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EPN Comments on EPA's White Paper "Availability of New Approach Methodologies (NAMs) in the Endocrine Disruptor Screening Program (EDSP)"

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Founded in 2017, the Environmental Protection Network (EPN) harnesses the expertise of more than 550 former Environmental Protection Agency (EPA) career staff and confirmation-level appointees from Democratic and Republican administrations to provide the unique perspective of former regulators and scientists with decades of historical knowledge and subject matter expertise.

I. Introduction

On January 19, 2023, EPA announced the availability of and solicitation for public comment on a draft White Paper entitled, "Availability of New Approach Methodologies (NAMs) in the Endocrine Disruptor Screening Program (EDSP)." The agency specifically requested that "the public provide comment on the clarity and completeness of the draft document. Given the strengths and uncertainties of these methods, EPA also requests the public provide comment on the draft conclusions that certain NAMs have been validated and may now be accepted by the EPA as alternatives for certain EDSP Tier 1 assays while others are useful for prioritization purposes and for consideration for use as other scientifically relevant information."

EPN is pleased to see that the agency is once again focusing attention on implementation of a program mandated in law when the Food Quality Protection Act (FQPA) legislation was signed by President Clinton in August 1996. The principal drafters of these comments have particularly extensive knowledge of the EDSP as they were all present at its birth and heavily involved in its conceptual development, validation, and implementation and have continued to closely follow its journey over these 26+ years.

The EPN comments will be divided into three parts. The first will focus on the technical/scientific aspects of the NAMs being developed to serve as alternatives/replacements for certain elements of the original Tier 1 screening battery plus some other tools. It will also address the clarity and completeness of the draft document in this aspect of the document. In addition, the comments in this part will provide a critique of what should be in the document, but isn't, especially given the passage of time since the FQPA mandate was issued. The second part will offer some insights and recommendations as to what EPN views as potential steps going forward. The third part contains EPN's recommendations regarding future research efforts.

II. Technical Comments. Comments on the scientific integrity and projected roles of the ER and AR Pathway Models, Thyroid Pathway Model, updated High-throughput H295R steroidogenesis assay, and aromatase assay.

EPA published the Endocrine Disruptors Screening Program for the 21st Century Work Plan ("EDSP21 Work Plan") in 2011 and updated it in 2014. The Work Plan described the agency's intention to pivot towards validation and more efficient use of computational toxicology methods and high-throughput (HT) *in vitro* assays and to place less reliance on *in vivo* models. The Work Plan envisioned a multi-level and integrated approach to determine whether, as before, a chemical has the potential to interact with specific

endocrine signaling pathways. In the near-term, computational methods would be used to prioritize chemicals for screening. The next stage would see replacement of the initial menu of validated*in vitro* screening (Tier 1) assays with validated HT *in vitro* assays. The results of this effort would also inform efforts to replace current *in vivo* Tier 1 assays. The agency's long-term goal is to replace all current Tier 1 screening assays (both *in vitro* and *in vivo*) with tools based upon advances in computational modeling and molecular biology and conducting rapid, low-cost, non-(whole) animal assays.

EPA has made limited progress on implementation of its management plan and, contrary to its stated intentions in 2011 and 2014, has not provided an integrated annual update of progress since 2014.

The Estrogen Receptor (ER) Pathway Model. A key component of this effort is the development and validation of a battery of 18 HT assays, that collectively, provide substantial coverage of elements associated with receptor-binding, resulting in either agonistic or antagonistic consequences. This battery, however, does not address phenomena not associated with receptor-binding as the Molecular Initiating Event (MIE), which presents constraints on its ability to replace all elements of the current Tier 1 screening battery other than the lower-throughput ER binding and the uterotrophic assays. The conclusion that the full HT battery could substitute for the uterotrophic study, which can capture some non-receptor binding manifestations of estrogen disruption, was based upon an in-depth analysis and comparison of the results from the 18-component HT battery with results from guideline and "guideline-like" uterotrophic assays.
EPN agrees with the agency's conclusion that the 18-element HT battery can replace the two lower-throughput ER binding assays and the uterotrophic study. EPN recommends that some additional information be included in this document as it is revised in response to public comments:

1) Discussion of the role(s) that certain subsets of the 18-element HT battery could/would play. What specific assays are in those subsets, and are they (as) adequate as the full battery? For each subset, is it adequate simply for prioritization and/or use as Other Scientifically Relevant Information (OSRI) in a Weight of Evidence (WOE) assessment? Is each/any of the subsets adequate to substitute also for the uterotrophic assay?
2) EPN recommends that EPA perform an analysis comparing the results of the full HT battery (or any subset) with the results from guideline or "guideline-like" female pubertal and fish short-term reproduction assays. EPN thinks one would want to have documentation of the battery's capacity to substitute for them as well.
3) EPN recommends that the White Paper include enhanced discussion of the assertion that the full HT battery has truly been validated. Validation exercises for NAMs like the HT battery are evolving away from longstanding guidance, in some ways, for good reason. There remains, however, significant skepticism among segments of the scientific community and some of the agency's stakeholders as to whether the newer validation approaches are adequate. The absence of contemporary consensus guidance on what constitutes adequate validation for these newer tools is a barrier to stakeholder and regulatory acceptance.
4) Furthermore, the White Paper should recognize that the 18 HT assays do not address all aspects of estrogenicity, such as the importance of estrogens in neural development.

The Androgen Receptor (AR) Pathway Model. As with the ER Pathway Model, the AR Pathway Model includes a battery of HT assays (originally 11, now expanded to 14) that, collectively, provide substantial coverage of elements associated with receptor-binding as the MIE. As with the ER model, this battery, however, does not address phenomena not associated with receptor-binding, which presents constraints on

its ability to replace any elements of the current Tier 1 screening battery other than the one lower-throughput AR binding assay. Comparison of the results in the 11-component battery compared poorly with the results for a number of chemicals studied in the Hershberger assay. Therefore, the battery was not proposed to serve as a substitute for the Hershberger test.

EPN agrees with the agency's conclusion that the 11-component HT battery can replace the lower throughput AR binding assay, but not the Hershberger study or any other *in vivo* screen. As before, we ask to have some additional information included in the White Paper as it is revised in response to public comments. We recommend that:

1) EPA should discuss whether the addition of three more assays to the 11-assay HT battery changes the conclusions with regard to comparison of results from the Hershberger studies.
2) EPA should describe the value of the 5- or 6- component subsets of either the original or updated full battery. Could they be used both as an alternative to the full battery in screening and/or prioritization and/or as OSRI in the WOE analysis? EPN's opinion is that the answer to these questions is "No" because one subset can identify agonists, the other antagonists, and if one employs only one subset, chemicals exhibiting the other characteristic would be missed.
3) The agency should perform an analysis comparing the results from the full HT battery and from either subset with results from guideline or "guideline-like" male pubertal and fish short-term reproduction assays. EPN thinks one would want to have documentation of the battery's capacity to substitute for them as well.
4) The White Paper should provide justification that the validation approach was adequate. (See Item #3 above as it applies here, too.)

Thyroid Adverse Outcome Pathway. Regrettably, but somewhat understandably, no *in vitro* screening assays, HT or otherwise, for thyroid-relevant targets have been developed, validated, or introduced into the EDSP to serve either in the prioritization process and/or implementation of the screening tier. EPA should ramp up the pace of its research program on developing and validating new tools to supplement and/or replace the three *in vivo* assays that now constitute the screening battery for thyroid effects in the EDSP.

Steroidogenesis. In the draft document, the agency states that work has not been completed on the development and validation of a HT version of the H295R steroidogenesis assay. Furthermore, the draft document is totally silent on the status of an HT aromatase assay. A cursory scanning of the literature would suggest that both tools have been developed and, allegedly, validated. See bibliographic citations at the end of these comments. A discussion of this apparent discrepancy is warranted.

Incidental editorial comments:

Page 17: "In September 2011, EPA published the Endocrine Disruptors Screening Program for the 21st Century Work Plan (U.S. EPA, 2012) ("EDSP21 Work Plan")." "2014 EDSP Comprehensive Management Plan." Neither version of the work plan is cited in the Reference section.

III. Relaunch the EDSP: EPA Should Mandate Further Screening and Testing That Will Lead to Regulatory Decisions.

While EPN generally commends the ground-breaking scientific research EPA has done to develop NAMs to prioritize and screen chemicals for their potential to affect the endocrine system, EPN thinks that the

agency urgently needs to have a plan for how to fulfill the statutory mandate in Federal Food, Drug, and Cosmetics Act (FFDCA) sec. 408(p). That law, enacted over 25 years ago, directs EPA to "determine whether [a pesticide chemical] may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or such other endocrine effect as the Administrator may designate."

It appears to EPN, however, that most of EPA's work has failed to address the specific actions—screening chemicals—mandated by the law. The agency has yet to take regulatory action to modify the use of any pesticide chemical or other compound based on data generated through its EDSP. Moreover, EPA's work paused, without explanation, the EDSP process for the first group of chemicals to enter the process, List 1. The agency has not issued Data Call-in notices (DCIs) for Tier 2 studies for the List 1 chemicals exhibiting signals in their Tier 1 screens. There are possible explanations for EPA's inaction, including limitations on agency resources and the urgency of other priorities. EPN also appreciates that, because List 1 pesticide chemicals have been evaluated in EPA's registration review program, the agency may already have taken regulatory action based upon adverse effects observed in the equivalent Tier 2 studies that had been required previously. It should be noted, however, that Tier 2 studies for fish and wildlife may not have been performed.[1] But to comply with the law, EPA still needs to screen pesticide chemicals for their potential to produce estrogen-related effects in humans.

EPN thinks that the agency's existing EDSP approach, developed with the advice of the expert input of the Endocrine Disruptor Screening and Advisory Committee (EDSTAC) and multiple SAP reports, is fundamentally sound and that EPA should continue to use it. Essentially, the EDSP has four components: 1) a set of tools for prioritization of chemicals [Tier 0]; 2) a battery of screening assays for high-priority chemicals [Tier 1]; 3) a battery of tests to evaluate chemicals that demonstrate bioactivity affecting the endocrine system in the screening assays [Tier 2]; and 4) if necessary, risk assessment and regulation of the chemicals. EPA has already identified two groups of chemicals—"List 1" and "List 2"—as priority chemicals. Further, EPA has required List 1 registrants to submit data from Tier 1 screening assays and has reviewed those results to determine which List 1 chemicals need further testing in Tier 2 studies. EPA should complete the process for List 1 and, with two modest changes—limiting initial DCIs for List 2 chemicals to active ingredients and substituting NAMs that were validated to replace Tier 1 assays—for List 2.

In EPN's view, the agency has the capacity—the testing methodologies and resources—by which it could immediately resume making progress toward identifying and, if necessary, regulating pesticide chemicals that pose a risk to human health or non-target animal species because of their endocrine effects. EPN thinks the current state of the science in testing chemicals for endocrine activity, and eventually the evolving research on new, high throughput *in silico* and *in vitro* systems, can enable the agency to meet this statutory duty to screen and test pesticide chemicals ever more efficiently.

EPN offers its thoughts on how that might best be done.

Step 1: Complete Tier 2 Testing of List 1 Chemicals. EPA should restart the EDSP effort by issuing DCIs

[1] Regulatory action does not require the understanding of the mode of action underlying the observed adverse effects to proceed. Time constraints prevented the drafters of these comments from comparing "hits" in the ER, AR, and thyroid screens with the results of existing Tier 2 studies for each of the List 1 chemicals to characterize the degree of success of the screens to predict effects in the tests.

to the registrants of the List 1 chemicals deemed to warrant further Tier 2 testing back in 2015. The justification for this first step is based on the findings in the Tier 1 screening studies for the List 1 chemicals. Data on 52 chemicals were evaluated. There was no evidence for potential interaction with any of the endocrine pathways for 20 chemicals. For another 14 chemicals—ones that showed potential interaction with one or more pathways—EPA already had enough information to conclude that these chemicals did not pose unreasonable risks to human health. For the remaining 18 chemicals, however, EPA judged that it needed additional testing to evaluate whether these chemicals could pose risks as a result of their ability to perturb endocrine systems.

Based on the Tier 1 results, EPA determined all 18 showed potential interaction with the thyroid pathway, 17 of them with the androgen pathway, and 14 with the estrogen pathway. Testing in all of the Tier 2 studies is necessary to assess these 18 chemicals.[2] Because the remaining 18 chemicals displayed a wide range of bioactivity affecting different endocrine pathways, EPN recommends that the next round of DCIs for List 1 chemicals require the full set of Tier 2 tests unless adequate data already exist.

Step 2: Screening and Testing of Active Ingredients on List 2.
Once it has issued the DCIs for Tier 2 testing of the 18 chemicals from List 1, EPA should issue DCIs to the registrants of the pesticide active ingredients included on List 2 to conduct Tier 1 screening assays.[3] The agency should resist the temptation to otherwise revise the existing List 2, using the new and updated tools cited in the draft White Paper, both because of the limitations of the HT ER and AR assays discussed in Part 1 and because taking that step would likely delay more substantive action for a year or more. Screening and review of the Tier 1 results for List 2 active ingredients will, itself, probably take several years. Then, EPA should issue DCIs for Tier 2 testing of any chemicals showing the ability to affect an endocrine pathway and for which there is a concern about unreasonable adverse effects. The generation and agency review of required Tier 2 data will likely take several more years. Altogether, EPN envisions steps 1 and 2 will take more than five years.

Based upon the science currently available, EPN recommends that all List 2 chemicals be subjected to screening for all three hormone systems (estrogen, androgen and thyroid), employing the screening assays validated at the time the DCIs are issued. If they are unchanged from today, that would mean the HT ER battery; the female pubertal and fish short-term reproduction studies, minus the uterotrophic assay for estrogen; the HT AR battery; Hershberger; male pubertal and fish short-term reproduction study for

[2] There is a cautionary tale here. Several chemicals in the group of 18 showed no effects on the estrogen system but did in one or both of the other two systems. And it is unclear which endocrine pathways showed "hits" for the 14 chemicals for which sufficient data were available to not go forward with Tier 2 testing. The science is already available to show that disturbances in the androgen and thyroid hormone systems can lead to significant adverse consequences, particularly in developing organisms. It would be irresponsible to ignore the possibility of missing something important by foregoing the androgen and thyroid screening.
[3] SDWA sec. 1431 authorizes EPA "to use the estrogenic substances screening program created in the Food Quality Protection Act to provide for testing of substances that may be found in drinking water if the Administrator determines that a substantial population may be exposed to such substances." In EPN's view, this statutory language grants authority to expand the scope of chemicals tested in the EDSP but, unlike the FFDCA, it does not impose a mandate to conduct screening of any chemicals. Because screening of drinking water contaminants is discretionary, EPN recommends that the agency not pursue them at this time. Further, if List 2 included any substances that are intentionally-added inert ingredients in pesticide products, EPA should not pursue them at this time. While intentionally-added inert ingredients are "pesticide chemicals" subject to the statutory mandate, the DCI process for requiring testing of such substances is very complex and resource intensive. EPA resources would be more productive if focused on active ingredients although this runs the risk of missing the potential toxicity of "inerts" for the formulated product.

androgen; and the female and male pubertal with thyroid function and amphibian metamorphosis studies for thyroid hormone, as well as the low-throughput (LT) steroidogenesis and aromatase assays. New or updated validated assays should be incorporated as soon as they become available.

Step 3: Prioritization for the next list (List 3).
Given the scope of the chemical universe covered by the 1996 amendments to FFDCA and Safe Drinking Water Act (SDWA), EPN thinks that prioritization of chemicals for screening and testing is essential. Without prioritization, EPA and the regulated community may expend limited resources screening and testing compounds that are not likely to be posing the greatest risks. Based on EPN's review of the draft White Paper, EPN thinks that the currently available ER and AR pathway model NAMs are not sufficiently comprehensive to set priorities for Tier 1 screening on their own. As detailed in Part 1 of these comments, the NAMs for estrogen activity were not specifically designed to identify or discriminate between antagonists and agonists. The two subsets for AR testing together do cover both agonism and antagonism, a useful enhancement over the original LT AR receptor binding assay, given that there appears to be a preponderance of antiandrogen-active substances in the chemical universe. EPN thinks the subsets (which equate to the full HT battery) could be used for priority setting. As EPA's draft White Paper acknowledges, the thyroid Adverse Outcome Pathway (AOP) is very complex, and progress on developing NAMs to cover thyroid effects has understandably been slow. Given the cost of setting up HT systems, in some cases, it may be more cost effective to use the LT assays in the Tier 1 battery.

EPA could begin by prioritizing all pesticide active ingredients chemicals, reserving action on intentionally added inert ingredients and other chemicals for subsequent cycles. The agency could issue DCIs under FIFRA sec. 3(c)(2)(B) requiring generation of data needed for screening of pesticide active ingredients.[4]

In all likelihood, EPN would expect registrants, working through their trade associations (e.g., Crop Life America, the American Chemistry Council), to form data generation consortia that would enable the use of economical HT approaches to conduct the required screening.

The agency could use the results of more robust HT ER and AR assays, together with its exposure predictions from the IBER and EXPOCAST models and other exposure information, to rank chemicals by potential for risky activity. Combining information on hazard potential—the Tier 0 results—with information on exposure—IBER and EXPOCAST outputs plus other exposure information, as available—should produce a prioritized list of chemicals that are likely to have greater concerns than would a prioritization scheme that considered only hazard or only exposure. (Once the screening of active ingredients is complete, EPA could turn its attention to screening all intentionally-added inert ingredients in pesticide products, then the drinking water contaminants, using a similar approach.)

Based upon the results of the Tier 1 screening of List 3 and evaluation of these and other data, EPA could decide which chemicals should be subjected to Tier 2 testing. EPN thinks the agency could choose to stage implementation of the program, beginning with evaluation of the estrogen hormone system in humans only.

[4] Previously, in a letter to Assistant Administrator Michal Freedhoff, EPN recommended that the agency focus on screening chemicals for both estrogenic and androgenic activity. Because of EPA's lack of progress on screening chemicals for either estrogen or androgen activity, EPN now favors a narrower focus for List 3 that, EPN hopes, will lead the agency to take regulatory actions to address risks to human health. EPN also listed three options for obtaining needed data—standard DCIs; a public-private partnership with pesticide industry trade associations, and government-funded testing. EPN now thinks it makes sense to use existing DCI authority to require the screening studies for pesticide chemicals.

If so, EPA could use the results from the full Tier 1, together with OSRI, to determine whether further testing in the Tier 2 traditional two-generation or, preferably, the extended one-generation rat reproduction study was needed to generate data suitable for human risk assessment.[5]

It should be noted that the extended one-generation rat reproduction study includes evaluation of parameters that provide insights on effects that may be the result of disturbances of the estrogen system, as well as the androgen and/or thyroid hormone systems. Once the evaluation of the estrogen-related Tier 1 data is complete and DCIs are issued for Tier 2 testing for chemicals which warrant it, while waiting for those data to be submitted, the agency could shift its attention to reviewing data from the Tier 1 studies for androgen and issue DCIs for Tier 2, as warranted. Then, while waiting for the Tier 2 data for androgen data to be submitted, EPA could start work on thyroid.

EPN expects that, in some cases, the agency will already have data from a rat reproduction study on an active ingredient that would be adequate to determine the chemical does not have potential to cause apical effects at an exposure level that could be used as a new, lower Point of Departure for human risk assessment. If not, EPA should issue DCIs for chemicals when further *in vivo* testing would be necessary to quantify potential human risks. To the extent such data lead EPA to predict greater risks to human health than were previously identified, the agency should take regulatory action to mitigate the risks.

It should be noted that EPN's recommendations regarding Step 3 reflect our view that the agency should allocate its resources to activities that will enable it to comply with the statutory mandate in the FFDCA. The law points to a particular concern with estrogenic effects and humans. While EPN recognizes that a chemical may be biologically active in other endocrine systems (e.g., androgen and thyroid), the law's focus on estrogen is specifically mandated. Similarly, even though EPN understands that an endocrine-disrupting chemical may pose risks to non-target animals (e.g., birds, fish, reptiles), the statute directs EPA to address compounds that may have effects in humans. Likewise, FFDCA sec. 408(p) addresses only "pesticide chemicals," not the many other types of chemicals that EPA regulates. Thus, it should be recognized that EPN's recommendation is a significant compromise to expedite screening and testing. However, we believe that holding out for a more perfect, inclusive approach will result in unacceptable delays in assessing and regulating chemicals.

IV. Future Research Directions

EPN expects that, while EPA is implementing steps 1 and 2, the agency will continue to pursue research to increase and improve the use of NAMs in setting priorities for screening [Tier 0] and in screening compounds for potential bioactivity [Tier 1]. This high-priority research needs to provide reliable, rapid, low-cost assays to set priorities and, if possible, replace LT screening assays. While EPN understands that there are many parts of the endocrine system, an EPA choice to focus on pesticide chemicals' potential estrogenic effects in humans should not be seen to preclude concerns about other chemicals, other endocrine pathways, and other species. EPN recognizes that placing an immediate focus on prioritizing, screening, testing, and regulating, if necessary, both active and intentionally-added inert ingredients for estrogenic effects in humans will require at least five years, and possibly more, while significantly

[5] EPN's 2021 letter to Dr. Freedhoff recommended that EPA develop and take comment on the criteria it would use to determine whether the results of screening warranted imposing a requirement for Tier 2 testing. EPN thinks that the current White Paper contains much useful explanation on how EPA would make such decisions.

compromising the economy of scale, particularly in the Tier 1 screening.

Because of the statutory mandate in FFDCA, we recommend the agency intensify its work on NAMs for the estrogen pathway. One of the weaknesses of the current NAMs is not taking into account metabolism. This should be addressed as metabolism can intensify or reduce the bioactivity of chemical substances, and the lack of metabolic activation can cause NAMs to fail to identify chemicals that are endocrine active. An additional limitation is the fact that the estrogen and androgen pathways are components of the hypothalamic-pituitary-gonadal (HPG) axis, and these pathways can interact and crosstalk through the HPG axis. For the foreseeable future, NAMs cannot address interaction through the HPG and hypothalamic-pituitary-thyroid axes.

EPN supports EPA's continuing work on the development of NAMs for other endocrine pathways. Agency research and validation work has developed a HT NAM battery for androgen-related effects, and it has made some progress on NAMs for the far more complicated thyroid pathway. While estrogen, androgen, and thyroid effects are important endocrine systems for continued research, EPA should also expand its research efforts to investigate other endocrine systems such as those involved in diabetes, obesity, and lipid metabolism, for instance. EDSTAC limited its recommendations to ER, AR and thyroid because it thought that the screening and testing for endocrine activity in these three systems alone were a sufficient challenge for the agency to implement, not that they were the only systems of concern.

EPA should also continue its work to understand how studies in cell cultures and whole animals of mammalian species can (and cannot) be used to predict effects in non-mammalian species and vice versa. This understanding can potentially reduce the costs of screening and testing.

EPA should also compare the results of the enhanced mammalian reproductive tests with the older two-generation tests to determine whether the updated protocols provide substantially more power to detect and characterize the adverse effects of endocrine activity.

Finally, EPA should continue its work on how to rank chemicals in terms of their potential for human and environmental exposures. All of that work will provide an essential foundation for the time when EPA expands its focus beyond pesticide chemicals that may affect estrogen in humans.

Relevant published papers:

Steroidogenesis: Haggard et al. 2018. High-Throughput H295R Steroidogenesis Assay: Utility as an Alternative and a Statistical Approach to Characterize Effects on Steroidogenesis. *Toxicol Sci.* 2018 Apr 1;162(2):509-534. doi: 10.1093/toxsci/kfx274.

Aromatase: Chen, et al. 2015. Cell-Based High-Throughput Screening for Aromatase Inhibitors in the Tox21 10K Library. *Toxicol Sci.* 2015 Oct; 147(2): 446–457. Published online 2015 Jul 3. doi: 0.1093/toxsci/kfv141.

These comments were prepared by Penny Fenner-Crisp, Bill Jordan, Gary Timm, and Les Touart on behalf of EPN.