



Food for Thought ...

Systems Toxicology

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Summary

The need for a more mechanistic understanding of the ways in which chemicals modulate biological pathways is urgent if we are to identify and better assess safety issues relating to a wide range of substances developed by the pharmaceutical, chemical, agri-bio, and cosmetic industries. Omics technologies provide a valuable opportunity to refine existing methods and provide information for so-called integrated testing strategies via the creation of signatures of toxicity. By mapping these signatures to underlying pathways of toxicity, some of which have been identified by toxicologists over the last few decades, and bringing them together with pathway information determined from biochemistry and molecular biology, a “systems toxicology” approach will enable virtual experiments to be conducted that can improve the prediction of hazard and the assessment of compound toxicity.

Keywords: systems toxicology, metabolomics, mechanistic toxicology, pathways of toxicity, toxicogenomics

The discussion leading toward Toxicity Testing in the 21st Century, prompted by the hallmark report by the US National Research Council (NRC, 2007), has raised expectations that modern high-content and high-throughput technologies might be able to bring about a revolution in regulatory toxicology (Hartung, 2008). Such expectations are nourished by top US agency representatives calling for change (Hamburg, 2011) and major funding programs for predictive *in vitro* systems (by NIH, FDA, DARPA and DTRA, totaling \$ 200 million in 2011-2012 alone). The technologies needed to make such a change happen appear to be largely at hand (van Vliet, 2011). Typically, they are used to phenotype substance effects (signatures of toxicity, SoT). Increasingly, attempts are made to link these to specific pathways of toxicity (PoT). Three major efforts are underway in the United States: First, the EPA’s ToxCast program¹ and the Tox-21 alliance formed with other agencies has taken an approach based largely on “off the shelf” pathway assays for high-throughput testing. Increasingly, however, other information sources are being included. Second, work undertaken at the Hamner Institute and propagated by the Human Toxicology Project Consortium addresses selected case study examples of PoT (Andersen et al., 2011). Third, the NIH-sponsored consortium to map the Human Toxome, headed by CAAT but including both Hamner and ToxCast in the consortium, has begun, in an unsupervised manner, to identify PoT starting from metabolomics and transcriptomics (Hartung and McBride, 2011).

These approaches are complementary and strongly overlapping. They will shape the way we characterize substances of regulatory interest in the future. Primarily, they enable the description of a footprint that the substance has left in the biological system. The SoT will be a mix of causal PoT, pathways of defense (PoD) inevitably induced in parallel, epiphenomena (EpiP), and artifacts (Basketter et al., 2012). The data subsequently will be put in context through PoT identification (Judson et al., 2012) and interpretation via a systems toxicology approach.

It is important to define what is meant by “systems toxicology,” which borrows heavily from “systems biology,” i.e., attempts to model the (patho)physiology of the body with computational tools. It is proposed here that we will need such modeling, both to identify putative PoT (to enable their experimental validation) and, ultimately, to make sense of the data, as well as to make predictions about the effect of a substance in humans.

1 Drivers for change

Toxicology testing is likely to witness a significant influx of new testing methodologies over the next few years, driven in part by ever increasing concerns regarding the impact of long-term exposure to a wide range of chemical and biological substances, and in part by the need to gain a deeper understanding of the

¹ <http://epa.gov/nct/toxcast/>



mechanisms by which these substances lead to toxicity (Hartung 2009ab, 2010, 2011). It is worth noting that a recent consensus workshop (Basketter et al., 2012) placed PoT-based approaches, together with integrated testing strategies, in the foreground of a roadmap for future systemic toxicity assessments.

Traditional *in vivo* tests and *in vitro* assays typically provide only limited mechanistic information, while new comprehensive screening studies are underway that aim to reveal the interactions of chemicals with biochemical pathways that control cell function, communication, and adaptation to environmental changes. They are based on a broad biological (initially) phenotypic profiling by either omics or high throughput screening (HTS) technologies. The latter requires large batteries of tests to characterize an individual substance, as each and every one reflects only a limited amount of information regarding biological characteristics. Therefore, as a tool to profile substances, HTS is either restricted to larger screening programs such as ToxCast or Tox-21 or to providing a comparison to a set of substances that manifest a property earlier identified as predictive. It is evident that chemicals can interact with these pathways, thereby altering their normal functions leading to toxicity and disease. The fundamental question, however, is whether the ability to analyze interactions at the pathway level can enable the prediction of adverse outcomes at the organism level. This will require first identifying which PoT are “involved” and then separating these from the noise of physiological variation and other (parallel and secondary) cellular responses to the substance studied. This raises the possibility of a qualitative hazard identification, whereby the analysis of the dose-response characteristics of the perturbations of these pathways, together with *in vitro*-to-*in vivo* extrapolation of the pharmacokinetics (Wetmore et al., 2011), could ultimately provide the basis for a so called “pathway-based hazard assessment.”

The National Academy of Sciences publication, *Toxicity Testing in the 21st Century: A Vision and a Strategy* (NRC, 2007), proposes a paradigm shift in toxicology from current animal-based testing towards the application of emerging technologies, including genomic, metabolomic, and proteomic approaches, as well as systems biology. This new paradigm would provide greater mechanistic insight into the ways in which many compounds, including pharmaceuticals, affect human health – an approach that has been advocated by many in the field for some time (Goldberg, 1983; Balls, 1998; Hartung, 2001; Coecke et al., 2007).

Public concern for risks associated with many consumer products is accelerating the adoption of new regulations that increasingly require new methods of testing. Demand for toxicological information on chemicals now exceeds the ability to produce relevant data based on the current regulatory animal tests. At present, there are about 80,000 chemicals on the market, and each year about 2,000 new chemicals are introduced, for which there is limited or no adequate toxicological information (US EPA, 1998a,b; Bakand et al., 2005; Grandjean and Landrigan, 2006; Rand, 2010). In the European Union, REACH (registration, evaluation, authorization, and restriction of chemicals) legislation (EC, 2006) aims to test 30,000 chemicals produced in quantities of more than one ton per year,

for which no adequate information exists on their toxicological properties. Toxicologists and regulators are still debating the expected costs and animal numbers required for the REACH program. The latest estimates show much higher costs and vertebrate animal numbers than initially expected (assuming the current requirements are followed), leading to concerns about the feasibility and ethics of such a large amount of *in vivo* toxicity testing (Rovida and Hartung, 2009). It is clear that the traditional approach does not accommodate the broad old and new testing needs. In order to reduce time, costs, and animal numbers, REACH foresees increased use of *in vitro* and *in silico* testing methods. However, such methods have been accepted only for the less demanding acute and topical toxicities, which represent a very small portion of test needs. It is clear that simple *in vitro* test systems or *in silico* approaches will not be able to reflect the complexity of the organism and its derangement by substances. The answers to this dilemma currently are addressed by three approaches:

- 1) Reflect the complexity of the organism in the *in vitro* test system, i.e., the current attempts to create a “human on a chip” with a number of 3-dimensional organotypic organ models combined with microfluidics, as promoted most recently through large scale funding programs in the United States (more than \$ 200 million available from NIH, FDA, DARPA, and DTRA).
- 2) Develop combinations of information sources in integrated testing strategies (ITS) that together map the hazard of interest. Here, the complementary nature of information sources and their integration represent a challenge. A notable example is the test battery developed in the European Commission Framework 7 funded Integrated Project, ReProTect, which showed promising early feasibility (Schenk et al., 2010). While the combination of tests here was still very simple, pioneering work shows the potential of data integration, e.g., by Bayesian networks (Jaworska and Hoffmann, 2010; Jaworska et al., 2011). The components of ITS typically are on the level of complex cellular functions assessed by relatively crude endpoints. This does not exclude the use of PoT-based assays, but an approach based solely on PoT information, as discussed here, moves the challenge to another level that likely will require increasingly specific ways of interrogating the biological systems’ response to pre-defined substances.
- 3) Molecularly defined PoT for mapping the Human Toxome, thus transforming assays from black boxes of complex endpoints to defined PoT.

There is a continuum between approaches (2) and (3): PoT-based assays can contribute to ITS and pure PoT-based systems will require integration. The distinction aimed for is that, in its current state, ITS typically uses the resolution of a mode of action, informed by cell physiology or affected cell function rather than molecularly defined PoT. The unclear terminology of “mode of action,” “mechanism of toxicity,” “molecular toxicology,” “adverse outcome pathway,” “pathway of toxicity,” etc. contributes to such confusion. It is suggested that the term PoT should be reserved for “molecularly defined descriptors of pathways of substance interference with biological systems.”

Note that, while we are addressing mainly PoT here, i.e., the molecular mechanisms of hazard manifestation, other aspects such as toxicokinetics (Basketter et al., 2012), exposure, etc. (Wetmore et al., 2012) need to be integrated in order to make them useful for risk assessment. However, this might not hold true where hazard can be excluded (or made sufficiently unlikely). As discussed earlier, (Hartung 2010b, Hartung and McBride, 2011) the beauty of a molecularly defined approach is that the perturbation of crucial PoT might be excluded, omitting any further assessment of the given hazard. This is in strong contrast to current practice, where an arbitrary effect level is established in high-dose experiments to apply safety factors and define use conditions. It should be clear that such practice leaves the impression that all chemicals are dangerous and that only restricted exposure keeps us safe. This impression, in turn, results in the well known chemophobia of the general public.

2 An integrated post-genomic approach to pathways of toxicity

Sequencing of the genome, annotation of genes, and gene chip developments, especially for transcriptomics, clearly have been the starting point for elucidation of toxic effects. Multi-omics research, including data integration and modeling, using both *in vivo* and cell-based assays, is beginning to gain traction as a viable approach in the development of a pathways-based mech-

anistic view of chemically induced bioactivity (Evans, 2000; Aardema and MacGregor, 2002; Craig et al., 2006; Parman et al., 2011). For instance, both medium- and high-throughput genomics, proteomics, and metabolomics measurements have been applied to identify and to understand molecular and biochemical pathways that control homeostasis (Heijne et al., 2005). It appears that either the early changes in response to a substance or the new homeostasis established after compensation for this interaction qualifies for analysis, while the dynamics of adapting to exposure require a high level of resolution and an even greater number of processes to dissect. Figure 1 shows an adaptation of a classical figure by Hans Seyle² (Seyle, 1956), who introduced the concept that a system under stress aims to establish a new homeostasis until exhausted. Translating this to a biological system stressed by exposure to chemicals, we see the chemico-biological interaction engaging PoT. Then, by PoD and other mechanisms (EpiP), a new homeostasis is established, with a deranged physiology. This holds true especially for high dose effects, where many derangements are to be expected. Note that this is the opposite of the high-dose/concentration approaches typical in toxicology, but it is likely to be far more relevant to the low-dose exposures of real life. The hypothesis is put forward that initial perturbations, when passing the no-effect levels by increasing concentrations of substances, will reflect the key PoT. However, it is possible that even lower concentrations are of interest, as effects might just be hidden by the compensatory capacity of the cell (PoD), while the PoT may already be at work. It will need to be shown whether sub-adversity levels of

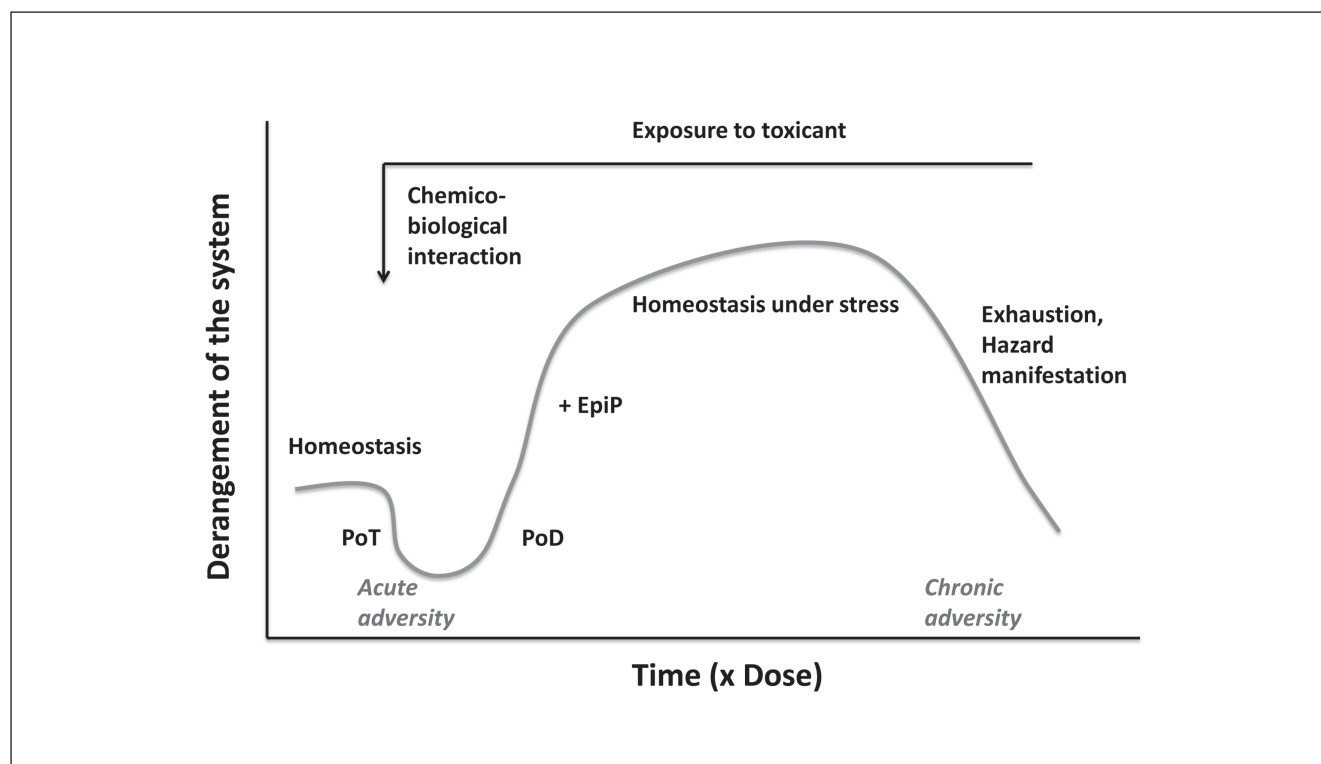


Fig. 1: System under stress aims to establish a new homeostasis until exhausted (Seyle, 1956)

² We would like to thank Dr Drew Ekman, EPA, for bringing this to our attention.



exposure qualify for PoT dissection. Certainly, when measuring at the new plateau established under stress homeostasis, it will be easier to determine the derangement of physiology but more difficult to deduce the underlying PoT.

Bioinformatics enables visualization of these pathways, as well as modeling of the system's behavior in response to, for example, chemical exposure. The lack of availability of adequate bioinformatics tools is a current bottleneck to fully integrating this information, especially from different platform technologies. However, new open source webtools (GeneNetwork³), as well as commercial solutions (Figure 2) for multi-omics data integration, are now becoming available and should facilitate the elucidation of pathways of toxicity.

A closer look into the different omics technologies as data-sources for systems toxicology is warranted (for more details see (van Vliet, 2011)).

3 Transcriptomics

Gene expression can rapidly change in response to chemical exposure, thereby providing a sensitive endpoint for toxicity. In addition, advances in microarray technology now include the

capability to survey the entire transcriptome, thus providing a global evaluation of cellular changes. The technology also has become more reliable and reproducible. Small changes in gene expression now can be reliably detected (Shippy et al., 2006), and extensive validation studies performed by the MicroArray Quality Control (MAQC) project demonstrated consistent results across multiple platforms and research sites (Shi et al., 2006). Under the label of toxicogenomics, new approaches increasingly are suggested for regulatory use concerning both health (MacGregor, 2003; Waters and Fostel, 2004; Boverhof and Zacharewski, 2005) and environmental effects (Ankley et al., 2006).

For systems toxicology applications, transcriptomic measures typically are performed across both dose and time. The dose-response characteristics are important for inferring dose-dependent transitions in what pathways are perturbed, while the time course measurements provide a better understanding of the pathways involved in the temporal transitions from the molecular initiating event to secondary and tertiary responses (Andersen et al., 2010). For chemicals or their metabolites that interact weakly with cellular macromolecules, multiple pathways may initially be perturbed, leading to a broader activation of pathways involved in the stress response, cell death by ap-

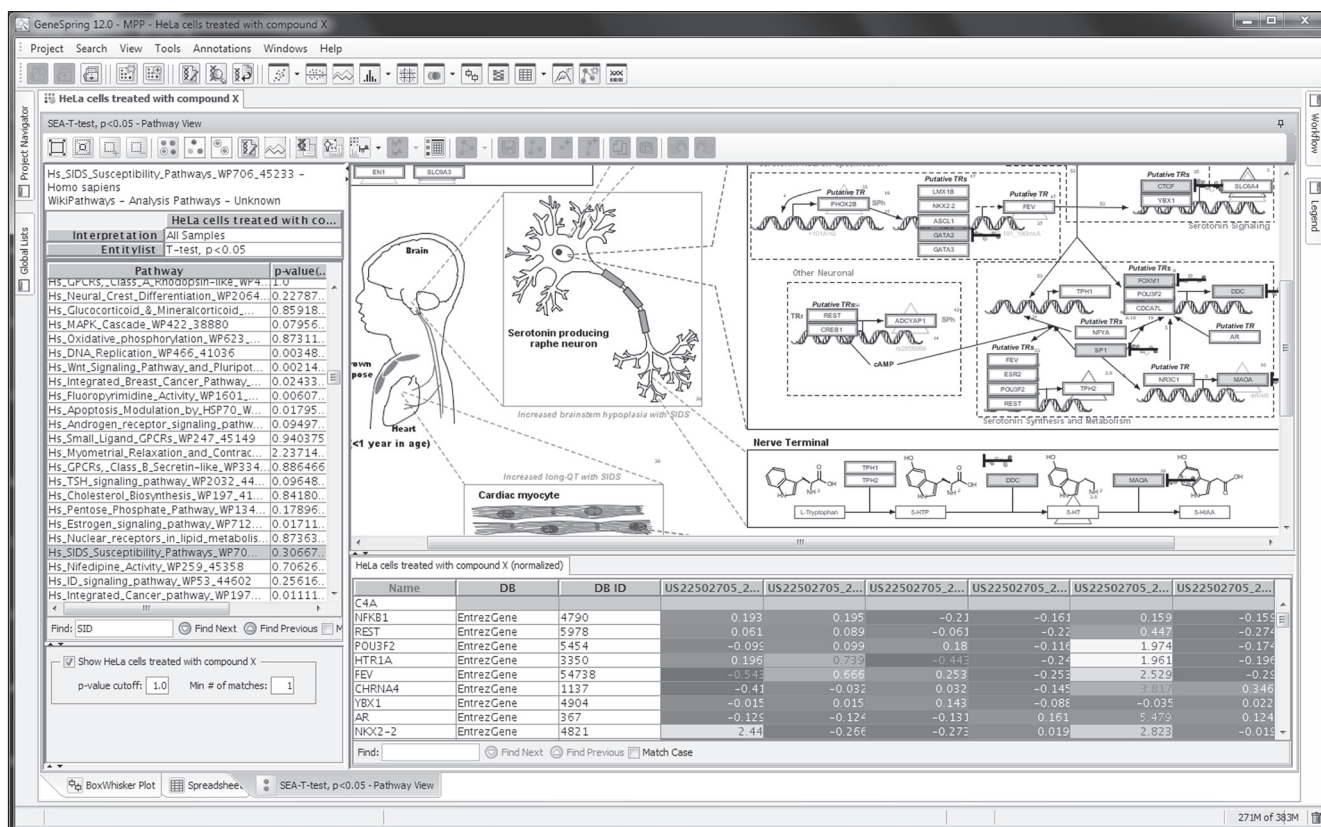


Fig. 2: Screen capture of the GeneSpring-Integrated Biology software platform (Agilent Technologies, Santa Clara, CA) showing the results of co-analysis of differential gene expression and metabolomics data

GeneSpring-IB is configurable for various multi-omics data analysis combinations. It imports raw data, performs a variety of statistical analyses, conducts pathway analyses, integration, and visualization.

³ www.genenetwork.org/webqtl/main.py

optosis or necrosis, inflammation, and tissue remodeling. For chemicals that interact with higher affinity to a single cellular macromolecule, a single pathway would be perturbed initially, followed by downstream activation of secondary and tertiary pathways that underlie the adverse response. In either case, a systems-level analysis provides an understanding of the key events in the mode-of-action that ultimately leads to toxicity. Transcriptomic studies often are complemented by proteomic and metabolomic approaches in order to functionally validate the observed changes in the transcriptome.

4 Proteomics

Proteomics studies in the field of toxicology have focused mainly on identifying biomarkers and refining a mechanistic understanding of molecular mechanisms of toxicity (Kennedy, 2002). The pharmaceutical industry has performed a large number of studies to identify biomarkers for liver and kidney toxicity (Wetmore and Merrick, 2004). Protein profiling in human blood and urine samples is particularly promising for the prediction of human toxicity (Thongboonkerd, 2008). Currently, the human blood plasma proteome is being mapped to gain more insight into disease and toxicity (Omenn et al., 2009). Proteomics analysis is increasingly applied *in vitro*, including applications based on stem cells (van Hoof et al., 2008), embryotoxicity in embryonic stem cells (Osman et al., 2010), and mechanisms of toxicity in primary cell cultures (Vendrell et al., 2010).

In vitro proteomics is proving to be a valuable method of identifying protein biomarkers for mechanisms of toxicity (e.g., oxidative stress, cell death, or energy metabolism). Proteomics is still less standardized than transcriptomics or metabolomics, however, and thus is more difficult to apply for PoT identification. The sensitivity of proteomics methods for identification of single proteins is lower than immunohistochemistry, enzyme-linked immunosorbent assay (ELISA) or Western blotting (Heijne et al., 2005). However, in contrast to those methods, proteomics allows the detection of changes in unexpected and unknown proteins – and often subsequent identification with mass spectrometry (MS). Typically, only water-soluble proteins within a specified mass and isoelectric point range will be analyzed.

5 Metabolomics

The number of metabolites in a biological system is estimated to be a few thousand, which is relatively small compared to the number of genes and proteins. Metabolomics, therefore, is considered a more approachable methodology than proteomics, particularly in translating results to phenotypic changes. Furthermore, as metabolic changes represent the final outcome of all physiological processes, metabolomics is a relevant approach to study toxicity (Robertson, 2005; Keun, 2006; van Vliet et al., 2008). At the same time, while transcriptomics and

proteomics only indicate possible derangements of physiology, a shift in metabolites indicates actual change in physiologic chemistry.

The principal technologies for metabolomics studies are nuclear magnetic resonance spectroscopy (NMR) and MS. NMR examines the proton spectrum of a sample, which represents a robust and quantitative measurement. Advantages of NMR are that it is non-invasive, requires no metabolite extraction procedure, and allows a relatively easy structural identification of metabolites. MS-based applications have increased over the last years, primarily due to the high sensitivity, specificity, and ability to detect and identify large numbers of metabolites (Dettmer et al., 2007).

In the case of MS, signals are characterized by mass, time of flight (TOF), and, if coupled with chromatography, a retention time. The first step is to break these “features” down into (identified) metabolites. To confirm the identities of metabolites relevant to a specific toxic perturbation, their accurate mass, retention time parameters, or NMR spectra can be compared to a database with annotated metabolites, such as METLIN (Smith et al., 2005), that contains thousands of metabolites. Identities of metabolites not annotated in a database can be confirmed by MS-MS fragmentation. Since this is quite laborious for metabolites not yet annotated in a database, it is useful to restrict the analysis to metabolites of relevance to the biological effect. This can be done, for example, by principal component analysis (PCA), identifying the most interesting signals correlated with the biological effects of interest. This means that signals correlating with treatment, concentrations used or responses developed over time, are used. At this stage, these principal components can be used to form a signature of toxicity (SoT), even without identification of the substance. The metabolites of interest then can be detected and their concentrations accurately quantified in future experiments. The metabolite signals representing their concentrations can be used to identify biomarkers and metabolic pathways, or to develop systems biology models (Lanza et al., 2010).

Statistical data analysis compares the different spectra to find significant differences in metabolite patterns and intensities, revealing clustering patterns that show differences or similarities between samples. Identification of the metabolites responsible for the clustering can provide metabolic fingerprints (SoT) useful in classifying compounds into toxicity classes or acting as biomarkers of toxicity. The latest software packages control the complete metabolomics process, including data detection, normalization, statistical analysis, metabolite identification, database search, and pathway identification.

Metabolomics toxicity studies have been performed both *in vivo* and *in vitro*. For example, an LC-MS based footprinting approach was used to investigate Valproate toxicity in human embryonic stem cells (Cezar et al., 2007). Using the same footprinting approach, several teratogens were tested in human embryonic stem cells (hES) to identify biomarkers for developmental toxicity and generate a preliminary prediction model (West et al., 2010). Metabolomics applications in cell cultures have been initiated only recently, and much is ex-



pected from this approach, especially applications in human stem cells. These are seen as highly promising, as they could provide insight into human toxicity pathways (Kleinstreuer et al., 2011).

6 Systems biology and toxicology

The underlying structure of systems biology and toxicology is a network.

The move towards systems toxicology and massively parallel techniques opens new opportunities but, at the same time, raises problems in deriving meaningful information out of the wealth of generated data. Such data is increasingly represented as networks, in which the vertices (e.g., transcripts, proteins, or metabolites) are linked by edges (correlations, interactions, or reactions, respectively). Networks can vary in their functionalities. Some are undirected graphs that enable only the study of structure; others, like the biochemical network, are characterized by interactions of varying strengths, strongly nonlinear dynamics, and saturating response to inputs (Wagner, 1996).

Network analysis has evolved into a very active and interdisciplinary area of research encompassing biology, computer science, social and information sciences. Many studies are highly theoretical, but they may eventually help in identifying SoT and PoT. The network research has three primary goals. First, it aims to understand statistical properties that characterize the network structure in order to suggest appropriate ways to measure these properties. This is very relevant to SoT identification. Second, it aims to create models of networks that can help us understand the meaning of these properties and how they interact with one another. Third, it aims to predict what the behavior of networked systems will be on the basis of measured structural properties. This direction can be very useful in elucidating PoT.

Structural analysis of networks has already led to new insights into biological systems, and it is a helpful method for proposing new hypotheses. Several techniques for such structural analysis exist, such as the analysis of the global network structure, e.g., scale-free networks, network motifs (i.e., small sub-networks that occur significantly more often in the biological network than in random networks), network clustering (modularization of the network into parts) and network centralities. Network centralities are used to rank elements of a network according to a given importance concept (Koschutski and Schreiber, 2008). The resilience of networks to the removal of their vertices is another important structural property. This has been the subject of a good deal of attention in the literature because it is regarded as a very promising method to link network structure to function (Rodrigues et al., 2011).

While the topology of biochemical networks is informative – for example, feedback loops are necessary for oscillatory dy-

namics – topology does not fully describe network behavior. Studies of the effects of structure on system behavior are still in their infancy. It remains to be seen what the crucial theoretical developments will be in this area. Progress on this front has been slower than progress in understanding network structure, perhaps because without a thorough understanding of structure an understanding of the effects of that structure is hard to come by. However, some important advances have been made (Milenkovic and Przulj, 2008).

Biological systems are networked at many levels: hormone regulations, signaling cascades, gene regulation by transcription factors or microRNA, for example. Increasingly, these systems can be modeled dynamically, though often in isolation – and they are difficult to combine. Fortunately, this challenge has been taken up by a broad scientific community in the life sciences, and toxicology can profit from many parallel or pioneering developments. This offers hope that solutions to understanding the interplay of network structure, function, and dynamics will emerge rapidly. Ultimately, virtual cells and organs need to be set up to integrate these networks, their interactions, and, for toxicology, their perturbation by exogenous substances (e.g., the German Virtual Liver Network⁴ or similar activities for liver⁵ and the virtual embryo⁶ at US FDA). Such systems increasingly allow simulations and virtual experiments to be carried out. We have learned that such biological networks typically have critical nodes, which largely reflect the derangement of the network. Often these are the crossings of different pathways, which may enable the simplification of some of the models.

Systems toxicology, with its new datasets and large scale data integration, will enable the exploration of properties of biological systems beyond what is currently possible. It will provide the prospects of investigating natural variation and stochastic effects and their role in defining phenotypes and the transitions between them. Harvard is conducting interesting research on how variation in gene expression patterns influences the development of disease states, arguing that one should move beyond a simple comparison of the means relative to variance (the t-test) to also considering how variance itself changes between phenotypes⁷. The study of inter-individual variability was recognized as a tremendous opportunity to resolve some questions regarding structure-function relationships in the endocrine systems and the functional significance of absolute versus relative changes (William, 2008).

To deliver on this potential, systems toxicology will need tools. There is an urgent need for novel tools for navigating, filtering, aggregating, visualizing, and assessing research content. In taking inspiration from systems biology, we grow more and more appreciative of collaborative, open source tools to accelerate interoperability and to leverage resources available to scientists. Recently, the Opentox computational toxicology platform has been interfaced with Bioclipse. The integra-

⁴ <http://www.virtual-liver.de/>

⁵ http://epa.gov/ncct/virtual_liver/

⁶ <http://epa.gov/ncct/v-Embryo/>

⁷ <http://www.iq.harvard.edu/blog/sss/archives/2012/01/>

tion takes advantage of semantic web technologies, thereby providing an open and simplified communication standard. Additionally, the use of ontologies (see also the contributions by Hardy et al. in this issue of ALTEX) ensures proper inter-operation and reliable integration of toxicity information from both experimental and computational sources (Willighagen et al., 2011).

A number of challenges in modeling biological networks remain:

- a) Few measurements are continuous, but we need dynamic/kinetic modeling; we have only snapshots of the dynamic system, which we need to combine with knowledge on (reaction) kinetics.
- b) Each of these networks has its own set of technologies to monitor them, and they are not necessarily compatible to measure in the same sample at the same time.
- c) Many systems are not completely known or measurable (only some network members can be followed).
- d) Many biological systems have a spatial (e.g., compartmentalization) or temporal (e.g., sequence and timing of events) aspect.
- e) Developmental aspects (establishment, maturation, (de-)differentiation, aging, etc.) take place in the models or represent measures of interest.
- f) Many relevant physiological processes involve interactions of different cell types or tissues, adding layers of complexity to the modeling.
- g) Inter-individual differences affect data acquisition.

h) The multitude of parameters and conditions creates problems of multiple testing, over-fitting, noise/signal ratios.

And this is only to model the physiological process. It does not yet include the interference of substances. The opportunity lies in using the measurement endpoints as input parameters for the systems biology model and simulating the effect on the dynamics of the system. Ideally, this includes points of interaction with the foreign substance and effect data on nodes in the networks.

Figure 3 depicts the larger perspective of moving from empirical approaches, which have given us the substances found to be problematic (reference substances) and the test systems to identify them, to a fully fledged mechanistic approach, as suggested in the vision of Toxicity Testing for the 21st Century. Because they have proven to predict a relevant number of reference substances, these predictive tests qualify for inclusion into ITS, which, for many reasons detailed elsewhere (Basketter et al., 2012), lend themselves to a probabilistic based condensation of information. Omics technologies offer a prime opportunity to refine existing tests and to provide information for ITS via the creation of SoT. The empiric character of SoT is evident. The vision put forward is that, by identifying the underlying PoT, this can be moved to a mechanistic level. Some mechanisms of toxicity (modes of action) have been identified in toxicology from the predictive tests (*in vivo* or *in vitro*) over the last decades. They will add to the array of PoT and confirm those identified in an unsupervised manner from SoT of omics technologies. Additional pathway information

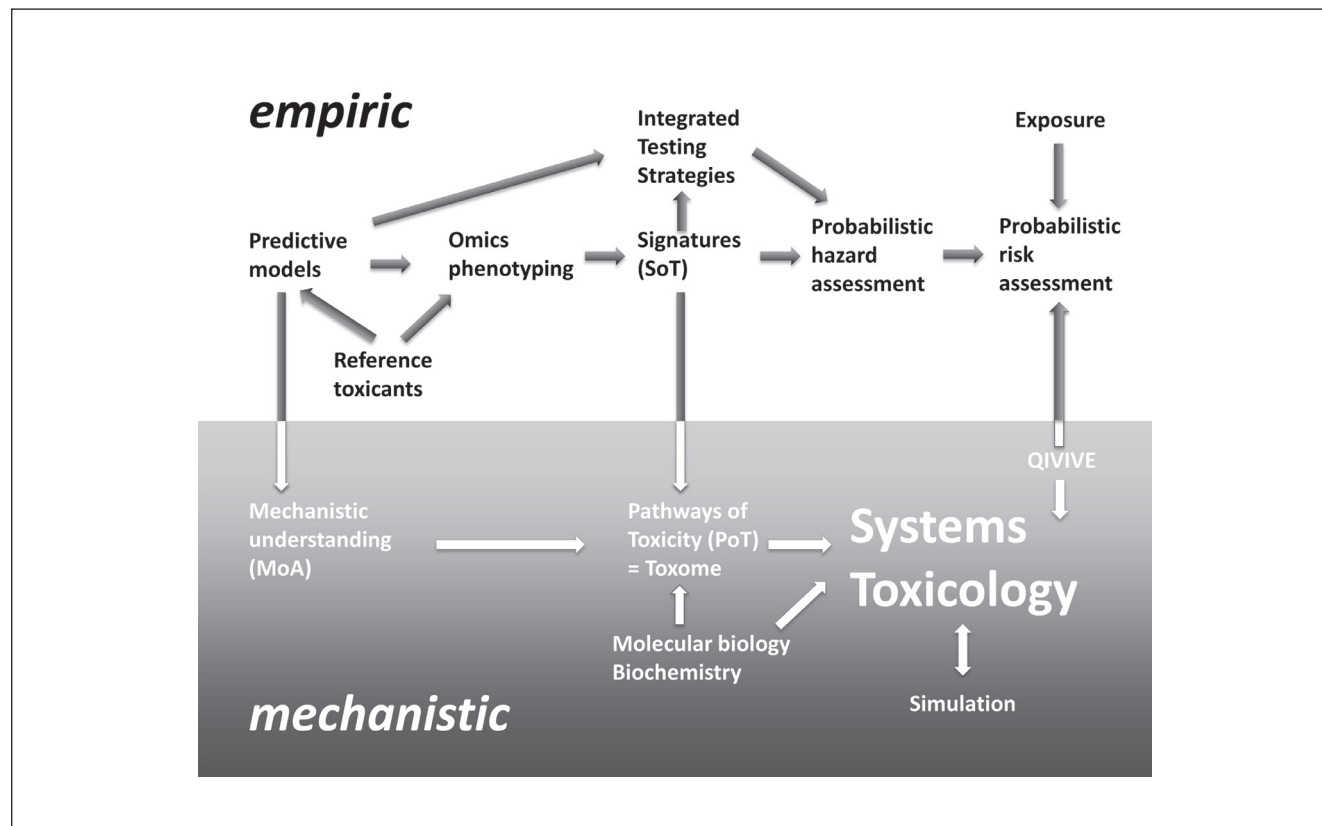


Fig. 3: Move from empirical to mechanistic approach in toxicity testing



comes from biochemistry and molecular biology. Together, these shall form the basis for systems toxicology, which by virtual experiments (simulation) can undergo reality checks, thus moving toward prediction of hazard. Last but not least, the components of quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) and exposure move us to the risk paradigm that ultimately is required.

Systems toxicology is an exciting new prospect on which to base our studies on information-rich methods and bioinformatics, which reflect the dynamics and complexity of physiology. The fact that we have our “disease agent,” i.e., the toxicant, at hand and can induce derangement by varying timing, conditions, concentrations, etc. as often as we want, distinguishes systems toxicology from similar approaches for clinical problems. The question arises: How can such a systems toxicology approach be quality controlled and validated? Again, toxicology is more advanced than other medical fields here, with experience in such quality assurance schemes as Good Laboratory Practice (GLP) and formal validation. Toxicology, therefore, should not wait as a bystander to embrace the systems biology developed in other scientific disciplines but should instead bring its specific opportunities and experiences to the table. This promises, in return, to advance toxicology to a true reflection of human toxicity.

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