Chad Sandusky, PhD  
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Physicians Committee for Responsible Medicine  
5100 Wisconsin Avenue, N.W., Suite 400  
Washington, DC 20016

RE: Response to Comments Regarding the Endocrine Disruptor Screening Program

Dr. Sandusky, Dr. Sullivan and Dr. Willet:

On July 16, 2008, the Physicians Committee for Responsible Medicine (PCRM) met with the Office of Management and Budget (OMB) to discuss its concerns with the Environmental Protection Agency’s Endocrine Disruptor Screening Program (EDSP). OMB provided these comments to the Agency and requested that the Agency provide a response to the comments for the docket. As such, EPA has treated the comments PCRM provided to OMB as late comments. Your original comments and the enclosed Agency response have been placed in the EDSP docket (Docket ID No. EPA-HQ-OPPT-2007-1080), and will also be available in the docket for the battery selection when the Federal Register Notice announcing its availability publishes.

Thank you for your interest in EPA’s EDSP. If you have additional questions, or require further assistance concerning the EDSP, please contact Bill Wooge on my staff at 202-564-8476.

Sincerely,

[Signature]

Frank Sanders, Director  
Office of Science Coordination and Policy

Enclosure
EPA Response to the Comments
From the Physicians Committee for Responsible Medicine (PCRM)
to the Office of Management and Budget (OMB)
March 26, 2009

On July 16, 2008, the Physicians Committee for Responsible Medicine (PCRM), an animal protection organization, met with the Office of Management and Budget (OMB) to discuss its concerns with the Environmental Protection Agency’s Endocrine Disruptor Screening Program (EDSP). PCRM identified three major concerns which are listed below with EPA’s response to each. OMB provided these comments to the Agency, and EPA will treat them as late comments on the battery because they pertain to the validation status and implementation of the test battery. EPA has added the comments and this document to the EDSP dockets.

1. “The EDSP Phase I is not likely to provide new regulatory information.”

In support of this assertion, PCRM argues that pesticides are among the most data rich chemicals in existence and that EDSP testing requirements are redundant because current tests include many of the same endpoints as the “presumptive EDSP Tier 2 tests.” PCRM states that chemicals tested in the two-generation reproductive effects test have already been subject to EDSP Tier 2 mammalian testing so the in vivo screens would provide little or no value for pesticides. PCRM also pointed out that several of the chemicals have been studied in detail for endocrine disrupting effects and some are ICCVAM reference chemicals.

**EPA Response:** EPA recognizes that several of the chemicals on the initial list have been studied in detail for endocrine disrupting effects. Some were reference chemicals in EPA’s or OECD’s validation programs as well as being on the ICCVAM list. EPA selected chemicals for the initial list on the basis of exposure, not hazard or completeness of data base. Consistent with the process used for pesticide re-registration, 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. For those that choose to rely on citations to existing data, EPA will evaluate the industry submissions and eliminate unnecessary testing to the extent that available functionally equivalent and other scientifically relevant information adequately address the objectives of the Tier 1 screening.

The Agency has written a paper entitled “EPA’s Approach for Considering Other Scientifically Relevant Information (OSRI) under the Endocrine Disruptor Screening Program.” This paper was developed by EPA to provide guidance to EPA staff and managers who will be reviewing the responses to Tier 1 Orders issued under the EDSP, and may also be of interest to parties considering whether to submit other scientifically relevant information to EPA. This paper provides general guidance and is not binding on either EPA or any outside parties. Anyone may provide other scientifically relevant information, and the Agency will assess the information for appropriateness on a case-
by-case basis, responding to the submitter in writing, and making EPA's determination publicly available. A copy of the approach paper has been placed in the docket for the Policies and Procedures for the Initial EDSP Screening (Docket ID number EPA–HQ–OPPT–2007–1080).

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

PCRM raised a number of points in support of this assertion, including that, at the time of the SAP review, validation of the individual assays was not complete and that the composition of the Tier 1 battery was not finalized.

**EPA Response:** Although PCRM correctly noted that two assays had not been validated as of the SAP review in March 2008, EPA noted this in its presentation to the SAP, and the SAP explicitly recognized this (SAP Report at 7). In addition, the H295R assay peer review has since been completed (June 2008). Although EPA did not ask for a consensus peer review panel opinion, the comments of one panel member are typical: “The H295R steroidogenesis assay is biologically and toxicologically relevant to the stated purpose. The assay would fit perfectly in the Tier 1 battery of assays to screen for endocrine disruptors. The assay has a series of strengths that would make it an excellent screening tool for endocrine disruptors of sex steroid hormone synthesis.”

Currently, the ER binding assay is the only Tier 1 assay for which validation is incomplete. Completion of validation for the ER binding assay is expected early in 2009. EPA will not require the ER binding assay if it is not successfully validated. PCRM is incorrect in stating that EPA’s proposed Tier 1 battery was not presented to the SAP. EPA identified its proposed battery as consisting of the following assays: (1) an androgen receptor binding assay, (2) estrogen receptor binding assays, (3) an estrogen transcriptional activation assay, (4) a cell-based assay using the H295R human adrenocortical carcinoma cell line, (5) the Aromatase assay, (6) the Amphibian (Frog) Metamorphosis assay (7) a Fish short-term reproduction screen (8) the Hershberger assay, (9) the Pubertal Female (10) the Pubertal Male (11) and the Uterotrophic Assay. The EPA final battery will be that which was proposed to the SAP for review and which the SAP accepted, with the possible exception of the ER binding assay, if ultimately it is not successfully validated.

**PCRM:** ECVAM and ICCVAM issued statements refuting the validation status of the uterotrophic assay.

**EPA Response:** OECD addressed all of the significant issues raised by The Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) and the European Center for the Validation of Alternative Methods (ECVAM). In response to ICCVAM’s concern about phytoestrogens, data from the validation program showed that phytoestrogens in feed were not a concern for rats if phytoestrogens were kept below 350 μg genestein equivalents per gram of feed. In response to questions that negative chemicals had not been demonstrated, OECD conducted an in depth analysis of a negative chemical, styrene, and also compared the results of the ER binding, transcriptional activation and uterotrophic assays for 60 compounds. Based on this in depth analysis, OECD concluded that the comparison
showed good correlation. OECD did not expect perfect correlation due to factors such as absorption and metabolism in in vivo systems. More specifically, OECD concluded that "The qualitative and semi-quantitative comparison of Uterotrophic Bioassay data with those from two screening assays demonstrate that the Uterotrophic Bioassay can well differentiate between chemicals with strong/weak estrogen receptor binding and agonist activity... Thereby it has to be taken into account that the in vitro tests belong to a lower level of the "OECD Conceptual Framework" and more weight has to be given to the results of the in vivo Uterotrophic Bioassay. In addition to the negative result obtained in the international validation program with dibutyl phthalate (the negative reference chemical tested), these data give strong evidence for good specificity of the Uterotrophic Bioassay."

PCRM: The Hershberger Test Guideline will not be accepted as final by OECD until 2009.

EPA Response: Although it is true that the Hershberger test guideline will not be accepted by OECD's National Coordinators of the Test Guideline Programme as final until its March 31-April 1, 2009 meeting, that is a separate question from whether the Hershberger castrate test procedure (which is what will be required as part of EPA's EDSP battery) has been validated. The Hershberger castrate test procedure has been peer reviewed and the reviewers (including the FIFRA SAP) concluded that draft test guideline covering that procedure is validated and can be used in its current form. OECD has not adopted the Hershberger test guideline as final because several member countries wanted to await the completion of validation and peer review of the test using a weanling version to see if it could be included as a substitute for the castrate adult version. The validation and peer review were completed in January 2009 and this issue will be discussed and decided by the National Coordinators of Test Guidelines, who meet in April. Thus, a final version of this test guideline will likely be available by the time EPA begins issuing Test Orders.

PCRM: The SAP said there were significant concerns about the specificity of some of the assays since no negative chemicals were tested in the pubertal, frog or fish assays and that criticism from the peer reviewers of seven EDSP assays raise serious questions about the validation status of the assays.

EPA Response: EPA responded in detail to all of the comments raised in each of the peer reviews in response-to-comment documents posted on the OSCP website, as well as those raised by public commenters. With respect to the concern raised by the peer review panel that EPA had failed to show specificity of the male and female pubertal assays, EPA has laid out its evidence for believing that these assays are specific. Although a similar concern was also noted by the SAP, both the majority of the peer review panels and the entire SAP ultimately recommended that these assays be included in the battery, notwithstanding their concerns on this issue, which were being addressed through a separate process. Estrogen was negative in the frog metamorphosis assay using the data interpretation guidance. Three separate negatives were used in the validation of the fish screen including potassium permanganate, octanol, and sodium perchlorate.
PCRM: PCRM stated that the SAP was concerned about the interpretation of results at high doses and said that the effects of body weight on organ weight had not been completely addressed in the pubertals.

EPA Response: While dose setting may at times be difficult, the dose setting for the screening assays is consistent with that used with other in vivo studies which typically set the top dose as the maximum tolerated dose. Despite its concern about the use of high doses, the SAP recommended the use of the assays but with appropriate caution in interpreting endocrine effects only at high doses. This is also where the strength of the battery comes into play: responses that are buttressed by the results of other assays will be accorded more weight by EPA than those that occur at only the high dose in a single assay.

The SAP did not conclude that validation of the pubertal assays is incomplete without refinement of the body weight analysis to account for reduction in body weight gain due to reduced feed consumption.\(^1\) EPA regards the SAP discussion of body weight analysis as an important suggestion for improvement of the assay, not notification of a fatal flaw. In EPA’s judgment, it would not be appropriate to delay implementation of the testing phase of the EDSP for this improvement since the appropriate analysis method has not been fully worked out and the effect of the correction is likely to be small.

PCRM: The SAP said there were serious concerns about the transferability of the hormone assays in the pubertal and amphibian assays.

EPA Response: The SAP’s comment appears to be directed at the transferability of the thyroid portions of the pubertal and amphibian assays rather than at the hormone assays per se. Hormone measurements are not part of the amphibian assay. There were mixed opinions among the assay peer reviewers on the level of interlaboratory variability in the results obtained in the amphibian metamorphosis assay, with some peer reviewers acknowledging that variability in observations were minimal and others expressing concern. The lack of consistency noted in most cases arises from specific and quantitative comparisons of the data acquired from individual test subjects as opposed to the overall outcome of the screen. As a screening assay, the intent is to interpret the results in a more general and qualitative manner. It is considered that the overall response of the assay was consistent across the laboratories in identifying potential thyroid system perturbation. Suggestions made to decrease potential variability and improve the consistency of the results between laboratories were accepted and considered in the revision of the test protocol. Furthermore, at the level of identifying agonism, antagonism, and other modes of thyroid action, the overall reproducibility of the assay was very good. None of the peer reviewers of the female pubertal assay questioned the overall ability of the assay to detect thyroid-active agents. Instead, individuals provided comments such as the following: “All studies using thyroid-active agents showed that the female pubertal assays detect alterations in thyroid function following exposure to compounds interacting to thyroid system.” and “It is especially

\(^1\) The SAP was not asked to comment on the specific individual assay validation as this was conducted through a separate process. Additional information about the Agency’s EDSP validation effort can be found at http://www.epa.gov/scipoly/oscpendo/pubs/assayvalidation/.
noteworthy that a so called ‘weak’ estrogen, methoxychlor, and a ‘weak’ thyroid disrupting compound DE-17 produced quantifiable and generally reproducible effects on the expected endpoints.” Transferability does not appear to be a significant issue for this assay. Similarly, for the male pubertal assay, none of the peer reviewers of the assay questioned the overall ability of the assay to detect thyroid-active agents. Typical comments were similar to the following: “The reproducibility and transferability of the assay is clearly demonstrated by the reproducibility of overall results across laboratories. While there was some variability with some endpoints between the laboratories, the overall weight of evidence and conclusions were consistent.”

As for the thyroid hormone measurements per se in the pubertal assays, EPA acknowledges the variability that was pointed out by peer reviewers. Nevertheless, the EPA believes that the hormone measurements in the pubertal assays provide added value despite the variability that is sometimes encountered and can be given appropriate weight in the weight-of-evidence evaluations of the entire Battery. As noted by one of the peer reviewers of the male pubertal assay, “There were inconsistencies in hormonal measurements between laboratories. This is likely due to biological variability but may also have to do with technique. Despite the inconsistencies, the overall trend was consistent across laboratories and the redundancy of endpoints reduces concern regarding any one specific measurement. Thus, while there is some variability associated with specific endpoints in this assay, the inclusion of multiple endpoints increases its reliability.”

**PCRM: The SAP questioned the reproducibility of the fish short-term reproduction assay, particularly the fecundity endpoint, a critical aspect that would have been addressed by adequate validation.**

**EPA Response: The SAP did not question, as purported, the reproducibility of the fish assay per se, but acknowledged that they heard public comment regarding the reliability of the assay. The specific quote is: “However, the Panel did hear some concern from public comment on the reliability of the fish short-term reproduction assay. This concern addressed the standardization of reproductive success by measurement of fecundity. However, it was noted that this component of the assay was an essential part of apical analysis of hypothalamic/pituitary/gonadal (HPG) activity.” The SAP did raise a concern that a false positive result could be obtained in the fish assay based on fecundity due to mechanisms other than those involving EAT activities. The Panel recommended that EPA be alert to non-endocrine mediated refinements of the fish assay to ensure fecundity effects are truly representative of EAT mechanisms and not generalized toxicity. The Panel went further to emphasize: “It should be recognized that the role of the fecundity assay is paramount for evaluations of the HPG axis.”**

**PCRM: The SAP noted that anti-estrogenicity is inadequately covered by the battery, antiandrogenicity even more poorly addressed and the HPG axis the least well supported in the battery.**

**EPA Response: PCRM has inaccurately characterized the SAP’s conclusions. The SAP compared the degree to which each mode of action was represented in the battery. They concluded that certain modes of action can be represented several times**
as they can be captured by in vitro, mode-of-action specific in vivo, as well as more apical in vivo assays, but that others cannot. For instance, the HPG axis can only be represented in assays using intact animals such as the fish and pubertal assays. Indeed, the SAP noted that EPA has made a good case for the pubertal assays as effective in detecting disturbances in the HPG axis. The SAP did not conclude this was a fatal flaw precluding adoption of the battery. Ultimately, the SAP’s consensus recommendation was that “[i]n summary, the proposed set of Tier 1 assays are appropriate to begin screening for disruptors of the EAT axes.” (SAP Rep at 11).

PCRM: In its review of the Tier 1 assays relating to estrogenic activity, the SAP concluded that EPA has failed to provide a thorough evaluation with a sufficient number of compounds, or the screen is too sensitive, or perhaps the battery lacks specificity. PCRM makes a similar allegation regarding the assays relating to the thyroid effects, pointing to the SAP’s conclusion that “it is not currently possible to estimate what (the) levels of false results might be….” PCRM concludes that a determination of false positive and false negative rates is part of validation and, as this is clearly missing from several of the assay assessments, these assays cannot properly be considered to be validated.

EPA Response: A demonstration of assay performance is an essential part of the validation process; however, the metric used is not the same for all assays since assays differ in nature and serve different purposes. As explained in EPA’s paper “Validation of Screening and Testing Assays for the Proposed EDSP,” a determination of false positive and false negative rates is important for assays that are replacing others or predicting toxic effects. Because EPA’s Tier 1 assays will operate in a battery and will only identify a chemical’s potential to interact with the endocrine system, rather than predict actual effects, the rate of false positives and negatives for individual assays in the battery is not an essential part of validation. Furthermore, for in vivo assays, the calculation of false positive and false negative rates requires an impractically large number of reference chemicals. Because the results of the assays in the battery will be considered together, false positives in one assay will be counterbalanced by the other assays in the battery.

PCRM: To improve the battery, the SAP recommended the inclusion of several additional in vitro methods.

EPA Response: EPA does not regard the SAP’s comments as a negative reflection on the battery but an acknowledgement that there is always room for improvement as science progresses. EDSTAC also recommended that EPA keep abreast of technical developments and incorporate the best validated assays in the battery. EPA is continuing to develop new methods and will validate them if they show sufficient promise.

3. “The EDSP Phase I essentially amounts to an ill-conceived validation exercise and is an inappropriate use of the DCI process.”

PCRM claims that requiring testing of the first chemicals is inappropriate because EPA has failed to explain how the data will be used within a regulatory framework, because
the information obtained from the testing will be of limited regulatory utility, and because the assays have not all been validated. PCRM claims that in essence, EPA is merely attempting to conduct a research program.

**EPA Response:** In implementing the EDSP, EPA is following the advice of EDSTAC which said that EPA should implement a two-tiered program. The first tier is designed to identify chemicals that have the potential to affect the estrogen, androgen and thyroid hormone systems. Tier 2 provides definitive data in several different taxa needed for risk assessment. The SAP has on two separate occasions reviewed EPA’s proposal for implementing the program and has advised EPA to continue on the Agency’s proposed course.

Requiring chemicals on the initial list to begin testing is not a validation exercise, nor a research program. All of the assays that EPA will require under the EDSP either have been validated, or will be validated before EPA requires order recipients to generate data. Contrary to PCRM’s allegation, EPA is not seeking validation of the battery *per se*. However, the SAP, in 1999, did advise EPA to examine the data from the initial list to determine how well the assays performed together as a battery so that unnecessarily redundant or poorly performing assays can be dropped or new ones added. The Agency has committed to do this after all Tier 1 data from the initial group of chemicals is available.