July 29, 2013

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Designated Federal Official
FIFRA Scientific Advisory Panel Staff
Office of Science Coordination and Policy (7201M)
Environmental Protection Agency
1200 Pennsylvania Ave., N.W.
Washington, DC 20460–0001

RE: FIFRA SAP Meeting: EDSP Tier 1 Weight of Evidence; Docket ID EPA-HQ-OPP-2013-0230.

Dear Mr. Bailey:

These comments are submitted on behalf of **People for the Ethical Treatment of Animals** (PETA), **Physicians Committee for Responsible Medicine** (PCRM), and **The Humane Society of the United States** (HSUS), national animal protection and scientific advocacy organizations, which together represent more than thirteen million members and supporters 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.

We appreciate the opportunity to comment on the documents that are the subject of this FIFRA SAP meeting, including the SAP Review of Weight of Evidence: Evaluating Results of EDSP Tier 1 Screening white paper and the additional Case study No. 5.

GENERAL COMMENTS

Use of the Weight of Evidence Approach (WoE) for Decision-making

The approach as presented by EPA for using WoE to determine whether or not a chemical interacts with the E, A, or T pathways is reasonably straightforward and transparent, but is lacking in one crucial element: how the results will be used to determine whether or not Tier 2 testing is warranted. EPA states on p. 11 that the focus of the white paper is on "the interpretative process for determining whether or not there is a chemical interaction with E, A, or T signaling pathways" and that the "document does not focus on the Tier 2 decision-making process itself." These two steps would seem to be inseparable. Will there be yet *another* process to determine if Tier 2 testing is warranted, and if so, what will this process consist of and when will it be available for both public and SAP review? The process described in this document is the evaluation of the data (strength and consistency), and not, in fact, the interpretation (meaning) of the data. Now would seem to be the time to illustrate with these case studies, how EPA proposes to take the results of its WoE analyses and use them to make decisions about Tier 2.

The case studies presented here offer the opportunity to illustrate those instances that EPA feels are likely to require additional testing, and which tests it would likely require. For example, would additional testing be required for those more equivocal cases such as where there are negative results in mechanistic studies but some positive effects *in vivo*, as in the case of Chemical N's potential interaction with the androgen pathway? Or would further testing likely be required to better characterize those cases where stronger positive evidence is present, as in the case of Chemical J's effect on steroidogenesis? Either situation may provide an opportunity for EPA to work with registrants to devise "Tier 1.5" approaches to avoid multi-generation, animal-intensive studies.

Use of an Adverse Outcome Pathway-Based Approach for Determining Interaction with the Endocrine System

The concept of adverse outcome pathways (AOP) or modes of action (MoA) is relevant and appropriate in the evaluation of EDSP data at different levels of biological organization (molecular, cellular, tissue/organ, organism) to determine whether or not a chemical interacts with E, A, or T signaling pathways. However, this approach also highlights inadequacies in the Tier 1 battery as it currently stands, with regard to understanding of intermediate events and linkages between upstream (e.g., receptor binding and gene activation) and downstream events (e.g., endpoints measured in the pubertal assays). In the future, we hope to see the focus shift away from the often equivocal results of *in vivo* assays to potentially more informative mechanistic cell and tissue-based assays, as they become available, particularly for the HPT axis which is currently lacking an in vitro suite of assays to evaluate effects on the thyroid pathway.

Decision Trees

We support the use of the decision trees described on pp. 27 & 28 for interpreting the results of the AMA and FSTRA. Indicating the increase in strength of the diagnostic utility of the endpoints along the left side of the chart for the FSTRA provides very useful and transparent information as to how observed effects may be given greater or lesser weight in a WoE approach. We recommend a similar decision tree be constructed for the multiple endpoints contained in the pubertal assays, some of which are more indicative of endocrine interaction than others.

It became obvious in reading the white paper, that a large amount of data was available for each case study, both in terms of Tier 1 assay data and OSRI. We suggest that rather than manually assimilating and interpreting all of this information, perhaps a computer program could be developed that applies decision trees and weighting factors for all the information in an integrated and standardized manner. This would avoid interpretation inconsistencies among reviewers and provide a more transparent approach. It would be much easier to manage the data as well.

Integration of Other Scientifically Available Information (OSRI) and Need for Further Testing

We are also pleased to see integration of the Tier 1 results with Other Scientifically Available Information (OSRI), including published literature studies and Part 158 studies. The Case Study

5, which depended solely on OSRI to determine potential effects, is a good example of how existing information can be used for decision-making and reducing animal use in duplicative testing. We urge EPA to make better and more consistent use of OSRI and provide better guidance to test order recipients in the future so that the OSRI submitted has a higher likelihood of satisfying the agency's data needs *without the need for Tier 1 testing*, as it did in Case Study 5.

It is important to note that with most of the case studies, the information available from the cited Part 158 studies appeared to be just as useful in helping to interpret results of the Tier 1 assays as what would be generated from the Tier 2 tests. This is especially relevant since, as was evidenced by the information presented for consideration for the June SAP regarding Tier 2 Ecotoxicity Tests, the proposed Tier 2 tests are not likely to be more accurate, reproducible or sensitive (with the exception of the extended one-generation rodent study) than any existing Part 158 studies. Furthermore, for those chemicals that tested positive, it appeared possible to calculate approximate NOAELs and LOAELs from the Tier 1 results and OSRI data. We urge EPA to take this under consideration and fully utilize all existing information, particularly for data-rich chemicals, such as pesticides, before ordering further testing.

In addition, any observed perturbations to the endocrine system must be balanced against the levels at which known, unrelated toxic modes of action for the chemical occur. It would make no sense to continue testing for endocrine effects when a chemical's ability to inhibit cholinesterase, for example, takes place at a lower dose than the endocrine effects.

Maximum Tolerated Doses and Use of Solvents

We continue to question the use of doses that begin to challenge the animal with overt toxicity and their relevance to real-world exposures. Along these same lines, for those chemicals with low solubility, use of a solvent to introduce them into aquatic test systems also seems to have no relevance to real-world exposures. Endocrine effects seen at high doses are difficult to interpret and could lead to more animal testing with little information gained.

Interpretation of Positive Results

We believe that the white paper should discuss more fully the assignment and interpretation of positive findings. There were some cases, as with Chemical J's effects in the steroidogenesis and aromatase assays, where results were clearly positive. However, there were other instances of positive findings that were not so clear-cut. In the case of Chemical N, effects in the male pubertal assay including delay in preputial separation, decrease in seminal vesicle and coagulating gland weights, and decrease in serum testosterone, were statistically significant only at the highest dose of 800 mg/kg/day. Other non-endocrine effects seen at the high dose, such as increased liver weight, and decreased serum nitrogen and alkaline phosphatase levels, were seen at lower dose levels as well, suggesting toxicity unrelated to the androgen pathway. Yet, EPA indicated that based on these findings, Chemical N was positive for the androgen pathway, despite no observed effects in the AR binding, the Hershberger, the steroidogenesis or the aromatase assays.

In cases where there are conflicting or equivocal results, it is not clear from the document's discussion whether EPA will require further evaluation. We suggest that a lack of observed effects in existing multi-generation reproduction studies conducted under Part 158 should preclude Tier 2 studies as they are unlikely to provide any additional information.

CHARGE QUESTIONS

Questions have been excerpted for brevity:

Charge 1.1. Please comment on whether the agency has transparently described the conduct and results of the individual Tier 1 studies and the OSRI for each of the case studies (Sections 6-9 of the white paper), and specifically whether the level of detail is sufficient to ensure that a study is reliable for determining the potential to interact with E, A, or T signaling pathways and the rationale for the preliminary study conclusion.

> The conduct of the individual studies has been transparently and adequately described. However, in the summary tables for the findings, there should be an additional column to explain the finding conclusion (e.g. to "equivocal," "negative," or "positive"), including the main data that support the conclusion and a brief rationale for the decision. Not all findings that are labeled with the same conclusion are identical (e.g. not all positives or negatives are supported by the same strength of evidence) and the summary should reflect this.

Charge 1.2. For each of the case studies, please comment on whether the performance criteria are clearly stated for the Tier 1 assays and, when results were not within the boundaries of the performance criteria, whether EPA has clearly expressed why the data are still considered reliable.

> Generally, yes.

Charge 1.3. The test guidelines for Tier 1 assays recommend that the organism is challenged by attaining sufficiently high treatment doses/concentrations. Difficult to test substances may be encountered in Tier 1 screening. Please comment on the agency's conclusion regarding the utility of the AMA data for Chemical S to still reliably evaluate its potential endocrine interaction in a WoE analysis.

> While some uncertainty may remain regarding testing, particularly in aquatic systems, of difficult to test substances, this solubility problem reflects a real-world issue. As long as every reasonable effort was made to maximize solubility of the test chemical, the results of the test should be considered valid, as it is highly unlikely that greater exposure would be possible under normal environmental circumstances. In this regard, use of solvents in aqueous test systems is also of questionable value.

Charge 2.1. Chemicals do not necessarily act by one adverse outcome pathway (AOP)…These case study analyses include a characterization of the treatment-dependent nature and severity of the overt toxicity as well as the specificity of the potential endocrine-related responses

coincident with the overt toxicity. These analyses inform the weight that is placed on certain Tier 1 responses in the presence of overt toxicity.

Charge 2.1.a. Chemical A can result in cholinergic toxicity given that its pesticidal mode of action is cholinesterase inhibition. In particular, overt toxicity was observed at high concentrations in the FSTRA. Although a number of endocrine responses were observed (e.g., decrease in female VTG, fecundity/fertility, GSI, male tubercles) at the highest concentration in the FSTRA, there was also pronounced overt toxicity…in male fish, overt toxicity was not observed at the intermediate concentration, possible endocrine responses were limited to two effects that lacked diagnostic specificity (i.e., altered GSI and histology).

Please comment on how the agency's has applied its decision logic to integrate an understanding of overt toxicity in the context of observed Tier 1 in vivo responses, and in particular, the agency's determination not to place weight on the FSTRA high concentration responses coincident with overt toxicity.

> It is appropriate to discount findings that could indicate a specific activity at doses that cause overt toxicity for two reasons: 1) it is not possible to discriminate specific from non-specific effects in the presence of overt toxicity, and 2) there is no practical utility to so-called "specific activity" findings at toxic doses, since the chemical will be regulated based on the most sensitive endpoint.

Charge 2.1.b. The pesticidal mode of action of Chemical S involves the uncoupling of mitochondrial oxidative phosphorylation and resulting in the depletion of ATP…At concentrations where no apparent overt toxicity occurred, there were no endocrine related responses in the FSTRA, and responses in female rats were limited to a 2 day delay in VO, and for male rats, a decrease in the weights of two androgen-dependent tissues. The majority of Tier 1 responses were decreases in the measured endpoints, which were largely expressed in the presence of overt toxicity, are consistent with a depletion of ATP and restricted caloric intake. Although male VTG was increased in fish this is likely an artifact of a single elevated response.

Please comment on how the agency's has applied its decision logic to integrate an understanding of overt toxicity in the context of observed Tier 1 in vivo responses, and in particular, on the agency's determination to place less weight on the Tier 1 in vivo responses in the presence of overt toxicity.

> See response to 2.1.a.
>
> In addition, consideration of more than one mode-of-action should also include comparison of any potential endocrine activity with the known prevalent mode of the chemical in question. For example, for Chemical A, in aquatic studies using fathead minnows, the neurological phenotype of scoliosis/lordosis was the most sensitive endpoint and for brook trout reduced growth rate in juvenile fish was the most sensitive.

Charge 2.1.c. Chemical N is a cyclic unsaturated ketone whose acute mode of toxic action is nonpolar narcosis (toxicologically induced and reversible stages of neural disruption, i.e. general anesthesia)…Unlike Chemicals A and S, the overt toxicity is not as pronounced for Chemical N. The responses in fish and rats at the high dose could be due to a compromised metabolic ability and inability to reduce chemical load.

Please comment on the agency's analysis in characterizing Tier 1 responses that are expressed at or near limit doses where some degree of overt toxicity occurs, and the extent to which such responses are considered in the WoE analysis.

It is appropriate to discount the weight of findings that at limit doses and/or that occur under some degree of overt toxicity, as described in the response to 2.1.a.

Charge 2.1.d The case study analyses described above all involve situations in which overt toxicity was observed coincident with Tier 1 responses.

Please comment on the agency's overall approach to characterizing Tier 1 responses coincident with overt toxicity and determining the weight to be given to such responses.

See 2.1.a and 2.1.b. above.

Charge 2.2. In certain case studies, there was a lack of anticipated complementary and redundant responses (within an in vivo assay or across assays) at different levels of biological organization (molecular, cellular, tissue/organ, and organism) indicative of a chemical interaction with an endocrine signaling pathway.

Please comment on the decision logic the agency has used to characterize these types of situations where there is a lack of robustness in terms of complementarity and redundancy, and the transparency and reasonableness of the approach.

Although there is uncertainty involved in circumstances where data are inconsistent or conflicting, the Agency's description of the evaluation for the case study chemicals was generally transparent and reasonable. Further characterization of endocrine pathways will help explain, support hypothesis-based testing, and decrease the uncertainty in these situations (which, if the case studies are a general indication, will be the majority of cases).

What is not clear is how this evaluation will be interpreted and used to direct possible further testing. The Agency should develop and make public for comment this critical piece of the WoE determination process.

Charge 2.3. In contrast to the situation described in question 2.2, Chemical J appears to interact with the estrogen signaling pathway in terms of complementarity and redundancy across multiple levels of biological organization as evidenced through altered steroidogenesis, resulting in decreased VTG in female fish which in turn translates to a higher-level response (e.g.,

reduced fecundity) in fish. However, this biological continuum was not observed in the Tier 1 rat female pubertal assays and the Part 158 mammalian data.

Please comment on the how the agency has characterized this endocrine interaction at different levels of biological organization across taxa, and the transparency and reasonableness of the conclusions drawn. Please include in your response, comments regarding the agency's conclusion about differences in sensitivities between taxa (i.e., fish and rats), regarding chemicals that appear to alter steroidogenesis.

The conclusion that chemical J perturbs steroidogenesis but is not likely to act via the estrogen or androgen receptors or the thyroid pathway directly seems reasonable and the reasoning is transparent. What is not clear is how the agency will interpret that information to inform risk assessment or to direct possible further testing. Any further testing, particularly in light of existing Part 158 data, should be limited to in vitro or short-term in vivo mechanistic assays that will further delineate the endocrine MOA (e.g. Tier 1.5).

Charge 2.4. Chemical A illustrates a situation where a molecular event has been initiated along a pathway via binding to the androgen receptor and by altered steroidogenesis, with corroborative evidence from the Hershberger assay. However, at a higher level of biological organization, an anti-androgenic response is not expressed within the context of the mammalian intact hypothalamic-pituitary-gonadal axis (based on the Tier 1 mammalian assays and the mammalian in vivo OSRI)…

Please comment on how the agency has integrated different sources of data along a biological continuum to characterize endocrine interactions of Chemical A and the transparency and reasonableness of the decision logic.

Again, the evaluation and integration of these findings is generally transparent and reasonable; what is not described is how these evaluations will be interpreted to decide further testing.

The Agency has not articulated how mode-of-action (MoA) information, in the absence of higher- level adverse effects, will be used; at the moment, decisions regarding risk for individual chemicals are driven by observations of adverse effects and therefore we would expect that the Agency would require no further testing in the case Chemical A.

One can envision that MoA information could inform cumulative risk analyses. MoA information could also be used to direct more specific testing (e.g. Tier 1.5) and to inform AOP development and therefore this information should be added to the appropriate data and knowledge-bases (e.g. OpenTox/AOP Wiki).

Charge 2.5. In some chemical situations, the in vitro Tier 1 data are negative. Nonetheless, this does not necessarily detract from a conclusion of a potential endocrine interaction in vivo either because a different molecular initiating event (MIE) may be occurring than what the in vitro assay evaluates or because an activated metabolite may be responsible for the in vivo effects…

Please comment on the how the agency has integrated different sources of data along a biological continuum to characterize this endocrine interaction and the transparency and reasonableness of the conclusion drawn.

It is not clear how the Agency will interpret these findings either to inform risk assessment or to direct further testing. Such findings do, however, highlight the need for a broader array of mechanistic tests, such as those contained in the ToxCast or CompTox battery of tests, that could inform the spectrum of possible MOA's for a given chemical.

Charge 2.6. In each of the cases studies, there was a lack of anticipated complementary and redundant responses indicative of a chemical's interaction with the thyroid signaling pathway...

Please comment on the how the agency has characterized this endocrine interaction at different levels of biological organization, and the transparency and reasonableness of the conclusion drawn.

Some of these findings are reflective of the inherent variability and uncertainty regarding the test methods or individual endpoints (e.g. hormone levels). The Agency's evaluation was generally transparent; however, in the case of Chemical A, the evaluation of the thyroid findings in the male and female pubertal seems inconsistent: on the one hand, the lack of dose-responsiveness of T4 in the male was considered negative, while a similar non-dose responsive finding in the female was considered equivocal.

In general, it is appropriate to conclude that a preponderant lack of consistency in findings indicates a negative response.

Charge 2.7. In the absence of Tier 1 data, OSRI was available for Chemical X that indicated effects on thyroid endpoints in the rat but the results were inconsistent within and among studies and there was no OSRI presented from amphibian studies. Because of studies that were not specifically validated to detect an interaction with the thyroid hormonal pathway, limited data, and ambiguous results, the potential for Chemical X to interact with the thyroid pathway cannot be excluded.

Please comment on the how the agency has characterized this endocrine interaction at different levels of biological organization, and the transparency and reasonableness of the conclusion drawn.

While the agency appears to have correctly characterized the potential for thyroid interaction, interaction with the estrogen pathway is much more clearly defined and supportable. EPA should consider how this informs risk assessment first, keeping in mind that there are potentially effects to the thyroid pathway. It may well be that any additional controls on this chemical for the estrogen pathway would be protective of the thyroid pathway as well.

Charge 3. Based on all of the case study analyses, please provide overall comments on how the

agency has employed its WoE guidance and characterized the evidence and conclusions and include in your response the following points:

a. How consistent and transparent the cases studies are in terms of documentation.

The Agency's evaluation of the data was generally consistent and transparent (for an exception, see response to 2.6).

In addition to the information presented in the summary tables, it would be helpful to have an additional column to explain the finding conclusion (e.g. to "equivocal," "negative," or "positive"), including the main data that support the conclusion and a brief rationale for the decision. Not all findings that are labeled with the same conclusion are identical (e.g. not all positives or negatives are supported by the same strength of evidence) and the summary should reflect this.

b. How adequately the agency has described the extent of complementarity and redundancy of responses and has integrated and interpreted diverse lines of evidence across different biological levels of organization and taxa to reach preliminary conclusions regarding endocrine interactions.

While the agency has summarized the findings and evaluated the data to an adequate extent, what remains to be articulated is the agency's interpretation of the data in terms of either informing risk assessment of in directing further possible testing. The agency should develop and make public for comment this critical piece of the WoE determination process.

c. How the agency has used OSRI data to further characterize the observations from EDSP Tier 1 assays in determining potential chemical interactions with the E, A, and T signaling pathways.

We appreciate the agency's attempt to include OSRI data to further characterize EDSP Tier 1 observations. However, it is not clear how OSRI data will be used to inform decisions regarding possible further testing; we urge the agency to fully consider the adequacy of OSRI data in this regard, particularly considering that the proposed Tier 2 tests are not likely to be more accurate, reproducible or sensitive (with the exception of the extended one-generation rodent study) than many of those tests already carried out.

In addition, OSRI should be used to a greater extent to inform whether and which Tier 1 testing is indicated (i.e., MoA information).

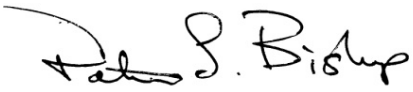
d. How the agency has considered the understanding of a chemical's mode of action and how that informs the weight that is placed on Tier 1 responses in the presence of uncertainties introduced by dose setting, overt toxicity, and portal of entry issues.

The concept of adverse outcome pathways (AOP) or modes of action (MoA) is relevant and appropriate in the evaluation of EDSP data at different levels of biological

organization (molecular, cellular, tissue/organ, organism) to determine whether or not a chemical interacts with E, A, or T signaling pathways. However, consideration of mode-of-action should also include comparison of any potential endocrine activity with known prevalent mode-of-action of the chemical in question. For example, if the chemical is a known neurotoxin at lower concentrations than the chemical displays any potential endocrine activity, further testing is not indicted.

In addition, evaluation of Tier 1 data with respect to MoA highlights inadequacies in the Tier 1 battery as it currently stands, with regard to understanding of intermediate events and linkages between upstream (e.g. receptor binding and gene activation) and downstream events (e.g. endpoints measured in the pubertal assays). In the future we hope to see the focus shift away from the often equivocal results of *in vivo* assays to potentially more informative mechanistic cell and tissue-based assays, as they become available, particularly for the HPT axis which is currently lacking an in vitro suite of assays to evaluate effects on the thyroid pathway.

Sincerely,



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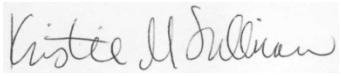

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