Use of Tox 21 Tools from Prioritization for Screening to Risk Assessment in the Context of Endocrine Disruption

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Summary

The EPA's Office of Pesticide Programs' approach to improving and transforming human health and ecological risk assessment and management is described with reference to screening for endocrine disruptors. Building on the NRC's vision for toxicity testing in the 21st century, OPP is moving to increase the efficiency and effectiveness of testing for risk assessment to inform decisions and reduce the costs of the process, both in dollars and in animals used. The approach is based on building libraries of adverse outcome pathways (AOPs) to use (Q)SAR models and cell-based assays to predict perturbations of normal cellular pathways leading to adverse outcomes. Dose response models are organized using computational systems biology models that describe the circuitry underlying each toxicity pathway and employ in vitro to in vivo predictions and pharmacokinetic models.

Keywords: Toxicity Testing in the 21st Century, alternatives to animal testing, endocrine disruption

The 1996 Food Quality Protection Act (FQPA, 1996) and the 1996 Safe Drinking Water Act (SDWA, 1996) amendments require the U.S. Environmental Protection Agency (EPA) both to develop a screening program for estrogenic activity and other hormonal effects and to test chemical substances that may be found in sources of drinking water for estrogenic effects, if substantial human populations may be exposed. The Endocrine Disruptor Screening, Testing and Assessment Committee (EDSTAC), established to advise the EPA in meeting these requirements, recommended that the agency not limit its efforts to those chemicals with potential estrogenic effects in humans but that it also should consider potential estrogenic, androgenic, and thyroid effects on human health, as well as effects on fish and wildlife from exposures to pesticides, commercial chemicals, and environmental contaminants. A summary of their recommendations with links to the report and related documents can be found on EPA's website.[1]

The EPA established its Endocrine Disruptor Screening Program (EDSP) as a Tiered Testing program. Tier 1 was designed to provide data to determine if a chemical has the potential to interact with the estrogen, androgen, or thyroid (E, A, or T) hormone systems. Its objective was to determine which chemicals should be considered for further testing. Tier 2 Testing is designed to provide data to determine if endocrine-mediated adverse effects occur and to provide the means to quantify dose-response relationships as a basis for hazard and risk assessment. The assays that make up the Tier 1 Screening battery tiers are summarized in Table 1. Additional information and the current status of the EDSP program can be found on the EPA's EDSP webpage.[2] The EPA has provided its vision on how to begin to utilize methods consistent with the NAS report on Toxicity Testing in the 21st Century. This plan, the EDSP 21 work plan, describes the EPA's future for how to approach screening chemicals for their ability to interact with the E, A, or T systems using computational or *in silico* models and molecular based high throughput assays.

Tab. 1: Endocrine Disruptor Screening Battery

In vitro	Estrogen receptor (ER) binding – rat uterus
	Estrogen receptor (hER) transcriptional activation – Human cell line (HeLa-9903) (OECD Test Guideline 455)
	Androgen receptor binding – rat prostate
	Steroidogenesis – Human cell line (H295R)
	Aromatase – Human recombinant
In vivo	Uterotrophic (rat) (OECD TG 440)
	Hershberger (rat) (OECD TG 441)
	Pubertal female (rat)
	Pubertal male (rat)
	Amphibian metamorphosis (frog) (OECD TG 231)
	Fish short-term reproduction (OECD TG 229)

Disclaimer: The views expressed in this paper are those of the author and do not necessarily reflect the official views of the U.S. Environmental Protection Agency.

1 http://www.epa.gov/endo/pubs/edspoverview/edstac.htm

2 http://www.epa.gov/endo/

Building on the vision of the National Academy of Sciences for Toxicity Testing in the 21st Century (NRC, 2007), the EPA's office of Pesticide Program (OPP) is developing processes that will allow a move away from extensive whole animal tests and towards the use of new, rapid, and mechanistically based 21st century tools. This involves not only screening large inventories of previously untested chemicals to prioritize the scope and order for further testing but also incorporating our developing understanding of adverse outcome pathways (AOP), which will enable the development of alternative testing approaches that do not rely on animals. These approaches will be based on an enhanced interpretation of how data from non-animal tests relates to toxicity in the intact animals, in humans, and in other species of interest.

The use of data from each alternative test has to be considered in context. A greater understanding is required of how the test measures relate to an adverse outcome before the test results can be used with confidence for risk assessment than is required to identify additional testing needs. In general, less certainty is required when the assay is used as an indicator of the chemical's ability to have biological activity, rather than to inform a decision about whether the chemical actually is causing an effect. In this context, the focus is on what additional testing may be needed. The language in the FQPA and SDWA legislation refers to the entire process – from determining the potential to the hazard assessment – as screening. This definition of screening is more proscriptive than how it is used in the context of high throughput assays for evaluating large numbers of chemicals or screening for prioritization and further testing.

The NAS vision (Fig. 1) provides a basis for identifying AOPs and their use in risk assessment, as it puts toxicity in the context of normal biological pathways which, if perturbed, can return to normal via adaptive responses such as homeostatic mechanisms. However, if the dose is sufficiently high, occurs for a sufficiently long period of time, or interacts with a critical target site, then the normal adaptive responses are not able to return the pathway to its normal biologic function, and an adverse outcome results. The key events of this can be described in terms of an adverse outcome pathway.

The EPA plans to work with the greater scientific community to develop AOPs and to use knowledge gained from them, as it becomes available, in an "Integrated Approach to Testing and Assessment" (IATA) to inform risk assessment (OECD, 2008). The EPA's approach for the use of TOX 21 tools via AOPs for endocrine disrupting chemicals (EDCs) and beyond can be found at its website.[3] In essence, IATA involves integrat-

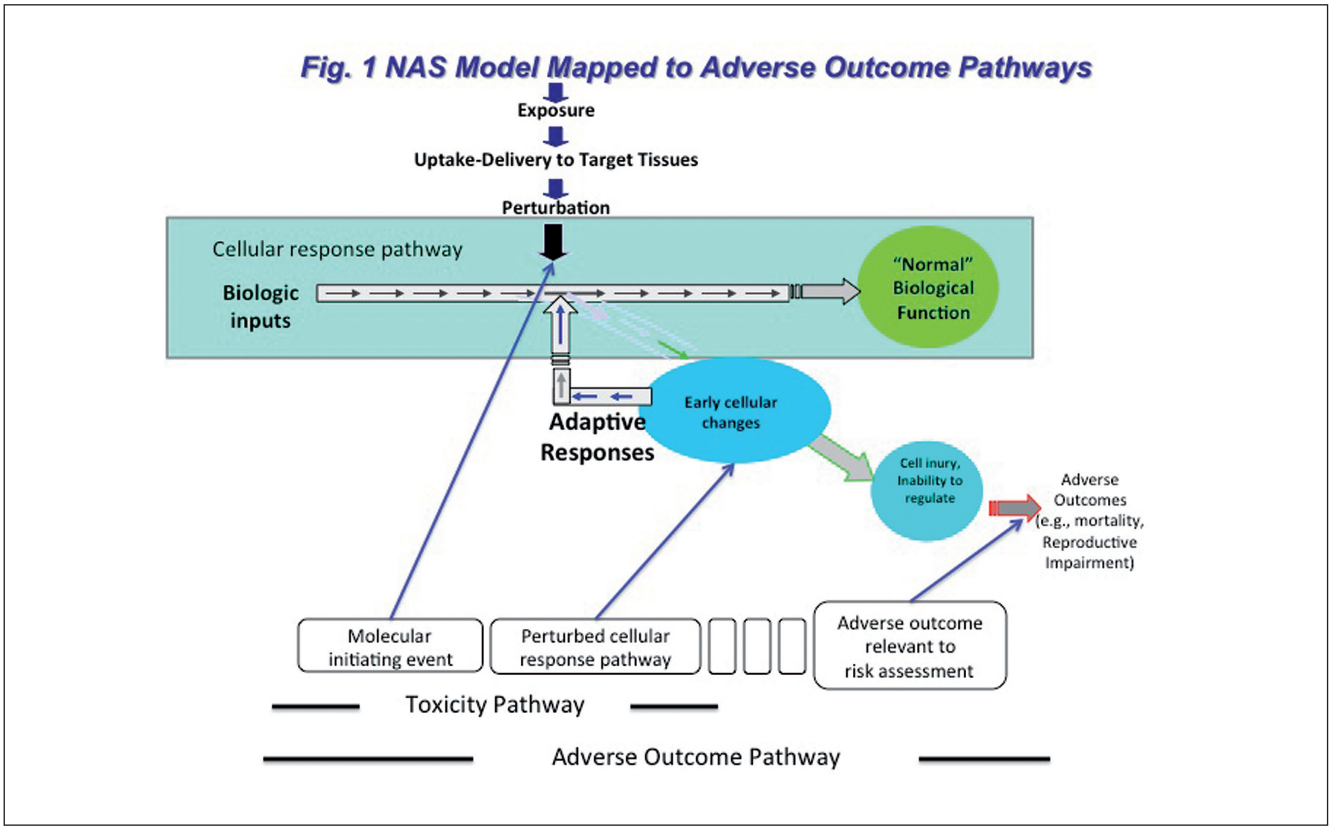

Fig. 1: NAS model mapped to adverse outcome pathways

[3] http://www.epa.gov/pesticides/science/testing-assessment.html

ing existing information, including the use of new technologies, to formulate plausible and testable hypotheses to target *in vivo* testing on chemicals and endpoints of concern.

OPP's goal for this paradigm shift from conventional animal testing to Tox 21 approaches via an integrated approach to testing and assessment is to increase the efficiency and effectiveness of the risk assessment process. The plan is to routinely use *in silico* and *in vitro* models to predict adverse consequences for critical toxicities at the levels of chemical structure, molecular, cellular, organ, individual, and population outcomes to understand the linkage of biological events that leads from exposure to adverse outcomes. AOPs can be combined to develop hypotheses to target testing and to inform risk assessment. As AOPs are developed, a future is envisioned where safety evaluations will not be based on the testing of every chemical using each of the required pesticide guideline tests, but rather there will be less reliance on animal studies, and tests will be tailored to likely outcomes based on an understanding of toxicity pathways.

The degree of "validation" required for the use of these new Tox 21 approaches is contextual (Judson et al., submitted). For example, FQPA requires the use of validated methods to screen for endocrine activity. The understanding of how the test measures relate to an adverse outcome may need to be greater before the test results can be used with confidence for risk assessment than for prioritizing large inventories of previously untested chemicals to stage follow up testing.

The speed at which we move to this future will depend not only on how well the approaches are "validated," which is dependent on the development of a scientific understanding of how the various *in silico* and *in vitro* Tox 21 approaches map to AOPs to inform risk assessment, but also on how well the new approaches are accepted by the various affected stakeholders. Thus, partnerships with various federal, state, international, and private organizations, together with interactions between scientists, decision makers, and the various stakeholders, are required to build the scientific basis for and the public acceptance of the approach to ensure that not only is it scientifically sound but that it is acceptable and tailored as well as appropriate for the purpose desired.[4]

[4] http://www.epa.gov/pesticides/science/testing-assessment.html#partnerships

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