BOSC
BOARD OF SCIENTIFIC COUNSELORS

December 19, 2023

H. Christopher Frey, Ph.D.
Assistant Administrator
Office of Research and Development
U.S. Environmental Protection Agency

Dear Dr. Frey:

On behalf of the Board of Scientific Counselors (BOSC), we are pleased to provide you a review report addressing the charge questions posed by the Office of Research and Development to the BOSC EPA Transcriptomic Assessment Product (ETAP) Panel.

The ETAP Panel was charged with assessing the documents supporting the development of transcriptomic-based reference values and the implementation of a new EPA Transcriptomic Assessment Product. This report represents the cumulative effort of the ETAP Panel's workgroups and the Executive Committee.

We anticipate that this report will assist ORD in evaluating the ETAP methodology and its impact on Agency decision making. We will be happy to provide any additional information concerning the review or answers to any questions you may have, and we look forward to working with you in the future on these programs.

Sincerely,

Paul Gilman

Paul Gilman, Ph.D.
Chair, BOSC

Lucinda Johnson

Lucinda Johnson, Ph.D.
Vice Chair, BOSC

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REPORT OF THE U.S. ENVIRONMENTAL PROTECTION AGENCY BOARD OF SCIENTIFIC COUNSELORS EPA TRANSCRIPTOMIC ASSESSMENT PRODUCT (ETAP) PANEL

RESPONSES TO CHARGE QUESTIONS

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Tom Tracy, Designated Federal Officer

July 11-12, 2023

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LIST OF ACRONYMS

AI	Artificial Intelligence
AOP	Adverse Outcome Pathways
API	Application Programming Interface
BMD	Benchmark Dose
BMDL	Benchmark Dose Level
BOSC	Board of Scientific Counselors
BW	Body Weight
CBI	Confidential Business Information
ECHA	The European Chemicals Agency
EPA	Environmental Protection Agency
eSTAR	Emerging Systems Toxicology for Assessment of Risk
FACA	Federal Advisory Committee Act
ETAP	EPA Transcriptomic Assessment Product
FDA	Food and Drug Administration
GIVIMP	Good *In Vitro* Method Practices
GO	Gene Ontology
HTTK	High-Throughput Toxicokinetics
HTTr	High-Throughput Transcriptomics
HTPP	High-Throughput Phenotypic Profiling
IATA	Integrated Approaches to Testing and Assessment
ICE	Integrated Chemical Environment
ITRC	Interstate Technology and Regulatory Council
IUCLID	International Uniform Chemical Information Database
IVIVE	*in vitro* to *in vivo* extrapolation
LC	Liquid Chromatography
LOAEL	Lowest Observed Adverse Effect Level
MS	Mass Spectrometry
NAM	New Approach Methods
NAS	National Academy of Sciences
NCCRP	New Chemicals Collaborative Research Program
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIH	National Institute for Health
NOAEL	No Observed Adverse Effect Level
NSF	National Science Foundation
OECD	Organization for Economic Cooperation and Development
OPPT	Office of Pollution Prevention and Toxics
ORD	Office of Research and Development
PECO	Population-Exposure-Comparator-Outcome
PFAS	Per- and polyfluoroalkyl substances
PFOA	Perfluorooctanoic acid

POD	Point of Departure
PPRTV	Provisional Peer-Reviewed Toxicity Value
QSAR	Quantitative structure-activity relationship
(Q)SAR	A collective term signifying QSARs and SARs collectively
QSUR	Quantitative Structure Use Relationships
RACT	Research Area Coordination Teams
RfD	Reference Dose
SAR	Structure-activity relationship
SEM	Systematic evidence mapping
SMARTS	Simplified Molecular-input line-entry system Arbitrary Target specification
TK	Toxicokinetic
TRV	Transcriptomic Reference Value
TSCA	Toxic Substances Control Act
UF_H	Uncertainty Factor to account for intraspecies variability
UF_A	Uncertainty Factor to account for interspecies differences
UF_D	Uncertainty Factor to account for database limitations
UF_S	Uncertainty Factor to account for duration
UF_L	Uncertainty Factor to account for LOAEL-to-NOAEL extrapolation
UVCB	Unknown or variable composition, complex reaction products, or biological materials
VOI	Value of Information

INTRODUCTION

The EPA Transcriptomic Assessment Product (ETAP) is a methodology proposed by the Office of Research and Development (ORD) at the US Environmental Protection Agency (EPA) to develop transcriptomic reference values (TRV). The scientific rationale underlying ETAP is provided in the EPA report entitled Scientific Studies Supporting Development of Transcriptomic Points of Departure for EPA Transcriptomic Assessment Products (ETAPs) (EPA 2023). EPA has a need to develop TRVs, defined as estimates of daily oral doses likely to be without appreciable risk of adverse effects following chronic exposure. The TRV is intended to protect both the individual and population from adverse effects. While a TRV is expressly defined as a chronic value in an ETAP, it may also be applicable across other exposure durations of interest including short-term and subchronic exposures. This generalization has been previously used by EPA in certain risk assessment applications [e.g., Provisional Peer-Reviewed Toxicity Value (PPRTV) assessments] where a chronic non-cancer reference value has been adopted as a conservative estimate for a subchronic non-cancer reference value when data quality and/or lack of duration-relevant hazard and dose-response data preclude direct derivation.

The ETAP is intended to be applied to data-poor substances with no existing or publicly accessible repeated dose toxicity studies or suitable human evidence available. ETAPs may be updated to incorporate new data or methodologies that might impact the estimated reference values or retired if traditional toxicity studies and an associated human health assessment are published.

The scientific peer review of the ETAP includes two main documents. The first document, entitled Scientific Studies Supporting Development of Transcriptomic Points of Departure for EPA Transcriptomic Assessment Products (ETAPs), details the scientific studies and analyses supporting development of transcriptomic points-of-departure for ETAP. The second document, entitled Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs), outlines the methods for deriving a transcriptomic-based toxicity value and developing an ETAP. A reporting template for ETAP and an example ETAP assessment are provided as embedded files in the second document for review.

The identified strengths, suggestions, and recommendations herein are informed by a review of the EPA's draft reports entitled, "Scientific Studies Supporting Development of Transcriptomic Points of Departure for EPA Transcriptomic Assessment Products (ETAPs)" and "Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs)," the EPA's presentations to the Committee, available scientific literature, and Committee members' experiences using a variety of NAM tools including those developed or used by the EPA.

In this report, Committee members provide specific Recommendations for priority actions by EPA as the Agency moves forward with implementing the ETAP. These Recommendations should be of the highest priority. The Committee also provides numerous Suggestions. The Committee's judgment regarding the priority for these Suggestions and estimates of the level of effort for each Suggestion are also provided to aid decision making. However, these Suggestions are subordinate to the Recommendations. Accordingly, Suggestions should be viewed as information for EPA to take under consideration, whereas Recommendations should be viewed as activities that the Committee agreed reflected the most critical opportunities to improve the ETAP.

CHARGE QUESTIONS AND CONTEXT

The ETAP Panel was charged with four questions as follows:

Q.1: Given the literature review and the data analysis performed in the documents, please comment on whether the approach outlined for transcriptomic benchmark dose analysis and gene set summarization following a 5-day *in vivo* exposure are clearly described and provide a scientifically supportable estimate of the point-of-departure for chronic toxicity for data-poor chemicals.

Q.2: EPA has proposed standard uncertainty factors to account for intraspecies variability (UF_H), interspecies differences (UF_A), database limitations (UF_D), duration (UF_S), and LOAEL-to-NOAEL extrapolation (UF_L) in the standard methods document. *Are the uncertainties in the derivation of the toxicity values clearly described, and are the uncertainty factors scientifically justified?*

Q.3: To facilitate timely development and release of ETAPs, EPA is proposing to have the standard methods document undergo peer-review. Individual ETAP reports based on these peer-reviewed methods would undergo internal technical and quality control review but not need to be individually peer-reviewed. *Please comment on this proposed approach*.

Q.4: To facilitate rapid development and review of each ETAP, the results from the systematic evidence mapping, 5-day transcriptomic study, and TRV derivation are compiled and reported in a standardized ETAP reporting template with minimal free-form text. The ETAP template and an example ETAP using empirical data are provided for your review. *Please comment on the extent to which the content and format of the reporting template and the example ETAP provide the important quantitative human health assessment information for a data-poor chemical, with suggestions for improvement if warranted.*

PANEL RESPONSES TO CHARGE QUESTIONS

Charge Question 1

Q.1. Given the literature review and the data analysis performed in the documents, please comment on whether the approach outlined for transcriptomic benchmark dose analysis and gene set summarization following a 5-day *in vivo* exposure are clearly described and provide a scientifically supportable estimate of the point-of-departure for chronic toxicity for data-poor chemicals.

Narrative

Based on the analyses presented to the panel, the panel concludes that the ETAP methodology, for the chemical and toxicological space explore, produces PODs that are consistent with those developed based on the results of the two-year chronic bioassay.

To answer charge question 1, the panel explored a number of questions, including the following:

- Were the analyses conducted and data used to assess the scientific evidence supporting the ETAP sufficiently complete, or were there critical analyses that could have been conducted but were not?
- Was the evidence presented sufficient to conclude with appropriate confidence that the 2700 gene set used in the ETAP method adequately represents the biological response space?
- Was the evidence of concordance in PODs between the ETAP method and results based on apical endpoints from chronic studies sufficiently strong and for a broad enough chemical space to justify the ETAP method?
- Are the methods used to develop BMDLs from the gene set data appropriate/accurate?
- Is the rollup of data from transcripts to genes to gene sets for BMD analysis scientifically justifiable?
- Were the statistical approaches applied to determine the parameter values appropriate and did they result in a set of optimal parameters likely to be appropriate for the expected application, e.g., a broader chemical space?

Strengths

- The observed concordance between the ETAP method and the more established method of determining a POD from 2-year chronic bioassays was strong and clearly sufficient to justify its use for data-poor chemicals.
- No data were excluded with respect to apical endpoints, and the analyses relied on the most sensitive endpoint as identified by the original authors.
- There was a careful analysis of the dose spacing to optimize the dose response information.

Suggestions

- The Panel suggests that the EPA continue to periodically evaluate the ETAP methodology as data for additional chemicals, for example chronic and subchronic animal data with corresponding transcriptomic data, become available, and consider their value to improve the method if appropriate.
- The EPA should re-assess the selection of the optimal combination of parameters, for example, those used in pre-modeling probe filter, BMD modeling and gene set summarization (Table 4.3) as additional data become available.

Recommendations

The Panel offers the following recommendations:

Recommendation 1.1: The Panel recommends that the Agency be more precise and consistent in the use of the terms "most sensitive" (related to gene sets) and "POD" in the Scientific Support Document. The ETAP Standard Methods document states Section 3.4.5.5): "The most sensitive Gene Onotology (GO) biological process class is identified based on the lowest median BMD across the tissues examined in either sex." Similar definition of the terms "most sensitive gene set", "most sensitive GO biological process class" and "transcriptomic POD" should be provided in the ETAP Scientific Support document (e.g., the point-of-departure (POD) should be consistently defined and used and "the lowest POD" should not be replaced with the term "most sensitive" Finally, the EPA states: "The ETAP is intended to be applied to data-poor substances with no existing or publicly accessible repeated dose toxicity studies or suitable human evidence." **The panel also recommends the EPA further clarify and clearly lay out the criteria for determining whether a chemical is data-poor and use that the term consistently in the ETAP documentation the panel reviewed.**

Recommendation 1.2: The ETAP VOI document states that *"In addition, strategically integrating the ETAP approach with other established methods, such as chemical categorization and read across, could further enhance the public health benefits, enabling the EPA to more rapidly address public health and environmental challenges (e.g., per- and polyfluoroalkyl substances)".* **In the ETAP Methodology documentation, the EPA should discuss how predictive methodologies and other sources of information (e.g., read across, QSAR; see VOI document) could be used in the process of prioritizing and selecting chemicals for the ETAP process.** For chemicals that may not be appropriate for ETAP because of predicted long half-lives or expected significant cross species differences EPA could consider using a different type of work product (e.g., PPRTV) that relies on a toxicogenomic POD but with a different adjustment factor than is used in the ETAP program, or using a different adjustment factor than is used in the ETAP and, if required or appropriate, submit the choice of the factor to a peer review with narrow scope.

Recommendation 1.3: The Panel recommends that the Agency clearly state what is meant by "apical endpoint" and document the range of endpoints that were the basis of the reported PODs used in the analysis presented in the document "Scientific Studies Supporting Development of Transcriptomic Points of Departure for EPA Transcriptomic Assessment Products." The tissues where transcriptomic data were collected are identified on an individual study basis. The tissue specific apical endpoint data should also be reported completely and on a tissue-specific basis. In particular, apical endpoint information similar to that shown in Table 4-1 (p.43) should be provided for all chemicals referenced in Figures 3-1 through 3-3.

Recommendation 1.4: The Panel recommends that the Agency ensure that the definition of TRV includes the concept of a generalized biological response not intended to represent a specific biological pathway or effect.

Recommendation 1.5: The Panel recommends adding a footnote to the PFOA results (1st paragraph of p. 33 of "Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs)") noting that in their most recent risk assessment for perfluorooctanoic acid (PFOA), EPA

(2023) relied on the results of epidemiological studies suggesting effects at exposures several orders of magnitude lower than effects observed in animal data as PODs for the reference dose (RfD). This note could clarify that, for the purposes of evaluating the correlation of the ETAP with the 2-year bioassay, the RfD derived from animal studies was the appropriate comparator.

Recommendation 1.6: The Panel recommends revising the Standard Methods for Development of EPA Transcriptomics Assessment Projects documentation as follows:

- The Background or Assessment Review section should include a statement of limitations for the underlying assumptions along with the appropriate intended application; i.e., as noted in other reviews (e.g., the NAS Silver Book), there are concerns with the Reference Dose being interpreted as a population threshold value even though it does not represent the current regulatory value. It should also note that the TRV based on results from the ETAP could be replaced later should more data become available for the chemical. The information in this section needs to be consistent with the other risk assessment approaches and documents the Agency releases.
- The Panel recommends developing a chart or graphic that explains the study design process for the 5-day studies used in ETAPs. Such a graphic or chart would help readers and end users to identify the points of attrition in determining the first lowest positive dose, the number of rats, the final dose spacing, tissue selection if non-default tissues are used, and other relevant details.
- Define the criteria for lowest dose selection.
- Define the criteria for inclusion of tissues beyond the standard list.
- Describe the process for tissue isolation and how this will be standardized across chemicals.
- Include in the template a precise definition of "data-poor" based on the revisions in response to Recommendation 1.1. EPA should then confirm that all uses of the term throughout the template are consistent with that definition.
- The Agency should provide additional details regarding the selection of acceptable purity for single chemicals/mixtures and formulations for testing.
- Clearly define what determines the most sensitive GO process in section 8.1.8.
- The statement: "The TRV is meant to protect both the exposed individual and population from effects other than cancer or related to cancer if a necessary key precursor event does not occur below a specific exposure level" (p. 9, 2nd paragraph; p. 31, last paragraph of "Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs)") should be shortened to "The TRV is meant to protect both the exposed individual and population from effects other than cancer."

Future Considerations

- EPA should consider conducting *in vitro* transcriptomic studies in multiple tissues (liver, kidney, brain, etc.) on chemicals for which there are in vivo ETAPs. The goal of these studies would be to eventually provide adequate validation data to support replacement of the in vivo ETAP with a battery of in vitro transcriptomic assays should they be found to be a better alternative based on science and other considerations (e.g. cost, time, reduce use of animals).
- As noted in the Recommendations, the EPA's statement that: "The TRV is meant to protect both the exposed individual and population from effects...related to cancer if a necessary key precursor event does not occur below a specific exposure level" (p. 9, 2nd paragraph; p. 31, last paragraph of "Standard Methods for Development of EPA Transcriptomic Assessment Products

(ETAPs)") should be shortened to remove reference to cancer endpoints. Significant effort to determine whether "a necessary key precursor event does not occur below a specific exposure level. "The panel agrees that research to understand the applicability of the ETAP to cancer endpoints would be highly valuable. For example, the EPA should consider developing an *in vitro* genotoxicity battery that could potentially be performed on a chemical with a completed ETAP to support the relevance of the TRV to the possible carcinogenicity of that chemical.

- When an adequate number and variety of chemicals have been evaluated in an in vivo ETAP, EPA should evaluate which tissues provide the most informative transcriptomic data for establishing a POD and which tissues have not been informative, and do not add significant value. For example, EPA could consider how often a POD for another tissue is significantly lower than that for the liver. The goal of these analyses would be to update the methodology if another set or more limited set of tissues or cell types to use is found to be more appropriate.
- As the cost of sequencing decreases, EPA could consider utilization of single cell sequencing technology as a way to address heterogeneity in the transcriptomic response across cells of a tissue. What are the most cost-effective set of tissues relevant to public health?
- The ETAP does not provide information on hazard and that information can be useful in decision making. EPA should consider complementary work that utilize in vitro information and gene sets from the toxicogenomic read outs that could be utilized outside the ETAP process to identify key characteristics (e.g., key characteristics of human hepatotoxicants) and other indicators of hazard for the tested compounds.

Charge Question 2

Q.2. EPA has proposed standard uncertainty factors (UFs) to account for intraspecies variability (UF_H), interspecies differences (UF_A), database limitations (UF_D), duration (UF_S), and LOAEL-to-NOAEL extrapolation (UF_L) in the standard methods document.

Are the uncertainties in the derivation of the toxicity values clearly described, and are the uncertainty factors scientifically justified?

Narrative

In the draft ETAPs "Standard Methods" document, EPA has proposed "standard" (i.e., default) uncertainty factors for development of ETAPs based on existing EPA guidance for selection of UFs for RfD development. A standard (default) composite (total) UF of 300 is proposed, based on the following standard (default) individual UFs:

- UF_H (intra-human variability) – 10.

- UF_A (animal-to-human) – 3. This includes a UF of 3 for toxicodynamic differences, and a UF of 1 (i.e., no adjustment) for toxicokinetic (TK) differences because interspecies TK differences are accounted for by body weight $(BW)^{3/4}$ allometric scaling.

- UF_s (subchronic-to-chronic duration) – 1. Although chronic TRVs are based on a 5-day (less than subchronic duration) study, a UF of 1 (no adjustment) is proposed because empirical data indicates concordance between PODs based on apical endpoints from chronic in vivo studies and PODs based on transcriptomic data from short-term studies.

- UF_L (Lowest Observed Adverse Effect Level [LOAEL]-to-No Observed Adverse Effect Level [NOAEL]) - 1. No adjustment is made because the PODs used for TRVs are BMDLs, and the UF is only used if the POD is a LOAEL.

- UF_D (Database Uncertainty Factor) – 10. This UF is applied when the toxicology database is incomplete, and toxicological endpoints that have not been studied are potentially more sensitive than the critical effect selected as the basis for the RfD (or TRV). Empirical data indicates that PODs (i.e., BMDLs) based on transcriptomic data from the 5-day studies agree with PODs (i.e., BMDLs) based on histopathological effects in chronic studies. However, other potential effects including reproductive, developmental, endocrine, neurological, or immune system toxicity are not necessarily accounted for in the 5-day transcriptomic studies, and these effects are potentially more sensitive than systemic effects indicated by histopathological changes.

The draft document states that in the "rare case that information is surfaced for a data-poor chemical that informs some aspect of a given area of uncertainty there may be an opportunity to reduce quantitative uncertainty applications". The panel recognized that the EPA should be prepared to also increase an uncertainty factor if the data that surfaces for a data-poor chemical supports the increase. However, the panel understands that because a key benefit of a standardized and accepted ETAP methodology is the elimination of the time and resources required for peer review of each assessment, any chemical-specific changes to the ETAP method, for example changing an uncertainty factor, would trigger a requirement for peer review and increase the time and cost of the ETAP assessment. Therefore, any departure from the standard defaults would be done sparingly, and if a peer review is triggered it could be narrow in scope and directed at the specific change for the chemical in question.

The Panel discussed whether the default UFs used for RfDs based on *in vivo* animal data are necessarily applicable to TRVs developed through the ETAP process and whether these default UFs can be assumed to capture all of the uncertainty associated with TRVs developed through the ETAP process. These points were discussed:

- Multiple health effects studies, including mechanistic studies, are usually available for chemicals for which RfDs are developed. However, chemicals evaluated through ETAPs will have only one standardized-protocol animal study and no human evidence. Since the ETAP represents a new EPA product, it is expected that the determination of the default UFs will eventually be reevaluated after a sufficient number of ETAPs have been performed to determine if the methodology should be revised.

- Some panel members noted that for some chemicals the EPA has determined that a UF_H greater than 10 may be appropriate to account for susceptible subpopulations, and that chemicals addressed through ETAPs may be novel, with no information as to whether they differ from previously regulated chemicals in this regard.

- For some types of chemicals (e.g., many PFAS), a Dosimetric Adjustment Factor based on $BW^{3/4}$ allometric scaling (equivalent to an adjustment of approximately 5-fold for rats) is not nearly sufficient to account for animal-to-human toxicokinetic differences. For this reason, RfDs developed by EPA and the states for such chemicals incorporate chemical-specific TK adjustments to account for the much slower clearance (e.g., much longer half-life) in humans compared to laboratory animals. This point is particularly relevant because EPA stated that the ETAPs process is likely to be used to develop TRVs for data-poor PFAS, as illustrated by the example ETAP developed by EPA, which was for a PFAS (perfluoro-3-methoxypropanoic acid).

In addition to this robust discussion about particular classes of chemicals that the proposed default uncertainty factors might not apply to, the panel discussed the practicality of identifying such chemicals when by definition little or no toxicity or toxicokinetic data exist for them. The panel also discussed the implications for the ETAP process if it recommended changes that shifted it from a standard process that produces PODs that do not require extensive and costly peer review to a flexible one that that requires peer review. The panel recognized the value of an efficient ETAP process that can be successfully applied to the vast majority of compounds, and declined to recommend a chemical-by-chemical consideration of departure from the standard default.

The panel recognized there are opportunities during the ETAP process for EPA's consideration of additional information on a candidate ETAP chemical that could either inform a decision whether to depart from the default ETAP uncertainty factor or provide useful data for any subsequent studies on the chemical. However, as discussed above, the Panel understands that departing from the default ETAP uncertainty factor could trigger an external peer review. Therefore, the panel makes two observations, one related to the $BW^{3/4}$ and one related to UF_s, but both are related to compounds with long half-lives:

- Predictive methods (computational approaches and/or read-across based on chemicals with similar structures or properties) can provide information as to whether a $BW^{3/4}$ adjustment is expected to be sufficient to account for interspecies TK differences. Evaluations using these methods could be performed during the literature review phase of the ETAP process and should therefore not increase the time needed to complete the ETAP.

- The proposed UF_S (subchronic/short-term-to-chronic duration) of 1 is based on the empirical observation that the PODs from the chronic in vivo studies that were reviewed are concordant with PODs from short-term transcriptomic studies. However, chemicals with long half-lives (~1 day or longer) in rats will not reach steady-state by the end of the 5-day transcriptomic study, meaning that the internal dose at the end of the 5-day study will be somewhat lower than in the chronic study. The collection of blood concentration data at the end of the ETAP exposure would provide useful data for the design of any subsequent experimental animal or *in vitro* studies on the chemical. Also, this may be a case where the EPA decides it is appropriate to depart from the default and may decide a peer review that is limited in scope is appropriate.

Strengths

- The default UFs are based on, and consistent with, existing EPA guidance and policy for selection of UFs in RfD development.
- The Panel agrees that the existing default UFs are appropriate and are unlikely to require chemical-specific revision in most cases.
- Toxicodynamic component of UF_A (animal-to-human) – 3: It is unlikely that data to indicate a UF other than 3 would be available for chemicals evaluated through the ETAP process.
- UF_L (LOAEL-to-NOAEL) -1: A strength of the ETAP approach is that it is based on a BMDL, and this UF is not needed when the POD is a BMDL.
- UF_D (to account for potentially more sensitive effects for which there are data gaps)- 10: The rationale provided by EPA justifies the use of 10 for this UF.

Suggestions

- During the scoping/literature review phase of the ETAP process, predictive methods (computational approaches and/or read-across based on chemicals with similar structures or properties) could be used for PFAS and other types of chemicals known to have large interspecies half-life differences to provide information as to whether a BW3/4 adjustment is expected to be sufficient to account for interspecies TK differences. The results of this predictive modeling could be included in the literature review section of the ETAP documentation.
- During the scoping/literature review phase of the ETAP process, predictive methods (computational approaches and/or read-across based on chemicals with similar structures or properties) should be considered to identify chemicals that may have longer half-lives (i.e., greater than ~ 1 day) in rats. For chemicals identified as potentially having long-half lives in rats, measurement of blood levels of the chemical on days 1 and 5 of the transcriptomic study could be performed to provide a preliminary half-life estimate that might be of value for any further in vivo or in vitro studies or in deciding whether to depart from the standard ETAP methodology.

Recommendations

The Panel offers the following recommendations:

Recommendation 2.1: **The EPA should state in the method documentation that they will periodically review the basis for the default uncertainty factors and if justified, make the necessary adjustments to the method.**

Recommendation 2.2: EPA should revise its statement that default UFs may be decreased when supported by chemical-specific information to say that default UFs may be increased or decreased in such situations.

Charge Question 3

> Q.3: To facilitate timely development and release of ETAPs, EPA is proposing to have the standard methods document undergo peer-review. Individual ETAP reports based on these peer-reviewed methods would undergo internal technical and quality control review but not need to be individually peer-reviewed. *Please comment on this proposed approach*.

Narrative

A structured set of scientifically justified individual analyses and procedures, applied equally to each chemical, comprise the ETAP methodology. Peer review and formal approval of the method obviates the need for external peer review of each ETAP chemical assessment. However, each ETAP assessment does undergo internal technical and quality control before completion. The panel agreed that additional external peer review would add little or no value and was contrary to the goals of the ETAP product, but did note some exceptional circumstances where EPA may determine a departure from the standard approach would produce a more scientifically sound assessment. The panel noted that EPA has recognized that that in rare cases there may be a need to change a default UF, but doing so would likely trigger the need for an external peer-review.

Strengths

- Relies on a standardized approach.
- Does not require any expert judgment.
- Follows existing and standardized protocols in study design and analysis.

Suggestions

The Panel suggests a periodic update and external peer review of the ETAP methodology and reporting so the process remains robust enough to continue to justify no peer review of the individual ETAP projects.

Recommendations

The Panel offers the following recommendation:

Recommendation 3.1: Do not add a routine, independent peer review process for individual ETAP products. There is limited or no value to peer review of individual ETAP products that are the product of a peer reviewed and approved standardized process without assessment or judgments. In an exceptional case, the EPA may decide to depart from the standard process, including a limited scope peer review, which would be consistent with this recommendation.

Charge Question 4

> Q.4: To facilitate rapid development and review of each ETAP, the results from the systematic evidence mapping, 5-day transcriptomic study, and TRV derivation are compiled and reported in a standardized ETAP reporting template with minimal free-form text. The ETAP template and an example ETAP using empirical data are provided for your review.
>
> *Please comment on the extent to which the content and format of the reporting template and the example ETAP provide the important quantitative human health assessment information for a data-poor chemical, with suggestions for improvement if warranted.*

Narrative

The ETAP method utilizes transcriptomic data, which is summarized using gene sets related to GO biological processes that are not directly or mechanistically linked to toxicity endpoints from previously regulated chemicals, e.g., apical effects in tissue. This will generate a large volume of data of interest to the scientific community and stakeholders, for this reason, the panel felt there would be considerable value in providing the raw data online in a form that is easily accessed, and citing the location in the document. In addition, because the ETAP methodology is a structured, stepwise process for deriving TRVs and PODs, each report should include all the necessary information to make it a standalone document. The panel recommends the addition of some standardized, brief, language to increase clarity and reduce the possibility of misinterpretation.

Strengths

- Standardized process and format to ensure consistency across reports.

- Systematic evidence mapping (SEM) to confirm that no data currently exists for the chemical. The SEM process has been previously peer-reviewed and published.
- Reference value derivation is consistent with existing risk assessment practices.

Suggestions

Consider adding the following to the standard ETAP method chemical report:

- Include a copy of the Data Quality Act checklist.
- Consider automation of report generation to avoid calculation errors.
- By definition, chemicals selected for ETAP have been determined to have no "suitable human studies" or any animal studies through the systematic review process. This systematic review should be documented. Such documentation will need to go beyond simply providing references and will need to provide justification as to why a study was considered sufficient or insufficient.

Recommendations

The Panel offers the following recommendations:

Recommendation 4.1: The EPA should add the following information to the individual reports:

- The Methods documentation includes the following regarding dose identification: "For the ETAP study design, a minimum of five dose levels plus control will be evaluated. In general, the lowest positive dose should be a full log10 lower than the next dose. The remaining doses should use half-log spacing." Since the actual doses used per chemical are not defined**, the criteria for selecting the lowest (or highest) dose should be included in the report for each chemical.** If the criteria used are always the same (e.g., highest dose is determined by limits of solubility) then the Methods document should clearly define those criteria, which removes the need to document this information individually for each chemical.
- The Methods document lists 12 standard tissues to be used for transcriptomic measurements: "kidney, liver, adrenal gland, brain, heart, lung, ovary (females), spleen, testis (males), thyroid, thymus, and uterus (females)." It also allows for isolation of RNA from additional tissues: "RNA may be isolated from other tissues and organs to increase the breadth of transcriptomic coverage, but it is not required." **Should additional tissues be tested or should any of the standard tissues not be tested; the inclusion/exclusion criteria determining the tissue selection for that chemical should be contained in the report.** The number of tissues tested could influence the "lowest median BMD across the tissues examined in either sex," which is used to define the POD, because of multiple testing considerations. For this reason, a caveat should be noted in the report when using something other than the 12 standard tissues.
- Table 3-1 from the Methods document outlines the general Population-Exposure-Comparator-Outcome (PECO) criteria that are to drive the systematic review. **The specific PECO criteria for the subject of the ETAP assessment should be documented in Appendix I along with the literature search results.**
- The evaluation of studies from the literature that pass the PECO criteria are defined in Section 3.2.4 of the Methods document. The criteria as described allow for expert judgment,

especially with epidemiologic studies where a determination must be made whether "exposure-response results are presented in sufficient detail." **The final ETAP report should include a list of all publications that pass the PECO filter, including studies deemed insufficient for use in POD/TRV derivation.**

- **Table 5-1, which summarizes the animal study parameters, should include the type of food and water used during the animal study, and samples of both should be saved for future testing.**
- **The template (section 5.2) should have standard documentation regarding the probes that are used and version of the sequencing platform.** This is needed in case these are changed in the future to make it clear when comparing documents whether different platforms were used.
- **Make all raw data (with filtered genes flagged but not deleted) and results from standard processing prior to TRV determination available to scientific community**. Provide a link to the publicly available data in Section 5.2 of the final report.

SUMMARY LIST OF CORE RECOMMENDATIONS

Recommendation 1.1: The Panel recommends that the Agency be more precise and consistent in the use of the terms "most sensitive" (related to gene sets) and "POD" in the Scientific Support Document. The ETAP Standard Methods document states Section 3.4.5.5): "The most sensitive Gene Onotology (GO) biological process class is identified based on the lowest median BMD across the tissues examined in either sex." Similar definition of the terms "most sensitive gene set", "most sensitive GO biological process class" and "transcriptomic POD" should be provided in the ETAP Scientific Support document (e.g., the point-of-departure (POD) should be consistently defined and used and "the lowest POD" should not be replaced with the term "most sensitive" Finally, the EPA states: "The ETAP is intended to be applied to data-poor substances with no existing or publicly accessible repeated dose toxicity studies or suitable human evidence." **The panel also recommends the EPA further clarify and clearly lay out the criteria for determining whether a chemical is data-poor and use that the term consistently in the ETAP documentation the panel reviewed.**

Recommendation 1.2: The ETAP VOI document states that *"In addition, strategically integrating the ETAP approach with other established methods, such as chemical categorization and read across, could further enhance the public health benefits, enabling the EPA to more rapidly address public health and environmental challenges (e.g., per- and polyfluoroalkyl substances)".* **In the ETAP Methodology documentation, the EPA should discuss how predictive methodologies and other sources of information (e.g., read across, QSAR; see VOI document) could be used in the process of prioritizing and selecting chemicals for the ETAP process.** For chemicals that may not be appropriate for ETAP because of predicted long half-lives or expected significant cross species differences EPA could consider using a different type of work product (e.g., PPRTV) that relies on a toxicogenomic POD but with a different adjustment factor than is used in the ETAP program, or using a different adjustment factor than is used in the ETAP and, if required or appropriate, submit the choice of the factor to a peer review with narrow scope.

Recommendation 1.3: The Panel recommends that the Agency clearly state what is meant by "apical endpoint" and document the range of endpoints that were the basis of the reported PODs used in the analysis presented in the document "Scientific Studies Supporting Development of Transcriptomic

Points of Departure for EPA Transcriptomic Assessment Products." The tissues where transcriptomic data were collected are identified on an individual study basis. The tissue specific apical endpoint data should also be reported completely and on a tissue-specific basis. In particular, apical endpoint information similar to that shown in Table 4-1 (p.43) should be provided for all chemicals referenced in Figures 3-1 through 3-3.

Recommendation 1.4: The Panel recommends that the Agency ensure that the definition of TRV includes the concept of a generalized biological response not intended to represent a specific biological pathway or effect.

Recommendation 1.5: The Panel recommends adding a footnote to the PFOA results (1st paragraph of p. 33 of "Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs)") noting that in their most recent risk assessment for perfluorooctanoic acid (PFOA), EPA (2023) relied on the results of epidemiological studies suggesting effects at exposures several orders of magnitude lower than effects observed in animal data as PODs for the reference dose (RfD). This note could clarify that, for the purposes of evaluating the correlation of the ETAP with the 2-year bioassay, the RfD derived from animal studies was the appropriate comparator.

Recommendation 1.6: The Panel recommends revising the Standard Methods for Development of EPA Transcriptomics Assessment Projects documentation as follows:

- The Background or Assessment Review section should include a statement of limitations for the underlying assumptions along with the appropriate intended application; i.e., as noted in other reviews (e.g., the NAS Silver Book), there are concerns with the Reference Dose being interpreted as a population threshold value even though it does not represent the current regulatory value. It should also note that the TRV based on results from the ETAP could be replaced later should more data become available for the chemical. The information in this section needs to be consistent with the other risk assessment approaches and documents the Agency releases.
- The Panel recommends developing a chart or graphic that explains the study design process for the 5-day studies used in ETAPs. Such a graphic or chart would help readers and end users to identify the points of attrition in determining the first lowest positive dose, the number of rats, the final dose spacing, tissue selection if non-default tissues are used, and other relevant details.
- Define the criteria for lowest dose selection.
- Define the criteria for inclusion of tissues beyond the standard list.
- Describe the process for tissue isolation and how this will be standardized across chemicals.
- Include in the template a precise definition of "data-poor" based on the revisions in response to Recommendation 1.1. EPA should then confirm that all uses of the term throughout the template are consistent with that definition.
- The Agency should provide additional details regarding the selection of acceptable purity for single chemicals/mixtures and formulations for testing.
  Clearly define what determines the most sensitive GO process in section 8.1.8.

Recommendation 2.1: The EPA should state in the method documentation that they will periodically review the basis for the default uncertainty factors and if justified, make the necessary adjustments to the method.

Recommendation 2.2: EPA should revise its statement that default UFs may be decreased when supported by chemical-specific information to say that default UFs may be increased or decreased in such situations.

Recommendation 3.1: Do not add a routine, independent peer review process for individual ETAP products. There is limited or no value to peer review of individual ETAP products that are the product of a peer reviewed and approved standardized process without assessment or judgments. In an exceptional case, the EPA may decide to depart from the standard process, including a limited scope peer review, which would be consistent with this recommendation.

Recommendation 4.1: The EPA should add the following information to the individual reports:

- The Methods documentation includes the following regarding dose identification: "For the ETAP study design, a minimum of five dose levels plus control will be evaluated. In general, the lowest positive dose should be a full log10 lower than the next dose. The remaining doses should use half-log spacing." Since the actual doses used per chemical are not defined**, the criteria for selecting the lowest (or highest) dose should be included in the report for each chemical.** If the criteria used are always the same (e.g., highest dose is determined by limits of solubility) then the Methods document should clearly define those criteria, which removes the need to document this information individually for each chemical.
- The Methods document lists 12 standard tissues to be used for transcriptomic measurements: "kidney, liver, adrenal gland, brain, heart, lung, ovary (females), spleen, testis (males), thyroid, thymus, and uterus (females)." It also allows for isolation of RNA from additional tissues: "RNA may be isolated from other tissues and organs to increase the breadth of transcriptomic coverage, but it is not required." **Should additional tissues be tested or should any of the standard tissues not be tested, the inclusion/exclusion criteria determining the tissue selection for that chemical should be contained in the report.** The number of tissues tested could influence the "lowest median BMD across the tissues examined in either sex," which is used to define the POD, because of multiple testing considerations. For this reason, a caveat should be noted in the report when using something other than the 12 standard tissues.
- Table 3-1 from the Methods document outlines the general Population-Exposure-Comparator-Outcome (PECO) criteria that are to drive the systematic review. **The specific PECO criteria for the subject of the ETAP assessment should be documented in Appendix I along with the literature search results.**
- The evaluation of studies from the literature that pass the PECO criteria are defined in Section 3.2.4 of the Methods document. The criteria as described allow for expert judgment, especially with epidemiologic studies where a determination must be made whether "exposure-response results are presented in sufficient detail." **The final ETAP report should include a list of all publications that pass the PECO filter, including studies deemed insufficient for use in POD/TRV derivation.**
- **Table 5-1, which summarizes the animal study parameters, should include the type of food and water used during the animal study, and samples of both should be saved for future testing.**
- **The template (section 5.2) should have standard documentation regarding the probes that are used and version of the sequencing platform.** This is needed in case these are changed in the future to make it clear when comparing documents whether different platforms were used.
- **Make all raw data (with filtered genes flagged but not deleted) and results from standard processing prior to TRV determination available to scientific community**. Provide a link to the publicly available data in Section 5.2 of the final report.

APPENDIX A: MEETING AGENDA

Day 1: July 11, 2023

Time	Duration	Topic	Speaker
9:00-9:10 am	10 minutes	Welcome	Maureen Gwinn
9:10-9:20 am	10 minutes	Introduction to the Panel	Tom Tracy
9:20-9:45 am	25 minutes	EPA ORD Portfolio Approach and Where ETAP Fits	Samantha Jones
9:45-10:00 am	15 minutes	Day 1 Agenda, Introduction of ETAP Team, and Charge to the Panel (Review Charge Qs)	Rusty Thomas
10:00-10:30 am	30 minutes	Break	
10:30-11:00 am	30 minutes	Science Support Introduction/Background	Alison Harrill
11:00-11:30 am	30 minutes	Literature Review	Leah Wehmas
11:30-12:00 pm	30 minutes	NTP Genomics Report Overview	Scott Auerbach
12:00- 1:00 pm	60 minutes	Working Lunch 12:00-12:30 pm Break 12:30-1:00 pm Discussion of Panel Roles and Responsibilities	
1:00-1:30 pm	30 minutes	Dose Response Methods and Parameter Refinement	Logan Everett
1:30-2:00 pm	30 minutes	Concordance Analysis with Inter-study Variability	Kelsey Vitense
2:00-2:10 pm	10 minutes	Summary	Alison Harrill
2:10-2:30 pm	20 minutes	Break	
2:30-3:30 pm	60 minutes	Facilitated Panel Q/A	Co-Chairs: Craig Rowlands and Katherine von Stackelberg
3:30– 4:30 pm	60 minutes	Public Comment Period	Facilitator: Tom Tracy
4:30 – 4:45 pm	15 minutes	Wrap Up	Annette Guiseppi-Elie
4:45 – 5:45 pm	60 minutes	Break up into Charge Question groups 1-4 and Initial Discussions (closed session)	Co-Chairs: Craig Rowlands and Katherine von Stackelberg

Day 2: July 12, 2023

Time	Duration	Topic	Speaker
9:00-9:10 am	10 minutes	Welcome Back	Chris Frey
9:10-9:20 am	10 minutes	Day 2 Agenda and Charge Qs	Rusty Thomas
9:20-9:35 am	15 minutes	ETAP Overview- Introduce MOPA as the example	Alison Harrill
9:35-10:00 am	25 minutes	Database Search and Systematic Evidence Map (SEM)	Avanti Shirke
10:00-10:30 am	30 minutes	*In vivo* Study Design	Leah Wehmas
10:30-11:00 am	30 minutes	Break	
11:00-11:20 am	20 minutes	Transcriptomic Dose Response Analysis	Logan Everett

11:20-11:50 am	30 minutes	Reference Value Derivation and Reporting	Jason Lambert
11:50-12:05 pm	15 minutes	Comparison of Transcriptomics Reference Doses (TRVs) with Reference Doses/Concentrations (RfD/Cs) and Summary	Alison Harrill
12:05-1:05 pm	60 minutes	Working Lunch 12:05-12:35 pm Break 12:45-1:15 Begin questions from Panel on ETAP Method	
1:05-2:00 pm	55 minutes	Continue Questions from Panel on ETAP Method	Co-Chairs: Craig Rowlands and Katherine von Stackelberg
2:00-4:00 pm	120 minutes	Break up into Charge Question Groups 1-4 (closed session)	Co-Chairs: Craig Rowlands and Katherine von Stackelberg
4:00-5:00 pm	60 minutes	Report out and Charge Question Discussions	Co-Chairs: Craig Rowlands and Katherine von Stackelberg
5:00- 5:15 pm	15 minutes	Wrap Up and Close Meeting	Rusty Thomas and Tom Tracy

APPENDIX B: MATERIALS

Material Provided in Advance of the Meeting

- Agenda
- Charge questions
- Draft report "Scientific Studies Supporting Development of Transcriptomic Points of Departure for EPA Transcriptomic Assessment Products (ETAPs)"
- Draft report "Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs)"

Material Provided During or After the Meeting

- PowerPoint presentation slides presented during the meeting
- ORD responses to BOSC follow-up questions