May 22, 2009

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Re: Information Collection Request (EPA ICR No. 2249.01) EPA-HQ-OPPT-2007-1081

Dear Mr. Rostker and Dr. Wooge:

These comments are submitted on behalf of the Alternatives Research and Development Foundation, the American Anti-Vivisection Society, Humane Society Legislative Fund, The Humane Society of the United States, People for the Ethical Treatment of Animals and the Physicians Committee for Responsible Medicine. The parties to this submission are national animal protection, health, and scientific advocacy organizations with a combined constituency of more than 12 million Americans who share the common goal of promoting reliable and relevant regulatory testing methods and strategies that protect human health and the environment while reducing, and ultimately eliminating, the use of animals.

On April 15, 2009, the Environmental Protection Agency (EPA; hereafter known as the Agency) submitted a new information collection request (ICR) to the Office of Management and Budget (OMB) regarding information collection activities associated with Phase I of its Endocrine Disruptor Screening Program (EDSP). At the same time, EPA published in the Federal Register its final Policies and Procedures for Initial Screening (74 FR 17560).

It is our understanding that these comments should not address the EDSP directly, but rather “to comment on the Agency’s practical utility justification of the collection activities and its related burden and cost estimates as they presented in the ICR.”

Therefore our comments are directed at the utility and cost of Phase I of the EDSP.

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I. Utility of Phase I of the EDSP: The EDSP Phase I is not likely to provide new regulatory information

A. Reliability and reproducibility of the assays to be used

We and others have pointed out on a number of occasions that the Tier 1 assays listed in the ICR have not been shown to be reproducible or sufficiently specific to adequately identify chemicals that are capable of interacting with estrogen, androgen or thyroid hormone receptors or systems.\(^2\)\(^3\)\(^4\) In response, the EPA has merely described the process it had taken to review the assays and concluded that the majority of the assays “had indeed completed the validation process.”\(^5\) Completing a validation process is not the same as having been validated. Our comments and those of others do not argue that many of the assays have not gone through a validation process; rather, we are arguing that the evaluations of these assays were not as unequivocally positive as the EPA has publically represented.

Since our specific concerns have been detailed elsewhere, we will not repeat them here. The EPA has provided a response to some of these concerns;\(^6\) however, several of the EPA’s responses highlight, rather than mitigate, our concerns. For example, in response to our concerns about inter-laboratory variability (reproducibility) of the amphibian metamorphosis assay and the male and female pubertal assays, the EPA acknowledged that, while different labs did indeed obtain different results, “the overall trend was consistent among laboratories.” This admission is disconcerting since for many Phase I chemicals, this will be the first time they have been run in the Tier 1 assays and, unless recipients of test orders all use the same few contract laboratories with experience running these assays, it is likely this will be the first time these assays will be run in some labs. In other words, the Phase I testing will likely not be performed in multiple, experienced labs, there will be no “overall trends” available for comparison, and consequently, interpretation of results is likely to be extremely difficult or impossible.

In response to our concerns about specificity (ability to distinguish true negatives from true positives) of several of the assays, the EPA argued that, “(b)ecause the Tier 1 assays will operate in a battery and will only identify a chemical’s potential to interact with the endocrine system, rather than to predict actual effects, the rate of false positives and negatives for individual assays in the battery is not an essential part of validation.” This reasoning is deeply flawed. Logically, if a battery consists of multiple assays of low specificity, the combined results will be heavily skewed toward false positives. For several of the assays, chemicals tested in the validation studies resulted in NO negatives (not even the negative controls were negative for some endpoints). What is the

\(^2\) Comments submitted by People for the Ethical Treatment of Animals et al., Crop Life America, the American Chemistry Council, the Center for Regulatory Effectiveness, available in Docket ID no. EPA-HQ-OPP-2008-0012.
\(^4\) Physicians Committee for Responsible Medicine (PCRM) Comments to OMB on the Endocrine Disruptor Screening Program (EDSP), available in Docket ID no. EPA-HQ-OPPT-2007-1080.
conceivable value of a collection of assays that are not capable of distinguishing positives from negatives?

Furthermore, it is disconcerting that the EPA has offered no discussion or guidance on interpretation. In response to a concern expressed regarding the draft ICR that “the agency has yet to provide guidance on how results of the individual assays will be interpreted…,” the EPA states that “the current [lack of] availability of final SEPs and WOEs for EDSP related determinations does not preclude the Agency form evaluating the potential interaction of a chemical with the endocrine system”. The EPA cites its extensive experience with WOE approaches in other assessment areas and suggests that this experience will translate to the EDSP, yet no one, including the Agency itself, has experience interpreting the result of the Tier 1 assays as a battery.

The ICR states that the EPA has “considered data from prototypes of the assays included in the current EDSP Tier 1 screen, along with other existing data in preparing the risk assessments of procymidone and vinclozolin; however, in the Tolerance Reassessment Progress and Risk Management Decision (TRED) for procymidone, no mention is made of data from a Tier 1-like assay. In fact, the TRED states: “In several studies, a number of testicular effects were observed at one or more dose levels in the developmental, multi-generation, and chronic toxicity studies in rats. When additional appropriate screening and/or testing protocols currently being considered under the Agency’s Endocrine Disruptor Screening Program (EDSP) have been developed, procymidone may be subjected to further screening and/or testing to better characterize effects related to endocrine disruption.” In other words, Tier 2 testing has already been performed for procymidone and did not contribute to the tolerance-setting decision, which was based primarily on carcinogenicity considerations. Vinclozolin, on the other hand, is a known modulator of androgen activity and has been thoroughly assessed in detailed studies resembling both Tier 1 and Tier 2 assays. Interestingly, the TRED for vinclozolin states “(h)owever, the human consequence of many of the low dose effects in male rats such as reduced ano-genital distance, areola and nipple development, and reduced prostate weight is unknown.” Ultimately, vinclozolin (and its primary active metabolite, 3,5-dichloroaniline) is also regulated based on its potential carcinogenicity (which is believed to be related to its anti-estrogenic activity) and not directly on data obtained from Tier 1- or Tier 2-like assays. Additionally, the EPA has never evaluated Tier 1 data for its intended purpose: to determine what, if any Tier 2 testing is needed for risk assessment.

The ICR 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

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8 www.epa.gov/pesticides/reregistration/procymidone/
9 www.epa.gov/pesticides/reregistration/vinclozolin/
and costs $380,000; one developmental toxicity study in two species uses 1,300 rats, 660 rabbits and
costs $127,000).

B. The chemicals to be tested in Phase I of the EDSP are already among the most data rich
chemicals in existence.

Of the 67 chemicals on the final list for Phase I testing, 58 are pesticide active ingredients and 9 are
High Production Volume (HPV) pesticide inert chemicals.\textsuperscript{10} For registration, pesticides currently are
often subject to dozens of separate animal tests, including reproductive and chronic/lifecycle studies
in rodents, fish and birds, as well as metabolism and pharmacokinetics studies.\textsuperscript{11} These tests kill
thousands of animals and include many of the same endpoints addressed in the presumptive EDSP
Tier 2 tests. Similarly, EPA’s HPV and ChAMP programs also provide for the collection of data
which may be germane to the assessment of potential reproductive toxicity.\textsuperscript{12}

For example, Reproduction and Fertility effects (OPPTS 870.3880) and Prenatal Developmental
Toxicity (OPPTS 870.3700) tests are required for both food-use and non-food-use pesticide
Technical Grade of the Active Ingredients (TGAI). The simple mechanistic data produced by the
Hershberger, Uterotrophic, the male and female pubertal assays will not provide additional
regulatory information; indeed, chemicals tested according to the current OPPTS 870.3880 have, in
effect, already been subject to EDSP Tier 2 mammalian testing. Thus, with the possible exception of
mechanistic screening for thyroid effects, \textit{EDSP Tier 1 screens would appear to provide little or no
value-added for pesticide chemicals.}

In addition, four of the chemicals included on this draft list (atrazine, butylbenzyl phthalate, di-\textit{n}-
butyl phthalate and linuron) are included in the Revised ICCVAM List of Recommended ED
Reference Substances. Atrazine has been well characterized in terms of its endocrine activity in
numerous \textit{in vitro} and \textit{in vivo} studies, including \textit{in vivo} studies and risk assessments already
conducted by the EPA.\textsuperscript{13} Similarly, butyl benzyl phthalate (BBP) has been shown to possess
endocrine activity in \textit{vitro} and \textit{in vivo} in numerous animal studies, including those already conducted
by the EPA.\textsuperscript{14,15} The anti-androgenic activity of di-\textit{n}-butyl phthalate (DBP) has been studied in
detail\textsuperscript{16,17} Both BBP and DBP have been associated with endocrine-related effects in humans.\textsuperscript{18}
Linuron is a well-characterized weak anti-androgen, and was used as a control in OECD validation

\textsuperscript{10} 74 FR 17579. April 15, 2009; EPA Final List of Initial Pesticide Active Ingredients and Pesticide Inert
Ingredients to be Screened Under the Federal Food, Drug, and Cosmetic Act.
\textsuperscript{11} 72 FR 60934, October 26, 2007: EPA 40 CFR Parts 9 and 158: Pesticides; Data Requirements for Conventional
Chemicals.
\textsuperscript{12} 65 FR 81657, December 26, 2000; EPA 40 CFR Part 799: Testing of Certain High Production Volume Chemicals
\textsuperscript{14} Gray, et al., 2000. Perinatal exposure to the phthalates DEHP, BBP and DINP, but no DEP, DMP, or DOTP alters
sexual differentiation I of the male rat. Toxicol. Sci. 58: 350-65
\textsuperscript{15} Aso, et al., 2005. A two-generation reproductive toxicity study of butyl benzyl phthalate in rats. J. Toxicol. Sci. 30
Spec No.:39-58.
\textsuperscript{16} Bredhult, C. et al., 2007. Effects of some endocrine disruptors on the proliferation and viability of human endometrial
\textsuperscript{17} Wang Y.B., et al. 2007 Monobutyl phthalate inhibits steroidogenesis by down-regulating steroidogenic acute
\textsuperscript{18} Marsee, K. et al., 2006. Estimated daily phthalate exposures in a population of mothers of male infants exhibiting
exercises for the Hershberger assay and as a control in the EPA’s own evaluation of the 15-day intact male assay.∗∗ Due to the abundance of existing endocrine-related data, it is unlikely that further testing using the presumptive Tier 1 or Tier 2 EDSP assays will provide any additional information regarding the endocrine activity of these chemicals.

We have previously brought this to the attention of both EPA and OMB. EPA responded that it “recognizes that several of the chemicals on the initial list have been studied in detail for endocrine disrupting effects…” and goes on to explain that “…registrants will have the option of citing to existing data to satisfy part or all of the Tier 1 Orders in addition to the option of conducting testing.” Under the final Policies and Procedures for Initial Screening, the EPA will now accept existing data and “(o)ther scientifically relevant information may either be functionally equivalent to information obtained from the Tier 1 assays—that is, data from assays that perform the same function as EDSP Tier 1 assays—or may include data that provide information on a potential consequence or effect that could be due to effects on the estrogen, androgen or thyroid systems,” suggesting that, for many pesticides, data from reproductive, fertility and developmental studies will suffice, since these address the “potential consequence” of endocrine disruption and in fact will comprise the EDSP Tier 2. In addition, the purpose of the Tier 1 is to identify chemicals for testing in Tier 2; therefore it is unnecessary to test chemicals for which Tier 2 data are available in the Tier 1 battery.

However, in the final Policies and Procedures, EPA significantly mitigates the notion that it will accept such existing data by stating: “EPA generally expects that if the chemical was used by EPA as a ‘‘positive control’’ to validate one or more of the screening assays, only the data submitted related to those assays for which the chemical was used to complete the testing as part of the validation effort would be sufficient to satisfy the Tier 1 Order,” indicating that the EPA intends to collect all data for the Tier 1 battery for each of the chemicals, regardless of whether the chemical has demonstrated estrogen, androgen or thyroid activity. In its Phase I exercise, EPA is requesting the testing of chemicals in a large battery of assays that are unlikely to yield any new information that will be useful in regulating those substances.

II. Cost and Practicality of the Tier 1 battery assays

EPA cost estimates in the ICR, while apparently thorough, are difficult to interpret in terms of actual impact, and appear to be at odds with other estimates (see Appendix). For example, Policies and Procedures for Initial Screening give a deadline of 24 months from issuance of the Order for a recipient to submission of the data and a final report, yet the annual burden calculated in the ICR assumes a “3 year duration of equal annual effort.” The current cost estimates for running the assays have been revised in the current ICR (Supplement F) and are closer to estimates that have been made

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21 http://www.epa.gov/scipoly/oscpendo/pubs/adult_male_peer_review_final.pdf
22 Comment submitted by People for the Ethical Treatment of Animals (PETA), et al., available in Docket ID no. EPA–HQ–OPPT–2004–0109.
23 Physicians Committee for Responsible Medicine (PCRM) Comments to OMB on the Endocrine Disruptor Screening Program (EDSP), available in Docket ID no. EPA-HQ-OPPT-2007-1080.
24 74 FR 17560. April 15, 2009; EPA Endocrine Disruptor Screening Program; Policies and Procedures for Initial Screening.
elsewhere (Appendix, Table 1), which estimate a cost as high as $938,000 per chemical. In addition, each chemical requires a minimum use of approximately 600 animals (Appendix, Table 2). However, given the uncertainties involved in generating these estimates and that most of these studies will require pilot studies in most of the labs (since the methodology is new), it is likely that the actual cost for running these assays, in terms of both dollars and animal lives, will be much higher.

The ICR assumes that “data generation will not be directly performed by the Order recipient. Instead, EPA assumes that data generation will be performed by a contract laboratory at the request of the order recipient” and that this will result in some reduction of cost. However, several of the tests require unique expertise or equipment (those requiring hormone or histopathological examination, e.g., the amphibian and fish tests) that only a very few (one or two) contract facilities possess. Logistically, it is difficult to see how 67 chemicals will be tested in these assays in the few available contract labs within the two- to three-year time frame.

Part 3(3)(a) of the ICR (Non-duplication) cites the use of harmonized test guidelines as a sign of the EPA’s “strong commitment to avoiding potential duplication.” Yet several of the methods used by the EDSP are expressly not harmonized test guidelines. For example, the EDSP protocol for androgen receptor uses rat prostate cytosol, while other protocols in development (including those at the OECD) use human androgen receptor, even though the isolation of the receptor is a major contributing factor to variability of the assay and the use of rat receptor contributes requires interspecies extrapolation. The same is true for the proposed estrogen receptor-binding assay in validation exercises at the EPA, which uses rat uterine cytosol. It is very likely that these methods will not be used internationally. An attempt to harmonize the EPA’s Fish Reproduction Assay with the Fish Screen in development at the OECD was rejected, in a large part due to stakeholders’ objections to the high variability of the fecundity and gonadal histopathology endpoints. Thus far, the male and female pubertal assays are used exclusively in the EDSP. Although a harmonized test guideline for the amphibian metamorphosis assays is in development at the OECD, agreement has not been reached on draft test guideline. The only harmonized test guidelines currently in the EDSP are the Uterotrophic, Hershberger, and ER transcriptional activation assays.

This section of the ICR also mentions that the EPA is a charter member of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). It is implied that this involvement will lead to the incorporation of methods that reduce, refine or replace the use of animals, and that this is related to reducing duplicative testing; however, these contentions are unsubstantiated since none of the methods in the current EDSP Tier 1 were validated by ICCVAM.

In that it is unlikely to yield any new regulatory information, the EDSP Phase I is an inappropriate use of resources and waste of a large number of animal lives.

III. The current Tier 1 battery should be replaced by a more considered, step-wise approach

While we agree with EPA’s use of a tiered screening program, we do not believe the EPA’s choice of assays for a Tier 1 battery is appropriate. Recognizing the need for a faster, more accurate, valid screening battery, we propose an alternative tiered strategy. The preliminary tier includes physical and chemical data, existing toxicological data including metabolism and pharmacokinetics information, 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 (Appendix, Table 3). While the framework is not intended as a tiered system, it 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.

A strategy 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 would be more logical, efficient, economical, and use fewer animals. Most of the Phase I chemicals have already been tested in ToxCast screens that include a large number of ER and AR binding and transcriptional activation assays, and nearly half of these showed no evidence of endocrine activity (Appendix, Figure 1). This and similar information must be evaluated for indications of the pathway with which a chemical is capable of interacting before any animal testing is performed, and any subsequent testing must be tailored appropriately.

Thank you for considering our comments.

Sincerely,

Catherine Willett, PhD
Science Policy Advisor
Regulatory Testing Division
People for the Ethical Treatment of Animals

Troy Seidle
Science Policy Advisor
Humane Society of the United States

Kristie Stoick, MPH
Research Analyst
Physicians Committee for Responsible Medicine

Sara Amundson
Executive Director
Humane Society Legislative Fund

Sue A. Leary
President
Alternatives Research & Development Foundation

Tracie Letterman, Esq.
Executive Director
American Anti-Vivisection Society
### Table 1: Cost Estimates of the EDSP Tier 1 from various sources

<table>
<thead>
<tr>
<th>EDSP Tier 1 assay cost estimates (USD)</th>
<th>APT 1998&lt;sup&gt;1&lt;/sup&gt; (median)</th>
<th>APT 2003&lt;sup&gt;2&lt;/sup&gt; (median)</th>
<th>EPA 2008&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Other estimates 2008 - 2009</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER Transcriptional Activation: human ERα</td>
<td>4,900</td>
<td></td>
<td>2,500 - 7,500&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2,500 - 7,500&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2,500</td>
<td>7,500</td>
</tr>
<tr>
<td>AR binding: rat cytosol</td>
<td>4,200</td>
<td>7,500</td>
<td>1,500 - 8,000&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1,500 - 8,000&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1,500</td>
<td>8,000</td>
</tr>
<tr>
<td>Steroidogenesis: rat testes</td>
<td>7,500</td>
<td>6,850</td>
<td>11,717</td>
<td>22,200 - 36,300&lt;sup&gt;6&lt;/sup&gt;</td>
<td>6,850</td>
<td>36,300</td>
</tr>
<tr>
<td>Aromatase - human placental and recombinant</td>
<td>8,175</td>
<td>19,808</td>
<td>37,600 - 61,400&lt;sup&gt;6&lt;/sup&gt;</td>
<td>8,175 - 61,400&lt;sup&gt;6&lt;/sup&gt;</td>
<td>8,175</td>
<td>61,400</td>
</tr>
<tr>
<td><strong>In vivo:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uterotrophic</td>
<td>26,000 - 67,500*</td>
<td>14,500</td>
<td>38,000 – 47,000&lt;sup&gt;5&lt;/sup&gt;</td>
<td>14,500 - 67,500&lt;sup&gt;5&lt;/sup&gt;</td>
<td>14,500</td>
<td>67,500</td>
</tr>
<tr>
<td>Hershberger</td>
<td>34,400 - 105,000*</td>
<td>23,880</td>
<td>52,400 - 85,500&lt;sup&gt;6&lt;/sup&gt;</td>
<td>27,579 - 105,000&lt;sup&gt;6&lt;/sup&gt;</td>
<td>27,579</td>
<td>105,000</td>
</tr>
<tr>
<td>Pubertal female plus thyroid function</td>
<td>34,700 - 81,000*</td>
<td>44,700</td>
<td>107,800 - 175,800&lt;sup&gt;6&lt;/sup&gt;</td>
<td>34,700 - 175,800&lt;sup&gt;6&lt;/sup&gt;</td>
<td>34,700</td>
<td>175,800</td>
</tr>
<tr>
<td>Pubertal male plus thyroid function</td>
<td>44,000</td>
<td>56,680</td>
<td>107,700 - 175,700&lt;sup&gt;6&lt;/sup&gt;</td>
<td>44,000 - 175,700&lt;sup&gt;6&lt;/sup&gt;</td>
<td>44,000</td>
<td>175,700</td>
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<tr>
<td>Adult male 15-day</td>
<td>68,000</td>
<td>67,900</td>
<td>165,000 – 212,000&lt;sup&gt;7&lt;/sup&gt;</td>
<td>67,900 - 212,000&lt;sup&gt;7&lt;/sup&gt;</td>
<td>67,900</td>
<td>212,000</td>
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<tr>
<td>Amphibian metamorphosis</td>
<td>17,000</td>
<td>34,894</td>
<td>89,000 – 105,000&lt;sup&gt;5&lt;/sup&gt;</td>
<td>34,894 - 105,000&lt;sup&gt;5&lt;/sup&gt;</td>
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<td>105,000</td>
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<tr>
<td>21-day fish (reproduction) screen</td>
<td>40,000</td>
<td>52,340</td>
<td>76,000 – 97,000&lt;sup&gt;5&lt;/sup&gt;</td>
<td>76,000 - 97,000&lt;sup&gt;5&lt;/sup&gt;</td>
<td>76,000</td>
<td>97,000</td>
</tr>
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</table>

$318,598 $938,000

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<sup>2</sup> Mandatory vs. optional endpoints


<sup>4</sup> Jeff Pregenzer, CeeTox, personal communication, May 6 - 8, 2008.

<sup>5</sup> 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.

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Table 2: Animals used in the Proposed Tier 1 Assays

<table>
<thead>
<tr>
<th>According to EPA as of Dec 2008</th>
<th>Animals used per chemical</th>
<th>Species</th>
<th>Theoretical mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER TA: CERI version (OECD TG 455)</td>
<td>endogenous human ERα</td>
<td>rat prostate cytosol</td>
<td>Estrogen agonists</td>
</tr>
<tr>
<td>AR binding; rat cytosol</td>
<td>?</td>
<td>rat prostate cytosol</td>
<td>Androgen agonists, antagonists</td>
</tr>
<tr>
<td>Steroidogenesis - H295R</td>
<td>human</td>
<td>Steroid synthesis (estrogen and testosterone)</td>
<td></td>
</tr>
<tr>
<td>Aromatase - human placental and recombinant</td>
<td>human</td>
<td>Steroid synthesis (estrogen)</td>
<td></td>
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<tr>
<td><strong>In vivo:</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Uterotrophic (OECD TG 440)</td>
<td>18</td>
<td>rat, mouse</td>
<td>Estrogen agonists, antagonists</td>
</tr>
<tr>
<td>Hershberger</td>
<td>18 - 36</td>
<td>rat, mouse</td>
<td>Androgen agonists, antagonists</td>
</tr>
<tr>
<td>Pubertal female plus thyroid function</td>
<td>45</td>
<td>rat</td>
<td>Estrogen agonists, antagonists, synthesis; HPG axis, HPT axis</td>
</tr>
<tr>
<td>Pubertal male plus thyroid function</td>
<td>45</td>
<td>rat</td>
<td>Androgen agonists, antagonists, testosterone synthesis; HPG, HPT axes</td>
</tr>
<tr>
<td>Adult male 15-day</td>
<td>60</td>
<td>rat</td>
<td>Androgen agonists, antagonists, testosterone synthesis; HPG, HPT axes</td>
</tr>
<tr>
<td>Amphibian metamorphosis</td>
<td>320</td>
<td>Xenopus laevis</td>
<td>HPT axis</td>
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<tr>
<td>Fish 21 day fish screen</td>
<td>72</td>
<td>fathead minnow</td>
<td>Estrogen and androgen agonists and antagonists, steroid synthesis, HPG, HPT axes</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
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<td></td>
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<td>578 - 596</td>
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Table 3: The OECD Conceptual Framework for Endocrine Disruptor Screening

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Physical and chemical properties</th>
</tr>
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<tbody>
<tr>
<td>Level 2</td>
<td>In vitro:</td>
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<tr>
<td></td>
<td>Estrogen and androgen receptor binding</td>
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<td>Thyroid hormone receptor binding</td>
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<td>Transcriptional activation</td>
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<td>Aromatase</td>
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<td>Steroidogenesis</td>
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<td>Arylhydrocarbon receptor binding</td>
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<td>QSARs</td>
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<td>High-throughput screens</td>
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<td>Thyroid function</td>
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<td></td>
<td>Fish hepatocyte vitellogenin</td>
</tr>
<tr>
<td>Level 3</td>
<td>In vivo:</td>
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<tr>
<td></td>
<td>Uterotrophic</td>
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<td></td>
<td>Hershberger</td>
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<td></td>
<td>Fish VTG</td>
</tr>
<tr>
<td>Level 4</td>
<td>Enhanced 407</td>
</tr>
<tr>
<td></td>
<td>Male and female pubertal assays</td>
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<td>Adult intact male</td>
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<tr>
<td>Level 5</td>
<td>1 and 2 generation reproduction</td>
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</tbody>
</table>
HTS results from 14 ToxCast assays directly related to E/A/T activity. Assay are grouped left to right as androgen (4 assays), estrogen (5 assays), thyroid (4 assays) and aromatase (1 assay) related. The black bars on the left side designate occurrence of a few selected endocrinopathies seen in multi-generation studies.