# *'Omics and Risk Assessment Science*
# *25-26 February 2013, Málaga*

Workshop Report No. 25

EUROPEAN CENTRE FOR ECOTOXICOLOGY AND TOXICOLOGY OF CHEMICALS

# *'Omics and Risk Assessment Science*
# *25-26 February 2013, Málaga*

Workshop Report No. 25

Brussels, September 2013

ISSN-2078-7200-25 (print)

ISSN-2078-7219-25 (online)

**ECETOC Workshop Report No. 25**


European Centre for Ecotoxicology and Toxicology of Chemicals
2 Avenue E. Van Nieuwenhuyse (Bte 8), B-1160 Brussels, Belgium.

*'Omics and Risk Assessment Science*

**CONTENTS**

# 1. SUMMARY

There have been two previous ECETOC workshops in 2007 and 2010 on "*The Application of 'Omics Technologies in Toxicology and Ecotoxicology: Case Studies and Risk Assessment*". The results were published in ECETOC Workshop Reports 11 and 19 respectively. Their main recommendations were:

1. Conduct studies in a more standardised form using reference chemicals.
2. Obtain a common and agreed definition of what constitutes a toxicologically relevant biochemical pathway.
3. Study the toxicity dose- and time-dependent transition on relevant biochemical pathways from normal variability through adaptive response, to adverse effect.

These recommendations seem to have been applied when using these 'omics technologies, but there is still some uncertainty in defining reliable toxicological effect pathways with validated key molecular events (expression of genes / proteins) that regulate the responses.

The aim of the 2013 workshop was to review the progress made since the 2010 workshop on the application of 'omics technologies to chemical safety and assess the potential impact of these new technologies on the risk assessment of chemical substances.

In particular the workshop addressed the following points:

1. Case studies of the application of 'omics data for risk assessment and regulatory (eco)toxicology.
2. Guidance to help to increase the intrinsic value of 'omics data and stimulate its use in (eco)toxicology.
3. Impact of 'omics sciences on the risk assessment process.
4. Can 'omics data contribute to the elucidation of (1) life stage / subpopulation sensitivity, (2) low-dose effects and (3) the effects of mixtures?

Over a dozen case studies were presented from industry, regulatory agencies and academia (several European and North American Universities). In turn, four parallel syndicates groups discussed the following topics:

1. Assessing the opportunities for 'omics to help improve (eco)toxicology.
2. Identifying what key data / knowledge is missing that would help improve (eco)toxicology.
3. Considering the main challenges for using 'omics in risk assessment in general.
4. Considering the specific challenges for using 'omics in human risk assessment.

One of the main conclusions of this workshop was that 'omics data are particularly valuable for understanding modes of action via exposure-associated differential gene expression patterns and that such data are now being used to improve the quality of the risk assessment process. Moreover, 'omics data are now also used for early recognition adverse outcome pathways from which mode of action may be inferred. There was general agreement that molecular 'omics information should ideally be connected to the outcome of apical studies (phenotypic anchoring). Analysis of the most sensitive pathway for transcriptomics (at several points in time) allowed for a reasonable approximation of the NO(A)EL of the individual compounds

studied. Bioinformatics was identified as a key area where efforts are needed since a great deal of data are being generated and require expert assembly and interpretation.

There are a number of opportunities for the use of 'omics data for risk assessment of chemicals. The combined analysis of transcriptomics, proteomics and metabolomics should provide a better understanding of underlying mechanisms. Using 'omics technologies in *in vitro* studies is an attractive way to investigate, and hopefully predict adverse outcome pathways. Combining such information with 'omics data derived from current *in vivo* studies in which the whole organism response to a toxicant is better represented, should help to validate the *in vitro* approach.

Progress is gaining pace and 'omics tools are being used to identify biomarkers and guide study design towards shorter more targeted studies. However, predicting adversity from 'omics data remains an important issue for the use of such data in the risk assessment process. A number of solutions have been discussed in this and previous workshops, e.g. use of Bradford Hill criteria, biological plausibility of associations, phenotypic anchoring, pathway / pattern development. The WHO IPCS MoA / Human Relevance Framework can be used in a weight of evidence approach to confirm MoA where the outcome is already known or to predict potential apical effects based on evidence of 'key events' (molecular and physiological changes) being triggered in an adverse outcome pathway (AOP).

Overall, it was concluded that with more standardisation in study protocols studies and a better understanding of the association of differentially expressed genes with modes of action the relevance of animal (or *in vitro*) data for human and environmental risk assessment will be improved by the inclusion of data obtained with 'omics technologies.

# 2. WORKSHOP OVERVIEW

## 2.1 Introduction

There have been two previous ECETOC workshops in 2007 and 2010 on "*The Application of 'Omics Technologies in Toxicology and Ecotoxicology: Case Studies and Risk Assessment*". The results were published in ECETOC Workshop Reports 11 and 19 respectively (ECETOC, 2008; 2010). Their main recommendations were:

a) Conduct studies in a more standardised form using reference chemicals.
b) Obtain a common and agreed definition of what constitutes a toxicologically relevant biochemical pathway.
c) Study the toxicity dose and time dependent transition on relevant biochemical pathways from normal variability through adaptive response, to adverse effect.

These recommendations seems to have been taken up, but there is still some way to go in defining reliable toxicological effect pathways with validated key molecular events (expression of genes / proteins) that regulate responses. This is despite a number of influential workshops e.g. SETAC Pellston Workshops on Adverse Outcome Pathways (AOPs), papers (e.g. Ankley *et al,* 2010) and the availability of commercial software / databases e.g. Ingenuity Pathway Analysis and NextBio that are continually, and extensively referenced / validated by literature reviews of published studies.

## 2.2 Workshop structure

The workshop was organised around case studies and syndicate discussion sessions where (1) prediction and biomarkers: adverse outcome pathways and MoA, (2) the use of 'omics information in a regulatory (eco)toxicologogy context and (3) dose-response characterisation with the 'omics technologies were discussed. Six case studies were presented as well as a session on future perspectives, systems biology and modelling. The discussions from the syndicate groups were recapitulated in a final plenary session where several recommendations were made and conclusions drawn. A total of 29 scientific experts from industry, academia and governmental agencies participated in the workshop, which was held in Malaga, Spain, on the 25th and 26th February 2013. A list of participants is given in Appendix A, and the programme is detailed in Appendix B. The workshop was limited to participation by selected industry experts and invited external scientists.

## 2.3 Workshop aims and objectives

The aim of the workshop was to review the progress made on the application of 'omics technologies to chemical safety and assess the potential impact of these new technologies on the risk assessment of chemical substances.

In particular the workshop addressed the following points:

- Case studies of the application of 'omics data for risk assessment and regulatory (eco)toxicology.
- Guidance to help to increase the intrinsic value of 'omics data and stimulate its use in (eco)toxicology.
- Impact of 'omics sciences on the risk assessment process.
- Can 'omics data contribute to the elucidation of (1) life stage / subpopulation sensitivity, (2) low-dose effects and (3) the effects of mixtures?

# 3. PRESENTATION SUMMARIES

## 3.1 Prediction and biomarkers: adverse outcome pathways and MoA

### 3.1.1 Using gene signatures to predict adverse outcome pathways in the liver

**J. Christopher Corton**

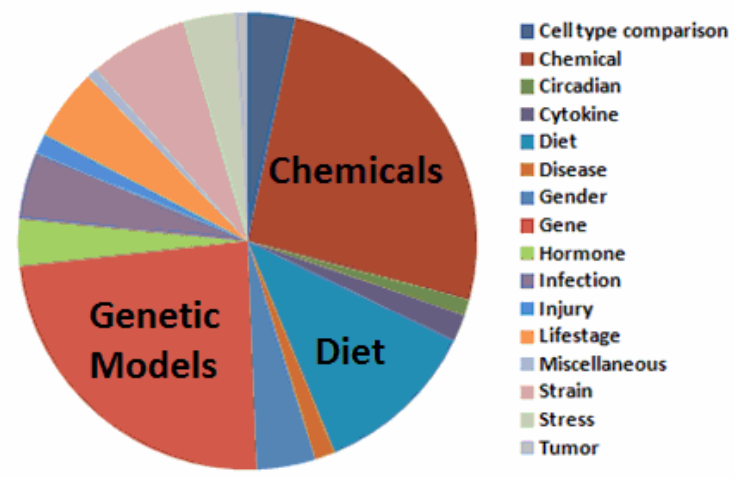
*US EPA, National Health and Environmental Effects Research Laboratory/ORD, Research Triangle Park, NC*

Exposure to many drugs and environmentally-relevant chemicals causes adverse outcomes in the rodent liver. Adverse outcomes such as cancer have been linked to molecular initiating events (MIE) and subsequent key events that define adverse outcome pathways (AOP). Identification of gene sets (signatures) that are predictive of either MIEs (e.g. transcription factor activation) or key events (e.g. cell proliferation) would be useful in quantitative assessment of AOP modulation after chemical exposure. A novel approach to identify signatures predictive of MIEs is discussed here. Manuscripts describing this work are currently being written (Oshida *et al*, in preparation).

Signatures for MIEs were derived by identifying genes that require an intact xenobiotic-responsive transcription factor (TF) for alteration. Signature genes were identified by comparing the microarray profiles of chemically-treated wild-type or corresponding TF-null mice. The signatures included those dependent on PPARalpha, CAR, PXR, AhR, Nrf2, FXR, and PERK as well as hormonally-regulated TFs (glucocorticoid receptor, oestrogen and androgen receptors). Genes were identified that were altered in wild-type but not TF-null mice after exposure to a chemical activator or control treatment in two (FXR, PXR, GR, PERK) or three (PPARα, CAR, and AhR) separate experiments. To pass the filters, genes had to be altered in wild-type but not TF-null mice in 2 out of 2 (PXR, FXR, GR, PERK) or 2 or 3 out of 3 (PPARα, CAR, AhR) comparisons. For Nrf2, genes had to be altered in wild-type but not Nrf2-null mice after exposure to the chemical CDDO-Im and in one or more of the Keap1-null (whole body) or Keap1-null (hepatocyte-specific) versus wild-type comparisons (in the absence of chemical treatment). Gender-dependent genes (including those that are putatively under control of the androgen receptor) were identified in six independent experiments comparing gene expression in untreated wild-type males versus untreated wild-type females. Genes had to exhibit consistent behaviour in at least four of the six comparisons.

In order to test the signatures for prediction of MIEs, an EPA database was constructed from a number of sources including Gene Expression Omnibus (GEO), ArrayExpress, and unpublished experiments. Only 430A or 430 2.0 Affymetrix chips were used in the analysis. Rosetta Resolver was used to identify genes which had a fold-change ≥ |±1.2| and a Benjamini-Hochberg false discovery rate (FDR) of ≤ 0.01. A total of ~800 gene lists (contrasts) were created. A commercially available genomic database (NextBio) was used to determine the similarity between contrasts and the derived signatures. A comparison between the contrasts in the EPA database and those in NextBio revealed an overlap of ~400 contrasts. Non-overlapping contrasts were uploaded into NextBio giving a total of 1933 contrasts. The composition of the resulting database is shown in Figure 1.

***Figure 1: The mouse liver genomic database***



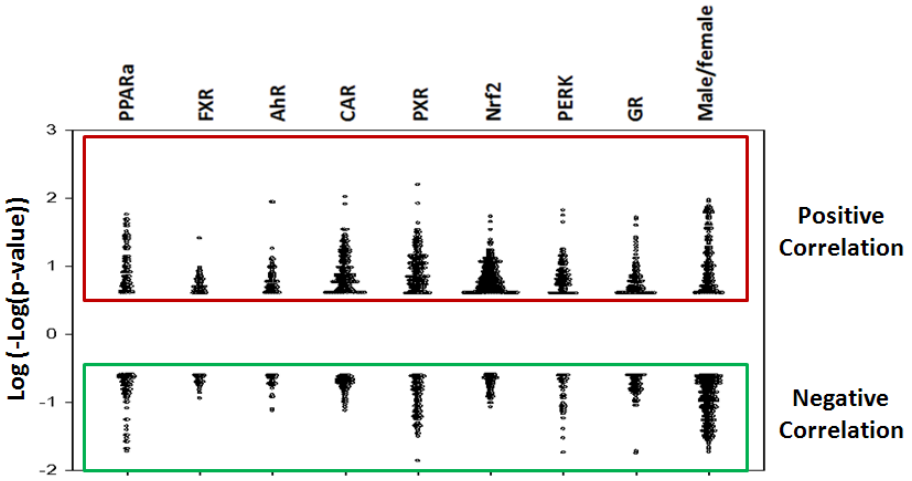

The ~1900 contrasts were categorised by factor being examined. The factors being assessed in mouse liver and mouse primary hepatocyte comparisons included chemical treatment, genetic knockout / knockdown models and other conditions that affect the outcome of hazard studies including diet and life stage.

In NextBio, all contrasts were compared to the signatures using a running Fisher test. The test calculates a p-value of the correlation in expression behaviour in the overlapping genes including an assessment of positive or negative correlation. The cut-offs used for determining whether there was a significant correlation between the contrast and the signature were p-value < 0.0001 (BH corrected) and ≥ 10% overlap between signature and contrasts.

The accuracy of the predictions were assessed a number of ways. 1) The significance of the activation of the signature in the wild-type and the corresponding TF-null mice was examined. In each case, the signature was activated by chemical exposure in wild-type but not the TF-null mice. For example, 8 chemicals and 5 synthetic triglycerides fed to wild-type but not PPARalpha-null mice were positive for the PPARα signature. 2) The behaviour of the known positives and negatives for each signature were examined by principle component analysis (PCA). Using the probe sets and the unfiltered fold changes, each contrast was examined by PCA using the three first principle components. For PPARalpha, AhR, CAR, PXR, GR, Nrf2 and masculinisation / feminisation, each set of signature genes clearly separated the known positives and the known negatives. FXR and PERK were not analysed due to the small number of known positive and negative controls. 3) Based on the behaviour of the known positives and negatives for each signature, the balanced accuracy for PPARalpha, CAR, AhR, GR, Nrf2, masculinisation and feminisation signatures ranged from ~90-98% with most being ≥ 95%. Thus, the signatures accurately predict the activation of the TF and can be used to predict modulation of the MIE under diverse conditions.

The impact of diverse stressors on the TFs in the mouse liver genome was comprehensively examined. The number of contrasts that resulted in activation or repression of the TF is shown in Figure 2.

***Figure 2: Contrasts in the mouse liver database with significant positive or negative correlation to the signatures for the indicated transcription factor. Each dot represents a contrast with positive (above) or negative (below) correlation with the signature for the indicated TF (p-value ≤ $10^{-4}$)***




The MIEs that were most often altered were CAR, Nrf2, PXR and either masculinisation (above) or feminisation (below) of the male / female signature. It was somewhat surprising that many contrasts resulted in suppression of the TF. The chemicals which activated or suppressed the TFs are currently being evaluated but preliminary assessment of the profiles appears to be consistent with known effects of chemical exposure on these TFs. For example, the chemicals that activated PPARalpha include chemicals that are known to activate PPARα including a number of prescribed or experimental hypolipidemic drugs (bezafibrate, C-775146, C-865520, C-868388, ciprofibrate, clofibrate, fenofibrate, WY-14,643), environmentally relevant compounds (DEHP, PFHxS, PFNA, PFOA, PFOS), and a number of other compounds (benzofuran, CDDO-Im, N,N-di-n-propylnitrosamine, galactosamine [GALN], TCDD) that might be activating PPARalpha secondary to increases in fatty acid mobilisation. Suppression of PPARalpha occurred after exposure to chemicals that are known to be cytotoxicants and/or cause inflammation in the liver, consistent with the literature (acetaminophen, bisphenol A, conconavalin A, 3,4-dichlorobenzene-1,2-diol, N-ethyl-N-nitrosourea, lipopolysaccharide, malathion, napthalene, silicon particles, tBuHQ, trovofloxacin). Thus, the analysis allows a comprehensive assessment of a diverse set of stressors and other factors on activity of TFs that play roles in liver cancer and other adverse outcome pathways in the liver.

In conclusion, 1) a gene expression compendium of contrasts from mouse liver or mouse primary hepatocytes was created including extensive annotation of the details of each comparison; 2) a genetic strategy was implemented to derive gene signatures that are useful for identifying chemicals, knockouts, diets, etc. that activate or repress the transcription factor; 3) the signatures were extensively validated using a number of techniques with known positive and negative factors; 4) an assessment of TF activation or suppression across ~1900 comparisons revealed a large number of factors that either activate or suppress the TF including chemicals, knockouts/knockdowns, diet and life stage. Future work will focus on expanding the analysis to other TFs and linking to phenotypic changes allowing better prediction of adverse outcome pathways in the liver.

*This extended abstract does not necessarily reflect the official views or policies of the US EPA. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.*

## 3.1.2 Contribution of 'omics to better understanding modes of action and definition of adversity in toxicological assays

**Marjoke Heneweer**[1]; Jane Botham[2]; Thomas Pfister[3]; Hans Ketelslegers[4]; Lauren K. Markell[5]; David Rouquié[6]; Winfried Steiling[7]; Volker Strauss[8] and Christa Hennes[9]

*[1]Shell International, The Hague, The Netherlands; [2]Syngenta, Bracknell, Berkshire, UK; [3]F. Hoffmann-La Roche, Basel, Switzerland; [4]ExxonMobil, Machelen, Belgium; [5] DuPont, Newark, DE, USA; [6]Bayer CropScience, Sophia Antipolis, France; [7]Henkel, Düsseldorf, Germany; [8]BASF, Ludwigshafen, Germany; [9]ECETOC, Brussels, Belgium.*

### Introduction

Scientifically sound discrimination between adverse and non-adverse effects has always been a key element in the evaluation of toxicological results. ECETOC (2002) addressed this issue in terms of classical biochemical and histopathological endpoints. It was concluded that there was a critical need for harmonised definitions. Furthermore, a generic, structured approach for evaluation of data was needed in order to identify treatment-related effects and to differentiate between adversity and non-adversity (Lewis *et al*, 2002).

However, 'omics technologies are now available to help in the exploration of toxicological MoAs and should contribute to the development of adverse outcome pathways (AOPs).

An ECETOC task force was established to review the current interpretation of adversity through literature and individual views. The aim is to formulate recommendations for the useful integration of these new technologies in toxicological testing. Additionally, the task force analyses the impact of such new data for the definition of adversity and its contribution to a better understanding of Modes of Action (MoAs).

### Adversity in the context of the current testing paradigm

In the current testing paradigm, *in vivo* tests are used to assess effects of chemical exposure on humans. Uncertainty factors are used to convert an assessment of an adverse effect in the animal model to an estimated dose with no adverse effect in humans.

'Omics technologies can contribute to a better understanding of MoA and adversity in both a qualitative and a quantitative manner. The qualitative approach involves exposure of animals to a reference compound and a new compound of interest. After exposure, qualitative differences in gene signatures obtained from the reference compound and the new compound can be observed.

When looking at quantitative applications, dose-response relations can be refined by adding molecular information to data obtained with more traditional methods. This additional molecular information can help with predicting the dose at which the eventual adverse effect of chemical treatment would occur.

Both the qualitative and the quantitative approach aim to predict an adverse outcome without the actual observation of the adverse effect.

## Adversity in the context of toxicity in the 21st century (Tox21)

The focus of Tox21 lies with high throughput *in vitro* assays and short *in vivo* studies to identify MoA. It aims to look at molecular endpoints and to move away from apical responses. Several initiatives have been taken in the light of Tox21, such as MoA/AOPs (OECD), ToxCast, Toxome and others.

***Adverse Outcome Pathway***

An Adverse Outcome Pathway (or MoA) describes a series of events that strongly link two anchors of the pathway. The first one being the initiating event and the second one being the adverse outcome at the organism or population level (Ankley *et al*, 2010). To gain confidence in the Adverse Outcome Pathway (or MoA), Bradford Hill criteria can be used to describe the events included in the pathway. These criteria require good descriptions of the following:

- Postulated AOP/MoA
- Key events
- Temporal association
- Dose-response for key events
- Possible other MoAs
- Strength, consistency and specificity of association of key event and apical endpoint
- Uncertainties / inconsistencies and data gaps
- Assessment and conclusion

It is important to realise that the relevance of each individual criterium depends on the aim of the AOP/MoA investigation.

AOP/MoA investigations can be applied to assess human relevance of observed apical endpoint in animal study or to predict the MoA of an unknown chemical. Furthermore, it can be used to design a strategy for targeted testing or to prioritise chemical testing. Also, grouping and classification and labelling of chemicals can be guided by information obtained from AOP/MoA investigations.

## Learnings and perspectives

- Phenotypic anchoring has been demonstrated and molecular technologies can provide mechanistic information on MoA.
- Shorter-term experiments using molecular technologies can provide predictive effective dose in long-term studies (Thomas *et al*, 2012).
- In order to assess relevance of postulated MoA, Bradford Hill criteria play an important role in identifying a clear causal link between 'intermediate' molecular key events and apical endpoint.
- Regarding ToxCast, use of 'omics technologies may result in relatively high probability of identifying hazardous properties of which the relevance to human health risk is not fully understood.
- Establishing a clear link between currently accepted regulatory studies and molecular technologies will contribute to acceptance by scientific and regulatory community.
- Task is large, therefore efforts need to be global and well-coordinated.

### 3.1.3 Applicability of next generation sequencing in toxicogenomics for identifying adverse outcome pathways and modes of action

**Jos Kleinjans**

*Maastricht University, The Netherlands*

Whole-genome transcriptome measurements are pivotal for characterising molecular mechanisms of chemicals and predicting toxic classes, such as genotoxicity and carcinogenicity, from *in vitro* and *in vivo* assays. In recent years, deep sequencing technologies have been developed that hold the promise of measuring the transcriptome in a more complete and unbiased manner than DNA microarrays. We applied this RNA-seq technology for the characterisation of the transcriptomic responses in HepG2 cells upon exposure to benzo[a]pyrene (BaP), a well-known DNA damaging human carcinogen. We demonstrate that RNA-seq detects *circa* 20% more genes than microarray-based technology but almost threefold more significantly differentially expressed genes. Functional enrichment analyses show that RNA-seq yields more insight into the biology and mechanisms related to the toxic effects caused by BaP, i.e. two- to fivefold more affected pathways and biological processes (van Delft *et al*, 2012).

Additionally, we demonstrate that RNA-seq allows detecting alternative isoform expression in many genes, including regulators of cell death and DNA repair such as TP53, BCL2 and XPA, which are relevant for genotoxic responses. Moreover, potentially novel isoforms were found, such as fragments of known transcripts, transcripts with additional exons, intron retention or exon-skipping events. The biological function(s) of these isoforms remain for the time being unknown. Finally, we demonstrate that RNA-seq enables the investigation of allele-specific gene expression, although no changes could be observed.

Our results provide evidence that RNA-seq is a powerful tool for toxicology, which, compared with microarrays, is capable of generating novel and valuable information at the transcriptome level for characterising deleterious effects caused by chemicals.

## 3.2 Use of 'omics information in a regulatory (eco)toxicology context

### 3.2.1 Use of 'omics information in risk assessment of new food safety risks: Proteomic effects induced by 3-MCPD and its dipalmitate ester in rat liver: is there a common mode of action?

Stefanie Sawada, Axel Oberemm and **Alfonso Lampen**

*BfR, Berlin, Germany*

Thermal treatment of fat-containing foodstuff in the presence of salt leads to formation of 3-monochloropropane-1,2-diol (3-MCPD) and its fatty acid esters. Results from rat studies indicate liver toxicity and a carcinogenic potential of 3-MCPD. Similar, but reduced histopathological effects were observed for 3-MCPD palmitic esters in a recent rat 90-days feeding study. In order to obtain first insights into toxicity mechanisms of 3-MCPD and its esters, a proteomic approach was initiated in a 28-days repeated-dose feeding study with male Wistar rats. Animals received equimolar doses of 3-MCPD (10 mg/kg body weight) and 3-MCPD-dipalmitate (53 mg/kg body weight), which did not cause clinical signs and clear pathological effects. Additionally, a lower dose of 13.3 mg/kg body weight of dipalmitate was applied, which did not induce any visible alterations. Liver samples were snap-frozen and analysed using two-dimensional gel electrophoresis / MALDI mass spectrometry. Ingenuity Pathways Analysis was used for data mining. In all treatment groups characteristic toxicologically relevant processes and pathways were induced, including fatty acid metabolism, Nrf2-mediated oxidative stress and LPS/IL1-induced inhibition of RXR function. Generally, molecular effects were more distinctive in the 3-MCPD dipalmitate treatment groups. Notably, downregulation of several isoforms of glutathione-s-transferase as well as peroxisome proliferator-activated receptor α was observed in the livers of animals treated with 3-MCPD dipalmitate only. General results indicate a similar toxicity of 3-MCPD and its dipalmitate with regard to the induction of oxidative stress. However, esters could potentially induce specific mechanisms of liver toxicity, which may contribute to different long-term effects compared to 3-MCPD.

The results and conclusions of this new 'omic study will be discussed in the context of their impact in the actual risk assessment of new contaminants in food safety.

## 3.2.2 Purpose oriented integration of genomic data in regulatory risk assessment

**M.E. (Bette) Meek**

*McLaughlin Centre for Population Health Risk Assessment, University of Ottawa, Canada*

Frameworks to systematically consider the weight of evidence for hypothesised modes of action (key events) in animals and their relevance to humans have promoted transparency in hazard characterisation and dose-response analysis as a basis for estimation of population risks associated with exposure to chemicals. These frameworks also encourage early consideration of mechanistic data to enable greater predictivity in risk assessment, facilitate peer input and review and identify critical research needs. Iterative use of such frameworks has been helpful in integrating research and assessment and in transitioning to the use of data from more progressive and predictive mode of action based testing strategies, including that from genomic profiling.

The World Health Organisation / International Programme on Chemical Safety mode of action / human relevance (MoA/HR) framework[1] has recently been updated to reflect evolving experience in its application and to incorporate recent developments in toxicity testing and non-testing methods. While the underlying principles have been retained, the scope of the framework has been extended to integrate information on different levels of biological organisation and to reflect evolving experience in a much broader range of potential applications. These applications are relevant not only to full risk assessment for individual chemicals but to evolving methods for priority setting and assessment to meet increasing demands to more efficiently and accurately assess and manage large numbers of substances.

They include read-across and assessment of groups of chemicals and combined exposures, based on greater reliance on genomic data. Envisaged broader application is illustrated in an integrative and iterative roadmap to address needs for assessment identified in formal problem formulation, as a basis to tailor appropriate extent of MoA/species concordance analysis. The roadmap, problem formulation and framework are iterative in nature, with feedback loops encouraging continuous refinement of 'fit for purpose' testing strategies and risk assessment.

The relationship between mode of action and the more recently defined 'adverse outcome pathway' (AOP) is also clarified: conceptually, the terms are synonymous, with both representing division of the path between exposure and effect into a series of key events (including early molecular initiating events) for both individuals and populations, though mode of action does not necessarily imply adversity of effect.

Broader application of the modified mode of action framework is considered in two contexts, including one for which it was originally developed, where the effects of chemical exposure are known (i.e. when, as a result of problem formulation, there is a desire to perform a mode of action / human relevance analysis for an observed toxicological effect). The modified framework can also be applied in hypothesising effects resulting from exposure to a chemical that is, with information on putative key events in established modes

[1] Meek ME, Boobis A, Cote I, Dellarco V, Fotakis G, Munn S, Seed J, Vickers C. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. *Toxicol Appl Pharmacol (Submitted)*.

of action from appropriate *in vitro* or *in silico* systems and other lines of evidence to predict and assess the likelihood of a potential mode of action and consequent effects. The implications of the considerable experience acquired in application of the framework in addressing documented (adverse) effects to inform the more limited knowledge base in these more predictive applications are addressed. This is illustrated in various case examples including the use of mode of action analysis in prioritising substances for further testing, in guiding development of more efficient testing strategies and in identifying critical data gaps and testing strategies in read-across. In this vein, mode of action considerations should inform further development of research strategies and data generation methods, as well as the development of biomarkers.

One of the case examples illustrates the potential contribution of well-designed genomic studies to consider species concordance and dose-response analysis. Cacodylic acid (dimethylarsinic acid) is a pesticide that causes dose-related increases in the incidence of bladder tumours at highest dose-levels in rats, but not mice. The parent compound undergoes reductive metabolism to a toxic metabolite, and observed damage to urinary epithelial cells correlates with this pathway. The levels of toxic metabolite are significantly increased at doses causing cytotoxicity, proliferative regeneration and bladder tumours. The weight of evidence from critically evaluated data from a wide range of assays both *in vitro* and *in vivo* indicates that the parent compound is not mutagenic, but that the active metabolite is clastogenic at high concentrations or doses. The concentration-response relationships for cytotoxicity associated with the active metabolite were similar in *in vitro* studies in bladder cells of rats and humans.

Application of considerations such as dose-response and temporal concordance (determined from benchmark dose-analyses of a range of *in vivo* studies of different durations), consistency / specificity and biological plausibility supports hypothesised key events in the mode of action including reductive metabolism and cytotoxicity and proliferative regeneration leading to bladder tumours. Qualitative and quantitative concordance analysis based on relevant kinetic and dynamic data indicated that these effects are relevant to humans and that quantitative differences would most likely be related to extent of delivery to the target organ of the toxic metabolite and variations in sensitivity of the bladder to damage induced by this metabolite. Chemical-specific adjustment factors could then be derived from a physiologically based pharmacokinetic model incorporating metabolic rates, enzyme affinities and distribution based on *in vitro* human data supported by *in vivo* data and quantitative reflection of the similarity in sensitivity to the active metabolite between the rat and human bladder in *in vitro* studies.

While the mode of induction of bladder tumours was deduced principally on the basis of key cytological and biochemical events in traditional mechanistic studies, this information was well supported by genomic data from experiments designed to address critical aspects of both the mode of action and species concordance analysis. The results of genomic studies indicated that similar networks were altered in rat and urothelial cells exposed to the active metabolite at doses similar to those in urine at which tumours were observed in the critical bioassays.

Similarly, the potential contribution of genomic data to read across for an early tier assessment of a new compound in a class for which the mode of action for critical effects is well documented is addressed.

## 3.2.3 A vision for modernising environmental risk assessment

**Daniel L. Villeneuve**[1] and Natàlia Garcia-Reyero[2]

[1]*US EPA, Mid-Continent Ecology Division, Duluth, MN*

[2] *Institute for Genomics, Biocomputing and Biotechnology, Mississippi State University, Starkville, MS, USA*

In 2007, the US National Research Council (NRC) published a Vision and Strategy for [human health] toxicity testing in the 21st century. Central to the vision was increased reliance on high throughput *in vitro* testing and predictive approaches based on mechanistic understanding of toxicity pathways as a more cost effective and efficient approach to chemical hazard assessment. The idea is to observe the initiation of toxicity rather than directly observing apical toxicity outcomes in whole organism toxicity tests. While this paradigm can potentially be applied to support either human health or ecological toxicity testing and hazard assessment, there are two broad scientific needs required to support this vision. First, there is a critical need to develop adverse outcome pathway knowledge, which defines scientifically-credible predictive linkages between responses or perturbations observed at low levels of biological organisation (e.g. molecular, biochemical, cellular), and adverse outcomes that they at trigger at a level of biological organisation relevant to risk assessment (e.g. the level of organs and organ systems for human health; the level of populations and ecosystem services for ecological health). Second, there is a need to develop models and extrapolation tools that can support quantitative extrapolation of responses measured at low levels of organisation or in highly simplified systems into an estimated probability of adverse outcome under different exposure scenarios (e.g. concentrations and durations of exposure). In the case of ecological risk assessment, there is an equally critical need for species extrapolation tools. 'Omics has an important role to play in both supporting the development of adverse outcome pathways and extrapolation tools, and in implementation of this new testing paradigm. Given the open-ended or unsupervised nature of 'omics analyses, 'omics are well suited to aid discovery of novel adverse outcome pathways and/or adaptive mechanisms which influence concentration-duration-response relationships. Due to the breadth of potential pathways that can be probed using 'omics approaches, on a target organ basis, 'omics can provide a breadth of pathway coverage on par with that of nascent high throughput *in vitro* screening batteries (e.g. Toxcast™). Thus, 'omics analyses themselves can be a viable source of high content screening data. Finally, 'omics approaches are uniquely suited to provide critical insights needed to understand and predict the response of complex systems (i.e. those composed of multiple interacting cell types, tissues, and organs) to multiple simultaneous or overlapping perturbations. Such insights are critically important for extrapolation of molecular screening results to effective predictions of outcome.

*This abstract does not necessarily reflect the official views or policies of the US EPA.*

## 3.3 Dose-response characterisation with the 'omics technologies

### 3.3.1 Application of transcriptomics in dose-response assessment of environmental chemicals

**Jason C. Lambert**[1] and Russell S. Thomas[2]

[1]*US EPA, National Center for Environmental Assessment, Cincinnati, OH*

[2]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC, USA*

Environmental media such as water, soil, and air could potentially contain hundreds of chemical contaminants with limited or non-existent toxicity data. Further, there are thousands of chemicals currently in commerce for which risk to human health has not yet been characterised. However, human exposure to a diverse array of chemicals commonly found in environmental media has been confirmed by a host of biomonitoring efforts (e.g. National Health and Nutrition Examination Survey in the United States; http://www.cdc.gov/nchs/nhanes.htm). Given the number of existent toxicologically uncharacterised chemicals and the rate of new chemical development, the capacity of the traditional human health risk assessment (HHRA) paradigm (NRC, 1983) is overwhelmed. This is due in large part to the database typically necessitated for comprehensive evaluation of health hazards and dose-response associated with exposure to chemicals. For many environmental chemicals there is often a paucity of human and/or experimental animal bioassay data available to support decisions in a HHRA context. Development of innovative assessment approaches is critical to better support chemical prioritisation, risk assessment, and remediation of contaminated environments across the globe. While several alternative data methodologies and approaches have been proposed over the past decade to address data gaps in risk or safety assessment of chemicals, a critical tenet of HHRA, quantitative dose-response assessment, has not yet received the attention that is warranted to meaningfully advance practical application. To this end, we have evaluated dose-response concordance between traditional apical (phenotypic) toxicity endpoints and perturbations in transcriptional activity for cancer and non-cancer effects, with the goal of identifying the potential for use of transcriptomic data in 'quantitative' chemical risk assessment. In our approach, traditional toxicological endpoints such as histopathology and organ weight changes and gene expression microarray analyses were performed in tandem on target tissues of mice exposed for 13 weeks to multiple concentrations of five different chemicals that were previously shown to be positive in 2-year rodent cancer bioassays. The 13-week histopathological and organ weight changes and, the tumour incidences from the original cancer bioassays were analysed using standard benchmark dose (BMD) methods to identify non-cancer and cancer points-of-departure, respectively. The dose-related changes in transcriptional activity following 13-weeks of exposure were also analysed using a BMD approach and the responses were grouped based on biological processes or signal transduction pathways. A comparison of transcriptional BMD points-of-departure with those for traditional non-cancer and cancer apical toxicity endpoints showed a high degree of correlation for specific cellular biological processes or signal transduction pathways (Thomas *et al*, 2011; 2012). A common concern expressed by participants during the third Málaga workshop was a lack of characterisation of temporal concordance between alterations at a molecular or cellular level and health or environmental outcomes. Our group has recently investigated this aspect in our short-term *in vivo* transcriptomics approach. Correlations between potential points-of-departure based on traditional apical (phenotypic) toxicity endpoints or

perturbations in transcriptional activity for cancer and non-cancer effects in target tissues of rats were conserved across a time course ranging from 5 days up to 13 weeks of chemical exposure (Thomas *et al*, 2013). This suggests that transcriptomic data derived from shorter-term *in vivo* exposures may be suitable for informing hazard and dose-response assessment of chemicals. Collectively, our work demonstrates positive dose-response correlations between traditional apical toxicity endpoints and transcriptional alterations across four target organs (bladder, liver, thyroid, and lung), multiple modalities of exposure (oral gavage, feed, and inhalation), and both sexes of two different species (mice and rats). While it is acknowledged that more chemicals need to be tested in our approach, the observations to date suggest that data derived from transcriptional analyses could inform non-cancer and cancer human health risk assessment when other data are lacking.

*This abstract does not necessarily reflect the official views or policies of the US EPA.*

### 3.3.2 Dose and time genomic responses to reproductive toxicants

**Aldert H. Piersma**

*RIVM, Bilthoven, The Netherlands*

The interpretation of alternative assay results in reproductive toxicology is hampered by the complexity of the models used and uncertainties about their biological applicability domain. Relatively apical end-points such as dysmorphogenesis in assays such as, the zebrafish embryo test and rodent whole embryo culture test, as well as cell differentiation in embryonic stem cell test, are not easily extrapolated to the *in vivo* counterpart i.e. pregnant animals. Furthermore, there is a level of subjectivity in current scoring systems, which usually involves expert judgment. The introduction of 'omics technologies allows a more objective assessment of effects. In addition, it provides mechanistic information about effects of test compounds that can potentially be used as a more informative basis for extrapolation to the *in vivo* situation and to hazard and risk in man. We have performed various concentration-response studies in different *in vitro* models for developmental toxicity that illustrate added value of the multiple concentration design and functional pathway analysis for understanding compound effects. We also showed that 'omics signatures for classes of chemicals can be used for potency ranking that is reminiscent of the *in vivo* situation. Challenges appearing are the question of adaptive versus adverse gene expression responses, and the opportunities of single gene groups as biomarkers of embryotoxicity versus adverse outcome pathway approaches that may offer a deeper insight into mechanisms of action. Although much of this work is as yet exploratory, it is anticipated that in the long run such approaches may contribute considerably to mechanistically informed hazard and risk assessment of chemicals and drugs.

- Dose-response provides crucial additional information about compound response as compared to single concentration data.
- Challenges include discrimination between adaptive and adverse responses (effect size and types).
- Pathway-defined gene expression responses ('fingerprints') are superior to single gene expression responses for data interpretation.
- Understanding biological relevance is more important than mathematical prediction (e.g. for extrapolation to man).
- Combined assays (battery approaches) may enhance information level over individual assays.

## 3.3.3 Exposure assessment using 'omics technologies

**Timothy W Gant**

*Centre for Radiation, Chemical and Environmental Hazards, Public Health England, Harwell Science Park, Oxfordshire, UK.*

'Omic technologies now have an established place in characterising adverse outcome pathways (early departures from normal homeostasis) resulting from chemical exposure that can act as indicators of a future pathophysiology. In doing so these technologies, particularly metabonomics, have delivered molecular epidemiology, where adverse outcome pathways rather than pathophysiology act as the outcome event for association with an exposure. In a similar manner the 'omic technologies have been extensively used in hazard identification and characterisation, and discernment of mechanism/modes of toxicity. To date though high throughput 'omic technologies have not had a large role to play in the characterisation of exposure. Now two types of omic technologies offer the possibility of exposure assessment; mass spectral methods for proteins and high throughput sequencing methods for DNA.

Adduct formation on DNA has been extensively studied as part of hazard characterization and as a mechanism that can lead to neoplasia. However as a method it has not been extensively employed in exposure biology. However the rapid development of mass spectrometry methods has made the large scale determination of these adducts possible. Adducts formed on DNA or protein can act as a marker of exposure. This means that for chemicals which form adducts on DNA or proteins this technology has the potential of acting as a high throughput method of exposure assessment. The time frame in which DNA or protein adducts can act as historical markers of exposure will depend on the adduct half-life or the half-life of the macromolecule to which it is attached.

Epigenetics, defined as DNA alteration that excludes base sequence change, can lead to the alteration of gene expression and potentially altered phenotype. This is an area of biology that is developing rapidly and impacting on hazard characterisation; it also has the potential to contribute to the assessment of exposure. There are three generally recognised areas of epigenetic modification, modification of the DNA cytosine base by a methyl (or related) group in the 5' position, modification of the tails of the histone proteins around which DNA is wound (acetylation in particular) and alteration of expression on miRNA species. The latter of these mechanisms involves the determination of expression of small non-coding regulatory RNA species the expression of which may be controlled by the former two mechanisms. miRNA have a role in controlling protein translation from the mRNA, and are thought to be important in modulating the transfer of epigenetic marks from one DNA strand to another. They can directly suppress the transcription of mRNA from protein coding genes.

In 2008 an ECETOC meeting set the scene for using 'omic technologies in retrospective assessment of exposure (ECETOC, 2009). A focus of this meeting was the use of macromolecule alteration as a biomarker of historic exposure such as DNA methylation and adducts on both protein and DNA. Since that meeting studies in monozygotic twins have started to show that DNA methylation markers can diverge during life even for these genetically identical individuals. This change is hypothesised to be due to environmental exposure to chemical hazards. Some molecular epidemiological studies have started to identify a relationship between alteration of DNA methylation patterns and environmental chemical exposures; for example arsenic.

Additionally, certain changes in epigenetic markers lead to altered gene expression and this may be a first point of departure from normal homeostasis towards an adverse outcome.

The very rapid development of high throughput sequencing methods has now reached the point where these methods can be used to quickly determine differential methylation on DNA while mass spectrometry can be used to assess differential histone methylation after chemical exposure. Both of these methods have been applied to show methylation changes on DNA and histones following arsenic exposure and indicate the potential utility of these 'omic methods in determining historical exposure (Pilsner *et al*, 2012; Chu *et al*, 2011).

These recent studies indicate that 'omic technologies are moving out of hazard identification and characterisation, and show potential for the assessment of exposure, and perhaps most pertinently historical exposure. Though possibilities have been demonstrated, a lot more data will need to be collected to show the true potential of the evaluation of epigenetic modification of DNA and protein by high throughput 'omic methods for exposure assessment.

### 3.3.4 Identifying No Observed Effect Levels (NOEL) and No Observed Adverse Effect Levels (NOAEL) through metabolomics and a sensitivity analysis relative to classical regulatory toxicity studies in rats

G. Montoya[1]; V. Strauss[1]; E. Fabian[1]; H. Kamp[1]; W. Mellert[1]; T. Walk[2]; R. Looser[2]; M. Herold[2]; G. Krennrich[1]; E. Peter[2] and **B. van Ravenzwaay**[1]

[1]*BASF SE, Ludwigshafen, Germany*

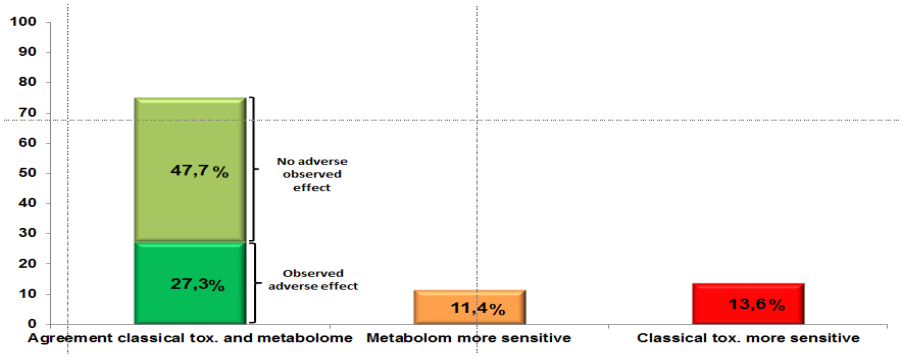
[2]*Metanomics, Berlin, Germany*

With the introduction of 'omics into toxicology questions have been raised concerning the sensitivity of these methods relative to classical regulatory toxicology studies. BASF's Experimental Toxicology and Ecology and metanomics have developed the MetaMap®Tox database which contains more than 500 reference compounds and their effects based on observations from rat studies (OECD 407 design [OECD, 1995]). The database combines the toxicological information of the substance with the plasma metabolome data obtained after 7, 14 and 28 days of treatment.

As we now frequently include metabolome analysis in regulatory studies, we have obtained a data set, which includes metabolome data at toxicological NO(A)EL doses. To obtain a measure of sensitivity of metabolomics versus classical toxicology, we have used these data to analyse metabolomics changes at toxicological NO(A)EL doses. We have done this using the following criteria and definitions. The toxicological NOAEL: (according to US EPA) an exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered as adverse, or as precursors to adverse effects. The metabolomics NOAEL: a consistent pattern of change associated with an adverse effect (ECETOC, 2008), where at least 5% of the parameters are statistically significantly changed. In MetaMap®Tox we have defined more than 100 of these patterns.

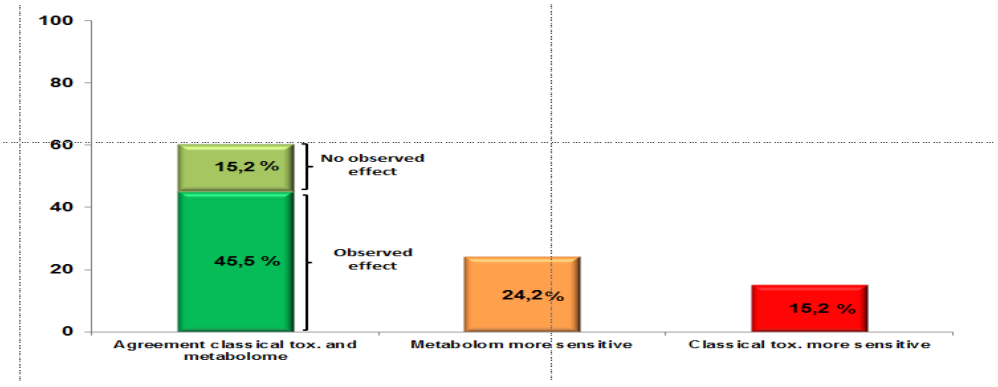
We have analysed 44 individual cases. Results show that in 75% of the cases sensitivity appears to be equal: i.e. toxicological effects are associated with consistent metabolome changes (according to the definition above), or there is an absence of both toxicological and metabolome changes. In 11% of the cases metabolomics was more sensitive than classical toxicology. In 14 % of the cases classical toxicology was more sensitive than metabolomics. Increased metabolome sensitivity was frequently associated compounds inducing a moderate to slight increase in liver weights (which were considered as being non-adverse). In cases were the classical toxicity study was more sensitive, this was two times associated with the lack of a consistent metabolome pattern in the database (selective Sertoli cell toxicity or crystal formation in the urinary bladder) (See Figure 3).

***Figure 3: A sensitivity analysis of metabolomics versus classical toxicological studies based on the no observed adverse effect level***




In a second analysis we compared the no observed effect level (NOEL) obtained in toxicological studies and the metabolome analysis. The NOEL definition being: an exposure level at which there are no statistically or biologically significant increases in the frequency or severity of any effect between the exposed population and its appropriate control for classical toxicology. For metabolomics: 5% or less of the parameters are statistically significantly changed. A total of 33 individual cases were analyses. Concordance between NOEL levels was noted in 61% of the cases. In 24% of the cases metabolomics was more sensitive, in 15% of the cases classical toxicology was more sensitive (See Figure 4).

***Figure 4: A sensitivity analysis of metabolomics versus classical toxicological studies based on the no observed effect level***




Comparing the NOAEL and NOEL evaluations, it can be seen that there is a better concordance if the NOAEL criteria are used. The reason for this is that according to the ECETOC criteria 'omics changes should only be considered as real if they are associated with a consistent pattern (pre-defined using reference substances with a known adverse mode of action). This requirement allows for the establishment of a biological plausible association, rather than relying only on a statistical association. The use of the ECETOC criteria for metabolomics therefore is highly recommendable.

## 3.4 Case studies

### 3.4.1 Case 1: Robustness and reproducibility of toxicogenomics-based human *in vitro* assays

**Raffaella Corvi** and CarcinoGENOMICS partners

*Systems Toxicology Unit, European Union Reference Laboratory for Alternatives to Animal Testing (EURL-ECVAM), IHCP, Joint Research Centre of the European Commission, Ispra, Italy*

During the last decade the field of toxicogenomics has expanded rapidly. However, to date there is still limited experience with the validation of toxicogenomics data from *in vitro* systems, especially with regard to the evaluation of their reproducibility. CarcinoGENOMICS, a project of the European Union, offered an excellent platform for the investigation of reproducibility of the 'omics-based tests in general and for the assessment of various bioinformatics approaches employed to tackle this issue. The major goal of carcinoGENOMICS was to develop and select appropriate 'omics-based *in vitro* methods for assessing the carcinogenic potential of compounds. The idea was to design a battery of mechanism-based *in vitro* tests covering major target organs for carcinogenic action e.g. liver, lung and kidney. 'Omics responses (genome-wide transcriptomics as well as metabolomics) were generated following exposure to a well-defined set of model compounds, namely genotoxic carcinogens, non-genotoxic carcinogens and non-carcinogens.

Among the several test methods evaluated in the initial phase of the project two test methods were identified as having the highest potential for distinction of genotoxic, non-genotoxic and non-carcinogenic substances. Both test methods are based on human cell lines, HepaRG for the liver and RPTEC/TERT1 for the kidney, which is in agreement with current thinking that humanised models should be more predictive for human toxicity than models based on animal cells. Among others, the objectives of the study presented here were: a) to preliminarily assess in a blinded inter-laboratory study test method transferability and between-laboratory reproducibility by testing three coded chemicals in three laboratories for each test model using the same agreed standard operating procedures (SOPs) and controlled conditions, and b) to develop dedicated bioinformatics tools to serve as a basis for future validations of 'omics-test methods.

To assess transferability and reproducibility 3 coded chemicals were tested by 3 laboratories in each test model. The lead laboratories also tested 15 additional chemicals (5 genotoxic and 5 non-genotoxic carcinogens, 5 non-carcinogens). For the HepaRG model the experimental design was: one dose testing (IC10 at 72 hours), 2 time points (24 and 72 hours), and 3 replicates. For the RPTEC/TERT1 kidney model, the experimental design was: one testing dose (IC10 at 72 hours), 3 time points (6, 24 and 72 hours), and 3 replicates.

Several bioinformatics approaches were identified to judge data reproducibility. These approaches ranged from evaluation of response gene lists, correlation analyses to multivariate statistical methods such as support vector machine classification and analysis of variance. Independently from the bioinformatics approaches applied, the HepaRG model generated reproducible transcriptomics results, with the exception of a single experiment in one laboratory. Regarding the RPTEC/TERT1 model, two laboratories showed

highly reproducible results, while one laboratory generated results which did not appear to be reproducible. This outcome was in line with experimental observations, due to problems related to the culturing of cells in one of the laboratories (much slower cell growth in comparison to the other laboratories). Interestingly, despite these results the three coded chemicals were classified in the correct classes by all laboratories, indicating that the prediction model is quite robust. Overall, these results present a proof of concept that such *in vitro* models may be considered suitable to be used for transcriptomics analysis. Moreover, the demonstration that the different bioinformatics tools led to consistent results and do not seem to be a major source of result variability is very reassuring, especially in view of regulatory use of transcriptomics data. The publication of the description of the different bioinformatics approaches used to evaluate the reproducibility of transcriptomics data will represent a guide for future users and set the basis for the validation of high-content test methods.

### 3.4.2 Case 2: TXG-Data to clarify relevance of neoplastic effects in rodent life time bioassays: a case study

**Roland Frötschl**

*BfArM, Bonn, Germany*

A selective 5-HT receptor agonist significantly increased the incidence of neoplastic lesions in intestinal mucosal epithelium in mice and caused hyperplasia in the intestinal mucosal epithelium in rat. The substance was negative in a standard core battery of genotoxicity tests and also negative in further follow up testing in *in vivo* comet assay and *in vitro* UDS test. It was concluded that the substance was not DNA reactive and also did not indirectly induce genotoxicity. Carcinogenic effects were seen as a non-genotoxic mechanism. However there was no convincing explanation for the mode of action for the carcinogenic effects observed. Carcinogenic effects were seen in high dose animals only but hyperplasia was observed as early as 14 days after start of treatment. There were no reliable local tissue exposure data and calculation of margin of exposure was limited to plasma levels only. The favoured hypothesis of diamine oxidase (DAO) inhibition as cause for hyperplasia and carcinogenic effects was not supported by data as no increase in polyamine levels was measured in intestinal mucosa and sufficient data supporting other hypothesis like chronic inflammation in mucosal epithelium due to local irritation of high doses of drug substance in intestinal mucosa did not exist.

Based on the data available assessment of clinical relevance of the carcinogenic effects in mice was not possible and additional data were needed to identify the mode of action for carcinogenic effects and enable a reliable weight of evidence approach and risk assessment.

To clarify the mode of action for carcinogenic effects in rodents a follow up testing strategy was pursued including short and medium-term repeated dose testing in mice and rat with toxicogenomic analyses of the jejunum, the major target organ of mucosal epithelium hyperplasia in mice and rat and carcinoma in mice. The main aims of the toxicogenomic study were analyses of gene expression changes to clarify the mode of action for carcinogenic effects and to investigate dose-dependence of gene expression changes for the existence of a NOAEL on gene expression level.

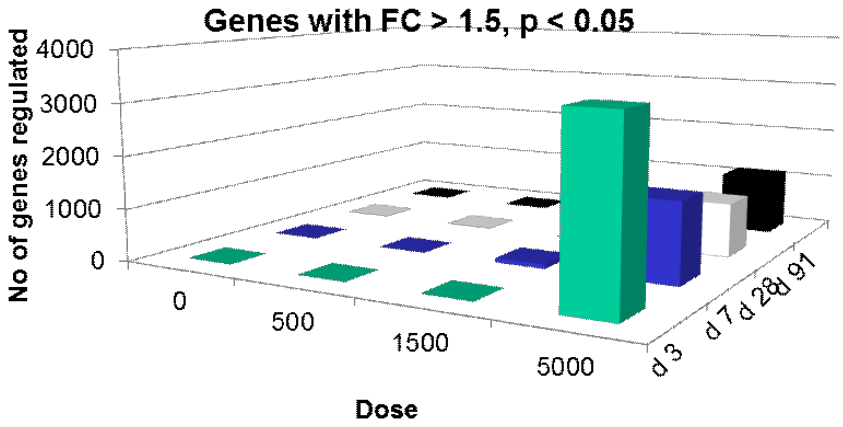
A 13-week repeated dose study in mouse and a 4 week repeated dose study in rat were conducted. Studies were performed as in feed studies to avoid any side effects that might be caused by gavage with daily dosing.

For the mouse study, 10 male mice were treated for every treatment and sampling time point. Treatment groups were dosed either with the active pharmaceutical ingredient (API) with 500, 1500 and 5000 fold the human dose based on body surface equivalence calculations or with vehicle control. For each dosing and control group 5 sampling time points were defined with sampling at day 0, 3, 7, 28, and 91. An additional oral treatment substudy was performed to investigate for short-term transcription kinetics. For this substudy oral treatment was via gavage to get exact treatment times and sampling points. For each sampling point 10 animals were treated. Sampling points were at 0, 1, 3, 6, 12, 27, and 51 h. Animals were treated at 0, 24, and 48 h. There was one vehicle control and one treatment group treated with the API with 750 fold the human dose based on body surface equivalence calculation or with vehicle control. Jejunum liver and colon tissues

were collected from every animal. Liver and colon samples were collected as backup. RNA was isolated from all jejunum samples and processed for hybridisation using Affymetrix mouse whole genome arrays (MOE 430 2.0). Data analysis was performed with GeneSpring software package. Expression profiles were first analysed by principle component analysis to identify outliers. Array results for each time point were normalised to the median of the respective control arrays. Gene lists were then stratified by setting a cut off value of raw expression signals to > 50 to filter for expressed genes and including only genes with present call in at least two thirds of repeat experiments. Further analysis of resulting gene lists was performed by one way ANOVA with a Benjamini and Hochberg false discovery rate test with $p < 0.05$. Then only genes with a fold change of ≥ 1.5 compared to controls were considered as significantly regulated.

Results of the short-term transcription kinetic study clearly show a strong early effect on gene expression with a quick adaptive response. At 3 hours the number of genes regulated reaches its peak with almost 2000 genes regulated significantly, followed by a constant decrease in number of regulated genes to around 530 at 51 hours. In the 13-week study the number of genes significantly regulated was highest with nearly 3400 genes for the high dose at the earliest time point of 3 days (Figure 5).

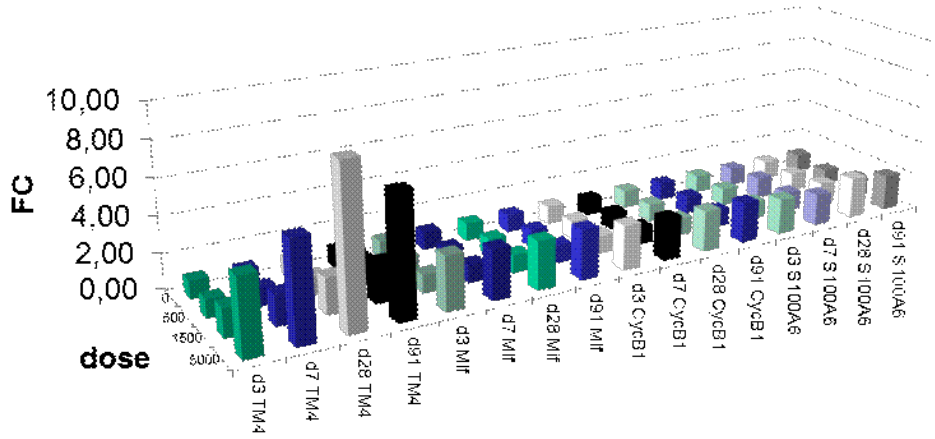
***Figure 5: Expression profile kinetics in mice over 13-week treatment. Sampling time points at days 3, 7, 28, 91 (d3, d7, d28, d91)***




At the low dose no significantly regulated genes were detected at any time point and for the mid dose a relatively low number of genes was found significantly regulated after 3 and 7 days of dosing only. After 28 or more days of dosing only in highest-dose group around 1000 genes remained significantly regulated whereas at lower doses gene expression had returned to control level. Genes regulated at the measured time points were analysed for pathways significantly regulated with the tool DAVID (http://www.david.abcc.ncifcrf.gov) (Huang *et al*, 2009a,b).

Among the genes regulated mostly genes belonging to the Gene Ontology category (GO) cell growth and proliferation, nucleic acid and protein metabolism, and cell stress remained up regulated at all time points indicating a mechanism of chronic cell injury and regenerative cell proliferation as a significant adverse effect of high doses of the API on mucosal epithelium in jejunum. Gene expression alterations were clearly dose-dependent with a clear NOAEL at the low dose (Figure 6).

***Figure 6: Expression profile kinetics of selected proliferation related genes in mice over 13-week treatment. TM4 – Transmembrane 4 superfamily member 4, Mif – macrophage migration inhibition factor, CycB1 – CyclinB1, S100A6 – calcyclin***




Comparing these expression data with histopathology of the jejunum mucosa, gene expression changes were stable also at 28 days plus of treatment correlated with morphological changes (induction of hyperplasia) in the mucosa. Therefore histopathology and gene expression confirmed the NOAEL for tissue alterations observed in the chronic toxicology studies.

In conclusion, chronic cell injury and regenerative cell proliferation as a significant adverse effect of high doses of the API on mucosal epithelium in jejunum should be considered to be a plausible reason for hyperplasia and carcinomas in intestinal mucosa.

When analysing genes involved in polyamine metabolism only DAO was downregulated efficiently for up to three days at the high dose, whereas all other polyamine metabolism genes remained stable or were upregulated including polyamine oxidase. This may also probably be the reason for not finding increased polyamine levels in mucosal tissues. However these were only short-term effects and expression levels of polyamine metabolism genes were back to control levels after 28 days of treatment. Additional studies on polyamine levels in intestinal mucosa showed no increase further confirming the expression data. Therefore expression profiling did not confirm DAO inhibition as a long-term effect and also not as a probable cause for mucosal hyperplasia via increase in tissue polyamine levels.

The results of the 13-week repeated-dose mouse study were confirmed by the results of the rat study. As expected from the less severe effects seen in the two-year carcinogenicity study in rats compared to mice the effects of the expression profiling were less pronounced in the rat than in the mouse study.

All additional *in vitro* and *in vivo* follow up studies supported the conclusion of expression profiling studies that local chronic cell stress and injury with induction of regenerative proliferation might be the most probable cause of hyperplasia and carcinogenic effects in rodent intestinal mucosa.

In conclusion, expression profiling was a valuable follow up study to generate and confirm an alternative hypothesis for the MoA of carcinogenic effects in mice intestinal mucosa. It was also possible to establish a

NOAEL for expression induction in these studies to confirm that for the proposed MoA a threshold can be assumed. These data highly correlated with the histopathological observation in the classical repeated-dose toxicity studies in rodents and other *in vitro* and *in vivo* follow up studies. The weight of evidence was therefore considered sufficient to conclude that carcinogenic effect in intestinal mucosa in mice was triggered by local chronic cell stress and injury with induction of regenerative proliferation and the NOAEL for morphological changes and gene expression alteration provided sufficiently high margins of exposure to exclude clinical relevance.

So far in eight cases preclinical (pharmaco)-toxicogenomics data have been submitted to BfArM to support applications for market authorisation or clinical trials. In these cases the data of expression profiling were considered valuable to strengthen the body of preclinical data and substantially add to the weight of evidence for the final benefit-risk assessment.

### 3.4.3 Case 3: Reflection of exposure to environmental stressors via the epigenome

**J. Kevin Chipman**

*School of Biosciences, The University of Birmingham, Edgbaston, Birmingham, UK*

'Omic responses in environmental research have focussed on the transcriptome and metabolome (e.g. Williams *et al*, 2011) with some approaches on the proteome. Such studies have enabled the detection of adverse outcome pathways that are associated with pollutant effects in the environment and which can be useful in monitoring. However, despite the well-established influence of the environment on the epigenome, the latter has received insufficient attention. Epigenome-environment interactions play a major role in the way in which organisms adapt and compensate in response to environmental stress and can also be important in induction of disease. Overlaid on the genome are epigenetic marks, particularly methylation of CpG islands (Esteller and Herman, 2002) that determine how the genome responds through regulation of transcription. It is well established that various environmental stressors including dietary deficiencies and exposure to a wide range of chemical pollutants, can modulate the epigenome (Bernstein *et al*, 2007). Furthermore, studies in genetically identical human twins have shown that epigenetic marks can accumulate over a life-time reflecting previous environmental exposures. It is proposed that epigenetic 'finger-printing' of organisms could identify classes of chemical contaminants to which they have been exposed throughout their life-time. Such epigenetic variability has been shown to occur in natural populations of the clonal fish *Chrosomus eos-neogaeus* (Massicotte *et al*, 2011). The adaptive changes observed may help identify populations vulnerable to environmental change. The aquatic environment is of particular interest because of the relative extent of pollution impact. In addition, the mode of action of environmental carcinogens involves both genotoxic and epigenetic changes, the latter being dose-dependent. In fish, changes in the epigenome are characteristic both of the effect of such chemicals and of the disease process. Fish taken from the environment with a high incidence of liver cancers were found to have a remarkable DNA global hypomethylation and gene-specific CpG island methylation changes in histologically normal tissue distal to tumours (Mirbahai *et al*, 2011). We have shown, through metabolomics, that these changes are driven by disruption of the 1-carbon metabolic pathway in the liver. The extent to which these characteristics are driven by stressors relating to diet, chemical pollution or disease is yet to be established. These findings, coupled with evidence of an 'epigenetic fingerprint' in human twins that reflect in-life exposures, suggest a novel opportunity for the use of life-long epigenetic memory in the monitoring of environmental stressors. The controversial and intriguing possibility of trans-generational epigenetic change influencing disease susceptibility (e.g. Skinner *et al*, 2010), with potential effects on biodiversity, is also of concern and an opportunity in monitoring programmes.

### 3.4.4 Case 4: Transcriptomics and ecological risk assessment: Case studies with fish exposed to bisphenol A and diethylstilbesterol

**Daniel L. Villeneuve**

*US EPA, Mid-Continent Ecology Division, Duluth, MN*

Bisphenol A (BPA) and diethylstilbesterol (DES) are endocrine active chemicals whose actions as oestrogen receptor agonists are well characterised. Bisphenol A, while comparatively weak as an oestrogen relative to 17β-oestradiol (E2) or 17α-ethynyl oestradiol (EE2), is a high production volume chemical that is widely detected in the environment. In contrast, DES, is currently produced and used more sparingly (e.g. as a veterinary pharmaceutical), but has potency similar to that of E2 and EE2. Our research team has employed transcriptomics and/or metabolomics, anchored to phenotypic outcomes, to enhance understanding of these chemicals and their impacts on fish reproduction (Adedeji *et al*, 2012; Ekman *et al*, 2012; Villeneuve *et al*, 2012). The research has also provided insights into the utility and application of transcriptomics to ecological risk assessment. The BPA case study demonstrated some of the challenges faced in trying to utilise transcriptomic response as a scientifically credible means to estimate hazard thresholds. Determination of a no observed transcriptional effect level (NOTEL) was complicated by the lack of standardised procedures for setting an appropriate response threshold and by the non-monotonic nature of the transcriptomic concentration-response relationship. In comparing the ovarian transcriptomic response to BPA in two fish species exposed in parallel, the overall shapes of the transcriptional concentration-response curves were remarkably similar. On a concentration-specific basis there was little overlap among the individual genes identified as differentially expressed among the two species tested. However, there were overlaps in the functional pathways that were affected, albeit at different exposure concentrations. This suggests that key aspects of the transcriptomic response to BPA may be conserved, but differences in sensitivity may influence the concentrations at which those effects occur in different species. The DES case study examined the persistence of a hepatic transcriptome response to DES exposure, following a period of depuration. Significant numbers of genes that were up-regulated following a 4-day exposure to DES were found to be significantly down-regulated following depuration. Thus, impacts on similar transcripts and pathways could be detected, but nature of the impact (up or down-regulation) can vary before and after exposure. Overall the results provide optimism that key aspects of transcriptional responses to chemical stressors may be conserved across species, within complex mixtures, and following cessation of exposure, albeit with caveats and additional influences in each case. Results also suggest a need to develop consensus regarding the assumptions and approaches for estimating hazard thresholds from 'omics data.

*This abstract does not necessarily reflect the official views or policies of the US EPA.*

### 3.4.5 Case 5: Toxicogenomics applications for investigation of genotoxicity: A genomic biomarker approach

**Raegan O'Lone**

*HESI, Washington, DC, USA*

Assessment of the human health relevance of positive *in vitro* chromosome aberration assays poses a challenge and requires additional mechanistic studies *in vitro* and *in vivo*. The Health and Environmental Sciences Institute (HESI) Application of Genomics to Mechanism-Based Risk Assessment Technical Committee has undertaken a program utilising a genomic biomarker approach to provide mechanistic insights into positive findings in *in vitro* chromosome damage assays. The programme's goals include qualification of the genomic biomarker approach via the US FDA biomarker qualification process. A qualified genomic biomarker approach would be anticipated to facilitate safety risk assessment for drugs and chemicals and limit use of laboratory animals.

### 3.4.6 Case 6: The use of proteomics for the identification of compounds inducing toxicity

**André Schrattenholz**

*ProteoSys AG, Mainz, Germany*

The phenotypic effects of toxic interventions result from complex combinations of genetic, epigenetic and environmental predispositions and conditions. The various types of responses of *in vitro* models need precise SOPs for sample generation, processing and storage, meta data tracking frameworks (e.g. ISA-TAB), as well as fine-tuned assessments by a detailed molecular analysis, namely from modern 'omics-technologies. The aim is to define sets of biomarkers exactly describing molecular initiating events and the downstream key events which eventually cause adverse outcomes relevant to human neuropathology.

Epigenetic and environmental are, to a large extent, present on the proteomic level. Proteins also show the most immediate molecular effects in time-resolved experiments. Examples will be shown that the kinetics of cellular *in vitro* systems requires special attention with regard to collection of proteomic data by mass spectrometry-based methods, with its own set of quantitative, statistical and bioinformatics necessities. Data types and formats, data models and ontologies and interface solutions for integration will be discussed.

In terms of molecular biomarkers of molecular initiating events, we see the background of genomic information providing merely a 'risk' profile on the basis of approximately 20,000 human genes, and transcriptomics contributing momentary snap shots of cellular response profiles (based on genomic predisposition). Next generation sequencing (NGS) is an emerging technology with the potential of quickly providing detailed information about adverse outcome pathways (AOPs).

For the modelling of key events of AOPs, kinetic considerations are pivotal: NGS does not deliver post-translational details and represents time scales of 12-24 or more hours after toxic intervention. It is proteomics which reflects the real momentary cellular reaction in terms of pathway detail. Molecular initiating events will be abundantly quick post-translational modifications. Other 'omics have different time scales: e.g. metabolomics will represent biochemical / molecular end-points.

Mathematical modelling will need to not only integrate these kinetic and sequential premises, but also recognise the fact that in these pathways there is a high degree of feed-back and feed forward. Last but not least, on the level of protein a key factor is ACTIVITY (of enzymes, channels, etc.) which is quickly modified and/or regulated by post-translational modifications like phosphorylation.

Therefore an integrated strategy is required, which starts with very fundamental aspects of quantitative statistical quality control of human *in vitro* models exposed to well selected toxic standard compounds (see SEURAT-1).

In the first stage a thorough statistical analysis of raw data will reveal whether quantitative data signatures are consistent and plausible across samples and conditions. This will help to exclude effects of contamination and unrelated biological activity. Clear SOPs for definition of biological and data acquisition criteria will result in the selection of validated data sets subsequently undergoing specific processing and statistics.

In the second stage the whole validated data set or selected subgroups of biomarker candidates will be searched according to biological criteria. In the toxicological projects investigated so far (SEURAT-1, Reprotect) one of the hall marks was oxidative stress, contributing to a cascade of specific post-translational modifications (oxidative, glycation), some of them directly accessible by mass spectrometry (e.g. N-formyl-kynurenin modification).

It should be noted that these procedures are highly iterative and integrative. It could mean that we decide to employ other technologies at earlier or later stages (e.g. oxy blots).

Having said this, the importance of a sample amount and aliquotation management for every 'omics project becomes clear.

Once appropriate statistical and bioinformatics procedures for every layer of 'omics analysis are established and once appropriate integration by appropriate meta data tracking and management are achieved the following conclusion can be reached:

- We are obviously dealing with a limited number of pathways.
- These pathways are most flexible and redundant feed-back systems.
- Certain layers of 'omics analyses reflect better or worse the kinetics of reactions in these pathways, stress and escape responses.
- Predictive modelling would require adequate incorporation of kinetic information and treatment of feed-back: special need for innovative mathematics.

## 3.5 Future perspectives / systems biology / modelling

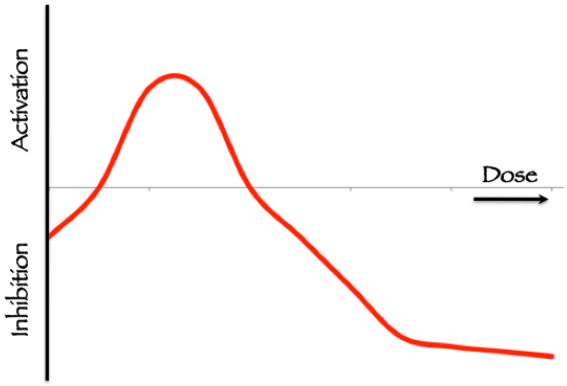
### 3.5.1 A systems toxicology approach to decipher hormesis in *Daphnia magna*

**Natàlia Garcia-Reyero**[1]; Jacob Stanley[2]; Tanwir Habib[3]; Lynn Escalon[2]; Mitch Wilbanks[2]; Jerre Simms[2]; Pornsawan Chappell[3]; and Ed Perkins[2]

[1]*Institute for Genomics, Biocomputing and Biotechnology, Mississippi State University, Starkville, MS, USA*
[2]*US Army Engineer Research and Development Center, Environmental Laboratory, Vicksburg, MS, USA*
[3]*Badger Technical Services, San Antonio, TX, USA*

There is a general assumption in toxicology approaches about the relationship between dose and response, or as it has been previously mentioned, "the dose makes the poison". This assumption is often used to determine the levels of exposure and doses below which chemicals should be harmless. Nevertheless, many compounds such as hormones and their mimics (e.g. endocrine disrupting-compounds) might present a non-monotonic dose-response curve. In this type of response, the shape of the curve reverses as the dose of the compound goes up (see Figure 7). These responses often follow either a U-shaped curve or an inverted U-shaped curve.

***Figure 7: A low dose of chemical triggers a response opposite to that of a high dose***

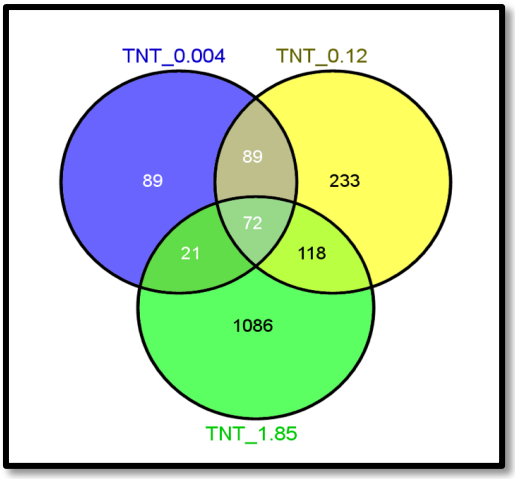



Hormesis is also a dose-response phenomenon characterised by a stimulation of an organismal response (such as growth or reproduction) at low doses of a chemical and inhibition (toxicity) at higher doses. It has been observed across a wide range of contaminants and organisms and is thought to be overcompensation to an alteration in homeostasis. While a hormetic response could be considered to have a beneficial effect at low concentrations, this statement should be carefully considered. An increase in growth or reproduction at low doses could be beneficial, but could also represent a change in the allocation of energy, with potential adverse effects. Here, we used an integrated, systems toxicology approach to decipher hormetic mechanisms in *Daphnia magna*.

*Daphnia magna* were exposed to the energetic compound trinitrotoluene (TNT) for 21 days and several endpoints such as number of neonates, length, growth and survival were measured. TNT elicited hormetic responses in reproduction and growth at very low concentrations. In order to elucidate the mechanisms

leading to hormesis, microarray analysis was performed at 0.004 (hormetic), 0.12 (sometimes hormetic), and 1.85 (toxic) mg/L TNT. There were a total of 271 differentially expressed genes (DEGs) at the lower dose, 512 at the middle dose, and 1,297 DEGs at the higher dose. Seventy-two genes were common among the three treatments.

***Figure 8**: **Venn diagram showing the differentially expressed genes on** **Daphnia magna** **exposed to 0.004, 0.12, or 1.85 mg/L TNT***




The lower dose was enriched in pathways such as fatty acid metabolism, AhR receptor signalling, or xenobiotic metabolism signalling, The middle dose was enriched in pathways such as AhR receptor signalling, NRF-2-mediated oxidative response, or PPARα/RXRα activation. The highest dose was enriched in pathways such as fatty acid metabolism, xenobiotic metabolism signalling, and PPARα/RXRα activation. Interestingly, some of these pathways such as fatty acid metabolism, PPARα/RXRα activation, xenobiotic metabolism signalling and NRF-2-mediated oxidative stress are common with other species exposed to TNT, such as rat, quail and fathead minnows.

Functional and transcriptional analyses identified concentration dependent differentially expressed genes that suggested the involvement of lipid metabolism in hormetic responses. In order to test the hypothesis that lipid metabolism was involved in the hormetic response, we performed lipidomics on the TNT exposed *Daphnia*. Lipidomic analysis supported the hypothesis that TNT exposure affected lipid metabolism, showing higher levels of phosphatidic acids, lysophosphatidylethanolamines and lysophosphatidylcholines at hormetic doses. This analysis leads us to conclude that TNT hormetic effects are related to increases in some polyunsaturated fatty acids and eicosanoids known to be involved in *Daphnia* growth and reproduction. This conclusion is consistent with observations that high levels of TNT and other nitroaromatics change fatty acid metabolism in fathead minnow, Bobwhite quail and rats.

# 4. REPORTS FROM THE SYNDICATE SESSIONS

## 4.1 Syndicate 1: Which opportunities does the group see for 'omics to help improve (eco)toxicology in the future?

Moderator: Neil Carmichael

*Rapporteur*: Natàlia Garcia-Reyero

Carlos Barata
Kevin Chipman
Jason Lambert
Aldert Piersma
André Schrattenholz

The following questions / concerns were discussed:

*1. How can we use 'omics to elucidate adverse effects?*

There was a long discussion about the need to define what adversity is in order to understand what can be considered an 'adverse effect'. More information about the upper bounds on normal ranges for basic biological function(s), as well as placing an increased emphasis on characterising homeostasis in an organism of interest, is needed. Research / data that helps inform what constitutes biological perturbations within a 'normal' range is key to understanding and defining what is adverse. Currently, there is not much information about homeostatic baselines or normal levels of biological function.

In order to understand adversity, we also need to truly understand gene and pathway functions. Dosimetry is also a challenge when defining adversity / normality. Adverse outcome pathways may entail non-monotonic dose-responses, multiple biological activity thresholds, and/or dose-dependent transitions in toxic mode of action. It is critical to link changes at the signal transduction pathway level to specific adverse outcomes, in order to understand and define potential hazards to human and ecological health qualitatively and quantitatively.

Integration of 'omics approaches/data into (eco)toxicology could be key to informing mechanisms of toxicity causally associated with adverse outcomes. Furthermore, understanding mechanisms is important for species extrapolation and to analyse relative species sensitivities. One of the benefits of 'omics technologies is that they can be used as a 'red flag' to suggest further, deeper analysis of chemical mixtures. Considering the potential complexity of signal transduction associated with chemical / non-chemical mixture exposures, the multiple 'omics platforms may be used in an integrated holistic approach to better inform identification of molecular key events or mechanism(s) of action: it is a non-biased (hypothesis-generating) approach. Because the toxicity of mixtures is often unknown, this approach could be extremely useful. Although 'omics approaches can help elucidate mechanisms of action, there is a need to build more mathematical models to maximise the predictability of chemical mixture toxicity based on 'omics data. These models may also inform

the influence of additivity, or deviations from additivity (e.g. synergism, antagonism), on adverse outcomes associated with mixtures of chemical and non-chemical stressors.

*2. Are 'omics technologies useful for species extrapolation of adverse effects?*

Many biological systems are highly conserved and operate very similarly across different species. Although very challenging, cross-species analysis of 'omics data can lead to insights that cannot be obtained from single species analysis. While biological signaling pathways affected by xenobiotic exposures are often conserved across species, the outcomes might be different. Several examples of cross-species comparisons were mentioned. For example, ibuprofen has been shown to affect the same signalling pathways in *Daphnia* and humans. But, while the pathways affected (such as carbohydrate metabolism, and especially glycolysis) might be the same, the (adverse) outcome is different, as ibuprofen seems to affect eicosanoid metabolism, which appears to disrupt signal transduction affecting juvenile hormone metabolism and oogenesis in *Daphnia* (Heckmann *et al*, 2008). It was mentioned that an important issue for cross-species extrapolation is the lack of pathway annotation for gene expression in many relevant species, which increases the difficulty of 'omics data interpretation. There was a general agreement on the importance of putting efforts into (gene) annotation of relevant species.

There was also general enthusiasm on the use of lower organisms as alternative species, as they have been shown to be extremely helpful to elucidate mechanisms of action and adverse outcomes in higher order species. While some models such as zebrafish embryo might not be useful for examining reproductive toxicity at the physiological level, they might still provide valuable information at the molecular level.

*3. Are systems biology and network analysis helpful in ecotoxicology?*

Network analysis and bioinformatics can help elucidate changes related to toxic responses as opposed to general stress responses. All participants considered them very promising tools for integrating the different 'omics technologies. There was a general agreement on the need to understand adverse outcomes at the pathway level and not so much at the gene level, as gene changes might not be so consistent, emphasising the need for basing the analysis at a gene expression pathway level versus expression of specific genes. That will also help avoid false positives by detecting effects on pathways and not just on genes. We need to understand how to separate 'housekeeping pathways' from the ones that are really related to toxicity.

There was also a suggestion to use nonlinear mathematics of dynamic systems to find key biomarker information in the ('omics) data. One mathematical outcome could be predictive attractors: Attractors are sets (of e.g biomarker quantities) towards which the variables of a dynamic system (e.g human in vitro model for kidney function), are moving dependent on toxic exposure. The intrinsic dynamic reactions to toxic exposure are mediated by redundant pathways which can react quite flexible to a variety of inputs, based on genetic and epigenetic predispositions. Attractors could be helpful to understand the actual risk situation after exposure. For prediction of biological consequences, time is a crucial dimension. The time scales of the different 'omics technologies are very different: genomics and transcriptional changes can be much slower than proteomics changes (i.e. oxidative post-translational modifications can occur in split seconds, while metabolic changes can take hours or days; see Table 1).

***Table 1: Oxidative post-translational modifications***

| Technology | Characteristics / Time scales |
|---|---|
| Genomics | Provides a 'risk' profile; approx. 20,000 in humans |
| Transcriptomics | Provides momentary status of blue prints of cellular response profiles (based on genomic predisposition): (backbones of) AOPs: adverse outcome pathways, kinetic considerations, no post-translational detail |
| Proteomics | Reflects the real momentary cellular reaction in terms of pathway detail (quick post-translational modifications), molecular initiating events and biochemical / molecular endpoints |
| Metabolomics | Rather biochemical / molecular endpoints |
| Epigenomics | AOPs, molecular initiating events |
| Lipidomics | End-point |

Another key factor is temporality, as at some time points toxicity is seen whilst at others repair mechanisms may be predominant. It is therefore crucial to characterise windows of temporality and percentage or availability of receptors and targets, as well as sensitive stages. As a final conclusion, all participants considered 'omics as a powerful and promising tool in (eco)toxicology, although many aspects such as the aforementioned need to be considered and new systems approaches including mathematical modelling and computational biology need to be developed and incorporated.

The mathematical modelling would need to integrate these kinetic and sequential premises. We also discussed the fact that in these pathways there is a high degree of feedback and feed-forward. Last but not least: the whole discussion so far has been about abundances of genes, transcripts, post-translational protein modifications, etc., but at the protein level a key factor is activity (of enzymes, channels, etc.), which is quickly modified by post-translational modifications like phosphorylation.

## 4.2 Syndicate 2: What (data) is missing, what would be the most important (data) to do and why to promote the use of 'omics in (eco)toxicology?

Moderator: Chris Corton

*Rapporteur*: Tim Gant

Ceri Morris
Raegan O'Lone
Ben van Ravenzwaay
Christoph Wierling

The following questions / concerns were discussed:

*1. Integration of 'omics data (systems biology approach)*

The group recognised the importance of a systems biology approach in tackling problems in predictive and mechanistic toxicology. There was a consensus that an understanding of the behaviour of all of the components of the cell under different conditions of exposure to chemicals with diverse modes of action would provide the information that could be turned to knowledge using appropriate bioinformatics tools. Because environmental chemicals perturb multiple targets, it is thought that systems biology approaches could help to narrow down the key events that lead to adverse outcomes.

There are several types of 'omics technologies that measure DNA, mRNA, protein and metabolites. However, there are few studies in which all 'omics methodologies have been applied, (e.g. five in total from the CEFIC-LRI consortium [Wilson *et al,* 2011]) on which to assess which, if any of these are most useful to advance toxicological understanding and risk assessment. The group expressed an interest in seeing more examples published where multiple systems approaches are used to objectively determine their added value to toxicity prediction over more traditional analyses in which one type of 'omics is examined at a time. Thus, it is not clear at the moment that having a full set of data improves predictivity and understanding, but there is probably going to be a need for more 'full set' data on compounds in order to make an assessment.

Application of systems biology is hampered to some extent by bioinformatics challenges for determining changes in abundance and combining data across different biological scales. More structured analyses are required to define standard procedures for data analysis (i.e. normalisation and statistics). Different methods between studies can render cross comparisons difficult. Furthermore, there is no clear way to define a differentially expressed gene, or indeed a pathway. Although a number of big studies have examined this issue (e.g. MAQC), there is still a need to critically examine bioinformatics methods to derive best practice. Pathways are a particular challenge as there is confusion over what is being examined. For example what constitutes a pathway and how are regulatory points in a pathway dealt with? Unbiased methods that require no objective input such as connectivity analysis need to be further evaluated (see below).

2. *The relative importance of the individual 'omics (proteomics, metabolomics, transcriptomics, (toxico)genomics) to address specific questions*

The group recognised the dominance of RNA expression profiling and to a lesser extent, metabolic profiling in assessing chemical hazards. There is little comprehensive proteomics data due to the technical challenges of assessing protein expression in different subcellular compartments. Given the emphasis on transcriptomics, large databases from expression profiling studies are available in public (e.g. GEO, ArrayExpress) or commercial (e.g. NextBio) databases. The challenge is to effectively use the data for prediction of chemical toxicity. One goal would be to use signature based approaches to predict chemicals (or other stressors) that alter molecular initiating events or activate key events in adverse outcome pathways. This type of approach would be useful to risk assessors and help to narrow down the complexity of the transcriptional responses linking events important for predicting toxicity. Another challenge is to use the transcript profile differences between two life stages (e.g. foetal versus adult) or animals with different lifestyles (e.g. different diets) and predict responses to chemicals. This approach is hampered by lack of data on the proteins that genes encode (i.e. what are the functions of the proteins) and how the proteins impact chemical exposure either through metabolism / transport or through modulation of a cellular function (e.g. changes in the potential for apoptosis).

Toxicologists should take advantage of approaches taken in the academic community to use gene expression profiling for pathway assessment and to build predictive models. The Connectivity Map effort at the Broad Institute is a notable example of a large effort to generate expression information on genes across several cell lines and 1000s of chemicals. The toxicology community would do well to coordinate efforts to capitalise on this approach, assessing expression of the same set of genes after exposure to environmentally relevant chemicals in cell lines routinely used by toxicologists.

Like transcriptomics, metabolomics faces problems in interpretation especially when there are no reference data to compare the profile to. The strength of using metabolomics to predict adverse outcomes would be strengthened if transcript profiling could be carried out in parallel. The field of predictive toxicology using metabolic profiling could capitalise then on the advances made in prediction using RNA expression profiling.

There is also a lack of data in what constitutes normal for all the 'omics technologies. The result of this is that comparisons are always being made against a control and there is no standardisation of controls between experiments making cross comparisons challenging. Additional analyses of historical 'omics profiles to establish baselines (such as the HESI baseline project) could be very valuable.

The group discussed pathway analysis as a way to be able to categorise chemical action. There are currently a number of tools available that allow the investigator to determine the degree to which pathways are altered by chemical exposure. Although pathway analysis has been quite useful in preliminarily identifying how a chemical may be causing pharmacology or toxicology effects, there is no universal definition of what is a pathway, and to what level of hierarchy pathway analysis is useful. A lack of definition hinders their use as standard biomarkers of adverse outcomes. Moreover, pathway analysis may not be as useful if we have no information about what the key points of regulation are in that pathway or genes that are rate limiting for metabolic flux through the pathway. Pathway analysis based on gene expression is hampered by our inability to accurately predict the effect of alterations in the levels of RNA on abundance of encoded proteins.

*3. Including µRNAs in the 'equation'*

Although investigators using genomics techniques mainly query mRNA expression, more studies are appearing in the literature in which microRNAs (miRNAs) are being examined. Overall the group thought the field has a long way to go before miRNAs will be routinely examined as biomarkers of exposure / effect or used as signatures to determine mode of action of a chemical. This particularly applies to circulating miRNAs where there are still no demonstrated examples of greater utility over established biomarkers. This field is still relatively young, and so it is quite possible that more predictive circulating miRNA biomarkers of adverse response will be found. In mechanistic assessment, one of the major problems is that there is a lack of understanding of what the targets of miRNAs are. Targets can be predicted through bioinformatics methods but these have limitations. The gold standard still remains molecular techniques to examine interactions between individual miRNAs and individual mRNAs.

The group agreed that an understanding of the expression and targets of miRNAs could be essential to link transcriptomics and proteomics data. Furthermore knowledge of miRNA expression could be of value in this field especially in being able to predict the biological impact of changes in mRNAs through expression of encoded proteins. It is important that miRNA data is generated from the same tissues as mRNA data.

In conclusion, the group felt that the application of 'omics in toxicology had progressed since the last meeting, but there was still some way to go before routine application was likely to be achieved in chemical risk assessment. In particular, a number of areas were identified by the workgroup as needing further studies including analysis of the most informative 'omics method, standardisation (or at least best practice) in bioinformatics methods, improved definition of a pathway to facilitate analysis, capitalising on databases of genomic information such as C-Map and understanding the role and function of miRNAs both as biomarkers and to connect transcriptomic and proteomic data sets.

## 4.3 Syndicate 3: Which challenges do we face to use 'omics data in risk assessment?

Moderator: Jos Kleinjans

*Rapporteur:* Dan Villeneuve

Ross Brown
Malyka Galay Burgos
Miriam Hampel
Benjamin Piña
Tokuo Sukata

The following questions / concerns were discussed:

*1. Linking 'omics to adversity*

One of the primary challenges in using 'omics data in risk assessment centres around the question of adversity. Risk assessments are intended to support evaluation of the probability or likelihood of an adverse outcome given a particular exposure scenario. In human health assessments, adverse outcomes may be defined as onset of disease or discomfort in individuals. In ecological assessments, adverse outcomes are typically defined as impacts on populations and/or the ecosystem services they provide. By their nature, 'omics endpoints pertain to low levels of biological organisation (i.e. molecular, biochemical), rather than the organ / organ system, individual, or population-level on which risk assessments and common definitions of adversity typically focus. Therefore, if 'omics are to be used effectively in a risk assessment context, scientifically credible linkages between 'omics responses and adverse outcomes that occur at a level of biological organisation relevant to risk assessment need to be established. The required level of certainty and confidence in those linkages is in turn defined by the relative levels of precaution versus risk that stake-holders are willing to accept, ideally as defined during problem formulation.

The syndicate group was asked to consider and discuss four inter-related questions pertaining to the linkage of 'omics to adversity:

1. *How to identify the 'toxic' fingerprint? [That is, how do we identify those 'omic responses that predict or precede an adverse outcome?]*
2. *How to define the toxic endpoint at low, environmentally-relevant doses?*
3. *How to validate predictivity: differentiating indirect biomarkers of exposure or effect from alterations that play a direct, causal role, in an adverse outcome?*
4. *How to deal with patterns recognisable at subtoxic (lower than phenotypic NOAEL) doses? Are they predictive, or irrelevant?*

There was consensus among the group that change alone does not necessarily equate to toxicity or adversity. There are many changes that occur in an organism, particularly dynamic changes measured at low levels of biological organisation, which can reflect normal or adaptive responses to stress that lie within an organism's homeostatic range. Anonymous 'change' is not sufficient for establishing a hazard threshold.

Rather, the changes observed ('omics fingerprint / profile) need to be linked in a scientifically-credible manner to a higher level outcome.

There are two primary approaches for establishing links between 'omics responses and adverse outcomes relevant to risk assessment, biological plausibility and statistical association. Biological plausibility relies on mechanistic understanding of the roles that genes, transcripts, proteins, and metabolites play in a biological system and how those roles contribute to function. When both the roles and normal operating range of those features are well understood, perturbation of those features can be used to infer something about the potential consequences to function. Because biological systems are complex, our understanding of those systems has limits, and false positives can be expected when employing 'omics, cumulative weight of evidence is a particularly important consideration relative to biological plausibility. Multiple independent observations that support similar conclusions in terms of expected effects on function provide stronger evidence than a single observation. In this sense, effects on 'pathways', however broadly defined, would generally be viewed as a stronger basis for predicting outcomes than changes in individual features. Likewise, linkage of an 'omics outcome with biologically consistent effects spanning multiple levels of organisation (i.e. multiple key events within an adverse outcome pathway; supporting evidence from multiple 'omics approaches) would provide greater confidence than a single 'omics approach alone.

An on-going impediment to the effective use of biological plausibility to support the use of 'omics responses in risk assessment are limitations in the functional annotation of genes/transcripts, proteins, and metabolites. In the case of ecotoxicology, annotation information is very limited or non-existent for many organisms that play critical roles in ecosystems. This is especially true of invertebrate and microbial organisms which both make up the base of many food chains and also contribute to essential biogeochemical processes that higher organisms rely on. However, meta-genomic profiling of microbial communities is being used to great effect to assess functions such as the competency of degraders. Such functional meta-genomics are likely to play an increasingly important role in assessing risks chemicals pose to some traditionally understudied components of ecosystems (i.e. microbial and micro-invertebrate communities). Even where there is adequate sequence homology to map genes / proteins from a species with poor annotation information to a species with rich annotation information, there can be significant deviations in the function of orthologous or paralogous features within those different organisms. Even for well annotated organisms, the level of functional detail and specificity afforded in typical annotation schemes (e.g. gene ontology terms, KEGG pathways, protein families) is often inadequate to support useful functional inference from 'omics results. Further, there is an on-going risk toward biasing interpretation toward what is well known and understood, and ignoring changes in less well annotated features, which may none the less play critical biological roles that influence and predict outcomes. Integration of different 'omic techniques, particularly those such as metabolomics which are not reliant on species-specific annotations, may compensate for some limitations in functional annotation. However, not surprisingly, the primary suggested solution to these limitations was more research. In particular, the capability to perform gene and protein knock-down and over-expression studies in certain model organisms may significantly enhance understanding of function. Additionally, more comparative research that defines both the similarities and differences in the function of specific features and/or pathways among different groups of organisms is needed. Finally, it was noted that the poorly defined and unannotated features measured in 'omics experiments should not be ignored. Those that show consistent patterns of response to stressors

(i.e. replicated among experiments, distinct temporal and concentration-response trends, etc.), in particular, should be brought to the attention of the scientific community for investigation.

This brings us to the second approach of statistical association. The group agreed that even in the absence of a known mechanistic or biological connection between an 'omic' response and an adverse outcome, statistical association between specific profiles / fingerprints and outcomes can support their application in risk assessment. However, such approaches can be expected to be much more data intensive as both the predictive utility and consistency of the profile needs to be established across many experiments and appropriate domains of applicability in terms of time course, strength of perturbation (dose), etc. need to be defined. In this respect, the group suggested that it may be possible to develop statistically robust profiles that signal a cell/cell type in distress that may translate across a wide variety of tissues, species, etc.

There was also a discussion as to whether irreversibility may represent an important criterion in determining whether a change in state, as measured by 'omic' response profile, even if it can't be linked mechanistically to a known adverse outcome, for associating adversity with a particular 'omic' response. Transient changes in the transcriptome, proteome, or metabolome can be expected as part of normal homeostatic responses to stressors. Likewise, irreversible changes in the transcriptome, proteome, or metabolome may also be adaptive. However, any adaptation has potential to be maladaptive in the face of changing conditions (e.g. environmental change, introduction of a new stressor) and/or incur other costs to the organism (e.g. energy expenditure). Thus, differentiation of homeostatic 'omic' response versus allostatic changes in 'omic states that contribute to allostatic load is necessary. Epigenetic changes would be one expected source of such allostatic changes in biological systems. While it remains unclear whether overall 'epigenetic load' could be equated with the concept of 'allostatic load', the group was in agreement that disruption of the mechanisms of epigenetic regulation themselves could be accepted as toxic, even if epigenetic change itself (i.e. increased methylation of DNA) may or may not be.

In summary, because 'omics measurements are both dynamic and made at low levels of biological organisation, linkage to adversity is likely to remain a major challenge to the broad use of 'omics data in risk assessment for quite some time. However, these same limitations are equally applicable to high throughput molecular screening, QSARs, and similar approaches that have been advocated as part of a 21st century approach to toxicity testing.

*2. The sensitivity of 'omics relative to classical (eco)toxicology*

A second topic addressed by the syndicate group was whether 'omics endpoints are more sensitive than classical apical (eco)toxicology endpoints. The answer, of course, is that it depends. Group members were aware of and could cite specific examples of studies that would seem to support the general idea that 'omics responses are more sensitive than apical responses. One example cited related to hepatotoxic effects of acetaminophen in rodents. In that case, 'omics were found to be more sensitive and reliable for identifying hepatotoxicity than pathology. There are examples from the ecotoxicological community, which demonstrate marked changes in gene expression at concentrations equal to or lower than those at which apical impacts were observed. By definition, any 'omics response will be more sensitive than the apical outcome of mortality, which is probably the most widely used endpoint in classical (eco)toxicology. However, the outcome of mortality aside, the attempt to evaluate the relative sensitivity of 'omics compared to other endpoints used in risk assessment revolves back to the issue of adversity and linkage to adversity.

The group was in general agreement that, broadly speaking, some kind of 'omic response could be expected, and can be observed, at concentrations lower, or durations shorter, than those needed to induce apical responses. However, that does not mean that those more sensitive responses have necessarily been demonstrated to have a clear predictive linkage, either through biological plausibility or statistically robust association, to the apical outcome of interest. In most cases, that has not been clearly established. Thus, the question of sensitivity can only be evaluated in a meaningful way once one defines whether or not a clear link to adversity is requisite of the 'omics response.

The answer to this question can also depend on the nature of the specific endpoints being compared and logistical constraints of experimental design. Certain endpoints are inherently more variable than others. At present, most 'omics experiments employ relatively small sample sizes due to the cost of 'omics analyses (particularly transcriptomics and proteomics). Therefore, in some cases, 'omics may be thought to have poorer sensitivity solely due to lack of statistical power. Consequently, a fair comparison of the relative sensitivity of the methods should compare analyses performed with similar sample sizes.

Finally, the temporal component of a perturbation-response relationship complicates the ability to compare sensitivity. Apical endpoints, particularly chronic sublethal apical endpoints can take considerable time to manifest. Sensitive 'omics responses associated with the initiation of a pathologic response may occur much earlier in the time course of exposure than the apical response can be measured. In a temporal sense this constitutes greater sensitivity in 'omic responses in terms of providing early warning for adverse pathological or other effects. By the time the apical response is evident, the 'omic response profile that reflects the pathology itself may be very different from the response that indicated the direct impact and initiation of that outcome. Given the dynamic fluctuations that can occur in transcript, protein, and/or metabolite abundance identifying the appropriate temporal windows at which to effectively compare sensitivity can be challenging. In monitoring organisms collected from the environment, and thus subject to chronic exposure, the dynamic nature of 'omics endpoints can be expected to contribute stochastic variability which may also influence the sensitivity with which responses predictive of adversity, or indicative of exposure, can be discriminated from biological noise.

In summary, there is a strong intuitive and theoretical basis to expect that 'omics responses should be more sensitive than apical outcomes. However, empirical demonstration of that difference in sensitivity is challenged by unequal statistical sample sizes, subjectivity in what differentiates "'omics noise" from an 'omics response that is predictive of outcome, and temporal disconnects between when an 'omics response and an apical response are most sensitively observed.

*3. 'Omics study guidance*

The final topic addressed by the syndicate group was that of whether guidance and/or standards related to 'omics studies are needed. The consensus among the group was that the field is evolving too quickly to allow for any significant degree of standardisation or identification of 'gold standard' methods. With that said, there is a need for general guidance that the community can follow. It was noted that there are a number of on-going efforts within the scientific community to provide some general frameworks and guidance for conducting 'omics studies and analysing results. Specific examples such as development of like MIAME standards (Minimum Information About a Microarray Experiment) and the Microarray Quality Control (MAQC) Project being coordinated by the United State Food and Drug Administration

(see MAQC Consortium, 2006; 2010) were discussed. Lots of progress is being made in standardising: i) practical study design incorporating 'omics in (eco)toxicology (i.e. including time-course, positive controls, concentration-response, etc.); ii) using 'omic' biomarkers to derive NOAELs; and iii) using mode of action / adverse outcome pathways in risk assessment. Several examples in each of these areas were illustrated in the case studies. Guidance regarding study designs for next generation sequencing studies is also being developed (e.g. DeLuca *et al*, 2012).

One broad recommendation at present is to employ multiple approaches (e.g. different types of normalisation, statistical tests, class prediction algorithms, etc.) and look for consensus among the results rather than relying on any single methodology. It was noted that, in general, the quality of 'omics work presented at the workshop, as a reflection of the field as a whole, has improved. Specifically, most studies included phenotypic anchoring and leveraged multiple types of complementary data. Additionally, many of the studies were much more hypothesis- and purpose-driven, and less open ended. Broadly speaking, the group felt that guidance was most needed in the area of data analysis as opposed to experimental design, which has improved significantly since the previous two workshops.

In summary, the field is evolving too quickly for the development of detailed standards to be practical. However, general guidance, particularly as it pertains to data analysis could still enhance the quality and utility of 'omics data for potential application to risk assessment.

## 4.4 Syndicate 4: 'Omics data in a human context

Moderator: Roland Frötschl

*Rapporteur*: Saskia van der Vies

Rafaella Corvi
Marjoke Heneweer
Alfonso Lampen
Jordi Mestres

The following questions / concerns were discussed:

*1. Improved understanding of human variability and its effects on response*

Variation in individual human responses to environmental toxicants can be caused by seemingly unrelated differences such as genetic polymorphisms, age, and gender, as well as co-existing diseases or infections. This large diversity in responsiveness among individuals makes it difficult to determine actual risk, particularly at the low doses to which most people are exposed. Age may be a determining factor for risk of toxicity and how toxicity may affect life at a later stage. Especially in developing individuals when physiological changes occur over a short period of time. Also pre-existing disease may increase human susceptibility to the effects of a toxicant and may result in manifestation of the disease in a person who may otherwise never have reached the disease state.

In order to determine the base line variability in a human population a coordinated effort is needed in combination with standardisation of methods e.g. MoA, 'omics and exposure, and determination of variability in (sub)population e.g. children, older people, men, women and ethnic groups would also be important. (Inter)national agreements on the accepted range of baseline variability (upper and lower boundaries) will also be needed.

For risk assessment the first choice should always be human data. However if the data is not available second choice can be *in vivo* animal data and *in vitro* data.

*2. Providing more accurate information on human exposure levels (can 'omics data be used as a (bio)marker of exposure?)*

It was felt that the major issue on providing more accurate data on exposure is the availability of the data. (Animal) data is often 'hidden' within companies and is presented in different languages. Also the type of data is often highly variable e.g. numbers, toxicological reports, various type of 'omics data. Standardisation of the data is often lacking despite the WHO guidelines. A second major issue is the need for read across, which is difficult since research is conducted using different (model) systems. What is needed is a systematic analysis to generate (all) standard toxicological data (endpoints) and at the same time 'omics data i.e. have all the data for one model system.

Information on a chemical may be considered incomplete because of the absence of information about particular routes of exposure, number of tested subjects, and accuracy of the data resulting in increase of

uncertainty of the extrapolations. In addition, quality and completeness of the data can vary substantially. The development of a database that will contain extensive classical toxicological data which will make possible to choose a compound and perform an 'omics analysis that can be correlated directly, is encouraging. In addition QSAR is a good starting point to decide whether or not to perform 'omics analysis. The group was unanimous in their support for 'omics technologies and its use to show that people have been exposed e.g. environmental exposures, diet and lifestyles are reflected in DNA methylation profiles of blood. In addition use of 'omics technologies have made it possible to identify new pathways that the 'traditional' toxicological analysis did not reveal. This has an effect on risk assessment i.e. to set the boundary values.

*3. Better understanding of the relevance of animal data to humans.*

It was felt that human data should be the first choice for risk assessors and regulators. However, when not available, second choice is animal data and then *in vitro* data. Preferably *in vitro* data should be generated with human cell models.

These cell models and human primary cell cultures will greatly benefit from standardisation and validation of the methodologies, generation of SOPs and OECD guidelines. 'Omics technology will help to better understand MoA and identify pathways in model systems. If it turns out that similar pathways exist in humans, the confidence in risk assessment is expected to increase. 'Omics technologies will also allow a better understanding of MoA and underlying molecular mechanisms, all of which is expected to result in a better prediction of the outcome upon exposure. Encouraging are new developments for (human) 'models' using humanised systems, co-cultures, flow cytometry, human cell lines 3D-models and Embryonic Stem cell Technology.

It was suggested that a workshop be organised in order to review the status of *in vitro* models and how far/near they are to the human organ level. This may help identify most advanced models and the models limitations.

# 5. CONCLUSIONS AND RECOMMENDATIONS

Since the 2010 ECETOC workshop the field of 'omics technologies has expanded further and is now being used in a more routine way. Therefore, more case studies, applications and regulatory experience have been obtained. One of the main conclusions of the 2010 workshop was that 'omics data are particularly valuable for understanding modes of action via underlying molecular patterns. The 2013 workshop clearly confirmed this application and provided some examples of how 'omics data are now being used for hazard identification. The 2010 workshop also indicated the potential of 'omics data to be used in a regulatory context, e.g. mode of action based risk assessment and facilitating read across / comparison to other substances. In the present workshop an example was provided in which proteomics data were used to address exactly these questions and demonstrated that such data are now being used to improve the quality of the risk assessment process. Moreover, 'omics data are now also used for early recognition of mode of action (also referred to as identification of adverse outcome pathways). An interesting example was provided to show how transcriptomic analysis can be used to reduce the uncertainty (false positive results) in clastogenicity tests.

The quality of study design, sample preparation and processing remains an issue, however with inter and intra laboratory comparisons a better understanding of the variability of 'omics data have now been obtained. An important observation was that despite significant variability between some laboratories in individual parameters, the overall pattern of chance allowed in all cases for the correct identification of the mode of action. There was general agreement that molecular 'omics information should be connected to the outcome of apical studies (phenotypic anchoring). Several presenters mentioned the Bradford Hill criteria (which are commonly used in epidemiological studies to assess the logical connection between epidemiological findings and the study hypothesis) are also applicable for 'omics data and *in vivo* toxicological findings.

One of the conclusions of the 2010 workshop was that 'omics data cannot yet be used in terms of quantitative information. This has changed drastically. Many of the presenters provided data in which the quantitative aspects played an important role. In three presentations the sensitivity issue of 'omics data at dose levels that represent a NO(A)EL in apical studies was addressed. In all cases 'omics NO(A)ELs could be obtained, indicating that a dose-response relationship starting at a zero response at low exposure levels is as common to 'omics data as it is to apical studies. It should be noted that this was mainly based on a pathway / pattern assessment and not necessarily on individual parameter analysis. Again the importance of phenotypical anchoring was stressed, because not all individual parameter changes are associated with an adverse outcome. Analysis of the most sensitive pathway for transcriptomics (at several points in time) allowed for a reasonable approximation of the NO(A)EL of the individual compounds studied. A quantitative analysis of metabolomics pattern data at NOAEL dose levels (using the ECETOC criteria provided in the Malaga I workshop[2]) in 28 day studies indicated that in *circa* 75% of the cases this was also a metabolomics

[2] Malaga workshop I: *"To derive a meaningful NOAEL it was recommended that only specific patterns of change (in any type of 'omics study) should be used to conclude that a potentially relevant biological effect is taking place. As changes in 'omics pathways do not necessarily imply that changes at cellular, individual or population levels will necessarily occur, these pathways need to be correlated to observable changes at the microscopic or macroscopic level. To use changes in an 'omics pattern for NOAEL purposes, it must be assured that the pathway identified is casually related to an adverse effect."*

NOAEL. Using only statistical parameters (i.e. NOELs) the concordance was 60% with an increase in sensitivity of metabolomics.

With transcriptomic analysis temporal aspects play an important role. Gene expression changes profoundly following exposure to a substance over time. This can now be connected to changes in pathways providing important information of molecular responses to toxicants.

There are a number of opportunities for the use of 'omics data for risk assessment of chemicals. Gene expression analysis through RNA sequencing is providing more, and apparently, better information than the array technologies. More information will be coming with respect to dose-time response relationships which will allow for a better understanding of the molecular pathways of cells in their process to adapt to a toxic insult. The combined analysis of transcriptomics, proteomics and metabolomics should provide a better, holistic, understanding of underlying mechanisms. As such 'omics data will have an advantage over *in vitro* studies in which the holistic aspects are far more difficult to take into account. It can be expected that quantitative information will start to play a more important role in the use of 'omics in risk assessment. With more knowledge becoming available, it may even be that species extrapolation will associated with less uncertainty, which could provide an opportunity to reduce mammalian toxicity testing. Pathway analysis appears to be the best approach to achieve consistency and relevance of 'omics data.

Taking into account the importance of pathway analysis, it was considered timely to provide clear criteria / definitions on how to derive and validate pathways based on 'omics data. Furthermore, the regulatory links between transcriptomics and proteomics are not yet fully understood. It may become necessary to include the effects of miRNAs in this process to obtain the desired understanding. The level of complexity seems to be one of the hurdles to discriminate between important and less important data. Identifying important regulatory nodes may help to reduce the complexity and allow for a focus on those changes that are truly relevant for an adverse effect. This would in fact be similar to the pathway approach described above, and would also be subject to the development of clear definitions.

Overall, one of the greatest challenges remains the fact that molecular responses are not necessarily adverse. Predicting adversity from 'omics data will remain the most important issue for the use of such data in the risk assessment process. A number of solutions have been discussed in this and previous workshops; e.g. use of Bradford Hill criteria, biological plausibility of associations, phenotypic anchoring, pathway / pattern development. The aforementioned temporal aspects, particularly with transcriptomic data, need to be better understood and linked to organism responses to identify which of these are adaptive, toxic or non-associated responses to an external stimulus.

Finally, to place 'omics data in a human context it was noted that human variability is not well known, and that compared to highly standardised laboratory research another level of complexity and uncertainty will be added. However, 'omics data have the potential to reveal pathways that may not be immediately evident in toxicological studies. With more studies and a better understanding of the molecular mechanisms, the relevance of animal (or *in vitro*) data for human risk assessment will be improved by the inclusion of data obtained with 'omics technologies.

# ABBREVIATIONS

| | |
|---|---|
| 3-MCPD | 3-monochloropropane-1,2-diol |
| 5-HT | 5-hydroxytryptamine |
| AhR | Aryl hydrocarbon receptor |
| ANOVA | Analysis of variance |
| AOP | Adverse outcome pathway |
| API | Active pharmaceutical ingredient |
| BaP | Benzo[a]pyrene |
| BCL2 | B-cell leukemia protein-2 |
| BfArM | Bundesinstitut für Arzneimittel und Medizinprodukte (German Federal Institute for Drugs and Medical Devices) |
| BfR | Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment) |
| BH | Benjamini-Hochberg |
| BMD | Benchmark dose |
| BPA | Bisphenol A |
| CAR | Constitutive androstane receptor |
| CDDO-Im | 2-cyano-3,12-dioxooleana-1,9-dien-28-oic imidazolide |
| Cefic-LRI | The European Chemical Industry Council Long-range Research Initiative |
| DAO | Diamine oxidase |
| DEG | Differentially expressed gene |
| DEHP | Di(2-ethylhexyl) phthalate |
| DES | Diethylstilbesterol |
| DNA | Deoxyribonucleic acid |
| E2 | 17β-oestradiol |
| EE2 | 17α-ethynyl oestradiol |
| EPA | Environmental Protection Agency |
| EURL-ECVAM | European Union Reference Laboratory for Alternatives to Animal Testing |
| FDR | False discovery rate |
| FXR | Farnesoid X receptor |
| GEO | Gene expression omnibus |
| GO | Gene ontology |
| GR | Glucocorticoid receptor |
| HESI | Health and Environmental Sciences Institute |
| HHRA | Human health risk assessment |
| IPCS | International Programme on Chemical Safety |

| | |
|---|---|
| KEGG | Kyoto Encyclopedia of Genes and Genomes |
| LPS/IL1 | Lipopolysaccharide-induced release of interleukin 1 |
| MALDI | Matrix-assisted laser desorption / ionisation |
| MAQC | Microarray quality control |
| MIE | Molecular initiating event |
| MoA | Mode of action |
| mRNA | Messenger ribonucleic acid |
| miRNA | Micro ribonucleic acid |
| NGS | Next generation sequencing |
| NOAEL | No observed adverse effect level |
| NO(A)EL | No observed (adverse) effect level |
| NOEL | No observed effect level |
| NOTEL | No observed transcriptional effect level |
| NRC | National Research Council |
| NRF-2 | Nuclear respiratory factor 2 |
| Nrf2 | Nuclear factor erythroid 2-related factor 2 |
| OECD | Organisation for Economic Cooperation and Development |
| PCA | Principle component analysis |
| PERK | Protein kinase RNA-like endoplasmic reticulum kinase |
| PFHxS | Perfluorohexanesulfonate |
| PFNA | Perfluorononanoic acid |
| PFOA | Perfluorooctanoic acid |
| PFOS | Perfluorooctane sulfonate |
| PPAR | Peroxisome proliferator-activated receptor |
| PPARα | PPARalpha |
| PXR | Pregnane X receptor |
| QSAR | Quantitative structure-activity relationship |
| RIVM | Dutch National Institute for Public Health and the Environment |
| RNA | Ribonucleic acid |
| RPTEC | Renal proximal tubule epithelial cell |
| RXR | Retinoid X receptor |
| SETAC | Society of Environmental Toxicology and Chemistry |
| SOP | Standard operating procedure |

| | |
|---|---|
| tBuHQ | 2,5-di(tert-butyl)hydroquinone |
| TCDD | 2,3,7,8-tetrachlorodibenzodioxin |
| TF | Transcription factor |
| TNT | Trinitrotoluene |
| TP53 | Tumour protein p53 |
| UDS | Unscheduled DNA synthesis |
| WHO | World Health Organisation |
| XPA | Xeroderma pigmentosum group A-complementing protein |

# BIBLIOGRAPHY

Adedeji OB, Durhan EJ, Garcia-Reyero N, Kahl MD, Jensen KM, Lalone CA, Makynen EA, Perkins EJ, Thomas L, Villeneuve DL, Ankley GT. 2012. Short-term study investigating the estrogenic potency of diethylstilbestrol in the fathead minnow (Pimephales promelas). *Environ Sci Technol* 46(14):7826-7835.

Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrano JA, Tietge JE, Villeneuve DL. 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem* 29(3):730-741.

Bernstein BE, Meissner A, Lander ES. 2007. The mammalian epigenome. *Cell* 128(4):669-681.

Chu F, Ren X, Chasse A, Hickman T, Zhang L, Yuh J, Smith MT, Burlingame AL. 2011. Quantitative mass spectrometry reveals the epigenome as a target of arsenic. *Chem Biol Interact* 192(1-2):113-117.

DeLuca DS, Levin JZ, Sivachenko A, Fennell T, Nazaire M-D, Williams C, Reich M, Winckler W, Getz G. 2012. RNA-SeQC: RNA-seq metrics for quality control and process optimization. *Bioinformatics* 28(11):1530-1532.

ECETOC. 2002. Recognition of, and differentiation between, adverse and non-adverse effects in toxicology studies. Technical Report No. 85. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2008. Workshop on the application of 'omics in toxicology and ecotoxicology: Case studies and risk assessment. Workshop Report No. 11. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2009. Use of markers for improved retrospective exposure assessment in epidemiology studies. Workshop Report No. 14. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2010. 'Omics in (eco)toxicology: Case studies and risk assessment. Workshop Report No. 19. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

Ekman DR, Hartig PC, Cardon M, Skelton DM, Teng Q, Durhan EJ, Jensen KM, Kahl MD, Villeneuve DL, Gray LE, Collette TW, Ankley GT. 2012. Metabolite profiling and a transcriptional activation assay provide direct evidence of androgen receptor antagonism by bisphenol A in fish. *Environ Sci Technol* 46(17):9673-9680.

Esteller M, Herman JG. 2002. Cancer as an epigenetic disease: DNA methylation and chromatin alterations in human tumours. *J Pathol* 196(1):1-7.

Heckmann LH, Sibly RM, Connon R, Hooper HL, Hutchinson TH, Maund SJ, Hill CJ, Bouetard A, Callaghan A. 2008. Systems biology meets stress ecology: linking molecular and organismal stress responses in *Daphnia magna*. *Genome Biol* 9(2):R40.

Huang DW, Sherman BT, Lempicki RA. 2009a. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4(1):44-57.

Huang DW, Sherman BT, Lempicki RA. 2009b. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res* 37(1):1-13.

Lewis RW, Billington R, Debryune E, Gamer A, Lang B, Carpanini F. 2002. Recognition of adverse and nonadverse effects in toxicity studies. *Toxicol Pathol* 30(1):66-74.

MAQC Consortium; Shi L, Reid LH, Jones WD, Shippy R, Warrington JA, Baker SC, Collins PJ, de Longueville F, Kawasaki ES, Lee KY, Luo Y, Sun YA, Willey JC, Setterquist RA, Fischer GM, Tong W, Dragan YP, Dix DJ, Frueh FW, Goodsaid FM, Herman D, Jensen RV, Johnson CD, Lobenhofer EK, Puri RK, Schrf U, Thierry-Mieg J, Wang C, Wilson M, Wolber PK, Zhang L, Amur S, Bao W, Barbacioru CC, Lucas AB, Bertholet V, Boysen C, Bromley B, Brown D, Brunner A, Canales R, Cao XM, Cebula TA, Chen JJ, Cheng J, Chu TM, Chudin E, Corson J, Corton JC, Croner LJ, Davies C, Davison TS, Delenstarr G, Deng X, Dorris D, Eklund AC, Fan XH, Fang H, Fulmer-Smentek S, Fuscoe JC, Gallagher K, Ge W, Guo L, Guo X, Hager J, Haje PK, Han J, Han T, Harbottle HC, Harris SC, Hatchwell E, Hauser CA, Hester S, Hong H, Hurban P, Jackson SA, Ji H, Knight CR, Kuo WP, LeClerc JE, Levy S, Li QZ, Liu C, Liu Y, Lombardi MJ, Ma Y, Magnuson SR, Maqsodi B, McDaniel T, Mei N, Myklebost O, Ning B, Novoradovskaya N, Orr MS, Osborn TW, Papallo A, Patterson TA, Perkins RG, Peters EH, Peterson R, Philips KL, Pine PS, Pusztai L, Qian F, Ren H, Rosen M, Rosenzweig BA, Samaha RR, Schena M, Schroth GP, Shchegrova S, Smith DD, Staedtler F, Su Z, Sun H, Szallasi Z, Tezak Z, Thierry-Mieg D, Thompson KL, Tikhonova I, Turpaz Y, Vallanat B, Van C, Walker SJ, Wang SJ, Wang Y, Wolfinger R, Wong A, Wu J, Xiao C, Xie Q, Xu J, Yang W, Zhang L, Zhong S, Zong Y, Slikker W Jr. 2006. The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. *Nat Biotechnol* 24(9):1151-1161.

MAQC Consortium; Shi L, Campbell G, Jones WD, Campagne F, Wen Z, Walker SJ, Su Z, Chu TM, Goodsaid FM, Pusztai L, Shaughnessy JD Jr, Oberthuer A, Thomas RS, Paules RS, Fielden M, Barlogie B, Chen W, Du P, Fischer M, Furlanello C, Gallas BD, Ge X, Megherbi DB, Symmans WF, Wang MD, Zhang J, Bitter H, Brors B, Bushel PR, Bylesjo M, Chen M, Cheng J, Cheng J, Chou J, Davison TS, Delorenzi M, Deng Y, Devanarayan V, Dix DJ, Dopazo J, Dorff KC, Elloumi F, Fan J, Fan S, Fan X, Fang H, Gonzaludo N, Hess KR, Hong H, Huan J, Irizarry RA, Judson R, Juraeva D, Lababidi S, Lambert CG, Li L, Li Y, Li Z, Lin SM, Liu G, Lobenhofer EK, Luo J, Luo W, McCall MN, Nikolsky Y, Pennello GA, Perkins RG, Philip R, Popovici V, Price ND, Qian F, Scherer A, Shi T, Shi W, Sung J, Thierry-Mieg D, Thierry-Mieg J, Thodima V, Trygg J, Vishnuvajjala L, Wang SJ, Wu J, Wu Y, Xie Q, Yousef WA, Zhang L, Zhang X, Zhong S, Zhou Y, Zhu S, Arasappan D, Bao W, Lucas AB, Berthold F, Brennan RJ, Buness A, Catalano JG, Chang C, Chen R, Cheng Y, Cui J, Czika W, Demichelis F, Deng X, Dosymbekov D, Eils R, Feng Y, Fostel J, Fulmer-Smentek S, Fuscoe JC, Gatto L, Ge W, Goldstein DR, Guo L, Halbert DN, Han J, Harris SC, Hatzis C, Herman D, Huang J, Jensen RV, Jiang R, Johnson CD, Jurman G, Kahlert Y, Khuder SA, Kohl M, Li J, Li L, Li M, Li QZ, Li S, Li Z, Liu J, Liu Y, Liu Z, Meng L, Madera M, Martinez-Murillo F, Medina I, Meehan J, Miclaus K, Moffitt RA, Montaner D, Mukherjee P, Mulligan GJ, Neville P, Nikolskaya T, Ning B, Page GP, Parker J, Parry RM, Peng X, Peterson RL, Phan JH, Quanz B, Ren Y, Riccadonna S, Roter AH, Samuelson FW, Schumacher MM, Shambaugh JD, Shi Q, Shippy R, Si S, Smalter A, Sotiriou C, Soukup M, Staedtler F, Steiner G, Stokes TH, Sun Q, Tan PY, Tang R, Tezak Z, Thorn B, Tsyganova M, Turpaz Y, Vega SC, Visintainer R, von Frese J, Wang C, Wang E, Wang J, Wang W, Westermann F, Willey JC, Woods M, Wu S, Xiao N, Xu J, Xu L, Yang L, Zeng X, Zhang J, Zhang L, Zhang M, Zhao C, Puri RK, Scherf U, Tong W, Wolfinger RD. 2010. The MicroArray Quality Control (MAQC)-II study of

common practices for the development and validation of microarray-based predictive models. *Nat Biotechnol* 28(8):827-838.

Massicotte R, Whitelaw E, Angers B. 2011. DNA methylation: A source of random variation in natural populations. *Epigenetics* 6(4):421-427.

Mirbahai L, Yin G, Bignell JP, Li N, Williams TD, Chipman JK. 2011. DNA methylation in liver tumorigenesis in fish from the environment. *Epigenetics* 6(11):1319-1333.

NRC. 1983. *Risk assessment in the Federal Government: Managing the process.* Committee on the Institutional Means for Assessment of Risks to Public Health, Commission on Life Sciences, National Research Council. National Academy Press, Washington, DC, USA.

OECD. 1995. OECD Guideline for the testing of chemicals. Test No. 407: Repeated dose 28-day oral toxicity study in rodents. Organisation for Economic Cooperation and Development, Paris, France.

Oshida et al. In preparation.

Pilsner JR, Hall MN, Liu X, Ilievski V, Slavkovich V, Levy D, Factor-Litvak P, Yunus M, Rahman M, Graziano JH, Gamble MV. 2012. Influence of prenatal arsenic exposure and newborn sex on global methylation of cord blood DNA. *Plos one* 7(5):e37147.

Skinner MK, Manikkam M, Guerrero-Bosagna C. 2010. Epigenetic transgenerational actions of environmental factors in disease etiology. *Trends Endocrinol Metab* 21(4):214-222.

Thomas RS, Clewell HJ, Allen BC, Wesselkamper SC, Wang NCY, Lambert JC, Hess-Wilson JK, Zhao QJ, Andersen ME. 2011. Application of transcriptional benchmark dose values in quantitative cancer and noncancer risk assessment. *Toxicol Sci* 120(1):194-205.

Thomas RS, Clewell HJ, Allen BC, Yang L, Healy E, Andersen ME. 2012. Integrating pathway-based transcriptomic data into quantitative chemical risk assessment: A five chemical case study. *Mutat Res* 746(2):135–143.

Thomas RS, Wesselkamper SC, Wang NCY, Zhao QJ, Petersen DD, Lambert JC, Cote I, Yang L, Healy E, Black MB, Clewell HJ, Allen BC, Andersen ME. 2013. Temporal concordance between apical and transcriptional points of departure for chemical risk assessment. *Toxicol Sci* 134(1):180-194.

van Delft J, Gaj S, Lienhard M, Albrecht MW, Kirpiy A, Brauers K, Claessen S, Lizarraga D, Lehrach H, Herwig R, Kleinjans J. 2012. RNA-Seq provides new insights in the transcriptome responses induced by the carcinogen benzo[a]pyrene. *Toxicol Sci* 130(2):427-439.

Villeneuve DL, Garcia-Reyero N, Escalon BL, Jensen KM, Cavallin JE, Makynen EA, Durhan EJ, Kahl MD, Thomas LM, Perkins EJ, Ankley GT. 2012. Ecotoxicogenomics to support ecological risk assessment: A case study with bisphenol A in fish. *Environ Sci Technol* 46(1):51-59.

Williams TD, Turan N, Diab AM, Wu H, Mackenzie C, Bartie KL, Hrydziuszko O, Lyons BP, Stentiford GD, Herbert JM, Abraham JK, Katsiadaki I, Leaver MJ, Taggart JB, George SG, Viant MR, Chipman KJ, Falciani F. 2011. Towards a system level understanding of non-model organisms sampled from the environment: A network biology approach. (C Von Mering, Ed). *PLoS Comput Biol* 7(8):20.

Wilson VS, Keshava N, Hester S, Segal D, Chiu W, Thompson CM, Euling SY. 2011. Utilizing toxicogenomic data to understand chemical mechanism of action in risk assessment. *Toxicol Appl Pharmacol* [Epub ahead of print].

# APPENDIX A: LIST OF PARTICIPANTS

| *Initial* | *Name* | *E-mail* | *Affiliation* |
|---|---|---|---|
| C. | Barata | cbmqam@cid.csic.es | Institute of Environmental Assessment and Water Research, Spain |
| R. | Brown | ross.brown@astrazeneca.com | AstraZeneca, UK |
| N. | Carmichael | neil.carmichael@orange.fr | Consultant, formerly ECETOC, France |
| M. | Chamberlain | markchamberlain@cxrbiosciences.com | CXR Biosciences, UK |
| K. | Chipman | j.k.chipman@bham.ac.uk | School of Biosciences, University of Birmingham, UK |
| C. | Corton | corton.chris@epa.gov | US EPA, USA |
| R. | Corvi | raffaella.corvi@jrc.ec.europa.eu | Joint Research Centre of the European Commission, Italy |
| C. | Elcombe | cliffelcombe@cxrbiosciences.com | CXR Biosciences, UK |
| R. | Frötschl | roland.froetschl@bfarm.de | Federal Institute for Drugs and Medical Devices (BfArM), Germany |
| M. | Galay Burgos | malyka.galay-burgos@ecetoc.org | ECETOC, Belgium |
| T. | Gant | tim.gant@hpa.org.uk | Health Protection Agency, UK |
| N. | Garcia-Reyero Vinas | nvinas@igbb.msstate.edu | Mississippi State University, USA |
| M. | Hampel | miriam.hampel@icman.csic.es | Spanish National Council for Scientific Research CSIC, Spain |
| M. | Heneweer | marjoke.heneweer@shell.com | Shell, The Netherlands |
| J. | Kleinjans | j.kleinjans@grat.unimaas.nl | Maastricht University, The Netherlands |
| J. | Lambert | lambert.jason@epa.gov | US EPA, USA |
| A. | Lampen | alfonso.lampen@bfr.bund.de | Federal Institute for Risk Assessment (BfR), Germany |
| B. | Meek | bette.meek@uottawa.ca | University of Ottawa, Canada |
| J. | Mestres | jmestres@imim.es | IMIM (Hospital del Mar Medical Research Institute), Spain |
| C. | Morris | morrisc10@cf.ac.uk | Cardiff University, UK |
| R. | O'Lone | rolone@hesiglobal.org | ILSI Health and Environmental Sciences Institute, USA |
| A. | Piersma | aldert.piersma@rivm.nl | RIVM, The Netherlands |
| B. | Piña | bpcbmc@cid.csic.es | Institute of Environmental Assessment and Water Research, Spain |
| A. | Schrattenholz | andre.schrattenholz@proteosys.com | ProteoSys, Germany |
| T. | Sukata | sukata@sce.sumitomo-chem.be | Sumitomo Chemical Europe, Belgium |
| S. | van der Vies | vdvies@few.vu.nl | VU University Medical Center, Amsterdam, The Netherlands |
| B. | van Ravenzwaay | bennard.ravenzwaay@basf.com | BASF, Germany |
| D. | Villeneuve | villeneuve.dan@epa.gov | US EPA, USA |
| C. | Wierling | wierling@molgen.mpg.de | Max Planck Institute for molecular Genetics, Germany |

# APPENDIX B: WORKSHOP PROGRAMME

***Monday 25 February 2013***

| | | |
|---|---|---|
| **12.00 – 12.45** | *Registration and lunch* | |
| **12.45 – 12.50** | Background | *Neil Carmichael*<br>*ECETOC* |
| **12.50 – 13.00** | Overview of the recommendations from the two previous workshops | *Ben van Ravenzwaay*<br>*BAS*F |

**1 – PREDICTION AND BIOMARKERS: ADVERSE OUTCOME PATHWAYS AND MOA**

| | | |
|---|---|---|
| **13.00 – 13.20** | Using gene signatures to predict adverse outcome pathways in the liver | *Chris Corton*<br>*US EPA* |
| **13.20 – 13.40** | Contribution of new technologies to better understanding modes of action and definition of adversity in toxicological assays | *Marjoke Heneweer*<br>*Shell* |
| **13.40 – 14.00** | Applicability of next generation sequencing in toxicogenomics for identifying adverse outcome pathways and MoAs | *Jos Kleinjans*<br>*University of Maastricht* |

**2 – USE OF 'OMICS INFORMATION IN A REGULATORY (ECO)TOXICOLOGY CONTEXT**

| | | |
|---|---|---|
| **14.00 – 14.20** | Use of 'omics in risk assessment of new food safety risks: Proteomic effects induced by 3-MCPD and its dipalmitate ester in rat liver: is there a common mode of action? | *Alfonso Lampen*<br>*BfR* |
| **14.20 – 14.40** | Purpose oriented integration of genomic data in regulatory risk assessment | *Bette Meek*<br>*University of Ottawa* |
| **14.40 – 15.00** | A vision for modernising environmental risk assessment | *Dan Villeneuve*<br>*US EPA* |
| **15.00 – 15.20** | *Coffee break* | |

### 3 – DOSE-RESPONSE CHARACTERISATION WITH THE 'OMICS TECHNOLOGIES

| | | |
|---|---|---|
| **15.20 – 15.40** | Application of transcriptomics in dose-response assessment of environmental chemicals | *Jason Lambert*<br>*US EPA* |
| **15.40 – 16.00** | Dose and time genomic responses to reproductive toxicants | *Aldert Piersma*<br>*RIVM* |
| **16.00 – 16.20** | Exposure assessment using 'omics technologies | *Tim Gant*<br>*Health Protection Agency* |
| **16.20 – 16.40** | Identifying No Observed Effect levels (NOEL) and No Observed Adverse Effect Levels (NOAEL) through metabolomics and a sensitivity analysis relative to classical regulatory toxicity studies in rats. | *Ben van Ravenzwaay*<br>*BASF* |

### CASE STUDIES

| | | |
|---|---|---|
| **16.40 – 16.45** | Introduction | *Malyka Galay Burgos*<br>*ECETOC* |
| **16.45 – 17.05** | Case 1: Robustness and reproducibility of toxicogenomics-based human *in vitro* assays | *Raffaella Corvi*<br>*JRC* |
| **17.05 – 17.25** | Case 2: TXG-Data to clarify relevance of neoplastic effects in rodent life time bioassays: a case study | *Roland Frötschl*<br>*BfArM* |
| **17.25 – 17.45** | Case 3: Reflection of exposure to environmental stressors via the epigenome | *Kevin Chipman*<br>*Birmingham University* |
| **17.45 – 18.05** | Case 4: Transcriptomics and ecological risk assessment: Case studies with fish exposed to bisphenol A and diethylstilbesterol | *Dan Villeneuve*<br>*US EPA* |
| **18.05 – 18.25** | Coffee break | |
| **18.25 – 18.45** | Case 5: Toxicogenomics applications for investigation of genotoxicity – A genomic biomarker approach | *Raegan O'Lone*<br>*HESI* |
| **18.45 – 19.05** | Case 6: The use of proteomics for the identification of compounds inducing reproduction toxicity | *André Schrattenholz*<br>*ProteoSys* |
| **19.05** | *End of Day 1* | |
| **20.30** | *Dinner* | |

***Tuesday 25 February 2013***

**4 – FUTURE PERSPECTIVES / SYSTEMS BIOLOGY / MODELLING**

| | | |
|---|---|---|
| **08.20 – 08.40** | A systems toxicology approach to decipher hormesis in *Daphnia magna* | *Natàlia Garcia-Reyero*<br>*Mississippi State University* |
| **08.40 – 09.00** | A systems approach to predicting the safety profile of small molecules | *Jordi Mestres*<br>*Hospital del Mar Medical Research Institute* |
| **09.00 – 09.20** | Recapitulation of Day 1 and introduction to syndicate sessions | *Jos Kleinjans*<br>*Maastricht University* |
| **09.20 – 11.00** | Syndicates | |

**Syndicate Sessions**

**Breakout Group Sessions**

**Syndicate 1 – Which opportunities does the group see for 'omics to help improve (eco)toxicology in the future?**
*Moderator: Neil Carmichael*
*Rapporteur: Natàlia Garcia-Reyero*

**Syndicate 2 – What (data) is missing, what would be the most important (data) to do and why to promote the use of 'omics in (eco)toxicology?**
*Moderator: Chris Corton*
*Rapporteur: Tim Gant*

**Syndicate 3 – Which challenges do we face to use 'omics data in risk assessment?**
*Moderator: Jos Kleinjans*
*Rapporteur: Dan Villeneuve*

**Syndicate 4 – 'Omics data in a human context**
*Moderator: Cliff Elcombe*
*Rapporteur: Saskia van der Vies*

| | | |
|---|---|---|
| **11.00 – 11.20** | *Coffee break* | |
| **11.20 – 12.10** | In plenary: Syndicate reports and discussion | *Chair: Aldert Piersma, RIVM*<br>*Audience and Speakers* |
| **12.10 – 12.30** | Summary / Conclusions | *Moderator: Ben van Ravenzwaay*<br>*BASF* |
| **12.30 – 14.00** | *Adjourn and lunch* | |

Close of Workshop

# APPENDIX 3: ORGANISING COMMITTEE

Ben van Ravenzwaay (Chairman)
BASF
Carl Bosch-Str. 38
D - 67056 Ludwigshafen
Germany

Rémi Bars
Bayer CropScience
355, rue Dostoïevski
F - 06903 Sophia Antipolis Cedex
France

Malyka Galay Burgos
ECETOC
Avenue E. Van Nieuwenhuyse, 2
B - 1160 Brussels
Belgium

Saskia van der Vies
VU University Medical Center
De Boelelaan 1117
1081 HV Amsterdam
The Netherlands

# ECETOC PUBLISHED REPORTS

The full catalogue of ECETOC publications can be found on the ECETOC website:
http://www.ecetoc.org/publications

**Responsible Editor:**
Dr. Alan Poole
ECETOC AISBL
Av. E. Van Nieuwenhuyse 2 (bte. 8)
B-1160 Brussels, Belgium
VAT: BE 0418344469
www.ecetoc.org
D-2013-3001-229

Established in 1978, ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) is Europe's leading industry association for developing and promoting top quality science in human and environmental risk assessment of chemicals. Members include the main companies with interests in the manufacture and use of chemicals, biomaterials and pharmaceuticals, and organisations active in these fields. ECETOC is the scientific forum where member company experts meet and co-operate with government and academic scientists, to evaluate and assess the available data, identify gaps in knowledge and recommend research, and publish critical reviews on the ecotoxicology and toxicology of chemicals, biomaterials and pharmaceuticals.