

AXLR8-2

ROADMAP TO INNOVATIVE TOXICITY TESTING



WORKSHOP SUMMARY & RECOMMENDATIONS
FOR FUTURE EU RESEARCH &
INNOVATION FUNDING

17 JUNE 2011

AXLR8 CONSORTIUM



Horst Spielmann, Vivian Kral & Monika Schäfer-Korting
Freie Universität Berlin



Troy Seidle, Emily McIvor & Andrew Rowan
Humane Society International/UK



Greet Schoeters
Centre for Advanced R&D on Alternative Methods
Flemish Institute for Technological Research

AXLR8 SCIENTIFIC PANEL

Julia Fentem	Unilever, Sharnbrook, UK
Maurice Whelan	European Commission Joint Research Centre, Ispra, IT
Nathalie Alépee	L'Oréal, Paris, FR
Jürgen Borlak	Fraunhofer Institute, Munich, DE
Manuel Carrondo	Instituto de Biologia Experimental e Tecnológica, Oeiras, PT
Harvey Clewell	Hamner Institutes for Health Sciences, Research Triangle Park, US
Steffen Ernst	Pfizer, Surrey, UK
Ellen Fritsche	University of Düsseldorf, DE
Barry Hardy	Douglas Connect, Zeiningen, CH
Jürgen Hescheler	University of Köln, DE
Robert Kavlock	Environmental Protection Agency, Research Triangle Park, US
Joanna Jaworska	Procter & Gamble, Brussels, BE
Jos Kleinjans	Maastricht University, NL
Hajime Kojima	National Institute of Health Sciences, Tokyo, JP
Robert Landsiedel	BASF, Ludwigschafen, DE
Carl-Fredrik Mandenius	Linköping University, SE
Michael Schwarz	University of Tübingen, DE
Flavia Zucco	Consiglio Nazionale delle Ricerche, Roma, IT

DISCLAIMER

The views expressed in this document are those of the authors and do not necessarily reflect the views of the authors' affiliated institutions or those of the European Commission. The AXLR8 Consortium, Scientific Panel, and European Commission are not responsible for any use that might be made of the information contained herein.

Feedback regarding this document is welcome and may be directed to info@axlr8.eu.

EXECUTIVE SUMMARY

AXLR8 is a co-ordination action funded by the European Commission Directorate General for Research & Innovation under the Health theme of the 7th Framework Programme (FP7) within the framework on 'Alternative testing strategies: replacing, reducing and refining use of animals in research'. AXLR8 is particularly aimed at accelerating a transition in Europe toward a more sophisticated approach to chemical and product safety assessment with the common goals of improved health and environmental protection, positioning the EU on the leading-edge of a rapidly developing global research area, and working toward replacement of animal testing. An essential element of the AXLR8 project is the organisation of annual workshops to provide a scientific platform for high-level information exchange and critical discourse among co-ordinators of EU-funded projects and independent European and international scientists on progress achieved in developing alternative testing strategies, as well as challenges, needs, and priorities for future EU research.

The second annual AXLR8 workshop (AXLR8-2) was held in Berlin, Germany from 22-25 May 2011 with a focus on developing a 'roadmap to innovative toxicity testing'. Among the more than 50 invited participants were representatives of projects funded by the FP6 and FP7 health and environment programmes, the heads of Member State centres on alternatives to animal testing, the leaders of international efforts to establish advanced molecular toxicology from the United States and Japan, and members of the AXLR8 Scientific Panel and Consortium. The workshop began with a public satellite meeting, providing an overview of current EU and global research efforts such as the joint initiative between DG Research & Innovation and the European Cosmetics Industry Association (COLIPA) aimed at 'replacement of *in vivo* repeated dose systemic toxicity testing' with the long-term target of 'safety evaluation ultimately replacing animal testing' (hereafter referred to as 'SEURAT'), and the work by regulatory and research agencies in the United States aimed at 'toxicity testing in the 21st century'. The satellite meeting was also devoted to innovative disease models based on mapping of molecular and cellular 'pathways' of human disease; to advanced methods funded by the German Ministry of Research and Technology such as the 'virtual liver' project and a multi-organ chip project; and to the European Medicines Initiative 'eTOX' project on the use of proprietary pharmaceutical industry data in bioinformatics.

The formal workshop programme included updates from FP6/7 projects, European companies and academic scientists, and international 'thought leaders'. Plenary presentations focused on the toxicity pathway concept in general, with case studies in the areas of reproductive toxicity and sensitisation (allergy). Building on the success of FP6/7 projects on 'alternative testing strategies' as well as the recent launch of the SEURAT-1 project in the area of repeated dose toxicity, workshop participants were divided into three breakout groups for a focused discussion of the scientific state-of-the-art and of knowledge gaps and priorities for future EU research funding. In the first breakout group, general building blocks for a pathway-based paradigm were discussed, while the other two groups examined reproductive toxicity and sensitisation as case studies.

The workshop concluded with an *in camera* (closed) meeting of the AXLR8 Scientific Panel aimed at refining a strategy and roadmap for future EU research in this area, with a clear focus on advancing the 'Europe 2020' goals of addressing major societal challenges through high-impact, results-driven research and robust integration of key technologies in the field of health (and environmental) protection. The results and recommendations of the AXLR8-2 workshop are briefly summarised below, and will be expanded upon in the report *Alternative Testing Strategies: Progress Report 2011*, to be released later this year.

There was a general view within and among breakout groups that limitations intrinsic to conventional high-dose *in vivo* studies limit their relevance and utility as tools for modern safety assessments aimed at protecting and improving human health (e.g., in relation to nanomaterials, endocrine disrupters, and environmental chemicals), and that the way forward requires a shift towards a pathway-based paradigm for safety assessment. In particular, assessment of a substance's toxic 'mode of action' is considered by the AXLR8 Scientific Panel and other authorities to be a cornerstone of '21st century' safety assessment (NRC, 2007; Berg et al., 2008; EPA, 2009). Development of a robust understanding of the networks of biological pathways—many of which are not yet described in full—and key events associated with chemical toxicity (Figure 1) can feed back into the innovation cycle to support 'greener', biocompatible chemistries, and can contribute to the study and treatment of human diseases (Gohlke et al., 2009), guiding research on fundamental biology and feeding into the product innovation cycle. By focusing on priority diseases with integration of human patient data, biomonitoring of healthy and diseased populations, and other modern exposure assessment tools, it should be possible to better understand population diversity and susceptibility, and perhaps achieve a closer alignment between human health and environmental risk assessments. Opportunities for synergistic partnerships between EU projects with high-impact initiatives such as Germany's Virtual Liver project, the Japanese METI-NEDO High Throughput Assay Systems project and the US Virtual Embryo, Tox21, and NexGen programmes were noted.

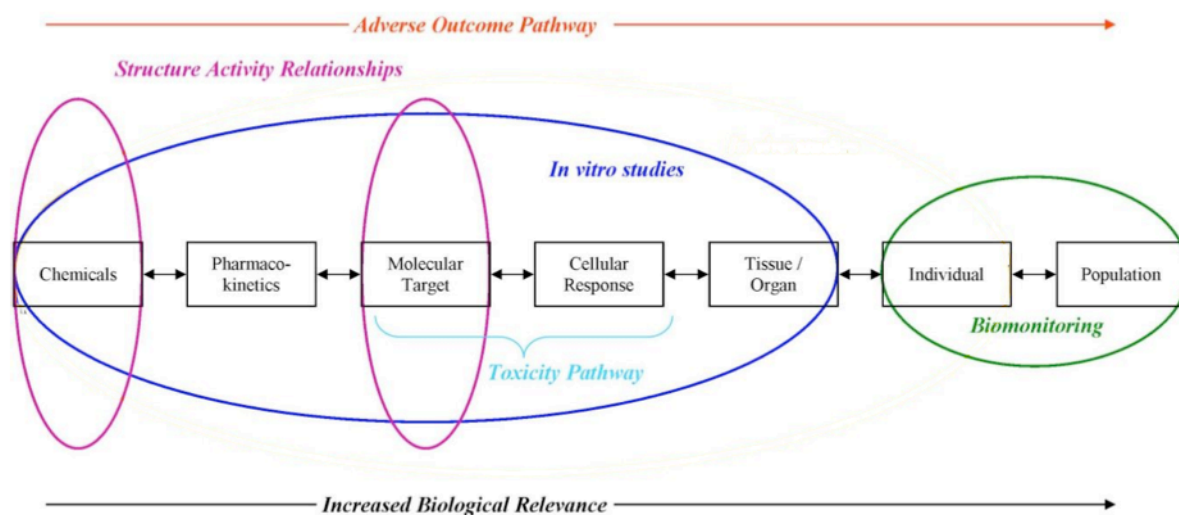


Figure 1 An illustration of the mechanistic pathway from a molecular initiating event through toxic outcome at the whole organism or population level (adapted from EPA, 2011).

Workshop participants underlined the importance of the higher level of human-relevant biological understanding that can be achieved by means of an integrated pathway and modelling approach to safety assessment, but noted that a substantial investment in targeted interdisciplinary research and related infrastructure will be required to fully develop each of the key 'building blocks' (illustrated in Figure 1) and demonstrate their functional integration before such benefits can be fully realised. It was also noted that early and active interactions with regulatory authorities, regulated industry, and civil society stakeholders will be necessary to achieve timely acceptance and integration of new testing tools and strategies as part of an evolutionary shift in the safety testing and assessment paradigm.

During its *in camera* session aimed at developing an innovative toxicity testing roadmap for consideration under the forthcoming Common Strategic Framework for Future EU Research and Innovation Funding, the AXLR8 Scientific Panel noted the substantial progress that has been made in Europe in the development of 'alternative testing strategies' as a product of

funding by DG Research & Innovation under FP6 and FP7 (Figure 2). The SEURAT-1 initiative was recognised as an important step towards new experimental and computational approaches to safety testing and assessment, and as a public-private partnership between the Commission and regulated industry, it represents a promising new funding model. Indeed, COLIPA’s direct financial support for this initiative was highly welcomed and favoured over, e.g., in-kind contributions from the private sector.

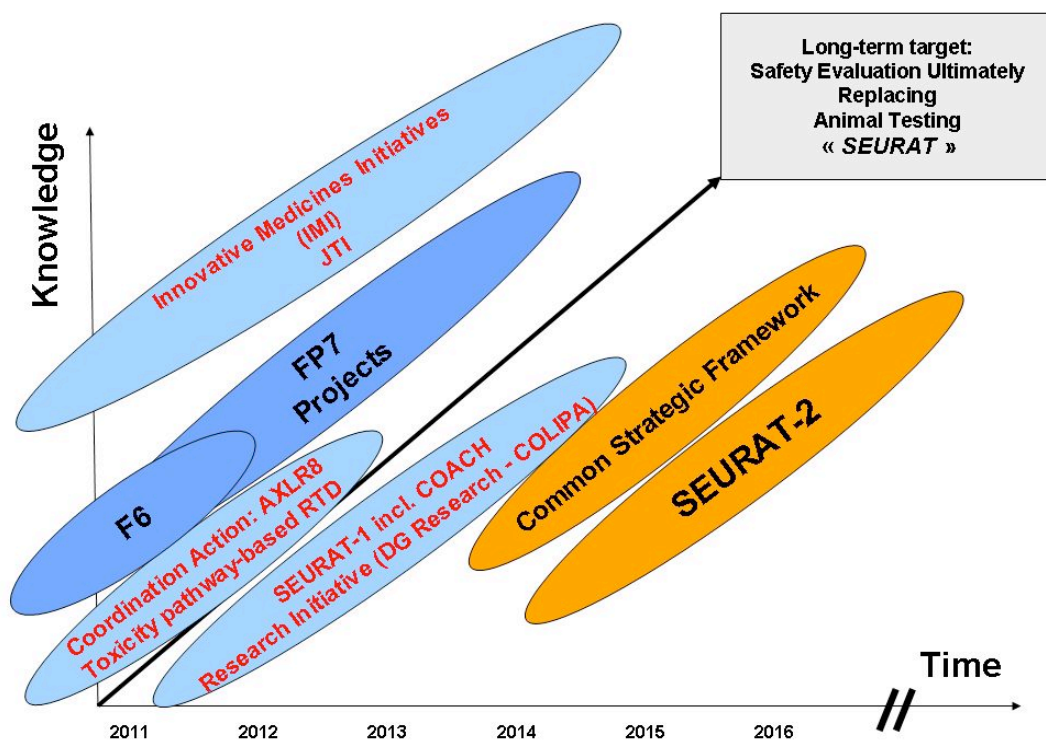


Figure 2 Illustration of the funding activities of the European Commission DG Research & Innovation within the Health theme of FP6 and FP7 in the context of policy needs, industry requirements and scientific challenges to develop ‘alternative testing strategies for replacing, reducing and refining the use of animals in research’.

■ programmes currently funded via FP6/FP7 ■ future funding programmes

Structurally, the Scientific Panel welcomed the ‘cluster’-type design of SEURAT-1, i.e., multiple project-level ‘building blocks’ organised around a central co-ordinating action (Figure 3). This approach facilitates intense scientific exchange and supervision of the research projects/areas¹ and governance of the cluster. Procedurally, it was recommended that up-front co-ordination and development of project plans and consortia around a central unifying vision—including clear scientific objectives and tangible milestones and deliverables at both project and cluster levels—is essential to ensure strategic alignment within and across projects and cohesion at cluster-level. Procedurally, it was suggested that the existing scientific experts panels could begin now with the development of a detailed roadmap to innovative toxicity testing within the scope of the pathway paradigm. It was also recognised that project co-ordination at cluster-level requires a permanent secretariat run by a group of experts with a multitude of skills at administrative,

¹ These objectives could also be pursued through creation of a centralised and highly networked ‘EU Centre for Molecular Toxicology’, where scientists from different disciplines could work at one location toward the same well-defined aim, and through which research and training could be co-ordinated in a focused way. Work not covered by the EU Centre could be subcontracted to external research groups or clusters as appropriate.

organisational and scientific levels. Additional administrative instruments to support and enforce cluster-level interactions should be explored in future programmes in areas such as data and knowledge exchange, management and exploitation of intellectual property, and communication and dissemination of research results.

The AXLR8 Scientific Panel considered that the fields of systems toxicology and medicine are primed to advance by a quantum leap, and the EU—as a leading innovator in the area of health research funding aimed at advancing the science of safety testing—is well positioned to play a major role in this dynamic and rapidly evolving research area. Not since the Human Genome Project has the EU been presented with such a tremendous opportunity to contribute to world-class scientific breakthroughs. Indeed, mapping the human ‘toxome’ is directly analogous to the human genome mapping of the 1990s, and has the potential to be a ‘game changer’, with substantial benefits foreseen in the areas of public health and environmental protection, economic growth and competitiveness, and animal welfare. Thus, to build on the momentum of successful FP6/7 projects such as ReProTect and Sens-it-iv, and to cover the full spectrum of health and toxicity concerns, the experts concluded that it would be essential to extend SEURAT-1 to its next phase, with integration of all relevant aspects of systems medicine into the core research strategy.

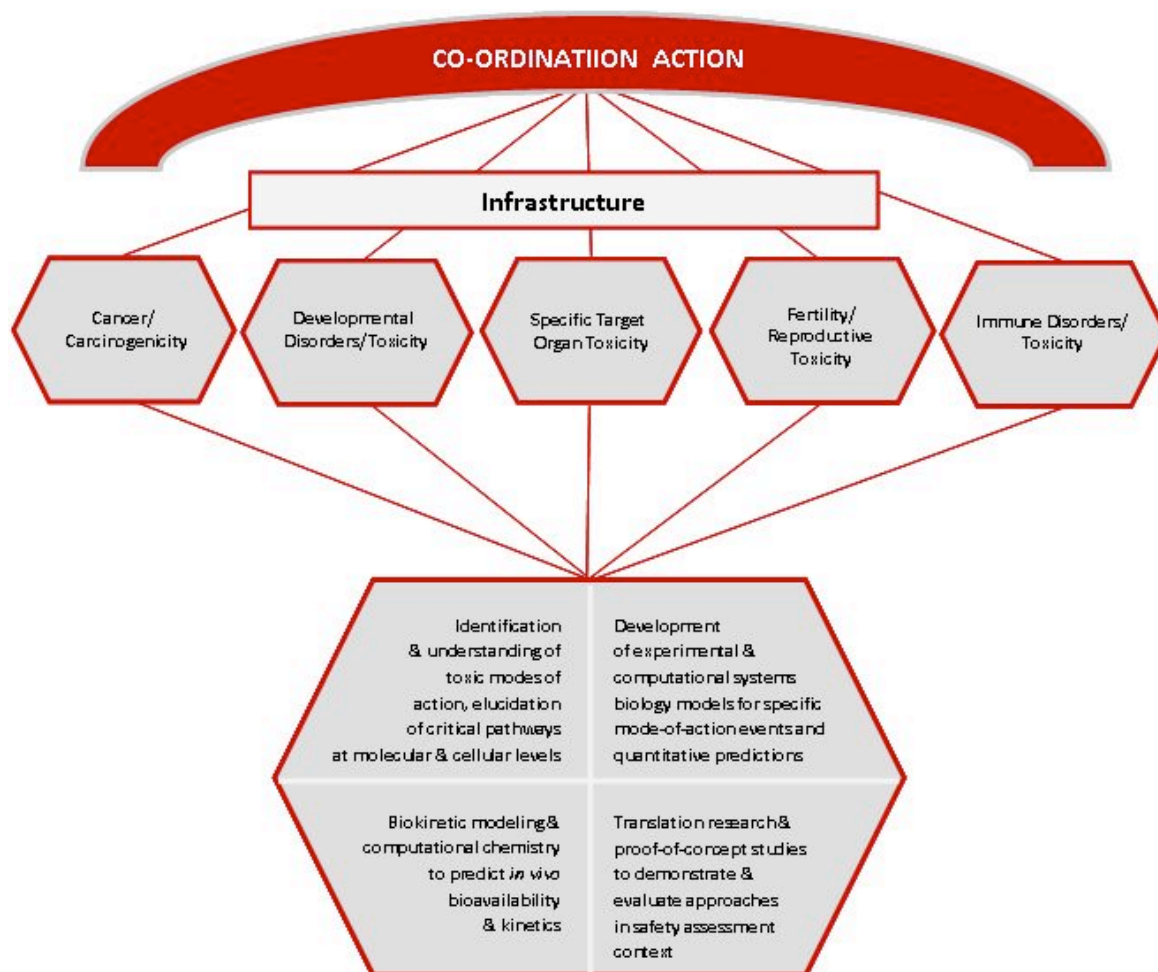


Figure 3 Illustration of the proposed SEURAT-2 structure. Six large-scale clusters encompassing five human health effect areas together with cross-cutting infrastructure would be developed under the direction of a central co-ordination action. There should be a strong focus in all clusters on core ‘building blocks’, illustrated here as four distinct projects/research areas; however, the exact number of projects per cluster should be determined on a case-by-case basis.

RECOMMENDATIONS FOR FUTURE EU RESEARCH & INNOVATION FUNDING

The AXLR8 Scientific Panel recommends swift and decisive action to develop an ‘innovation flagship-level’ interdisciplinary research effort that builds upon the results of European FP6/7 projects and the emerging results of SEURAT-1, but on a much larger scale given the magnitude of work that is still needed to achieve a full paradigm shift in toxicological safety assessment. The general concept is illustrated in Figures 2 and 3, and is denoted here as ‘SEURAT-2’. Taking into account the positive experience of SEURAT-1, SEURAT-2 should be established as a public-private partnership between the Commission, Member States and regulated industry.

As successfully introduced in SEURAT-1, the key element of SEURAT-2 should be the ‘cluster’, comprising a group of typically 4-6 individual research projects focused on a particular area. A total of 6 clusters should be funded, organised around five priority health concerns, i.e., cancer/carcinogenicity, fertility and developmental health/toxicity, specific target organ toxicities, and immune disorders/toxicity (including sensitisation), which have been identified elsewhere as requiring additional research resources (Adler et al., 2011). An additional cluster is envisaged to address infrastructure and servicing needs, including knowledge management, high-throughput screening platforms, bioengineering, communications, training and outreach. Overall management of the six clusters should be handled by a central co-ordination action. Based on the SEURAT-1 model, a funding level of 50 million € per cluster could be envisioned, and taken together with a co-ordination action with 3-4 full-time personnel, a total budget of 325 million € would seem appropriate.

In contrast to previous research, there should be a strong focus in all clusters on the following cross-cutting themes and development of the core ‘building blocks’ of technical capabilities and models for a common toolbox:

- Identification and understanding of toxicological modes-of-action associated with adverse health effects and disease in humans², including elucidation of critical perturbations/pathways at the molecular and cellular levels.
- Development of experimental, theoretical and computational models that capture specific mode-of-action events at different scales (molecular, cell, tissue, organ, organism), underpinned by a systems biology approach to integrate models and make quantitative predictions.
- Expansion and refinement of ‘physiologically-based biokinetic’ (PBBK) modelling and computational chemistry methods to predict *in vivo* bioavailability, biotransformation and bioactivity of exogenous chemicals.
- Translational research and proof-of-concept activities to realise fit-for-purpose methods and tools for toxicological hazard and potency prediction, and the demonstration and evaluation of these in a safety assessment context.

It is recommended that the SEURAT-2 central co-ordination action would be responsible for articulating initial scientific objectives, milestones and deliverables at both cluster and project levels. To achieve the optimal collection of projects within a cluster, it may be necessary to carry out two successive peer reviews, i.e., the first focusing on scientific excellence and then on programmatic relevance at cluster-level, in order to maximise potential synergies both within and between projects and clusters. As projects are

² Identifying modes of action in other species, e.g., rodents, could be useful for understanding the mechanistic basis for species differences and for bridging the gap between emerging human *in vitro* data and the results of ‘legacy’ *in vivo* experiments.

established, contract agreements should be put in place to clearly define responsibilities and relationships within and among projects, including at the cluster level. Consideration should be given to the use of contracts as opposed to grant agreements in some cases for specific research or service needs. The co-ordination action should establish an independent scientific panel to continually monitor progress at both project and cluster levels, stimulate communication between projects and clusters, define and co-ordinate future tasks and long-range planning of the cluster, and ensure general accountability of all partners toward the common goals. The scientific panel should include the co-ordinators of all projects and further external experts, including international experts. The running of the co-ordination action should be managed by a permanent administrative and scientific secretariat. It is recommended that the role of the co-ordination action be reinforced with effective tools to ensure alignment among projects and enforcement of milestones and deliverables, and that sufficient flexibility be provided to make mid-stream course corrections as needed, for example by bringing in new projects mid-stream when a gap is identified.

'Value added' collaborations among established research teams (e.g., the US Tox21 initiative, Japanese institutes, etc.) in key areas should be encouraged to share the workload, develop synergies without duplication, and together reach for an ambitious, global objective that would be impractical to pursue on a regional basis. This should allow for recruitment of international partners as appropriate, as well as joint funding calls with Member State and international agencies and funding bodies. The European Commission's own Joint Research Centre could make an important contribution owing to its extensive programme in safety assessment methodology and its role in translating scientific results into support to policy-making. Targeted multidisciplinary partnerships should also be encouraged, given that a solution for more predictive and animal-free safety assessment needs the mobilisation of the best scientists in their fields, many of whom would not traditionally apply their work to toxicology. Funding for SEURAT-2 should, as a matter of principle, be used to support research that does not involve the use of living animals.

REFERENCES

- Adler S, Basketter D, Creton S, et al. Alternative (non-animal) methods for cosmetics testing: current status and future prospects - 2010. Arch Toxicol. 2011; 85, 367-85.
- AXLR8 Consortium. Alternative Testing Strategies – Progress Report 2010, www.axlr8.eu/axlr8-2010-progress-report.pdf
- Berg N, De Wever B, Fuchs HW, et al. Toxicity in the 21st century – working our way towards a visionary reality, 2010, www.ivtip.org/images/IVTIP_publication-final.pdf
- EPA [US Environmental Protection Agency]. The Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals, US EPA, 2009.
- EPA. Use of New Computational and Molecular Tools. FIFRA Scientific Advisory Panel Consultation, 24-26 May 2011. Washington, DC: EPA.
- NRC [US National Research Council]. Toxicity Testing in the 21st Century: A Vision and Strategy. Washington, DC: National Academies Press, 2007.
- Gohlke JM, Thomas R, Zhang Y, et al. Genetic and environmental pathways to complex diseases. BMC Syst Biol. 2009; 3, 46-61.
- NexGen – Advancing the Next Generation of Risk Assessment, www.epa.gov/risk/nexgen
- Tox21 – Memorandum of Understanding, research, develop, validate and translate innovative chemical testing methods that characterize toxicity pathways, www.epa.gov/ncct/Tox21
- Virtual Embryo Project – A computational framework for developmental toxicity, www.epa.gov/ncct/v-Embryo
- Virtual Liver Network, www.virtual-liver.de