

SEURAT: Vision, Research Strategy and Execution

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"The goal of mode-of-action, human biology-based testing is not to generate batteries of tests to provide a prediction of animal toxicity test results for various endpoints. Instead, these methods are intended to determine regions of exposure that will not cause any adverse responses in exposed human populations."

Boekelheide, K, Andersen, M E (2010): A Mechanistic Redefinition of Adverse Effects – a Key Step in the Toxicity Testing Paradigm Shift.- ALTEX 27, 4/10: 243-252. Text taken from p. 248.

Introduction

The SEURAT initiative - Safety Evaluation Ultimately Replacing Animal Testing - was introduced in 2008 by the Health Directorate of the European Commission's Directorate General for Research and Innovation (DG RTD). The aim was to devise and implement a comprehensive EU research programme that will drive a major overhaul in the chemical safety assessment paradigm, ensuring the greatest protection of human health without having to experiment on animals. The initiative is expected to take many years, perhaps decades, and will require significant resources. However, rapid advances in life sciences and a strong desire among stakeholders to embrace change suggest that SEURAT is indeed feasible.

The first execution phase, **SEURAT-1**, was successfully launched in January 2011. It comprises a cluster of five complementary research projects, a data handling and servicing project and a coordination action and is co-financed by the FP7 Health Programme and the European Cosmetics Association (*Colipa*), through a new model of public-private partnership. Over the next 5 years, over 70 research institutions will work together towards the replacement of repeated dose systemic toxicity testing on animals. The ultimate aim of this first cluster is to deliver a proof-of-concept to show how the latest scientific tools and knowhow can be combined to deliver decision support systems for safety assessment.

In this paper we endeavour to further elaborate SEURAT, by proposing a *Vision* - describing what a future safety assessment paradigm should comprise, a *Strategy* - defining the underpinning scientific concepts and approach, and an *Execution* plan - that outlines the main elements of the research programme to be undertaken. In this context

we also describe the research priorities of the first execution phase, **SEURAT-1**, and propose how the results and momentum can be carried forward to the next phase, **SEURAT-2**.

The Vision

The SEURAT vision is to fundamentally change the way we assess the safety of chemicals, by superseding traditional animal experiments with a predictive toxicology that is based on a comprehensive understanding of how chemicals can cause adverse effects in humans.

The vision foresees safety assessment frameworks that optimally combine a range of reliable and robust experimental (*in vitro*) and computational tools in a purposeful manner to deliver the relevant information needed for decision making. These predictive toxicology tools will associate substances of concern with a new taxonomy of toxicological hazard categories, and they will predict the likelihood of any adverse health effects as a function of exposure, for different sub-populations. The uncertainty of these predictions will be sufficiently characterised as to facilitate effective risk management and communication, with the appropriate degree of precaution. The predictive tools will be widely available, affordable, and reliable so that every substance destined for commerce will be sufficiently evaluated in good time, at a reasonable cost, and in a consistent manner. To facilitate trade and the global market, safety assessment frameworks will be established and harmonised at international level, allowing them to be implemented in all jurisdictions. The knowledge gained from safety assessment of new substances will be fed back into the product development process, thereby improving human safety evaluation, driving innovation, increasing consumer choice, promoting sustainability, and improving industrial competitiveness.

The Strategy

*The SEURAT strategy is to adopt a toxicological mode-of-action framework to describe how any substance may adversely affect human health, and to use this knowledge to develop complimentary theoretical, computational and experimental (*in vitro*) models that predict quantitative points of departure needed for safety assessment.*

The mode-of-action framework (Boobies et al., 2008) is based on the premise that any adverse human health effect caused by exposure to an exogenous substance can be described by a series of causally linked biochemical or biological key 'events' that result in a pathological endpoint or disease outcome. An 'adverse-outcome-pathway' is a very similar concept proposed by the computational toxicology community (Ankley et al., 2010), where the linking of a chemical with a pathway that leads to an adverse human health or ecological outcome is determined by its ability to trigger the associated 'molecular initiating event'. Another related framework is that of 'toxicity pathways'

introduced by the NRC (*Krewski et al., 2010*), where in this case the description of toxicological processes tends to focus on early events at the molecular level. Thus one can consider toxicological pathways as critical upstream elements of a more expansive mode-of-action or adverse-outcome-pathway description of how a chemical can compromise human health.

Mode-of-action theory is still emerging but there are already a number of important principles that have shaped the SEURAT research strategy. The first is that every toxicant can be associated with one or more mode-of-action categories. To facilitate this, however, a suitable ontology that describes all the possible modes of toxicological action needs to be developed by harvesting and organising the wealth of knowledge and information available from the literature on well studied chemicals and pharmaceuticals. Systematically checking 'reference' chemicals against mode-of-action categories will help challenge and refine the mode-of-action ontology as it emerges, and will identify a wide range of key biological events and pathways that should be represented in relevant experimental (*in vitro*) and computational models.

The framework assumes that many modes-of-action share common key biomolecular or biological events. Thus it is the particular chain of causally-linked events that makes a mode-of-action unique (*Figure 1*). In the case where a substance is promiscuous and could trigger multiple modes-of-action, the concentration and persistence of the substance at the initiation sites will dictate the modes-of-action that will tend to dominate. Thus, for example, chronic low-dose effects are likely to be quite different from high-dose acute effects. Special consideration needs to be given therefore to characterising dose-response relationships, to describe how and when mode-of-action transitioning may occur for a single substance, depending on factors such as exposure dynamics, site of action, genetic and epigenetic predisposition or inherent phenotypic vulnerabilities.

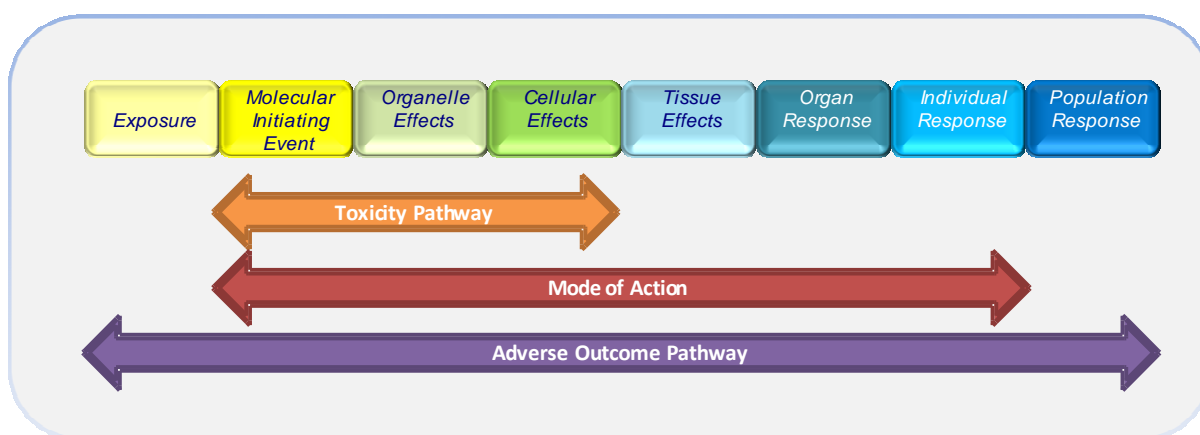


Figure 1 Schematic illustration of a sequence of events contributing to an Adverse Outcome Pathway, including the Mode of Action and Toxicity Pathway as sub-sequences.

Another principle that must be considered concerning mode-of-action theory is that many key events and pathways are common to many cell types throughout the human body.

Thus although the same substance can cause different pathological outcomes in different tissues, the upstream event, such as mitochondrial inhibition or generation of reactive oxygen species, may be common to the modes-of-action triggered at each site. On the other hand, certain modes-of-action involve key events or pathways which are associated with specific biological functions expressed by particular cell types. The presence of metabolising enzymes in liver cells which may bioactivate exogenous chemicals to produce toxic metabolites, or the presence of cell membrane transporters required for the uptake of certain toxicants are examples illustrating cell-type specific toxicity. Similarly, the presence of receptors for neurotransmitters in neuronal cells which can be targeted by toxicants is another example of cell specific properties that can be implicated in a toxicological mode-of-action.

Although many toxicological modes-of-action are conserved across mammalian species, there will likely be many situations where for example, rodent or tumour derived cell lines will fail to capture essential aspects of human biology. Attention needs to be given therefore to the development of experimental models based on properly conditioned human primary cells or differentiated stem cells. In addition, modelling a toxicological mode-of-action in a holistic fashion will require the emulation of downstream events that manifest themselves as pathology at the tissue level. Simple cell-based *in vitro* models will not be sufficient for this purpose and thus 3D tissue models will be needed to reproduce the more apical biological processes or endpoints. These 3D tissue models will be produced experimentally in bioreactor systems, or virtually using computational biology approaches. Such models will not only allow the qualitative association of a chemical with one or more modes-of-action, but will also serve to quantify dose-response relationships. Complementing the cell and tissue models, computational chemistry, quantitative structure-activity relationships (QSAR) and chemoinformatics tools will provide

Table 1. Objectives of SEURAT-1

Selection of well studied chemicals with evidence of chronic systemic toxicity.
Hypothesis driven approach to elucidating modes-of-action and identifying associated key events and biomarkers.
Emphasis on <i>in vitro</i> models that capture important modes-of-action directly relevant to human physiology.
Exploit stem cell technology to develop <i>in vitro</i> systems with cellular diversity to model higher level functions.
Development of fit-for-purpose <i>in vitro</i> assays suitable for HTS implementation.
Use of bioreactors to engineer tissue comprising multiple cell types to model complex toxicological processes.
Biokinetic modelling to extrapolate between <i>in vitro</i> test concentrations and repeated dose organ exposure <i>in vivo</i> .
Computational toxicology to associate chemicals with molecular initiating events and describe metabolism
Use of high content analysis tools including 'omics to describe modes-of-action at the molecular level.
Systems biology approaches to model mode-of-action dynamics at the molecular scale for quantitative analysis.
Proof-of-concept exercise to demonstrate a mode-of-action based integrated test system to predict sub-chronic liver toxicity
Feasibility study to show how test data can be used in a safety assessment context

the means to understand and predict key biochemical events such as protein binding and metabolic transformation. However, these advanced experimental and computational approaches may be limited if they are overly reductionist or simplistic, thus failing to capture aspects such as hormonal regulation, tissue innervation, immune surveillance, blood circulation and metabolic turnover.

An important aspect of the SEURAT strategy will be the emphasis placed on understanding and predicting the *in vivo* biokinetics of exogenous chemicals. Quantifying the dose in different target tissue compartments as a function of time and exposure conditions, will be a fundamental requirement of any predictive toxicology paradigm. The expectation is that most chemicals are not likely to be harmful to only one specific cell or tissue type, but that in fact most apparent specificity-of-action can be explained by the bioavailability of the chemical at different anatomical sites, dictated by how it is absorbed, distributed, metabolised and excreted in the human body. Experimental and computational tools to profile chemicals, for example, in terms of their affinity to bind to proteins, their metabolic stability, and their ability to diffuse or be transported across biological barriers, will provide the necessary input for physiologically based bio-kinetic models that will ultimately predict chemical fate *in vivo*.

Establishing a comprehensive description of the mode-of-action domain is a challenging but necessary element of the strategy that will require the use of advanced discovery and modelling tools. Identifying the key biological events and biomarkers that comprise a particular mode-of-action, and elucidating the relationship between these events will benefit greatly from high content functional analysis tools such as transcriptomics, proteomics, and molecular imaging. High throughput screening can play an important role in generating reference data using more traditional assay formats, whereas microelectronic and optical biosensing technology will be necessary to monitor the dynamic response of biological models in a non-invasive manner. Aiming at a more quantitative description of a mode-of-action and in particular, defining the array of conditions that must be met to progress towards an adverse outcome, or that might result in system recovery, will require mathematical models of sufficient complexity. Systems biology theory and tools will provide a strong basis for these models that will need to take different phenomenological aspects into account, such as the stochastic nature of many biological systems.

As the mode-of-action framework becomes more established, and the range of validated models grows, an increasing number of chemicals can be profiled to establish to which mode-of-action categories they belong. This will then facilitate read-across within categories and provide the basis for ultimately predicting hazard threshold values, akin to *in vivo* no-effect levels. Initially, assessment frameworks exploiting such predictions are likely to apply quite conservative uncertainty factors. However, as prediction algorithms are improved and validated, and the description and quantification of the uncertainty is more thoroughly addressed, it is likely that factors can be more optimally defined. Feasibility (proof-of-concept) studies will help pull together the various components of a testing strategy in a purpose-driven fashion, and will be an important instrument for engaging the regulatory community and promoting uptake of SEURAT approaches.

The Execution

The First Step, SEURAT-1

The first execution phase, entitled **SEURAT-1**, has a broad and highly ambitious work programme that aims to prove the scientific and technological concepts underpinning the SEURAT strategy. The main objectives of the **SEURAT-1** Research Initiative are summarised in Table 1. The overall emphasis is on the identification and elucidation of modes-of-action related to repeated dose systemic toxicity in humans, and the development of experimental and computational models that effectively capture the related pathways and key biological events. A set of reference chemicals is being compiled which have been thoroughly investigated regarding their chronic toxicological action in animals, and in humans if possible, and this information will be used to propose an initial mode-of-action framework to which the various research activities can refer. The chemicals will also be supplied throughout the cluster as controls for *in vitro* model characterisation and assay development.

Significant effort will be invested in basic research concerning both embryonic and induced pluripotent stem cells, of human origin, with the intention of devising optimal maintenance and differentiation protocols that deliver large quantities of well characterised, stable, and reproducible cell lineages, which express the important phenotypic properties and functions found *in vivo*. An important goal of this work is the production of a comprehensive set of genetically engineered stem cell derived models which express light-producing enzymes or proteins on triggering of certain signalling or metabolic pathways (*Figure 2*). Bioreactor technology will be employed to engineer 3D tissue constructs *in vitro* in an attempt to capture the intricate interactions between different cell types present in an organ that must work in unison to maintain homeostasis and function. It is anticipated that such systems will be required to represent more complex modes-of-action and to move towards more predictive systems from which chemical activity/effect levels can be derived.

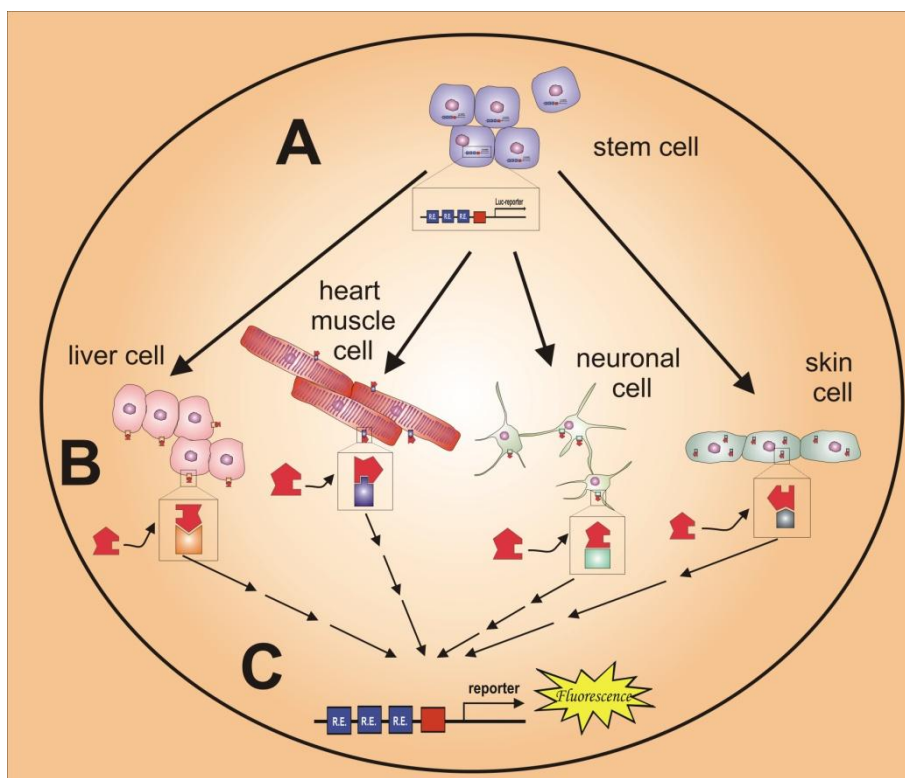


Figure 2: The use of genetically engineered stem cells derived *in vitro* models for toxicity screening A: Cell lines are generated from human stem cells carrying reporters for relevant signalling or metabolic pathways associated with different modes-of-action B: Reporter cells are differentiated into cells of different lineages related to different target tissues of the liver, heart, muscle, central nervous systems, skin, etc. C: Differentiated reporter cells are incubated with test chemicals (red blocks) which may interact with different "receptors" (coloured blocks) thus triggering reporter activity that is monitored by measuring luminescent light.

Transcriptomics and proteomics (*in vitro*) are being employed to dig deeper into the underlying molecular processes associated with selected modes-of-action, and this data and information will be used to guide the definition of systems biology models which capture the process dynamics and allow quantitative analysis and prediction of adverse pathway perturbation. As the mode-of-action framework is refined, and more key biological events are identified, new biomarkers of effect will be investigated that can be incorporated into assay systems. In addition, the intention is to exploit novel biosensing and imaging techniques to more effectively detect biomarkers, thus improving on more traditional read-out approaches.

Apart from the systems biology modelling, computational toxicology methods will also be applied in two specific areas, namely, biokinetic profiling and structure-activity relationships. The biokinetic profiling will centre on the use of Physiologically Based BioKinetic (PBBK) approaches to model both the *in vivo* and *in vitro* fate of exogenous chemicals, in terms of their adsorption, distribution, metabolism and excretion. This will allow *in vivo* to *in vitro* exposure extrapolation for a limited set of chemicals for which the relevant intrinsic/extrinsic properties are known, or can be determined (e.g. lipid-water partitioning coefficient, protein binding affinity, metabolic clearance rate). Regarding Structure-Activity Relationships (SAR), the attention here will focus on finding

associations between the structural features of a chemical and its ability to trigger the key biomolecular events that initiate toxicological responses that may lead to adverse health outcomes. Forming chemical categories based on combined structure-activity descriptors will ultimately facilitate more rapid and robust hazard profiling of chemicals and read-across between chemicals which have a similar mode-of-action.

The **SEURAT-1** Research Initiative will deliver many important computational and experimental tools, and related knowhow, that will be critical components in predictive toxicology approaches. To demonstrate the potential of these tools and how they can be assembled in an integrated manner, the cluster will undertake a proof-of-concept exercise to demonstrate how a mode-of-action based testing strategy can be used to predict aspects of repeated dose target organ toxicity. In addition, a feasibility study will also be carried out to show how test data derived from such systems can be used in a safety assessment context. In doing so, the intention is to engage regulatory scientists and stakeholders in a practical dialogue aimed at building confidence in the tools, identifying important sources of uncertainty, and deciding on how to best progress the field to foster uptake and acceptance of the new methodology.

Next Steps, SEURAT-2 and Beyond

Successful completion of **SEURAT-1** will lay the foundation for follow-on efforts in **SEURAT-2** that will broaden the toxicological, chemical, and regulatory domains addressed, as illustrated in Table 2. The mode-of-action framework will have been well established, but will be limited in scope, covering mainly repeat-dose toxicity associated with primary organs. Thus the mode-of-action ontology will need to be further expanded by harvesting existing

Table 2. Objectives of SEURAT-2

<p>Broaden the toxicological mode-of-action ontology to cover other adverse effects</p> <p>Address the issue of population diversity regarding predisposition and susceptibility</p>
<p>Expand the inventory of cell models to cover other tissues and physiological processes</p> <p>Develop stably transfected stem cell models for reporter gene assays sensitive to key pathways</p>
<p>Cover a greater diversity of chemical type, structure and class.</p> <p>Broaden the chemical domain to cover an extensive range of industrial sectors</p>
<p>Scale up testing using HTS and HCS to generate more research data on a large set of reference chemicals</p> <p>Implement standalone computational workflows for virtual screening/profiling</p>
<p>Refine biokinetic and systems biology models to give more accurate and comprehensive predictions for a larger chemical space</p> <p>Assemble tools to realise integrated toxicological hazard prediction systems for a wide range of regulatory endpoints</p>
<p>Undertake a comprehensive evaluation and demonstration programme based on typical safety/risk assessment scenarios</p>

knowledge, and generating new knowledge where gaps exist, to cover other adverse health effects linked for example to cancer and reproduction.

Exploration of this broader toxicological domain will need a more extensive range of cell models and engineered tissues that capture important biological processes and function, that can be used not only to investigate and confirm modes-of-action, but which could also be used as a component of an integrated test system. Genetically engineered stem cell models that can be used for event-specific gene-reporter assays will facilitate this greatly, for example. **SEURAT-2** will have to consider a larger number of chemicals taken from a wider chemical space, in order to cover more diverse physicochemical properties, modes-of-action, and related health effects. By tackling a wider chemical space, **SEURAT-2** will also be relevant for a number of different industrial sectors and legislative areas.

Broadening the toxicological, chemical, and regulatory domains to be addressed in **SEURAT-2** will require the generation of high quality in vitro datasets on large numbers of reference chemicals. Therefore experimental activities will need to be scaled up through the exploitation of High Throughput and High Content Screening (HTS/HCS) platforms, including in situ biosensing, imaging and 'omics. Moreover, computational tools will require further development, refinement and integration to broaden their applicability domain and improve their predictive power. On completion of **SEURAT-1** it is likely that biokinetic modelling of Adsorption, Distribution, Metabolism and Elimination (ADME) of exogenous chemicals will not yet be sufficiently developed and thus **SEURAT-2** will need to invest further in this area if overall progress is not to be hindered. In addition, systems biology modelling will also have to be further improved and expanded in order to effectively link processes at the molecular, cellular, tissue, organ and organism levels, in order to make accurate quantitative predictions of in vivo effects from, for example, in vitro data. All this will need to be supported by the definition and implementation of computational workflows that formalise processing steps and decision making logic for a more consistent application of assessment methodology in a context specific manner. Such workflows will ultimately drive the assessment process, commencing with virtual screening steps to use existing information and chemoinformatics to associate a chemical with specific mode-of-action based hazard categories, followed by targeted in vitro testing and computational analysis to elaborate dose-response relationships and to predict quantitative points of departure, such as no-effect levels. This should provide the basis to undertake a comprehensive programme of evaluation and demonstration, to consider a range of safety/risk assessment scenarios that can be effectively tackled with the new methodology. Moreover, key elements and methods might be subject to more systematic validation, if required to facilitate scientific and regulatory acceptance.

Uptake and application of SEURAT methodology for safety assessment will begin modestly on a proof-of-concept level within **SEURAT-1**, but will continually expand in both depth and scope throughout **SEURAT-2** and beyond. Possible application areas in the relatively near future include satisfying Classification, Labelling and Packaging (CLP) requirements, or supporting a weight-of-evidence analysis or read-across in a Chemical Safety Assessment under REACH. It is likely that novel tools and safety assessment frameworks deriving from SEURAT will be initially implemented and evaluated in parallel to more traditional approaches. This will identify any shortfalls, build confidence, and

define good practice for better safety evaluation that will ultimately replace animal testing.

The transition to **SEURAT-2** represents the expansion and application of the concepts and tools proven in **SEURAT-1**. It will require a substantial scaling up of efforts to engage a wider section of the scientific community in a critical mass of complimentary collaborative-research projects. It is recommended that this be facilitated through the establishment of a dedicated research programme for innovative toxicity testing and safety assessment within the Common Strategic Framework for EU Research and Innovation. This should not only provide the necessary funding, but also the right instruments to support and coordinate large-scale strategic actions complimented by smaller targeted projects, and lever the resources and expertise of industry through public-private partnerships. In addition, it is imperative that the new EU research programme be positioned squarely within an international context, to join forces with complimentary initiatives in the USA, Canada, Japan and elsewhere, and to facilitate the work of international organisations such as the OECD and WHO.

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