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

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Abstract

The US EPA plans to initiate its Endocrine Disruptor Screening Program in early 2009. The EPA has designed its program in two tiers, consisting of *in vivo* and *in vitro* screens and tests; the results of Tier 1 screens would trigger further Tier 2 testing. However, the proposed screening battery has been criticized for its cost, length, and low specificity. We present the costs, in animal numbers and dollars, of the full Tier 1 battery as proposed by EPA, and a suggested revised intelligent testing strategy that would save animals and resources and result in more efficient screening and characterization of the endocrine-disrupting potential of manufactured chemicals. The preliminary tier includes physical and chemical data, existing toxicological data, and *in vitro* and (Q)SAR 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, chemical-specific strategy for further screening or testing. Such a strategy would greatly reduce the use of animal testing for both Tier 1 and 2, while maintaining a high level of sensitivity for identification and classification of endocrine disrupting chemicals.

Table 1: EPA Proposed Tier 1 Assays Over Time

July, 2007 (FR notice)	March, 2008 (SAP presentation)	June, 2008 (SAP summary)	December, 2008 (EPA Implementation Update)	Validation Status According to EPA, Dec 2008
In vitro:	***In vitro:***	***In vitro:***	***In vitro:***	
ER binding or TA	ER binding	ER binding	ER binding	Not ready
	ER TA		ER TA	Valid, OECD Draft TG 455
Androgen binding or TA	AR binding	AR binding	AR binding	EPA validation
	AR TA			
Steroidogenesis	Steroidogenesis	Steroidogenesis	Steroidogenesis: sliced testes	Validation terminated
		Aromatase	Steroidogenesis: H295R	Valid, OECD Draft TG
			Aromatase	EPA validation
In vivo:	***In vivo:***	***In vivo:***	***In vivo:***	
Uterotrophic	Uterotrophic	Uterotrophic	Uterotrophic	Valid, OECD TG 440[1]
Hershberger	Hershberger	Hershberger	Hershberger	Valid, OECD Draft TG
Pubertal female	Pubertal female	Pubertal female	Pubertal female	EPA validation
		Pubertal male	Pubertal male	EPA validation
AMA	AMA	AMA	AMA	EPA validation, OECD Draft TG
Fish screening	Fish screening	Fish short-term reproduction	Fish short-term reproduction	EPA validation, OECD Draft TG
Alternates:	***Alternates:***		Adult male 15-day	EPA validation
Aromatase: placental	Aromatase: human placental			
Pubertal male	Pubertal male			
Intact adult male rat	Intact adult male rat		***Alternates:***	
	In utero through lactation		*In utero* through lactation	No longer under consideration as Tier 1: too expensive

ER = estrogen receptor; AR = androgen receptor

The individual assays of the Tier 1 battery have not been properly validated

At the FIFRA SAP meeting held to discuss the Tier 1 battery on March 12, 2008, validation of the individual assays was not complete and the composition of the Tier 1 battery had not been finalized. At the time of the SAP meeting, eight assays that had been through some sort of assessment were reviewed. Of the *in vivo* tests to be used in the EDSP Tier 1, the Uterotrophic assay has been accepted for international use by the OECD (Test Guideline 440)[ii], in spite of persistent and unresolved concerns regarding the variability of the assay and the validation procedure. In fact, both the European Centre for the Validation of Alternative Methods (ECVAM) and the US Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) have issued statements refuting the validation status of this method.[iii,iv,viii]

The Hershberger, Amphibian Metamorphosis and Short-term (21-day) Fish Reproduction Assays are currently being evaluated by the OECD. Review of revised test guidelines for these methods is expected in March or April of 2009.

The EPA performed its own peer reviews of seven additional assays (the male and female pubertal, adult male 15-day, fish short-term reproduction, amphibian metamorphosis, and the androgen receptor binding and aromatase assays).[ix][2][3][4][5][6][7] The Peer Review Reports for the animal assays were generally equivocal regarding the validation status; nevertheless, the EPA has claimed that these peer reviews constitute validation, irrespective of the substantive criticisms mentioned in the reviews. Similarly, the EPA's Meeting Minutes of the FIFRA SAP of March 25-26 that were released on June 11, 2008[viii], concludes that the assays comprising the Tier 1 battery are valid and adequate for screening, in spite of numerous public comments and the SAP's own arguments to the contrary.

For example, the SAP concludes that "significant concerns remain about the specifics of the male pubertal assay" and pointed out that in both pubertal assays, there were no negative compounds (in fact, no compounds were negative in the fish short-term or amphibian metamorphosis assays either), concluding that "this fact stands as a major limitation to the Tier 1 battery." The Panel pointed out that the effect of body weight on organ weight endpoints in the pubertal assays has not been adequately addressed (in other words, validation of the pubertal assays is incomplete). The Panel had serious concerns regarding the transferability of the hormone assays required in the pubertal and amphibian assays – hormone assessment is a primary element of these assays, and inter-lab reproducibility is an essential element of a validated assay. The Panel noted that the amphibian assay requires expensive and large specialized equipment, which limits the assay's transferability. In addition, the Panel questioned the reproducibility of the fish short-term reproduction assay, particularly for the fecundity endpoint, a critical aspect that would have been addressed by adequate validation.

The Center for Regulatory Effectiveness (CRE) has submitted an Information Quality Act Request for Correction regarding the validation status of the Amphibian Metamorphosis Assay. The CRE provides several pages of examples from the peer reviewers' statements supporting the conclusion that the assay has not been validated and asks the EPA to "correct its many public statements that the Amphibian Metamorphosis Assay is reproducible and properly validated." Similar arguments could be made regarding EPA's conclusions of its other sponsored peer reviews, including both the male and female pubertal assays.

[ix] Peer Review Report of the pubertal male (http://www.epa.gov/endo/pubs/pubertal_male_peer_review_final.pdf, accessed March 14, 2009)
[2] Peer Review Report of the pubertal female: (http://www.epa.gov/endo/pubs/pubertal_female_peer_review_final.pdf, accessed March 14, 2009)
[3] Peer Review Report of the adult male 15-day: (http://www.epa.gov/endo/pubs/adult_male_peer_review_final.pdf, accessed March 14, 2009)
[4] Peer Review Report of the fish short-term reproduction: http://www.epa.gov/endo/pubs/fishassay_peer_review_013008.pdf, accessed March 14, 2009)
[5] Peer Review Report of the amphibian metamorphosis assay: (http://www.epa.gov/endo/pubs/ama_peer_review_121907.pdf, accessed March 14, 2009)
[6] Peer Review Report of the androgen receptor binding: (http://www.epa.gov/endo/pubs/ar-binding_peer_review_122007.pdf, accessed March 14, 2009)
[7] Peer Review Report of the aromatase assay: (http://www.epa.gov/endo/pubs/aromatase_peer_review_012208.pdf, accessed March 14, 2009)
[ii] Uterotrophic Bioassay in Rodents: a short-term screening test for oestrogenic properties (Original Guideline, adopted 16th October 2007), Chemicals Testing: OECD Guidelines for the Testing of Chemicals – Section 4: Health Effects.
http://caliban.sourceoecd.org/vl=481498/cl=16/nw=1/rpsv/cw/vhosts/oecdjournals/1607310x/v1n4/contp1-1.htm
[iii] ESAC Statement on the status of validation of the uterotrophic assay. February, 2005. (http://ecvam.jrc.it/index.htm)
[iv] Letter from ICCVAM to Jerry Smrchek (EPA), US National Coordinator to the OECD. February, 2007.
http://iccvam.niehs.nih.gov/methods/endocrine/OECDdocs/Uterotrophic/ICCVAM_UB_Ltr_Smrchek.pdf
[viii] SAP Minutes No. 2008-03. A set of scientific issues being considered by the Environmental Protection Agency regarding: the Endocrine Disruptor Screening Program Proposed Tier 1 Screening battery. June 11, 2008, EPA. http://www.epa.gov/scipoly/sap/meetings/2008/march/minutes2008-03-25.pdf

The Tier 1 Battery has not been validated

In general comments made in response to the report, the SAP itself concluded that anti-estrogenicity is inadequately covered by the battery (neither the uterotrophic nor the female pubertal assays have been validated for this purpose), and that anti-androgenicity is even more poorly addressed, while it considers the hypothalamic/pituitary/gonadal axis effects the "least well supported" in the Tier 1 battery. The Panel also noted serious concerns regarding results obtained at the high doses used in the Tier 1 assays and suggested using lower doses; however, to follow this recommendation, validation studies would need to be performed at lower doses.

Interestingly, in evaluating the redundancy of the Tier 1 for estrogenic activity, the Panel concludes: "Given an 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 the 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," essentially concluding that the battery has not been validated. The Panel 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.

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).

Introduction

The Endocrine Disruptor Screening Program (EDSP) was created through a revision to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) in 1998 that requires the EPA to propose an assay or battery of assays that have been shown to reliably characterize the risks posed by estrogenic chemicals to human health. The agency has expanded that mandate to include chemicals that affect the androgen and thyroid systems in humans and other animals.

The EPA has devised a two-tiered screening program: Tier 1 screening assays (the Tier 1 Battery) are intended to "identify substances that have the potential to interact with the EAT [estrogen/androgen/thyroid] hormonal systems…," and the results would then be used to trigger Tier 2 testing (the Tier 2 Battery, proposed to consist mainly of reproductive and developmental toxicity studies) which is intended to characterize the potential risks of those substances. The constituent assays of the proposed Tier 1 battery differ slightly every time the EPA posts a list (**Table 1**). However, based on the assays listed in the summary of the FIFRA Scientific Advisory Panel (SAP) meeting held in March, 2008,[i] and on those assays the EPA considered valid by December of 2008, the Tier 1 is likely to consist of 4 *in vitro* assays [an estrogen receptor transcriptional activation assay, validated at the Organization for Economic Co-operation and Development (OECD); an androgen receptor binding assay validated by the EPA; the H295R steroidogenesis assay validated by the EPA and through the OECD; and an aromatase assay validated by the EPA] and 6 *in vivo* assays (the uterotrophic, OECD TG 440; Hershberger, being validated through the OECD; male and female pubertal assays validated by the EPA; the amphibian metamorphosis assay, validated by the EPA and in process at the OECD; and the fish short-term (21-day) reproduction assay, validated by the EPA and in process at the OECD).

Phase I of the EDSP, which is now proposed to begin sometime during 2009, involves Data Call-In (DCI) requests comprised of the entire Tier 1 Battery for 73 chemicals, including 64 pesticide active ingredients and nine high production volume (HPV) chemicals that are also inert ingredients in pesticide formulations. These chemicals were announced as a draft list in 2007; the EPA has informally stated that number of chemicals on the final list will be 69; however, the EPA has not yet officially released this information.

[i] SAP Minutes No. 2008-03, of the FIFRA SAP Meeting held March 25-26, 2008 to review and consider the EDSP proposed Tier 1 screening battery (www.epa.gov/scipoly/sap/meetings/2008/march/minutes2008-03-25.pdf).

Additional shortcomings of the current Tier 1

- Does not consider physiochemical data, existing toxicological data or QSAR modeling
- Contains several *in vivo* assays that measure the same or highly related endpoints, i.e.:
  - the Uterotrophic and Female Pubertal
  - the Hershberger, Male Pubertal and 15-day Intact Male
- Contains *in vivo* assays that require 2 – 4 weeks to perform
- Requires enormous quantities of each chemical (especially the aquatic assays)
- Is not consistent with assays undergoing international validation, i.e.:
  - OECD protocols use human recombinant AR (hrAR), but the EPA's AR binding protocol uses rat cytosol whereas OECD's protocol uses human recombinant AR

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

Table 2: Animals used in the Proposed Tier 1 Assays

According to EPA as of Dec 2008	Animals used per chemical	Species	Theoretical mechanism
In vitro:			
ER TA: CERI version (OECD TG 455)		endogenous human ER*α*	Estrogen agonists
AR binding: rat cytosol		rat prostate cytosol	Androgen agonists, antagonists
Steroidogenesis - H295R		human	Steroid synthesis (estrogen and testosterone)
Aromatase - human placental and recombinant		human	Steroid synthesis (estrogen)
In vivo:			
Uterotrophic (OECD TG 440)	18	rat, mouse	Estrogen agonists, antagonists
Hershberger	18 - 36	rat, mouse	Androgen agonists, antagonists
Pubertal female plus thyroid function	45	rat	Estrogen agonists, antagonists, synthesis; HPG axis, HPT axis
Pubertal male plus thyroid function	45	rat	Androgen agonists, antagonists, testosterone synthesis; HPG, HPT axes
Adult male 15-day	60	rat	Androgen agonists, antagonists, testosterone synthesis; HPG, HPT axes
Amphibian metamorphosis	320	*Xenopus laevis*	HPT axis
Fish 21 day fish screen	72	fathead minnow	Estrogen and androgen agonists and antagonists, steroid synthesis, HPG, HPT axes
Total:	**578 - 596**		

Table 3: Various Cost Estimates for the Proposed Tier 1 Assays

EDSP Tier 1 assay cost estimates (USD)	APT 1998[1] (median)	APT 2003[2] (median)	EPA 2008[3]	Other estimates 2008 - 2009	Low	High
In vitro:						
ER Transcriptional Activation: human ER	4,900			2,500 - 7,500[4]	2,500	7,500
AR binding: rat cytosol	4,200	7,500	7,066	1,500 - 8,000[4]	1,500	8,000
Steroidogenesis: rat testes	7,500	6,850	11,717	22,200 - 36,300[6]	6,850	36,300
Aromatase - human placental and recombinant		8,175	19,808	37,600 - 61,400[6]	8,175	61,400
In vivo:						
Uterotrophic	26,000 - 67,500*	14,500	20,068	38,000 – 47,000[5]	14,500	67,500
Hershberger	34,400 - 105,000*	23,880	27,579	52,400 - 85,500[6]	27,579	105,000
Pubertal female plus thyroid function	34,700 - 81,000*	44,700	56,725	107,800 - 175,800[6]	34,700	175,800
Pubertal male plus thyroid function		44,000	56,680	107,700 - 175,700[6]	44,000	175,700
Adult male 15-day	68,000	67,900	67,900	165,000 – 212,000[5]	67,900	212,000
Amphibian metamorphosis	17,000		34,894	89,000 - 105,000[5]	34,894	105,000
Fish gonadal recrudescence (21-day fish screen)	40,000		52,340	76,000 – 97,000[5]	76,000	97,000
					$318,598	**$938,000**

[1]EDSTAC Final Report, August 1998. EPA, Endocrine Disruptor Screening Program. http://www.epa.gov/endo/pubs/edspoverview/finalrpt.htm (accessed March 9, 2009).

*mandatory vs optional endpoints

[2]Applied Pharmacology and Toxicology, Inc. May 23, 2003 (http://www.apt-pharmatox.com/pdf/2003EDSP-CostReport.final.pdf, accessed March 12, 2009).

[3]According to the Chemicals Producers and Distributers Association, presented at the AIC Annual Conference, San Antonio, TX, May 6 - 8, 2008.

[4]Jeff Pregenzer, CerTox, personal communication, 2009: cost estimates per chemical are based on number of chemicals assayed: ERa binding: 1 chemical = $6,845, 16 or more chemicals = $1050; AR binding: 1 chemical = $7563, 16 or more chemicals = 1500; ER or AR transactivation: 1 chemical = 7,760, 16 or more = $2,550.

[5]Applied Pharmacology and Toxicology, Inc., March 10, 2008 Comments on EPA's Information Collection Request ("the ICR") developed for theAgency's Endocrine Disruptor Screening Program; Draft Policies and Procedures for Initial Screening, 72 Fed. Reg. 70861 (December 13, 2007).

[6] Estimated from the EPA 2008 estimates using the multipliers determined by APT in the March Comments referenced above. APT determined that the EPA underestimated assay costs by between 1.9- and 3.1-fold.

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

3. MECHANISTIC *IN VIVO* SCREENS

4. TARGETED TESTING

EVIDENCE ANALYSIS

Read-across → Exposure/ Expected use → ADME information → Hazard Assessment

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 the 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 518 - 536 animals and cost between $324,000 to more than $938,000 USD per chemical*** (conservatively estimated with no range-finding or repeat experiments; **Tables 2** and **3**). ***Phase I of the EDSP is therefore likely use over 37,000 animals and to cost anywhere from $22,356,000 to in excess of $67,700,000.***

Most of the costs listed in Table 3 are estimates for running the assays only, and do not include other associated costs, such as paper work, management, technical and clerical costs, which ***could add another 100 – 300% to the cost estimates listed***. 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 3.1-fold. Some industry estimates are even higher (e.g. the Chemicals Producers and Distributers Association, presented at the AIC 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.

A more effective approach

Recognizing the need for a faster, more accurate, valid screening battery, we propose an alternative tiered strategy here. The preliminary tier includes physical and chemical data, existing toxicological data, and *in vitro* and (Q)SAR 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, chemical-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.

This strategy is reflected in the Organization for Economic Cooperation and Development (OECD) Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals (framework), which is organized into 5 levels (**Table 4**). While the framework is not intended as a tiered system, the framework nevertheless suggests a logical approach to the sequential and targeted gathering of data. Level 1 assays sort and prioritize chemicals for testing based on existing information. Level 2 consists entirely of *in vitro* assays that address possible mechanisms of action. Not until Level 3 are animal tests involved as *in vivo* mechanistic tests. Chemicals can be screened and prioritized using the fastest, least expensive methods, and the number of animal tests performed overall is greatly reduced.

An organization similar to the OECD framework that includes preliminary tiers that first assess physiochemical and pre-existing toxicological data, plus *in silico* and a much broader range of *in vitro* mechanistic assays (**Table 5**) would be more logical, efficient, economical, and use fewer animals (**Diagram**).

Table 4: OECD Conceptual Framework

Level 1	Physical and chemical properties Human and environmental exposure Hazard (available toxicological data)
Level 2	***In vitro:*** Estrogen and androgen receptor binding Thyroid hormone receptor binding Transcriptional activation Aromatase Steroidogenesis Arylhydrocarbon receptor binding QSARs High-throughput screens Thyroid function Fish hepatocyte vitellogenin
Level 3	***In vivo:*** Uterotrophic Hershberger Fish VTG
Level 4	Enhanced 407 Male and female pubertal assays Adult intact male
Level 5	1 and 2 generation reproduction

Application of an Intelligent 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, multi-faceted 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,[11] European,[12] and International[13] proposals for the detection of endocrine-active chemicals.

[11] National Research Council. Toxicity Testing in the Twenty-first Century: A Vision and a Strategy. Committee on Toxicity and Assessment of Environmental Agents, National Research Council. ISBN: 0-309-10989-2, 146 pages (2007).
[12] Bradbury SP, et al. 2004. Meeting the scientific needs of ecological risk assessment in a regulatory context. Environ Sci Technol 38, 463A-470A.
[13] OECD. 2002. Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals. Organization for Organization for Economic Cooperation and Development, Environment Directorate, Paris.

Table 5: 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	hrERα (2 different protocols)	Validation starting early 2008	Estrogen agonist, antagonist
ER transcriptional activation	5 methods under development using human ERα, and one using ERβ	HeLa cell-based protocol has been validated and OECD Test Guideline is awaiting approval; all others in validation studies	Estrogen agonist, antagonist
AR binding	Rat rAR (binding domain is identical to human AR)	Prevalidation under ReProTect (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	hrTH	Prevalidation studies in Japan	Thyroid hormone agonist
TH transcriptional activation		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/NHEERL/CeeTox			
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]			
DiscoverRX/NIH Chemical Genomics Center			
ER and AR binding and transcriptional activation assays currently being validated in high-throughput screening format[10]			
T Screen	thyroid hormone dependent cell proliferation	Proof of principle, method used to test flame retardants[11]	Thyroid hormone function

1. Bradbury S et al. 2000. A computationally based identification algorithm for estrogen receptor ligands: part 1. Predicting hERalpha binding affinity. Toxicol Sci. 58(2):253-69.
2. Klopman G and SK Chakravarti. 2003. Structure-activity relationship study of a diverse set of estrogen receptor ligands (I) using MultiCASE expert system. Chemosphere. 51(6): 445-59.
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5. The 13th International Workshop on Quantitative Structure-Activity Relationships (QSARs) in the Environmental Sciences, June 8 - 12, 2008, Syracuse, New York: http://www.syrres.com/qsar2008/QSAR%202008%20Abstract%20Book%20-%20Complete.pdf
6. Vinggaard AM et al. 2008. Screening of 397 chemicals and development of a quantitative structure–activity relationship model for androgen receptor antagonism. Chem Res Toxicol. 21(4):813-23.
7. OECD. 2007. Guidance Document on the Validation of (Q)SAR Models. Organization for Economic Cooperation and Development, Environment Directorate, Paris. http://www.oecd.org/dataoecd/55/35/38130292.pdf
8. EPA's Pesticide Program Dialogue Committee Work Group on 21st Century Toxicology/New Integrated Testing Strategies teleconference, September 4, 2008.
9. Wilson, VS., Judith E. Schmid, JE, David P. Blakeman, DP, Jeffrey F. Pregenzer, JF, McKim JM, Jr, Goldman, JM, and L. Earl Gray, Jr. 2007. Evaluation of an *In Vitro* Pre-screening Strategy to Prioritize Environmental Chemicals for Further Testing. Androgen and Estrogen Receptor Mediated Activity. Presented at the 46th Annual Meeting of the Society of Toxicology, Charlotte, North Carolina.
10. Olsen, K. Presentation at NTP Request for Information Meeting: High Throughput screening approaches to toxicology, September 11 – 12, 2008. NIEHS, Research Triangle Park, NC.
11. Hamers T et al. 2006. *In vitro* profiling of the endocrine-disrupting potency of brominated flame retarnts. Toxicol. Sci. 92(1):157-73.