



The Promise and Challenges of 21st Century Toxicology

by Terry Shistar, Ph.D.

Recent advances in estimating real world chemical interactions and exposure through computer models, known as “computational toxicology,” offer some promise for identifying chemicals that adversely affect the endocrine system and have other toxic effects. They also present critical challenges for integrating standards for precaution, transparency, and effective public involvement. It has been 20 years since Congress passed the *Food Quality Protection Act of 1996*, and the Environmental Protection Agency (EPA) has not fulfilled its obligation to screen pesticides for endocrine disrupting effects. To help meet the requirements of the act, EPA, nearly a decade ago, sponsored a National Research Council (NRC)/National Academy of Sciences report, *Toxicity Testing in the 21st Century – A Vision and a Strategy* (2007), which recommended the use of “computational toxicology.”

What is computational toxicology?

Computational toxicology uses computer models to combine data generated by a variety of real world tests, both *in vivo* (in organisms) and *in vitro* (in glass containers), with theoretical knowledge based on factors like structural relationships to chemicals

with known toxicological properties. These models replace risk assessments based on testing of actual organisms with “toxicity-pathway-based risk assessments” based on virtual organisms having virtual tissues composed of virtual cells that interact with virtual chemicals. Exposure estimates are also based on computer models of how toxic chemicals and their metabolites reach cells in the body where they can affect physiological processes. The assessment of virtual risk produced by this process is anticipated to replace conventional risk assessment over the next decade or two. Meanwhile, EPA is considering its use as a tool for chemical screening or prioritizing reviews.

The Uses of Computational Toxicology

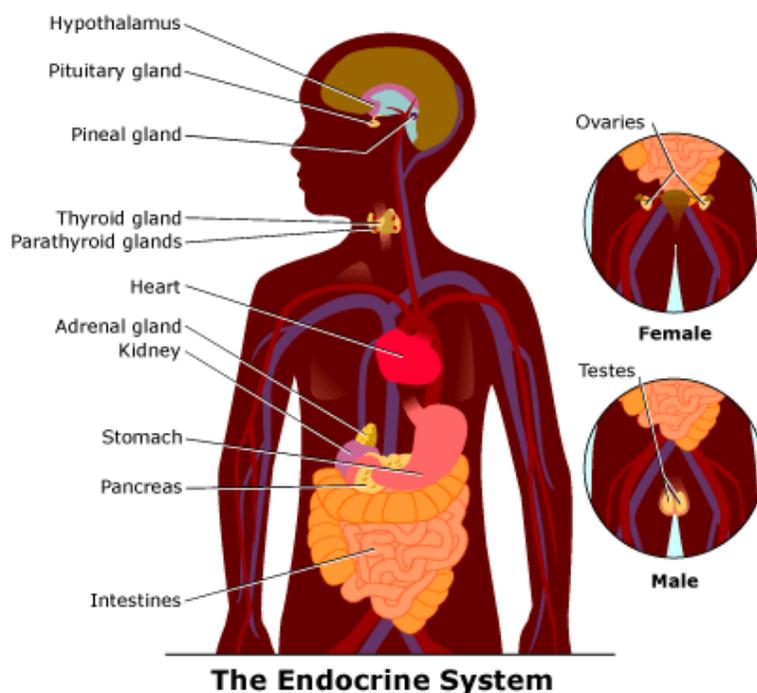
The role that computational toxicology might play in evaluating potential endocrine disrupting chemicals (EDCs) differs in the views of EPA, the European Union (EU), and a group of independent scientists.

Environmental Protection Agency. The EPA strategy that emerged from the 2007 NRC report recommendations envisions computational toxicology as replacing conventional toxicology, eliminating currently used uncertainty factors.¹ The thinking is that this new approach will replace traditional toxicology after a transition pe-

riod in which computational models will be used “to predict chemicals most likely to cause hazards of concern for humans. . . and enable risk assessors to determine the specific effects, *in vivo* data, and exposures that would be most useful to assess, quantify, and manage.” EPA foresees the transition taking 10 to 20 years.²

In particular, EPA sees the screening of chemicals for endocrine disrupting potential as an important application of computational toxicology. Screening is the first step –“Tier 1” in the testing program for endocrine disrupting chemicals (EDCs) that EPA identified in its protocol released in 2014.³ Tier 1 identifies chemicals with the potential to interact with estrogen, androgen, or thyroid hormonal pathways and assesses the need for Tier 2 testing to further characterize those effects by using *in vivo* studies to establish dose-response relationships for any “potential adverse effects” for a risk assessment. The agency does not appear to accept what endocrinologists call an inverse dose response curve (U-shaped dose response curve or non-monotonic dose response curve), characterizing effects seen at minute or very low doses, instead of a diagonal straight line graphic that shows higher dose exposures associated with elevated effects (higher “dose makes the poison” theory). In fact, EPA’s Scientific Advisory Panel reinforces this thinking with its statement that, “Monotonic dose-responses are assumed to be dominant in the assays.”⁴

European Union (EU) Chemical Review Law. The EU’s *Registration, Evaluation, Authorisation and Restriction of Chemicals* (REACH) regulations allows consumers to learn from any supplier whether its products contain officially recognized *Substances of Very High Concern* (SVHC) (substances that have been identified as carcinogens, mutagens or reproductive toxicants, that are persistent and bioaccumulative, or that warrant similar concern). Access to information on SVHCs in products is viewed as a powerful tool for promoting the substitution of harmful chemicals with safe alternatives.⁵ Some uses of SVHCs may require prior authorization from the European Chemicals Agency (ECHA), and applicants for authorization will have to include plans to replace the use of the SVHC with a safer alternative (or, if no safer alternative exists, the applicant must work to find one). As of June 2014, 155 SVHCs are on the candidate list for authorization.⁶



Article 13(1) of REACH states that information on intrinsic properties of substances may be generated by means other than tests, provided that the conditions set out (Annex XI) are met. In particular for human toxicity, it requires information to be generated whenever possible by means other than vertebrate animal tests. This includes using *in vitro* methods, qualitative or quantitative structure-activity relationship (QSAR) models, or information from structurally related substances (grouping or “read-across”).⁷

It is not clear how effective alternative testing methods have been for regulation, since non-governmental organizations in 2011 called on the Member States and the Commission to agree that, in the absence of data from animal studies, it is necessary to accept regulation on the basis of *in vitro* test methods. The risk assessments performed under REACH incorporate exposure assessment in a conventional risk assessment protocol, and regulation of chemicals under REACH may consist of reducing exposure.

In summary, REACH encourages non-animal studies, but requires manufacturers to demonstrate the adequacy of their methods. Alternative methods and strategies (computational toxicology and *in vitro* testing) are used, but it is not clear how effective they are as a basis for regulation.

Tiered Protocol for Endocrine Disruption (TiPED)

The Tiered Protocol for Endocrine Disruption (TiPED), developed by a multi-disciplinary group of independent scientists, is a testing methodology using a tiered approach for evaluating chemicals for endocrine disruption. It consists of five testing tiers ranging from broad *in silico* (computer simulation) evaluation through specific cell- and whole organism-based assays. Like “green chemistry,” it approaches risk reduction through the elimination or reduction of the hazardous chemical in favor of a safer alternative, rather than allowing a hazardous chemical agent, but attempting to mitigate risk by reducing exposure. This is consistent with the method for evaluating materials allowed in organic production and processing under the *Organic Foods Production Act* (OFPA), but contrary to the regulatory approach taken by EPA, which depends heavily on exposure assessment and management.

TiPED incorporates computational methods as a first step in identifying safer chemicals. Chemicals that “pass” Tiers 1-3 without detecting EDC activity are further studied by *in vivo* experiments—Tier 4 in fish and amphibians and Tier 5 in mammals—to reduce the likelihood of false negatives, since the use of whole animals can identify EDC effects caused by mechanisms that target different functions of the endocrine system—including developmental disruptions that may not manifest themselves until much later in life— or identify EDC effects even though the mechanism may not be known. Because mammals differ from fish and amphibians in hormones and pathways, it is important to include testing in mammals that is used only when prior tests detect no EDC activity.⁸ To reduce the likelihood of false negatives, TiPED protocol states that, “Power analyses should be performed in preparation for the full assay.”⁹

In applying TiPED to known endocrine disruptors, the researchers have found that some—e.g., bisphenol A and phthalates—are identified by computational studies, while others—such as perchlorate and atrazine— might not be identified until Tiers 3 or 4. They found that, “The proposed assays are clearly robust enough that these chemicals would not make it to market, providing supportive evidence that the TiPED screens will be sufficient to identify putative EDCs.”¹⁰

Promises and Challenges

Computational toxicology promises to eliminate the logjam in screening a large number of pesticides for their endocrine disrupting properties. In addition, it presents a way to screen industrial

chemicals coming on to the market, and could be used in overhauling the *Toxic Substances Control Act* (TSCA) review process. In theory, this approach makes the maximum use of existing data and minimizes the extensive animal testing conducted under current toxic chemical regulatory testing protocols. However, as is the case with current toxic chemical regulatory schemes, new models do not inherently address the need for a precautionary regulatory approach to toxic chemical approval.

The comparison of the different ways in which computational toxicology could be used by EPA under the *Federal Insecticide, Fungicide, and Rodenticide Act* (FIFRA) and TSCA, by ECHA under REACH, and under TiPED protocol illustrates some of the problems that might arise in EPA’s proposed use for screening pesticides for EDCs. Potential problems include:

- **Reduced transparency for the public.** First of all, reliance on computer models can reduce transparency in regulation. Animal testing looks for actual effects on actual animals. Computational toxicology extrapolates estimates of actual effects from study results on related chemicals or effects inferred from results on cells in *in vitro* testing. This may not be transparent to the general lay public. Only those few with training in these methods will be able to understand and comment on their use. The chemical industry has always challenged the extrapolation of toxicological testing on laboratory animals to the human population, so it is expected that EPA’s ability to restrict, cancel, or suspend the use of a pesticide based on results of comprehensive computational models will be questioned.

Objectives of EPA’s 2014 workplan for the Endocrine Disruptor Screening Program¹¹

1. Prioritization

The near-term goal (less than 2 years) is to use existing data, *in silico* (computer simulation) models, and *in vitro* HTP [high throughput] assays to determine the relative order in which non-pesticide chemicals and pesticide active ingredients going through registration review should be screened.

2. Screening (Tier 1)

The intermediate-term goal (2-5 years) is to replace current validated *in vitro* screening assays with validated *in vitro* high throughput screening computer-based assays; use the results to inform and target current *in vivo* estrogen- or androgen-specific screening assays; and, where possible, reduce the use of animals for screening purposes.

3. Replacement

The long-term goal (over 5 years) is to consider full replacement of the *in vivo* screening assays with validated *in vitro* HTP assays and eliminate the use of animals for screening purposes.

- **Lack of attention to complexities.** The extreme reductionist approach, depending on computer models with an unknown range of applicability, poses a problem for dependence on computational toxicology as the sole source of toxicity information. Particularly concerning is EPA's view that it could "eliminate currently used uncertainty factors." In fact, dependence on computational toxicology can increase uncertainty. Whenever relying on computer models, caution is essential to avoid the phenomenon of "garbage in, garbage out" (GIGO). Computer models must be based on sound science and have solid data as inputs.

The creators of TiPED point out that, although computational methods have a place, reliance on them alone would create many false negatives. They state, "The complex biology of endocrine disruption means that *no single assay nor single approach* [emphasis in original] can be used to identify chemicals with EDC characteristics. Instead, a combination of approaches is necessary, including computational methods as well as both *in vitro* and *in vivo* testing. . . Today's *in vitro* and computer models do not incorporate the complexity that this involves. For this reason, *in vivo* assays will also be necessary."¹²

- **Sacrificing precaution for a simpler testing scheme.** Under REACH, chemical manufacturers are required to both avoid animal testing and justify the need for the chemical based on the availability of safer alternatives. This adds an additional layer of

protection that is not present in EPA's proposed methodology.

A Perspective on the Bigger Picture

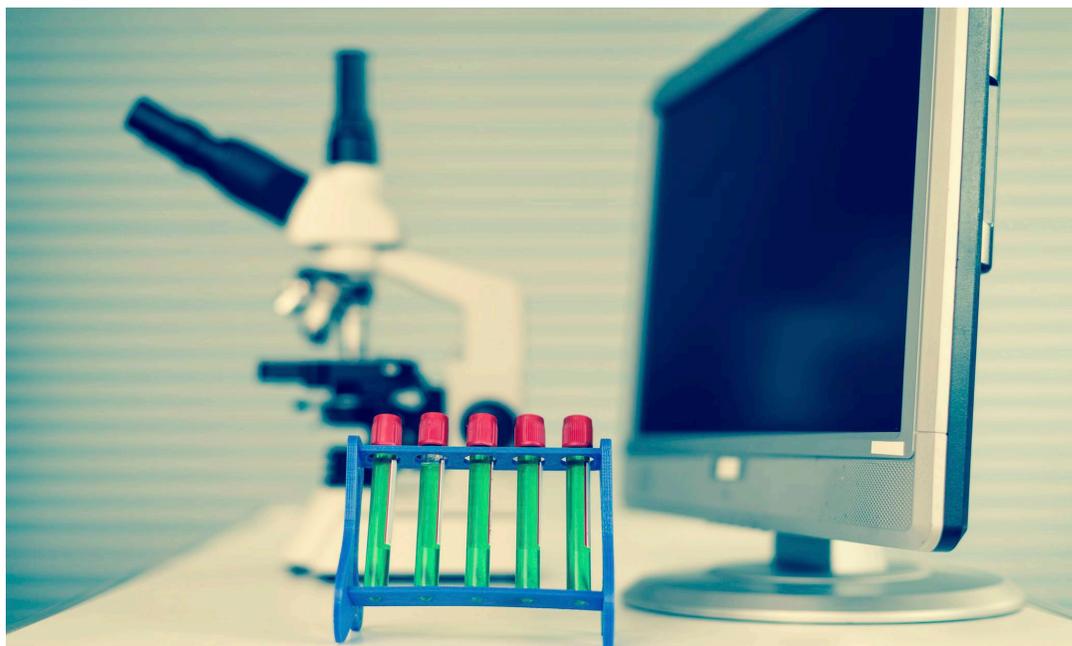
In order to be protective, ["computational toxicology"] must be used in concert with other methods and embedded in a regulatory system that allows chemicals to be removed from the market when hazards or safer alternatives are demonstrated.

Much of the emphasis in proposals for using computational toxicology is focused on evaluating new chemicals – probably because taking existing chemicals off the market is such a daunting task. However, the current situation allows humans and all other organisms to be exposed daily to many chemicals that should not be present in the environment. Any methods of evaluating chemicals that are used must be embedded in a regulatory system that allows for the removal of EDCs and other problematic chemicals.

In addition to the need to evaluate and eliminate hazardous chemicals, the framework in which chemicals are evaluated needs to change. A good model is the *Organic Foods Production Act* (OFPA), since the law creates a default bias against synthetic chemical use – natural materials are acceptable unless shown to be hazardous, and synthetic materials are unacceptable unless it is determined that there is an absence of harm (in chemical life cycle analysis) – and the material is essential to and compatible with an organic management system, as defined by law and certified by a third-party. As in the TiPED protocol, harm is evaluated regardless of exposure. Synthetic chemicals should not be allowed to be used unless they are essential, and unless their use is sustainable.

Summary/Conclusion

The computer-based methods encompassed by the term "computational toxicology" offer great promise for reducing exposure to EDCs and other toxic chemicals. In order to be protective, however, they must be used in concert with other methods and embedded in a regulatory system that allows chemicals to be removed from the market when hazards or safer alternatives are demonstrated. The methods should be used with a precautionary approach – in other words, if a chemical "fails" a computer model (or *in silico* test), it should not be allowed to be marketed. However, materials that "pass" such tests should move on to *in vitro* and *in vivo* tests to ensure that the complexity of endocrine and other physiological functions is fully considered.



Endnotes

1. Michael Firestone, Robert Kavlock, Hal Zenick, Melissa Kramer, and the U.S. EPA Working Group on the Future of Toxicity Testing, 2010. *The U.S. Environmental Protection Agency Strategic Plan for Evaluating the Toxicity of Chemicals. Journal of Toxicology and Environmental Health, Part B*, 13:139–162.
2. Michael Firestone, Robert Kavlock, Hal Zenick, Melissa Kramer, and the U.S. EPA Working Group on the Future of Toxicity Testing, 2010. *The U.S. Environmental Protection Agency Strategic Plan for Evaluating the Toxicity of Chemicals. Journal of Toxicology and Environmental Health, Part B*, 13:139–162.
3. EPA Office of Chemical Safety and Pollution Prevention, 2014. *Endocrine Disruptor Screening Program Comprehensive Management Plan*.
4. EPA, 2013. Transmittal of the Meeting Minutes of the FIFRA SAP Meeting Held June 25-28, 2013 on the Scientific Issues Associated with the “Proposed Endocrine Disruptor Screening Program (EDSP) Tier 2 Ecotoxicity Tests.”
5. European Environmental Bureau, 2010. *The Fight to Know: Substances of Very High Concern and the Citizens’ Right to Know Under REACH*.
6. http://en.wikipedia.org/wiki/Registration,_Evaluation,_Authorisation_and_Restriction_of_Chemicals
7. European Chemicals Agency, 2011. *Guidance on information requirements and chemical safety assessment*, Chapter R.2: Framework for generation of information on intrinsic properties. http://echa.europa.eu/documents/10162/13643/information_requirements_r2_en.pdf
8. T. T. Schug, R. Abagyan, B. Blumberg, T. J. Collins, D. Crews, P. L. DeFur, S.M. Dickerson, T. M. Edwards, A. C. Gore, L. J. Guillette, T. Hayes, J. J. Heindel, A. Moores, H. B. Patisaul, T. L. Tal, K. A. Thayer, L. N. Vandenberg, J. C. Warner, C. S. Watson, F. S. vom Saal, R. T. Zoeller, K. P. O’Brien and J. P. Myers, 2013. *Designing endocrine disruption out of the next generation of chemicals. Green Chem.*, 2013, 15, 181-198.
9. Schug et al, 2013. See also the PAY article “Precaution: Science and Policy” in the Spring 2015 issue for a discussion of statistical power.
10. T. T. Schug, R. Abagyan, B. Blumberg, T. J. Collins, D. Crews, P. L. DeFur, S.M. Dickerson, T. M. Edwards, A. C. Gore, L. J. Guillette, T. Hayes, J. J. Heindel, A. Moores, H. B. Patisaul, T. L. Tal, K. A. Thayer, L. N. Vandenberg, J. C. Warner, C. S. Watson, F. S. vom Saal, R. T. Zoeller, K. P. O’Brien and J. P. Myers, 2013. *Designing endocrine disruption out of the next generation of chemicals. Green Chem.*, 2013, 15, 181-198.
11. EPA Office of Chemical Safety and Pollution Prevention, 2014. *Endocrine Disruptor Screening Program Comprehensive Management Plan*.
12. T. T. Schug, R. Abagyan, B. Blumberg, T. J. Collins, D. Crews, P. L. DeFur, S.M. Dickerson, T. M. Edwards, A. C. Gore, L. J. Guillette, T. Hayes, J. J. Heindel, A. Moores, H. B. Patisaul, T. L. Tal, K. A. Thayer, L. N. Vandenberg, J. C. Warner, C. S. Watson, F. S. vom Saal, R. T. Zoeller, K. P. O’Brien and J. P. Myers, 2013. *Designing endocrine disruption out of the next generation of chemicals. Green Chem.*,