First joint meeting of the German and Swiss Societies of Cell Biology

University of Konstanz, 24.-27. March 2009

In an extremely lively and well-organized meeting, the university of Konstanz welcomed between 500 and 600 cell biologists during 3 days. The meeting had a very broad scientific basis, and not a specific focus on the use of cells for alternative methods. However, it provided a very good opportunity to learn more about high-throughput methods, high content imaging, signalling pathways and systems biology, which are becoming more and more prominent in basic cell biology, but also form the basis for the definition of toxicity pathways as defined by the 21st century vision of the national research council of the US. One of the sessions was particularly devoted to stem cells. It was opened by **K.-H. Krause** (Geneva) who demonstrated how such cells may be used for testing of drugs and how complicated 3-dimensional and electrically-active neural tissue can be generated from such cells. **Alan Trounson** (San Francisco) then introduced into the activities of the California stem cell consortium that is using 1 billion dollars/year to push stem cell technologies to applications in medicine and drug profiling. **Ed Baetge** (San Diego) gave the point of view of a small pharmaceutical company, that tries to develop stem cell based therapies for diabetes. The session was complemented by the Zeiss honorary lecture held by **Rudi Jaenicke** (Boston) who gave a deep insight into induced pluripotent cells (iPS) as opposed to embryonic stem cells (ESC). Interestingly, the speakers seemed to agree, that the potential of iPS cells as alternative to ESC looks good, but is too early to be judged yet. They were more sure, that toxicity testing and examination of disease mechanisms will be one of the first actual uses of cells differentiated from ESC or iPS.

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Checking on the direction: 2008 EPAA Annual Conference

Brussels, 3rd November 2008

Research is the pre-condition for any progress towards 3Rs, both in the short and the long term. At its 2008 Conference in Brussels on 3rd November 2008, the EPAA ran a check on whether research activities supported in the EU were heading in the right direction. Under the title "Research into alternative approaches: are we on the right track?", a high-profile list of speakers and some 300 active participants in the audience reviewed where EPAA had got to, where it was going, and where it could usefully modify its focus. The overall conclusion was that real progress has been made by EPAA – but that there are still many challenges ahead.

Setting the scene

The scene for the conference was set by two European Commissioners. **Janez Potocnik**, Commissioner for Science and Research, said: "The progress made over the last year is impressive". He singled out the EPAA's work on research as "an important element in implementing the '3Rs' of reduction, refinement and replacement in many scientific and industrial areas". He also noted that "the five EPAA working groups have cranked up their engines and intensified their efforts over the last years". The result of the workshop on "New Perspectives of Safety" was to identify truly novel approaches for the characterisation of the potential hazards of chemicals and drugs, and to provide a better view of which areas of science and technology should be exploited to create new approaches to safety assessment. An important message emerged: the need to move "from correlations towards causalities".

But, he went on, "in some areas – such as in the 'full replacement of animals in safety testing' – we are not there yet". The Commissioner stressed the need to coordinate research activities better, to avoid doing the same things twice. The activities and results also need to be monitored, evaluated, disseminated and, in particular, brought to application by rel-

evant stakeholders, he added. "The EPAA is well-placed to help us to do this job."

Günter Verheugen, Vice-President of the European Commission, and responsible for Enterprise and Industry, insisted that overall, the EPAA has become the main platform for the Commission, industry and stakeholders to identify areas where pragmatic and concrete measures are being agreed to promote the 3Rs in the area of regulatory compliance. Cooperation between authorities and industry, across different sectors, has become a key driver for change. The EPAA now has an impact in Europe and beyond, and alternative approaches remain high on the political agenda, including in the regulatory dialogue with Europe's main partners. The example of the EPAA is certainly one of the elements that reinforce EU policy towards its trading partners.

Jacques Leclaire, Director of Life Sciences Research of L'Oréal, which is taking over the industry co-chairmanship of the EPAA Steering Committee in 2009, affirmed EPAA's response to the need for a multidisciplinary approach, but stressed that the work "needs tenacity and continued commitment". He underlined that EPAA's focus was scientific, and that "science cannot be commanded".

2008 activities – more challenges ahead...

Bernward Garthoff of Bayer, the EPAA co-chairman in 2008, outlined the key EPAA achievements in 2008. In his view, the challenges are to remain focused, improve communication, go beyond low-hanging fruits, and allow time for EU and company staff to contribute.

Coenraad Hendriksen spoke as a member of the EPAA Mirror Group – an advisory committee consisting of experts from different societal interests and academia. In his view, EPAA is on track both in its direct output, and in its indirect impact – notably through the implementation of 3Rs culture among industry, regulators, and academia. This, he said, was reflected by the increase of the "3Cs": commitment, communication and common sense. But he felt performance could be raised still further through more specific targets and more closely defined indicators for assessing the results. He insisted on the need to take education on board in the context of a tighter focus on dissemination.

Research: today's achievements and tomorrow's perspectives

Michael Schwarz of the University of Tübingen examined whether 3Rs science today offered a solid basis for the future. There have been some replacement successes for a limited number of endpoints, but "we do not have tests accepted for full replacement of key areas" – and will not have for the foreseeable future, he projected, in particular in the area of systemic effects. By contrast, the major impact in the short- to medium-term will be through reduction and refinement.

Hennicke Kamp of BASF, the Co-chair of EPAA's WG2 (research), assessed how far EPAA was bridging the gaps, by using the multi-stakeholders and multi-sector set up, using the potential of a number of projects for reapplication and cross-fertilisation. Among the challenges he raised was assuring acceptance of test methods through OECD – as seen with the extended one-generation study, sometimes a lengthy exercise.

New perspectives on safety testing were reviewed by **Ian Kimber** of the University of Manchester, who depicted a "hugely complicated landscape driven by regulatory and political pressures", and predicted that it would take a lot of time and money and effort to combine the many necessary disciplines and move ahead: tissue engineering, mathematical modelling, stem cells, systems biology, computational chemistry. "Are we doing things in the right way with the right people and right resources at present?", he demanded, urging efforts to bring more people into toxicology from other disciplines that never look at it or think of it. In his view too, EPAA was well placed to do the job.

Research strategies to implement the 3Rs principles

Manuel Hallen of the European Commission's DG Research set out the research strategy of the EU Health Programme in relation to the replacement of animal tests in systemic toxicity. He felt that EU research in this direction was "probably still on the right track", but exhorted the scientific community to make use of all analytical tools, including those beyond toxicology, to reach what would be a long-term target of full replacement. New research projects on repeat dose systemic toxicity will be launched in a joint research initiative with industry partners.

Elke Anklam of the European Commission's DG Joint Research Centre examined integrated testing strategies from the point of view of the Commission. She said this was the current approach to meeting the challenge of human hazard assessment using alternative methods, by combining expertise and resources across the Institute for Health and Consumer Protection. Optimally, this approach combines data from diverse sources (*in vitro*, *in silico*, read-across, ...) and is driving targeted testing where data are lacking. In her view, a major challenge lies in the integration of the data and its interpretation in relation to specific regulatory questions.

Joanna Jaworska of Procter & Gamble offered an industry viewpoint on integrated testing strategies. The approaches already in use depend on the application domain, the endpoints and the purpose, she observed, and although today they do not allow full replacement, they do contribute to minimising the number of *in vivo* studies. But progress in integrated testing strategies depends on overcoming methodological, organizational and technological challenges, she insisted. "EPAA offers the opportunity to look at different approaches and learn across sectors", she concluded.

Raymond Tice of NICEATM-ICCVAM provided a US perspective with a review of US research programmes and screening activities. Among the priorities he identified were the need to integrate *in vitro*, experimental animal, and human data on substances, from all sources. He also spoke of the needs for advances in bioinformatics to process the extensive data being generated, and for extensive and committed international cooperation. Above all, there was a need to validate the resulting testing strategies, in terms of reliability and relevance, for mak-

ing regulatory decisions. But he made clear he is well aware of the huge challenge: "A gene is not a pathway! A pathway is not a cell! A cell is not an organ! An organ is not an organism! An organism is not a species! Individual organisms and species differ in sensitivity to toxicants!"

Roundtable: what more could we do?

In a roundtable moderated by **Phil Botham** of Syngenta, the question of whether EPAA is on the right track was discussed by a panel and by the audience. A range of views were expressed over whether the short-term EPAA programme is compatible with longer-term goals. **Gerrit Schüürmann** of the Helmholtz Centre for Environmental Research believed that finding ways of reducing animal use could be accelerated by computational chemistry.

Emilio Benfenati of the Istituto Mario Negri pointed out that developing replacement tests was not an entirely scientific process, and needed the involvement of regulators and other stakeholders for success. **Decio Eizirik** of the Université Libre de Bruxelles, who took also part in the EPAA workshop on new perspectives on safety, felt that "There is no way to assess risk based on the current technologies, but this is the right time to initiate the work necessary to achieve this goal. We are getting a clearer idea of where to go". He favoured training more people in new technologies and the exploitation/interpretation of results to advance the process. He also pointed out that the expectations of politicians are too high and that the 2013 deadline for cosmetics will not be met since scientific results cannot be delivered on demand.

Raymond Tice warned against a closed-end approach. "This is a research programme, so we can't say when it will bring complete results", he stressed. But better screening should certainly make it more possible to exclude from testing those substances that present no real hazard. Highlighting the merits of wider communication and coordination between different paths, he suggested that EPAA might bring key players together every year for an update and brainstorming session among scientists, regulators, researchers, and toxicologists.

Jacky Van Gompel of Johnson & Johnson expressed caution in defining the right balance. Tests with high sensitivity are needed to protect human health, he recognised, but if they produced too many false positives, the result would be to damage research. **Arnd Hoeveler** of the European Commission's DG Research believed that EPAA had correctly identified some priorities, and "We can see we are on the right track", but he urged EPAA to consider offering incentives to attract researchers into the area and to act on the long term.

Measuring progress also received attention in the discussion. Collecting data on the overall number of animals used in safety testing is not necessarily a good indicator, Botham suggested: EPAA's real goal is to help deliver alternative safety tests in line with the 3Rs principle. Garthoff pointed out that EPAA should continue to lay the groundwork for the long term, even if its action programme had initially been set up only for five years.

Botham proposed that the focus on alternatives should be to provide better methods for risk assessment: "We have a lot of new technologies which could be used to identify hazards – but if not used wisely could amount to more and more sophisticated technology to get answers to the wrong questions", he warned. His views were shared by other speakers who emphasised the need to assess true risk rather than just hazard.

The conference report and the 2008 progress report are available on the EPAA website at: http://ec.europa.eu/enterprise/epaa/4_2_conf_2008.htm

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EPAA 2008 Poster competition winners

The objective of the 2008 EPAA poster session was to give researchers an opportunity to explain their work on alternatives to animal testing in a language understandable also to policy makers and the public at large.

Based on these criteria, the EPAA award was granted to the team led by **Mathieu Vinken** of the Department of Toxicology, Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel (VUB), for Establishment of an *in vitro* model of liver cell death. The prize was travel expenses and participant fees for the VII World Congress on Alternatives & Animal Use in the Life Sciences in Rome in 2009.

The team led by **Carl Westmoreland** (Unilever) won the best industry poster recognition award for Assuring Safety without Animal Testing: New Risk Assessment Approaches for Skin Allergy and Cancer.

The abstracts of the winning posters are presented below.

All abstracts and posters from the competition are available for downloading and viewing on the EPAA website: http://ec.europa.eu/enterprise/epaa/4_events/ann_conf_2008/abstracts_book.pdf

1 Establishment of an *in vitro* model of liver cell death

Vinken, M.[1], Decrock, E.[1], De Vuys,t E.[2], Leybaert, L.[2], Vanhaecke, T.[1] and Rogiers. V.[1]
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1.2 Results

Historically, the liver has gained particular attention by toxicologists, as it represents a key target organ of chemical-induced cell injury. Damage to hepatocytes, the most important cells in the liver, may burgeon into the onset of cell death. The lat-

ter mainly occurs *via* a well-orchestrated process called "apoptosis". A number of protocols have been described to study liver cell death *in vivo*, including the direct administration of cell death-evoking toxicants to animals and the use of genetically modified animals. Such experiments not only raise serious ethical questions, but are also of limited scientific value. Indeed, apoptotic cells are barely detectable *in vivo*, as they are rapidly removed by surrounding (non-hepatocyte) cells. Such constraints can be overcome by using *in vitro* experimentation. Our group has recently developed an *in vitro* system to study liver cell death. Basically, this system consists of primary cultures of hepatocytes that are exposed to a combination of chemicals with clear-cut cell death-eliciting properties. The model was characterized by testing a battery of well-known cell death markers. Using a number of biochemical techniques, we could demonstrate that the entire time course of apoptosis can be monitored in our experimental setting, whereby all typical apoptotic features are manifested. Based on our findings, it can be concluded that a reliable *in vitro* model of apoptotic liver cell death was developed. This model can specifically be used in early drug development to screen for effective anti-apoptotic molecules and more generally to test anti-apoptotic properties of drugs. The advantage of the model particularly lies in the fact that it replaces an *in vivo* model which, scientifically spoken, does not provide convincing results, by a robust *in vitro* alternative which also allows a clear characterization of the pathways involved. It should be considered as an additional tool to improve and enlarge the set of alternative methods available in modern drug development.

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2 Assuring Safety without Animal Testing: New Risk Assessment Approaches for Skin Allergy and Cancer

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2.1 Results

Assuring consumer safety without the ability to generate animal test data on novel ingredients is a considerable challenge. However, through the application of new technologies and the further development of risk-based approaches for safety assessment, we remain confident it is ultimately achievable.

Recent changes in EU legislation [i.e. 7th Amendment to the EU Cosmetics Directive; EU Chemicals Regulation (REACH)] have made developing alternative approaches to assure the safety of consumer products a key business need. A substantial research programme was therefore initiated by Unilever in 2004, aimed at critically evaluating the feasibility of a new conceptual approach (Fentem et al., 2008) based upon the following key components:

1. Developing new risk assessment approaches
2. Developing new biological (*in vitro*) and computer-based (*in silico*) models
3. Evaluating the applicability of new technologies for generating data that can be interpreted for risk-based safety assessment (e.g. omics, informatics).

We have focussed initially on risk-based approaches for skin allergy (sensitisation), since this is an important consumer safety endpoint for home and personal care products and an endpoint where animal data (e.g. mouse local lymph node assay data) are often needed currently to perform risk assessments. A new conceptual framework for skin sensitisation risk assessment that does not require the generation of new animal data is being evaluated. We are also exploring the value of utilising new consumer exposure modelling techniques, existing *in vivo* (animal and human) data and new in *silico/in vitro* hazard characterisation approaches to inform the risk assessment framework.

A similar rationale is being applied to develop a new risk assessment approach for cancer that does not require the generation of new animal test data. Novel biological insights are being generated that will be capable of informing the risk-based approach and we are investigating the applicability of several 'omics' and other analytical technologies for constructing, visualising and interrogating biological networks. By combining exposure and dose considerations together with a greater understanding of the influence of biological thresholds, we are striving to integrate *in vitro* test data into a more informed decision making process.

These two new risk assessment approaches are part of Unilever's ongoing effort to develop novel ways of delivering consumer safety and represent our strategy to ultimately achieve full replacement for both skin allergy and cancer endpoints.

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