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

Terry W. Schultz, Claire L. Mellor, Katarzyna R. Przybylak, Sylvia E. Escher, Richard Judson, Ivanka Tsakovka, Andrea-Nicole Richarz and Mark T.D. Cronin

TOPICS

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Similarities & Uncertainties

Applicability Domain & Source substance

New Approach Methodologies (NAMs)

Conclusion

THIS CASE STUDY

- **was designed to illustrate specific issues associated with read-across and use of NAMs, and stimulate discussion on the topics**

- **while evaluated via the RAFF, was developed prior to release of the RAAF**

- **is not intended to be related to any ongoing regulatory discussions of PFAAs**

INTRODUCTION

Based on similarities in:

1)chemistry

2)toxicokinetics, especially clearance

3)toxicodynamics, especially peroxisome proliferator-activated receptor (PPARα and/or PPARγ) activation

An analogue approach (i.e., one source substance) is proposed to fill data gaps in the small series (i.e., C7-C10).

CATEGORY MEMBERS

Perfluoroheptanoic acid	PFHpA	375-85-9	C7F13O2
Perfluorooctanoic acid	PFOA	335-67-1	C8F15O2
Perfluorononanoic acid	PFNA	375-95-1	C9F17O2
Perfluorodecanoic acid	PFDA	335-76-2	C10F19O2

PREMISE

PFAAs are direct-acting toxicants with similar modes of action (MoA).

MoA is most likely a combination of PPARα/ PPARγ interactions, leading to oral repeated-dose hepatotoxicity.

Symptoms, centrilobular hepatocellular hypertrophy correlated with higher liver weights, are via perturbations to fatty acid uptake, lipogenesis and fatty acid oxidation.

There are *in vivo* data of sufficient quality and quantity for PFOA to be a source chemical.

STRUCTURAL, CHEMICAL PROPERTY AND CHEMICAL CLASS SIMILARITIES

Annex I Tables 1 - 3

All 4 analogues are structurally highly similar.

All 4 analogues have physico-chemical/molecular properties that are either constant or show a trend in values related to C-chain length.

All the PFAAs included in the category have common constituents in the form of:

1) a single key substituent, -CO_2H,

2) structural groups, -CF_3 and -CF_2-,

3) extended structural fragment -CF_2CO_2H.

TOXICOKINETIC SIMILARITY

Annex I Tables 4 & 5

While the toxicokinetic understanding of PFAAs is incomplete, PFOA is well-studied.

Experimental data support the idea that intermediate size PFAAs are readily orally absorbed, poorly eliminated and not metabolized with similar distributions and similar elimination mechanisms among rodent species.

Elimination (e.g., ½ life) is key to establishing toxicokinetic similarity.

TOXICODYNAMIC SIMILARITY

Annex I Tables 6 – 8

PFAAs give rise to a standard set of symptoms, including liver toxicity.

Hepatocellular injury is accompanied by oxidative stress and inflammatory response, as well as alteration in lipid transport and metabolism.

While the molecular mechanism of PFAA-induced liver toxicity is not completely characterized, numerous studies suggest that PFAAs suppress immunity and induce fatty acid transport and metabolism due to PPARα/PPARγ interactions.

Identifying most likely initiating event (e.g., PPAR activity) is key to establishing toxicodynamic similarity.

THE WEAKER ASPECTS OF THE SIMILARITY ARGUMENT

There are two weak aspects of the similarity argument:

1) **Similarity in elimination rates**
2) **The mechanistic plausibility of PPAR activation as the most likely initiating event leading to repeated-dose liver toxicity**

SOURCE SUBSTANCE

The well-studied PFOA was selected as the single source analogue.

The NOAEL for PFOA of 0.06 mg/kg bw/day based on hepatocyte necrosis (males) and hepatocellular hypertrophy and increased liver weight (females) was used as the basis of the predictions.

The agreement of *in vivo* data for undecyl and dodecyl derivatives with that reported for PFOA increase the strength-of-evidence.

APPLICABILITY DOMAIN

To assure that toxicokinetic and toxicodynamic effects are similar to accept the read-across, the applicability domain was restricted to the category of C7-C10 analogues.

NAM RESULTS-1

Toxicogenomic studies of PFAAs reveal the largest group of induced genes is those involved in transport and metabolism of lipids, particularly fatty acids.

The largest groups of suppressed genes are those related to inflammation and immunity.

***In vitro* binding studies with 11 PFAAs to human PPARγ ligand binding domain (*h*PPARγ-LBD) and their activity on the receptor in cells show that the binding affinity increased with carbon number from C4 to C11 and then decreased slightly.**

NAM RESULTS-2

The USEPA ToxCast program screened the PFAA molecules C6-C11 in up to 800 separate *in vitro* assays.

They noted an increasing trend in cytotoxicity with C-atom chain length as measured in a set of assays.

Specific activity was seen in several target classes (e.g., PPAR, PXR/CAR, FXR, ER, AR, cell stress pathways and a number of enzymes and G-protein-coupled receptors).

PFOA had the strongest evidence for PPAR activity in concentration ranges not associated with cytotoxicity.

NAM RESULTS-3

Within the COSMOS project of SEURAT-1, PFAAs were screened with a variety of *in silico* profilers.

The potential for full PPARγ agonism is predicted for the higher molecular weight analogues by a virtual screening procedure, including docking with filtering by four PPARγ pharmacophores.

No potential for binding was found with profilers for 12 other nuclear receptors.

IMPROVING THE WEAKER ASPECTS OF THE SIMILARITY ARGUMENT

Taken collectively, the NAM data support the premise that the molecular mechanism of action inducing repeated-dose liver toxicity of PFAAs is PPAR-linked; most likely a combination of PPARα/ PPARγ interactions.

NAM FINDINGS

Reduce the data uncertainty and increase the strength-of-evidence associated with the fundamentals toxicodynamic similarity.

Reduce the uncertainty associated with mechanistic relevance and completeness of the read-across.

Do NOT alter uncertainty associated with toxicokinetic similarity.

WE CONCLUDE

- **This group of PFFAs represents an analogue approach to read-across.**
- **To reduce uncertainties associated with toxicokinetics and toxicodynamic to an acceptable level, a small congeneric series (i.e., C7-C10) of straight-chain PFAAs is proposed as the read-across category.**

WE FURTHER CONCLUDE

- **Read-across should be conducted from perfluoronated octanoic acid (PFOA).**
- **The NOAEL for PFOA of 0.06 mg/kg body weight/day based on hepatocyte necrosis (males) and hepatocellular hypertrophy and increased liver weight (females) may be read across to fill the data gaps for the three other analogues in the category.**

DATA GAP FILLING

Derivative	90-day NOAEL (mg/kg bw/d)
PFHxA	20-200
PFHpA	ND
PFOA	0.06
PFNA	ND
PFDA	ND
PFUA	0.1
PFDoA	0.1

WITH HIGH CONFIDENCE

Derivative	90-day NOAEL (mg/kg bw/d)
PFHxA	20-200
PFHpA	ND
PFOA	0.06
PFNA	ND
PFDA	ND
PFUA	0.1
PFDoA	0.1

0.06

WITH LOW CONFIDENCE

Derivative	90-day NOAEL (mg/kg bw/d)
PFHxA	20-200
PFHpA	ND 0.06??
PFOA	0.06
PFNA	ND
PFDA	ND
PFUA	0.1
PFDoA	0.1

Thank You
for Your Attention