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"Toxicity Testing in the 21st Century" and reforming the science of EPA's Endocrine Disruptor Screening Program

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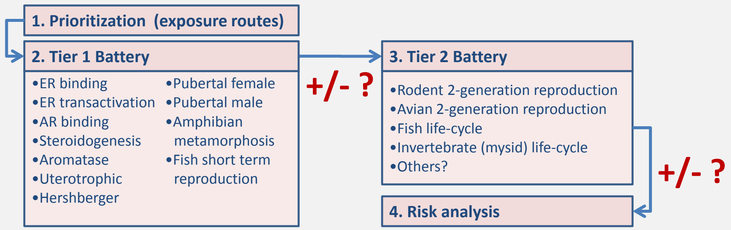
Introduction

In light of recent developments in biotechnology and computational toxicology, there is an unprecedented opportunity to improve risk-based regulatory decision-making by modernizing outmoded testing approaches that continue to rely on time-consuming, resource-intensive animal-based studies that are of questionable relevance to humans. At the behest of the Environmental Protection Agency (EPA), the National Research Council (NRC) devised a new approach to toxicity testing that recognizes the impact of these scientific developments. The result of this effort, NRC's 2007 publication, *"Toxicity Testing in the 21st Century: A Vision and A Strategy,"* outlines a transformative paradigm shift in toxicology from an observational to a predictive science. Based largely on non-animal test methods, this shift favors high-throughput methods capable of managing large numbers of chemicals and mixtures at relatively low cost. Consistent with NRC's recommendations, we present an integrated testing strategy (ITS) applied to the EPA Endocrine Disruptor Screening Program (EDSP) that would result in more efficient screening and characterization of the endocrine-disrupting potential of manufactured chemicals while reducing reliance on the use of animals.

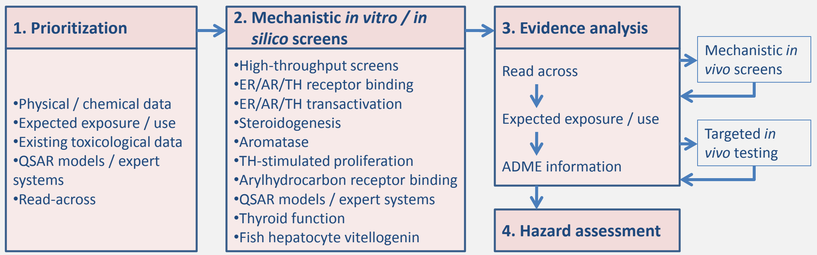
EPA EDSP / Current approach

Created in 1998 through a revision to the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), the EDSP is organized into two tiers of test batteries that aim to **characterize the risks posed by estrogenic, androgenic and thyroid (EAT) chemicals to human and environmental health**. The finalized Tier 1 battery (5 *in vitro* and 6 *in vivo* assays) aims to identify substances that have the potential to interact with the EAT hormonal systems by discerning mechanistic information about test chemicals. Although EPA has not defined how this will take place, the Agency has stated that it will determine whether a chemical should proceed to Tier 2 testing based on a weight-of-evidence assessment of the results of Tier 1 assays. The putative Tier 2 battery, consisting of developmental and reproductive toxicity tests in several vertebrate species, is designed to identify and establish dose-response relationships for any adverse endocrine-related effects. It is unclear what combination of Tier 1 results will trigger Tier 2 testing. Additionally, although the Office of Management and Budget (OMB) instructed EPA to "promote and encourage test order recipients to submit Other Scientifically Relevant Information (OSRI) *in lieu* of performing all or some of the Tier 1 assays" and to "accept OSRI as sufficient to satisfy the test orders to the greatest extent possible," it is unclear how existing test data will be used to waive portions of Tier 1 and Tier 2 testing. For Phase I of the EDSP, test orders have been issued for 67 chemicals (58 pesticide active ingredients and nine High Production Volume (HPV) chemicals used as pesticide inert ingredients) based on their exposure potential.

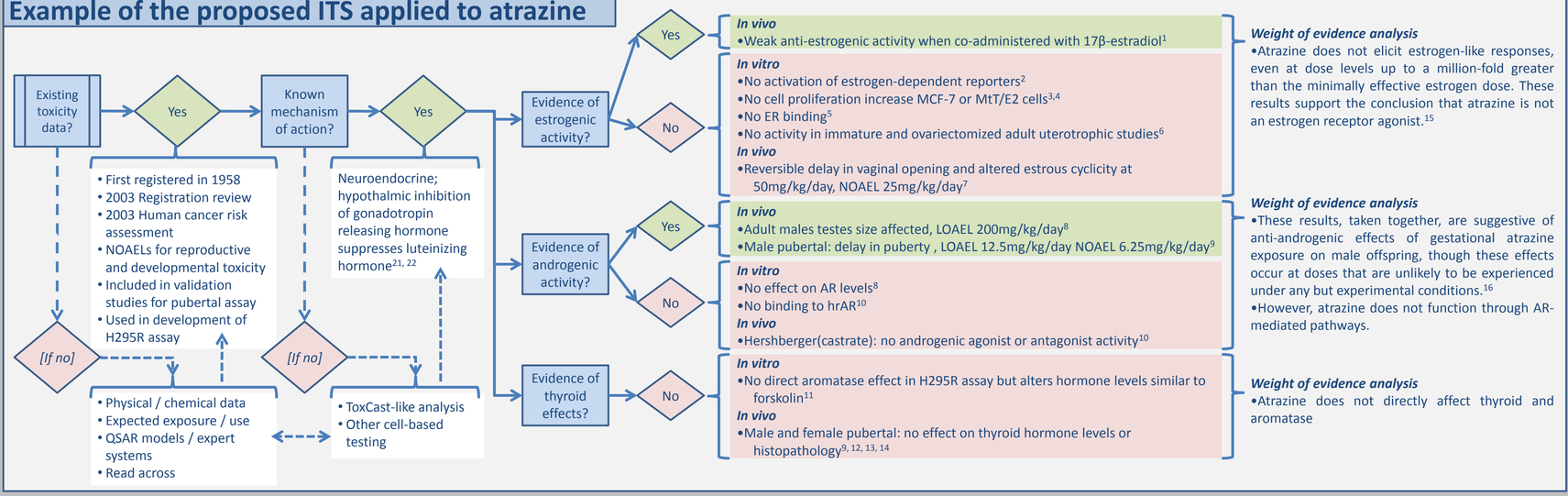


EPA EDSP / Proposed Integrated Testing Strategy (ITS)

Rather than a default application of the full battery of Tier 1 assays, we propose a more efficient and potentially more useful integrated approach. All existing relevant toxicological information for a chemical, reproductive and developmental information in particular, is considered alongside information generated using a series of *in vitro* mechanistic assays, QSAR and other physical information, to determine what, if any, further testing is warranted. Such an approach, illustrated below, would meet NRC's standards to "provide wider coverage of chemicals of concern, reduce the time needed for generating toxicity test data required for decision-making, and use animals to a far smaller extent,[17]" while increasing the amount of available human-relevant information on the nature and dimensions of risk needed to make "well-grounded decisions" necessary for regulation aimed at protecting public health.[19] At right, this proposed ITS is applied to atrazine, one of the already heavily tested Phase I pesticides, to illustrate the system of information collection using existing data from toxicity tests and OSRI.



Example of the proposed ITS applied to atrazine



Evaluation of existing data for atrazine, Phase I chemicals

Atrazine is a pesticide active ingredient that has been on the market for more than 50 years and is one of the most widely used agricultural products worldwide. As a result, atrazine has been thoroughly tested in a wide range of vertebrate species using a variety of methods, including protocols similar if not identical to those required under both the Tier 1 and Tier 2 testing battery of the EDSP (such as reproductive and chronic / lifecycle studies in rodents, fish and birds, as well as a range of metabolic and pharmacokinetic studies). The Tier 1 uterotrophic assay, for example, measures uterine growth in rats or mice following exposure to a test chemical by evaluating two endpoints, body weight and uterine weight, to determine the test chemical's potential estrogenic activity. As shown in the table below, information on many of the endocrine-related endpoints examined in EDSP Tier 1 and Tier 2 tests already exist in the pool of data generated during the process of atrazine's pesticide registration.

EDSP Tier 1 assay	Endpoints in EDSP assay	Pesticide data requirements related to those endpoints
Uterotrophic (rats / mice)	Body weight	OPPT 870.4100: Chronic toxicity
	Uterine weight	OPPT 870.3800: Reproduction and fertility effects
	Vaginal histopathology	
Hershberger (rats/mice)	Prostate weight	OPPT 870.3800: Reproduction and fertility effects
	Seminal vesicle weight	
	Levator ani /bulbocavernosus weights	
	Cowpers glands weights	
	Glans penis weight	

It has been established that atrazine delays puberty and sexual development in both male and female rodents and has long-term effects in adult male testes; detailed studies on mechanism of action, including hormone activities and organ histology, indicate **this is an indirect effect on the endocrine system through the central nervous system**.[21, 22] For most endpoints included in the Tier 1 tests, LOAEL and/or NOAELs have been established. Atrazine does not affect thyroid hormone-dependent processes in rodent or in amphibians. Atrazine does not appreciably affect development or sexual differentiation in amphibians or fish as assessed by protocols similar to the amphibian metamorphosis or fish short-term reproduction Tier 1 assays. Atrazine has also been tested in a number of estrogen receptor binding and transcriptional activation assays both *in vitro* and *in vivo*; there is no evidence that atrazine binds or activates the estrogen receptor.[15] Likewise, there is no evidence that atrazine affects AR binding or activation.

Therefore, existing data satisfy most or all of the EDSP requirements and there is no conceivable justification for further testing of atrazine as part of the EDSP.

Summary

As illustrated using available relevant data for the EDSP Phase I chemical atrazine, an integrated scheme incorporating existing data from *in vitro* and *in vivo* tests is capable of fulfilling information requirements in place of new Tier 1 *in vivo* tests. Since EPA issued the first EDSP test orders in October 2009, PETA and PCRM have submitted reviews to EPA of the currently available toxicity testing information for **14** of the 67 Phase I chemicals. Consistent with the example provided here for atrazine, many of these 67 Phase I chemicals have already been examined for endpoints that would be sought by Tier 2 testing, eliminating the need for further tests using animals. This review has found that :

•Phase I chemicals, pesticides and HPV chemicals, have already been tested in Tier 2 tests, including multi-generation mammalian reproductive tests and in some cases assessment of effects on fish and bird reproduction and development.
•Other relevant human observational and epidemiological data and mechanistic *in vitro* and *in vivo* data are also available for most Phase I chemicals.
•For many of these chemicals, the mechanism of action and primary toxicological pathways have already been characterized.
•Chemicals can then be screened and prioritized in a consistent stepwise manner starting with high throughput *in vitro* and *in silico* characterization, completing an evidence-based analysis before moving on to additional testing.

While we were able to identify existing data on many of the endpoints of interest to the EDSP for atrazine and many other chemicals, this process also demonstrates that an ITS approach can be useful for testing chemicals for which no toxicity data yet exists. The conceptual framework of the ITS is such that the mix of tests, in keeping with NRC's vision, "includes *in vitro* tests that assess critical mechanistic end points involved in the induction of overt toxic effects, rather than the effects themselves, and targeted *in vivo* tests that ensure adequate testing of metabolites and coverage of endpoints.[17]" If EPA decides in the future to expand the EDSP to include many more than these initial 67 chemicals, making the change to an ITS approach will be essential, as"[i]n the long run, using upstream indicators of toxicity from high-throughput assays based on toxicity pathways can be more sensitive and hence more protective of public health than using apical endpoint observations from assays in small numbers of live rodents.[18]"

Reshaping the use of existing information on Phase I chemicals in place of conducting a full, standardized suite of new tests for each is a more pressing issue. Of eleven chemicals reviewed so far, EPA has rejected all OSRI submissions for seven of them and accepted OSRI for only one assay each for four others. Rather than taking a true weight-of-evidence approach to evaluating OSRI, EPA seems to be instead evaluating OSRI for the exact endpoints and measurements used in Tier 1 protocols. This narrow definition of OSRI is likely to lead to excessive, redundant and wasteful testing.

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References available on request