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Commentary on “Toxicity Testing in the 21st Century: A vision and a Strategy”

Robert F Phalen

Abstract

Toxicity Testing in the 21st Century: A Vision and a Strategy (NRC, 2007) presents a bold plan for chemical toxicity testing that replaces whole-animal tests with cell-culture, genetic, other *in-vitro* techniques, computational methods, and human monitoring. Although the proposed vision is eloquently described, and recent advances in *in-vitro* and *in-silico* methods are impressive, it is difficult to believe that replacing *in-vitro* testing is either practical or wise. It is not clear that the toxicity-related events that occur in whole animals can be adequately replicated using the proposed methods. Protecting public health is a serious endeavor that should not be limited by denying animal testing. Toxicologists and regulators are encouraged to read the report, carefully consider its implications, and share their thoughts. The vision is far too important to ignore.

This reviewer writes from a broad perspective, having served 14 years on the Institutional Review Boards for both human and animal research at a major research university and medical center, and having performed basic and applied inhalation toxicology research involving several species of laboratory animals, cell systems, and computer models for 30 years. This experience has clearly demonstrated the intimate relationship between laboratory animal studies and human studies, as well as the importance of having the very best possible toxicology data available for regulatory and public health purposes. Upfront, two aspects of the NRC Committee (The Committee on Toxicity Testing and Assessment of Environmental Agents) report¹ appear to be troublesome: (1) placing a high value on reducing the use of animals and (2) pressing for cutting the cost and time involved in regulatory toxicity testing. Neither of these goals seems to be compatible with improving the value of toxicity assessment of chemical agents. In summary, the report proposes a future for regulatory toxicology (the ‘vision’) that involves replacing whole-animal studies with a combination of cell culture, genetic and other *in vitro* methods, computer models, and poorly specified human monitoring. Certain response pathways in cells, termed ‘toxicity pathways,’ are to be the focus of *in vitro* testing. These pathways will be used to predict diseases with the aid of a generation of

emerging computational models. The report appears to have been overly influenced by pressure to discourage animal studies, despite their proven utility. Also, the vision may well increase the cost of regulatory toxicology assessments by possibly requiring vast amounts of new data using unproven methods. However, the report describes important new emerging technologies that can augment current testing approaches. Incorporating such new technologies in toxicity testing is well defended in the report. Still, the vision is not adequately defended as (1) being necessary and/or feasible, (2) leading to improvements in the protection of human and nonhuman animal health, and (3) being cost effective. The current rapid evolution of toxicity testing seems to be going well, so it may be premature to consider the proposed new master plan. A proposed long-term goal, to eventually replace *in vivo* testing with *in vitro* testing, appears to this reviewer to be unwise, and possibly fatally flawed. The report does acknowledge that novel classes of agents, such as those associated with

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nanomaterials and biotechnology products, will require maintaining 'some whole-animal tests into the foreseeable future' (NRC, 2007, p. 47).¹

Toxicologists and other informed readers should be able to follow the NRC Committee's logic, description of the vision, and the key scientific issues and details with ease. Practicing toxicologists should examine this report, as it is likely to have an impact on influential parties that affect future funding opportunities and establish requirements for regulatory data. A good place to start reading the report is the Appendix, which presents valuable biographic sketches of the report's 22 authors. Knowledge of the training, experience, and current pursuits of the Committee members will help the reader to understand the strengths (and weaknesses) of the report. The report was sponsored by the U.S. Environmental Protection Agency via a contract with the National Academy of Sciences, but the conclusions and recommendations are those of the authors and do not necessarily reflect those of the involved agencies.

In spite of its problems, the report makes interesting reading, and it has several strengths. It eloquently presents a case for augmenting toxicity testing by exploiting many of the new and impressive developments in genetics, cell biology, and physiologic modeling: Developments that, no doubt, are destined to add greatly to understanding the actions of chemical toxicants and significantly contribute to protecting animal, human, and ecosystem health. The vision for the future of toxicology relies heavily on the extensive availability human, and transgenic laboratory animal cell lines, and to the credit of the report, the inherent artificial nature of cells in culture is acknowledged. Cultured cells do not have the complex realistic chemical environment that is dynamically provided by the whole animal, and which both modifies and responds to the cell's status. Unfortunately, a solid solution to this severe limitation of cell cultures is not provided by the Committee. The additional problem of extrapolating from subcellular and cellular scales of biological complexity to the whole organism scale (including normal functioning and disease states) is acknowledged, but also not critically evaluated. Also acknowledged is the current inability of *in vitro* assays, including those other than cell cultures, to mirror the complex metabolic environment in the integrated whole animal. Validation of the vision and the need for new animal and human studies for such validation of the *in vitro* assays are discussed. However, the extent and exact nature of such new whole

animal and human studies are not well described. The emerging *in vitro* tools for toxicologists and their promise are more clearly described by experts on the Committee than are the limitations and challenges involved in adapting these tools to chemical toxicity testing for regulatory use. The report discusses implementation of the vision, including many of the research needs, needed perceptual changes (by scientists, regulators, legislators, industry and the public), very substantial institutional changes, and cost requirements for (a) improving and (b) adapting the emerging new tools to regulatory needs.

The weaknesses of the report's vision for the future of toxicity testing are substantial, and this reviewer believes that many of these weaknesses may be insurmountable. A few examples will be described here. First, the apparent assumption that disease processes in complex whole mammals can, even in theory, be understood without extensive on-going whole-animal research seems to be seriously flawed. The Committee proposes identifying key toxicity pathways in cells, which can be used to adequately predict whole-animal responses to chemical-agent exposures. Such a bottom-up reductionist approach is not even very successful in the physical sciences, let alone the biological sciences.² Whole animals are fundamentally different and behave in more complex manners than can reliably be predicted from data in cells, even when kinetic models are used in order to extrapolate the data. Whole animals respond to stress in many ways including hormone secretion, changes in cell replication, changes in metabolism, etc. The current system of integrated *in vitro*, *in vivo*, and *in silico* laboratory approaches complemented by appropriate epidemiologic and clinical data has evolved to be remarkably effective for protecting health. The current system, based on the experience and insight of tens of thousands of scientists, works well, and it does not need to be largely replaced by unproven methods. Countless potentially hazardous chemical agents have been dropped from development programs, and even withdrawn from use, on the basis of either *in vivo* or *in vitro* testing: To drop, or even substantially limit, the *in vivo* tests could be a serious mistake. An intact mammal consists of about 100 trillion cells of about 200 distinct types that are highly coordinated and interdependent. Most, if not all diseases, involve the participation of numerous cell types, significant modifications of chemical environments throughout the body, countless adaptive mechanisms, and the eventual failure of corrective physiological mechanisms. It does not seem to be cost effective to

maintain and use sufficient numbers of cultures, of preferably human cells, for all of the relevant types involved in the important diseases. To attempt to duplicate this complexity and integration using cell cultures and computer models may never be possible. A well-designed whole-animal study, by contrast, includes all of the cell types and all of the countless interactions among cell types, tissues, organs, and organ systems. Consider a chemical that must be tested in 100 cell types, each with 50 potential toxic pathways, with 50 different modulating hormones and other internal environmental factors, with 5 genetic variations for each cell type, at 3 doses of the tested chemical, and for 3 exposure durations. One must hypothetically set up, use, and evaluate about 100 million separate cell culture tests, which, if even possible, could be enormously time consuming and costly. Superior information might be efficiently obtained from the study of just 300 mice. Interestingly, the report mentions in several places that validation of the cell-level studies will actually require conducting new animal studies. The number of such studies could be enormous, generating a new parallel realm of animal usage.

As previously mentioned, *in vitro* techniques are inherently artificial, as the dynamic physiological environment of the body cannot be replicated outside of the intact living body. Consider the testing of mixtures in cell cultures, which is at best a formidable task, and at worst not manageable. Mixtures not only interact chemically at many points but they also often trigger varied physiologic defensive mechanisms that lead to currently unpredictable whole-animal responses. The testing of many types of mixtures very clearly requires the use of laboratory animals. Also, it may not be possible to detect false-positive and false-negative toxicity results for many chemical agents within the limits and constraints of the vision. Therefore, one could expect many promising and/or useful chemicals to be prohibited or withdrawn from use, and unacceptably toxic ones to be put into widespread use by regulators who do not have access to sufficient *in vivo* data.

To illustrate another problem, consider an aerosol consisting of a broad size distribution of nanosilver particles plus an antibiotic or a pesticide. It is not possible now, or in the foreseeable future, to evaluate the effects of such an aerosol without extensive inhalation studies.³ The initial detailed pattern of deposition in the respiratory tract, and the subsequent post-deposition phenomena are so complex as to not be currently predictable. Some portion of the

deposited material may travel directly to the brain via the olfactory nerve,^{4,5} a poorly understood pathway that is not yet included in the existing computer models. Whole-animal studies are most likely essential for studying many of the future complex, multicomponent and engineered nanomaterials that have unknown distribution in the body, and subsequent potentially widespread effects. This example is but one of many that seem to reach beyond the limits of the Committee's vision for the future of regulatory toxicity testing.

Another problem with the vision can be understood by reference to the Nuremberg Code (<http://ohsr.od.nih.gov/guidelines/nuremberg.html>), which describes the criteria that were developed after World War II for defining crimes against humanity. The Nuremberg Code was used during the 1940s trials of Nazi scientists who performed human experimentation. Item number 3 of the Code states that 'The experiment should be so designed and based on the results of animal experimentation and a knowledge of the natural history of the disease or other problem under study that the anticipated results will justify performance of the experiment.' This clearly prohibits (on ethical grounds) intentionally exposing humans to potentially toxic chemicals prior to performing sufficient animal studies. The logic behind this aspect of the code is that all possible adverse events must be explored in whole-animal studies prior to permitting human exposures. It should be understood that laboratory animal studies are conducted only when justified as determined by an ethics review committee, and even then only when using means to prevent unnecessary suffering as well as the use of excess numbers of animals.⁶

The last chapter of the report (Chapter 6), covering 'Prerequisites for Implementing the Vision in Regulatory Contexts,' 'anticipates continual change over the next 2-3 decades.' Such change includes: 'far reaching shifts in orientation and perception ...'; 'congressional funding of agencies to implement the vision, ...'; 'large expenditures of money ...' (possibly more than hundreds of millions of dollars); and the development of test methods that 'are in early stages of development ...' and 'others that will be used eventually (that) are not yet on the drawing board or even imagined.' In practical terms, implementation of the proposed vision may not be affordable or feasible, especially for those test methods that are not 'even imagined.'

To conclude, the report is certainly worthy of being examined and contemplated by all interested parties.

Each reader can assess the value and feasibility of the vision on the basis of their own experience and understanding of toxicity testing and emerging regulatory needs. This reviewer is convinced that the report's vision is interesting and of value, but that it is seriously flawed. Perhaps pressure to eventually eliminate all animal research has contributed to the flaws. However, it is clear that many of the new approaches that are described will eventually play significant roles in improving decisions (including regulatory ones) regarding the potential risks of chemical substances. It seems rational to consider the vision as an addition to, rather than a substantial replacement for, the methods by which chemical substances are evaluated for their potential toxic effects.

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