U.S. EPA's use of Read-Across in Provisional Peer-Reviewed Toxicity Value Assessments

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Understanding and Applying Read-Across for Human Health Risk Assessment Workshop

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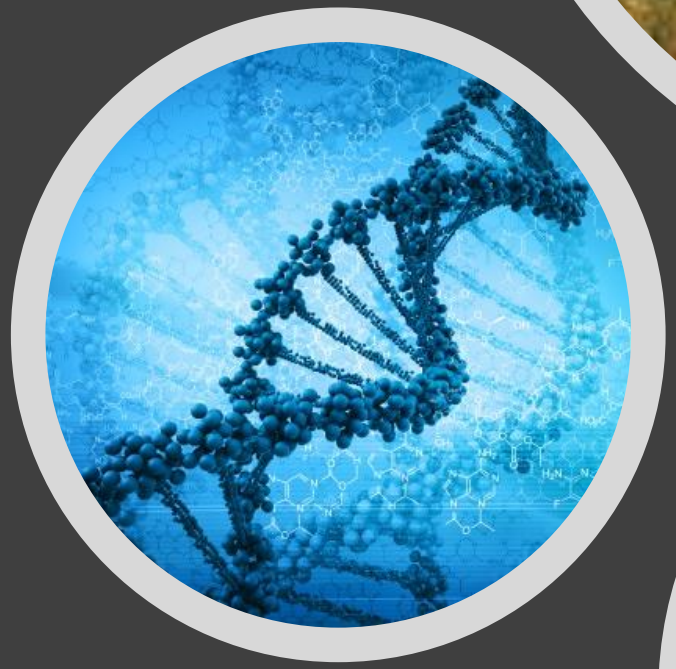

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The author has no conflicts of interest to disclose

Outline of this presentation

- What are PPRTVs?
- Advent of Appendix Screening Values: Expert-Driven Read Across
- *n*-Heptane example
- p,p'-DDD example (integrating ToxCast bioactivity)
- Limitations and challenges

Provisional Peer-Reviewed Toxicity Values

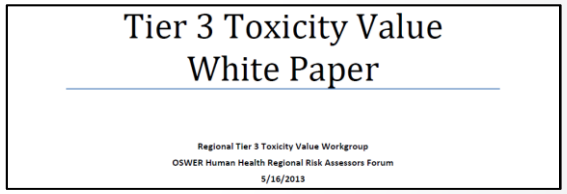
- Annual goal: Derive provisional human health reference values for 12 priority chemicals for OLEM/Regions when such values are not available from IRIS
- OSWER Directive 9285.7-53 (Dec. 5, 2003) established a hierarchy for selecting Human Health Toxicity Values for use in Superfund Risk Assessments

Tier 1 ➡ IRIS

Tier 2 ➡ PPRTV

Tier 3 ➡ Other Tox Values

OSWER 9285.7-86 (2013)

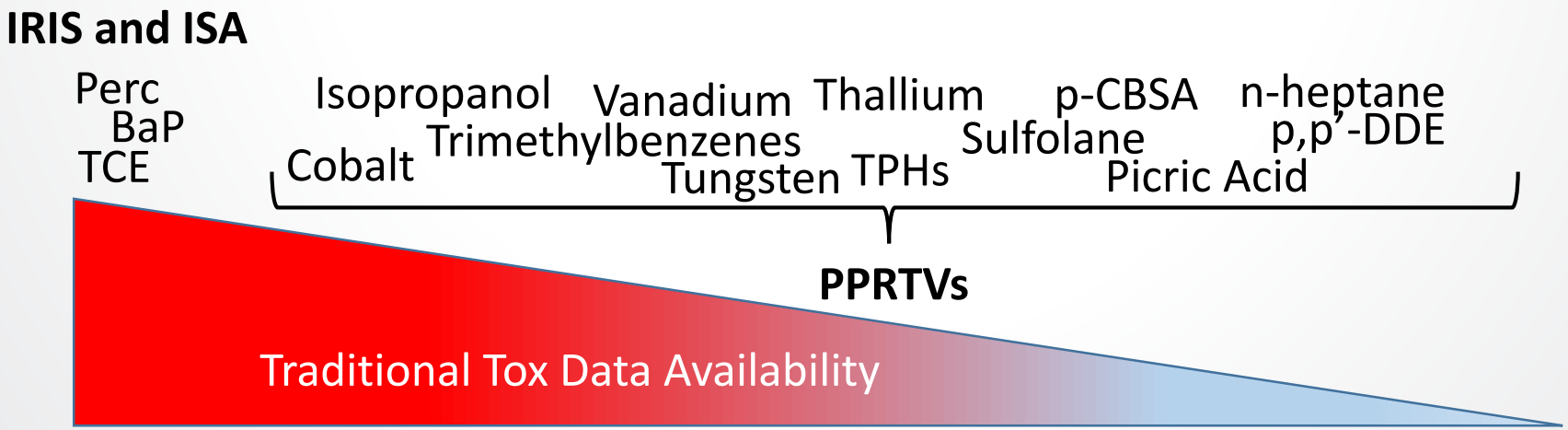

Tier 3 Toxicity Value
White Paper

Regional Tier 3 Toxicity Value Workgroup
OSWER Human Health Regional Risk Assessors Forum
5/16/2013

ATSDR MRLs
CalEPA
PPRTV appendixes

Provisional Peer-Reviewed Toxicity Values

- For over two decades, the PPRTV program has developed human health assessments for chemicals with highly variable hazard databases
- Includes development of subchronic and chronic non-cancer reference values (RfVs) and cancer values for chemicals of interest to OLEM/Regions
- A data-rich PPRTV assessment might provide up to six provisional values
- PPRTV 'appendixes' were a key development circa 2007



Expert-driven Read Across

- Approach is based on evidence integration and synthesis to select the best analogue chemical based on three proposed similarity contexts/categories (Wang et al., 2012)
- Similarity Contexts are:
  - (1) structural (including physchem),
  - (2) metabolic, and
  - (3) toxicity-like

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journal homepage: www.elsevier.com/locate/yrtph

Application of computational toxicological approaches in human health risk assessment. I. A tiered surrogate approach

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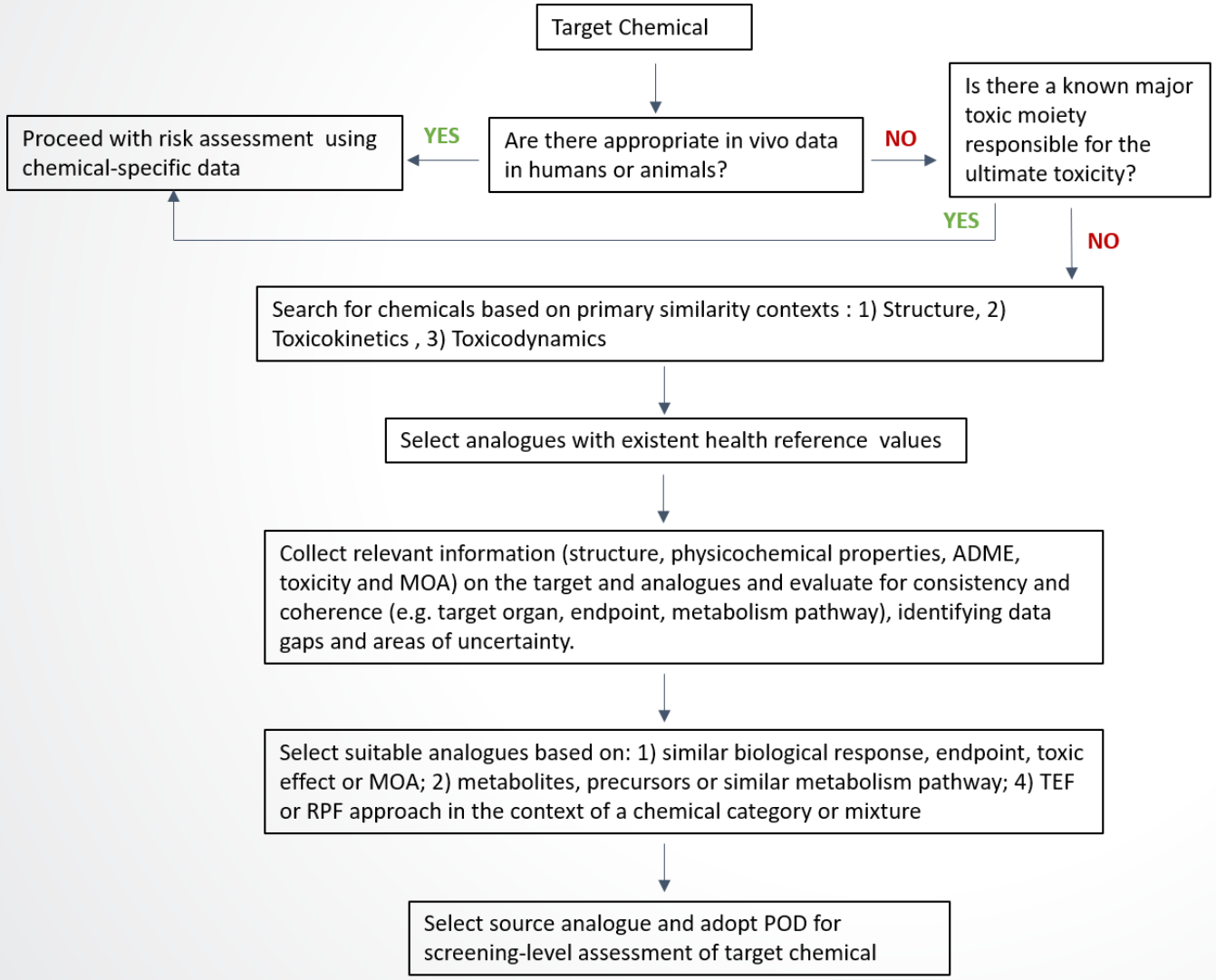
http://www.sciencedirect.com/science/article/pii/S0273230012000323

- Analogue chemicals are selected based on evidence across all three similarity contexts (i.e., analyses are integrated not sequential/linear)
- The POD(s) from the selected analogue is used as a surrogate to derive screening PPRTVs for a data-poor target chemical of concern

General Expert-Driven Read Across Workflow



Adapted from Wang et al., (2012)

n-Heptane (CASRN 142-82-5)

- Saturated aliphatic hydrocarbon
- Used as a non-polar solvent and also as a standard in gasoline engine testing
- Requested for human health assessment
- Poor hazard and dose-response database

Physicochemical Properties of *n*-Heptane	
Property (unit)	**Value**
Physical state	Liquid
Molecular weight (g/mol)	100.21
Vapor pressure (mmHg at 25°C)	46
Henry's Law constant (atm-m^3/mol at 25°C) (estimated)	2.27
Solubility in water (g/L at 25 C)	0.0034
Octanol-water partition constant (log K_{ow})	4.66

Identify Structural Analogues of *n*-Heptane

- Identify commonalities in structural/physicochemical properties between potential surrogates *that have available toxicity values*, and target chemical of concern

Table A-1. Physicochemical Properties of *n*-Heptane (CASRN 142-82-5) and Structural Analogs[a]

Chemical	*n*-Heptane	*n*-Hexane	*n*-Nonane
Structure			
CASRN	142-82-5	110-54-3	111-84-2
Molecular weight	100.21	86.18	128.26
DSSTox similarity score (%)[b]	100	85.7	87.5
ChemIDplus similarity score (%)[c]	100	82.7	84.6
Melting point (°C)	−90.6	−95.3	−53.5
Boiling point (°C)	98.5	68.7	150.8
Vapor pressure (mm Hg at 25°C)	46	151.3	4.45
Henry's law constant (atm-m^3/mole at 25°C)	2.27 (estimated)[a]	1.71 (estimated)[a]	4 (estimated)[a]
Water solubility (mg/L)	3.4	9.5	0.22
Log K_{ow}	4.66	3.9	5.65
pKa	NA	NA	NA

[a]Data was gathered from PHYSPROP database for each respective compound unless otherwise specified (U.S. EPA, 2012b).
[b]DSSTox (2015).
[c]ChemIDplus Advanced, similarity scores (ChemIDplus, 2016).

NA = not applicable.

Identify Toxicokinetic Similarities

- Identify commonalities primarily in metabolism (metabolic precursors, metabolites), but also absorption, distribution, and excretion, between potential analogue chemicals that have available toxicity values, and target chemical of concern

Table A-2. Summary of Metabolites for *n*-Heptane (CASRN 142-82-5) and Structural Analogs

Chemical	Route	Species	Metabolites in Urine	References
n-Heptane	Inhalation (1,800 ppm for 6 h)	Rat/M	2-heptanol (46.3), 3-heptanol (35.2), γ-valerolactone (11.5), 2-heptanone (3.5), 3-heptanone (1.5), and 4-heptanone (1.2), 2,5-heptanedione (0.8) over 24 h[a]	Perbellini et al. (1986)
n-Hexane	Inhalation (1,000 ppm for 8 h)	Rat/M	2-hexanol (57), 2,5-hexanedione (33), 3-hexanol (6), and 1-hexanol (3), 2-hexanone (1) over 24 h[a]	Fedtke and Bolt (1986)
n-Nonane	Oral (800 mg/kg-d)	Rat/M	γ-valerolactone (38.6), 2-nonanol (17.9), 3-nonanol (10.7), 4-nonanone (6.8), δ-heptanolactone (6.5), 1-heptanol (5.7), 4-nonanol (3.5), 5-methyl-2-(3-oxobutyl) furan (3.2), δ-hexanolactone (2.8), 2,5-hexanedione (1) over 48 h[b]	Serve et al. (1995)

[a]Percentage of total metabolites in urine.
[b]Relative abundance of metabolites in urine.

Identify Toxicity/Bioactivity Similarities

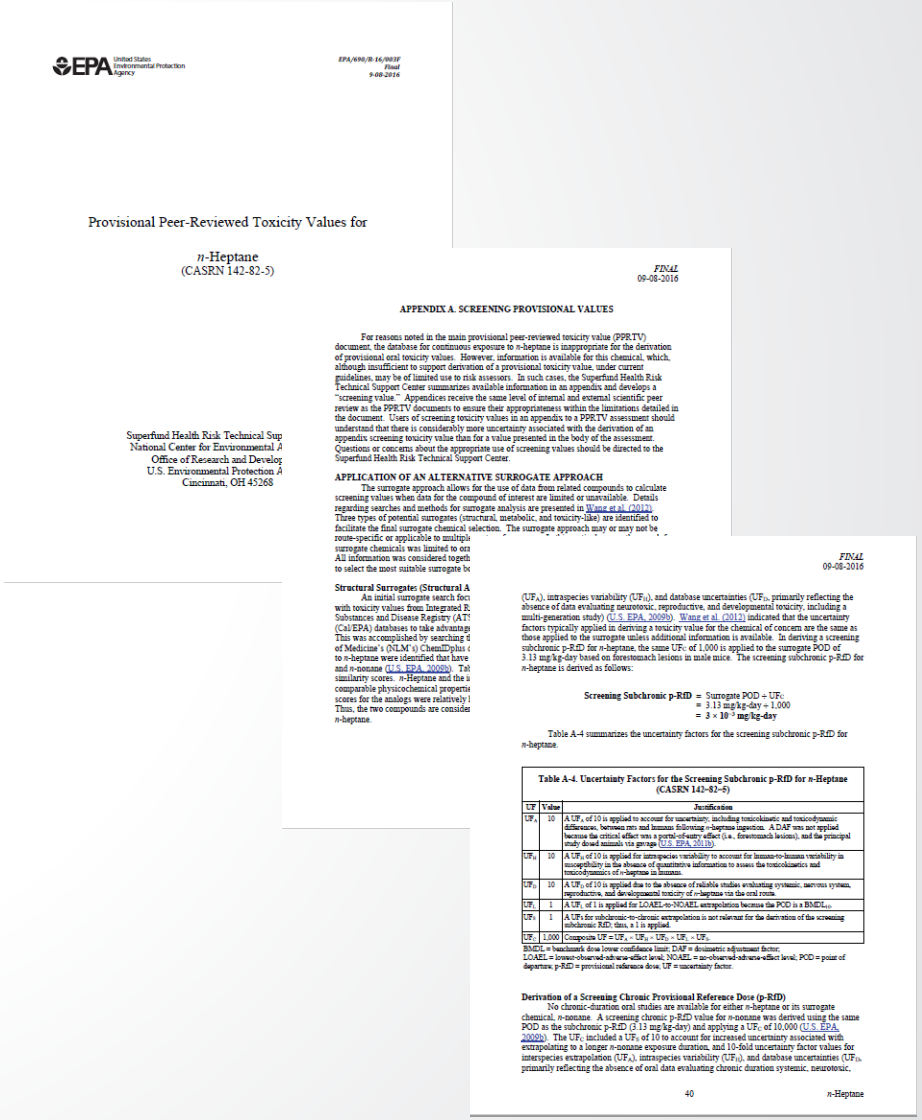
- Identify commonalities in toxicity (e.g., effect levels, target organs/tissues) and/or bioactivity between potential analogue(s) (with available toxicity values) and data-poor target chemical of concern.

Table A-3. Comparison of Available Repeated-Dose Oral Toxicity Data for *n*-Heptane (CASRN 142-82-5) and Structural Analogs

Chemical	*n*-Heptane	*n*-Hexane	*n*-Nonane
Structure			
CASRN	142-82-5	110-54-3	111-84-2
Repeat-dose toxicity—oral, subchronic			
POD (mg/kg-d)	NA	785	3.13
POD type	NA	LOAEL	$BMDL_{10}$
Subchronic UF_C	NA	3,000	1,000
Subchronic p-RfD (mg/kg-d)	NA	3×10^{-1}	3×10^{-3}
Critical effects	Irritative and proliferative forestomach lesions and potential effects in the liver, kidney, and adrenal glands at a dose of 2,860 mg/kg-d. Lack of neurotoxicity at doses up to 2,860 mg/kg-d based on histological evaluation (13-wk rat study).	Decreased MNCV associated with peripheral neuropathy	Proliferative forestomach lesions with varying degrees of hyperplasia and hyperkeratosis of the squamous epithelium
Other effects		Hind-limb paralysis accompanied by evidence of peripheral neuropathy and testicular effects based on histopathology at a dose of 2,843 mg/kg-d (90-d rat study)	Additional effects in principal study: histopathological lesions in the duodenum (rats) and rectum (rats and mice) at doses ≥1,000 mg/kg-d; nasal and pulmonary lesions, possibly due to aspiration (rats and mice). Increases in liver and lung weights at a dose of 5,000 mg/kg-d and dose-related increases in adrenal gland and ovary weights at doses ≥1,000 mg/kg-d. No significant neurohistopathology or neurobehavioral abnormalities reported in rats or mice at doses up to 5,000 mg/kg-d
Species	NA	Rat (M)	Mouse (M) and Rat (F)
Duration	NA	8 wk	90 d
Route (method)	NA	Oral (gavage)	Oral (gavage)

Evidence Integration and Synthesis: *n*-Nonane as selected analogue

- Similarity Context 1: high structural similarity to *n*-Heptane (>84%)
- Similarity Context 2: *n*-Nonane is metabolized *in vivo* similarly to *n*-Heptane (higher relative amounts of the 2- and 3-alcohol and γ-valerolactone metabolites formed, compared to the neurotoxic γ-diketone compounds from *n*-Hexane candidate analogue)
- Similarity Context 3: *n*-Nonane-induced proliferative forestomach lesions are similar to the lesions observed after oral *n*-Heptane exposure (as compared to unique *n*-Hexane-induced neurotoxicity)



EPA/690/R-16/003F
Final
9-08-2016

Provisional Peer-Reviewed Toxicity Values for

n-Heptane
(CASRN 142-82-5)

Superfund Health Risk Technical Sup
National Center for Environmental A
Office of Research and Develop
U.S. Environmental Protection A
Cincinnati, OH 45268

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09-08-2016

APPENDIX A. SCREENING PROVISIONAL VALUES

For reasons noted in the main provisional peer-reviewed toxicity value (PPRTV) document, the database for continuous exposure to *n*-heptane is inappropriate for the derivation of provisional oral toxicity values. However, information is available for this chemical, which, although insufficient to support derivation of a provisional toxicity value, under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in an appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the PPRTV documents to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there is considerably more uncertainty associated with the derivation of an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center.

APPLICATION OF AN ALTERNATIVE SURROGATE APPROACH

The surrogate approach allows for the use of data from related compounds to calculate screening values when data for the compound of interest are limited or unavailable. Details regarding searches and methods for surrogate analysis are presented in Wang et al. (2012). Three types of potential surrogates (structural, metabolic, and toxicity-like) are identified to facilitate the final surrogate chemical selection. The surrogate approach may or may not be route-specific or applicable to multipl
surrogate chemicals was limited to ora
All information was considered togeth
to select the most suitable surrogate be

Structural Surrogates (Structural A
An initial surrogate search foc
with toxicity values from Integrated R
Substances and Disease Registry (ATS
(Cal/EPA) databases to take advantag
This was accomplished by searching f
of Medicine's (NLM's) ChemIDplus
to *n*-heptane were identified that have
and *n*-nonane (U.S. EPA, 2009b). Tab
similarity scores. *n*-Heptane and the
comparable physicochemical propertie
scores for the analogs were relatively
Thus, the two compounds are consider
n-heptane.

FINAL
09-08-2016

(UF_A), intraspecies variability (UF_H), and database uncertainties (UF_D), primarily reflecting the absence of data evaluating neurotoxic, reproductive, and developmental toxicity, including a multi-generation study) (U.S. EPA, 2009b). Wang et al. (2012) indicated that the uncertainty factors typically applied in deriving a toxicity value for the chemical of concern are the same as those applied to the surrogate unless additional information is available. In deriving a screening subchronic p-RfD for *n*-heptane, the same UF_C of 1,000 is applied to the surrogate POD of 3.13 mg/kg-day based on forestomach lesions in male mice. The screening subchronic p-RfD for *n*-heptane is derived as follows:

$$\text{Screening Subchronic p-RfD} = \text{Surrogate POD} \div UF_C = 3.13\ \text{mg/kg-day} \div 1{,}000 = 3 \times 10^{-3}\ \text{mg/kg-day}$$

Table A-4 summarizes the uncertainty factors for the screening subchronic p-RfD for *n*-heptane.

Table A-4. Uncertainty Factors for the Screening Subchronic p-RfD for *n*-Heptane (CASRN 142-82-5)

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied to account for uncertainty, including toxicokinetic and toxicodynamic differences, between rats and humans following *n*-heptane ingestion. A DAF was not applied because the critical effect was a portal-of-entry effect (i.e., forestomach lesions), and the principal study dosed animals via gavage (U.S. EPA, 2011b).
UF_H	10	A UF_H of 10 is applied for intraspecies variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of *n*-heptane in humans.
UF_D	10	A UF_D of 10 is applied due to the absence of reliable studies evaluating systemic, nervous system, reproductive, and developmental toxicity of *n*-heptane via the oral route.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a $BMDL_{10}$.
UF_S	1	A UF_S for subchronic-to-chronic extrapolation is not relevant for the derivation of the screening subchronic RfD; thus, a 1 is applied.
UF_C	1,000	Composite $UF = UF_A \times UF_H \times UF_D \times UF_L \times UF_S$

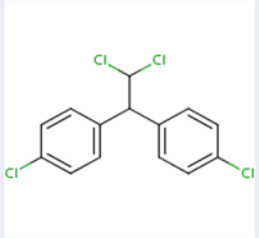
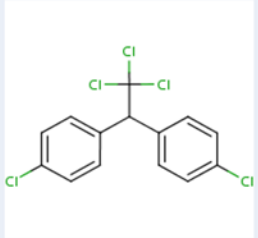
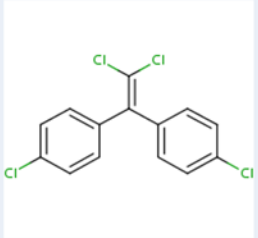
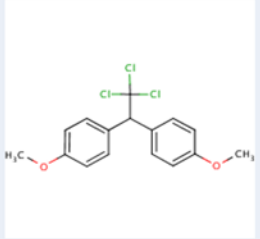
BMDL = benchmark dose lower confidence limit; DAF = dosimetric adjustment factor; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose; UF = uncertainty factor.

Derivation of a Screening Chronic Provisional Reference Dose (p-RfD)

No chronic-duration oral studies are available for either *n*-heptane or its surrogate chemical, *n*-nonane. A screening chronic p-RfD value for *n*-nonane was derived using the same POD as the subchronic p-RfD (3.13 mg/kg-day) and applying a UF_C of 10,000 (U.S. EPA, 2009b). The UF_C included a UF_S of 10 to account for increased uncertainty associated with extrapolating to a longer *n*-nonane exposure duration, and 10-fold uncertainty factor values for interspecies extrapolation (UF_A), intraspecies variability (UF_H), and database uncertainties (UF_D), primarily reflecting the absence of oral data evaluating chronic duration systemic, neurotoxic,

40 *n*-Heptane

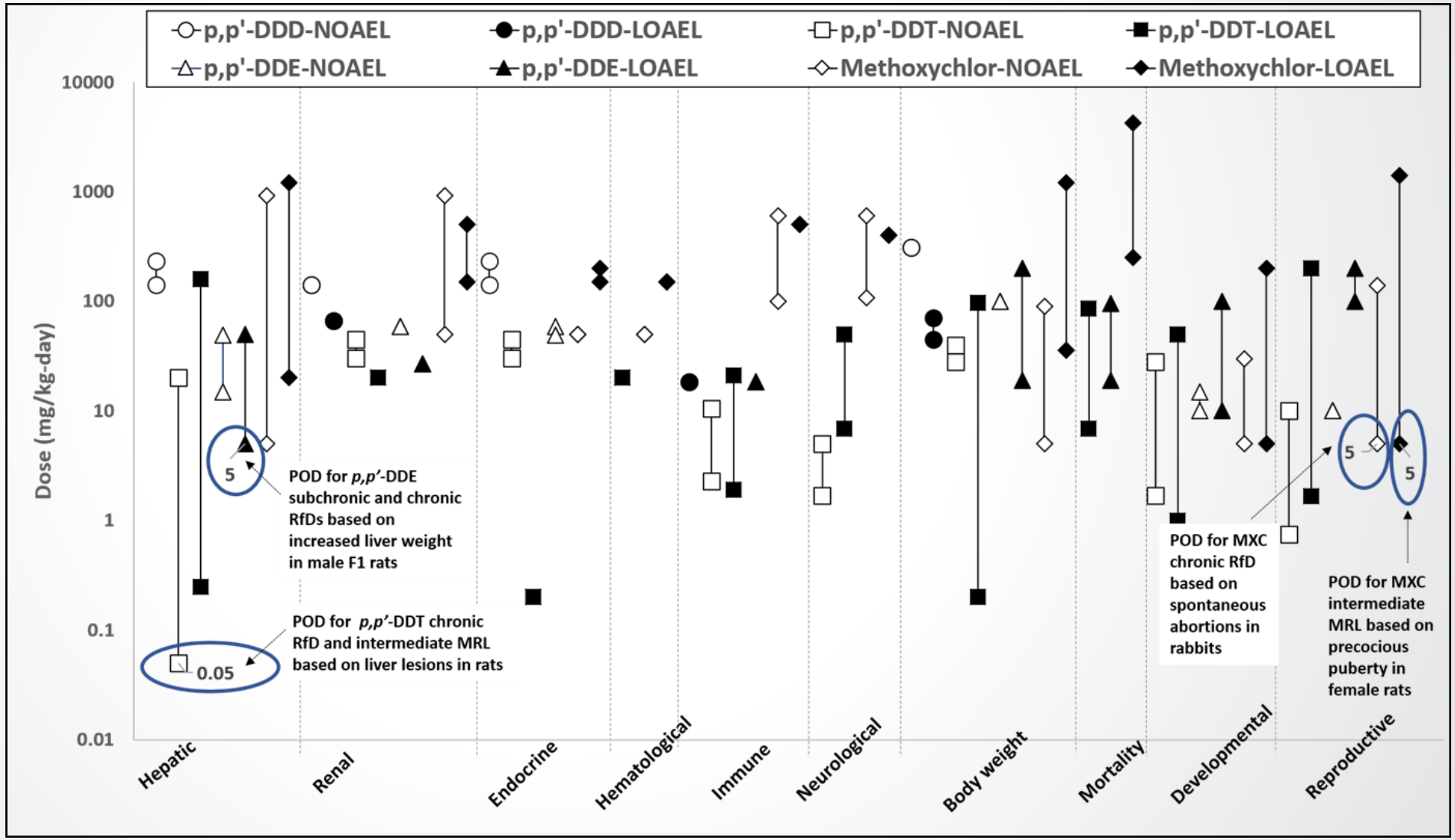
Identify Structural Analogues of p,p’-DDD

Table 1. Structural Analogues of p,p’-DDD				
	Target Chemical	Analogues[a]		
Name	p,p'-Dichlorodiphenyl dichloroethane (p,p’-DDD)	p,p'-Dichlorodiphenyl trichloroethane (p,p’-DDT)	p,p'-Dichlorodiphenyl dichloroethylene (p,p’-DDE)	p,p’-Dimethoxydiphenyl trichloroethane (Methoxychlor)
CASRN	72-54-8	50-29-3	72-55-9	72-43-5
Structure				
ChemIDplus similarity score (%)	100	77	67	65
DSSTox similarity score (%)	100	96	61	52

[a]Analogues represent a set of structurally similar chemicals identified using two publicly available similarity databases (ChemIDplus and DSSTOX) prefiltered on the basis of availability of health reference values for non-cancer oral toxicity from regulatory agencies, including ATSDR (2002a, b) and U.S. EPA (2017 b, c).

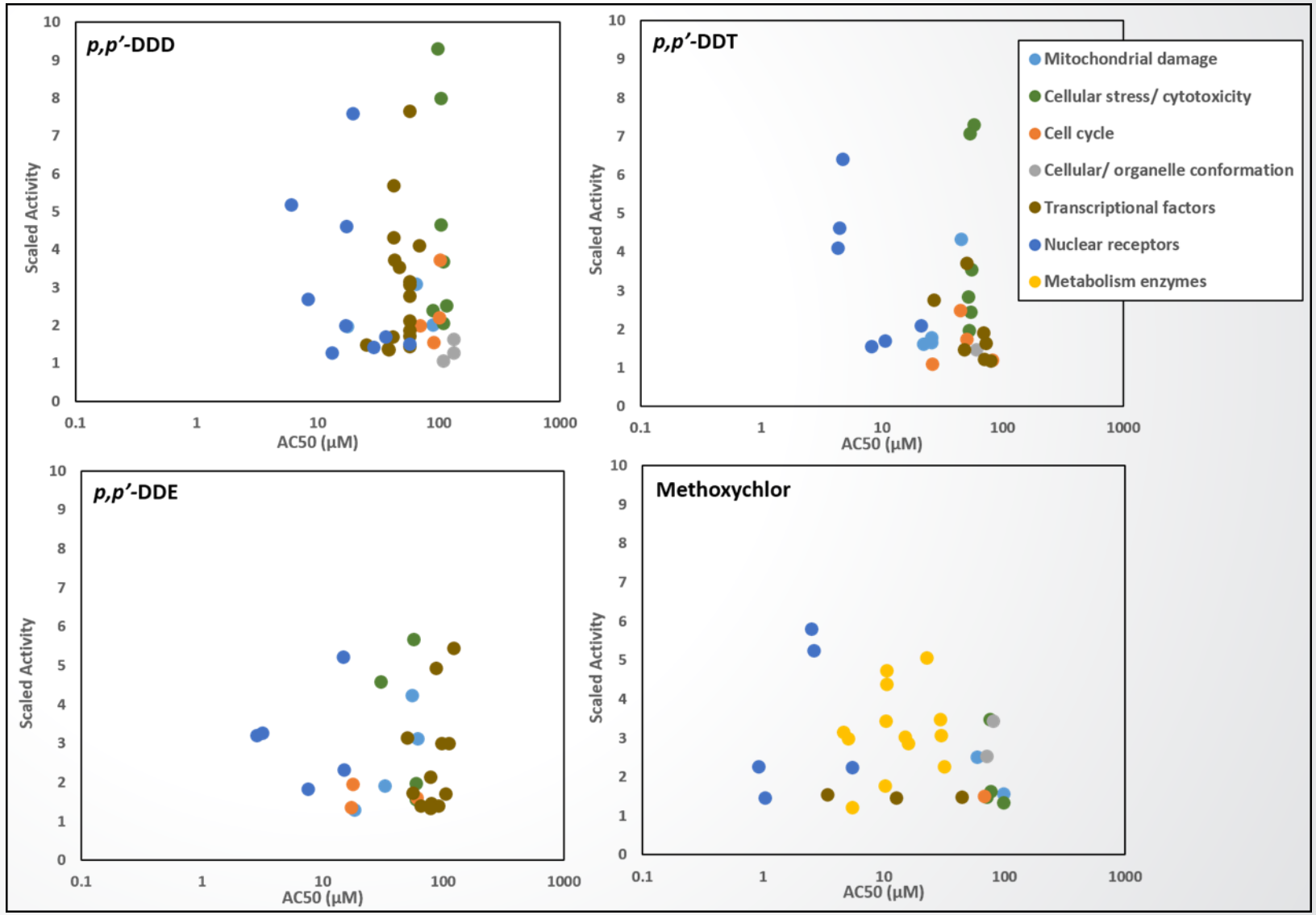
Putative Toxicity Targets for p,p'-DDD and Analogues

- Liver
- Reproductive



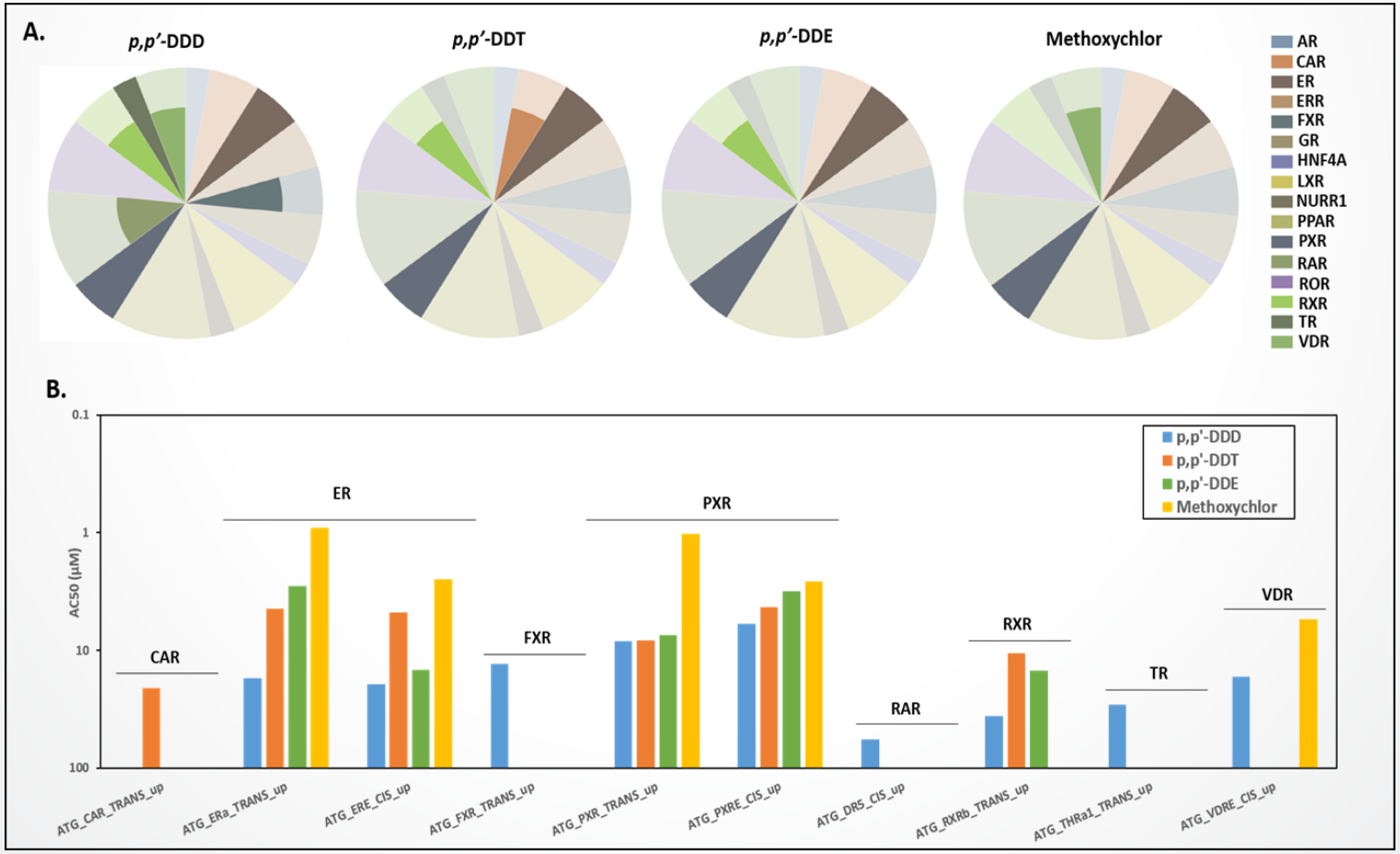
Analysis of Similarities in Bioactivity between p,p'-DDD and Analogues

- *p,p'*-DDD and analogues exhibit similarities in cell-specific responses and target gene pathways in *in vitro* ToxCast assays conducted in Human Liver Cells



Comparative receptor activation between p,p'-DDD and Analogues

- *p,p'*-DDD and analogues exhibit similar upregulation of Steroid/Xenobiotic-sensing Nuclear Receptors in *in vitro* ToxCast assays conducted in Hepatoma HepG2 Cells



Summary of Comparative ER/AR Bioactivity

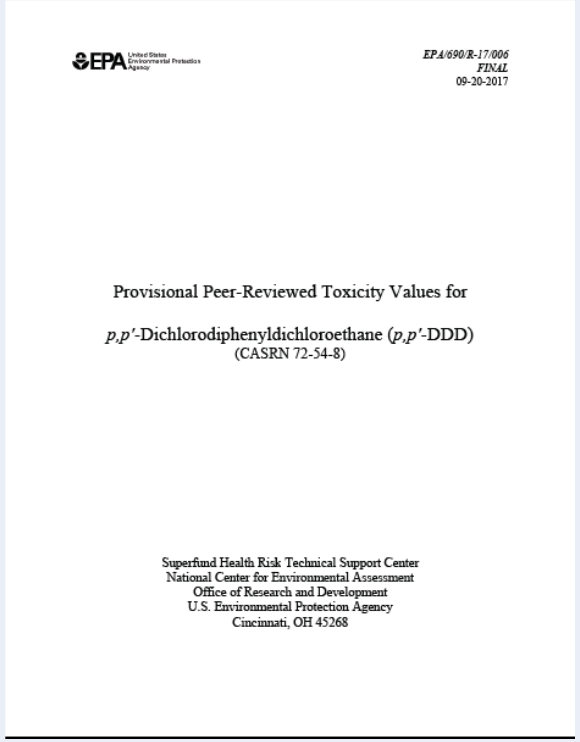
- p,p'-DDD and analogues exhibit similar estrogenic and anti-androgenic activities in *in vitro* ToxCast assays and model predictions for the ER and AR across multiple tissues and cell lines

Table 2. ToxCast Bioactivity Summary and Model Prediction Scores (AUC values) for ER and AR activities[a]

	p,p'-DDD	*p,p'*-DDT	*p,p'*-DDE	Methoxychlor
ER assays				
Active/Total Assays (%)	7/18 (39)	11/18 (61)	8/18 (44)	14/18 (78)
AC50 values (μM)	Range = 14.0 - 32.4 Median = 18.7	Range = 3.3 - 59.8 Median = 6.1	Range = 3.5 - 46.2 Median = 16.5	Range = 0.9 - 44.2 Median = 4.6
Agonist activity AUC value (95% CI)[b]	0.0715 (0.0342-0.0738)	0.190 (0.181-0.231)	0.0679 (0.0614-0.0963)	0.254 (0.247-0.260)
Antagonist activity AUC value (95% CI)	0	0	0	0
AR assays				
Active/Total Assays (%)	4/11 (36)	3/11 (27)	4/11 (36)	3/11 (27)
AC50 values (μM)	Range = 31.0 - 62.8 Median = 44.8	Range = 17.8 - 72.0 Median = 47.0	Range = 7.0 - 58.7 Median = 29.6	Range = 29.3 - 40.8 Median = 34.2
Agonist activity AUC value (95% CI)	0	0	0	0
Antagonist activity AUC value (95% CI)	0.0973 (0.0649-0.124)	0.0642 (0.0318-0.108)	0.251 (0.234-0.291)	0.0429 (0.0364-0.0465)

[a]Data were sourced from Judson et al. (2015) and Kleinstreuer et al. (2016). [b] 95% CI for the ER activity model were sourced from a subsequent publication to the Judson et al., (2015) study (Watt and Judson, 2018).
Abbreviations: AUC = area under the curve score ranging from 0−1. An AUC value of 0 indicates that the chemical is inactive; CI = confidence interval.

Table 3. Evidence Integration Summary and Conclusion

Similarity Context	Summary of Findings	Evidence Integration conclusions
Structure and physicochemical properties	• *p,p'*-DDD and identified analogues (*p,p'*-DDT and *p,p'*-DDE and methoxychlor) demonstrate similarities in basic structural features (chlorinated diphenylalkane structure) • *p,p'*-DDT and *p,p'*-DDE also share key functional groups (*p,p'*-chlorine substituents) and physicochemical properties important for bioavailability (lipophilicity and low BCF values) with *p,p'*-DDD	• *p,p'*-DDT is selected as a suitable source analogue for the assessment of non-cancer oral toxicity of *p,p'*-DDD based largely on toxicokinetic similarities, with supportive information from *in vivo* toxicity testing, structural similarity evaluations and *in vitro* bioactivity from HTS assays
Toxicokinetics	• *p,p'*-DDT is a metabolic precursor of *p,p'*-DDD and both chemicals show similarities in toxicokinetics (Absorption, Distribution and Metabolism [ADME]) in humans and experimental animal models (preferential partitioning into fat, similar metabolism and excretion pathways and prolonged elimination rates) • Other analogues demonstrate differences in ADME in comparison to the target. *p,p'*-DDE is less metabolically active; methoxychlor is metabolized differently and appears to be less bioaccumulative	 EPA United States Environmental Protection Agency EPA/690/R-17/006 FINAL 09-20-2017 Provisional Peer-Reviewed Toxicity Values for *p,p'*-Dichlorodiphenyldichloroethane (*p,p'*-DDD) (CASRN 72-54-8) Superfund Health Risk Technical Support Center National Center for Environmental Assessment Office of Research and Development U.S. Environmental Protection Agency Cincinnati, OH 45268
Toxicodynamic	• Consistency and coherence across health effects in experimental animals for non-cancer oral toxicity among the analogues point to putative toxicity targets for *p,p'*-DDD (primarily liver and reproductive toxicity) • Similarities in *in vitro* bioactivity profiles from ToxCast assays between the target and analogues with respect to cell-specific responses and target gene pathways provide mechanistic plausibility for the liver and reproductive effects associated with this group of chemicals	

Strengths, Limitations, and Challenges of Expert-Driven Read Across

Strengths

- Provides an opportunity to develop human health values where none would be possible based on traditional risk assessment approaches and practice
- Approach is flexible, nimble, and evergreen

Limitations

- Is not high-throughput per se
- Current approach is dependent on analogue space with existent health values

Challenges

- Toxicokinetics (e.g., metabolism) is a critical similarity context that is often highly data-poor
- Increase throughput…

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