Transcriptomic Reference Value (TRV) Derivation and Reporting

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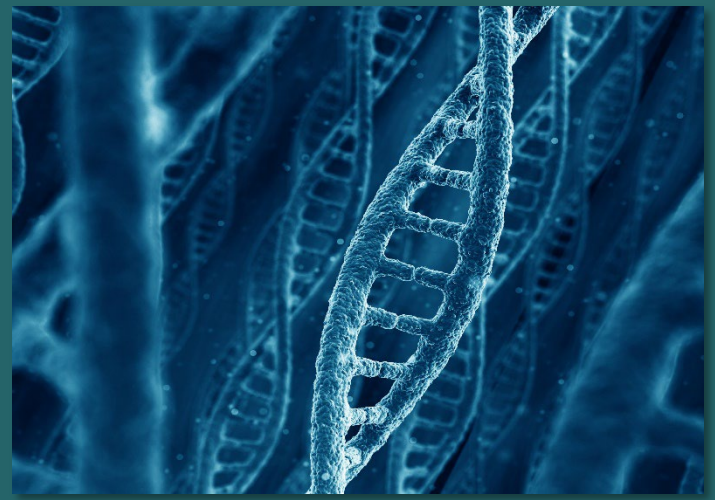

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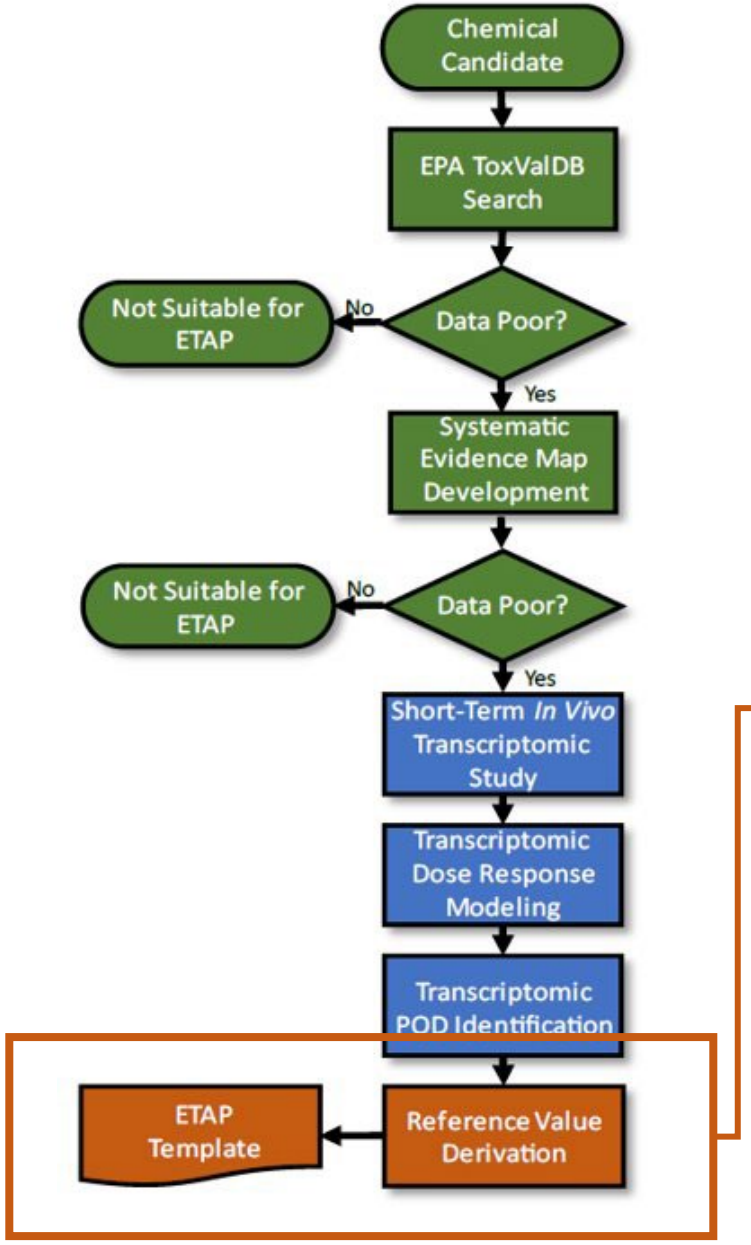


Outline

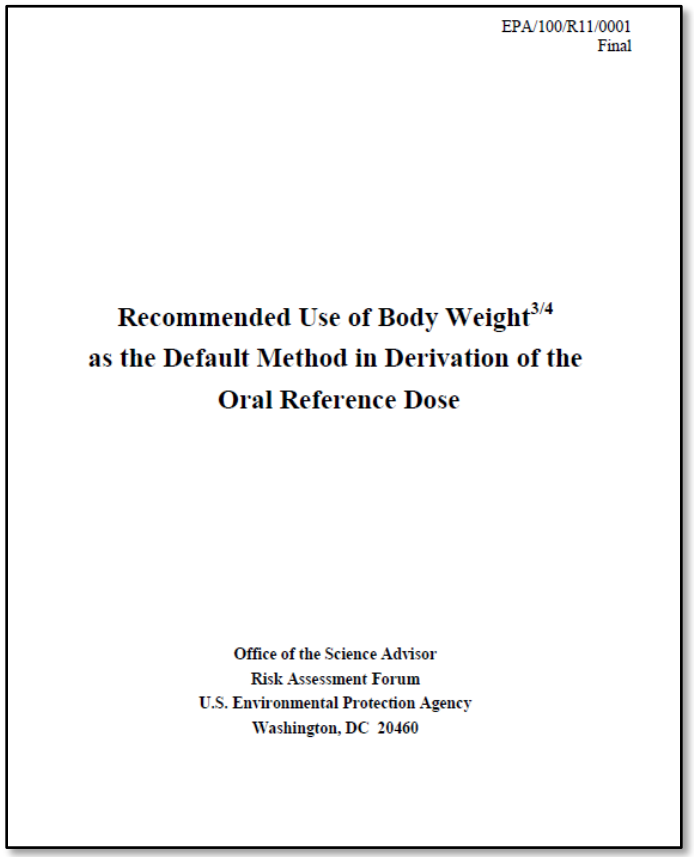
- Transcriptomic-Based POD Identification Quick Review
- Definition of a Transcriptomic POD for Human Health Assessment Application
- Human Equivalent Dose Conversion of Transcriptomic-Based PODs
- Derivation of a Transcriptomic Reference Value (TRV)
  - Areas of Quantitative Uncertainty
  - What does a TRV represent?
- Quality Assurance and Reporting for ETAP
  - Technical approach leading to transcriptomic-based PODs
  - Documentation and reporting for ETAP/TRV(s)

Definition of a Transcriptomic POD for Human Health Assessment Application

- **Point-of-Departure (POD)**: The dose-response point that marks the beginning of a low-dose extrapolation. This point can be the lower statistical bound on a dose for an estimated incidence or a change in response level from a dose-response model (e.g., BMD), or a NOAEL or LOAEL for an observed incidence, or change in level of response.
- The transcriptomic POD is defined as the administered dose at which there were no coordinated transcriptional changes that would indicate a toxicity of concern.
- The transcriptomic POD is not associated with a specific hazard. It does not necessarily discriminate between non-cancer or cancer effects, adverse or adaptive responses, nor is it used to infer mechanism or mode-of-action.

Considerations in Cross-Species Dose Conversion of Transcriptomic-Based PODs

- In traditional EPA human health risk assessment practice, candidate PODs from experimental animal studies must be converted to a human equivalent dose (HED).
- Hierarchical approach to HED conversion:
  - Physiologically-Based Toxicokinetic Modeling
  - Chemical-specific cross-species toxicokinetic data (e.g., animal and human clearance)
  - Cross-species allometric body weight scaling
- Chemicals in ETAP process are data-poor.


EPA/100/R11/0001
Final

Recommended Use of Body Weight$^{3/4}$
as the Default Method in Derivation of the
Oral Reference Dose

Office of the Science Advisor
Risk Assessment Forum
U.S. Environmental Protection Agency
Washington, DC 20460

ETAP Method for Cross-Species Dose Conversion of Transcriptomic-Based PODs

- Study specific terminal BW (by sex) for animal species (i.e., rat).
- Default adult human BW as per the EPA Exposure Factors Handbook (Table 8-1). https://www.epa.gov/expobox/exposure-factors-handbook-chapter-8

$$BMDL_{HED} = BMDL \times DAF = BMDL \times \frac{BW_{Animal}^{1/4}}{BW_{Human}^{1/4}}$$

where,

BMDL = animal assay-based POD (in ETAP…the lowest GO BP class BMDL).

DAF = dosimetric adjustment factor = ratio of $BW^{1/4}$ of animal to human.

POD_{HED} Conversion for the Example Chemical (MOPA)

Endpoint	Sex	Organ	BMDL (mg/kg-day)	Terminal Rat Body Weight (kg)	Dose Adjustment Factor (DAF)	$BMDL_{HED}$ (mg/kg-day)
Transcriptional changes	Female	Uterus	0.121	0.227	0.231	0.0279

$$BMDL_{HED} = BMDL \times \frac{BW_{Rat}^{1/4}}{BW_{Human}^{1/4}} = 0.121\ mg/kg-day \times \frac{0.227\ kg^{1/4}}{80\ kg^{1/4}} = 0.0279 mg/kg-day$$

- The $BMDL_{HED}$ of 0.0279 mg/kg-day is identified as the POD for derivation of a Transcriptomic Reference Value (TRV) for perfluoro-3-methoxypropanoic acid (MOPA)

Derivation of a Transcriptomic Reference Value (TRV) in ETAP

- A transcriptomic POD (i.e., $BMDL_{HED}$) is then used in the calculation of a TRV using the same five areas of uncertainty as used in traditional human health assessment.
- Based on EPA human health assessment guidance and practice, an uncertainty factor represents one of several, generally 10-fold, default factors used in operationally deriving a non-cancer reference value.
- The default factors typically used cover a single order of magnitude (i.e., 10^1); by convention, in the EPA, a value of 3 is used in place of one-half power (i.e., $10^{0.5}$) when some aspect of the uncertainty is accounted for or not comprehensively addressed.
- A standardized set of uncertainty factors are proposed due to the carefully prescribed design of the animal studies and data analysis procedures.

Derivation of a Transcriptomic Reference Value (TRV)

The UFs considered in an ETAP are intended to account for:

1) Uncertainty in extrapolating animal data to humans (i.e., interspecies uncertainty) (UF_A);
2) Variation in susceptibility among members of a human population (i.e., inter-individual or intraspecies variability) (UF_H);
3) Uncertainty in extrapolating from a LOAEL rather than from a NOAEL (UF_L);
4) Uncertainty in extrapolating from data obtained in a study with less-than-lifetime exposure (i.e., extrapolating from subchronic to chronic exposure) (UF_S);
5) Uncertainty associated with an incomplete database (UF_D).

$$TRV = \frac{BMDL_{HED}}{UF_A \times UF_H \times UF_L \times UF_S \times UF_D}$$

UF_A – Animal-to-Human Interspecies Uncertainty Factor

- The interspecies UF accounts for the extrapolation of laboratory animal data to humans, and it generally is presumed to include both toxicokinetic (TK) and toxicodynamic (TD) considerations.
- Quantitatively, TK and TD are assigned a default of 3 each (i.e., $10^{0.5} \times 10^{0.5} = 10^{1}$).
- For TK, allometric cross-species scaling is applied to convert an animal POD to a $POD_{HED.}$
- For TD, sufficient comparative cross-species data are not expected to be available in an ETAP.

❖ *In the derivation of a TRV, a UF_A of 3 is applied in conjunction with calculation of a POD_{HED}*

UF_H – Intraspecies Variability Uncertainty Factor

- The intraspecies UF is used to account for the possibility that the evidence considered is not representative of the dose-response relationship in subgroups of the human population that are most sensitive to the health hazards of the chemical being assessed.
- Sufficient evidence to demonstrate protection of all susceptible subgroups is not expected to be available in an ETAP.

❖ *In the derivation of a TRV, a UF_H of 10 is applied.*

UF_L – Uncertainty in Extrapolating From a LOAEL to a NOAEL

- In traditional human health assessment practice, when dose-response data are not amenable to BMD modeling, effect levels (e.g., LOAEL, NOAEL) are identified.
- This UF is traditionally applied when the lowest tested dose in a given study induces a statistically and/or biologically significant change in anatomy/physiology, compared to control(s).
- BMD modeling is used in the ETAP to identify transcriptomic PODs.

❖ *In the derivation of a TRV, a UF_L of 1 is applied when a GO BP BMDL value is successfully identified for one or more classes using the ETAP method*

UF_S – Subchronic-to-Chronic Uncertainty Factor

- In traditional EPA assessment practice, the UF_S is applied in the derivation of a chronic reference value if toxicity data are based on a subchronic duration study.
- Multiple studies have demonstrated concordance between gene set-based transcriptional PODs from shorter-term studies and apical PODs from chronic studies (Leah's and Logan's presentation).
- Error in concordance between 5-day transcriptomic BMDs and apical effect BMDs from chronic studies was approximately equivalent to the combined inter-study variability (Kelsey's presentation).
- Observed differences in 5-day transcriptomic and chronic apical BMDs are largely driven by inter-study variability rather than systematic differences.

❖ *In the derivation of a TRV, a UF_S of 1 is applied.*

UF_D – Database Uncertainty Factor

- The database UF is intended to account for the potential for deriving an underprotective reference value as a result of an incomplete characterization of the chemical's toxicity.
- In traditional EPA assessment practice, the UF_D is applied as a function of the overall landscape of what is known and not known about a chemical's toxicity.
- A complete database of toxicity information is not expected to be available in an ETAP.

❖ *In the derivation of a TRV, a UF_D of 10 is applied.*

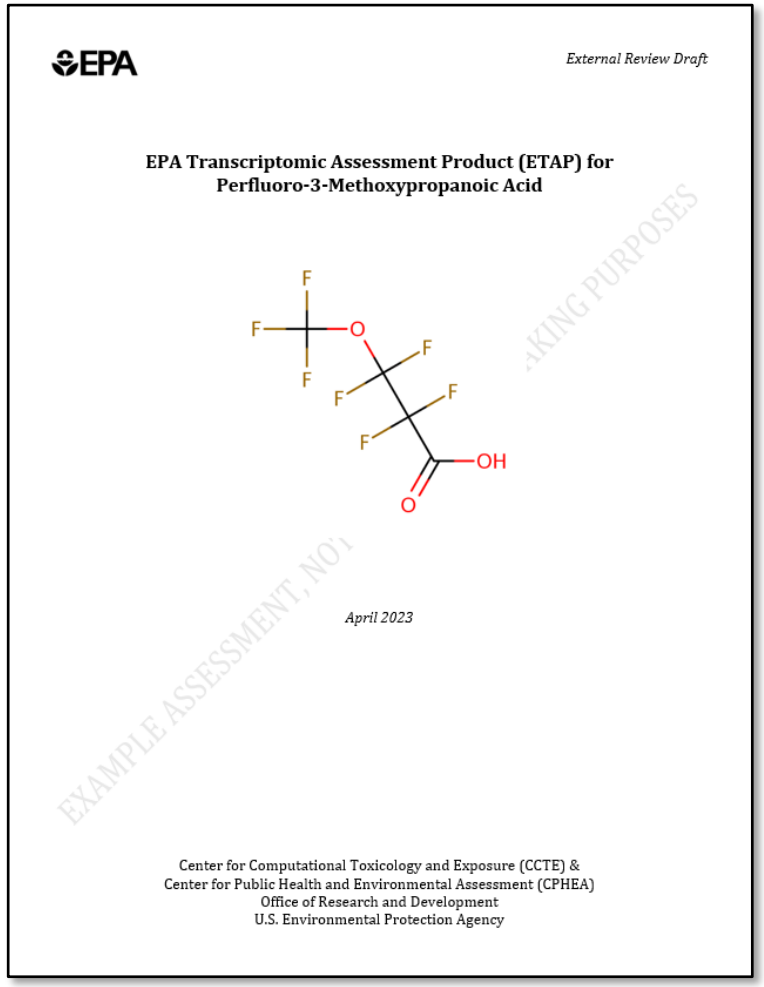
Derivation of the Transcriptomic Reference Value (TRV)

The qualitative rationale(s) and quantitative application of each UF will be standard within the ETAP process:

$$TRV = \frac{BMDL_{HED}}{UF_A(3) \times UF_H(10) \times UF_L(1) \times UF_S(1) \times UF_D(10)}$$

$$TRV = \frac{BMDL_{HED}}{Composite\ UF\ (300)}$$

ETAP and TRV for Example Chemical (MOPA)


EPA

External Review Draft

EPA Transcriptomic Assessment Product (ETAP) for Perfluoro-3-Methoxypropanoic Acid

April 2023

Center for Computational Toxicology and Exposure (CCTE) &
Center for Public Health and Environmental Assessment (CPHEA)
Office of Research and Development
U.S. Environmental Protection Agency

$$TRV = \frac{BMDL_{HED}}{Composite\ UF\ (300)}$$

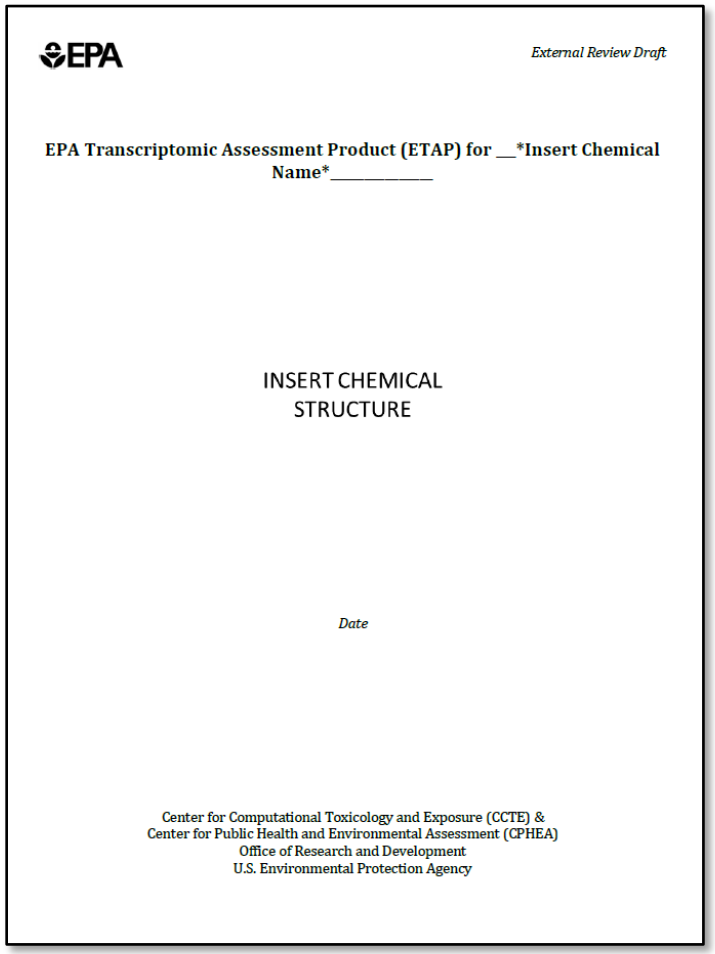
$$TRV = \frac{0.0279\ mg/kg{-}day}{300} = 0.00009\ mg/kg{-}day$$

- MOPA TRV is:
  - ~equivalent to the chronic RfD for PFPrA (0.0001 mg/kg-day)
  - ~3X lower than the EPA chronic RfD for PFBS (0.0003 mg/kg-day)
  - ~30X higher than the chronic RfD for GenX (0.000003 mg/kg-day)

What is a Transcriptomic Reference Value (TRV)?

- A TRV is defined as an estimate of a daily oral dose that is likely to be without appreciable risk of adverse effects following chronic exposure.
- 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.
- While a TRV is expressly presented as a chronic value in an ETAP, it may also be applicable across other exposure durations of interest including short-term and subchronic.
- ETAP assessments may be updated to incorporate new data or methodologies that might impact the reference values, or, retired if traditional toxicity studies and an associated human health assessment are published.

ETAP Reporting Template



EPA

External Review Draft

EPA Transcriptomic Assessment Product (ETAP) for __*Insert Chemical Name*_____

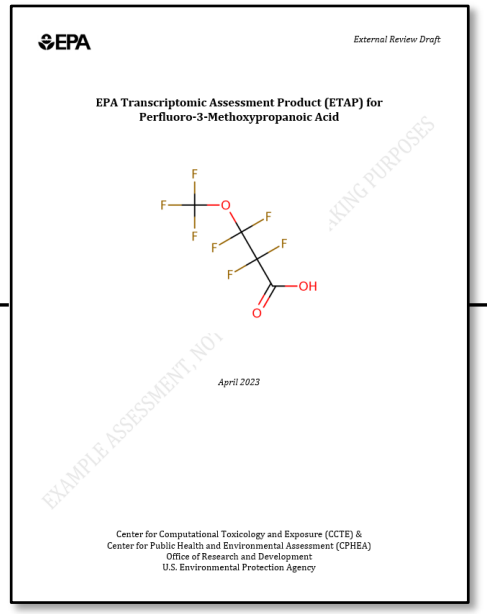
INSERT CHEMICAL STRUCTURE

Date

Center for Computational Toxicology and Exposure (CCTE) &
Center for Public Health and Environmental Assessment (CPHEA)
Office of Research and Development
U.S. Environmental Protection Agency

- Highly standardized assessment template
- Minimal free-form text and no subjective interpretation
- Six sections:
  - Background
  - Assessment Review
  - Chemical Identity and Physical Properties
  - Literature Survey
  - Animal Study
  - Human Equivalent Dose and Transcriptomic Reference Value
- Detailed results for each study provided in the appendix

ETAP Quality Assurance and Technical Review Process



Audit of Data Quality

Technical Review

Post Final ETAP

ETAP Quality Assurance Process

- Quality Assurance
  - All ETAP activities and testing covered under a standard EPA Category A Quality Assurance Project Plan (QAPP).
  - Each individual ETAP will undergo independent Audit of Data Quality (ADQ) by CCTE Quality Assurance (QA) team.
  - ETAP ADQ Process
    - Final study reports received from EPA contractors.
    - CCTE QA team evaluates documentation using ADQ checklists developed for each process.
      - Analytical Chemistry: chemical purity and stability
      - Animal Study: dosing solution preparation and analysis, animal dosing, tissue collection and preparation
      - Transcriptomics: RNA preparation, RNA sequencing, data pipelining and QC, data analysis and modeling

MOPA Audit of Data Quality Example Check List

	QUESTION	Y	N	N/A	COMMENT(S)
Research Notebooks and QA Documentation					
1.	Does the researcher maintain an approved research notebook? Indicate the type in comments. Approved methods to document research activities currently consist of paper, ELN software, or the Microsoft OneNote application	X			OneNote notebook is titled "I-CCED-0032409-QP-1-0". Please see separate notebook review checklist for details.
2.	Do research notebooks contain a log of daily research activities, observations, and conclusions, and reference information (e.g. SOPs, computer file names, location, etc.) for project records/study files that are stored in other media (e.g., forms, instrument print-outs, computers)?	X			See above note.
3.	Was there an approved Quality Assurance Project Plan associated with the animal component?	X			QAPP ID: I-CCED-0032409-1-2
4.	Were methods specified in the QAPP used?	X			
Test Substance Characterization and Test System					
5.	Are the chemicals uniquely identified and correctly transcribed throughout the data package to the summary of results?	X			
6.	Is there a description of the physical state and appearance of the test chemical?	X			Section 4 (starting page 9) of the ILS Final Report (ILS Study Number 50104.101.02). Referred to as the final report in this checklist. **Note:** Final report was not available during initial review. Checklist was completed using the ILS Draft report on 2/27/2023 then verified when Final report was available on 3/6/2023.
7.	Is the lot or batch number recorded for the test chemical?	X			See above
8.	Is the identification and composition of the test vehicle used available?	X			Section 4 (page 10) of the final report
9.	If applicable, is chain-of-custody documentation complete?	X			Complete throughout the project.
10.	Was data available for species and strain of animals used?	X			Section 5 (page 10) of the final report
11.	Were age, body weight data, and sex recorded?	X			Section 6.3 (page 15) of the final report
12.	Was the test environment including cage conditions, ambient temperature, humidity, and light/dark periods recorded?	X			Section 5.2 (starting on page 11) of the final report

MOPA Audit of Data Quality Results and Recommendations

- The review, verification, and validation processes for the analytical chemistry support were methodical, detailed, and throughly documented.
- Chain of Custody documentation was complete throughout the MOPA ETAP.
- The use of “Readme” files to give locations and descriptions of folder contents enabled efficient navigation of the electronic files.
- Documentation generally flowed well between multiple contractors and EPA staff.
- Four calculation errors were identified, corrected and noted as points of emphasis for future studies. These points of vulnerability were shared with staff to ensure the future reliability of documents that are crucial to the timeline and outcome of the ETAP QA Process.
- The processes generating each of the key documents should undergo periodic audits to ensure the accuracy of the key reports.

ETAP Technical Review Process

- Technical Review
  - Each ETAP will be reviewed by a minimum of two ORD technical experts.
- ETAP Release
  - Following successful completion of ADQ and technical review, individual ETAPs will be publicly posted to an EPA website.

Summary

- Transcriptomic points-of-departure are defined as the dose with no coordinated transcriptional changes that would indicate a potential toxicity of concern, but not linked to a specific hazard.
- The ETAP employs a set of uncertainty factors that are consistent with traditional human health assessment guidance and practice, but the uncertainty factors are applied in a standardized way for each chemical due to the carefully prescribed design of the animal studies and data analysis procedures.
- A TRV is defined as an estimate of a daily oral dose that is likely to be without appreciable risk of adverse effects following chronic exposure.
- The results of the ETAP are reported in a standardized assessment template with minimal free-form text and no subjective interpretation.
- The ETAP will undergo an internal technical review by two ORD experts and an independent Audit of Data Quality (ADQ) by QA staff prior to release.