by body builders/sculptors, but the associated risk is of uncontrolled thermogenesis leading to multi organ failure which is a particular concern with higher doses or after acute overdoses. There were several deaths from occupational DNP exposure in munitions workers between 1910 and 1920. Subsequently, widespread unregulated use of DNP occurred in the USA before medical use was prohibited in 1938 because of adverse effects including fatalities. Of particular recent concern has been the increasing numbers of cases of DNP toxicity, including fatal poisoning, reported to UK poisons centres in 2012 and 2013. This prompted various actions including warnings to users and educational work directed at key user groups (e.g. in gyms). While there was subsequently a reduction in numbers of enquiries and deaths, a further increase occurred in 2015. Of particular concern is the higher proportion of women involved in this later outbreak, suggesting that the substance is now being used for weight reduction rather than confined to predominantly male body builders.

While unlicensed weight loss agents may be attractive to users their benefits are often poorly defined and there is little information about potential risks. DNP appears to be particularly hazardous and further steps are needed to minimise cases of severe toxicity.

Notes:

S030 Can we perform *in vitro* to *in vivo* extrapolation with Genetic Toxicology data?

Author: A. Doherty
Affiliation: AstraZeneca

Abstract Body: In recent years the genetic toxicology meetings have showcased a number of examples of in vitro risk assessment in both its annual meeting and in its data workshops of UKEMS and IGG. There is a significant push within the genetic toxicology world to model gene tox in vitro and in vivo data and determine if this may be of use in establishing point of departures or thresholds. We have considered in vitro data from the pharmaceutical industry and bench mark dosing that we conducted alongside the in vivo testing. For a compound series with known genotoxic mechanism in the in vitro micronucleus assay we have experienced some cases where we could fit the model to bench mark dose (BMD) using PROAST and other compounds where we could not. We can also compare these data and BMD_{10} to the in vivo micronucleus data ultimately seen. Other industry groups are also using quantitative genotoxicity data to add weight of evidence to risk assessments. The cosmetics and personal care industries have presented data at IGG meetings using POD/BMD_{10} to explore the concept of exposure based risk assessment. There is concern within the industry that where BMD_{10} or other threshold levels to be applied in the field of genotoxic impurities, we could end up with much lower levels required than would have been case is currently used with the TTC (threshold of toxicological concern).

Notes:

S031 Investigating the impact of carbon nanotube physico-chemical properties towards their potential genotoxicity *in vitro*

Author: M. Clift
Affiliation: Swansea University Medical School

Abstract Body: Carbon nanotubes (CNTs) represent an advantageous component for a plethora of industrial- and human-based applications. Yet there remain heightened (public) concerns as to their (inevitable) human exposure, of which, the potential health hazard is limited. Currently, scientific understanding of the ability for CNTs to elucidate an adverse biological effect is equivocal. Nonetheless, it is apparent that the biological impact of CNTs is strongly related towards their specific physico-chemical characteristics[1,2]. A mechanistic understanding of how CNT physico-chemical characteristics may cause genotoxicity, is currently lacking. Thus, primary aim of this study was therefore to systematically evaluate the potential genotoxicity of CNTs with varying physical properties, including length, stiffness, morphology and elemental constitution. Further to this, a secondary objective was to elucidate whether or not *in vitro* multi-cellular systems could pose as advantageous model cultures, beyond the commonly used epithelial cell monocultures, to decipher CNT genotoxicity *in vitro*. At sub-lethal concentrations ([0.005-0.02mg/mL]), known to cause a (pro-)inflammatory response[3], all CNTs studied, independent of their characteristics caused significant (p<0.05) cell proliferation (EdU assay) in the bronchial epithelial cell-line 16HBE14o$^-$ after 24hrs suspension exposure only. Exposure of the same panel of CNTs to a triple cell co-culture model of the human alveolar airway barrier[4] however, showed a significant increase (p<0.05) in cell proliferation at both 4 and 24hrs in a concentration-dependent manner. Yet, there was no absolute differences between the physical and chemical attributes of the CNTs tested. Such similarities between the effect-trend were also seen between mono- and co-culture scenarios when investigating the ability for CNTs to inflict DNA damage (COMET assay) after 4hrs exposure at [0.02mg/mL] in the absence of the FPG enzyme. However, the influence of physico-chemical characteristics was evident with, surprisingly, the shortest CNTs elucidating the greatest DNA damage across the two culture systems. Analysis of the role of oxidative stress (*i.e.* in the presence of the FPG enzyme) in the DNA damage noted showed significant differences between the mono- and co-culture formats, with the % DNA tail between 3-7 fold higher in the co-culture model compared to monocultures. Again the short CNTs were paramount in causing DNA damage over long CNTs and bundled CNTs. These findings indicate the dominant role of oxidative stress within the co-culture. Investigation of the oxidative potential of the ability for CNTs to cause reactive oxygen and nitrogen species (ROS/RNS) *via* EPR, the DCFH-DA assay and a RNS kit further highlighted the oxidative potential of all nanofibers, albeit heightened in an acellular environment compared to within either the mono- or co-culture system. Further analysis is ongoing to deduce the role of oxidative stress in these scenarios and its association with the pro-inflammatory effects previously noted[3]. Currently, from the findings noted above the ability for the physico-chemical characteristics of CNTs to contribute towards any measureable genotoxicity *in vitro*, through means of oxidative stress, can be ranked as; short>bundled >tangled>long. The results further show that multi-cellular systems show a different picture compared to monocultures, highlighting the need to conduct future nanomaterial hazard assessment with these systems.

Notes:

S032 Development of a novel in vitro tool for determining carcinogenicity.

Author: G. Jenkins
Affiliation: Swansea University Medical School

Abstract Body: Traditionally, compounds have been tested for carcinogenicity using short term DNA damage (genotoxicity) tests. The battery of test used has changed over the years, but the aim has always been to use DNA damage induction as a surrogate for carcinogenic potential, based on the undisputed link between mutation and cancer. Compounds testing positive in a battery of in vitro tests are often then taken forward into animal studies to confirm the genotoxicity effects, and sometimes the potential for carcinogenicity also. Obviously with a ban on testing cosmetics in animals, alternative approaches are being considered. Indeed, there have been significant strides taken in Europe in particular to reduce the reliance on animals in safety testing. DNA damage can be a useful predictor of cancer induction risk, however, it is known that many compounds are carcinogens without the key induction of DNA damage. These non-genotoxic compounds have been difficult to detect in vitro and have numerous underlying mechanisms that can be a challenge to identify in vitro. We have explored the possibility of developing a wide ranging testing programme for carcinogenicity that is purely performed in vitro. This testing system is aimed at detecting both genotoxic and non-genotoxic carcinogens. We have coupled cytotoxicity testing (relative population doubling) with DNA damage assessment (micronucleus assay) in both TK6 and MCL5 cells for compounds either not requiring or requiring metabolic activation. In addition, we have used data from these initial studies to design multiple lines of investigation to consider if these compounds alter the following cell biological features: cell cycle distribution, oxidative stress, mitochondrial effects, cell signalling errors and changes to cell morphology. These assessments utilise flow cytometry, fluorescent reporters, a Seahorse bioanalyser, realtime PCR, Western blotting and InCell analysis respectively. The data is then integrated together to obtain an overview of the effect of each chemical on each endpoint. This integration allows close scrutiny of mechanisms of action and can identify the specific method with the highest sensitivity.
Using a collection of 16 chemicals including genotoxic and non-genotoxic carcinogens, and compounds that are non-carcinogens and some that are misleadingly described as carcinogens, we have tested our approach. We have shown that by integrating these lies of enquiry we are able to correctly classify each of the different chemicals as a carcinogen or not. Crucially, multiple positive results were obtained in the different tests for each compound and there were key links between some endpoints measured (e.g. G2 cell cycle checkpoint and cell size). The use of cell signalling and InCell analysis was particularly useful in identifying non-genotoxic carcinogens.
On the basis of this large body of data obtained by our group over the previous 4 years we are able to propose a novel alternative to animal testing for carcinogens in vitro with the capability of detecting different types of carcinogen through integrated cell biology assessments.

Notes:

S033 Exploiting the promise of advanced 2D and 3D cell systems for selection of safe and efficacious drugs

Author: M. Ingelman-Sundberg
Affiliation: Karolinska Institute

Abstract Body: Liver biology and function, drug-induced liver injury (DILI) and liver diseases are difficult to study using current in vitro models such as primary human hepatocyte (PHH) cultures, as their rapid de-differentiation restricts their usefulness substantially. Thus, we have developed and extensively characterized an easily scalable 3D PHH spheroid system in chemically-defined, serum-free conditions. Using whole proteome analyses, we found that spheroid cultures were similar to liver in vivo and even retained their inter-individual variability. Furthermore, spheroids remained phenotypically stable and retained morphology, viability, and hepatocyte-specific functions for culture periods of at least 5 weeks. We show that under chronic drug exposure, sensitivity of hepatocytes drastically increased and toxicity of a set of hepatotoxins was detected at clinically relevant concentrations. An interesting example was the toxicity of fialuridine for which hepatotoxicity was mimicked after long-term repeated dosing in the spheroid model, not possible to detect using previous in vitro systems. Additionally, we provide proof-of-principle that PHH spheroids can reflect liver pathologies such as cholestasis, steatosis and viral hepatitis. Combined, our results demonstrate that the 3D PHH spheroid system developed can present a versatile and promising in vitro system to study liver function, liver diseases, drug targets and chronic DILI. We have also developed a dedifferentiation model of hepatocytes in 2D cultures where we have studied the importance of non-coding RNAs for the process. The results show the importance of several different types if ncRNAs in the control of hepatic gene expression.

Notes:

S034 Hazard identification and risk assessment based on epidemiology and animal data, the example of perfluorinated compounds.

Author: T. Fletcher
Affiliation: Public Health England

Abstract Body: Perflourinated compounds, principally PFOA (perfluorooctanoic acid) and PFOS (perfluoroctane sulphonate), have been risk assessed based on both experimental and human evidence. For example IARC has recently classified PFOA as a possible carcinogen (Category 2B) based on limited animal and human evidence. Animal evidence provides a source for estimating no effect levels, but where the same biological effects are apparent, epidemiology suggests that people may be more sensitive than rodents. In epidemiology, given their long half lives in humans and stable serum levels, biomarkers have been the exposure indicators of choice in many epidemiological studies. They are attractive indicators, reflecting individual exposure histories, however they can also be vulnerable to confounding factors, which can determine inter-individual differences in excretion rates. The most convincing evidence derives from associations, which can be demonstrated from both contrasts in measured serum levels and contrasts in intake or exposure. These contrasting approaches to assessing exposure will be illustrated from the epidemiology studies carried out in the "C8 studies" in the Mid Ohio valley, USA, where a community was exposed to varying degrees in different water districts, to PFOA in drinking water supplies. Several exposure-disease associations were identified, including raised cholesterol, and cancers of the kidney and testes.

Notes:

SO35 Responding to new psychoactive substances in Europe: early warning and risk assessment

Author: M. Evans-Brown
Affiliation: European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)

Abstract Body: In recent years a large open market in substances which mimic the effects of controlled drugs such as cannabis, MDMA, cocaine, ketamine, and heroin, has developed across Europe. Now, a huge range of potent substances can be made on a large scale by legitimate chemical companies in China and India, rapidly shipped to Europe, were they are either sold directly on the illicit market or packaged into products—such as 'legal highs', 'research chemicals', and 'food supplements'—which are then sold off the shelf in the high street and the web. Known as new psychoactive substances, the EU Early Warning System (EWS), operated by the EMCDDA, monitors more than 570—more than double the number of substances controlled under the United Nations international drug control convention. In 2015, 100 new substances were detected for the first time on the European drug market. While in 2014 almost 4 tonnes of new substances were seized across Europe—many of which are vastly more potent than their controlled counterparts. These seizures were dominated by synthetic cannabinoids and cathinones which are sold as legal replacements to cannabis and illicit stimulants, respectively. They also include a huge range of other drugs, including benzodiazepines and exceptionally potent narcotic analgesics—such as fentanils which may be sold as heroin. Mirroring this increased availability of new substances is both a growth in their use and reports of a range of serious harms. In the latter case these include acute poisonings which present to hospital emergency departments—sometimes on a large scale—and sudden deaths. There is also evidence that new substances are driving changes in the patterns of drug injection in Europe. This is particularly evident with some of the new stimulants. These changes have been linked to serious drug-related infectious disease such as HIV and hepatitis C as well as bacterial infections. In some cases these have manifested as outbreaks which can place substantial demands on healthcare. It's the job of the EMCDDA to identify signals of such serious harms and react through a range of early warning responses including public health alerts and risk assessment — since the beginning of 2014, 34 public health alerts have been issued to our partners, while 7 risk assessments were conducted by our Scientific Committee. In this presentation I'm going show how the EMCDDA detects and manages these signals. Using case studies, I'll show you how we collect and use data from both our partners across Europe as well as from our monitoring of open source information. Finally, I will present the risk assessments process.

Notes:

Early Career Oral Communications Abstracts

EC001 Using in-vitro toxicity assays to track soil contaminants

Authors: P.M.E. Probert [1], M.P. Cooke [1], M. Dunn [1], M.C. Wright [1]
Affiliations: Newcastle University[1]

Abstract Body Soil contamination, which poses a significant risk to the environment and human health, is often assessed by quantitative measurement of known contaminants. This approach however neither reflects the effects of contamination, alone or in combination, on human health nor identifies previously unidentified toxic chemicals. We hypothesised that extracts from soil could be prepared and tested in cell based assays in order to screen for toxic effects. Multiple soil samples were therefore taken from around the boundary of a functioning waste site and from 3 distant control sites from which aqueous, alcohol and organic extracts were generated. The effect of these extracts on a range of toxic endpoints was then determined. Initial screening showed that of the generated extracts, aqueous extracts 1 and 2 were the most cytotoxic: they significantly inhibited liver progenitor cell proliferation and induced apoptosis. Cell death was preceded by activation of 5' AMP-activated protein kinase (AMPK) and depletion of glucose from culture media. High glucose concentrations protected against cell death as did AMPK inhibitor compound C. Screening for mitochondrial toxicity indicated that aqueous extracts 1 and 2 were potent inhibitors of mitochondrial respiration and induced glycolysis, potentially by inhibiting complex V activity. These data demonstrate that extracts can be generated from soil and tested in cell-based toxicity screens. In this work, this approach demonstrated that soil from around a waste site contains chemicals which had toxic effects in-vitro. Use of this approach at contaminated locations would enhance our understanding of the potential effects of contamination upon disease.

Notes:

EC002 Investigating the *In vitro* utility of keratin-18 forms as biomarkers of drug-induced liver injury (DILI)

Authors: J.D. Lea [1], T.K. van der Made [2], S.A. French [1], D.J. Antoine [1]
Affiliations: University of Liverpool[1], Radboud University[2]

Abstract Body Keratin-18 is a filamentous protein expressed in epithelial cells. During necrotic cell death keratin-18 is released in an unmodified form (K18), whereas during apoptotic cell death keratin-18 undergoes caspase processing resulting in the formation and release of caspase-cleaved keratin-18 (ccK18). Hence, the profile of keratin-18 forms is indicative of the apoptotic/necrotic cell death profile.

Clinical and in vivo studies have demonstrated that K18 and ccK18 show potential as sensitive and specific novel mechanistic biomarkers of DILI. However, knowledge of the in vitro utility of keratin-18 forms as DILI biomarkers is lacking.

Using HepG2 cells and primary human hepatocytes (PHH), we induced apoptosis in a dose- and time-dependent manner using staurosporine or APAP (determined by caspase activation and flow cytometry). Significant intracellular ccK18 expression (detected by immunofluorescence microscopy and Western blotting) and ccK18 release (detected by ELISA) occurred in a dose- and time-dependent manner.

In 2^{M} staurosporine-treated PHH, intracellular ccK18 expression was increased 14-fold vs control at 24h (*n=4, p<0.05*). In staurosporine-treated HepG2 cells, time-matched intracellular and extracellular ccK18 levels correlated with caspase activity (intracellular: r^2*= 0.83*, p*<0.0001>*, extracellular: r^2*=0.76, p<0.0001*) and percentage apoptosis (intracellular: r^2*<=0.53, p<0.01,* extracellular: r^2 *0.55, p<0.01*). Co-treatment of cells with the caspase inhibitor Z-VAD.fmk inhibited apoptosis and ccK18 expression and release.

Our studies demonstrate that ccK18 has in vitro utility as a marker of apoptosis during DILI. The findings demonstrate the translatability of ccK18 and support its use as a novel mechanistic biomarker of DILI.

Notes:

EC003 An MIE atlas

Authors: T.E.H. Allen [1], S. Liggi [1], J.M. Goodman [1], S. Gutsell [2], P.J. Russell [2]
Affiliations: University of Cambridge[1], Unilever[2]

Abstract Body The adverse outcome pathway (AOP) framework for risk assessment aims to build understanding of toxicity across all levels of biological organisation. The molecular initiating event (MIE) can be thought of as the gateway to the AOP - the initial chemical interaction. Chemistry is key to understanding the MIE. What is it about molecules that allow them to do this?
In this project answers to this question have been explored in a number of cases. This has included the characterisation of a number of MIEs across several well understood toxicants using literature searches. This work led to a greater understanding of what an MIE is and how it should be described, including a new unified definition of the MIE: the initial interaction between a molecule and a biomolecule or biosystem that can be causally linked to an outcome via a pathway. Principles for model construction have been developed, linking a molecule's properties with its activities more closely than ever before, and a manner in which understanding gained from these models can feed back into AOP resources has been presented. Finally a number of models based on these principles have been developed, providing a platform for the screening of novel chemicals to establish which MIEs they may be able to activate, and hence the toxicities they may elicit.
This work represents the first steps in the development of an MIE-based approach to human safety risk assessment. These efforts represent a foundation to develop the area of alternatives to animal based toxicity testing.

Notes:

EC004 Switching cells to galactose results in metabolic reprogramming and profound changes in cellular signalling, the mitochondrial proteome and ultrastructure

Authors: G.J. Miles [1], C. Langlais [1], D. Dinsdale [1], N. Morone [1], M. MacFarlane [1], K. Cain [1]
Affiliations: MRC Toxicology Unit[1]

Abstract Body Switching cells to galactose results in dependence on mitochondrial oxidative phosphorylation (OXPHOS) and an enhanced sensitivity to mitochondrial toxins. Thus, switching cells to galactose is widely used as a cell-based pre-clinical assay to investigate/detect unknown drug-induced mitochondrial liabilities. We have used this model of inducing a metabolic switch from glycolysis to OXPHOS to investigate the interplay between sensitivity to cell death, cellular bioenergetics and mitochondrial dynamics using leukaemia-derived cells maintained in galactose-supplemented media. Inhibition of glycolytic ATP production by culturing cells in galactose media does not alter the basal cellular ATP content due to profound upregulation of basal OXPHOS. Cells maintained in galactose-supplemented media are exquisitely sensitive to classical mitochondrial toxins and evaluating the mode of cell death reveals that mitochondrial toxicity results in a necrotic cell death phenotype. Intriguingly, switching cells to galactose to investigate mitochondrial toxicity not only induces metabolic reprogramming to rely on OXPHOS but induces profound changes in mitochondrial function, the mitochondrial proteome and ultra-structure. Importantly, although galactose-maintained cells are highly sensitive to classical mitotoxins, in contrast they exhibit decreased sensitivity to canonical inducers of caspase-dependent apoptotic cell death. Our analysis reveals that switching to an OXPHOS phenotype changes the cellular protein expression of pro/anti-apoptotic proteins, which in turn modifies sensitivity to drug-induced cell death. Our findings show that cellular switching to OXPHOS results in profound changes not only in cellular bioenergetics but also in key cell signalling pathways that may mask the identification of potential drug-induced mitochondrial liabilities.

Notes:

Oral Abstracts

O001 microRNA-122 provides sensitive detection of paracetamol-induced acute liver injury and dysfunction -- a large prospective study of 985 patients

Authors: J.I. Clarke [1], B. Francis [1], J.W. Dear [2], D.J. Antoine [1]
Affiliations: University of Liverpool[1], University of Edinburgh[2]

Abstract Body Drug-induced liver injury (DILI) is widely acknowledged as an important human health concern. Currently used biomarkers, such as alanine aminotransferase (ALT), may be deemed inadequate and therefore provide an emerging need for novel biomarker development; microRNA-122 (miR-122) is thought to hold several advantages over ALT. Most importantly, miR-122 has been shown to detect DILI at earlier timepoints where ALT elevations are delayed. The objective of this work was to elucidate whether miR-122 is able to sensitively detect liver injury in a high number of patients, and furthermore to determine predictive capabilities. <P>miR-122 levels were analysed in serum from paracetamol overdose patients (N = 985) at their first presentation to hospital; almost 20% of which (50 patients) initially had acute liver injury (ALI) ruled out, based on ALT levels, but later went on to develop injury. Receiver operator characteristic curve (ROC) analysis will provide an assessment of the ability of miR-122 to detect/predict liver injury. <P>ROC values show miR-122 was able to detect ALI with an AUC of 0.96 and gave predictions of oncoming liver injury with an AUC of 0.73; thus appearing to be successful at identifying patients with ALI, as well as predicting those that will spontaneously recover vs. those who require pharmaceutical intervention. <P>This data shows miR-122 as an advantageous biomarker that holds high clinical utility due to successful performance on a large patient cohort. Hereby this work continues to exemplify miR-122 as a sensitive and specific marker for the detection and prediction of hepatic injury in man.

Notes:

O002 Neurotoxicity of novel psychoactive substances

Authors: P.S. Hanson [1], K. Matthias [1], S.H.L. Thomas [1], P.G. Blain [1], C.M. Morris [1]
Affiliations: Newcastle University[1]

Abstract Body A large number of new recreational drugs, termed novel psychoactive substances (NPS) and sometimes called 'legal highs' have recently been identified, with more than 100 notified in Europe during 2014. It is often believed that these NPS are legal and therefore safe to use despite the method of purchase and many now being controlled substances. The prevalence of NPS use is slowly becoming available, however information regarding their safety and long-term effects on human health is sparse. The potential effects NPSs can have on the developing brain were examined using human neuronal stem cell (hNSCs). The synthetic cannabinoid receptor agonists (SCRAs) Win 55,212-2 mesylate (WIN-55) and STS-135, were examined to determine a dose response relationship, along with anandamide (endogenous cannabinoid) and cannabis, to compare their effects with natural cannabinoids. Changes in protein expression were examined by exposing the hNSCs for 1 week to WIN-55 or STS-135, prior to differentiating into mature neuronal cells. The results suggest that SCRAs are more toxic than cannabis and anandamide, with WIN-55 being the most toxic showing toxicity at 500nM. Western blotting analysis of exposure to SCRAs shows decreases of 27% in cannabinoid receptor 1 and 14% in metabotropic glutamate receptor 6 expression suggesting an increase in adenylate cyclase activity and potential changes in synaptic plasticity. Preliminary results suggest that some SCRAs may have neurotoxic effects that are more potent than those of cannabis. Further research to characterise these effects in more detail and to examine the relevance to human recreational use is needed.

Notes:

O003 Optimisation of air-liquid interface cultures of human bronchial epithelial cells and their use in the investigation of potential health effects of diesel exhaust particles and cerium dioxide nanoparticles.

Authors: K. Meldrum [1], R. Smith [2], T.D. Tetley [3], M.O. Leonard [2]
Affiliations: Imperial College London/Public Health England [1], Centre for Radiation, Chemical and Environmental Hazards[2], Imperial College London[3]

Abstract Body Over recent years, there has been an increase in diesel vehicle use and together with new developments, such as additional use of fuel catalysts, concerns have been raised regarding their potential health effects. Cerium dioxide nanoparticles (CeO2NPs) are active ingredients within fuel catalysts and little information is available as to the hazards they may pose to healthy or respiratory compromised individuals. Central to the response the lung initiates towards inhaled material, the epithelial cells play a pivotal role. Air-liquid interface (ALI) cultures of human primary bronchial epithelial cells (HPBECs) were developed and optimised as a model of airway epithelium. To optimise ALI differentiation conditions, we investigated various combinations of medium constituents. Final medium composition was decided upon differentiation marker expression. Levels of MUC1, a marker of mucin production, were increased by 512 fold at day 14. Levels of KRT14, a marker of basal cells, were decreased by 2 fold at day 12 as measured by RT-qPCR. CeO2NPs release into the environment arises as a component of diesel exhaust particulates (DEP). Therefore we initially investigated responses of these optimised cultures to the DEPs. The cultures were exposed to DEPs (0.1-100<micro>g/ml) followed by recovery period of 24hours. ALIs were then analysed for specific mediators. Initial results indicated increases in CYP1A1 expression after 24hours of recovery, this expression decreased in a dose dependant manor. We have also initiated further exposures of DEP in combination with CeO2NPs in order to complete analysis for markers of respiratory disease, this data will be presented.

Notes:

O004 A novel role for the microRNA biogenesis apparatus in the repair of double-stranded breaks

Authors: B. Hawley [1], W.T. Lu [1], S. Moxon [2], M. Malewicz [1], M. Bushell [1]
Affiliations: MRC Toxicology Unit[1], The Genome Analysis Centre[2]

Abstract Body DNA damage, particularly double stranded breaks (DSBs), can be deleterious to a cell and require immediate resolution. The genome requires constant maintenance and a plethora of different pathways to detect and repair the break with minimal loss of genetic information. Recently, components of the microRNA biogenesis machinery, Drosha and Dicer, have been shown to be important in DSB repair. Furthermore, what appears to be newly generated small RNAs proximal to a break site have also been reported
Using inducible endonucleases and ionising radiation (IR), we have further characterised the role of the miRNA biogenesis apparatus in DNA repair. Specifically, we show that depletion of Drosha and Dicer but not miRNA effector proteins affects the recruitment of critical repair factors to damaged foci. Crucially, depletion of these prevents 53BP1 focus formation as early as 5 minutes after IR. Persistence of foci after toxic insult suggests a deficiency in resolution. We have performed repair pathway outcome experiments, as well as a more novel resection assay to show that loss of Drosha results in diminished error-free repair. These results suggest the miRNA biogenesis apparatus, but not the miRNA pathway *per se* is critical for the precise and non deleterious repair of DSBs.
We are currently finalising the precise step at which these proteins are involved in the repair process, as well as detecting these small RNAs in an endogenous situation using a robust sequencing strategy to ultimately determine where RNA fits into this fundamental pathway previously considered a purely DNA-protein process.

Notes:

O005 Early studies assessing the cardioprotective properties of metformin during sunitinib-induced cardiotoxicity

Authors: R. Kuburas [1], I. Haussmann [1], H. Maddock [2], H. Sandhu [2]
Affiliations: Coventry University [1], Coventry University[2]

Abstract Body Introduction: Anticancer therapies such as Sunitinib, a multi-tyrosine kinase inhibitor are associated with an increased risk of cardiotoxicity. In contrast the anti-type-2 diabetic drug Metformin is associated with cardioprotective properties. In this study we investigated the effects of Metformin on Sunitinib-induced cardiotoxicity. Methods: Female Sprague-Dawley rat (120g-150g) hearts were Langendorff perfused with vehicle (normoxic control), Sunitinib (1µM) ± Metformin (50µM) for 120 minutes after 20 minutes of stabilisation. Haemodynamic data of heart rate (HR), left ventricular developed pressure (LVDP) and coronary flow (CF) was measured (n=4). Heart tissue was stained with triphenyltetrazolium chloride (TTC) to measure the level of infarct to risk ratio (I/R) size (n=4). Results: Sunitinib (1µM) administration significantly reduced HR when compared with vehicle control over the 120 minute time period ($P<0.001$). Metformin (50µM) administration reduced LVDP when compared with vehicle control ($P<0.05$). Co-treatment with Sunitinib (1µM) ± Metformin (50µM) was shown to further increase CF when compared to treatment with Metformin alone ($P<0.001$). TTC staining revealed that Sunitinib caused a significant increase in I/R (±SEM) compared to vehicle (vehicle=13±1%; Sunitinib=54±10%, $P<0.001$). Metformin administration revealed no significant difference in I/R compared to vehicle control (Metformin=22±7%). Co-administration with Metformin attenuated Sunitinib-induced I/R (Metformin±Sunitinib=24±3%, $P<0.01$). Conclusion: These data suggest that administration of Metformin protects the heart against Sunitinib-induced cardiac injury. More studies are required to investigate these findings in further detail. Further investigation into the mechanism of action of the protection afforded by Metformin against Sunitinib-induced cardiac injury will aid the understanding of multi-tyrosine kinase inhibitor associated cardiotoxicity.

Notes:

O006 Phototoxicity Testing for Agrochemicals: A Proposed Human Risk Assessment Framework and Case Studies

Authors: Fiona Macleod , M. Aggarwal [1], M. Corvaro [1], A. Morriss [1], J. Mehta [1]
Affiliations: Dow AgroSciences[1]

Abstract Body Phototoxicity testing is required by EU pesticide regulations if the active substance has a UV/visible molar extinction/absorption coefficient (MEC) of >10 L x mol-1 x cm-1 in the wavelength range 290-700 nm (EU No 283/2013). The relevance of this hazard characterisation requirement is unclear as the number of confirmed cases of pesticide-induced human phototoxicity is very limited or may be non-existent. Currently, the only available regulatory test guideline is OECD 432 (*in vitro* 3T3 Neutral Red Uptake (NRU) Phototoxicity Test 2004) which is known to have a high false positive rate. Despite EU regulations stating that "A positive result shall be taken into account when considering potential human exposure", there is no guidance on how to utilise positive results in human exposure assessments. <P> Our goal is to take a first step towards exploring a framework for human (operator, bystander/resident, re-entry worker and consumer) exposure and risk assessment for phototoxicity. The proposed framework utilises dermal absorption data (e.g., OECD TG 428) and exposure models (e.g., EFSA models) for exposure assessment. The framework for human phototoxicity risk assessment can be divided into three basic steps: 1) establish a reference concentration (RfC) for phototoxicity, 2) estimate potential exposure to skin and internal exposure (via dermal and oral route), and 3) overall risk assessment. Two case studies of the active substances of a fungicide and an herbicide are provided to show how this approach can be applied to chemicals that test positively for phototoxic potential in the 3T3 NRU test.

Notes:

O007 Toxicological evidence underpinning the assessment of the carcinogenicity of alcoholic beverages by the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

Authors: B.M. Gadeberg [1], F.D. Pollitt [1], K. O'Leary [2], K. Vassaux [3], L. Rushton [2], A.R. Boobis [2], D.H. Phillips [4]
Affiliations: Public Health England[1], Imperial College London[2], Independent Consultant for Imperial College London[3], King's College London and Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment[4]

Abstract Body Recent advice from the UK Chief Medical Officers (CMOs) that men and women should limit alcohol consumption to 14 units per week received extensive press coverage. Alongside this advice, the Committee on Carcinogenicity (COC) published its statement on alcohol and cancer risk, following extensive work by PHE/CRCE and Imperial College London. This work considered the findings of IARC and the newly published literature, and the evidence from animal and in vitro studies were drawn together with epidemiological data and mechanistic assessment. The COC concluded that even low levels of alcohol intake below 1.5 units per day increases the risk of oral cavity, pharyngeal, oesophageal and female breast cancers. Above 1.5 units per day risk of laryngeal and colorectal cancer increases and above 6 units per day there is increased risk of liver and pancreatic cancer. All types of alcoholic beverage increase the cancer risk suggesting that ethanol, as the common constituent, is the causative agent. Supporting this conclusion is mechanistic data. Acetaldehyde, classified by IARC as "carcinogenic in humans" (Group 1), results from the first step in the metabolism of alcohol, the oxidation of ethanol catalysed by alcohol dehydrogenase 1B (ADH1B). ADH1B is expressed in the tissues in which increased cancer incidence is apparent and there is evidence for genetic polymorphisms in ADH1B affecting cancer risk. In this presentation we elaborate on the evidence and its assessment that supported the new advice from the CMOs and is a good example of the role of public health toxicology.

Notes:

O008 Assessment of reactive metabolite-induced mitochondrial toxicity and generation of personalised <I> in vitro</I> models as a tool to determine susceptibility to idiosyncratic hepatotoxicity

Authors: A. Ball [1], A. Alfirevic [1], J. Lyon [2], A.E. Chadwick [1]
Affiliations: University of Liverpool[1], GlaxoSmithKline[2]

Abstract Body The anti-androgen, flutamide, is strongly associated with idiosyncratic hepatotoxicity amongst patients. However, flutamide undergoes extensive first-pass metabolism to its primary metabolite, 2-hydroxyflutamide. Although flutamide is a known direct mitochondrial toxicant, there has been little investigation into the potential mitochondrial toxicity of 2-hydroxyflutamide as a cause of idiosyncratic hepatotoxicity. We hypothesise that both parent compounds and reactive metabolites can cause mitochondrial toxicity and that interindividual variation in mitochondrial DNA (mtDNA) underlies the idiosyncratic nature of hepatotoxicity.
We have demonstrated that 2-hydroxyflutamide, as well as flutamide is a direct mitochondrial toxicant in HepG2 cells by use of ATP assays in both glucose and galactose-conditioned cells to enhance susceptibility to mitochondrial toxicants. We have also shown for the first time that 2-hydroxyflutamide is a potent inhibitor of mitochondrial respiratory complex I in permeabilised HepG2 cells using Seahorse XFe96 extracellular flux analysis.
Knowledge of specific toxicological targets of compounds will aid the identification of critical polymorphisms in mtDNA which may account for interindividual differences in susceptibility to compound toxicity. Due to the dual influence of mtDNA and genomic DNA, the study of the phenotypic consequences of mtDNA variation is best studied against a stable nuclear background through the generation of transmitochondrial cybrids; by the fusion of the mtDNA of interest with Rho-zero (<rho>0) cells, in which the endogenous mtDNA is depleted. We are currently using HepG2 <rho>0 cells for the generation of transmitochondrial cybrids to enable any correlation between mtDNA variation and susceptibility to idiosyncratic hepatotoxicity to be established.

Notes:

Poster Abstracts

P001 A NOVEL METHODOLOGY TO TEST DRY DISLODGEABLE FOLIAR RESIDUE OF AGROCHEMICAL SPRAY FOR *IN VITRO* DERMAL ABSORPTION USING HUMAN SKIN

Authors: M. Aggarwal [1], W. Maas [2], P. Fisher [3], N. Morgan [4], R. Parr-Dobrzanski [4], M. Soufi [5], C. Strupp [6], C. Wiemann [7]

Affiliations: Dow AgroSciences[1], TNO Triskelion, [2], Bayer CropScience[3], Syngenta [4], DuPont de Nemours GmbH[5], ADAMA MAH BV Amsterdam NL Schaffhausen Branch[6], BASF Oesterreich GmbH[7]

During agrochemical use in field, dermal exposure may occur to undiluted product during mixing and loading or to the diluted spray during application (*i.e.,* operators). However, once the spray has dried, re-entry workers can enter the field and may be exposed to dried residues present on foliage. To determine systemic exposure upon dermal exposures, dermal absorption (DA) studies are typically performed using product and representative in-use spray(s). Currently, a methodology to test dry residue for DA is lacking, therefore, the highest DA value, typically for the spray is conservatively used for re-entry worker risk assessments.

In this study, a novel methodology was developed to test dry residue of spray for *in vitro* DA using human skin. The standard EU re-entry worker risk assessment model can used to calculate the target skin dose. The dry residues were created on Teflon-coated septa by air drying the target volume of spray. Based on transfer efficacy, the doses on septa were adjusted. These septa were used to transfer the dose to the pre-wetted human skin membranes mounted on flow-through diffusion cells and DA study was performed according to OECD TG.

Initial results from 7 compounds indicate the degree of DA from the dry residues was noticeably lower than that obtained with corresponding sprays. In summary, a novel methodology is being developed to test dry dislodgeable foliar residues from agrochemical spray dilutions for DA within the standard OECD 428 study design in order to provide data that would be relevant for re-entry worker risk assessments.

Notes:

P002 APPLICATION OF THE COMET ASSAY TO DETECT METAL-INDUCED DNA STRAND BREAKS IN CULTURES OF THE MARINE SPONGE *HYMENIACIDON PERLEVE*

Authors: R.U. Akpiri [1], R.S. Konya [2], N.J. Hodges [1]

Affiliations: The University of Birmingham[1], University of Port Harcourt

Because of their wide distribution, sessile filter feeding lifestyle and ability to bioaccumulate pollutants marine sponges represent an ideal tool for monitoring the marine environment. In the current study field collected and cryo-preserved marine sponge (*Hymeniacidon Perleve*) cells cultured in synthetic seawater rapidly formed viability aggregates approximately thirty microns in size that remained stable in culture for up to 10 days as assessed by their ability to reduce the dye MTT. Treatment of these aggregates with non-toxic concentrations (as assessed by the MTT assay) of cadmium chloride (0-1 mg/l), nickel chloride (0-0.4 mg/l) and sodium dichromate (0-0.4 mg/l) for 12 hours resulted in statistically significant dose-dependent accumulations of metal levels in sponge tissues as assessed by inductively coupled plasma mass spectrometry (ICP-MS). Furthermore, to the best of our knowledge we have utilised for the first time the alkaline comet assay to detect DNA-strand breaks in marine sponge cells and demonstrated that treatment with non-toxic concentrations of all three metals for 12 hours results in a dose-dependent increase in DNA single stranded breaks

In conclusion, we have developed a novel in vivo model based on culture of cryopreserved sponge cells that is compatible with the alkaline comet assay. Genotoxicity in marine sponges measured by the comet assay technique may be a useful tool for bio-monitoring research and risk assessment in aquatic ecosystems.

Notes:

P003 DECONVOLUTING TARGET AND CHEMISTRY RELATED TOXICOLOGY IN THE RAT THROUGH THE UTILIZATION OF 'INACTIVE' AND 'ACTIVE' PAIRED ENANTIOMERS

Authors: M. Anderton [1], R. MacDonald [1], K. Maratea [1], D. Sutton [1], E. Leonard [1], J. Basak [1], G. Hawthorne [1], J. Rose [1], A. Hird [1]

Affiliations: AstraZeneca[1]

Discovery safety aims to understand whether toxicology findings are related to the biological target or the chemical. A strategy that can build evidence of target toxicity is the use of enantiomers. Enantiomers have identical molecular weights and sequence of bonded atoms but differ in the three-dimensional orientation which can result in differences in potency against the primary biological target. AZ1 and AZ2 have remarkably similar kinase and secondary pharmacology selectivity but differ in primary target potency by approximately 1500 fold. Ten rats received either a single 1h intravenous administration of vehicle, the active enantiomer (AZ1) or the 'inactive' enantiomer (AZ2). Liver, haematology and clinical pathology samples were collected 5h following the start of the infusion. AZ1 and AZ2 had similar total and free levels of compound but differed in pharmacological cover over the biological target. AZ1 was associated with mild hepatocyte apoptosis, elevated AST (18 fold) and ALT (10 fold) whilst the 'inactive' enantiomer had only minimal/rare hepatocyte apoptosis and only minimal increases in AST (2 fold) and ALT (2.5 fold) only. The different toxicology correlated well with differences in pharmacological activity as determined by blood haematology. AZ1 was associated with a 54% and 92% reduction in WBC count and lymphocytes, respectively, whereas AZ2 had no effect on WBC or lymphocytes. The use of paired enantiomers can serve are a useful tool to evaluate biological and chemical toxicology, particularly when a marker of pharmacological activity can be incorporated.

Notes:

P004 EVALUATION OF A CHEMICAL GENETIC APPROACH TO DETERMINE GPCR FUNCTION

Authors: S.M. Brooke [1], R. Prihandoko [1], A.B. Tobin [1]

Affiliations: MRC Toxicology Unit[1]

We have developed a chemical genetic approach to study the function of the M^1 muscarinic receptor in vivo. This method involves mutating the receptor to remove receptor responsiveness to its natural ligand acetylcholine (ACh) while simultaneously engender activity to an otherwise inert compound Clozapine-N-oxide (CNO). Prior to the generation of a knock-in mouse model expressing the mutant receptor (termed M^1 RASSL) we have characterised the pharmacological properties of CNO *in vitro*. Using inositol phosphate accumulation assay, here we show that CNO was able to cause inositol phosphate accumulation with EC50 of -5.61 +/- 0.23 and -5.29 +/- 0.27 at the human and mouse wild-type M^1 muscarinic receptors, respectively. The compound produced maximal response ~30% of the response produced by ACh and hence behaved as a partial agonist. This partial agonism was further evident in the mouse M^1 receptor which showed CNO functionally antagonise ACh response when the two ligands were co-added. These data suggest that CNO, unlike previously thought, possesses pharmacological activities at the M^1 muscarinic receptors. It is therefore important to select the right dosage for *in vivo* studies in order to avoid toxic or unwanted off target effects.

Notes:

P005 The utility of QSARs in predicting acute fish toxicity of pesticide metabolites: a retrospective validation approach

Authors: Natalie Burden , S.K. Maynard [1], L. Weltje [2], J.R. Wheeler [3]

Affiliations: Syngenta[1], BASF SE[2], Dow AgroSciences[3]

EC Regulation 1107/2009 requires that registrants establish whether pesticide metabolites pose a risk to the environment. Fish acute toxicity tests may therefore be carried out. The number of metabolites can be considerable, thus this could use many vertebrates. EFSA's recent "Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters" outlines opportunities to apply non-testing methods, e.g. Quantitative Structure Activity Relationship (QSAR) models. A scientific evidence base supporting the use of QSARs in predicting acute fish toxicity of pesticide metabolites could reduce the numbers of animals used.

This work aims to provide this evidence base through retrospective data analysis. Experimental fish LC_{50} values for 150 metabolites were extracted from the Pesticide Properties Database (http://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm). QSAR calculations were performed for these metabolites using US EPA's ECOSAR software. The lowest predicted LC_{50} value was compared with experimental LC_{50} values. The relationship between the predicted and experimental values was assessed.

There is a significant correlation between predicted and experimental fish LC_{50} values (Spearman r_s = 0.6253, $p < 0.0001$). For 61% of metabolites, predicted values are equal to or lower than their experimental values. Applying data quality and experimental variability considerations increases the proportion of sufficiently predictive estimates to 90%. For all outliers except one there is a plausible explanation for the obtained ECOSAR prediction. As such there is value in further refining the QSAR approach, to improve the method and enable its future incorporation into regulatory guidance.

Notes:

P006 Adverse effects of anti-tuberculosis drugs on HepG2 cell bioenergetics

Authors: Wayne Carter , E. Elmorsy [1], S. Attalla [1], E. Fikry [1], L. Nwidu [2], E. Elmorsy [1]

Affiliations: Departments of Forensic Medicine and Clinical Toxicology, Mansoura University, [1], Department of Pharmacology and Toxicology, Niger Delta University[2]

Tuberculosis is an intractable chronic infection. Disease treatment with anti-tuberculosis (TB) drugs remains challenging due to drug-induced hepatotoxicity. The toxic effect of the anti-TB drugs rifampicin (RIF), isoniazid (INH), and pyrazinamide (PZA) either alone or in combination was investigated in HepG2 cells. Assays of intracellular ATP levels at 4, 24, and 48 hour post-exposure to gradient concentrations of RIF, INH, and PZA were conducted. Drug-induced effects on mitochondrial membrane potential, mitochondrial complex I & complex III activity, and cellular lactate production were also assessed. Decreased intracellular ATP levels were dose-dependent and correlated with drug exposure duration. Approximate 24 hour IC50s were 0.5 mM, 70 mM, and 84 mM for RIF, INH, and PZA, respectively. Twenty-four hours post-drug treatment, significant reductions of mitochondrial membrane potential ($p = 0.0005$), mitochondrial complex I & III activities ($p = 0.0001$ and $p = 0.0003$, respectively), and increased lactate production ($p < 0.0001$) were observed. Drug combinations used to mimic cumulative drug treatments induced a synergistic inhibition of mitochondrial complex I activity. Collectively, this study highlights the potential hepatotoxicity of commonly employed anti-TB drugs and provides a mechanistic insight into their hepatotoxicity.

Notes:

P007 Age is a Factor of the Extent of Anti-Cancer Sunitinib Therapy Induced Cardiotoxicity: Studying Haemodynamic Parameters and Cardiotoxicity Specific Microrna Expression Involved

Authors: S.L. Cooper[1], C.J. Mee[1], A. Hussain [1], H.L. Maddock [1], H. Sandhu [1]

Affiliations: Coventry University[1]

Introduction: Sunitinib is a potent chemotherapy agent. However, adverse cardiovascular events have been reported. Here we investigate the influence of age on the level of Sunitinib-induced cardiotoxicity by detecting injury development and expression profiles of cardiotoxicity linked microRNAs in rats aged 9 week, 1 year or 2 year old.

Methods: Langendorff perfused hearts were treated with Sunitinib (1μM) for 120 minutes and either (a) stained with triphenyl-tetrazolium chloride (TTC) to assess the level of infarct or (b) analysed by real-time PCR for cardiac injury related microRNAs (miR-1, miR-27a, miR-133a and miR-133b). Haemodynamic data was collected from all hearts.

Results: TTC staining revealed that Sunitinib treatment of both aged and young rat heart increased infarct size (2 year old rats = 24.01±0.9% ($p=0.006$), Vehicle = 7.51 ± 0.65; 1 year old rats = 32.04±1.31%, ($p<0.0001$), Vehicle=10.88 ± 0.2041, 9 week old rats = 40.20±2.0 ($p<0.001$) Vehicle = 5.4 ± 0.74. 35 minutes of Sunitinib perfusion showed a significant decrease in Left Ventricular Developed Pressure compared to vehicle (33±12% decrease, $p=0.04$) in 2 year old rats. Sunitinib increases the expression of all the cardiac injury related microRNAs assessed in this study. Data expressed as mean ± S.E.M., n=6 for TTC and n=6 for PCR.

Conclusion: This study emphasises the strain that age puts on the cardiac system when treated with Sunitinib. Here we show for the first time that age may have an impact on the extent of Sunitinib-induced cardiotoxicity, when undertaking both pathological, functional and molecular level investigations.

Notes:

P008 Re-shaping acute toxicity testing of agrochemical formulations by combining the GHS ATE formula and *in vitro* approaches

Authors: M. Corvaro [1], S. Gehen [1], K. Andrews [1], C. Arasti [1], R. Chatfield [1], F. MacLeod [1], H. Mikolajczak[1], J. Moore [1], J. Mehta[1]

Affiliations: Dow Agrosciences[1]

An acute toxicology six-pack of studies is currently required for the registration and Classification and Labelling (C&L) of agrochemical formulations. The UN GHS (Globally Harmonised System for C&L) provides the opportunity to calculate the classification category based on the Acute Toxicity Estimate (ATE) of each individual component within the mixture, without performing additional in vivo studies. A retrospective analysis was performed for 225 agrochemical formulations in order to A) identify main classification drivers and prioritise research on methods alternative to in vivo testing; B) evaluate accuracy of the GHS ATE approach, comparing to data from existing in vivo toxicity studies. For the acute systemic toxicity end-points (oral, dermal and inhalation), the main hazard concern was acute oral toxicity (despite being less relevant from an exposure perspective). A classification threshold of 2000 instead of 5000 mg/kg bw should be used for agrochemical formulations. The GHS ATE formulae showed high accuracy and specificity (both > 85% and up to 99.5%) for prediction of the estimated LD50 compared to 4 classification systems GHS, CLP, EPA and ANVISA. Using the GHS ATE formula together with physico-chemical characteristics related to exposure as a criterion for triggering the systemic toxicity study, would lead to a potential animal use reduction of up to ~48% (currently implemented in limited geographies). For the topical effects (skin/eye irritation; skin sensitisation), variability of the reference in vivo dataset was highlighted also for agrochemical formulations (see Adriaens et al., 2014). Predictions using the GHS ATE formulae resulted in an overall accuracy of ~70%. However, combination of GHS ATE with results from accepted *in vitro* methods and the use of Adverse Outcome Pathways (AOP) provides the opportunity to move beyond animal testing. Taken together, the combination of ATE, AOP and *in vitro* tools provides a reliable, animal-free and alternative to traditional acute toxicity testing.

Notes:

P009 Toxicogenomics in Agrochemicals: Developing a Predictive Systems Toxicology Platform

Authors: M. Corvaro[1], N. Elango[1], K. Johnson[2], B. Veeramani[1], S. Sriram[1], M. LeBaron[2], J. Raymond[1], K. Yang[1], R. McEwan[1], J. Bino[1], R. Rasoulpour[1]

Affiliations: Dow Agrosciences[1], The Dow Chemical Company[2]

Abstract Body The global toxicology requirements for agrochemical active substance registration includes a very animal intensive program using four different species for hazard characterisation. Toxicogenomics (TxG; e.g. RNA profiling) may represent a novel approach for molecule development. In fact, TxG allows generation of early knowledge on the molecular events of agrochemical interactions with biological systems, potential prediction of toxicity outcomes, and development of Mode of Action (MoA) hypotheses. In addition, it represents a translational tool to examine human relevance. However, to-date, use of TxG for screening or regulatory use has been limited. In this collaborative effort, a proof of concept project has been designed to generate and analyse RNAseq-based liver transcriptome profiles following a rat oral 28 day treatment, with compounds (chemicals and agrochemicals) with established modes of action and/or liver toxicity outcomes. RNAseq data were combined with publicly available rat liver microarray profiles (TG Gates and Drug Matrix) to identify bioinformatics processes that would most accurately group molecules by liver toxicity, chemical structural similarity and/or MoA. Bioinformatics processes examined include unsupervised clustering, machine learning, connectivity mapping and a novel gene network-based analysis. Results indicated RNAseq profiles can accurately group molecules by structural similarity and mode of action. These findings demonstrate the utility of liver transcriptome profiling to predict rat liver toxicity. These results confirm that collaborative development of tools such as TxG can increase confidence in the safe use of agrochemicals.

Notes:

P010 Disruption of the Immunodominant T cell epitope of Green Fluorescent Protein (GFP): impact on immunogenicity

Authors: R.J. Dearman[1], I. Kimber[1], T. Eyes[1], A. Doig[1]
Affiliations: University of Manchester[1]

Labelling cells with a genetically encoded fluorescent signal such as GFP is a powerful tool for monitoring biological events both *in vitro* and in vivo. However, enhanced GFP (EGFP, commonly used variant) can be immunogenic in animal models, impacting on the integrity and reliability of experimental studies, resulting in the elimination of cells expressing EGFP. The aim of these investigations was to develop an EGFP construct with fluorescent functionality but reduced cell-associated immunogenicity in BALB/c strain mice. EGFP was expressed in *Escherichia coli* and saturation mutagenesis was used to generate a library of EGFP variants where the major histocompatibility complex (MHC) class I anchor site in the dominant epitope (described previously for BALB/c mice) was mutated. The library was screened for fluorescent EGFP variants with anchor site mutations which should display reduced immunogenicity based on computational prediction. The EGFP mutation tyrosine 200 to threonine (Y200T) variant was selected and murine A20 B cell lymphoma A20 cells were stably transfected with either EGFP wild type (WT) or Y200T. BALB/c mice were immunised by subcutaneous injection with 10^6 or 10^7 cells expressing EGFP or EGFP Y200T and serum analysed at day 14. The Y200T mutation significantly reduced the anti-EGFP IgG response. Interestingly, immunisation with recombinant WT or Y200T EGFP protein each resulted in relatively high levels of IgG antibody. Thus, through a single mutation the immunodominant T cell epitope in EGFP can be disrupted and tolerance to cell associated protein enhanced, representing a novel strategy for improving tolerance to heterologously expressed proteins.

Notes:

P011 Subvisible protein aggregates result in T helper (Th) 1 skewing of immune responses

Authors: R.J. Dearman [1], K. Ratjani [1], J. Derrick [1], R. Thorpe [2], M. Wadhwa [2], I. Kimber [1]
Affiliations: University of Manchester[1], National Institute of Biological Standards[2]

Abstract Body The production of anti-drug antibodies against biotherapeutics can cause immunogenicity, impacting upon drug safety and efficacy. Protein aggregation is one factor that can enhance immunogenicity. Here, we have explored the relationship between protein aggregation and immune activation. A humanised scFv was used as a reference biologic and ovalbumin (OVA) as a second reference protein. Aggregates in the subvisible particle size range were prepared using heat stress or stir stress; both methods resulted in scFc aggregates of ~2^{m} in size, whereas stir stress resulted in a more heterogenous population with a peak at ~0.8^{m}. Intraperitoneal immunisation of BALB/c strain mice with 1mg/ml of aggregated or monomeric scFv induced similar IgG and IgG1 antibody titres. In contrast, both stir-stressed and heat-stressed aggregate preparations stimulated significantly higher levels of anti-scFv IgG2a antibody than did the monomer. Parallel results were obtained with heat-stressed OVA aggregates and control monomeric OVA. Anti-OVA IgG and IgG1 antibody titres were similar in sera from monomer and aggregate-immunised mice, whereas IgG2a was significantly higher in aggregate-immunised mice. Importantly, titres were identical regardless of whether monomer or aggregate was used as substrate in the analysis. Furthermore, cytokine profiles in supernatants taken from splenocyte-dendritic cell co-cultures were consistent with Th1-type skewing by the OVA aggregate preparation, whereas the monomer induced preferential Th2 activation (measured as a function of interferon- and interleukin 13 expression, respectively). Th1-type responses are typically mounted against intracellular bacteria and viruses, suggesting that the aggregated protein may stimulate polarised Th1 responses by mimicking repetitive pathogenic structures.

Notes:

P012 The combination of measuring oxygen consumption and extracellular acidification with the Glu/Gal assay improves the detection of mitochondrial toxicants.

Authors: J. Eakins [1], C. Bauch [1], B. Park [1], S. Bevan [1], H. Woodhouse [1], C. Dilworth [1], P. Walker [1]
Affiliations: Cyprotex Discovery Ltd[1]

Abstract Body Drug induced mitochondrial dysfunction has been implicated in various organ toxicities resulting in the withdrawal of drugs or black box warnings limiting their use. The development of highly specific and sensitive *in vitro*assays in early drug development would assist in detecting compounds which effect mitochondrial function. Here we report the combination of two *in vitro* assays for the detection of drug induced mitochondrial toxicity. The first assay measures cytotoxicity after 24 hr incubation of test compound in either glucose or galactose conditioned media (Glu/Gal assay). Compounds with a greater than 3-fold toxicity in galactose media compared to glucose media imply mitochondrial toxicity. The second assay measures mitochondrial respiration, glycolysis and a reserve capacity using the XF^{e} flux analyser (Seahorse Biosciences). Incorporating a mitochondrial stress test also allows the detection of potential mechanistic responses observed within one hour following exposure to test compound. In order to assess these assays a total of 72 known drugs and chemicals were used and screened through both assays. Dose response data was normalised to 100x C^{max} giving a specificity, sensitivity and accuracy of 100%, 81% and 92% respectively for this combined approach.

Notes:

P013 Experience With *In Vitro* EPISKIN ™ Corrosivity Testing in a CRO

Authors: David Esdaile , J. Hargitai [1]
Affiliations: CiToxLAB Hungary[1]

The *in vitro*skin corrosion test using reconstructed human epidermis (RHE) has been an OECD guideline method since 2004. It has been a principle method for skin corrosivity testing for classification and labelling under REACH, and has been a typical first step before live animal dermal testing to prevent skin corrosives from being applied to animals. There are several RHE models used, each of which uses a slightly different test protocol and prediction model. The EPISKIN™ model follows the same exposure periods as the OECD 404 live rabbit "Acute Dermal Irritation/Corrosion" method of 3 minutes, 1 hour and 4 hours; a positive corrosive response at these times allow classification as GHS category 1A, 1B or 1C. In the EPISKI™ protocol a positive response is defined as a reduction of skin viability to < 35% of the control, as measured by MTT reduction. The *in vivo* study is not generally used for corrosivity classification today on animal welfare grounds. The *in vitro* RHE methods other than EPISKIN™ only allow partial classification into the 3 positive grades of 1A, 1B and 1C. The data from testing about 150 substances shows that the EPISKIN™ method makes a clear classification in almost every case. When plotting all viability data appropriately, it can be seen that the lowest incidence of all viability results is at 35%, demonstrating that it is very unlikely to have a borderline result in this protocol. Approximately 80% of results are noncorrosive, ~10% are 1C and most of the remaining are classified 1B.

Notes:

P014 Updated guidance on risk assessment of chemical carcinogens by the UK Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

Authors: B.M. Gadeberg [1], F.D. Pollitt [1], S. Robjohns [1]
Affiliations: Public Health England[1]

The Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) provides independent advice on carcinogenic risk to UK government departments and agencies, and is supported by Public Health England and Imperial College London.
The COC is currently revising its guidelines on carcinogenicity testing strategies and risk assessment of chemical carcinogens, and is developing a series of guidance statements on specific topic areas. This poster, which ties in with a number of the symposia at this meeting, presents this useful resource for chemical risk assessors.
The statement on points of departure and potency estimates emphasizes use of the benchmark dose, especially in preference to use of the T25, or alternatively a NOAEL/LOAEL as the basis for a risk assessment. This statement also updates the COC view on the Threshold of Toxicological Concern recognising it as a pragmatic means of assessing if a chemical is of low concern or not when the data are not sufficient to support a full risk assessment.
Guidance on risk characterisation methods strongly recommends the use of the Margin of Exposure approach, rather than low dose extrapolation from animal studies, for genotoxic carcinogens to indicate the level of concern where exposure to such chemicals is unavoidable. Guidance such as this supports and informs the approaches used by UK government, e.g. PHE uses the MOE approach when assessing risk from benzo[a]pyrene in contaminated land.
Other guidance statements in the series, e.g. on biomarkers, and alternatives to the two-year bioassay, will also be discussed.

Notes:

P015 SMAC mimetics induce liver-specific NF- B activation *in vivo* and protect against hepatotoxicity

Authors: G.M. Hayward [1], P.M. Probert [2], S.K. Meyer [2], T. Chernova [1], P.G. Blain [2], H.M. Wallace [3], M.C. Wright [2], M. MacFarlane [1]
Affiliations: MRC Toxicology Unit[1], Newcastle University[2], University of Aberdeen[3]

Abstract Body Inhibitor of **AP**optosis (IAP) proteins are negatively regulated by the mitochondrial pro-apoptotic protein, SMAC, through a tetrapeptide binding-motif. A class of drugs mimicking this motif have been developed as anti-cancer agents and have further highlighted the role of IAP signalling in regulating cell death. These SMAC mimetics (SMs) activate the non-canonical NF- B pathway, induce TNF secretion and selectively promote cell death in tumour cells; an effect potentiated in a TNF - rich microenvironment. However, due to this mechanism of action, SMs have the potential to induce toxicity in non-tumour cells residing in this environment. Many hepatotoxins result in TNF release from macrophages in the liver. As liver regeneration is also TNF - dependent, SMs may have the potential to induce significant toxicity under conditions of liver injury. To explore this, we performed an *in vivo*> dose-escalation study in male adult C57BL/6 wild-type mice to ascertain the effect of the clinically-relevant, cIAP1/2-specific, bivalent SM (TL-32711) on the liver. No toxicity was observed except at the highest dose, where there was limited necrosis and apoptosis. To determine whether TL-32711 could induce NF- B activation *in vivo* in a tissue-specific manner, we used mice expressing an NF- B reporter gene (mice^{luc}) allowing for quantification of NF- B activity via *in situ* live imaging. Mice^{luc} were dosed with a non-toxic concentration of TL-32711, either alone or in combination with the known hepatotoxins, - naphthylisothiocyanate (ANIT) or carbon tetrachloride (CCl4). Strikingly, TL-32711 alone induced significant liver-specific NF- B activation *in vivo* and protected against ANIT and CCl4-induced hepatotoxicity. The potential mechanisms underlying this amelioration of hepatotoxicity will be presented.

Notes:

P016 The international regulatory need for tests and information to develop an Integrated Approach to Testing and Assessment (IATA) of non-genotoxic carcinogens.

Authors: M.N. Jacobs [1], A. Colacci [2], K. Louekari [3], M. Luijten [4], B. Hakkert [4], M. Paparella [5], P. Vasseur [6]
Affiliations: CRCE/PHE[1], Center for Environmental Toxicology and Risk Assessment, Environmental Protection and Health Prevention Agency Emilia Romagna Re[2], ECHA[3], RIVM[4], Environment Agency Austria[5], University of Lorraine[6]

Abstract Body Regulatory requirements for carcinogenicity testing of chemicals vary according to product sector and regulatory jurisdiction; however, a standard approach starts with a battery of genotoxicity (GTx) tests. If any of the in vivo GTx tests are positive, then under several regulatory schemes a lifetime rodent cancer bioassay may be requested. For most jurisdictions, there are no specific requests to obtain information on non-genotoxic mechanisms of carcinogenicity, and there are no OECD approved screening methods. When the *in vitro* GTx battery is negative, usually no further long term/carcinogenicity testing will be requested in various legislations, e.g. REACH. Consequently non-genotoxic carcinogens (NGTxC) might remain unidentified and therefore the risks they may pose to human health will not be managed. It has been estimated that 10-20% of recognized human carcinogens classified as Class 1 by the International Agency for Research on Cancer act by non-genotoxic mechanisms. A panel of tests covering multiple biological traits will be needed, which alternative methods could provide. Here we examine what NGTxC are, the current international and European regulatory requirements and their limitations with respect to NGTxCs, and how an Integrated Approach to Testing and Assessment (IATA) could be developed to assist regulators in their assessments of NGTxCs. With a strong international drive to reduce animals testing and costs, it is essential that proper and robust methods for addressing non-genotoxic modes of action are developed and used.

Notes:

P017 Can the *In vitro* Glu/Gal mitochondrial assay be used to predict rat *In vivo* toxicity outcome?undefined

Authors: Stefan Kavanagh , P. Newham [1]
Affiliations: AstraZeneca R&D[1]

Abstract Body Mitochondria are crucial for many cellular processes. Many drugs withdrawn from the market, or with labelling restrictions imposed, have been shown to disrupt mitochondria. Consequently, many pharmaceutical companies assess compounds for mitochondrial toxicity preclinically, with the Glucose/Galactose assay being the highest throughput.
Information enabling the prediction of preclinical toxicological outcome based on *in vitro* mitochondrial data is limited, making it difficult to quantify mitochondrial risk. Consequently, compounds are either discarded unnecessarily during the discovery phase or enter clinical development with inherent mitochondrial liabilities which might contribute to sub-optimal therapeutic windows and late stage attrition.
AstraZeneca have tested >6,700 compounds in the Glucose/Galactose assay. We cross referenced this dataset against all compounds in our preclinical toxicology database to identify examples where *in vivo* rat toxicity and *in vitro* mitochondrial data existed. We reviewed *in vitro* mitochondrial toxicity potency (Galactose IC^{50}) *in vivo* dose-limiting toxicity (DLT) and exposures (C^{max}) to evaluate whether trends existed that might enable the prediction of *in vivo* toxicity for new compounds based on *in vitro* Glucose/Galactose data.
A key finding of our analysis was that, when C^{max} (total) exceeded Galactose IC^{50} no significant difference was observed in the number of groups where significant toxicity was recorded compared with No Adverse Effect Level (NO(A)EL) groups. A greater proportion of toxic groups were expected in this cohort. Interestingly, dose groups where C^{max} was less than the Galactose IC^{50}, significantly more NO(A)EL groups were observed than toxic groups, warranting further investigation on a larger set of compounds.

Notes:

P018 PH dependent model of human hepatic metabolism

Authors: R.A. Kelly [1], S. Webb [1], A. Chadwick [2]
Affiliations: Liverpool John Moores University[1], University of Liverpool[2]

Abstract Body Drug induced mitochondrial dysfunction is an understated mechanism of toxicity responsible for the withdrawal of several drugs from the market. Recently, off-target toxicities of this nature have become more widely acknowledged, with more effort being assigned to better understanding the underlying mechanisms of mitochondrial toxicity. Unfortunately, current methods have poor predicative capabilities due to 1) outdated tools, 2) discordance between animal models. The mitochondria are an extremely complex system which begs for computational assistance. As such, current in silico models of mitochondrial bioenergetics already exist. However, a comprehensive model that incorporates oxidative phosphorylation, Tricarboxylic acid cycle, ion transport and glycolysis has yet to be constructed for the purpose of assessing mitochondrial toxicity. Furthermore, the inclusion of dynamic buffering of protons and metal cation binding followed by resulting rapid conversion of biochemical species has also yet to be modelled. In this study, we have constructed an *in silico* model of human liver glycolysis, incorporating pH-dependent enzyme kinetics and reaction equilibria to compute the time course of pH changes. The model can be coupled with extracellular acidification (ECAR) data from extracellular bioenergetic flux analysis. This model is the first step to dynamically modelling cellular bioenergetics that is able to quantify proton uptake and release by all biochemical reactions in the metabolic pathway and the effects of the resultant pH change on thermodynamics and reaction kinetics; a necessary feature to assess mitochondrial toxicity.

Notes:

P019 Assessment of the effect of mitochondrial DNA polymorphisms and mutations on drug sensitivity

Authors: J.C. Komen [1], M. Minczuk [2], H. Garside [1]
Affiliations: AstraZeneca, DSM, Discovery Safety UK[1], MRC Mitochondrial Biology Unit[2]

Abstract Body Mitochondrial toxicity is implicated in the withdrawal of many drugs from the market or has resulted in labelling restrictions. Although potential toxic effects are being monitored during drug development the ideosyncratic nature of mitochondrial toxicity is still not well understood and complicates setting up assays and models predicting mitochondrial safety. Variation in the multy-copy circular mitochondrial DNA (mtDNA) sequence between individuals has been associated with increased toxicity for certain drugs. The mtDNA encodes critical components of the Oxidative Phosphorylation System (OXPHOS) responsible for generating the majority of cellular ATP, as well as tRNAs and rRNAs required for translation of these OXPHOS components inside mitochondria. Certain ancestral polymorphic variants (haplotypes) in human mtDNA have enriched in various populations due to adaptive advantages and these populations can be divided in haplogroups. Other mtDNA variations are deleterious, i.e. mutations, causing mitochondrial diseases most often as a result in impaired energy (ATP) generation. The variation in mtDNA gives rise to structural and regulatory changes in mitochondrial homeostasis between individuals, which may affect drug sensitivity. In order to study this *in vitro* we have generated human osteosarcoma cell lines (143B) containing different mtDNA haplotypes in the same nuclear DNA background. This was achieved by fusing enucleated lymphoblasts with mtDNA-depleted cells. In addition, 143B cell lines containing different mutations in either MT-ATP6 (m.8993T>G), or mt-tRNAVal (m. 1624C>T) were also obtained. Basic mitochondrial function was studied using a Seahorse XF96e bioanalyzer and showed an increased spare respiratory capacity in cells with m.8993T>G mutation. Cell viability in glucose or galactose medium the presence of the 'gold standard' mitochondrial toxicants rotenone, antimycin A, oligomycin A and FCCP was determined. No significant differences in IC50 values were observed. In conclusion, we believe we have generated valuable cell models for the *in vitro* study of the effect of mtDNA variations on drug sensitivity. Using the classical mitochondrial toxins used thus far no differences in toxicity could be observed between the cell lines. In future studies we hope screen more drugs using our cell lines and also look at subacute effects of compounds e.g. by studying changes mitochondrial biogenesis.

Notes:

P020 Diesel exhaust particulate associated chemicals elicit inhibitory effects on human airway epithelial derived antiviral and Th1 type T-cell signals.

Authors: S. Macchiarulo [1], K. Meldrum [1], T.W. Gant [1], M.O. Leonard [1]
Affiliations: Public Health England[1]

Abstract Body Chemicals associated with fuel combustion and particulate matter generated from diesel engines are considered a key component of inhaled pollutant material, which impact respiratory health particularly in those with disease conditions such as asthma. Airway epithelial cells represent the primary biological contact point for this material and are active participants in directing tissue responses. Important mechanistic information regarding how these cells respond to diesel exhaust particulate chemical extracts (DEPE) remains under-developed. Using *in vitro* cultures of human primary bronchial epithelial cells (HPBECs) we examined global mRNA expression changes using an RNA-Seq poly(A) library based sequencing method (Illumina HiSeq 2000) in response to treatment with DEPE (0.5-20μg/ml residual mass). Analysis revealed a significant cluster of transcript alterations demonstrating a potent inhibition of epithelial signals normally positioned to promote antiviral and Th1 T-cell type inflammation. These included CXCL10, CXCL11 and IFIT1, changes which have been implicated in asthmatic disease. Further exploration revealed inhibition of basal levels of CXCL10 protein at doses within the range experienced in polluted urban environments. This inhibition of CXCL10 was also observed with IFN and viral mimetic treatment. We also investigated polycyclic aromatic hydrocarbons (fluoranthene, benzo[b]fluoranthene, benzo[a]pyrene and Benzo[e]pyrene) for their effect on CXCL10 and revealed similar responses to those observed with DEPE. Interestingly, using the aryl hydrocarbon receptor (AHR) inhibitor CH223191, we observed an attenuation of DEPE induced inhibition of CXCL10. These studies provide additional information as to the potential contributors and molecular initiating events underlying the effects of chemical pollutants in respiratory disease.

Notes:

P021 Could smokers' socio-demographic and housing factors affect and influence the choice between smoking cessation therapies?

Authors: S. Leone [1], R. Carrozzino [2], M.A. Martelli [3], F. Mattioli [3]
Affiliations: Post Graduate School of Clinical Toxicology and Pharmacology, University of Genoa [1], San Paolo Hospital[2], University of Genoa [3]

Abstract Body Introduction The published data suggest that interventions which combine pharmacotherapy and behavioural support increase success rates of smoking cessation compared to minimal intervention or usual care; however, a standardized behavioural psychotherapy programme has not been assessed yet. Our main aim was to assess if socio-demographic and housing characteristics of smokers attending an Italian smoking cessation centre, could have influenced the choice between varenicline therapy and psychological support only. Our secondary aims were: i) to evaluate the 6-month abstinence rates (ARs), confirmed by comparing exhaled air carbon monoxide concentrations, in smokers according to whether they took varenicline or received only psychological support; ii) to assess the most frequently reported adverse drug reactions (ADRs) by the varenicline group, mainly focusing on psychiatric events; iii) to evaluate the differences between men and women with regard to specific varenicline-related ADRs. Methods 144 smokers were enrolled; all of them received the same psychological support programme. They were evaluated by a team of clinical experts, who advised them to opt for either one quitting method or the other; then the smokers chose themselves a treatment option of either varenicline plus psychotherapy (VAR: 78 patients) or psychotherapy alone (PSY: 64 patients). Results Socio-demographic and psychological characteristics of patients have significantly influenced the treatment choice; the 6-month ARs were 35.9% versus 10.9% ($p<0.01$) in those using varenicline versus psychotherapy, respectively; 57.7% of the patients reported at least one adverse event. Conclusions The analysis of socio-demographic factors and psychological characteristics of patients seems to be necessary to offer them the most effective therapy in order to achieve good abstinence rates. Therefore, this study confirms the data about the efficacy and safety of varenicline. Our screening methods and exclusion criteria seem to be valid aids to achieving good therapeutic outcomes with a low risk of occurrence of severe psychiatric events.

Notes:

P022 Comparative Systems Toxicology Assessment of the Tobacco Heating System 2.2 and reference cigarettes (3R4F), on human organotypic respiratory tissue cultures.

Authors: P. Leroy [1], C. Mathis [1], A. Iskandar [1], S. Frentzel [1], A. Elamin [1], K. Trivedi [1], E. Garcia [1], A. Knorr [1], N.V. Ivanov [1], J. Hoeng [1], M.C. Peitsch [1]
Affiliations: Philip Morris International R&D[1]

Abstract Body With the development of new tobacco products that have the potential to reduce individual risk of harm in comparison to smoking cigarettes, a careful and robust assessment strategy is required. We developed and performed large scale *in vitro* toxicological studies using human organotypic respiratory epithelial cultures allowing direct aerosol exposure at the air-liquid interface. Various endpoints were measured to investigate the impact of an acute exposure to different dilutions of the aerosol generated by a Tobacco Heating System (THS2.2) versus mainstream smoke from 3R4F cigarette (from the University of Kentucky). These comprehensive studies comprised on average 5 experimental repetitions, using different production batches of tissue cultures (from the same cell donor) and different generation of smoke/aerosol. Numerous endpoints were measured ranging from nicotine/carbonyls content in the aerosol/smoke, adenylate kinase, CYP1A1/1B1 activity, release of inflammatory markers, determination of cilia beating activity, and assessment of morphological features by histology. By using appropriate statistical models and by considering various sources of variability (e.g. aerosol generation, batch of cells), consistent results were obtained between experimental repetitions for the same product. Nicotine and carbonyls data also confirmed the inherent products variability during aerosol/smoke generation and support the need of multiple exposure runs to extend the generalizability and the confidence in the study results. For all endpoints, the results support a reduced impact of THS2.2 aerosol exposure on respiratory epithelial tissue cultures compared to cigarette smoke at similar nicotine concentration, with the confidence that inherent product, test system and methods variabilities have been taken into account.

Notes:

P023 Designing novel mRNA-based therapeutic approaches for non-toxic delivery of genetic material

Authors: L.A. Mulley [1], A. Wilczynska [1], C. Betts [2], D. Williams [2], M. Bushell [1]
Affiliations: MRC Toxicology Unit[1], Astra Zeneca[2]

Abstract Body The delivery of RNA as a therapeutic molecule to treat human disease has only recently been made possible. Incorporation of modified nucleotides into the RNA molecule now allows evasion of the innate immune system. *In vitro* transcribed (IVT) mRNA is not integrated into the genome and has quicker turnover than DNA drug delivery, allowing better control over dosage and potentially increasing safety. However, major issues must be resolved before mRNA therapeutics can be considered as viable in the clinical setting: limiting adverse effects triggered by introduction of IVT mRNA, delivery to off-target tissues and consistency between batches of IVT mRNA.
The lack of consistency when producing IVT mRNA is caused by variation in length of the poly(A) tail - this will affect both stability and translatability of the transcript. We intend to overcome these issues by testing alternative ways of introducing poly(A) tails and other stability elements onto the 3' end of the mRNA.
Modified nucleotides have been shown to reduce the immune response activated upon introduction of RNA into cells. Tailoring nucleotide modifications and incorporation into the 5' UTR, 3'UTR, coding region, or combination of these within the mRNA can help to circumvent any toxic side effects,
MicroRNAs are a type of small non-coding RNAs that can bind and silence target mRNAs. The expression of microRNAs is often restricted to certain cell types. We will present our preliminary data and proposed strategies to utilise miRNA-mediated gene regulation as a way of modulating therapeutic mRNA levels in target and off-target tissues.

Notes:

P024 Are Inhaled Iron Oxides Human Lung Carcinogens?

Authors: Camilla Pease , T. Rucker [1], T. Birk [1]
Affiliations: Ramboll Environ[1]

Abstract Body Evidence will be presented regarding epidemiology, toxicology and lung bioavailability as to whether iron oxides are human lung carcinogens. Observed lung tumours in rats result from a generic particle overload effect and local inflammation that is rat-specific under the dosing conditions of intratracheal instillation. This proposed mode of action, is not relevant to real-life human exposure. However, emerging differences are seen between 'bulk' iron oxides (particles where 70% are >100nm) and 'nano' iron oxides (>95% fall in the range 1-100nm). Evidence suggests 'bulk' iron oxides are not genotoxic/mutagenic, whereas for 'nano' iron oxide it is conflicting. Genotoxicity was not observed in an in vivo acute oral dose study and 'nano' iron oxides are considered safe and are being investigated for biomedical uses; there is no specific in vivo genotoxicity study on 'nano' iron oxides via inhalation. Hypothetically, with the larger surface area of 'nano' iron oxide particulates, toxicity could be exerted via the generation of reactive oxygen species (ROS) in the cell. However, ROS generation as a basis for explaining rodent lung tumourigenicity is only apparent if free iron from intracellular 'nano' scale iron oxide becomes bioavailable at significant levels inside the cell. This would not be expected from 'bulk' iron oxide particulates. Furthermore, human epidemiological evidence from a number of studies suggests that iron oxide is not a human carcinogen, and therefore based upon the complete weight of evidence, we conclude that 'bulk' iron oxides are not human carcinogens.

Notes:

P025 High content analysis for prediction of human drug-induced liver injury across several pharmaceutical companies within the IMI MIP-DILI consortium

Authors: M. Persson [1], H. Aerts [2], J. Edsbagge [3], H. Gerets [4], P. Hewitt [5], J. Hornberg [1], S. Juhila [6], M. Karjalainen [6], C. Lowatt [7], N. Mesens [8], P. Newham [1], L. Richert [9], A. Thelin [10], R. Weaver [2]
Affiliations: AstraZeneca[1], Servier[2], Takara Bio Europe[3], UCB Pharma[4], Merck[5], Orion Pharma[6], GlaxoSmithKline[7], Janssen Pharmaceuticals[8], KaLy-Cell[9], H. Lundbeck[10]

Abstract Body High content analysis (HCA) for cell health is a commonly used technique in the pharmaceutical industry to assess molecular and cellular toxicological properties of small molecules, and to predict liability for human drug induced liver injury (DILI). Several companies in the European Federation of Pharmaceutical Industries and Associations (EFPIA) routinely use HCA assays in their primary safety screens, however, since EFPIA partner assays vary, we sought to address key questions regarding cell types used, end-points measured and overall assay performance. An HCA-focused group within the Innovative Medicines Initiative (IMI) Mechanism-based Integrated Systems for Prediction of Drug-induced Liver Injury (MIP-DILI) consortium compared the various DILI HCA assays. A training set of compounds (14) and a larger test set of compounds (69 DILI positive and 22 DILI negative drugs) were been tested in the various HCA assays. Data for the training and test set of compounds revealed that the various assays identify roughly half of the DILI positive compounds (~50% sensitivity), with only 10% false positives (~90% specificity). Endpoints such as mitochondrial membrane potential and oxidative stress boost predictivity compared to cell death alone. There was good correlation of results across companies and across assays with an overall scoring concordance of 85%. These results highlight the value of HCA for cell health analysis in drug discovery, and that, despite variations in the HCA setups, pharmaceutical companies tend to take similar decisions for the same compounds.

Notes:

P026 Measurement of Cyp2b1 protein induction in laser dissected FFPE liver samples by Nano-LC Mass Spectrometry

Authors: S.M. Plummer [1], F. Rao [2], R. Currie [3]
Affiliations: MicroMatrices Associates Ltd[1], Dundee Cell Products[2], Syngenta[3]

Abstract Body Cytochrome p450 gene induction is an early associative key event in the mode of action (MOA) of non-genotoxic carcinogens that act via activation of nuclear hormone receptors such as the constitutive androstane receptor (CAR). Cyp protein measurement in formalin fixed paraffin embedded (FFPE) samples has utility for retrospective read-across approaches for risk assessment. However, this task is challenging in FFPE tissue due to difficulties in applying traditional methods such as Western blotting to these samples. To address this issue we have developed a method for the direct measurement of Cyp2b1 protein induction in laser dissected FFPE samples using mass spectrometry (LC MS/MS). We measured Cyp2b1 protein changes (relative to control) in laser dissected FFPE liver sections derived from Wistar rats treated with a known CAR activator -- a pyrazole carboximide succinate dehydrogenase inhibitor (SDHI1). Proteins were extracted from the liver sections using the Qiagen Q proteome kit and trypsin digested prior to performing LC MS/MS using an Orbitrap mass spectrometer. The protein yield was in the range 0.7-1.4 ug/mm2 tissue. Fold change values were calculated from intensity levels of unambiguously identified peptides that mapped uniquely to Cyp2b1. SDHI1 caused a 60 fold induction of Cyp 2b1 ($P<0.05$). We also detected significant changes to Cyp3a1 and several other proteins many of which are regulated by CAR/PXR. There was no induction of Cyp1a1 or Cyp4a1. The Cyp protein induction reflected the biochemical data (PROD, EROD activity). The results suggest that the use of proteomics has utility for unambiguous assessment of Cyp protein induction.

Notes:

P027 Global cross-company data-sharing on the housing of non-rodents during the recording of cardiovascular telemetry data on toxicology studies

Authors: H. Prior [1], A. Bottomley [1], J. Cordes [2]
Affiliations: NC3Rs[1], Pfizer Inc[2]

Abstract Body A cardiovascular assessment of new chemical entities and biologics in a non-rodent species is a regulatory requirement prior to first administration in humans. This is generally performed as a separate safety pharmacology study and/or part of the toxicology program. An international working group and contacts within the safety pharmacology community, including pharmaceutical and biotechnology companies and CROs, has shared data on the housing of non-rodents during recording of cardiovascular telemetry data in toxicology studies. This project was facilitated by the NC3Rs and the Safety Pharmacology Society. Data showed that 94% of facilities socially house non-rodents (dogs, minipigs and non-human primates (NHP)) before and between recording sessions on toxicology studies, however, during the cardiovascular telemetry recording 62% individually house the animals. Separation during recording may introduce unwanted stress, even when an individual is within sight/touch of another animal. Major barriers for social housing were (% respondents): damage to the jacketed equipment (70%), food consumption recording (67%), limitations of hardware (58%) and temperament of animals (50%). However, companies who socially house have found solutions to these issues and some reported that non-rodents are more cooperative, less stressed and have better baseline cardiovascular parameters when socially housed. Currently, 36% and 50% respondents socially house dogs and NHPs respectively during telemetry recording and the majority of remaining companies indicated that they would consider social housing if the barriers can be overcome. Best practice, processes and validation data shared during this project are being used to develop recommendations and increase adoption of this refinement worldwide.

Notes:

P028 The potential of hLiMTs and HepaRG spheroids to improve the *in vitro* prediction of drug-induced liver injury (DILI) using a repeat dose high content screening (HCS) approach.

Authors: S. Ravenscroft [1], S. Bevan [1], B. Park [1], H. Woodhouse [1], J. Eakins [1], C. Bauch [1], P. Walker [1]
Affiliations: Cyprotex Discovery Ltd[1]

Abstract Body Drug induced liver injury (DILI) is a leading cause of attrition during drug development. Repeat dose drug exposures are often required for the manifestation of such toxicities, however, current preclinical *in vitro*models focus primarily on restrictive two-dimensional (2D) cell culture formats with limited longevity. Here, human liver microtissues (hLiMTs), encompassing primary human hepatocytes and kupffer cells and HepaRG spheroids have been compared for their ability to respond to known DILI compounds. Both 3D models displayed uniform size, shape, ATP content and longevity beyond 21 days in culture. Reduced glutathione (GSH) content, reactive oxygen species (ROS) formation and mitochondrial dysfunction are commonly observed responses to toxic compounds and are implicated in DILI. Following a 14 day repeat dose exposure to a panel of *in vivo* DILI categorized compounds (19 positives, 4 negatives), fluorescent probes were incorporated into each model. Multiplexed images were acquired using the confocal mode of an ArrayScan™ XTI HCS reader (ThermoScientific) followed by cellular ATP measurement using CellTiter-Glo (Promega) to determine cytotoxicity. The minimum effect concentration (MEC) was selected for each compound and a 10-fold C^{max}cut off applied. hLiMTs correctly predict 87% of the compound panel with pioglitazone and metformin displaying false positive outcomes and fialuridine resulting in a false negative outcome. HepaRG spheroids, however, correctly predicted 96% of the compounds tested. This study shows how using a single organotypic 3D liver model per well and automated multiplexed confocal HCS can be used as part of an early screening cascade, and further demonstrates that HepaRG spheroids present a viable tool for the improved *in vitro* to *in vivo* translation of the potential for novel compounds to elicit DILI.

Notes:

P029 Target organ profiles in toxicity studies supporting human dosing: does severity progress with longer duration of exposure?

Authors: R. ROBERTS [1], P. Duffy [1], R. Knight [1], R. Callander [2], M. Jacobson [2], A. Boobis [3]
Affiliations: ApconiX[1], AstraZeneca[2], Imperial College London[3]

Abstract Body We have previously reported the profile of target organs (defined as organs showing histopathological changes) in rodent and non-rodent toxicity studies conducted prior to first-time-in-man (FTiM) for 77 AstraZeneca candidate drugs (CDs). Here, we test the assumption that toxicity is exacerbated by dosing duration by comparing the incidence and severity of target organ toxicities in these â‰¤6 week FTiM studies with those observed in subsequent subchronic/chronic (â‰¥3 month) studies. Looking at the effect of dosing duration on severity (pathological score) and incidence (percentage of animals within the group) for the 39 CDs that met the criteria for inclusion (comparable doses between FTiM and subchronic/chronic studies), new toxicities appeared for 31 target organs but existing ones resolved for 29 target organs. Increased severity was more frequent for rodent (16 target organs) than for non-rodent (4 target organs). Most notable changes were a large increase in severity/incidence in liver and in non-rodent lung in contrast to a large decrease in severity and incidence for kidneys/ureter and for the non-rodent thymus. Overall this analysis shows that, even with continued exposure, target organ toxicities of CDs are as likely to show partial or complete recovery as they are to progress in severity. These findings are surprising and suggest a much more complex picture than previously assumed. For the key target organs studied here, there appeared to be a high level of adaptation to prolonged exposure for some CDs and in some tissues, but without a clear pattern that could form the basis of a predictive model at the present time. This has great significance for human risk assessment with differing potential impact depending on the sphere of toxicology.

Notes:

P030 Drinking water fluoridation and PHE monitoring report

Authors: S. Robjohns [1], H.E. Smith [1], J. Morris [1], N. Young [1]
Affiliations: Public Health England[1]

Abstract Body Fluoride is naturally present in most water supplies. In some areas of England the level is adjusted upwards to improve dental health. Approximately, 6 million people receive fluoridated drinking water in England. Public Health England (PHE) has a statutory requirement to monitor the efficacy and potential health effects of water fluoridation in England every four years.

Approximately 50% of ingested fluoride is excreted via the urine and 50% is stored in bone. Long-term exposure to levels greater than those found in drinking water in England, can increase the risk of bone fracture and skeletal fluorosis (bone abnormalities and joint pain). As the kidney is exposed to relatively high fluoride concentrations compared to other organs it has been suggested as a potential target for toxicity. Other adverse health effects have been suggested, such as developmental neurotoxicity, and hypothyroidism, although no convincing evidence supports this.

The 2014 PHE monitoring report compared indicators of health in fluoridated and non-fluoridated areas, which included both dental and non-dental health outcomes.

Key findings included:

- Reductions of tooth decay and hospital admissions for dental extraction in children in fluoridated compared with non-fluoridated areas
- No evidence of adverse non-dental health outcomes (including hip fracture, kidney stones, all-cause mortality, Down's syndrome, bladder cancer, osteosarcoma and all cancer)
- PHE is required to report further monitoring by 2018. This poster summarises the evidence and rationale for the inclusion of specific health effects and the findings from the recent PHE monitoring study.

Notes:

P031 Molecular initiating events in toxicity pathways using organotypic human *In vitro* models

Authors: P.J. Russell [1], P. Fowler [1], S. Scott [1], S. Ravenscroft [2], C. Bauch [2], P. Walker [2]
Affiliations: Unilever Safety & Environmental Assurance Centre,[1], Cyprotex,[2]

Abstract Body Within the new paradigms for toxicology risk assessment, understanding the interactions between chemical exposure and biology is paramount in being able to make pragmatic safety decisions based on sound mechanistic understanding. Pathways-based approaches such as AOPs are proposed as a framework for developing new approaches to assessing risk from chemical exposure. Key to these approaches is the molecular initiating event (MIE), which has been defined as 'the initial interaction between a molecule and a biomolecule or biosystem that can be linked to an outcome via a pathway'. Of equal importance is the need to represent human biology accurately enough in *in vitro* models to allow meaningful mechanistic interactions. The present study aims to develop and refine appropriate human organotypic *in vitro* models, using a 3D Cardiac spheroid model comprising iPSC human cardiomyocytes, fibroblasts and endothelial cells, linking an understanding of MIEs to changes in molecular pathways to tissue level toxicity. A combination of novel tissue culture methods and molecular level analysis techniques have been employed. The 3D cardiac model has been exposed to cyclophosphamide at physiologically relevant concentrations, the transcriptome and proteome of this system is mined for appropriate changes in biological pathways. This data is then mapped to the levels at which changes are seen biologically (metabolic efficiency, organelle health, membrane permeability) and which are indicative of downstream toxicity. Links between MIE's, molecular pathways and relevant toxicological effects to human organs, when combined with exposure predictions, will increase the mechanistic understanding for the risk assessment of chemicals.

Notes:

P032 Molecularly Imprinted Polymer Sensor as an alternative to binding assays

Authors: J.M.N. Settipani [1], P.J. Russell [2], S. Piletsky [1]
Affiliations: University of Leicester[1], Unilever Safety and Environmental Assurance Centre[2]

Abstract Body Within the Adverse Outcome Pathway (AOP) framework, identifying and developing in-vitro assays representing Molecular Initiating Events (MIE) and enabling the screening and filtering of compounds initiating the AOPs is one of the key challenges. One of the major type of MIEs is based on receptor ligand interactions. Although studying these interactions has been possible for years, the in-vitro assays currently available are expensive and time-consuming, mainly because they rely on natural receptors as recognition material. Natural receptors are expensive, unstable, and difficult to produce and to store. Therefore, the need for the development of alternative binding assays is important. Among the existing alternatives to natural receptors, Molecular Imprinted Polymers (MIP) are one of the most promising. MIPs can be defined as the construction of ligand selective recognition sites in synthetic polymers. Used in combination with transducing technologies such as Surface Plasmon Resonance (SPR) that enables real time monitoring of binding interactions, MIP sensors are a promising alternative to current binding assays. In order to prove this concept, beta-2 adrenergic receptor, a well-known receptor with recognised interest in toxicology, was chosen as a target.

Notes:

P033 Metabolic reprogramming of HepG2 cells alters mitochondrial function and expression of Bcl-2 family proteins that regulate both autophagy and cell death

Authors: K. Frame [1], G.J. Miles [1], J. Lyon [2], K. Cain [1], M. MacFarlane [1]
Affiliations: MRC Toxicology Unit[1], GlaxoSmithKline[2]

Abstract Body We are examining the galactose-conditioned HepG2 cell model, which is employed as a pre-clinical assay for detecting mitotoxicity in drugs with previously unknown mitochondrial liabilities. Though a glycolytic phenotype benefits the cell by increasing metabolic flux down the pentose phosphate pathway, thus increasing the biogenic capacity of the cells, it may disguise mitotoxicity of compounds during pre-clinical drug testing. Galactose-conditioned cells exhibit a switch to a metabolic profile dependent on oxidative phosphorylation for generating ATP, rendering cells exquisitely sensitive to mitochondrial perturbation. We now demonstrate that metabolic reprogramming in HepG2 cells not only causes significant changes in cellular bioenergetics but also major changes in the expression of proteins involved in mitochondrial function, autophagy and apoptotic cell death regulation. These findings highlight the importance of determining the mode of cell death when examining the sensitivity of HepG2 cells, grown under different metabolic conditions, to canonical inducers of cell death and classical mitotoxins. Our data also reveals that there is significant cross-talk between altered cellular bioenergetics and the regulation of autophagy, which should be considered when employing the galactose-conditioned HepG2 cell model for detecting unknown mitochondrial liabilities.

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