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Emerging Toxicity Models from Emerging Scientists

Patrick Allard, Ph.D.[*]

Department of Environmental Health Sciences and Institute for Society and Genetics, University of California, Los Angeles; Los Angeles, CA

Abstract

Toxicology has seen a recent influx of new talents from other fields attracted by the application of their expertise to pressing questions of toxicological and environmental relevance. This transition has opened the door to innovative and exciting scientific opportunities but has also generated a new set of questions and challenges. In this viewpoint article, I will highlight some of the drivers and hurdles encountered by the recent new breeds of toxicologists.

A field at a crossroad: a historical push to develop new toxicity models

Toxicology has seen changes in its practice that, arguably, few other fields have seen in recent years. For the last several decades, toxicologists and regulators have recognized significant limitations inherent to conventional toxicology approaches such as their inadequacy in the face of the large number of compounds: food additives, drugs, pesticides, personal care products and many others, that need toxicity assessment. In the United States alone, approximately 60,000 chemicals with little toxicity data, were declared exempt from further testing when the Toxic Substances Control Act was enacted in 1976 [1]. Concomitantly, mounting pressure from scientists and animal protection groups called for a rethinking of our approach to animal testing. The European Union in particular has seen a tremendous drive towards reduction in animal use which crystalized around the concept of the 3Rs (Replacement, Reduction and Refinement) promoted by Russell and Burch in their seminal 1959 publication [2]. Several other acts followed such as the Cosmetic directive of 1976 [3] subsequently supplanted by the Cosmetic regulation of 2013 which paved the way for the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and its requirement to test a substance only once in animals and only if an alternative test cannot be used [4]. In the United States, a roadmap for the field was proposed in 2007 in the National Academy of Science's report "Toxicity testing in the 21st century: a vision and a strategy" which highlighted in part the need for the development of novel toxicity strategies for faster, cheaper and relevant toxicity assessment [5].

These pressures on toxicologists came at a time when significant progress in cellular and molecular biology and other biological sub-specialties were made. With the advent of the 'Omics era, high throughput technologies and of systems biology, scientists could move

[*]Dr Patrick Allard earned his Ph.D. in Biology from McGill University and completed his post-doctoral fellowship in Genetics and Developmental Biology at Harvard Medical School. Since 2008, Dr Allard has applied his expertise to the design of novel genetic assays to capture reproductive and multigenerational toxicity effects.

beyond a case-by-case approach and work both on a comprehensive and mechanistically detailed level. These advances were particularly appealing to toxicologists who could begin to approach the number of chemicals to test but also provide insight on their mode of action while potentially extracting toxicity rules based on chemical structure as well as test positivity. Thus, while historically toxicologists came into the field from biology, chemistry and pharmacology, today's toxicologists often emerge from fields as diverse as genetics, engineering, mathematics and computer sciences. For many however, this transition is not without its challenges.

A field of opportunities… and hurdles

At first, mobilizing scientific and technological advances from other fields can appear as a straightforward way to move the field Toxicology into the 21st century. Decades of research on organisms such as yeast, *C. elegans*, Drosophila or Zebrafish have provided a deep understanding of their biology which serves as a solid foundation to the genetic and molecular tools that can be applied to toxicological questions. Furthermore, toxicity assays in small animal models allow the detection of chemicals' impact on intricate cellular and developmental processes while working in a setting comparable to *in vitro* assays such as 96-well or even 384-well plates for *C. elegans*. Thus, the advantages of assays designed around the tools available in these organisms are tremendous. For instance, by taking advantage of their small size and external development, toxicity screens in Zebrafish can rapidly assess the teratogenicity of a large number of compounds over a wide range of concentrations [6]. In *C. elegans*, an assay relying on the expression of a GFP-tagged transgene following chromosome segregation errors during meiosis allows the automated, large scale detection of chemicals that are germ cell toxicants [7]. In both cases, the complexity of the endpoints measured would make the screening of a high number of chemicals in conventional rodent tests tedious, expensive and unethical, thus highlighting the need for the development of alternative toxicity models. Despite these significant advantages, these new models have to take into account the issue of relevancy. Despite a relatively high degree of genetic conservation, the pharmacokinetic parameters are expected to be substantially different between alternative organisms and mammalian models. This matter is even more salient when considering that rodent models also show partial predictivity towards human endpoints. This was famously illustrated by the case of thalidomide for which rodent models show almost complete insensitivity but triggers severe limb, otic, ocular and heart teratogenicity in humans [8]. Thus, several factors can hinder the transition of new talents working on bringing new models into the field: (1) the perceived lack of relevance of an evolutionary distant model organism coupled with (2) an incomplete understanding from established toxicologists of what these new models can offer and (3) the need for these emerging scientists to rebuild stature and gain recognition from their (new) peers as they move into a different field.

Not all emerging toxicity models face the same obstacles however. For instance, recent discoveries in stem cell and cell culture biology have opened new and exciting avenues. An increasing number of cell types can be generated *in vitro* from cells of murine or even human origin circumventing issues of evolutionary conservation. Furthermore, these differentiated cells can be co-cultured with other cell types in a 3D matrix with the aim of

approximating a functioning organ. The issue of metabolisms differences can also be minimized by exposing the cells to the parent compounds as well as to their known metabolites. But stem cell approaches also have limitations that are inherent to their derivation. Stem cells culture is expensive as it requires the addition of growth factors to promote differentiation. Furthermore, the production of some of cell types can be inefficient (low yield), complex and lengthy. For example, hepatocyte-like cells can be derived in vitro but require a 25-day long procedure [9]. Mature germ cells can be produced but only by transplanting *in vitro* differentiated primordial germ cells into a live animal's gonad and following their development for more than 4 weeks[10]. Finally, there is also the concern that despite the proper expression of differentiation markers specific to the cell type of interest, these *in vitro* differentiated cells may not behave as their *in vivo* counterparts.

While each model carries both clear strengths and obvious caveats, they allow the fast inquiry of toxicity pathways in a way that was not conceivable just a few decades ago. This has led to the generation of toxicity data at a pace never before seen. Thus the advent of high throughput technologies also created two interconnected issues: (1) being able to handle and make sense of the tremendous amount of data generated by the multitude of assays within the context of the particular model used but also (2) be able to extend the meaning of the results to human endpoints. Although elegant examples of alternative model organisms toxicity data directly informing epidemiological studies have been published [11], on a broader scale, much work is required to confidently interpret the outcome of diverse toxicity assays towards human endpoints. This is a crucial area where new talents are particularly needed, especially around the concept of "Adverse Outcome Pathway" which provides a conceptual framework bridging several levels of biological organization from the initiating molecular event to the adverse outcome and have great potential as tools for human risk assessment[12].

Converging on an answer

The answer to some of the issues facing transitioning talents may be found in yet another new breed of toxicologists. While "toxico-geneticists" and "toxico-high throughput biologists" are needed to develop innovative toxicity approaches, computational toxicologists are also necessary to manage and interpret the vast amount of toxicity data generated and build predictivity models for human endpoints. In the United States, a significant effort in that direction has been made by the Environmental Protection Agency's National Center for Computational Toxicology and their Toxicity Forecaster (ToxCast) program which aims at prioritizing chemicals and reducing the number of animal-based toxicity tests [13]. The promises of computational sciences for the field are vast. Computational approaches do not have to be confined to the interpretation and extrapolation of data from a single model. Instead they can integrate numerous alternative toxicity models into a powerful predictivity analysis that weighs the strengths and weaknesses of each model for the endpoints of interest. With an inclusive take on toxicity models, endpoints that were seemingly unapproachable on a large scale basis, female reproductive or multigenerational endpoints for instance, can now be investigated through emerging toxicity methods and be incorporated in a detailed and comprehensive picture of chemical toxicity. And this is where

the utility of new talents in toxicology and the relevance of emerging toxicity models truly reside.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. United States Congress. Toxic Substances Control Act. 1976. 15 U.S.C. §2601 et seq
2. Russell, WMS.; Burch, RL. Methuen; London: 1959. The Principles of Humane Experimental Technique. ISBN 0900767782
3. Council Directive 76/768/EEC of 27 July (1976) on the approximation of the laws of the Member States relating to cosmetic products
4. Regulation (EC) No 1907 (2006) of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency
5. National Research Council Toxicity Testing in the 21st Century. A Vision and a Strategy. The National Academies Press. 2007
6. Padilla S, Corum D, Padnos B, Hunter DL, Beam A, Houck KA, Sipes N, Kleinstreuer N, Knudsen T, Dix DJ, Reif DM. Zebrafish developmental screening of the ToxCast™ Phase I chemical library. Reprod Toxicol. Apr; 2012 33(2):174–87. [PubMed: 22182468]
7. Allard P, Kleinstreuer NC, Knudsen TB, Colaiácovo MP. A *C. elegans* screening platform for the rapid assessment of chemical disruption of germline function. Environ Health Perspect. Jun; 2013 121(6):717–24. [PubMed: 23603051]
8. Vargesson N. Thalidomide-induced limb defects: resolving a 50-year-old puzzle. Bioessays. Dec; 2009 31(12):1327–36. doi: 10.1002/bies.200900103. [PubMed: 19921660]
9. Hannan NRF, Segeritz CP, Touboul T, Vallier L. Production of hepatocyte-like cells from human pluripotent stem cells. Nature Protocols. 2013; 8:430–437. doi:10.1038/nprot.2012.153.
10. Hayashi K, Ogushi S, Kurimoto K, Shimamoto S, Ohta H, Saitou M. Offspring from oocytes derived from in vitro primordial germ cell-like cells in mice. Science. Nov 16; 2012 338(6109): 971–5. doi: 10.1126/science.1226889. [PubMed: 23042295]
11. Fitzmaurice AG, Rhodes SL, Lulla A, Murphy NP, Lam HA, O'Donnell KC, Barnhill L, Casida JE, Cockburn M, Sagasti A, Stahl MC, Maidment NT, Ritz B, Bronstein JM. Aldehyde dehydrogenase inhibition as a pathogenic mechanism in Parkinson disease. Proc Natl Acad Sci U S A. Jan 8; 2013 110(2):636–41. [PubMed: 23267077]
12. Vinken M. The adverse outcome pathway concept: a pragmatic tool in toxicology. Toxicology. Oct 4.2013 312:158–65. doi: 10.1016/j.tox.2013.08.011. Epub 2013 Aug 23. [PubMed: 23978457]
13. Dix DJ, Houck KA, Martin MT, Richard AM, Setzer RW, Kavlock RJ. The ToxCast program for prioritizing toxicity testing of environmental chemicals. Toxicol Sci. Jan; 2007 95(1):5–12. [PubMed: 16963515]