Toxicology in the 21st century – Working our way towards a visionary reality

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Abstract

In November 2009 the In Vitro Testing Industrial Platform (IVTIP) organized a meeting entitled 'Toxicology in the 21st century – working our way towards a visionary reality'. Participating delegates included scientists, key opinion leaders, developers and users of 3Rs-related tests and testing strategies. This paper summarizes the discussions with respect to the conditions required to move the vision towards an applicable reality. It should not be considered as a comprehensive review of technologies that could be relevant for moving the in vitro testing and risk assessment field forward.

Overall, the US National Research Council (NRC) vision and strategy for toxicity testing in the 21st century was unanimously considered as the right approach to enable future toxicity testing without animal experimentation. Many elements of this vision were identified in the European initiatives aimed at the development of non-animal based methods. However, the need for concerted actions moving the current state-of-the-art towards a thorough, reliable and systematic approach to future toxicity testing was made evident by the discussions.

Among the difficulties and hurdles on the way forward, the lack of physiologically relevant, metabolic competent and robust in vivo, ex vivo and in vitro models of both healthy and diseased people was frequently mentioned. In addition, there was a call for immediate implementation of emerging technologies and paradigms considered to be essential for transferring the vision into the reality of a toxicity-testing system assessing biologically significant perturbations in key pathways which are relevant for human biology. While the unique strengths of each of the available and emerging technologies was recognized, integration of available data and emerging technologies to integrated testing strategies (ITS) was highlighted as the preferred way forward. Method harmonization and standardization, as well as procedures and guidelines for putting together ITS, were urgently requested in order to facilitate proper implementation and acceptance.

There was an urgent call for better coordination of the efforts that are ongoing or initiated in the 3Rs arena at national and international level. Education, training, communication and dissemination were addressed. It was recognised that the EPAA, through its 'Platform for Communication and Dissemination', has a very important and central role in this area.

1. Toxicity testing in the 21st Century: the National Research Council vision

Globally, growing societal and ethical concerns, current research demands and the mentioned European legislation have accelerated the development of new visions and strategies for toxicity testing. The most recent exponent of this process is the report by the US NRC entitled *Toxicity Testing in the 21st Century: A Vision and a Strategy* (National Research Council, 2007) ('21C') which advocates the implementation of scientific and technological progress for acquiring in-depth understanding of the physiological and toxicological processes related to toxicological endpoints. This understanding should allow the evaluation of biologically relevant compound-specific perturbations in key toxicity pathways by using new methods (e.g. computational biology) and a representative but comprehensive array of in vitro tests based on human biology. Being based on human biological systems, the emerging tests are considered to be more relevant than the traditional animal-based tests.

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The report envisions the incorporation of the identified biological pathways in a panel of human cell based in vitro high throughput (HT) and content tests for measuring a defined set of well characterized events representing relevant mechanistic endpoints following exposure to multiple doses of a chemical.

Dose–response modelling using systems biology models for the perturbed biological pathways in combination with pharmacokinetic models are to provide the information required for in vitro to in vivo extrapolation from relevant doses used in the in vitro tests rather than from high doses typically used in animal studies.

The US Environmental Protection Agency (EPA) launched ToxCast™ (http://www.epa.gov/nct/toxcast/) to develop a cost-effective approach based upon state-of-the-art HT screening bioassays for rapidly prioritising the toxicity testing of large numbers of chemicals. In its current state it has measured more than 400 molecular events induced by over 300 chemicals. The growing database is to be used for building statistical and computational models to forecast potential chemical toxicity in humans. This project will beyond doubt help us on the way to a ‘21C’ toxicity testing strategy. It should be discussed though to what extent ToxCast fulfills the main principles of the ‘21C’ vision: (i) in-depth understanding of physiological and toxicological processes, (ii) relevant key toxicity pathways, and (iii) in vitro tests based on human biology. Indeed, the limited use of -omics technologies may not give the mechanistic understanding that is required to select the most relevant key toxicity pathways. Furthermore, bioassays and endpoints employed by the ToxCast project were selected primarily on the basis of cost more than on the basis of their mechanistic relevance for human biology.

2. Toxicity testing in the 21st Century: the European reality

While there is a ‘top–down’ development of new toxicity tools in the USA driven by authorities, the European 3Rs strategy is a ‘bottom–up’ approach where industry and academia respond to legislative pressure, e.g. the European Directive 2010/63/EU implemented to afford protection to laboratory animals and encourage 3Rs development, and the 7th Amendment to the Cosmetics Directive facing out animal testing of cosmetics and toiletries in the EU and placing an EU-wide marketing ban on products that do not comply with the testing requirements. Nevertheless, many elements of characterizing the NRC vision and strategy can be found in the initiatives aimed at the development of non-animal based methods for screening, including early decision making and risk assessment that have been and currently are supported by the European Commission (EC) and Industry.

There is ample documentation showing that pathway approaches, state-of-the-art biological methods and in vitro toxicity have been pursued and pushed in Europe for more than a decade (Kimber, 2000; Lotti and Nicotera, 2002). Under Framework Program (FP) 6 projects were funded that follow -omics based strategies enabling the identification of key pathways of toxicity (e.g. Sens-it-iv (www.sens-it-iv.eu), carcinoGENOMICS (www.carcinogenomics.eu)). In addition, most projects dealing with cell-based assays do emphasize the necessity of physiologically relevant, metabolic competent and robust assays (ftp://ftp.cordis.europa.eu/pub/ftp7/docs/alternative-testing-progress-report-2009_en.pdf). These approaches and views were expanded further in the FP7 funded projects focusing on systemic toxicity.

The cosmetic industry addressed the 7th Amendment to the Cosmetics Directive deadline in March 2009 by developing methods for eye and skin irritation, genotoxicity and acute toxicity based upon a solid understanding of the mechanisms of action and relevant key pathways. For skin sensitization, the mechanism is now relatively well established (Jowsey et al., 2006; Karlberg et al., 2008) and for skin irritation a number of in vitro models some of which use 3D reconstructed skin have been developed and accepted by the European Centre for the Validation of Alternative Methods (ECVAM) for Phase II pre-validation (http://ecvam.jrc.e-c.europa.eu/).

To bridge the gap between the last stage of the Cosmetics Directive (deadline scheduled for March 2013) for complex endpoints (e.g. repeat-dose toxicity, reproductive toxicity, toxicokinetics) and the predictive power of currently available or emerging replacement strategies for assessing these endpoints, the European Cosmetics Association ‘COLIPA’ has initiated a joint programme with the EC to develop alternative methods for systemic toxicity testing (FP7-HEALTH-2010-4.2.9.1-6). As in the NRC vision, the primary focus of this programme is on how to acquire knowledge about key issues useful for risk assessment based upon in vitro methods rather than employing animal in vivo methods.

In the chemical sector, REACH is urging industry to use animal testing only as the last resort. The proposed strategy includes maximum usage of existing in vivo and in vitro data (e.g. through data sharing), application of the read-across approach and non-testing methods (e.g. chemical categorizing using (quantitative) structure–activity relationship (Q)SAR modelling). Additional in vivo and in vitro testing should be performed in an intelligent way with the aim of filling knowledge gaps that are specifically in need of address for decision making.

The Innovative Medicines Initiative (IMI) is a collaboration between the pharmaceutical industry and the EC (http://imi.europa.eu/index_en.html). The IMI aims at finding solutions to address research bottlenecks in the drug development process. Although not the primary focus, new 3Rs approaches have emerged. For pre-clinical genotoxicity testing of drugs, a number of in vitro screening models were implemented to determine toxic effects of drugs tested at concentrations relevant to the expected plasma concentration instead of the highest dose which is likely to be non-toxic. In addition, mechanistic-based toxicity tests have improved the pre-clinical filter for CNS, cardio- and hormonal drugs.

3. Difficulties and hurdles to consider and overcome on the way forward

The strategy of the ‘21C’ vision builds upon an in-depth understanding of the physiological and toxicological processes in humans related in vivo to toxicological endpoints, to identify the relevant key pathways and components of these pathways involved in the responses to toxin exposure, and to establish for each key event representative high quality in vitro models. The difficulties and hurdles that are to be considered and overcome on the way forward were summarized in Table 1.

3.1. Lack of human-specific methods with adequate physiological relevance

The ideal cell system for mechanistic studies and toxicity testing will comprise cells expressing in vitro the machinery that in vivo is required to elicit a relevant toxic response. Since we still do not understand the impact of the in vivo microenvironment and cell–cell interactions on the mechanisms driving cell differentiation and dedifferentiation, it is yet not realistic to pursue an in vitro model or test that copies entirely what happens in vivo. However, a physiologically relevant in vitro system can be established if the in vivo processes are understood sufficiently. It is anticipated that ‘relevance’ may have to be defined for each toxic endpoint and compound classes to be tested. It is also anticipated that the cell culture conditions required to support essential mech-
from important drawbacks which have to be solved before the pose of 21C toxicity testing, the vast majority of these tests suffer with a wide spectrum of cells covering various tissues. For the purposes of healthy and diseased conditions in both healthy and diseased people.

A targeted approach for identifying key pathways and networks requires the availability of physiologically relevant, metabolic competent and robust in vivo, ex vivo and in vitro models of both healthy and diseased people.

Solid in vivo human data are available for pharmaceuticals, but are rare for other compounds. However, minimally invasive techniques from the pharmaceutical sector (e.g. micro-dosing and tracing studies, acquisition of blood cells) for ex vivo work have a role to play across industry sectors in evaluating the relevance of identified biological pathways by providing insight into mechanisms and metabolic pathways in normal and diseased people. Similarly, precision cut tissue slices have been demonstrated to be powerful tools for acquiring knowledge about in vivo process and pathways.

3.2. Poor reliability due to the absence of method harmonisation and standardisation

An issue often faced while performing cell-based tests is intra- and inter-laboratory variability in spite of rigorous compliance with the SOP. The reasons for this variability are often undefined but it is generally accepted that the cell cultures, analytical processing, technical error and differences in qualitative judgment.

3.3. Insufficient knowledge about pathways and key events of toxicity within organisms/biological systems

While pathways have been identified by in vivo and in vitro studies as potentially relevant for in vitro toxicity testing, our current understanding of the processes underlying healthy and diseased conditions in humans in vivo is still insufficient to confidently identify the key pathways for toxicological endpoints.

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4. Issues related to human specific in vitro methods were addressed earlier

4.1. Poor understanding of the differences between adverse and adaptive reactions

The new technologies have made it imperative to understand the mechanistic differences in adverse and adaptive responses to compound exposure.

In vitro assays allow for a full dose response curve to be generated and thus cover both adaptive and adverse effects. Thus, a low dose of a chemical may cause a response which does not lead to the demise of the cell per se but causes changes in signal pathways to counteract the effect of the chemical (an adaptive response). This should not be equated to a toxic dose effect which causes irreversible cell injury. It was highlighted however that there is uncertainty about the impact of doses that cause adaptive responses when these doses are imposed on the system for longer periods.
4.2. Few high quality markers and marker signatures with predictive power

There is a general need for markers and marker profiles with adequate power to predict toxicity (including potency) and, in the case of pharmaceuticals, efficacy. Gene-cluster modelling has increased our understanding of the mechanisms of action driving the clinical conditions (e.g., allergy, cancer) and diagnostic markers have been identified (Gohlke et al., 2009). Similarly, gene-cluster modelling has been performed before and after exposure of human-specific in vitro test systems to toxic and non-toxic compounds in an effort to identify new markers and marker profiles for toxicity.

The relevance of the identified diagnostic markers for toxicity testing remains to be established though. Furthermore, the marker signatures resulting from human-specific in vitro test systems remain to be optimized and adapted for prediction of a specific endpoint related to a specific clinical condition. It is anticipated that these evaluation cannot be performed with confidence until human-specific methods with adequate in vivo relevance have been established.

It is important to consider that markers and marker profiles are likely to be less acceptable as surrogate endpoints for safety assessment than for efficacy testing or clinical management.

4.3. Determining thresholds on the background of adverse responses

Risk assessment has been based upon 'no effect levels' (chemical and cosmetic industry) or 'no adverse effect levels'(pharmaceutical industry) in animal studies.

As mentioned above, the high sensitivity of -omics technologies has made it possible to detect responses at very low doses of a compound (Hartung, 2009). Consequently, the current animal-based perception of 'no adverse effect levels' has been put under pressure. The consequences are very evident in the area of genotoxicity, where thus far any effect of a non-pharmaceutical compound on biological in vivo systems results in a 'no go'.

4.4. New technologies and paradigms to be implemented

There was a call for immediate implementation of emerging technologies and paradigms which were considered essential for transferring the vision into the reality of a toxicity-testing system assessing biologically significant perturbations in key pathways which are relevant for human biology (Table 1).

4.5. Physiologically relevant cell and tissue-based assays

As described in the previous sections, the challenges faced by developers of cell and tissue-based assays are substantial.

There has been a lot of progress in the development of models for topically applied chemicals and this has mainly been made possible by the advances using 3-dimensional skin cultures, some of which are accepted by ECVM as alternative models for skin corrosion and irritation. A number of non-validated models are used to predict endpoints in epithelial barriers (intestinal, ocular, oral, vaginal and airway). However, it is still a long way for many other toxicological endpoints (e.g. sensitization, repeated-dose toxicity, systemic toxicity).

Implementation of the novel 'body on a chip' technologies would allow for testing toxicants in a more realistic environment while using human cellular material. 'Organs' are mimicked by culturing cells from human tissue in small chambers which are interconnected. Although promising, this technology still needs further development to be of suitable use required for toxicity testing. If 'human chips' become successful, it is most likely that they will be applied to the '21C vision'.

Incorporation of microfluidics enables the investigation of dynamic systems such as the liver or lymph nodes. These models incorporate continuous flow conditions and may be a better model for the actual organ. They also allow cells to be cultured in arrangements which are supportive of cell–cell interactions and, at the same time, can accommodate longer range interactions and take into account a variety of fluid dynamic parameters of in vivo relevance, such as protein and macromolecule-induced shear stress, residence time of the circulating fluid in specific compartments and fluid flow rates.

Used iteratively with PBPK models, microfluidic and, similarly, allomoterically scaled fluidic models of multiple body systems, can be used for the detailed examination of toxicologically relevant mechanistic events when supported with high content and omics technologies.

4.6. Method harmonisation and standardisation

Factors that are critical for reproducibility and reliability of assays should be addressed systematically. It is known that culture medium and supplements can have a huge impact on the outcome of the assays. Therefore, culture media, supplements and other biological reagents should be carefully selected and standardised. The robustness of an in vitro assay will be apparent when determining the effect of media and reagents on the outcome.

Retrospective weight of evidence would be one tool for harmonizing how people perform specific tests and to assure good quality of the data. This would help to identify flaws in the analytical processes, technical error and qualitative judgment.

Finally, the exploitation by the in vitro testing community of emerging nano-biotechnologies facilitating the real time monitoring of cellular activity and processes reflecting the quality of the cell culture (e.g. emerging sensor-based tools measuring adhesion, metabolic activity) would provide objective tools for eliminating variations in the performance of cell-based tests. Currently used simple marker sets to confirm maintenance of the phenotype are not sufficient as protein expression not necessarily correlates with functionality (Winton et al., 1998; Berger et al., 1999).

4.7. Toxicogenomics for increasing predictivity

Toxicogenomics provides a library of generic expression profiles for different classes of toxicity that allows the characterization of an unknown compound based upon the profiles with which it fits. Genomics is currently used on a large scale for pathway analysis and marker identification, and its ability to reduce the need for animal studies is already being examined. However, the concept has not yet been fully implemented in toxicity testing strategies and risk assessment.

Carcinogenicity testing is in this respect an interesting case study. The use of toxicogenomics for identifying the mechanisms of action of genotoxic and non-genotoxic carcinogens has resulted in training sets for carcinogens and non-hepatotoxic non-carcinogens (Johnson et al., 2004; Van Delft et al., 2004). In addition, the FDA has invited the submission of genomics data for control compounds. It also brought together toxicogenomic experts for interpreting the submitted data. The learnings of this case study should be implemented as soon as possible on other toxicological endpoints.

It was pointed out that standardization of data analysis is needed since different approaches (e.g. gene fold-change cut-off, data curation, and meta-analysis) can give different outcomes.
4.8. Systems biology approach

The systems biology approach intends to bring together -omics databases obtained from exposed and unexposed cellular or animal models, and clinical samples, and to establish relevant associations using newly developed computational technologies. The paradigm is to use the system biology based methods for describing molecular pathways involved in the human endpoint of interest.

The power of systems biology is again best exemplified in the area of carcinogenesis. The complex network of processes leading to cancer can be set out in a generic model to simulate the process of carcinogenesis (Hanahan and Weinberg, 2000). Data from different studies and analytical platforms were integrated in this network to achieve a more detailed understanding of toxicity-related responses in cells, organs or a system. This way, more reliable predictors of toxicity can be identified.

Similar models for other toxicological endpoints (e.g. immunotoxicity, reproductive toxicity, liver and kidney toxicity) must be developed or refined to enable accurate prediction from the data they yield. One has to bear in mind though that more in vitro and in vivo data need to be generated irrespective of the endpoint. In order to assure the reliability of the in vitro data, the in vitro essays used for human hazard identification in vivo, should be validated. With respect to in vivo studies, it is important to underline that data from well defined (transgenic) animal models could be used as well as data from human clinical trials.

4.9. PBPK/Multiscale modelling

The absence of key processes in the biokinetics that govern the exposure of the target tissue of the organism in vivo makes data from in vitro studies not directly applicable to the in vivo situation. PBPK modelling would establish a meaningful link between in vivo and in vitro (Verwey et al., 2006).

PBPK modelling is a process that enables hypothesis generation, and creates model-driven experimentation. PBPK takes into account physiological and biological changes, as well as the diseased states and work related conditions. Human inter-individual variations can be built into the model, together with the uncertainty of a prediction. Physiological data for many species are available in the primary physiological literature, as well as in a number of compendia (Kararli, 1995; Brown et al., 1997; International Commission on Radiological Protection, 2002).

The technique also requires information about the relevant properties of the compounds that are to be predicted. These properties should enable quantitative prediction of Absorption, Distribution, Metabolism and Excretion (ADME). While distribution and excretion still are very dependent on data obtained in in vivo studies, in vitro assays for absorption and metabolism are emerging and should be implemented together with e.g. (Q)SAR modelling.

The technique is sufficiently developed to serve as a replacement for in vivo pharmacokinetic studies during drug discovery. Its extension to incorporate the prediction of in vivo therapeutic effects and toxicity is less well-developed, but has potential in the long-term to effect a significant reduction in animal use (Thomas, 2009). Huge potentials are also hiding in the fact that it can be combined with other technology platforms into an integrated testing strategy. One example is metabolomics for pattern recognition analysis of body fluids and tissues to differentiate between patients and healthy volunteers (Griffin, 2003). It was also successfully linked to other biologically-based modelling (Blaauboer et al., 2000).

4.10. The Toxicological Factors Analysis and Classification System (TFACS) paradigm

This approach is based on looking at potential points of failure at different stages in the pathway. Categories of potential failure may include chemical character, toxicity pathways, targeted testing and dose responses, and extrapolation (Reason, 1990).

When applied to toxicity assessment, each category has a failure point. Normally, this failure point is neutralized by other preventive mechanisms but occasionally all failure points add up and the pathway leads to critical ‘adverse’ effects occurring at the cellular or molecular level.

Existing data can be assessed using TFACS to help further understand and map out the latent and active failures from toxicant exposure.

4.11. Waiving animal testing with Integrating Testing Strategies (ITS)

It is anticipated that a more in-depth understanding of the relation between toxicity and biological pathways will make it possible to prevent animal testing by a combination of tests that assess the key elements of the perturbation potential of the test compound. In vitro and in silico methods can be used to accomplish this. If sufficient scientific justification is provided it may be possible to waive an animal test.

When selecting the battery of in vitro and in silico methods addressing key steps in the relevant biological pathways (the building blocks of the ITS) it is important to employ standardized and internationally accepted tests. Each block should be producing data that are reliable, robust and relevant (the alternative 3R elements) for assessing the specific aspect (e.g. biological pathway) it is supposed to address. If they comply with these elements they can be used in an ITS.

A joined EPAA – ECVAM workshop in November 2008 on overcoming barriers to validation of ITS made it clear that implementation of the ITS approach has to consider the product type, R&D stage and the regulatory context (Kinsner-Ovaskainen et al., 2009).

4.12. A new strategy for assessing Reliability, Robustness and Relevance (the alternative 3R elements)

In 2004 ECVAM proposed a modular approach to make the process for assessing the validity of tests more flexible, while maintaining its high standards (Hartung et al., 2004). At the meeting, this approach was presented as a sequence of seven steps, each representing an ‘information container’ that has to be ‘filled’ in order to provide enough confidence that the test will predict correctly. The first four steps assess the reliability of the test (pre-validation) which, if successful, forms the basis for the next phase (validation) demonstrating its relevance. It was noted that there is a strong interrelation between reliability and relevance as marked by the gradual transition between both tests properties (Fig. 1).

Also during the 21st century, standards for demonstrating scientific validity of a test or test system will provide the guarantee that the produced data are useful for hazard identification and eventually risk assessment. New strategies for assessing not only reliability and relevance, but also robustness, must be developed in concert with regulatory dialogue to ensure a minimal risk of non-acceptance. The importance of such standards for methods that are not intuitively understandable cannot be stressed enough.

Together, the reliability, robustness and relevance elements should provide increased confidence in the toxic events and biological pathways believed to be relevant for the toxicological endpoint of concern.
5. Conditions needed to move forward

5.1. Credible leadership assuring concerted projects

Industry and the European Commission have been, and still are, investing huge amounts of money in development and implementation of 3Rs strategies. Although coordination efforts were initiated by the European Commission (e.g. FP coordinator meetings) and the EPAA (e.g. the Platform for Science), there was a clear call from the audience for a credible leadership with the capacity to assure alignment of ongoing activities and initiation of concerted actions, e.g. a global human toxicology project.

This project intends to mobilize diverse stakeholders and find the support necessary to bring the ‘21C’ vision for toxicity testing to life. A multi-stakeholder consortium has been developed to lead the early steps in the transformation of toxicity testing for human health risk assessment (http://www.therhammer.org/docs/HTOX_flyer_8-21-09.pdf).

5.2. Validation of the building blocks of ITS required

To date there are no existing procedures and guidelines for putting together and validating an ITS. Obviously, this constitutes a hurdle for the implementation by regulatory agencies. Does it make sense to validate a strategy that builds upon tests for hazard identification which change over time, but is to be used for risk assessment? One needs to incorporate new thinking into risk assessment. Regulators are receptive to new technologies but specific knowledge domain (e.g. MCSym). However, in most cases specific knowledge is required to use these tools. Training should be provided to improve applicability and the quality of the output.

Data sharing was considered as the most direct way to the reduction of animal numbers in research and safety assessment, and has the focus of the EPAA Platform of 3Rs in Regulation (www.epaa.eu.com).

5.3. Education and training

The new approaches for risk assessment are going to require training for risk assessors. Furthermore, it is very important to inspire young scientists to gain interest in all the aspects of alternatives to animal testing. European projects should therefore initiate contact with younger scientists.

The disadvantage of Europe is that the groups are not as big as in the U.S. TRISK (Toxicology Risk Assessment Training, www.trisk-project.eu) is a European training programme established across European Member States developed to train toxicologists in areas of risk assessment. This is a promising way of training younger scientists but it does not include training of systems biology yet.

The Fund for the Replacement of Animals in Medical Experiments (FRAME) runs an ‘experimental design’ training school focussing on the reduction of animal usage. Similar training schools are under development for refinement and replacement.

5.4. Availability of applications to everyone

Toolboxes and software are available and some are in the public domain (e.g. MCSym). However, in most cases specific knowledge is required to use these tools. Training should be provided to improve applicability and the quality of the output.

Data sharing was considered as the most direct way to the reduction of animal numbers in research and safety assessment, and has the focus of the EPAA Platform of 3Rs in Regulation (www.epaa.eu.com).

5.5. Measures to improve the acceptance and implementation of 3Rs strategies by regulatory authorities

The lack of acceptance and implementation of 3Rs strategies by the regulatory authorities was considered an issue that will increase in size as a result of the ‘21C’ toxicity testing strategy. This issue has been, and still is, addressed by the EPAA Platform of 3Rs in Regulation (www.epaa.eu.com) as exemplified by two EPAA-ECVAM workshops on the validation of ITS.

In addition, the EPAA Platform of Communication and Dissemination has as a goal to facilitate the information flow related to alternatives to animal testing from the scientific community to the regulatory authorities (www.epaa.eu.com).

5.6. Financial support

It was agreed by delegates at the meeting that a substantial amount of money is needed to support the realisation of the ‘21C’ vision. It was not discussed as to the source of the finances, but there was a consensus to recommend financing of science driven activities. The finances should be handled by an independent body not involved in organisational or political concerns in analogy with the NIH in the U.S.A.

6. Conclusion

The In Vitro Testing Industrial Platform (IVTIP) organized this one day meeting to discuss the latest US EPA strategic plan for evaluating the toxicity of chemicals with scientists, key opinion leaders, developers and users of 3Rs-related tests and testing strategies. All delegates agreed this was the right approach to enable a thorough, reliable and systematic approach to future toxicity testing without the use of animals.

While discussing the needs of industry (in general) it became clear that human specific cell-based methods are paramount. For most target organs it is still unclear how to establish in vitro cultures with adequate in vivo functionality. This is a prerequisite for acquiring mechanistic insight into toxicological events that has in vivo relevance, and for biomarker identification.

The importance of method harmonization and standardization was recognized for implementation and acceptance by regulatory authorities. Unfortunately, human primary cell-based test methods often suffer from unexplained intra- and inter-laboratory variability. This issue may be addressed e.g. by implementation of state-of-the-art nano-biotechnologies.
The implementation of the -omics technologies was considered essential for making the ‘21C’ vision a reality. These technologies can provide the data that give insight in the pathways and key events of toxicity within organisms and biological systems, and thus can feed into systems biology techniques. They can though also be used as a powerful predictive tool (e.g. toxicogenomics for carcinogenicity testing).

An obvious consequence of the implementation of -omics technologies is that we have to find new ways to establish the ‘no-effect-level’ of compounds. Indeed, the sensitivity of these technologies makes it possible to monitor the effect of low concentrations of compound on both adaptive and adverse responses. As yet, it has to be established how to apply this additional information while setting thresholds for hazard identification and risk assessment.

Several new technologies and new paradigms were suggested as being important for driving the ‘21C’ vision to reality. While each of the new approaches had their unique strength, integration of old and new was considered as the preferred way forward.

Finally, the challenges related to validation of the methods were addressed. It is important to keep in mind that in vitro tests do not have fewer limitations than in vivo tests. Their limitations have to be carefully defined. The applicability domain of a model should always be carefully considered.

7. Endorsement

All delegates endorsed the emerging concept of the ‘21C’ vision as the right approach to enable a thorough, reliable and systematic approach to future toxicity testing. It was stressed though that in the European context the vision had to reach out for ‘full replacement of animal studies’.

A number of critical issues and concerns hampering the realization of this vision were presented. One such topic, the design and use of Integrated Testing Strategies was discussed at the IVTIP 2010 Spring meeting (May 19th, 2010) in Geneva, Switzerland.

Speakers

Bhogal N., Frame, UK; Boekelheide K., Division of Biology and Medicine, Brown University, Providence, Richmond, USA; Hartung T., CAAT, John Hopkins Public School of Health, Baltimore, USA; Kleinjans J., University of Maastricht, The Netherlands; Kreysa J., European Commission, JRC, Ispra, Italy; Maxwell G., Unilever, UK (representing COLIPA); Schoeters G., CARDAM, Geel, Belgium; Van Cauteren H., Pharmaparacelcus, Belgium; Yang R., Colorado State University, USA.

IVTIP members

Advancell, Spain; Agenolab GmbH, Germany; Beiersdorf A.G., Germany; BioDetectionSystems N.V., The Netherlands; BioMedzet Life Science GmbH, Austria; Biopredic, France; BioTeSys GmbH, Germany; Biovator, Sweden; British American Tobacco, United Kingdom; Cardam, Belgium; CellSystems GmbH, Germany; CIT, France; EggCentris, Belgium; Epithelix Sàrl, Switzerland; Euroderm GmbH, Germany; Gaiker, Spain; Genencor International b.v., The Netherlands; Harlan Laboratories Ltd., United Kingdom; Henkel, Germany; Huntingdon Life Sciences Ltd., United Kingdom; Kirkstall Ltd., United Kingdom; Leo Pharma, Denmark; L’Oréal, France; Natura, France; Novozymes A/S, Denmark; Novo Nordisk A/S, Denmark; Progenika, Spain; Scantox, Denmark; TNO, The Netherlands; Vitrocell Systems GmbH, Germany; ZF Biolabs, Spain.

Appendix A

IVTIP. 21C autumn meeting

“ Toxicology, in the 21st century – working our way towards a visionary reality.” Nov. 26, 2009, Antwerp (Edegem), Belgium

The meeting will kindly be hosted by the IVTIP member company CARDAM, Geel, Belgium (http://www.cardam.eu)

Moderator: Dr. Koen Van Deun, Toxicology consultant, Reflector Consulting

Welcome
Erwin Roggen/Bart De Wever, IVTIP Board

Part. 1: The vision and its perception by different industries

Toxicity Testing in the 21st Century: The vision and Distinguishing Adaptive versus Adverse Effects

Kim Boekelheide, Division of Biology and Medicine, Brown University, Providence, Richmond, USA

Views and Opinions

Gavin Maxwell, Unilever, representing COLIPA

Herman Van Cauteren, Pharmaparacelcus, Belgium

Nirmala Bhogal, Frame, UK

Discussion

Part. 2: Possible impact of the vision on regulations

Assuring human safety in the 21st Century

[1] Validation, a necessary burden?
Joachim Kreysa, European Commission, JRC, Ispra, Italy


Thomas Hartung, CAAT, John Hopkins Public School of Health, Baltimore, USA

Discussion

Part. 3: The technological reality

[1] Cell and tissue-based assays: perspectives and gaps
Greet Schoeters, CARDAM, Geel, Belgium

[2] Discussion

[3] From -omics to System Biology
Jos Kleinjans, University of Maastricht, The Netherlands

[4] Discussion

Raymond Yang, Colorado State University, USA

[6] Discussion

Part. 4: How far did we come?

Round Table Round up

References
