OBAMA-BIDEN TRANSITION PROJECT

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December 10, 2008

Lisa Jackson
Presidential Transition Team
Washington, DC 20270

Also sent by e-mail to: Lisa.Jackson@ptt.gov

Dear Ms. Jackson:

Congratulations on being a part of an exciting new chapter in American history as members of President-Elect's campaign transition team. We are writing on behalf of People for the Ethical Treatment of Animals and the Physicians Committee for Responsible Medicine, and our 2 million-plus members and supporters, to alert you to an impending testing program that is in critical need of immediate revision.

The endocrine disruptor screening Program (EDSP) is one of the largest testing programs ever devised by the Environmental Protection Agency (EPA). Unfortunately, the testing program as it is currently designed is ineffective and will generate data that will not ultimately be useful in determining risk. Briefly, we have the following concerns regarding the EDSP and suggestions for an improved screening battery which are presented in more detail in the attached appendix:

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

- Several of the assays in the Tier 1 battery have high false positive rates. In fact, no chemical that has been tested in the battery so far has been negative in all tests. This drastically limits the usefulness of the Tier 1 battery as a screen since the purpose of a screen is to distinguish potentially hazardous chemicals in need of additional testing.

- The EPA has made it clear at the informational meeting regarding policies and procedures (November 23, 2007) the FIFRA Scientific Advisory Panel held to review the Tier 1 battery (March 25 – 26, 2008), and most recently a meeting of the Pesticide Program Dialogue Committee (PPDC, October 7, 2008) that it is requesting complete data sets for all of the Tier 1 assays *even though the agency has provided no explanation regarding how the data obtained will be used*.

- Both pesticides and High Production Volume (HPV) chemicals undergo extensive animal testing, including the very same tests proposed as the EDSP Tier 2 Battery; therefore, it is unlikely that the Phase I exercise will provide any information relevant to regulating these chemicals and will only result in needless duplication and waste of resources and animal lives.

- Taken together, these elements suggest that the EDSP, and the Phase I exercise in particular, will generate (1) data that will not be useful in making hazard determinations or even in prioritization of chemicals for further testing, and (2) in the case of the Phase I chemicals, will provide no new regulatory information.

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- We propose that the EDSP be redesigned to follow a more effective approach, outlined in a report by the National Research Council (NRC), *Toxicity Testing in the 21st Century: A Vision and a Strategy,*[1] that more closely follows the Conceptual Framework for endocrine disruptor screening devised by the Organization for Economic Cooperation and Development.[2] This approach would include preliminary tiers that first assess physiochemical and pre-existing toxicological data, plus *in silico* and a much broader range of *in vitro* mechanistic assays, and would be more logical, efficient, economical, and use fewer animals.

We hope you will consider and act on these recommendations as quickly as possible. In addition, we would like to meet with you to discuss these issues at your earliest convenience, at which time we will provide more detailed information. We can be reached using the contact information below.

Sincerely,

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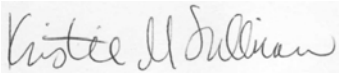

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[1] NRC, *Toxicity Testing in the 21st Century: A Vision and a Strategy*. National Academies Press, Washington, DC, 2007.
[2] OECD. 2002. Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals. Organization for Organization for Economic Cooperation and Development, Environment Directorate, Paris. http://www.oecd.org/document/58/0,3343,en_2649_34377_2348794_1_1_1_1,00.htm, Aaccessed 9 December, 2008.

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APPENDIX: INFORMATION DETAILING THE SHORTCOMINGS OF THE EPA'S EDSP AND RECOMMENDATIONS FOR AN IMPROVED SCREENING BATTERY

History of the Endocrine Disruptor Screening Program (EDSP)

The EDSP was created through a revision to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) in 1998. FIFRA 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 (chemicals that interact with any of these three hormone systems are collectively known as endocrine-active or endocrine-disruptive chemicals).

The EPA has devised a two-tiered screening program: Tier 1 screening assays (the Tier 1 Battery) to detect chemicals of concern, and Tier 2 tests to characterize the potential risks of those chemicals. The proposed Tier 1 Battery putatively consists of 11 individual assays (although this list is not yet final). Phase I of the EDSP, which is now proposed to begin in early 2009, involves Data Call-In (DCI) requests using 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 number of chemicals on the final list is rumored to be 69; however, the EPA has not yet released this information.

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

Over the past two decades, it has become apparent that testing methods, especially those used in regulatory testing programs, benefit from validation exercises that define the relevance (how the test relates to the biological effect of interest), robustness (resistance to small changes in protocol), reliability (the sensitivity and specificity) and reproducibility of the method. This enables the methods to be transferred from lab-to-lab, and for the data generated to be widely accepted. Three organizations that have provided internationally recognized recommendations for validation of regulatory testing protocols; the European Centre for the Validation of Alternative Methods (ECVAM)[3], the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)[4], and the Organization for Economic Cooperation and Development (OECD).[5] While the validation criteria described by each of these organizations differ slightly, the elements that are shared between the approaches define the basic requirements of validation.

The following elements should be adequately addressed in any validation exercise: (1) the rationale for the test, including: the scientific basis, choice of endpoints, regulatory purpose, and need in light of existing tests; (2) the relevance of the test method (the relationship between the test method

[3] ECVAM. General Guidelines for Submitting a Proposal to ECVAM for the Evaluation of the Readiness of a Test Method to Enter the ECVAM Prevalidation and/or Validation Process. ECVAM/JRC (accessed 9/2/2008).
[4] ICCVAM. 2003. ICCVAM Guidelines for the Nomination and Submission of New, Revised and Alternative test Methods. NIH Publication no. 03-4508. National Institute of Environmental Health Sciences, Research Triangle Park, NC.
[5] OECD: 2005. Guidance Document 34: Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment. Organization for Economic Cooperation and Development, Paris.

endpoint and the biological phenomenon of interest) expressed as a Data Interpretation Procedure (OECD) or Prediction Model (ECVAM); (3) the performance of the test relative to the existing regulatory test and relative to the response in the species of concern using a an appropriate set of reference chemicals; and (4) the transferability and reproducibility of the test within and among laboratories. The evaluation of individual assays of the EDSP Tier 1 fails on many of these points as detailed below.

The original mandate of the EDSP authorized in the Federal Food, Drug and Cosmetics Act (FFDCA) section 408(p) requires the EPA to develop an assay or battery of assays that have been shown to *reliably characterize* the risks posed by estrogenic chemicals to human health. The EPA then expanded its interpretation of this mandate to include androgen and thyroid effects (in addition to estrogen) in humans as well as effects in wildlife.[6] The stated purpose of the Tier 1 battery is to "identify substances that have the potential to interact with the EAT [estrogen/androgen/thyroid] hormonal systems…"

At the FIFRA Scientific Advisory Panel (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. In fact, each time the EPA publishes a list of the proposed Tier 1 assays, it is slightly different. 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)[7], 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.[8,9]

The Hershberger and Amphibian Metamorphosis Assays are currently being evaluated by the OECD. Review of a revised Hershberger test guideline is expected at the end of 2008, and will therefore not be completed until 2009 at the earliest.

The EPA released results of its own peer reviews of seven additional assays (the male and female pubertal, adult male rat, fish short-term reproduction, amphibian metamorphosis, and the androgen receptor aromatase assays) between November 2007 and February 2008. While EPA's validation of these seven assays took three months, validation studies and peer reviews carried out by organizations specializing in this procedure (ECVAM, ICCVAM, the OECD) take a minimum of 2 –

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[6] US EPA Endocrine Disruptor Screening and Testing Advisory Committee Final Report, August 1998. Published by the EPA.

[7] 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

[8] ESAC Statement on the status of validation of the uterotrophic assay. February, 2005. (http://ecvam.jrc.it/index.htm)

[9] 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

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3 years, and often much longer. The EPA is claiming that these peer reviews constitute validation, irrespective of the 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[10], 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, in general comments the SAP itself concluded that anti-estrogenicity is inadequately covered by the battery (the 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.

The high false positive rate of several individual assays limits the usefulness of the battery as a screen.

Regarding individual assays, the Panel admitted "significant concerns remain about the specificity of the male pubertal assay" and pointed out that in both pubertal assays, there were no negative compounds, 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 lab-to-lab 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 lab-to-lab 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. Finally, no compounds were negative in the fish short-term or amphibian metamorphosis assays.

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.

In addition to validation of individual assays, the Tier 1 battery as a whole must be evaluated. A decision scheme to inform the use of results from the Tier 1 battery has not been announced, much

[10] 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

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less tested with known positive and negative chemicals. If this inflated false-positive rate is not addressed, Tier 2 will be unnecessarily burdensome in terms of resources and animal lives.

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 is 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 a critical part of the validation process, and this is clearly missing from several of the assay assessments.

In public comments, several additional concerns were raised, including the high variability of results from the pubertal assays, the high cost, special equipment and large numbers of animals used in the fish and amphibian assays, the low specificity of several of the assays (with the likely result that nearly all chemicals will be positive in one or more of the Tier 1 assays), and the lack of decision-making guidance provided by the agency. Most of these concerns would be addressed by proper validation.

Therefore, contrary to the EPA's summary of the SAP review, significant elements of validation are missing from most, if not all, of the assays to be included in the Tier 1 battery, and the battery has yet to be evaluated as a whole; however, a battery containing a collection of assays of such questionable selectivity, is unlikely to function successfully as a screening tool.

The EDSP Phase I is not likely to provide new regulatory information

Pesticides are among the most highly data-rich substances in existence. For registration, pesticide active ingredients and final products are usually subject to dozens of separate animal tests, including reproductive and chronic/lifecycle studies in rodents, fish and birds.[11] These tests kill thousands of animals and include many of the same endpoints addressed in the presumptive EDSP Tier 2 tests. Similarly, US EPA's Chemical Challenge Program also provides for the collection of data which may be germane to the assessment of potential reproductive toxicity.[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 OPPTS 870.3880 have, in effect, already been subject to EDSP Tier 2 mammalian testing. In fact, EPA representatives at the recent PPDC meeting (October 7, 2008) indicated that the 2-generation reproduction test proposed for the EDSP Tier 2 is likely be very similar to, if not exactly the same as, the current Reproduction and

[11] 72 FR 60934, October 26, 2007: EPA 40 CFR Parts 9 and 158: Pesticides; Data Requirements for Conventional Chemicals.
[12] 65 FR 81657, December 26, 2000; EPA 40 CFR Part 799: Testing of Certain High Production Volume Chemicals

Fertility Effects test currently required for pesticide chemicals. Thus, with the possible exception of mechanistic screening for thyroid effects, 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-*n*-butyl phthalate and linuron) are included in the Revised ICCVAM List of Recommended ED Reference Substances.[13] Atrazine has been thoroughly tested in terms of its potential for endocrine activity in numerous *in vitro* and *in vivo studies*, including *in vivo* studies and risk assessments already conducted by the EPA.[14] In fact, the use of atrazine has been prohibited in Europe.[15] Similarly, butyl benzyl phthalate (BBP) has been shown to possess endocrine activity in *vitro* and *in vivo* in numerous animal studies, including those already conducted by the EPA.[16,17]

The anti-androgenic activity of di-*n*-butyl phthalate (DBP) has been studied in detail.[18,19] Both BBP and DBP have been associated with endocrine-related effects in humans.[20] Linuron is a well-characterized weak anti-androgen, and was used as a control in OECD validation exercises for the Hershberger assay[21,22] and as a control in the EPA's own evaluation of the 15-day intact male assay.[23] 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.

A more effective approach

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 (an assay for transcriptional activation through the estrogen receptor has just been validated at the OECD; the rest of these methods are currently in validation at the OECD) and the steroidogenesis assay (an assay using H295R cells which is being developed by the EPA).

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[13] http://iccvam.niehs.nih.gov/meetings/6thWC/posters/HattanSubstance.pdf, accessed 9 December 2008.
[14] Gammon, D.W, et al., 2005. A risk assessment of Atrazine use in California: human health and ecological aspects. Pest. Manag. Sci. 61: 331-55.
[15] Sass and Colangelo, 2006. European Union bans Atrazine, while the United states negotiates continued use. Int. J. Occup. Environ. Health. 12:260-7.
[16] 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
[17] Aso, et al., 2005. A two-generation reproductive toxicity study of butyl benzyl phthalate in rats. J. Toxicol. Sci. 30 Spec No.:39-58.
[18] Bredhult, C. et al., 2007. Effects of some endocrine disruptors on the proliferation and viability of human endometrial endothelial cells. Reprod. Toxicol. 23:550-9.
[19] Wang Y.B., et al. 2007 Monobutyl phthalate inhibits steroidogenesis by down-regulating steroidogenic acute regulatory protein expression in mouse Leydic tumor cells (MLTC-1). Toxicol. Environ, Health. A. 70:947-55.
[20] Marsee, K. et al., 2006. Estimated daily phthalate exposures in a population of mothers of male infants exhibiting reduced anogenital distance. Environ. Health. Perspect. 114: 805-9.
[21] Owens, et al., 2007. The OECD program to validate the rat Hershberger bioassay to screen compounds for *in vivo* androgen and anti-androgen responses: phase 2 dose-response studies. Environ. Health. Perspect. 115:671-8.
[22] Tinwell, H., et al., 2007. Evaluation of the anti-androgenic effects of flutamide, dDE, and Linuron in the weanling rat assay using organ weight, histopathological and proteomic approaches. Toxicol. Sci. 100:54-65.
[23] http://www.epa.gov/scipoly/oscpendo/pubs/adult_male_peer_review_final.pdf

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An approach in which the initial tier is comprised of mechanistic screens follows both the NRC vision of toxicity testing for the twenty-first century and the OECD Conceptual Framework. The NRC vision includes a move away from apical, whole-animal testing and toward mechanistic, in vitro testing methods. This approach is based on the years of experience with whole animal testing that shows animal testing produces data that is highly variable, often ambiguous and difficult to apply to hazard and risk determinations. In addition, most of the animal tests, including those proposed for the EDSP, were devised in the early and mid-twentieth century. Since then, there has been a revolution in our understanding of underlying biological and toxicological mechanisms as well as the technology available for designing testing methods. Highly reliable and reproducible, completely non-animal based screens have been devised that are also amenable to high-throughput screening. While it is not yet possible to completely replace animal testing with non-animal methods, the message of the NRC report is that full replacement is the goal, and in the meantime, any effective chemical hazard assessment program should incorporate as much of this new biological understanding and technology as possible.

The OECD Conceptual Framework for screening potential endocrine disrupting chemicals incorporates this approach to a much greater degree than the current EPA EDSP. The framework outlines 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.

We recommend that the EDSP be restructured to more closely follow the Conceptual Framework, incorporating as many actual mechanistic based screens as currently exist. The EPA should refocus its efforts to complete the validation of the many in vitro methods that are in development, and validate a battery of these mechanistic screens as the Tier 1 of the EDSP.

We also suggest that the EDSP be based on an Intelligent Testing Strategy (ITS).[24] An ITS is 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," 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 trends in toxicological assessment both in the US and internationally.[25]

[24] Bradbury SP et al. 2004. Meeting the scientific needs of ecological risk assessment in a regulatory context. Environ Sci Technol 38, 463A-470A.
[25] Collins F et al. 2008. Transforming Environmental Health Protection. *Science* 319: 906-7.