Application of the ECHA Read-Across Assessment Framework (RAAF) to the SEURAT-1 Read-Across Case Study* on Perfluoroalkyl Acids

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* Read-Across for 90-Day Rat Oral Repeated-Dose Toxicity for Selected Perfluoroalkyl Acids: A Case Study

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This presentation is an illustrative example and not necessarily a final definitive hazard assessment.

Outline

- Process for RAAF assessment of case study
- Brief overview of RAAF
- PFAA case Read-Across (RA) Hypothesis relative to RAAF
- Applicable RAAF RA Scenario and Assessment Elements (AEs)
- Assessment of PFAA case using RAAF
  - Summary of explanation and key evidence
  - Any outages as per RAAF requirements
  - Resulting read-across score for AE before and after addition of New Approach Methods (NAM) data
- Conclusion on PFAA case and RAAF
- Discussion

Process for RAAF Assessment of PFAA Case Study

- Case study report previously developed by SEURAT
- Reviewed case study report (read-across hypothesis, presented data, and read-across justification)
- Identified most appropriate RAAF read-across scenario (driving RAAF evaluation)
- Associated most relevant information in case study report to address each element of the RAAF evaluation
- Followed RAAF descriptions to ‘score’ each element
  - First only considering traditional data
  - Then adding NAM data into the consideration
- Evaluated impact of NAM data on the read-across

RAAF Overview

Scientific assessment or evaluation based on 3 key features of the read-across hypothesis:

- **Number of source chemicals**:
  - Analogue Approach
  - Category Approach
- **Nature of substance (toxicant) responsible for effect:**
  - Common substance
  - Different substances
- **For Category Approach (i.e. multiple sources):**
  - Variation in strength of effect
  - Strength of effect does not change

Combinations of these 3 features results in 6 possible RAAF Read-Across Scenarios

RAAF Overview

6 possible RAAF Read-Across (RA) 'Scenarios'

1. Analog approach / Common toxicant (via biotransformation)
2. Analog approach / Different toxicants (no biotransformation)
3. Category approach / Common toxicant / Variation in strength of effect across sources
4. Category approach / Different toxicants / Variation in strength of effect across sources
5. Category approach / Common toxicant / No variation in strength of effect across sources
6. Category approach / Different toxicants / No variation in strength of effect across sources

RAAF Overview

- Each RAAF Read-across **Scenario** has a defined set of Assessment Elements (**AE**) to be considered
- Each **AE** addresses a key scientific feature of that Scenario to judge the validity and reliability of the read-across
  - Key features must be explained in the read-across justification **and** supported with scientific evidence
- Evaluation of the **AE** results in an Assessment Option (**AO**) outcome (and associated score) chosen by assessor
  - AO scores ≥ 3 are acceptable
    - 5= high confidence; 4= medium confidence; 3= just sufficient confidence
  - AO score 2 = not acceptable in current form (more info needed)
  - AO score 1 = not acceptable

All AEs must have an AO ≥ 3 for the read-across to be accepted

PFAA Read-Across (RA) Hypothesis

- Category is defined as C7-C10 PFAAs
  - Data from flanking analogues C6 and C11,12 are included in WOE
- Data gaps for 90-day studies for C7, C9, and C10 are proposed to be filled by read-across from C8
  - Repeat dose data are available for C6, C8, C11, and C12 exhibiting liver toxicity as critical effect; however, strength of effect varies across members with potency C8>C11-12>C6
- Data exist to establish no/low metabolism of PFAAs

RAAF Read-Across Scenario #4

Category approach/Different toxicants/ Variation in effect

- C8 must be established as the worst-case category member for extrapolation to both C7 and C9,C10

Applicable RAAF Assessment Elements (AEs)

Category approach / Different toxicants causing same effect / Variation in strength of effect across category members

- Identity/characterization of substances
- Structural similarities and allowable differences
- Link of structural similarities/differences to prediction
- **Consistency of effects in data matrix**
- Adequacy of source data to meet information requirements
- **Identity of substances to which test organism is exposed**
- ***Common underlying mechanism, qualitative aspects***
- ***Common underlying mechanism, quantitative aspects***
- **Exposure to other substances not linked to prediction**
- **Occurrence of other effects than those covered by the hypothesis**
- Bias that influences the prediction

Assessment of PFAA Case Study using RAAF

Common Assessment Elements (relevant to any read-across scenario)

RAAF AE	RAAF AO Score	Reason /Change to address RAAF
C.1 Identity and characterization of substances	2	Category members identified but no purity/impurity information. Add impurity profile.
C.2 Structural similarities and allowable differences	5	Structural similarities defined (carboxylic acids with Fl saturated alkyl chain) and boundaries set by alkyl chain length.
C.3 Link of structural similarities/differences to prediction	5	Sufficient evidence provided to link structure to predicted property (liver toxicity in rats after repeated exposure).
C.5 Adequacy of source data to meet information requirements	4	Study design details are in cited source only. Add design details to case study report text.
4.6 Bias that influences the prediction	5	Based on category definition, no potential category members were omitted.

Assessment of PFAA Case Study using RAAF

Scenario Specific Assessment Elements (relevant to category approach)

- **Comparing biological properties across category members**

RAAF AE	RAAF AO Score	Reason /Change to address RAAF
C.4 Consistency of effects in data matrix	3	Detail in text demonstrates liver is consistent low dose target. Add more detail to ordered data matrix to allow direct determination of consistency across all endpoints from matrix .
4.5 Occurrence of other effects than those covered by the hypothesis	4	Detail in text does mention other effects of PFAAs. Increase description/discussion of other effects of PFAAs to better characterize occurrence across category, at what dose levels, etc.

Assessment of PFAA Case Study using RAAF

Scenario Specific Assessment Elements (relevant to different toxicants causing same effect)

- **Addressing metabolism and potential impact of any non-common metabolites on the effect**

RAAF AE	RAAF AO Score	Reason /Change to address RAAF
4.1 Identity of substances to which test organism is exposed	4	Lack of metabolism of PFAAs explained. Add metabolism data or METEOR predictions for other PFAAs in addition to C8 (only included)
4.4 Exposure to other substances not linked to the prediction	5	Text addresses that only the toxicity of the parent is of concern and that the impact of any impurities would be very small and explanation is provided as to how parent structure elicits predicted property (in other AEs)

Assessment of PFAA Case Study using RAAF

AE 4.2 *Common underlying mechanism, qualitative aspects*

Data Set	RAAF AO Score	Reason / Change to address RAAF
Traditional data only	5	Explanation based on traditional data demonstrates PFAA absorption, protein binding and distribution, renal reabsorption enhancing bioaccumulation, FA-like binding to PPAR and perturbation of lipid metabolism/transport leading to liver toxicity
NAM data added	5	NAM data demonstrates PFAAs: are identified by in silico profilers as PPAR agonists and nuclear receptor binders, induce genes involved in lipid metabolism/transport in toxicogenomics studies, up-regulate several PPAR/CAR/PXR genes in HepaRG cells, induce activity in ToxCast assays that target PPAR and PXR/CAR
RESULT: NAM data adds WOE to increase confidence in the MOA		

Assessment of PFAA Case Study using RAAF

AE 4.3 *Common underlying mechanism, quantitative aspects*

Data Set	RAAF AO Score	Reason / Change to address RAAF
Traditional data only	3	Traditional data demonstrates trends across chain lengths (clearance decreases with increasing chain length, increasing PPAR activity with increased chain length up to C9, repeat dose NOAELs for C8 are lower than for both C6 and C11-C12) suggesting peak potency at C8.
NAM data added	5	NAM data demonstrates that C8 PFAA: has stronger signal than C7 or C9-C10 for up-regulation of several PPAR/CAR/PXR genes in HepaRG cells, had most evidence for PPAR activity and was only PFAA with consistent PXR activity at non-cytotoxic concentrations in ToxCast assays on C6-11 PFAAs.
RESULT: NAM data provides more TD evidence that C8 is most potent PFAA in the category		

Conclusions of RAAF Evaluation of PFAA Case Study

- For key AEs, after NAM data added all AO scores were >3
- RAAF would conclude PFAA read-across is:
  - Traditional data: acceptable w/ just sufficient confidence
  - NAM data added: acceptable w/ high confidence
- Supported by in vivo data repeat dose NOAELs which demonstrate C6 >>> C11 and C12 > C8 PFAA, confirming read-across is conservative (i.e. C8 'worst-case')
- Some residual uncertainty remains with regard to TK differences between C8 and C9-C10 PFAA
- Possible helpful additional NAM data – comparative protein binding and in vitro renal transporter data

Conclusions of RAAF Evaluation of PFAA Case Study

- RAAF was useful for organizing and examining data that supports the read-across justification
- RAAF – based templates could be very useful tool for guiding construction and presentation of read-across cases

Discussion

- Are structured assessments helpful to combining lines of evidence? Should frameworks be WOE-based? Endpoint-specific?
- How to derive confidence levels for combined evidence? Do mechanistic data increase confidence? Can it be used to compensate some uncertainties?
- How to deal with remaining uncertainty? Are different approaches needed depending on problem formulation?
- What are potential difficulties and barriers for application of NAM? What are expected confidence levels of NAM for different regulatory applications?