

Application of an Integrated Testing Strategy to the US EPA Endocrine Disruptor Screening Program

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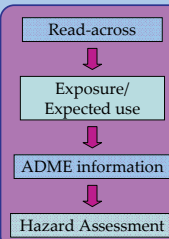
1. PRIORITIZATION

- Physical and chemical data
- Expected exposure/use
- Existing toxicological data
- QSAR models and expert systems
- Read-across

2. MECHANISTIC IN VITRO/IN SILICO SCREENS

- High-throughput screens
- ER/AR/TH receptor binding
- ER/AR/TH transactivation
- Steroidogenesis
- Aromatase
- TH-stimulated proliferation
- Arylhydrocarbon receptor binding
- QSAR/Expert systems
- Thyroid function
- Fish hepatocyte vitellogenin

EVIDENCE ANALYSIS



3. MECHANISTIC IN VIVO SCREENS

4. TARGETED TESTING

A more effective approach

Recognizing the need for a faster, more accurate screening battery, we propose an alternative tiered strategy. The preliminary tier includes physical and chemical data, existing toxicological data, and *in vitro* and QSAR methods that are either validated or nearly validated. The results of this alternative Tier 1 can be used in a weight-of-evidence approach to 1) identify priority chemicals and 2) design an intelligent, chemist-specific strategy for further screening or testing. Such a strategy would greatly reduce the use of animal testing for identification and classification of endocrine disrupting chemicals.

An additional benefit of this approach is that it can easily incorporate new technology, such as advances in QSAR modeling and the much broader range of *in vitro* mechanistic assays in development (Table 4). Such an approach would be more logical, efficient, economical, use fewer animals (Diagram) and be in keeping with the NRC's "Toxicity Testing in the 21st Century"¹¹ and EPA's 2009 Strategic Plan.¹²

¹¹ Committee on Toxicity Testing and Assessment of Environmental Agency, National Research Council 2007. Toxicity Testing in the 21st Century: A Vision and Strategy. National Academies Press, Washington, DC.
¹² Environmental Protection Agency, 2009. U.S. Environmental Protection Agency Strategic Plan for Endocrine Disruptors. Office of the Science Advisor, Science Policy Council. U.S. Environmental Protection Agency, Washington, DC. August 2009.

Cost of the Current Tier 1, in animals and USD

For the initial phase of the EDSP, every chemical that receives a Data Call-In notice from EPA must be tested in all of the assays that will eventually comprise the Tier 1 battery. The currently proposed Tier 1 battery would use a minimum of 578 - 596 animals and cost between \$49,475 to more than \$91,044,835 USD per chemical (conservatively estimated with no range-finding or repeat estimates) (Table 2 and 3). Phase 1 of the EDSP is therefore likely to cost over 40,000 animals and to cost anywhere from \$29,444,835 to in excess of \$69,936,611.

The costs listed in Table 3 are estimates for solely for the conduct of the assays, and do not include other associated costs, such as paper work, management, technical and clerical costs, which could add another 10-30% to the cost estimates listed (for estimates of these costs, see Table 3, 3rd column). The 2007 - 2008 estimates from Applied Pharmacology and Toxicology, Inc. (presented in their March 10, 2008 Comments on EPA's Information Collection Request (the "ICR") developed for the Agency's Endocrine Disruptor Screening Program; Draft Policies and Procedures for Initial Screening, 72 Fed. Reg. 70861 (December 13, 2007)) take into account some of these extra costs. APT determined that the EPA underestimated actual costs by between 1.9- and 31.6-fold. Some industry estimates are even higher (e.g. the Chemical Producers and Distributors Association, presented at the A/C Annual Conference, San Antonio, TX, May 6 - 8, 2008).

It is important to note here, that over time, with increased demand and efficiency of scale, *in vitro* methods are likely to become much cheaper.

Application of an Integrated Testing (IT) Strategy to the EDSP

IT strategies are based on prioritization, screening, and targeted testing, using multiple tools and existing data in a stepwise and iterative process. Prioritization narrows the field to chemicals of concern, multifaceted tools screen for potential hazards, and a weight-of-evidence approach, including exposure, grouping of chemicals and "read-across" between related chemicals, is then applied to determine which, if any, further targeted testing is required for risk assessment. Unlike the current EDSP tiered system, this approach is consistent with recent American, European,¹³ and International¹⁴ proposals for the detection of endocrine-active chemicals.

¹³ Committee on Toxicity Testing and Assessment of Environmental Agency, National Research Council 2007. Toxicity Testing in the 21st Century: A Vision and Strategy. National Academies Press, Washington, DC.
¹⁴ European Commission, 2005. Scientific and Technical Guidance on the use of the OECD 2002 Consensus Framework for the Testing and Assessment of Endocrine-Disrupting Chemicals. Organisation for Economic Co-operation and Development.

Table 1: EPA Proposed EDSP Assays

Tier 1	Validation Status as of June 2009
In vitro	
ER binding	EPA validation, peer review April 2009
ER/TA	OECD Draft TG 455
AR binding	EPA validation, peer review December 2007
Steroidogenesis: H295R	EPA validation, peer review June 2008
Aromatase	EPA validation, peer review January 2008
In vivo	
Uterotrophic	OECD TG 440
Hershberger	OECD Draft TG 441
Pubertal female	EPA validation, peer review November 2007
Pubertal male	EPA validation, peer review November 2007
Amphibian metamorphosis	EPA validation, peer review December 2007
Fish short-term reproduction	EPA validation, peer review January 2008
Adult male 15-day	EPA validation, peer review October 2007
Tier 2	
Mysid 2-generation	Estimated December 2010
Fish 2-generation	Estimated December 2010
Amphibian Growth/Reproduction	Estimated December 2010
Avian 2-generation	Estimated December 2011
Mammalian 2-generation	OECD TG 416

ER = estrogen receptor; AR = androgen receptor
US Environmental Protection Agency, semi-annual report concerning the status of activities under the Endocrine Disruptor Screening Program (EDSP) as provided under Clean Air Act (CAA) 112 (a) (1) (7) of the Settlement Agreement in NRC v. EPA, No. C-99-0370 (WHA (DC), Calif. 17 June 2009)

Table 3: Cost Estimates for the Proposed Tier 1 Assays

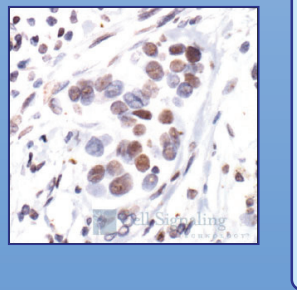
Assays as of June 2009	EPA ICR 2009 ¹				Other estimates	High	Low
	Assay cost ²	Other costs ³	Assay cost	Assay cost only			
In vitro							
ER binding	9,539	6,677	1,000 - 7,000 ⁴	1,000	9,539		
ER/TA	9,539	6,677	2,500 - 7,500 ⁴	2,500	9,539		
AR binding	10,335	7,249	1,500 - 8,000 ⁴	1,500	10,335		
Steroidogenesis: H295R	15,657	10,960	22,200 - 36,300 ⁴	15,675	16,300		
Aromatase	24,881	17,467	37,600 - 61,400 ⁴	24,881	61,400		
In vivo							
Uterotrophic	25,178	17,624	38,000 - 47,000 ⁴	25,178	47,000		
Hershberger	32,740	23,618	52,400 - 85,500 ⁴	32,740	85,500		
Pubertal female	66,966	46,877	107,800 - 175,800 ⁴	66,966	175,800		
Pubertal male	66,915	46,841	107,700 - 175,700 ⁴	66,915	175,700		
Amphibian metamorphosis	48,000	33,600	89,000 - 105,000 ⁴	48,000	105,000		
Fish short-term reproduction	84,424	59,097	76,000 - 97,000 ⁴	76,000	97,000		
Adult male 15-day	67,900	165,000 - 212,000 ⁴	67,900	212,000			
Analytical chemistry	9,120	6,384		9,120	9,120		
Total	439,474			439,475	1,044,233		

¹ US EPA, EPA Information Collection Activities, Submission to OMB for Review and Approval, Comment Request for Tier 1 Screening of Certain Chemicals Under the Endocrine Disruptor Screening Program (EDSP) as provided under Clean Air Act (CAA) 112 (a) (1) (7) of the Settlement Agreement in NRC v. EPA, No. C-99-0370 (WHA (DC), Calif. 17 June 2009)
² EPA, Information Collection Activities, Submission to OMB for Review and Approval, Comment Request for Tier 1 Screening of Certain Chemicals Under the Endocrine Disruptor Screening Program (EDSP) as provided under Clean Air Act (CAA) 112 (a) (1) (7) of the Settlement Agreement in NRC v. EPA, No. C-99-0370 (WHA (DC), Calif. 17 June 2009)
³ EPA, Information Collection Activities, Submission to OMB for Review and Approval, Comment Request for Tier 1 Screening of Certain Chemicals Under the Endocrine Disruptor Screening Program (EDSP) as provided under Clean Air Act (CAA) 112 (a) (1) (7) of the Settlement Agreement in NRC v. EPA, No. C-99-0370 (WHA (DC), Calif. 17 June 2009)
⁴ EPA, Information Collection Activities, Submission to OMB for Review and Approval, Comment Request for Tier 1 Screening of Certain Chemicals Under the Endocrine Disruptor Screening Program (EDSP) as provided under Clean Air Act (CAA) 112 (a) (1) (7) of the Settlement Agreement in NRC v. EPA, No. C-99-0370 (WHA (DC), Calif. 17 June 2009)

Additional shortcomings of the current approach

- Does not consider
 - o physicochemical data,
 - o existing toxicological data
 - o QSAR modeling
- o *in vivo* assays require 2 - 4 weeks to perform
- o Requires enormous quantities of chemical
- o Inconsistent with international protocols, i.e.:
 - o human recombinant AR and ER

Characteristics that are not suitable for a screen of tens of thousands of chemicals



The Tier 1 Battery has not been validated

The performance of the Tier 1 Battery as a whole has not been assessed. Evaluation of the individual assays did not include a standard reference set of chemicals. For several of the assays, no chemical was negative for all endpoints, suggesting that these assays may have extremely low specificity; at any rate the specificity could not be determined. EPA's Scientific Advisory Panel itself concludes: "Given the initial evaluation of the preliminary data from the Tier 1 assays there may be a tendency towards positives. This may be due to the fact that EPA has not provided a thorough evaluation with a sufficient number of compounds, or the screen is too sensitive, or perhaps the battery lacks specificity."¹⁵ The SAP makes a similar statement regarding the assessment of thyroid effects: "However, it is not currently possible to estimate what (the) levels of false results might be...; determination of false-positive and false-negative rates is part of validation, and this is clearly missing from several of the assay assessments."

EPA's Information Collection Request states that "(c)hemicals that go through Tier 1 screening and are found to have the potential to interact with the estrogen, androgen, or thyroid hormone systems will proceed to the next stage of the EDSP where EPA will determine which if any of the Tier 2 tests are necessary based on the available data." As described above, many of these assays have demonstrated low selectivity and high variability, which, combined with a lack of experience or guidance for interpretation of combined results, is very likely to lead to a large number of false positive determinations and therefore a large number of chemicals unnecessarily progressing to Tier 2 testing, which is extremely animal-intensive and expensive (one standard 2-generation reproductive toxicity test uses 2,600 rats and costs \$380,000; one developmental toxicity study in two species uses 1,300 rats, 600 rabbits and costs \$227,000).

To improve the Tier 1, the Panel suggested the inclusion several additional *in vitro* methods human recombinant ER and AR binding and transcriptional activation assays (all of which are currently in validation through the OECD) and the steroidogenesis assay (an assay using H295R cells which is being developed by the EPA).

¹⁵ SAP Minutes No. 2008-03. A set of scientific issues being considered by the Environmental Protection Agency regarding the Endocrine Disruptor Screening Program (EDSP) Tier 1 Screening Battery, June 11, 2008. EPA. <http://www.epa.gov/epa/scp3/p20080611minutes08-03-25.pdf>

Table 4: *in vitro/silico* methods under development

QSAR/Expert Systems	Several expert systems incorporating QSAR modeling for estrogen- and androgen-binding are being developed throughout the world. ^{1,2,3,4,5,6} One such system was recently subjected to expert review to demonstrate application of the OECD guidelines for validation of QSARs. ^{7,8}		
OECD activities			
ER binding	hERo (2 different protocols)	Validation starting early 2008	Estrogen agonist, antagonist
ER transcriptional activation	4 methods under development using human ERo, and one using ERβ	in validation studies	Estrogen agonist, antagonist
AR binding	Rat AR (binding domain is identical to human AR)	Prevaluation under RePoTest (EC)	Androgen agonist, antagonist
AR transcriptional activation	4 methods under development using human AR	Two methods nearing completion of validation studies; the other two validation starting in 2008	Androgen agonist, antagonist
Thyroid hormone (TH) binding	hTH	Prevaluation studies in Japan	Thyroid hormone agonist
TH transcriptional activation	hTH	Under development by Japan	TH agonist, antagonist
Aromatase	Human placental aromatase	Validation studies complete; peer review in 2008	Estrogen synthesis
Steroidogenesis		Validation studies complete; peer review in 2008	Steroid synthesis (estrogen and testosterone)
US EPA/NIH/ER/CeTox			
ER and AR binding and transcriptional activation pre-screening strategy to prioritize chemicals with respect to agonist/antagonist activity; solubility and cytotoxicity assessments were also performed on all chemicals. Pre-validation studies using 117 chemicals have been completed. ⁹			
Discover/RNH Chemical Genomics Center			
ER and AR binding and transcriptional activation assays currently being validated in high-throughput screening format. ^{10, 11}			
T Screen	thyroid hormone dependent cell proliferation	Proof of principle, method used to test flame retardants ¹²	Thyroid hormone function

¹ Boudreau J, 2004. A computationally based identification of estrogen receptor ligands. *Proc Natl Acad Sci USA* 101:14233-14238.
² Boudreau J, 2004. A computationally based identification of androgen receptor ligands. *Proc Natl Acad Sci USA* 101:14239-14244.
³ Boudreau J, 2004. A computationally based identification of thyroid hormone receptor ligands. *Proc Natl Acad Sci USA* 101:14245-14250.
⁴ Boudreau J, 2004. A computationally based identification of thyroid hormone receptor ligands. *Proc Natl Acad Sci USA* 101:14251-14256.
⁵ Boudreau J, 2004. A computationally based identification of thyroid hormone receptor ligands. *Proc Natl Acad Sci USA* 101:14257-14262.
⁶ Boudreau J, 2004. A computationally based identification of thyroid hormone receptor ligands. *Proc Natl Acad Sci USA* 101:14263-14268.
⁷ OECD, 2005. Guidance Document on the Validation of QSARs. *OECD Series on Chemical Safety*, No. 23. Paris: OECD.
⁸ OECD, 2005. Guidance Document on the Validation of QSARs. *OECD Series on Chemical Safety*, No. 23. Paris: OECD.
⁹ EPA, 2008. Endocrine Disruptor Screening Program (EDSP) Tier 1 Screening Battery. EPA/600/R-08/001. Washington, DC: EPA.
¹⁰ EPA, 2008. Endocrine Disruptor Screening Program (EDSP) Tier 1 Screening Battery. EPA/600/R-08/001. Washington, DC: EPA.
¹¹ EPA, 2008. Endocrine Disruptor Screening Program (EDSP) Tier 1 Screening Battery. EPA/600/R-08/001. Washington, DC: EPA.
¹² EPA, 2008. Endocrine Disruptor Screening Program (EDSP) Tier 1 Screening Battery. EPA/600/R-08/001. Washington, DC: EPA.