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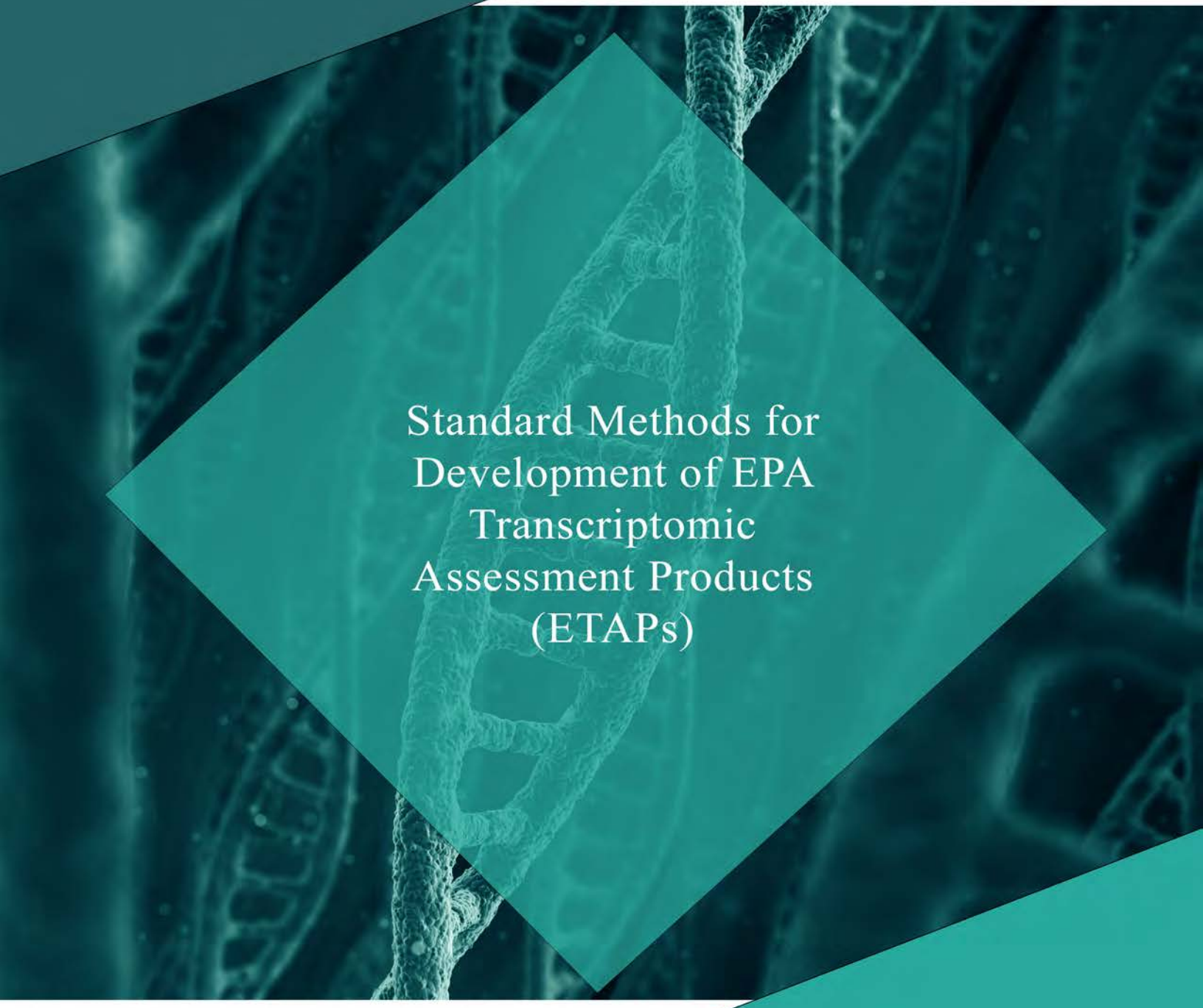

Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs)

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Office of Research and Development

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Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs)

May 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

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ABBREVIATIONS

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
ACToR	EPA Aggregated Computational Toxicology Resource
ADME	Absorption, Distribution, Metabolism, and Excretion
ANOVA	Analysis of Variance
ATSDR	Agency for Toxic Substances and Disease Registry
BCTD	Biomolecular and Computational Toxicology Division
BMD	Benchmark Dose
BMDL	Benchmark Dose Lower Confidence Bound
BMDU	Benchmark Dose Upper Confidence Bound
BMR	Benchmark Response
BOSC	EPA Board of Scientific Councilors
CASRN	Chemical Abstracts Service Registry Number
CBI	Confidential Business Information
CCED	Chemical Characterization and Exposure Division
CCTE	Center for Computational Toxicology and Exposure
CPAD	Chemical and Pollutant Assessment Division
CPHEA	Center for Public Health and Environmental Assessment
CPM	Counts Per Million
CTBB	Computational Toxicology and Bioinformatics Branch
DAF	Dosimetric Adjustment Factor
DDEF	Data-Derived Extrapolation Factors
$DDEF_{AK}$	Data-Derived Extrapolation Factor Animal Kinetics
$DDEF_{HK}$	Data-Derived Extrapolation Factor Human Kinetics
$DDEF_{HD}$	Data-Derived Extrapolation Factor Human Dynamics
DTT	Division of Translational Toxicology
ECOTOX	EPA Ecotoxicology Knowledgebase
ECHA	European Chemicals Agency
EU JRC	European Union Joint Research Centre
EDTA	Ethylenediaminetetraacetic Acid
EPA	U.S. Environmental Protection Agency
ETAP	EPA Transcriptomic Assessment Product
ETTB	Experimental Toxicokinetics and Toxicodynamics Branch
FDR	False Discovery Rate
GO	Gene Ontology
HAWC	EPA Health Assessment Workspace Collaborative
HED	Human Equivalent Dose
HERO	Health and Environmental Research Online
HPV	High Production Volume
HSDB	Hazardous Substances Data Bank
IRIS	Integrated Risk Information System
IUCLID	International Uniform Chemical Information Database
LOAEL	Lowest Observed Adverse Effect Level
LOEL	Lowest Observed Effect Level
MAD	Median Absolute Deviation
MeSH	Medical Subject Heading
NAM	New Approach Methodology
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health

NLM HSDB	National Library of Medicine Hazardous Substances Database
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Cooperation and Development
ORD	Office of Research and Development
OW	Office of Water
PBPK	Physiologically Based Pharmacokinetic
PCA	Principal Component Analysis
PECO	Population, Exposure, Comparator, and Outcome
PK	Pharmacokinetic
POD	Point of Departure
PPRTV	Provisional Peer-Reviewed Toxicity Value
QSAR	Quantitative Structure Activity Relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals Regulation (EU)
RfC	Reference Concentration
RfD	Reference Dose
RMSD	Root Mean Squared Difference
RNA	Ribonucleic acid
RNA-seq	RNA Sequencing
SEM	Systematic Evidence Map
SIDS	Screening Information Data Set
TEAB	Toxic Effects Assessment Branch
TEST	EPA Toxicity Estimation Software Tool
TD	Toxicodynamic
TK	Toxicokinetic
ToxValDB	US EPA Toxicity Value database
TRI	Toxic Release Inventory
TRV	Transcriptomic Reference Value
UF	Uncertainty Factor
UF_A	Animal-to-Human Interspecies Uncertainty Factor
UF_D	Database Uncertainty Factor
UF_H	Intraspecies Variability Uncertainty Factor
UF_S	Subchronic-to-Chronic Duration Uncertainty Factor
UF_L	LOAEL-to- NOAEL Uncertainty Factor

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1. PURPOSE AND APPLICABILITY

Current estimates of the size of worldwide and domestic chemical inventories are substantial, with increasing trends in future chemical production and release. Relatively few of the chemicals in commerce, as well as those found in the environment, various waste streams, and the human body, have traditional toxicity data or human health assessments. Given historical, current, and future trends in chemical production and the disparity in toxicity testing data and human health assessments, the U.S. Environmental Protection Agency (EPA) is frequently faced with making decisions with limited or no data when evaluating potential human health risks.

This document details the methods used to develop transcriptomic reference values (TRV) for use in EPA Transcriptomic Assessment Products (ETAP) by the Office of Research and Development (ORD), EPA. 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). The 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 intended to protect both the individual and population from adverse 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 defined as a chronic value in an ETAP, it may also be applicable across other exposure durations of interest including short-term and subchronic. 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. 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.

2. OVERVIEW AND PRINCIPLES OF THE METHOD

The ETAP consists of three primary components with associated processes and decision points within each component. The three primary components consist of: 1) initial database searches and systematic evidence map development; 2) short-term *in vivo* transcriptomic study for point-of-departure (POD)[1] identification; and 3) assessment development and reporting (Fig. 2-1). The main concepts of the ETAP are that the underlying methods and data analysis procedures are highly standardized and structured, and the decision context is narrowly focused on data poor substances. As a result, only the standard methods for the ETAP will be externally peer-reviewed and subject to public comment through the EPA Board of Scientific Counselors (BOSC), while the individual assessments will receive internal peer review by at least two ORD technical experts and quality control evaluation by ORD staff prior to public release. The combination of standardized methods and streamlined review process is intended to facilitate the rapid development, execution, and release of the assessments.

The first component of an ETAP is identifying potentially relevant toxicological studies. Candidate substances for ETAP are screened for publicly available repeated dose toxicity data using the US EPA ToxVal database (ToxValDB). If no suitable studies are identified in the ToxValDB, then systematic evidence map (SEM) methods are used to identify and organize the research available on a specific substance (Thayer et al. 2022a; Thayer et al. 2022b). For the ETAP, a SEM is developed to identify and evaluate the literature base associated with the candidate substance for mammalian *in vivo* repeated dose toxicity studies or suitable human evidence. Resources searched include databases of published research (*e.g.*, PubMed, Web of Science, ProQuest) as well as repositories of studies that may not have been peer-reviewed, such as those summarized in European Chemicals Agency (ECHA) registration dossiers or EPA's ChemView database. In addition, searches may be conducted to discern whether studies exist in such regulatory reporting databases but are classified as confidential business information (CBI). If such studies exist, then inquiries are made to determine whether they can be made available to the public. Based on the SEM, chemicals confirmed to have no publicly available mammalian *in vivo* repeated dose toxicity studies or suitable human studies may be eligible for development of an ETAP.

[1] In human health risk assessment practice, a point-of-departure (POD) represents the dose-response point that marks the beginning of a low-dose extrapolation. This point can be the lower bound on dose for an estimated incidence or a change in response level from a dose-response model (*e.g.*, Benchmark Dose; BMD), or a NOAEL or LOAEL for an observed incidence, or change in level of response. For BMD values, this is typically the BMD lower confidence bound (BMDL).

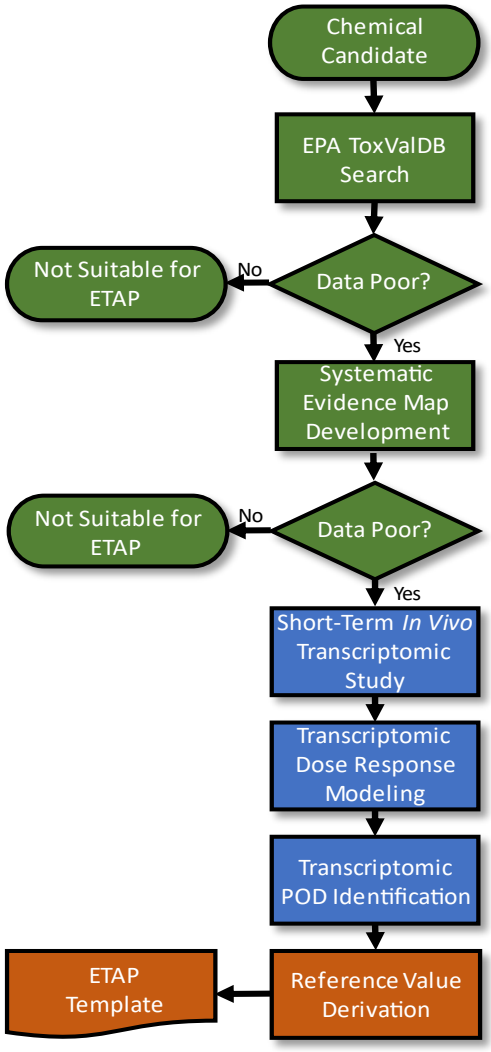

Figure 2-1. Flow chart depicting the three main components and associated processes in developing an ETAP. The green-colored processes and decision points are associated with the initial database searches and systematic search of the literature (evidence map). The blue-colored processes are associated with the short-term in vivo transcriptomic study and POD identification. The orange-colored processes are associated with the assessment product development and reporting.

The next component of an ETAP is performing a 5-day *in vivo* rat study and identification of the POD using transcriptomics. Transcriptomics is the characterization of gene expression changes in a cell, tissue, organ, or organism of interest. Transcriptional changes can provide a quantitative assessment of disruptions to signaling pathways, biological processes, and molecular functions by a chemical substance and the doses at which these disruptions occur (Thomas et al. 2007). The transcriptomic POD is derived from the transcriptomic BMD and BMDL and is defined as the experimentally determined dose at which there were no coordinated transcriptional changes that would indicate a potential toxicity of concern. Multiple studies have demonstrated good concordance between short-term transcriptomic BMD values (when grouped by gene sets based on pathway,

biological process, or molecular function) and phenotypic apical effect BMD values from traditional, rodent toxicity studies [reviewed in (EPA 2023)]. For *in vivo* repeated dose studies of 5-day duration, transcriptomic BMD values from the most sensitive gene set have been demonstrated to be concordant with both non-cancer and cancer phenotypic responses in subchronic and chronic toxicity studies in rodent models. The concordance between transcriptional and apical responses was robust across different exposure durations, exposure routes, species, sex, target tissues, physicochemical properties, toxicokinetic half-lives, and technology platforms. The concordance between the transcriptomic BMD values with non-cancer and cancer apical BMD values was approximately equivalent to the observed inter-study variability in the repeated dose toxicity studies (EPA 2023).

In the ETAP, a 5-day repeated dose design in both male and female rats is used as the basis for the transcriptomic study. Transcriptomic measurements are performed using targeted RNA sequencing in kidney, liver, adrenal gland, brain, heart, lung, ovary (females), spleen, testis (males), thyroid, thymus, and uterus (females). Transcriptomic BMD modeling is performed consistent with the NTP Approach to Genomic Dose-Response Modeling (NTP 2018), with adaptations for the targeted RNA-sequencing gene expression platform used in this method (EPA 2023). To select the transcriptomic POD, the BMDL from the most sensitive gene ontology (GO) biological process class in the most sensitive sex (male or female) and across all the tissues examined is identified. No determination of a specific type of hazard caused by the substance nor mechanistic interpretation of the gene expression changes is performed. The selected transcriptomic BMDL is converted to a Human Equivalent Dose (HED) using an oral dosimetric adjustment factor (DAF) based on allometric scaling (EPA 2011a).

The final step is the development of the assessment and reporting the results. The transcriptomic POD obtained from the 5-day *in vivo* oral exposure study is used in the derivation of a TRV through application of uncertainty factors (UFs) that are consistent with traditional human health assessment guidance and practice (EPA 2022). The values of the individual UFs and the overall composite value are the same across the individual assessments due to the standardized nature of the studies and data analysis procedures. The 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 from the systematic evidence mapping, 5-day transcriptomic study, and TRV derivation are compiled and reported in a standardized ETAP reporting template.

3. METHODS

3.1. CANDIDATE SUBSTANCE INITIAL SCREENING

Candidate substances for ETAP are initially screened for any mammalian *in vivo* repeated dose toxicity studies using a search of the US EPA ToxVal database (ToxValDB)[2]. If no suitable studies are identified from the ToxValDB, then a SEM is initiated using the methods published by Thayer and colleagues and described below to confirm or refute the absence of studies (Thayer et al. 2022a; Thayer et al. 2022b). Only substances that have no apparent publicly available mammalian *in vivo* repeated dose toxicity studies or suitable human evidence are further considered for an ETAP.

3.2. SYSTEMATIC EVIDENCE MAP DEVELOPMENT

3.2.1. POPULATIONS, EXPOSURES, COMPARATORS, AND OUTCOME (PECO) CRITERIA

PECO criteria (Morgan et al. 2018; Thayer et al. 2022a; Thayer et al. 2022b) are used to focus the research questions, search terms, and inclusion/exclusion parameters in a systematic review (Table 3-1). Studies that did not meet the PECO criteria but contain relevant supporting information are categorized (or "tagged") as potentially relevant supplemental material during the literature screening process (Table 3-2).

[2] ToxValDB is a database designed to store a wide range of public toxicity information while maintaining the linkages to original source information so that users can access available details. ToxValDB collates publicly available toxicity dose–effect related summary values typically used in risk assessments. These include POD data collected from data sources within ACToR and ToxRefDB, and no-observed and lowest-observed (adverse) effect levels (NOEL, NOAEL, LOEL, LOAEL) data extracted from repeated dose toxicity studies submitted under REACH (Regulation for Registration, Evaluation, Authorisation and restriction of chemicals in the EU). Also included are reference dose and concentration values (RfDs and RfCs) from EPA's IRIS and Provisional Peer-Reviewed Toxicity Values (PPRTV) assessments. Acute toxicity information is extracted from a number of different sources, including OECD eChemPortal, ECHA, NLM HSDB (Hazardous Substances Data Bank), ChemIDplus via EPA TEST (Toxicity Estimation Software Tool), and the EU JRC (Joint Research Centre) AcutoxBase. Finally, data from the eChemPortal and the EU COSMOS project also are included in ToxValDB. The ToxVal database is available through the EPA CompTox Chemicals Dashboard at: https://comptox.epa.gov/dashboard.

Table 3-1. Summary of PECO elements and associated evidence is as described in Thayer *et al.* 2022.

PECO element	Evidence
Populations	**Human**: Any population and lifestage (occupational or general population, including children and other sensitive populations). **Animal:** Non-human mammalian animal species (whole organism) of any lifestage (including fetal, early postnatal, adolescents and adults).
Exposures	**Relevant forms:** [substance X] (CAS number) Other forms of [chemical X] that readily dissociate (*e.g.*, list any salts, etc.). Known metabolites of interest, including metabolites used to estimate exposures to [chemical X]. **Human:** Any exposure to [chemical X] via [oral or inhalation] route[s]. Studies will also be included if biomarkers of exposure are evaluated (*e.g.*, measured chemical or metabolite levels in tissues or bodily fluids), but the exposure route is unclear or likely from multiple routes. Other exposure routes, such as those that are clearly dermal, are tracked during title and abstract screening and tagged as "potentially relevant supplemental material." **Animal:** Any exposure to [chemical X] via [oral or inhalation] route[s] of >1 day duration, or any duration assessing exposure during reproduction or development. Studies involving exposures to mixtures will be included only if they include an experimental arm with exposure to [chemical X] alone. Other exposure routes, including [dermal or injection], are tracked during title and abstract as "potentially relevant supplemental material."
Comparators	**Human:** A comparison or referent population exposed to lower levels (or no exposure/exposure below detection limits), or exposure for shorter periods of time, or cases versus controls, or a repeated measures design. However, worker surveillance studies are considered to meet PECO criteria even if no statistical analyses using a referent group is presented. Case reports or case series of > 3 people will be considered to meet PECO criteria, while case reports describing findings in 1–3 people will be tracked as "potentially relevant supplemental material." **Animal:** A concurrent control group exposed to vehicle-only and/or untreated control (control could be a baseline measurement, *e.g.*, acute toxicity studies of mortality, or a repeated measure design).
Outcomes	All health outcomes (cancer and non-cancer). In general, endpoints related to clinical diagnostic criteria, disease outcomes, biochemical, histopathological examination, or other apical/phenotypic outcomes are considered to meet PECO criteria.

Table 3-2. Major categories of potentially relevant supplemental material

Category (Tag)	Description
Mechanistic endpoints	Studies that do not meet PECO criteria but report measurements that inform the biological or chemical events associated with phenotypic effects related to a health outcome. Experimental design may include *in vitro*, *in vivo* (by various routes of exposure; includes all transgenic models), *ex vivo*, and *in silico* studies in mammalian and non-mammalian model systems. Studies using New Approach Methodologies (NAMs; *e.g.*, high throughput testing strategies, read-across applications) are also categorized here. Studies where the chemical is used as a laboratory reagent (*e.g.*, as a chemical probe used to measure antibody response) generally should not be tagged.
Classical pharmacokinetic (PK) or physiologically based pharmacokinetic (PBPK) model studies	**Classical Pharmacokinetic or Dosimetry Model Studies:** Classical PK or dosimetry modeling usually divides the body into just one or two compartments, which are not specified by physiology, where movement of a chemical into, between, and out of the compartments is quantified empirically by fitting model parameters to ADME (absorption, distribution, metabolism, and excretion) data. This category is for papers that provide detailed descriptions of PK models but are not physiologically-based pharmacokinetic (PBPK) models. The data are typically the concentration time-course in blood or plasma after oral and/or intravenous exposure, but other exposure routes can be described. **Physiologically Based Pharmacokinetic or Mechanistic Dosimetry Model Studies:** PBPK models represent the body as various compartments (*e.g.*, liver, lung, slowly perfused tissue, richly perfused tissue) to quantify the movement of chemicals or particles into and out of the body (compartments) by defined routes of exposure, metabolism, and excretion, and thereby estimate concentrations in blood or target tissues. A defining characteristic is that key parameters are determined from a substance's physicochemical parameters (*e.g.*, particle size and distribution, octanol-water partition coefficient) and physiological parameters (*e.g.*, ventilation rate, tissue volumes).
Pharmacokinetic (ADME)	Pharmacokinetic (ADME) studies are primarily controlled experiments, where defined exposures usually occur by intravenous, oral, inhalation, or dermal routes, and the concentration of particles, a chemical, or its metabolites in blood or serum, other body tissues, or excreta are then measured. These data are used to estimate the amount absorbed (A), distributed (D), metabolized (M), and/or excreted (E). ADME data can also be collected from human subjects who have had environmental or workplace exposures that are not quantified or fully defined. ADME data, especially metabolism and tissue partition coefficient information, can be generated using *in vitro* model systems.
Non-PECO animal model	Studies reporting outcomes in animal models that meet the outcome criteria but do not meet the population criteria in the PECO (non-human mammalian models).
Non-PECO route of exposure	Epidemiological or animal studies that use a non-PECO route of exposure, *e.g.*, injection studies or dermal studies if the dermal route is not part of the exposure criteria.
Susceptible populations	Studies that help to identify potentially susceptible subgroups, including studies on the influence of intrinsic factors (*e.g.,* sex, lifestage, or genotype) to toxicity, as well as some other factors (*e.g.*, health status). These studies are often co-tagged with other supplemental material categories, such as mechanistic or ADME. Studies meeting PECO criteria that also address susceptibility should be co-tagged as supplemental.

Human exposure and biomonitoring (no health outcome)	Information regarding exposure monitoring methods and reporting that are unrelated to health outcomes, but which provide information on the following: methods for measuring human exposure, biomonitoring (*e.g*., detection of chemical in blood, urine, hair), defining exposure sources, or modeled estimates of exposure (*e.g*., in occupational settings). Studies that compare exposure levels to a reference value, risk threshold or assessment points of departure are also included in this category.
Mixture study	Mixture studies use methods that do not allow investigation of the health effects of exposure to the chemical of interest by itself [*e.g*., animal studies that lack exposure to chemical of interest alone or epidemiology studies that do not evaluate associations of the chemical of interest with relevant health outcome(s)].
Case reports or case series	Human studies that present an investigation of a single exposed individual or group of ≤ 3 subjects that describe health outcomes after exposure but lack a comparison group (*i.e.,* do not meet the "C" in the PECO) and typically do not include reliable exposure estimates.
Records with no original data	Records that do not contain original data, such as other agency assessments, informative scientific literature reviews, editorials, or commentaries.
Posters or conference abstracts	Records that do not contain sufficient documentation to support study evaluation and data extraction.

3.2.2. LITERATURE SEARCH STRATEGIES

3.2.2.1. Database Search Term Development

The literature search focuses on the substance identifiers (name, synonyms, or trade names) with no date or language limits. Substance synonyms are identified by using synonyms in the EPA's CompTox Chemicals Dashboard[3] indicated as "valid" or "good". The preferred chemical name, Chemical Abstracts Service Registry Number (CASRN), DSSTox substance identifier (DTXSID), and synonyms are used by EPA information specialists to develop search strategies tailored to each of the databases listed below.

3.2.2.2. Database Searches

The three databases listed below are queried for literature containing the chemical search terms, and all retrieved records are stored in the Health and Environmental Research Online (HERO) database[4]. Full details of the search strategy for each database are presented in the substance specific ETAP.

[3] The EPA CompTox Chemicals Dashboard is available at: https://comptox.epa.gov/dashboard/

[4] EPA's HERO database provides access to the scientific literature behind EPA science assessments. The database includes more than 3 million scientific references and associated data from the peer-reviewed literature used by EPA to develop reports that support critical agency decisions-making and regulations.

- PubMed (National Library of Medicine)[5]
- Web of Science (Clarivate)[6]
- ProQuest (Clarivate)[7]

After deduplication in HERO[8], records are imported into SWIFT Review[9] software (Howard et al. 2016) to identify those references most likely to be applicable to a human health risk assessment. In brief, SWIFT Review has pre-set literature search strategies ("filters") developed by information specialists that can be applied to identify studies that are more likely to be useful for identifying human health content from those that likely do not (*e.g.*, analytical chemistry methods). The filters function like a typical search strategy: studies are tagged if the search terms appear in title, abstract, keyword or medical subject headings (MeSH) fields. The applied SWIFT Review filters focus on lines of evidence: human, animal models for human health, and *in vitro* studies. The details of the search strategies that underlie the filters are available online[10]. Studies not retrieved using these filters are not considered further. Studies that include one or more of the search terms in the title, abstract, keyword, or MeSH fields are exported as a Research Information Systems (RIS) file for further screening in DistillerSR[11], as described below.

3.2.2.3. Other Resources Consulted

The literature search strategies described above are designed to be broad; however, as with any search strategy, studies may be missed for assorted reasons (*e.g.*, specific substance is not mentioned in title, abstract, or keyword content; inability to capture "grey" literature not indexed in the databases listed above). Thus, in addition to the database searches, the sources below are used to identify studies that may have been missed. Records that appear to meet the PECO criteria are uploaded into DistillerSR, annotated with respect to source of the record, and screened using the methods described in Section 3.2.3. Other sources consulted include:

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[5] The PubMed database is available at: https://pubmed.ncbi.nlm.nih.gov/
[6] The Web of Science database is available at: https://www.webofscience.com/
[7] The ProQuest database is available at: https://www.proquest.com/
[8] Deduplication in HERO involves first determining whether a matching unique ID exists (*e.g.*, PMID, WOSid, or DOI). If one matches one that already exists in HERO, HERO will tag the existing reference instead of adding the reference again. Second, HERO checks if the same journal, volume, issue and page number are already in HERO. Third, HERO matches on the title, year, and first author. Title comparisons ignore punctuation and case.
[9] SWIFT-Review is an interactive workbench of tools to assist with problem formulation and literature prioritization. SWIFT is an acronym for Sciome Workbench for Interactive computer-Facilitated Text-mining. The workbench is available at: https://www.sciome.com/swift-review/
[10] Swift-Review filters are available at: https://www.sciome.com/swift-review/searchstrategies/
[11] DistillerSR is a web-based systematic review software used to screen studies available at: https://www.evidencepartners.com/products/distillersr-systematic-review-software.

- Manual review of the reference list from final or publicly available draft assessments [*e.g.*, EPA Integrated Risk Information System (IRIS), Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles] or published journal review articles specifically focused on human health. Reviews may be identified from the database search or from ToxValDB.

- Manual review of the reference list of studies judged as PECO-relevant after full-text review.

- Electronic queries of European Chemicals Agency (ECHA) registration dossiers to identify data submitted by registrants[12].

- Electronic queries of EPA ChemView database[13] to identify unpublished studies, information submitted to EPA under Toxic Substances Control Act (TSCA) Section 4 (chemical testing results), Section 8(d) (health and safety studies), Section 8(e) (substantial risk of injury to health or the environment notices), and FYI (voluntary documents). Other databases accessible via ChemView include EPA's High Production Volume (HPV) Challenge database and the Toxic Release Inventory (TRI) database.

- Electronic queries of NTP database of study results and research projects[14].

- Electronic queries of the Organisation for Economic Cooperation and Development (OECD) Existing Chemicals Database and eChemPortal[15,16].

- Manual review of the list of references in ECOTOX database for the substance(s) of interest[17].

3.2.2.4. Confidential Business Information

The methods described above are intended to identify evidence that is in the public domain, but additional existing information may not be publicly available. To avoid mislabeling substances as data poor, searches of Confidential Business Information (CBI) databases may also be conducted to confirm data availability status. Although the results of CBI studies cannot be considered in many assessment products (including IRIS, PPRTV, ATSDR), confirmation of a true lack of data is an important consideration when determining whether to initiate new toxicological studies. In certain cases, CBI information may be utilized to determine whether an ETAP should be developed.

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[12] ECHA registration dossiers available at: https://echa.europa.eu/information-on-chemicals/information-from-existing-substances-regulation
[13] EPA ChemView database is available at: https://chemview.epa.gov/chemview/
[14] NTP data and resources are available at: https://ntp.niehs.nih.gov/data/index.html
[15] OECD Existing Chemicals Database is available at: https://hpvchemicals.oecd.org/ui/Default.aspx
[16] OECD eChem Portal is available at: https://www.echemportal.org/echemportal/substance-search
[17] EPA's ECOTOX Knowledgebase is available at: https://cfpub.epa.gov/ecotox/

3.2.3. SCREENING PROCESS

The studies identified from database searches and SWIFT Review are housed in the HERO system and imported into DistillerSR for title/abstract and full-text screening. Both title/abstract and full-text screening are conducted by two independent reviewers. Records that meet PECO criteria during title and abstract screening are considered for full-text screening. At both the DistillerSR title/abstract and full-text review levels, screening conflicts are resolved by discussion between the primary screeners with consultation by a third reviewer (if needed) to resolve any remaining disagreements. For citations with no abstract, the articles are initially screened based on all or some of the following: title relevance (title should indicate clear relevance), and length (articles two pages in length or less are assumed to be conference reports, editorials, or letters). During title/abstract or full-text level screening in DistillerSR, studies that did not meet the PECO criteria, but which could provide supporting information are categorized (or "tagged") as supplemental information. Supplemental material is tagged using a "check all that apply" approach and reviewers resolve conflicts on the specific tags applied to studies.

Results of the screening process are presented in study flow diagrams and made publicly available in HERO to see full reference details. The study flow diagrams are also made available in an interactive literature tree format using EPA's version of Health Assessment Workspace Collaborative (HAWC)[18], a free and open-source web-based software application designed to manage and facilitate the process of conducting literature assessments.

3.2.4. EVALUATION OF WHETHER AVAILABLE STUDIES MAY PLAUSIBLY BE USED FOR POD AND REFERENCE VALUE DERIVATION

Studies that meet PECO criteria after full-text review are briefly summarized in DistillerSR. For animal studies, the following information is captured: chemical form, study type [*i.e.,* acute (< 24 hours), short term (1-30 days), subchronic (30-90 days), chronic (>90 days[19]), reproductive, developmental], duration of exposure, route, species, strain, sex, dose or concentration levels tested, dose units, health outcome(s) and specific endpoint(s) assessed, and a summary of findings at the health outcome level [*i.e.,* null or NO(A)EL/LO(A)EL based on author-reported statistical significance with an indication of which specific endpoints were affected].

For epidemiologic studies, the following information is summarized, when available: chemical form, population type (*e.g*., general population-adult, occupational, pregnant women, infants and children, etc.), study type (*e.g*., cross-sectional, cohort, case-control), short free text description of study population, sex, major route of exposure (if known), description of how exposure

[18] EPA's Health Assessment Workspace Collaborative (HAWC) is available at: https://hawc.epa.gov

[19] EPA considers chronic exposure to be more than approximately 10% of the life span in humans. For typical laboratory animal species, this can lead to consideration of exposure durations of approximately 90 days to 2 years. However, studies in duration of 1 - 2 years are typical of what is considered representative of chronic exposure rather than durations just over 90 days.

was assessed, health system and specific outcome assessed, and a summary of findings at the health system level (null or an indication of any associations found and a description of how the exposure was quantified in the analysis). Studies are extracted into DistillerSR or HAWC by one team member and checked by at least one other team member. These study summaries, referred to as a literature inventory, are presented in HAWC or Tableau visualization software,[20] and are also available as an Excel file.

Studies in the literature inventory are analyzed with respect to suitability for the identification of an inhalation or oral POD, with preference given to the following:

- Animal studies with chronic or subchronic exposure durations.
- Animal study designs that assess effects of exposure on reproduction or development.
- Non-human mammalian studies using a species that is generally considered a relevant human surrogate.
- Animal studies with a broad exposure range and multiple exposure levels. These can provide information about the shape of the exposure-response relationship [see the EPA Benchmark Dose Technical Guidance, §2.1.1 U.S. EPA (2012b)] and facilitate extrapolation to more relevant (generally lower) exposures. However, single dose studies can be considered for reference value derivation if they test phenotypic health outcomes unexamined in multidose studies testing similar levels or for informing acute toxicity hazard(s).
- Human studies for which quantitative exposure measurements are available and exposure-response results are presented in sufficient detail (*e.g.*, standardized mortality rate or relative risks, numbers of cases/controls). Studies based exclusively on duration of exposure analyses (*i.e.*, longer versus shorter exposure duration) are typically not considered suitable for dose response unless additional information on exposure can be incorporated. Epidemiological studies that use biomarker measurements in tissues or bodily fluids as the metric for exposure are only considered suitable for dose-response analysis if data or PBPK models are available to extrapolate between the reported biomarker measurement and the level of exposure.

For both animal and human studies, the nature of the outcomes/endpoints assessed and whether these are interpretable with respect to potential adversity is considered. Typically, apical or clinical measures ("phenotypic") are preferred over other endpoints for dose response. However, "mechanistic" endpoints can be useful in dose-response analyses when they can be reasonably

[20] Tableau is available at: https://www.tableau.com

established as predictive of, or strongly associated with, phenotypic outcomes interpreted as adverse.

3.3. EVIDENCE MAP REVIEW AND PRE-STUDY EVALUATION

The results of the evidence map are reviewed prior to initiation of the *in vivo* transcriptomic studies. Chemical substances may be eligible for an *in vivo* 5-day transcriptomic study and development of an ETAP if they meet one of the two following criteria: 1) confirmed to have no publicly available mammalian *in vivo* repeated dose toxicity studies or suitable human studies; or 2) the only available *in vivo* repeated dose studies have critical deficiencies and are considered *uninformative* using the study evaluation methods described by Thayer and colleagues (Thayer et al. 2022a; Thayer et al. 2022b).

3.4. 5-DAY *IN VIVO* TRANSCRIPTOMIC STUDIES AND ANALYSIS

3.4.1. DOSE FORMULATIONS AND PRE-ADMINISTRATION ANALYSIS

3.4.1.1. Chemical Purity

Substances evaluated in an ETAP are typically procured from a commercial source, synthesized, or obtained from a reliable third party. The purity of the chemical substance is typically provided by the commercial source and also evaluated independently using the most appropriate analytical method [*e.g.*, liquid chromatography-mass spectrometry (LCMS), gas chromatography-mass spectrometry (GCMS)]. Quantitative structure activity relationship (QSAR) models may be used to identify the probable physical form, acidity, and analytical method (Lowe et al. 2021; Mansouri et al. 2018; Mansouri et al. 2019). For most studies, purity of test articles of >95% or greater is acceptable. For studies with a purity <95%, the purity of the chemical substance is documented accordingly.

3.4.1.2. Vehicle Selection and Stability

For oral gavage studies[21], a set of dosing vehicles are evaluated for chemical solubility and stability. The vehicles may include 1:1:8 Kolliphor:ethanol:deionized water, deionized water with ≤2% Tween® 80, corn oil, deionized water as well as other options depending on physicochemical properties of the substance. The solubility is assessed visually and/or through analytical measurements. If an aqueous vehicle is used, the pH of the solution with test chemical should be determined, as too low or high of a pH can adversely affect the animal.

[21] Current application is limited to oral gavage studies. Certain toxicological responses are route and dosing regimen specific. As a result, other routes of exposure may be considered in the future. Extrapolations to other routes and dosing regimens may potentially be considered for gap-filling under specific circumstances.

3.4.1.3. Dose Identification

The approach used to select the dose range for the study will depend on a number of factors that may be specific to the substance of interest. Given the intended application of ETAP for data poor substances, neither *in vivo* repeated dose toxicity data nor suitable human evidence will likely be available. If existing acute toxicity data are available for the substance of interest, the selection of the dose range should incorporate these studies. If no acute toxicity data are available, *in silico* approaches (*e.g.*, QSAR modeling) or pilot tolerability/dose range finding studies with limited numbers of animals may be used to inform the selection of the dose range. 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 $\log_{10}$ lower than the next dose. The remaining doses should use half-log spacing.

3.4.2. ANIMAL HUSBANDRY AND EXPOSURE

Male and female Sprague Dawley (Crl:CD IGS, Charles River Laboratory) rats are purchased at 6 – 8 weeks of age. Upon receipt, the animals are placed on a standard, purified laboratory diet and reverse osmosis treated drinking water *ad libitum.* After a 7- to 14-day quarantine and acclimation period, the animals are weighed and randomly assigned by weight to chemical exposure and control groups. Only clinically healthy animals are used in the study. The target age for initiating exposure is 8 - 10 weeks. For oral gavage studies, at least four male and four female rats per dose group receive the vehicle alone or test article in vehicle via gavage (5 or 10 ml/kg) for five days. Animals are weighed daily prior to administration and are observed twice daily, once during administration and once in the late afternoon, at least six hours apart, for assessment of moribundity and mortality. Formal clinical observations are performed on the first day post-dosing and prior to necropsy. Moribund animals or animals exhibiting overt clinical toxicity are removed from the study.

The temperature in the experimental animal room is maintained at a target of 22°C (± 3°C) with a relative humidity that is ideally between 50-60% but is at least 30% and preferably not to exceed 70% other than during room cleaning. Lighting is artificial with a sequence of 12 hours light, 12 hours dark. Animals are housed individually or caged in small groups of no more than three animals of the same sex in accordance with local institutional animal care and use requirements. The facility will be accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and will follow published Public Health Service animal care and use guidelines (NASEM 2011).

3.4.3. TISSUE COLLECTION

Optional blood samples may be collected at a specific time interval (*e.g.*, 2 hr) following the first dose to provide estimates of toxicokinetic properties for certain chemicals. Treated and control animals are necropsied approximately 24 hours after the last exposure. Carbon dioxide asphyxiation is used as the method of euthanasia, with death confirmed by a secondary method such as exsanguination or cervical dislocation. At the time of necropsy, blood is collected [using potassium ethylenediaminetetraacetic acid (EDTA) as an anticoagulant] via cardiac puncture. Following

collection, plasma is isolated and stored at approximately -80°C. While previous studies have demonstrated that transcriptional responses from the liver and kidney could be used as sentinels for phenotypic responses in other tissues (EPA 2023), a larger number of tissues will be dissected to increase the breadth of biological responses evaluated. The dissected tissues will include kidney, liver, adrenal gland, brain, heart, lung, ovary (females), spleen, testis (males), thyroid, thymus, and uterus (females). Other tissues may also be dissected to increase the number of organs for potential transcriptomic evaluation. Tissue samples are typically collected within ten minutes of termination. Tissue samples are sectioned (approximately 5 mm^3) and aliquoted into at least two sample tubes. At least one of the samples from each tissue in each animal are preserved in RNAlater™ (Thermo Fisher Scientific) at 4°C overnight and then frozen at approximately - 20°C for up to 3 weeks before transferring to approximately -80°C. The remaining sample from each tissue is frozen immediately in liquid nitrogen and stored at approximately -80°C.

3.4.4. RNA ISOLATION AND TRANSCRIPTOMIC MEASUREMENTS

For each tissue undergoing transcriptomic analysis, total RNA is extracted from one of the aliquots stored in RNAlater™ using a standard approach for RNA isolation. At a minimum, RNA should be isolated and transcriptomic measurements performed on the kidney, liver, adrenal gland, brain, heart, lung, ovary (females), spleen, testis (males), thyroid, thymus, and uterus (females). RNA may be isolated from other tissues and organs to increase the breadth of transcriptomic coverage, but it is not required. The quantity and purity of the RNA (*e.g.*, absorbance at 260 and 280 nm, absorbance at 260 and 230 nm, RNA integrity number) are determined and documented. The isolated total RNA is used to perform targeted RNA sequencing (RNA-seq) using the BioSpyder TempO-Seq rat S1500+ assay according to manufacturer's instructions. No specific RNA purity or integrity criteria are applied to the RNA samples as the TempO-Seq assay has been designed to provide high quality gene expression measurements on whole cell lysates, purified RNA, and formalin-fixed paraffin embedded tissue. Each sample is sequenced to a target read depth of at least 1 million mapped reads per sample.

3.4.5. TRANSCRIPTOMIC DATA ANALYSIS

3.4.5.1. Sequence Alignment

Raw sequencing reads (FASTQ files) are aligned to known probe sequences listed in the TempO-Seq probe manifest to compute a matrix of read counts for each probe in each sample. Initial quality checks are performed post-alignment to identify samples with insufficient sequencing depth or input RNA to yield reliable results. Each FASTQ file is aligned to the TempO-Seq probe manifest using HISAT2 (Kim et al. 2015; Kim et al. 2019). The alignment results are imported directly into SAMtools (Li et al. 2009) to compute probe-level counts for each individual FASTQ file. Samples are examined for additional quality statistics and those not meeting minimum quality standards are removed from the analysis. Samples that do not pass quality checks may be subjected to reprocessing for RNA isolation and RNA-seq. Quality metrics include (Harrill et al. 2021a):

- Sequencing depth (*i.e.,* total number of mapped reads). Samples with < 10% of target depth are removed from further analysis.
- Fraction of uniquely mapped reads. Samples with < 50% of reads uniquely mapped to known probes are removed from further analysis.
- Probe coverage (*i.e.,* total probes with at least 5 reads). Samples with < 1,200 covered probes are removed from further analysis.
- Signal distribution (*i.e.*, the minimum number of probes that capture 80% of total mapped reads in the sample). No cutoff is applied, but this metric is considered when evaluating potential outlier samples (see below).

3.4.5.2. Sample Normalization

Prior to performing downstream gene expression across samples, probe counts for each sample are normalized to adjust for differences in sequencing depth. For each exposure regimen in each sex and tissue, raw probe counts for all samples (including matched controls) are normalized within each sample as follows:

- All probes with a mean read count < 5 are removed, as these probes lack sufficient signal for reliable analysis.
- Each remaining probe is normalized to Counts Per Million (CPM) which is probe count * 1,000,000 / sum of all remaining probe counts in sample.
- CPM values are transformed to $\log_2$ scale with added pseudo-count of 1 to prevent taking log of zero counts and ensuring a positive value for dose response modeling.

To identify potential outlier samples or batch effects, a principal component analysis (PCA) is performed on subsets of samples corresponding to: 1) all samples corresponding to same substance, tissue, and sex, including matched vehicle controls ("chemical exposure PCA"); and 2) all matched vehicle controls corresponding to the same tissue and sex ("vehicle PCA"). Samples not meeting the sequencing quality metrics (*e.g*., < 50% of uniquely aligned reads) are excluded prior to PCA analysis. Outlier samples are identified based on the following considerations:

- Individual samples separated from all remaining samples on either principal component #1 (PC1) or principal component #2 (PC2) by >2x the span of all other samples on the corresponding PC are considered strong outliers and removed from further analysis.
- Individual samples separated by <2x the range of all other samples are considered moderate outliers, and additional exclusion criteria are considered:
    - Vehicle samples that appear as moderate outliers on both a chemical exposure PCA and vehicle PCA are excluded unless multiple controls from the same group appeared as outliers.
    - Moderate outlier samples with lower quality than corresponding tissue samples by one or more sequencing quality metrics (*e.g*., percentage of uniquely mapped reads) are excluded.

- Samples that appear as moderate outliers in both PC1 and PC2 with a relatively large Euclidean distance from all other remaining samples are excluded.
- Moderate outlier samples that are especially distant from corresponding replicates or similar doses are excluded.

When multiple outlier samples are present on the same PCA, they are only removed if each outlier sample corresponds to a different dose group, as these are unlikely to represent any reproducible dose-dependent effect. A minimum number of two samples that pass quality control and outlier detection is required for each dose level; individual dose levels not meeting this criterion will be excluded from subsequent analysis. A minimum number of three vehicle control samples that pass quality control and outlier detection is required to proceed with dose response modeling for a given tissue, sex, and exposure regimen.

3.4.5.3. Dose Response Analysis

Once the sequencing data are aligned and normalized, and all low quality and outlier samples removed, dose response modeling is performed. Each data set consists of the series of remaining replicates for all concentrations of a single chemical and matched vehicle controls in the same sex and tissue. The dose-response modeling is performed independently on each probe and for each data set using the peer-reviewed BMDExpress software version 2.3 (Phillips et al. 2019; Yang et al. 2007). The dose response analysis procedures are consistent with the NTP Approach to Genomic Dose-Response Modeling (NTP 2018), but have been adapted for the specific gene expression platform used in this method (EPA 2023):

- Normalized Log_2(CPM) with added pseudo-count of 1 is used as input.
- For each data set (specific combination of exposure, sex, and tissue), the analysis of variance (ANOVA) pre-modeling test is used to confirm that at least one probe has significant response with a false discovery rate (FDR) < 0.05. If no probes have a significant response, the particular sex and tissue combination is determined to be inactive for the chemical and dose range tested.
- Pre-filtering of probes suitable for dose-response modeling is performed using a William's trend test ($p < 0.05$) and a mean absolute fold-change relative to vehicle controls of 1.5x or greater in at least one dose.
- Model fitting and BMD determination are performed on each probe passing the pre-filtering criteria:
  - The following dose-response models are used in the analysis – linear, second degree polynomial, power, Hill, second degree exponential, third degree exponential, fourth degree exponential, and fifth degree exponential.
  - Models are run assuming a constant variance.
  - For the power model, power is restricted >=1.

- The model with the lowest Akaike information criterion (AIC) is selected as the best-fit model except in cases where the "k" parameter for the Hill model is less than one-third the lowest dose. In cases for which the "k" parameter for the Hill model is out of bounds, the Hill model is excluded from the final selection (Rowlands et al. 2013; Thomas et al. 2013b).
- The Benchmark Response (BMR) is set to 1.349 * standard deviation of replicate vehicle control samples (Thomas et al. 2007). Based on EPA guidance, a BMR of 1 standard deviation for continuous data approximates a 10% increase in risk for normally distributed effects when the direction of the effects is known (EPA 2012). However, for most gene expression changes, the direction is not known *a priori*. To provide an equivalent 10% increase in risk, a BMR of 1.349 * standard deviation is required (Thomas et al. 2007).
- The BMD, BMDL, and BMD upper confidence bound (BMDU) are calculated for each probe.
- Only probes meeting the following BMD modeling criteria are included in the next step for gene set summarization (EPA 2023; NTP 2018):
  - BMD < highest dose used in the study
  - Model fit p-value > 0.1
  - BMD/BMDL < 20

3.4.5.4. Gene Set Summarization

BMD results for each exposure/sex/tissue are aggregated into Gene Ontology (GO)[22] biological process classes to identify BMD values. The gene set summarization process is performed as follows:

- Probes are mapped to associated genes. For genes with multiple probes, the BMD/BMDL values from valid probes are averaged. Probes mapping to multiple genes are excluded.
- Genes with conflicting probes are flagged for further review. Using the default setting in BMDExpress, conflicting probes are defined as those with a correlation cut-off of < 0.5 across doses.
- The BMD values for the individual genes are aggregated into GO biological process classes using the current annotations available in BMDExpress.
- GO classes containing fewer than 3 genes with valid BMDs meeting the above criteria are removed from the analysis.

[22] Additional information on Gene Ontology (GO) knowledgebase may be accessed at: http://geneontology.org/

- The BMD and BMDL for each GO class are calculated as the respective medians of corresponding values from the associated genes.

3.4.5.5. POD Identification

The most sensitive GO biological process class is identified based on the lowest median BMD across the tissues examined in either sex. If the median BMD from the most sensitive GO biological process is more than 3-fold below the lowest positive dose, a 'no value' ETAP is declared, and the dose range tested is reported. A follow-up study with an extended dose range may be considered. If the median BMD from the most sensitive biological process is less than 3-fold below the lowest positive dose or within the tested dose range, the median BMDL associated with the identified GO biological process class is selected as the POD. The transcriptomic POD is defined as the administered dose at which there were no coordinated transcriptional changes that would indicate a potential toxicity of concern. The coordinated transcriptional changes used to identify the POD do not necessarily discriminate between non-cancer or cancer effects, adverse or adaptive effects, nor are they used to infer a mechanism or mode-of-action. If there is more than one GO biological process class with identical median BMD values across tissues and sexes, the GO class with the most sensitive median BMDL is selected and used as the POD. If there is more than one GO biological process class with identical median BMD and BMDL values, each GO class is reported. If no tissue in either sex passes the pre-modeling filter nor produces at least one valid GO class, a 'no value' ETAP is declared and the dose range tested is reported. A follow-up study with an extended dose range may be considered.

3.5. HUMAN EQUIVALENT DOSE

The selected transcriptomic BMDL is scaled to a Human Equivalent Dose (HED) using an oral dosimetric adjustment factor (DAF) based on interspecies $BW^{3/4}$ allometry (EPA 2011a). The $BMDL_{HED}$ is calculated using the following equation:

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

The BW_{Rat} is the study-specific mean terminal rat body weight for the sex that is associated with the POD. The BW_{Human} is the reference human body weight of 80 kg (EPA 2011b). The $BMDL_{HED}$ represents the POD used to derive the TRV. The $BMDL_{HED}$ is also provided in the ETAP to enable users to calculate values for varying risk assessment applications such as a margin of exposure (EPA 2000, 2012), and to evaluate potential health risks from chemical mixtures (EPA 2000). Context specific applications are dependent upon multiple factors, including the statute or legislative mandate/purview involved, the exposure situation being addressed, the hazard and dose-response data available and associated uncertainties, and the fit-for-purpose needs of the decision-maker.

3.6. TRANSCRIPTOMIC REFERENCE VALUES

Biological process-based, transcriptomic PODs obtained from the 5-day *in vivo* oral exposure studies, described in Sections 3.4 and 3.5 of this document, may be used in the derivation of TRVs through application of uncertainty factors (UFs). The UFs are consistent with traditional human health assessment guidance and the fit-for-purpose rationale(s) considered for quantitative application of each factor are provided below.

3.6.1. UNCERTAINTY FACTORS

As a common practice in human health risk assessment of oral exposures, UFs are used in deriving RfD values from PODs estimated using experimental data (EPA 1994, 2002). UFs are intended to account for: 1) unknown or imprecise measures of variability in sensitivity among the members of the exposed human population (*i.e.*, interhuman or intraspecies variability, UF_H); 2) the uncertainty in extrapolating animal data to humans (*i.e.*, interspecies variability, UF_A); 3) the uncertainty in extrapolating from data obtained in a study with less-than-lifetime exposure to lifetime exposure (*e.g.*, extrapolating from subchronic to chronic exposure, UF_S); 4) the uncertainty in extrapolating from a LOAEL rather than from a NOAEL (UF_L); and 5) the uncertainty associated with deficiencies or knowledge gaps in the chemical-specific database (UF_D).

In current EPA human health risk assessment practice, in the absence of chemical-specific data supporting quantitative application of uncertainty, standard UFs of 10 are recommended, with 3 used in place of half-power values (*i.e.*, $10^{0.5}$) if some aspect of uncertainty is accounted for, or if uncertainty is not comprehensively addressed. An UF of 1 is applied if either the uncertainty is not relevant (e.g., UF_L of 1 because the POD is a BMD value), or if qualitative evidence comprehensively characterizes an area of uncertainty. Within the scope of an ETAP, the initial step in the process for selecting and pre-qualifying chemicals occurs through systematic evidence mapping to ensure that only data poor substances are considered. 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 application(s). Scientific support for application of UFs to PODs in the derivation of reference values should be clearly documented, with the qualitative and quantitative rationale defined explicitly.

3.6.1.1. Intraspecies Variability Uncertainty Factor (UF_H)

The intraspecies UF_H is applied to account for variation in susceptibility within the human population (interindividual variability) and the possibility (given a lack of relevant data) that the database available is not representative of the exposure/dose-response relationship in the subgroups of the human population that are most sensitive to the health hazards of the substance being assessed. As the reference dose is defined to be applicable to "susceptible subgroups," this UF is used to account for uncertainty in that regard. The reduction of the intraspecies UF_H from 10 should be considered only if data are sufficiently representative of the exposure/dose-response data for the

most susceptible human population(s) (*e.g.*, early and late lifestages). The UF_H may be presumed to entail aspects of both TK and TD, thus providing an opportunity to integrate traditional and/or NAM-based information that might support reduction in the UF or quantitative application of a data-derived extrapolation factor (DDEF) for human TK ($DDEF_{HK}$) and/or human TD ($DDEF_{HD}$) for the UF_H (EPA 2014).

For transcriptomic PODs identified in the ETAP, a UF_H of 10 is applied. However, if information is available that informs intraspecies variability or unique sensitivities or susceptibilities of relevance to human populations (*e.g.*, toxicokinetic and/or toxicodynamic variation[s] in human populations), then expert judgment may be used to consider the weight of the evidence to support application of a $DDEF_{HK}$ and/or $DDEF_{HD}$ in place of the standard UF_H of 10. However, should human intraspecies information be identified during the evidence mapping phase, consideration should be given to transitioning such a substance to another assessment product line outside of ETAP.

3.6.1.2. Animal-to-Human Interspecies Uncertainty Factor (UF_A)

The interspecies UF_A is applied to account for the extrapolation of laboratory animal data to humans, and it generally is presumed to include cross-species TK and TD uncertainties. With chemical-specific data that informs cross-species scaling of TK (*e.g.*, clearance or plasma $T_{1/2}$), the TK half of the UF_A may be reduced from a 3 (*i.e.*, $10^{0.5}$) to a 1 through the development and application of a dosimetric adjustment factor (DAF) that accounts, in general, for differences in TK between animals and humans. In the absence of chemical-specific TK data, a DAF may be applied to a transcriptomic POD obtained from *in vivo* animal oral exposure study designs using standard EPA guidance and practice, such as $BW^{3/4}$ allometric scaling (EPA 2011a). This results in the derivation of a POD human equivalent dose (POD_{HED}, such as a transcriptomic $BMDL_{HED}$).

The UF_A is intended to also account for differences in TD-related species sensitivity between the laboratory animals used for testing and humans. Seldom are there chemical-specific data available to inform TD differences between species, and one-half the standard 10-fold interspecies UF_A (*i.e.*, $10^{0.5}$) is assumed to account for such differences. Unless data support the conclusion that the laboratory test species is more or equally as susceptible to a chemical substance as are humans, and in the absence of any other specific TK or TD data, *a UF_A of 3 (in conjunction with calculation of a POD_{HED}) is applied for the ETAP.*

3.6.1.3. Subchronic-to-Chronic Duration Uncertainty Factor (UF_S)

EPA defines a chronic duration as repeated exposure by the oral, dermal, or inhalation route for more than approximately 10% of the life span in humans, corresponding to more than approximately 90 days to 2 years in typically used laboratory animal species (EPA 2002, 2011a). Subchronic duration is defined as repeated exposure by the oral, dermal, or inhalation route for more than 30 days, up to approximately 10% of the life span in humans (more than 30 days up to approximately 90 days in traditional laboratory animal species) (EPA 2002, 2011a). In traditional risk assessment practice, if no chronic duration study is available, information from a subchronic

study may be used to support the derivation of an RfD with the application of a UF_S of 10 to the subchronic POD.

Duration extrapolation in the context of an ETAP is informed by multiple previous studies that have demonstrated dose-concordance between traditional apical effect-based PODs derived from longer-term (*i.e.,* chronic) duration studies and gene set-based transcriptomic PODs derived from shorter-term studies (EPA 2023). The concordance was robust across species, sexes, routes or modes of exposure, and technological platforms. In the analysis performed to inform the choices and parameters used in the transcriptomic dose response modeling process, the error in the concordance of the 5-day transcriptomic BMDs with the apical effect BMDs from chronic rodent bioassays was approximately equivalent to the combined inter-study variability associated with the 5-day transcriptomic study and the chronic rodent bioassay (EPA 2023). This demonstrates that the observed differences between the 5-day transcriptomic and chronic apical BMDs are largely driven by inter-study variability in the BMDs, rather than systematic differences. As a result, *when using 5-day transcriptomic PODs for noncancer health effect domains in the ETAP, an UF_S of 1 is applied for considerations of duration in the derivation of a TRV.*

3.6.1.4. Lowest Observed Adverse Effect Level (LOAEL)-to-No Observed Adverse Effect Level (NOAEL) Uncertainty Factor (UF_L)

The current EPA approach for dose-response assessment prioritizes the application of BMD modeling to identify potential PODs for effects. However, in traditional human health risk assessment practice, when dose-response data are not amenable to BMD modeling, point estimates such as LOAELs and NOAELs are identified as potential PODs. A LOAEL is defined as the lowest exposure level at which there are statistically and/or biologically significant increases in frequency or severity of adverse effects between an exposed population and a corresponding control group. A NOAEL is the highest dose level tested at which the specified adverse effect is not produced. Generally, a LOAEL-to-NOAEL uncertainty factor (UF_L) is applied to derive a non-cancer reference value using an apical effect LOAEL if a NOAEL is unavailable. This UF_L is employed to estimate an exposure level below the LOAEL expected to be in the range of a NOAEL. Importantly, the underlying biology leading to and/or resultant of cell, tissue, or organ/system level toxicity invariably involves changes in gene expression. Selecting the BMDL for the "most sensitive" gene set is not necessarily associating transcriptional events with a specific adverse event per se, rather, it is thought to be a dose that approximates a NOAEL.

The gene set summarization of the gene expression changes is described in Section 3.4.5.4 and is suggested as the minimum unit of transcriptional activity to be used in the identification of a POD. That is, BMDLs for single genes are not recommended for POD identification; rather, only those groupings of genes that constitute a GO biological process class in accordance with the criteria outlined in 3.4.5.4 are considered for potential POD (*e.g.*, GO biological process-based BMDL) identification. *When GO biological process-based BMDL values are successfully identified for one or more classes using methods consistent with the ETAP, an UF_L of 1 is applied*.

3.6.1.5. Database Uncertainty Factor (UF_D)

In traditional human health risk assessment, the UF_D is intended to account for the potential for deriving an under-protective RfD as a result of an incomplete characterization of the substance's toxicity via the oral exposure route. In addition to identifying data gaps in toxicity information, review of existent data may also suggest that a lower reference value might result if additional data are available. Consequently, in deciding to apply this factor to account for deficiencies in the available data set and in identifying its magnitude, the assessor should consider both the data lacking and the data available for health outcome domains, tissues, or organ systems as well as life stages. In the context of the ETAP, previous studies have demonstrated that GO biological process-based transcriptomic BMD values following 5 days of exposure are in agreement with BMD values for histopathological effects in two-year chronic rodent bioassays (EPA 2023). Responses in other health effect domains, such as developmental, reproductive, endocrine, neurotoxicity, or immunotoxicity, may not necessarily be accounted for in 5-day *in vivo* transcriptomic studies. Therefore, *an UF_D of 10 should be applied to account for data gaps in the derivation of a TRV for an ETAP.*

3.6.1.6. Derivation of the Transcriptomic Reference Value

Using the $BMDL_{HED}$ from the most sensitive GO biological process class across tissues from both sexes, the standard calculation of the TRV is summarized based on the following equation; however, the exact calculation may vary in unusual circumstances based on the considerations discussed above:

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

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

The 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 individual and population from adverse 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. This approach has been previously used by EPA in certain risk assessment applications (*e.g.*, PPRTV assessments) wherein 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.

3.7. ETAP REPORTING

The summary results from the systematic evidence mapping, 5-day *in vivo* transcriptomic study, and TRV are to be reported in a standardized ETAP reporting template (Section 5). Use of the Organization for Economic Cooperation and Development's (OECD) Omics Reporting Templates (Harrill et al. 2021b; OECD 2022) as an appendix to the ETAP reporting template is recommended once the reporting template guidance is finalized.

3.8. INTERNAL AND EXTERNAL REVIEW OF ETAPs

The methods for developing the ETAP outlined in this document have been internally reviewed by ORD scientists and management. The methods have also been externally peer-reviewed by the EPA Board of Scientific Counselors and subject to public comment.

Due to the extensive review of the standardized methods and to facilitate the rapid development, execution, and review of the studies and product, the individual ETAPs for a specific substance will receive internal peer review by at least two ORD technical experts and will be published on a publicly available EPA ORD website (TBD). The individual ETAPs will undergo review for quality control and consistency with the standard method but will not receive independent external peer review.

4. COMPARISON OF TRANSCRIPTOMIC REFERENCE VALUES WITH TRADITIONAL RfDs

The formal statistical evaluation of the concordance between the traditional and transcriptional results has primarily been focused on the BMD values (EPA 2023). However, since the reference value is ultimately used to evaluate chemical risks, a comparison of available traditional RfD and TRV values provides some understanding of the relative level of protection afforded by the ETAP. In total, seven of the 14 chemicals that were used in the concordance evaluation in the EPA report (EPA 2023; Gwinn et al. 2020) had EPA IRIS, EPA chronic PPRTV, or EPA Office of Water (OW) reference values (Table 4-1). Notably, the critical effect in four of the seven chemicals were in species other than rat, which is the species utilized for ETAP. For six of seven chemicals, the TRV was lower than the RfD or provisional RfD (p-RfD), with perfluorooctanoic acid as the only chemical with a slightly higher TRV (3.1E-05 mg/kg-day versus 2.0E-05 mg/kg-day). Among the chemicals in Table 4-1, the median absolute ratio[23] was 2.9 ± 1.4 (Median Absolute Deviation; MAD).

Table 4-1. Comparison of Transcriptomic Reference Values (TRV) and Traditional RfD/provisional-RfD (p-RfD) Values for 7 of the 14 Chemicals Used on the Concordance Evaluation

Chemical	TRV (mg/kg-day)	RfD/ p-RfD (mg/kg-day)	TRV-to RfD Ratio	Source, Sex, Species, Study Type
Acrylamide	1.6E-04	2.0E-03	0.08	IRIS 2010[24]; Male Rats; Chronic
Di(2-ethylhexyl) phthalate	1.1E-02	2.0E-02	0.55	IRIS 1987[25]; Female Guinea Pigs; Subchronic-Chronic
Hexachlorobenzene	2.4E-05	8.0E-04	0.03	IRIS 1988[26]; Male and Female Rats; Chronic
Furan	3.5E-04	1.0E-03	0.35	IRIS 1987[27]; Male Mice; Subchronic
Perfluorooctanoic acid	3.1E-05	2.0E-05	1.55	OW 2016[28]; Male Mice; Developmental
Tris(2-chloroisopropyl) phosphate	6.7E-03	1.0E-02	0.67	PPRTV Chronic 2012[29]; Male Mice; Subchronic
Pentabromodiphenyl ether mixture (DE71)	4.1E-04	2.0E-03	0.21	IRIS 1987[30]; Male Rats; Subchronic

[23] The absolute ratio between a and b is defined as maximum{a/b, b/a}.
[24] Acrylamide IRIS Assessment: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=286
[25] Di(2-ethylhexyl) phthalate IRIS Assessment: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=14

In addition to the seven chemicals used to refine the dose response analysis parameters, a total of 20 additional chemicals were identified from the literature review (EPA 2023) that had EPA IRIS or EPA chronic PPRTV assessments (Table 4-2). A subset of the 20 chemicals had multiple time points, species, or tissues with reported transcriptomic POD values. The transcriptomic POD values were adjusted to a HED using the default body weights for the species, strain, and sex used in the study (EPA 1988). While the study designs and transcriptomic BMD analyses were not standardized as outlined in the preceding methods, the TRV was calculated using the composite UF of 300 to evaluate the general robustness of the approach and provide additional understanding of the relative level of protection that may be afforded by the ETAP. A total of 22 of the 47 combinations used different species for the transcriptomic studies than the study used to derive the RfD or RfC. A total of 28 of the 47 (~60%) combinations had TRVs that were more sensitive than the RfD/RfC; however, the relative sensitivity of the TRVs based on the open literature may be different compared with more standardized methods. The median absolute ratio was 2.3 $\pm$ 1.1 (MAD). The maximum absolute ratio was 59-fold for 2,2',4,4'-tetrabromodiphenyl ether where transcriptomic changes were measured in the rat liver after 5 days and the critical effect in the IRIS assessment was neurobehavioral changes in mice following a single dose administration. By comparison, the absolute ratio between the TRV and RfD for 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether was only 1.64-fold even though the transcriptomic changes were also measured in the rat liver after 5 days and the critical effect in the IRIS assessment was also neurobehavioral changes in mice following a single dose; however, the RfD for 2,2',4,4'-tetrabromodiphenyl ether used a composite UF of 3,000 to account for database uncertainties, while the RfD for 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether had only a composite UF of 300. In addition to the bromodiphenyl ethers, the TRV value for naphthalene was approximately 19-fold higher based on the mouse lung compared with the RfC. However, the RfC was based on adverse effects in the nasal epithelium in mice. When the TRV value for naphthalene was based on the nasal epithelium in rats, it was only 1.75-fold higher than the RfC. For those combinations that used different species for the transcriptomic studies, the median absolute ratio was 3.2 $\pm$ 1.3 (MAD), while those that used the same species had a median absolute ratio of 1.5 $\pm$ 1.1 (MAD). Overall, the results suggest that the TRV provides a similar level of protection relative to the traditional RfD, p-RfD, and RfC values.

[25] Di(2-ethylhexyl) phthalate IRIS Assessment: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=14
[26] Hexachlorobenzene IRIS Assessment: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=374
[27] Furan IRIS Assessment: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=56
[28] Perfluorooctanoic acid EPA OW Drinking Water Health Advisory: https://www.epa.gov/sites/default/files/2016-05/documents/pfoa_health_advisory_final_508.pdf . The new interim drinking water health advisory was not used since it has not yet been finalized.
[29] Tris(2-chloroisopropyl) phosphate PPRTV Assessment: https://cfpub.epa.gov/ncea/pprtv/chemicalLanding.cfm?pprtv_sub_id=1954
[30] Pentabromodiphenyl ether IRIS Assessment: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=184

Table 4-2. Comparison of Transcriptomic Reference Values (TRV) and Traditional RfD, p-RfD, or RfC Values for 20 Chemicals Identified in the Literature Review

Chemical	TRV (mg/kg-day or mg/m³)	Exposure Duration (d)	Sex, Species, Tissue	Reference	RfD or RfC (mg/kg-day or mg/m³)	Source, Sex, Species, Study Type	TRV-to-RfD Ratio
Acrylamide	1.1E-03	15	Male Mice, Lung	(Chepelev et al. 2018)	2.0E-03	IRIS 2010[31], Male Rats, Chronic	0.55
Acrylamide	4.9E-04	15	Male Rats, Thyroid	(Chepelev et al. 2017)	2.0E-03	IRIS 2010[32], Male Rats, Chronic	0.25
Acrylamide	2.7E-04	31	Male Mice, Hardarian Gland	(Chepelev et al. 2018)	2.0E-03	IRIS 2010[33], Male Rats, Chronic	0.13
Acrylamide	1.3E-03	31	Male Rats, Thyroid	(Chepelev et al. 2017)	2.0E-03	IRIS 2010[34], Male Rats, Chronic	0.67
Acrylamide	2.4E-03	31	Male Rats, Testis	(Recio et al. 2017)	2.0E-03	IRIS 2010[35], Male Rats, Chronic	1.20
Allyl alcohol	6.3E-04	1	Male Rats, Liver	(Johnson et al. 2020)	5.0E-03	IRIS 1987, Male Rats, Subchronic	0.13
Allyl alcohol	4.2E-04	4	Male Rats, Liver	(Johnson et al. 2020)	5.0E-03	IRIS 1987, Male Rats, Subchronic	0.08
Allyl alcohol	1.8E-03	8	Male Rats, Liver	(Johnson et al. 2020)	5.0E-03	IRIS 1987, Male Rats, Subchronic	0.37
Allyl alcohol	3.3E-03	15	Male Rats, Liver	(Johnson et al. 2020)	5.0E-03	IRIS 1987, Male Rats, Subchronic	0.67
Allyl alcohol	5.0E-03	29	Male Rats, Liver	(Johnson et al. 2020)	5.0E-03	IRIS 1987, Male Rats, Subchronic	1.01
Benzo[a]pyrene	9.4E-05	3	Male Mice, Liver	(Moffat et al. 2015)	3.0E-04	IRIS 2017[36], Rats, Developmental	0.31
Benzo[a]pyrene	9.9E-04	28	Male Mice, Lung	(Moffat et al. 2015)	3.0E-04	IRIS 2017[37], Rats, Developmental	3.29

[31] Acrylamide IRIS assessment: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=286
[32] Acrylamide IRIS assessment: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=286
[33] Acrylamide IRIS assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=286
[34] Acrylamide IRIS assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=286
[35] Acrylamide IRIS assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=286
[36] Benzo[a]pyrene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=136
[37] Benzo[a]pyrene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=136

Bromobenzene	7.9E-03	1	Male Rats, Liver	(Johnson et al. 2020)	8.0E-03	IRIS 2009[38], Male Mice, Subchronic	0.99
Bromobenzene	6.8E-03	4	Male Rats, Liver	(Johnson et al. 2020)	8.0E-03	IRIS 2009[39], Male Mice, Subchronic	0.85
Bromobenzene	3.6E-02	5	Male Rats, Liver	(Thomas et al. 2013b)	8.0E-03	IRIS 2009[40], Male Mice, Subchronic	4.45
Bromobenzene	3.4E-03	8	Male Rats, Liver	(Johnson et al. 2020)	8.0E-03	IRIS 2009[41], Male Mice, Subchronic	0.43
Bromobenzene	3.6E-02	14	Male Rats, Liver	(Thomas et al. 2013b)	8.0E-03	IRIS 2009[42], Male Mice, Subchronic	4.46
Bromobenzene	9.7E-04	15	Male Rats, Liver	(Johnson et al. 2020)	8.0E-03	IRIS 2009[43], Male Mice, Subchronic	0.12
Bromobenzene	2.0E-02	28	Male Rats, Liver	(Thomas et al. 2013b)	8.0E-03	IRIS 2009[44], Male Mice, Subchronic	2.52
Bromobenzene	3.1E-03	29	Male Rats, Liver	(Johnson et al. 2020)	8.0E-03	IRIS 2009[45], Male Mice, Subchronic	0.38
Bromobenzene	4.2E-02	90	Male Rats, Liver	(Thomas et al. 2013b)	8.0E-03	IRIS 2009[46], Male Mice, Subchronic	5.25
Chloroprene[a]	1.4E-02	5	Female Mice, Lung	(Thomas et al. 2013a)	2.0E-02	IRIS 2010[47], Male and Female Rats, Female Mice, Chronic	0.68
Chloroprene[a]	4.7E-02	15	Female Mice, Lung	(Thomas et al. 2013a)	2.0E-02	IRIS 2010[48], Male and Female Rats, Female Mice, Chronic	2.33

[38] Bromobenzene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=1020
[39] Bromobenzene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=1020
[40] Bromobenzene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=1020
[41] Bromobenzene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=1020
[42] Bromobenzene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=1020
[43] Bromobenzene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=1020
[44] Bromobenzene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=1020
[45] Bromobenzene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=1020
[46] Bromobenzene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=1020
[47] Chloroprene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=1021
[48] Chloroprene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=1021

Dichloroacetic acid	3.5E-02	6	Male Mice, Liver	(Cannizzo et al. 2022)	4.0E-03	IRIS 2003[49], Male and Female Dogs, Subchronic	8.67
Furan	6.6E-04	21	Female Mice, Liver	(Jackson et al. 2014)	1.0E-03	IRIS 1987[50], Male Mice, Subchronic	0.66
Furan	3.6E-05	90	Male Rats, Liver	(Dong et al. 2016)	1.0E-03	IRIS 1987, Male Mice, Subchronic	0.04
Myclobutanil	1.8E-02	14	Male Rats, Liver	(Bhat et al. 2013)	2.5E-02	IRIS 1988[51], Male Rats, Chronic	0.71
Myclobutanil	2.0E-02	14	Male Rats, Testis	(Bhat et al. 2013)	2.5E-02	IRIS 1988[52], Male Rats, Chronic	0.81
Naphthalene	5.8E-02	91	Female Mice, Lung	(Thomas et al. 2011)	3.0E-03	IRIS 1998[53], Male and Female Mice, Chronic	19.22
Naphthalene[a]	5.2E-03	91	Male Rats, Nasal epithelium	(Clewell et al. 2014)	3.0E-03	IRIS 1998[54], Male and Female Mice, Chronic	1.75
Pronamide	1.8E-03	90	Male Rats, Liver	(Bianchi et al. 2021)	7.5E-02	IRIS 1987[55], Dogs, Chronic	0.02
Propiconazole	2.8E-02	30	Male Mice, Liver	(Bhat et al. 2013)	1.3E-02	IRIS 1988[56], Male Dogs, Chronic	2.15
p-Toluidine	5.1E-03	5	Male Rats, Liver	(Dunnick et al. 2017)	4.0E-03	PPRTV 2012[57], Female Rats, Chronic	1.27
Tetrachloroethylene	2.5E-02	1	Male Mice, Kidney	(Zhou et al. 2017)	6.0E-03	IRIS 2012[58], Humans, NA	4.17
Triadimefon	2.9E-02	30	Male Mice, Liver	(Bhat et al. 2013)	3.0E-02	IRIS 1987[59], Rats, Chronic	0.96
Trichloroethylene	1.0E-04	1	Male Mice, Kidney	(Zhou et al. 2017)	5.0E-04	IRIS 2011[60], Mice and Rats, Developmental	0.20

[49] Dichloroacetic acid IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=654
[50] Furan IRIS Assessment: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=56
[51] Myclobutanil IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=342
[52] Myclobutanil IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=342
[53] Naphthalene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=436
[54] Naphthalene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=436
[55] Pronamide IRIS Assessment: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=95
[56] Archived IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=282
[57] p-Toluidine PPRTV Assessment at: https://cfpub.epa.gov/ncea/pprtv/recordisplay.cfm?deid=339175
[58] Tetrachloroethylene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=106
[59] Archived IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=131
[60] Trichloroethylene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=199

1,2,3-Trichloropropane	1.8E-03	91	Female Mice, Liver	(Thomas et al. 2011)	4.0E-03	IRIS 2009[61], Male Rats, Chronic	0.44
1,2,4-Tribromobenzene	5.1E-03	5	Male Rats, Liver	(Thomas et al. 2013b)	5.0E-03	IRIS 1987[62], Male Rats, Subchronic	1.03
1,2,4-Tribromobenzene	5.1E-03	14	Male Rats, Liver	(Thomas et al. 2013b)	5.0E-03	IRIS 1987[63], Male Rats, Subchronic	1.03
1,2,4-Tribromobenzene	6.8E-03	28	Male Rats, Liver	(Thomas et al. 2013b)	5.0E-03	IRIS 1987[64], Male Rats, Subchronic	1.36
1,2,4-Tribromobenzene	1.9E-03	91	Male Rats, Liver	(Thomas et al. 2013b)	5.0E-03	IRIS 1987[65], Male Rats, Subchronic	0.38
2,2',3,3',4,4',5,5',6,6'-Decabromodiphenyl ether	1.2E-02	5	Male Rats, Liver	(Shockley et al. 2020)	7.0E-03	IRIS 2008[66], Male Mice, Singe dose	1.64
2,2',4,4'-Tetrabromodiphenyl ether	5.9E-03	5	Male Rats, Liver	(Shockley et al. 2020)	1.0E-04	IRIS 2008[67], Male Mice, Singe dose	58.89
2,3,4,6-Tetrachlorophenol	2.6E-02	5	Male Rats, Liver	(Thomas et al. 2013b)	3.0E-02	IRIS 1988[68], Male and Female Rats, Subchronic	0.88
2,3,4,6-Tetrachlorophenol	8.7E-03	14	Male Rats, Liver	(Thomas et al. 2013b)	3.0E-02	IRIS 1988[69], Male and Female Rats, Subchronic	0.29
2,3,4,6-Tetrachlorophenol	1.4E-02	28	Male Rats, Liver	(Thomas et al. 2013b)	3.0E-02	IRIS 1988[70], Male and Female Rats, Subchronic	0.46

[61] 1,2,3-Trichloropropane IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=200
[62] 1,2,4-Tribromobenzene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=196
[63] 1,2,4-Tribromobenzene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=196
[64] 1,2,4-Tribromobenzene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=196
[65] 1,2,4-Tribromobenzene IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=196
[66] 2,2',3,3',4,4',5,5',6,6'-Decabromodiphenyl ether IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=35
[67] 2,2',4,4'-Tetrabromodiphenyl ether IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=1010
[68] 2,3,4,6-Tetrachlorophenol IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=108
[69] 2,3,4,6-Tetrachlorophenol IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=108
[70] 2,3,4,6-Tetrachlorophenol IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=108

2,3,4,6-Tetrachlorophenol	1.2E-02	91	Male Rats, Liver	(Thomas et al. 2013b)	3.0E-02	IRIS 1988[71], Male and Female Rats, Subchronic	0.40
[a]Comparison of the TRV was made to the RfC value since the transcriptomic POD was based on an inhalation exposure.							

[71] 2,3,4,6-Tetrachlorophenol IRIS Assessment at: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=108

5.STANDARD TEMPLATE FOR ETAP REPORT

The basic format of the ETAP conforms to a standardized template with minimal free form text. The ETAP standard template is provided as an embedded PDF document. **Double click** on the text below to access the standardized template.

Standard ETAP Template

6. EXAMPLE ETAP FOR PERFLUORO-3-METHOXYPROPANOIC ACID

The example ETAP for perfluoro-3-methoxypropanoic acid was developed based on real data using the standard methods outlined in this document. The example was provided to illustrate implementation of the standard ETAP method and not for regulatory decision-making purposes. The chemical selected as the example is a data poor per- and polyfluoroalkyl substances (PFAS) that was identified as a priority by EPA Program Offices, EPA Regions, or state agencies. The example ETAP for perfluoro-3-methoxpropanoic acid is provided as an embedded PDF file. **Double click** on the text below to access the example ETAP.

Example ETAP

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