



The European Partnership
for Alternative Approaches to Animal Testing

Final Report

Workshop on Computational Chemistry and Systems Biology

**“Harnessing the Chemistry of Life:
Revolutionising Toxicology”**

5-7 July 2010
Hotel Amigo, Brussels, Belgium

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1. Organisation of the meeting

The workshop was organised to test the assertion that leading edge computational chemistry and systems biology appropriately engaged and working closely together on well-defined problems can revolutionize toxicology and provide novel non-animal approaches to safety assessment. Computational chemistry encompasses all aspects of using computers in chemistry including chemoinformatics. As a case study to examine this assertion in greater detail, the chosen focus for the workshop was the prediction of hepatic toxicity given the importance of the liver in the metabolism of small molecules and the importance of hepatotoxicity across many of the industry sectors represented within the European Partnership for Alternative Approaches to animal testing (EPAA).

The workshop brought together computational chemists (many of whom are not currently directly involved in research related to toxicology), systems biologists, those experienced in modelling other organs or with specific liver expertise and toxicologists from Europe and USA and from academia, government and industry. The objectives were:

- To articulate clearly the challenges that face toxicologists in developing viable approaches for safety assessment of chemicals and drugs without the use of animals,
- To bring together experts in computational chemistry and other relevant disciplines to explore jointly how recent advances in computational chemistry – when aligned with developments in biology – can be harnessed to address the challenge of developing new paradigms for safety assessment,
- To identify what the needs are for delivering against this challenge to revolutionize toxicology, including the requirement for engagement with other scientific disciplines,
- To agree a high-level Strategic Plan to provide a detailed roadmap for progress to be made.

It was recognised that those attending represented only a sample of the expertise available in these fields but it was felt that this group, the majority of whom had not worked together before, would provide a good test of the assertion. Publishing the outcomes would subsequently widen the debate, to focus and encourage engagement

in research to accelerate developments towards the ultimate goal of predictive toxicology based on non-animal models.

The meeting delegates are listed in Appendix 1. Prior to the meeting background reading was suggested to orient thinking and provide context for the discussions. The references are listed in Appendix 2.

The workshop was structured to comprise five sessions over three days 5th-7th July 2010. The Agenda is attached as Appendix 3:

1. Agreeing the challenge and setting expectations – an opportunity for the group to work together for the first time
2. The state of the science:
 - How do chemicals cause adverse effects in the liver?
 - Systems Biology.
 - Computational Chemistry.
3. Syndicate discussions and plenary to address the question - *“with the skills and talents around this table what can we do to drive new innovation in liver toxicology”*
4. Second syndicate discussion to address the questions – *“what needs to happen, what will a plan look like?”*
5. Final plenary and wrap up.

2. Introductory Session

Carl Westmoreland (Unilever and joint chair of the workshop organising committee) described the work of the EPAA. He described this unique partnership between the European Commission and industry which has the objective of promoting the development and implementation of new 3Rs methods (to Replace, Reduce, Refine animal testing) and modern alternative approaches in the field of safety testing. Its activities include how to get the best out of research to drive innovation and create new opportunities. This is the work of the ‘Platform on Science’ which complements parallel platforms addressing:

- Streamlining validation and regulatory acceptance.
- Improving information and dissemination.

One of the key objectives of the Platform on Science is to engage scientists from groups previously unconnected with ‘alternatives’ to introduce new thinking to address science gaps. He explained that “alternatives“ research has typically tried to find one *in vitro* test that will give the same result as an animal test. The challenge for the workshop was to consider new ways of doing the science rather than looking for 1:1 replacements of the current animal methods. The focus now should be on what information is actually required to ensure that new products can be manufactured and used safely.

Colin Humphris (on behalf of EPAA) described the work of the EPAA’s New Perspectives workshop held in April 2008 (web-link to report



http://ec.europa.eu/enterprise/epaa/2_activities/2_1_science/new_perspectives.pdf

). At this event a number of innovative new approaches in toxicology were proposed, including those arising from fully harnessing the power of computational chemistry and chemoinformatics. The modelling of cells or even the liver was considered a challenging but realistic long term goal by the invited experts.

Ian Kimber (University of Manchester) focussed on the reality of what toxicologists face in their day-to-day work and decision making. He framed the challenge for this workshop; *“in a world without animals, how would we assess the potential of chemicals and drugs to cause adverse health effects following repeated systemic exposure?”* He pointed out that:

- Toxicology is the art of identifying the unexpected,
- The dose makes the poison,
- Toxicity tests are fallible.

He described the broad scope of toxic endpoints that usually need to be considered and the multiplicity of factors that are able to affect the development of adverse health effects (see Appendix 4).

He defined three needs:

We must:

1. align the best that modern science and technology have to offer with the needs of toxicology and safety assessment,
2. encourage the best minds to engage with the unique challenges that advancing the 3Rs brings to biology and chemistry,
3. encourage wide recognition among the international scientific community that research aligned with delivering animal welfare benefits is both legitimate and exciting.

Gianni Dal Negro (GlaxoSmithKline) focused on the liver as a case study for this Workshop. He emphasized that the liver is the most important organ involved in removal of xenobiotics derived from the environment or produced in the body during normal or pathological metabolism and that liver toxicity is amongst the most common adverse effects seen with xenobiotics.

He described the structure and functions of the liver including the importance of hepatic metabolism which is important for the activation of some drugs and can itself also lead to the formation of toxic substances. Perturbations in hepatic structure or function are very important as they can have significant ramifications for both the innate and adaptive immune systems. The structural complexity of the liver means that there are many possible sites for toxic interactions and a large number of known processes (and feedback processes) leading to toxic endpoints. There are also gaps in our knowledge leading to unpredictable effects.



He concluded:

- The liver is a complex organ consisting of different cell types having different functions,
- Current approaches to safety assessment still have many gaps that need to be urgently filled including the incidence of unexpected severe liver toxicity in humans,
- None of the alternative methods currently available can be used as a stand-alone tool to predict the hepatotoxic potential of a new molecule.

In this context he saw the challenge for the Workshop as combining computational tools and systems biology in new ways that describe the interactions between a xenobiotic and endogenous molecules and cells enabling improved prediction of hazard.

The resulting plenary discussion centred on the scale of this challenge. It was compared with major past challenges; *getting a man to the moon (and back!)*, or *the human genome project*. A number of important considerations were noted:

- Computational chemistry today can predict small molecule interactions reasonably reliably over time periods ranging from fractions of a second to seconds but has otherwise considerable limitations. Toxicology considers responses from days to years (for repeat dose systemic toxicity). Acute toxicity may be more amenable to modelling than chronic effects.
- We would be seeking to model across a huge range of spatial scale; from molecular interactions, through macromolecules to cells, tissues and organs and health effects in an entire organism.
- There are many possible modes/mechanisms of action, at many sites, in different types of cells and tissues. Animals are such attractive models for toxicologists because they are fully integrated systems. There are many challenges to our understanding of chemical reactivity that should be addressed by computational chemistry and molecular modelling in areas such as metabolite prediction, enzyme mechanisms, xenobiotic metabolism and P450 enzyme reactions.
- Reactive and potentially toxic metabolites are often key to hepatotoxicity. 80% of drugs that are toxic are not intrinsically toxic but activated in the body.
- We may be able to model specific interactions but the fundamental problem is connecting the bottom up with the top down across the spatial and temporal range i.e. computational chemistry with systems biology. It requires an integrated approach rarely achieved in the past.
- Do we need to know everything about a cell or organ to predict toxicology or only the parts that can go wrong? What is the level of granularity needed to make predictions that would allow us to confidently assure safety of consumers/patients/workers? We may be able to use information theory to enable us to extract and analyse only the relevant information. This however begs further questions:
 - What loss of accuracy can we accept?
 - How do we describe an adverse effect in the model?
 - How do we build confidence that we can get it right?

- The focus is the balancing of innovation and safety, whilst coping with unexpected events, knowing the tissue, the dose where it matters, and the fallibility of existing test methods and data which will be cited for comparison.
- Modelling will require good toxicological data and whilst vast amounts exist it is often not in a consistent form and therefore difficult to share. This is the reason for programmes such as TOX21¹ and TOXCAST² in the US which are developing ontologies and vocabularies to enable wider sharing of data. An integrated knowledge based approach will be essential.
- Although we are able to compute phase partition behaviour for small molecules in for instance oil/water, this becomes a far more challenging issue in the body where membrane and cellular structures are critical. It is key to the understanding of the effective dose at the site of interaction.
- We need good, quantitative early markers and reference materials for the different toxic responses in the liver which can be modelled.
- There was concern about the ability to estimate binding affinities between small molecules and proteins.
- Related existing research should be taken into account:
 - The successful activities recently modelling the heart (e.g. www.comlab.ox.ac.uk/chaste) have been far from trivial. The heart has a single primary function (a muscle pump), the liver (as described in the introductory session) has many.
 - Other projects (not necessarily currently closely associated with toxicology) envisaging a virtual liver including The Virtual Liver Network in Germany (http://www.ra.cs.uni-tuebingen.de/forschung/virtualliver/welcome_e.html) which aims to explore the physiology and functionality of the liver and is currently entering its third phase.
 - Recognition of the value of existing *in vitro* and *in silico* (QSAR) approaches in addressing today's needs to reduce and replace animal use in current regulatory safety assessment.

Despite the reservations expressed, there was a general agreement that cells are chemical objects and that we should and can move beyond simple molecular understanding. Building across the scale from molecular interaction to cellular response in an integrated way may well provide a basis for predictive modelling of toxicity in a complex organ like the liver.

¹ <http://www.epa.gov/ncct/Tox21/>

² <http://www.epa.gov/ncct/toxcast/>

3. State of the Science

How do chemicals cause adverse effects in the liver? H Jaeschke, University of Kansas

Hartmut Jaeschke focussed on the detailed understanding of Acetaminophen (Paracetamol) action in the liver as an example of small molecule effects.

He described the complexity of the intercellular mechanisms. A number of pathways exist which yield safe detoxification in the liver. This is why it is safe to use Paracetamol at therapeutic doses. At higher doses, and if detoxification mechanisms become overloaded, covalent binding with mitochondrial proteins can lead to the creation of reactive intermediates leading to toxic endpoints via mitochondrial dysfunction, eventual mitochondria membrane collapse and cell death.

The experimental conditions in the laboratory can significantly affect the extent of toxicity observed with Acetaminophen *in vitro*. Most published *in vitro* studies with Acetaminophen-treated hepatocytes have used 21% ambient air oxygen levels whereas oxygen levels in the liver *in vivo* range from 9 – 3% across the liver lobule.

A number of environmental and health factors affect whether Acetaminophen-induced mitochondrial dysfunction leads to either cell death or mitochondrial regeneration. To study this, and other aspects of hepatotoxicity *in vitro* reliably for humans the cell lines used should reproduce *in vivo* behaviour competently. The pros and cons of primary hepatocyte culture and the use of hepatoma cell lines was discussed as well as a relatively new cell line (HepaRG) from liver stem cells are proving to be useful in *in vitro* studies of hepatotoxicity.

It was recognised that it is important to focus on the mechanisms of toxicity rather than the complexity of the whole biological system. This had been important in the modelling work on the heart.

It was noted that just because something is reactive it does not necessarily imply that it will kill cells. Millions of people use Acetaminophen safely. What else do we need to know and how will we characterise relevant toxic events in models? The key unit to model is probably the cell in which there are approximately 500 mitochondria. There was a sense however that an ability to model at mitochondrial level would be a significant advance.

Systems Biology: I Shah, US EPA

The overall challenge is the need to review the safety of too many chemicals for which we have too little data. We have animal (rodent) models but these provide toxicity data only at high doses; they are costly, slow and uncertain. Little information exists at low and very low doses which is the normal human experience. We are trying to estimate a dose response curve from high dose rodent data only. This has led to the need for effective tiered and prioritised testing strategies, correlative approaches (QSARs) and also pressure to reduce dependence on animals.

The anticipated future directions will focus on improved *in vitro* assays to provide detailed understanding of toxicity pathways and from these the required dose response estimation. It was emphasised that the eventual use of any information on liver toxicity should be of use in the context of a risk assessment decision. Imran Shah exemplified his talk based on the model for adaption and or injury by Andersen,



Dennison, Thomas & Conolly, 2005. He reviewed the various modelling possibilities at the different scales; from environmental exposure to the internal dose, to molecular changes and interactions, to cellular responses, and finally to tissue responses. In the process a substantial literature was reviewed (Appendix 5). The models have to be capable of linking events at very different scales and show the potential for systems biology to link the molecular to the tissue level. The Physiome project for the heart was cited as an example of these approaches although the greater structural and functional complexity of the liver was noted.

The multi-scale modelling approach building from the physical, and molecular chemistry (binding affinities etc) and complemented by systems biology at cellular and tissue scale was contrasted with the use of empirical approaches including QSARs between the scales. These empirical approaches will continue to be important in the short term to fill key gaps in our detailed knowledge.

The literature for liver modelling was reviewed and the success of cell-based modelling to reduce the complexity highlighted, which focuses on the molecular pathways to cell changes, which lead to cell death and toxicity at the tissue level. Modelling approaches should focus on the cell as a unit to address the complexity of the task i.e. 'cells are the unit of function'. Tissue lesions can be viewed as events that are propagated by cellular pathways.

He noted that the problem with the bottom-up multi-scale approach is that you create the whole forest rather than the critical toxic processes and pathway. A combined experimental and integrated modelling approach was therefore proposed with *in vitro* experimentation designed to inform and focus the modelling specifically.

Computational Chemistry: Jonathan Essex, Southampton University

Jon Essex reviewed the state of the science for computational modelling of molecular interactions at a number of scales.

For small molecules, modelling works well and it is possible to create structural representations of the constitution, 3D shape and molecular surfaces. Descriptors can be created that correlate well to properties.

It becomes more complex when considering the interactions of small molecules and macromolecules (such as proteins, lipids, DNA) where shapes and conformation change on binding. Judgement is needed to estimate the free energy changes and binding affinity. Scoring systems can be used with variable reliability. Progress is being made on predicting interactions and how reactive they may be through a combination of quantum and molecular mechanics.

At the protein-protein level the availability of experimental data is driving advances. Predicting the conformational changes may not be right every time but they can add value when no other information exists and in deciding whether or not the proteins can interact.

For large molecular assemblies including mixtures of proteins/lipids and DNA it has proved possible to assess the influence of the environment on function (such as transport properties and molecular aggregation) at a coarse-grained level. The cytoplasm of *E.coli*. has been modelled showing how the protein-protein interactions and cellular congestion affect protein folding.



He concluded that there is a need for automated methods to link the different modelling methods, and to link this directly to experimental procedures. New software engineering processes combined with high performance computing are becoming available, meaning that this could now be possible.

4. Syndicate Session 1 and Plenary Discussions.

Two Syndicates addressed the question “*with the skills and talents around this table what can we do to drive new innovation in toxicology (liver)*”

The discussions recognised that we face a multi-scale challenge in seeking new approaches to predicting toxicity in the human liver which will have to bridge the languages of chemistry and biology.

Today the following is achievable;

- Existing data probably is sufficient to enable structural alerts of hepatotoxicity. This needs to be available in consistent forms to enable data mining.
- We have a range of modelling techniques available although of variable accuracy. Today most modelling as applied to toxicology is correlative (QSARs etc). We have Pharmacokinetic models, a good understanding of the 3D tissue structure and liver function, together with models for induction and repression of P450.
- This should enable a broad understanding of the distribution of chemicals within the liver structure, the conversion to reactive metabolites, where chemicals and metabolites may react or bind, and markers for hepatotoxicity.
- We can compute where small molecules are likely to interact and the types of interactions.

It was clear that the science is missing in a number of areas:

- We need common chemical and toxicological ontology's to describe the processes across the range of scales both spatially and temporally. We need a definition of toxicology in terms of doses, adaptive effects and adverse effects (including cell death) that can be incorporated into the models.
- We lack the ability to predict binding affinities of small molecules to macromolecules reliably and consistently.
- We have a poor understanding of effective tissue doses and also the processes/pathways at low or very low doses.
- We need good *in vitro* models of effects at low doses, effects of metabolites, effects of complex mixtures, effects of metabolites. In particular it will be important to identify suitable cell lines (and the potential of stem cell technology) to inform the modelling work.
- There is probably a need for new computer hardware and software to model at the tissue level.

The outline workflow that emerged comprised four steps:

1. Identifications of metabolites (and metabolites of metabolites) and their reactivity, through a combination of computational chemistry, *in vitro* experimentation and enzyme expression profiling.

2. Identification of the proteins (and potentially other intracellular targets such as DNA, lipids etc) each metabolite affects, again through computational chemistry and *in vitro* work using purified proteins.
3. Identification of the pathways affected by these proteins, through *in vitro* cell assays and systems biology.
4. Find which cell functions are affected by these pathways, by defining the boundaries of normal function, and understanding of the physiology and systems biology.

Through the discussion the pivotal role of the hepatic mitochondrion in hepatic toxicity became clear. The possibility of modelling to this scale as a realistic (although stretching) target for computational chemistry was clarified. There was recognition that this could only be achieved through a very tight integration of the chemical and biological modelling, building on data from *in vitro* and *in vivo* experimentation specifically designed to inform the modelling. This point was developed in the second syndicate session.

5. Syndicate Session 2 and Plenary Discussion

The second syndicate sessions focussed on deepening thinking on the way forward towards the final goal to develop a comprehensive model to predict the relationship between chemical structure, dose and induction of different types of toxicity in the liver. Specifically, the groups were asked to consider what needs to happen and what would a plan of action look like with regards to:

- for Group A
 1. Organotypic, human-relevant *in vitro* liver models,
 2. The data: Capturing what's available, databases, ontologies. What would we like to have (*in vitro* working hand-in-hand with model building)?
- For Group B
 1. Models for metabolism/reactivity and interactions with proteins,
 2. How could we go from chemical intervention → cellular response? Bringing chemistry and biology together.
 3. A case study: Mitochondria.

The outputs were shared in plenary.

There was complete agreement that a key aspect will be the derivation of suitable experimental *in vitro* data. A metabolically competent cell line such (e.g. HepaRG) and continued investment in stem cell technology is essential to achieve this. The key considerations will be:

- The chosen *in vitro* system must reflect 'important' aspects of liver function,
- Data from *in vitro* system must be designed to inform the *in silico* system,

- To this end it is essential that the modellers work with experimentalists to define what data the experimental model should generate,
- The 'biological endpoints' that should be examined first that will feed into models.

Appropriate *in vitro* systems should:

- Allow measurements at a single cell level including image-based assays and flow cytometry to allow data to be gathered on dose responses over time,
- Recapitulate relevant aspects of hepatic metabolism,
- Recapitulate most important pathways for: Proliferation, contact inhibition, stress, apoptosis and mitochondrial functions,
- Provide quantitative readouts of xenobiotic-induced effects, including:
 - Cytokine production
 - Mitochondrial function
 - Apoptosis
 - Proliferative state
 - Signalling pathways
 - Cell 'interactions'
 - Small molecule distribution.

A range of modelling activity will be needed:

- To predict selectivity e.g. Phase I reaction types, region-selectivity effects and polymorphism of CYP450s,
- Inherent reactivity of small molecules,
- Reactivity and interactions with proteins. A step-change (from qualitative to quantitative) is necessary in these predictions – A chemical (or its metabolite) may be reactive, but that doesn't necessarily imply that, under the conditions of exposure, it will be toxic. The most 'dangerous' metabolites may be those that are very short-lived and which only computerised modelling will predict.
- To predict (quantitatively) how individual compounds will be metabolised in the human liver and to be able to predict the reaction kinetics,
- To provide an understanding of reactive metabolites; identification, reactivity, predictive sites of reactions on proteins, possibilities of inhibition on CYP450s, types of reactions, impact on DNA and covalent binding,
- There is also a need to improve quantitatively current models for:
 - Linking docking and quantum and molecular mechanical modelling of enzymatic reactions
 - Transition state modelling
 - Effects of genetic variation and variation in metabolic ability
 - Protein dynamics and flexibility
 - Effects of genetic variability on selectivity and reactivity of Phase I and Phase II enzymes.
- To link computational chemistry and systems biology seamlessly so that protein modification and its effects on reaction pathways can be understood. To achieve this, large-scale simulations need to be developed involving many macromolecules in realistic cell/organelle environments to provide the mechanistic, thermodynamic and kinetic understanding to bridge between the understanding of small molecule interactions and the overall biological system.

The models need to be capable of predicting:

- Relationships between alterations in specific pathways and hepatocyte metabolism/function,
- Effects of loss of any specific protein and cell metabolism function,
- Relationship between cell function and 'toxicity'. Do we know enough about the distribution within normal/'healthy' cells of many of the parameters associated with toxicity pathways?
- Relationship between structure of the chemical or drug and P450-reactive products,
- Where and how an arbitrary reactive product will react,
- Effects of different human enzymatic/protein variants on hepatocyte function,
- The relationships between chemical structure and tissue distribution (extracellular and intercellular) with transport, and plasma protein binding.

A strong recommendation emerged for a case study to model the effects of small molecules on liver mitochondria. This was seen as a new "grand challenge" for science which would go well beyond existing research programmes to model liver and toxicity. It would help focus the overall activity with a clear strategic objective. The case study would consider the hepatocyte mitochondria and model the chemical interactions and systems biology to provide a predictive capability of value to predictive toxicology for chemicals, pharmaceuticals and other areas of health. This would help us frame safety assessment more effectively in the future. Computational chemistry and systems biology would be closely linked under a pilot case study building on the understanding of Acetaminophen-induced hepatotoxicity to provide the necessary insights into:

- Enzymology
- Electron transport and transport chains
- Membrane pore formation
- Tricarboxylic acid cycle
- Distribution
- Gene expression

In many cases, data would need to be generated with in vitro systems with the specific goal of informing the evolution of mathematical models (i.e. the in vitro results themselves may not directly impact on replacement of animal tests). An important element of this development would be a central repository of data which would provide:

- A list of chemicals that have well-characterised hepatotoxic effects
- Knowledge maps of hepatotoxicity field
- Compound-dose-cell response relationships
- Ontologies for classifying, describing and quantifying effects:
 - Standard experimental protocol
 - Curated data on hepatotoxicity
- Tools for data capture and literature curation
- Standards
- Stored models of hepatocyte/liver
- Rodent histopathology to chemical treatment, time course, dose etc
- Single cell data and multiscale data.

There is a need to understand the extent to which such a repository exists today.

6. Final Plenary and wrap up

There was recognition that the current gap between the molecular modelling (computational chemistry) and the cell/ tissue modelling (systems biology) can be closed.

Efficacy and safety of drugs and chemicals remains a longer term target, however a case study of liver mitochondria will allow the exploration of the potential of computational chemistry and the scope to bring together chemistry and biology. In addition this would be the relevant to personalised medicine and increased understandings for fundamental biology. This was seen as an early, valuable and highly relevant application of systems biology.

More is needed to articulate the work flow related to the liver mitochondria case study, to identifying appropriate teams to undertake the modelling and provision of experimental *in vitro* data, and the processes by which they could work seamlessly together. It was also important that this work takes account of and builds on related research already underway around the world to model and understand liver function and toxicology.

Significant added value was seen in bringing academia, the Commission and industry together to make this happen and to ensure access to data. The proposed tight linkage between molecular modelling and systems biology and iterative experimental design and data generation across such an integrated team is something not attempted before. It was felt that the trickiest part may be bringing together experimentalists and computational scientists. It needs to be a single team with a common goal suitably incentivised to work together. A modular programme distinguished by the convention scientific disciplines and labels would be less effective.

7. Conclusions

The main objective of this Workshop was to consider how recent advances in computational chemistry, when aligned with expertise in systems biology, could be harnessed to provide the basis for new truly innovative approaches to safety assessment that will obviate the need for animals.

The Workshop brought together experts in computational chemistry, systems biology, toxicology and other scientists at the leading edges of their fields. It examined the proposal that a solution for truly animal free, predictive safety assessment lies at this interface of sciences and the mobilisation of the best scientists in their respective fields, many of whom would not traditionally apply their work to toxicology.

The workshop was devoted primarily to the invited participants working in syndicate groups to define how best the assembled scientific expertise could be exploited for the development of significant new opportunities in toxicology using hepatotoxicity as a case study. The liver was chosen to focus the discussion on an organ which is of particular importance to the industry sectors represented within the EPAA.

The syndicate sessions were very lively, enthusiastic and productive, and the discussions were both energetic and creative. There was strong agreement that integrated research should be undertaken that:

1. considered modelling transport and interactions from molecular to cellular/organelle levels (mitochondria specifically)
2. was tightly integrated with in vitro stem cell experimentation designed specifically to inform this modelling activity
3. coupled directly to systems modelling from cellular to organ level
4. took account of mechanistic understandings of toxic responses in the liver
5. and that established an appropriate infrastructure for computation data basing and sharing.

A truly integrated approach where modellers, chemists and biologists define and engage jointly on integrated research with shared goals will provide an exciting platform for exploring future approaches to safety assessment without animals. In this regard, it would build on current attempts around the world that model the liver. Importantly it has the potential to go beyond these to deliver an approach which was fit-for-purpose for predictive toxicology.

The next steps are to publish the detailed outcomes from the workshop in the peer-reviewed literature to broaden the scientific debate, whilst a dedicated team drawn from workshop participants works on the key strategic principles from the workshop to define a workable research and dissemination plan for EPAA.

Colin Humphris
On behalf of the Workshop Participants
& the Organising Committee
February 2011

Appendix 1

Workshop Attendees

Invited Participants

Gabriele Cruciani	University of Perugia
Jon Essex	Southampton University
Johann Gasteiger	Universitaet Erlangen-Nuernberg
Barry Hardy	OPENTOX, Douglas Connect
Volkhard Helms	Universitaet Saarland
Hartmut Jaeschke	The Liver Center, University of Kansas, USA
Joanna Jaworska	P&G
Richard Lavery	Institut de Biologie et Chimie des Protéines, Lyon
Andrew Leach	GSK
Jean-Pierre Lepoittevin	Strasbourg University
Pedro Mendes	Manchester University
Adrian Mulholland	Bristol University
Benoit Perthame	Pierre and Marie Curie Université
Blanca Rodriguez	Oxford University
Imran Shah	US EPA
Steve Wiley	Environmental Molecular Sciences Laboratory Pacific Northwest National Laboratory, USA
Andrew Worth	JRC

Organising Committee

Carl Westmoreland	Unilever
Ian Kimber	Manchester University
Jon Essex	Southampton University
Gianni Dal Negro	GSK
Nathalie Alepee	L'Oreal
Juergen Buesing	DG Research
Irene Manou	EPAA Secretariat
Colin Humphris	EPAA

Appendix 2

Workshop Pre-reading

1. Computational modelling of CYP450s for toxicity prediction. G. Cruciani *Expert Opinion On Drug Metabolism & Toxicology (submitted paper)*
2. Intracellular Signalling Mechanisms of Acetaminophen-Induced Liver Cell Death, Hartmut Jaeschke and Mary Lynn Bajt *Toxicological Sciences 89(1), 31–41 (2006)*
3. Mechanisms of Immune-Mediated Liver Injury; David H. Adams, Cynthia Ju, Shashi K. Ramaiah, Jack Uetrecht, and Hartmut Jaeschke, *Toxicological Sciences 115(2), 307–321 (2010)*
4. Virtual Tissues in Toxicology Imran Shah, John Wambaugh, *Journal of Toxicology and Environmental Health, Part B, 13:314–328, 2010*
5. The Many Roles of Computation in Drug Discovery, William L. Jorgensen, et al. *Science 303, 1813 (2004)*

Appendix 3

Workshop Agenda



Workshop on Computational Chemistry and systems biology
“Harnessing the Chemistry of Life: Revolutionising Toxicology”
5-7 July 2010, Hotel Amigo, Brussels, Belgium

Programme

Time	Monday 5 th July
3.00 pm	Check-in and Registration
4.00 pm	Session 1 Setting the Challenge and Expectations Moderator: Carl Westmoreland <ol style="list-style-type: none">1. Introduction and “tour de table”2. “EPAA perspective – revolutionising toxicology” - Carl Westmoreland, Unilever3. “Why Computational chemistry?” - Colin Humphris4. Setting the Scene “The challenges of predictive toxicology” - Prof. Ian Kimber, Manchester University, UK5. “Case Study – an appropriate focus on the liver” - Gianni Dal Negro, GSK6. Refining the agenda for the next two days - Discussion with the Delegates
7.00 pm	Buffet dinner at the hotel – evening of informal discussion

Time	Tuesday 6 th July
9.00 am	<p>Session 2 State of the Science Briefing Moderator: Colin Humphris</p> <ol style="list-style-type: none"> 1. Computational chemistry: State-of-the-art small molecules interacting with biological systems – Prof. Jon Essex, Southampton University, UK 2. State-of-the-art Biological modelling - Imran Shah, Environmental Protection Agency, USA 3. Liver toxicity, how do chemicals cause adverse effects in the liver? - Prof. Harmut Jaeschke, The University of Kansas Medical Centre, USA <p>Plenary Discussion</p>
12.00	<p>Session 3 Syndicate sessions introduction: Questions and process Moderator: Prof. Ian Kimber</p>
12.30 pm	Lunch
1.45 pm	<p>Syndicate session 1 (2 parallel syndicates)</p> <p><i>“How best can we align computational chemistry with systems biology and biological modelling to develop significant new opportunities in toxicology?”</i></p>
3.45 pm	Tea and coffee
4.15 pm	Feedback from the Syndicate session 1 in plenary
7.00 pm	Dinner in Brussels

Time	Wednesday 7 th July
9.00 am	<p>Session 4 Syndicate session 2 (2 parallel syndicates) Moderator: Carl Westmoreland</p> <p><i>“What are the key gaps in our knowledge that need to be addressed to model and predict toxic insults?”</i></p> <p><i>“What are the major theoretical and practical hurdles to be negotiated in modelling systemic toxicity?”</i></p>
11.00 am	<p>Session 5 Wrap-up conclusions and recommendations in plenary Moderator: Carl Westmoreland</p>
1.00 pm	Close and lunch

Appendix 4

The Scope of Toxicology

Adrenal toxicity	Altered cellular differentiation
Allergy	Altered cellarmigration
Autoimmunity	Altered gene expression
Bone Marrow toxicity	Cytotoxicity
Carcinogenesis	Induction of apoptosis
Cardiotoxicity	Inhibition of apoptosis
Chromosomal aberrations	Inflammation
Dermal toxicity	Perturbation of cell division
Developmental toxicity	Perturbation of production/secretion
Embryo toxicity	Receptor agonism
Endocrine disruption	Receptor antagonism
Gastrointestinal toxicity	Stimulation of cell division
Genetic toxicity	
Haemotoxicity	
Hepatotoxicity	
Immuntotoxicity	
Neurotoxicity	
Ocular toxicity	
Renal toxicity	
Reproductive toxicity	
Skeletal damage	

Factors affecting the Manifestation of Toxicology

Species
Strain/Ethnicity and Race
Age
Gender
Nutritional status
Hormonal status
Metabolic stress
Pregnancy
Stress
Environmental factors
Biological clock
Intercurrent disease

Exposure:

Dose
Route
Duration
Timing

Appendix 5

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