Health Canada Santé Canada

Canada

Integrated Approaches to Testing and Assessment under Canada's Chemicals Management Plan: Proposed Phenols Case Study for Review Cycle #3

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Ottawa, ON, Canada

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YOUR HEALTH AND SAFETY... OUR PRIORITY.

Project Team

Health Canada Santé Canada

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The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

Collaborative Case Study - Motivation

Health Canada (HC) and U.S. EPA Collaboration

- Many remaining priorities for assessment under the Chemicals Management Plan (CMP) are considered data poor.

- Health Canada (HC) has an interest in establishing proof of concept for the integration of new approach methodologies into risk assessments and priority setting moving forward.

- The U.S. EPA National Center for Computational Toxicology (NCCT) has been actively exploring different contexts where HTS data can been effectively exploited, including:
  - Screening and prioritization;
  - Endocrine Disruption Screening Program (EDSP);
  - Systematic development and evaluation of chemical categories and their associated read-across.

- A collaborative case study is under development in order to gain experience for moving this methodology forward for decision making both broadly as well as more specifically within Canada's CMP.

Collaborative Case Study – Objectives/Elements

Purpose of Case Study

- The purpose of the case study is to provide insights to further develop guidance related to grouping and IATA approaches.

- Illustrative purposed only. Not to be interpreted as a regulatory decision by HC or EPA

The proposed case study will investigate several key elements:

1) Utility of analogues, QSARs and HTS data to support IATA-based evaluation of estrogenicity:

- Examine estrogenicity at the EDSP tier 1 level
- Systematic approaches for identifying source analogues with empirical data
- Support preliminary examination of estrogenicity for CMP Phenols

2) Comparison of *in vitro* bioactivity against effect levels derived from traditional animal studies

- Where available use *in vitro* bioactivity data, in concert with high-throughput toxicokinetic information and reverse dosimetry, to estimate oral equivalent dose (OED)
- Present and compare OEDs with traditional *in vivo* effect levels

Collaborative Case Study - CMP Phenols

- There are multiple phenols to be addressed under the third phase of the CMP
- A number of these substances are considered to be data poor and lack traditional toxicity data
- Certain phenols:
    - are high volume substances of widespread use,
    - have the potential for direct exposure through consumer products.
- A human health related concern with phenols is that they can have the potential to be estrogenic.

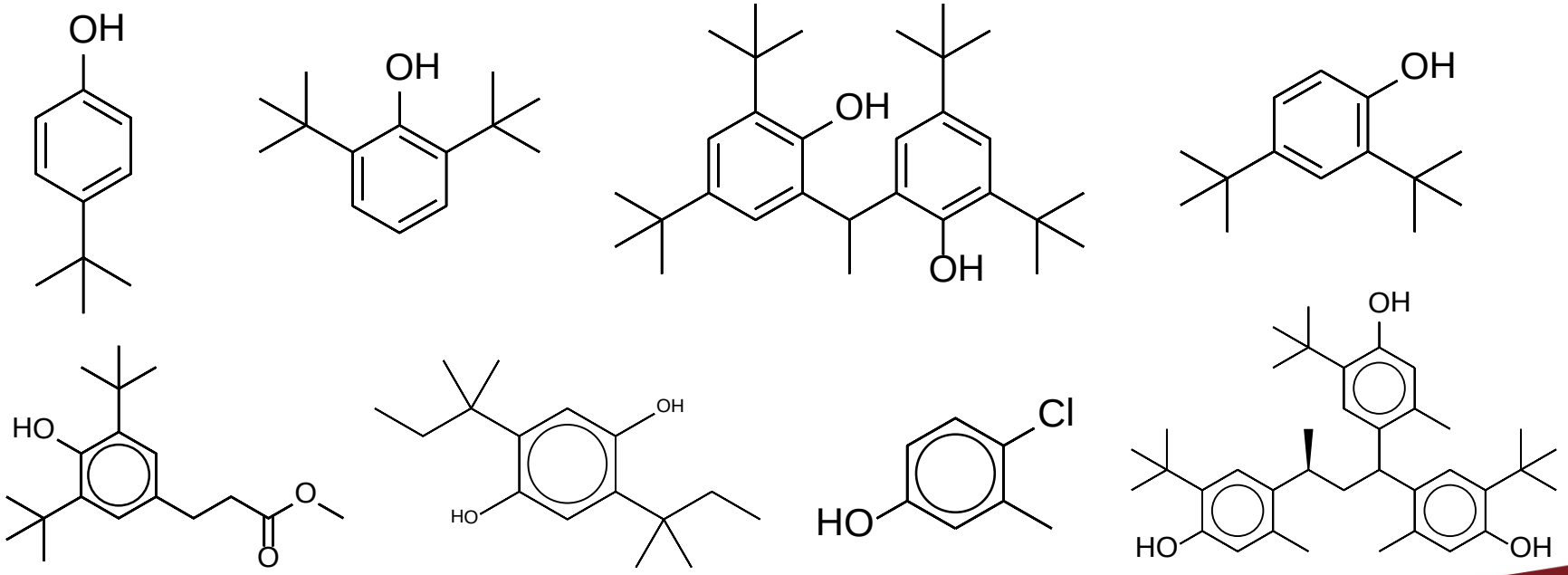
Collaborative Case Study - CMP Phenols

Hypothesis

- The group of CMP phenolic compounds contain substituents at various positions relative to the hydroxyl group.

- The type of substituent(s) and position(s) relative to the hydroxyl group is hypothesized to have an impact on the estrogenic potential and potency.

Examples of CMP Phenols



Analogue Selection – Analogue Sources

- Investigate computational approaches for identifying structurally related phenols with estrogenicity data.
- Analogue search will be conducted using:

1) Collaborative Estrogen Receptor Activity Prediction Project (CERAPP) evaluation dataset[1]

- ➢ High quality QSAR-ready dataset
  - Structure curation and standardization
  - Experimental data collected and cleaned
- ➢ ER pathway *in vitro* literature data reviewed and substances categorized
  - Data sources: Tox21; FDA EDKB; METI DB; ChEMBL DB
  - ER Binding
  - ER Agonist – reporter gene / transcriptional activation
  - ER Antagonist – reporter gene / transcriptional activation

2) U.S. EPA EDSP Program Data Availability List

- Uterotrophic
- Pubertal Female
- Fish short-term reproduction assay (FSTRA)

3) NICEATM UT Database of Guideline Studies (Kleinstreuer et al. 2015)

[1] Available from: https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data

Analogue Selection – Analogue Search Methodology

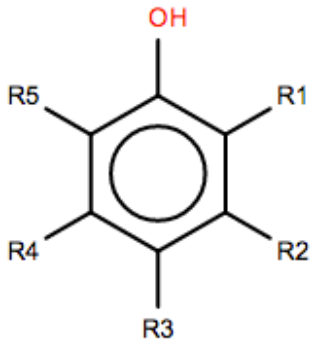
Hypothesis

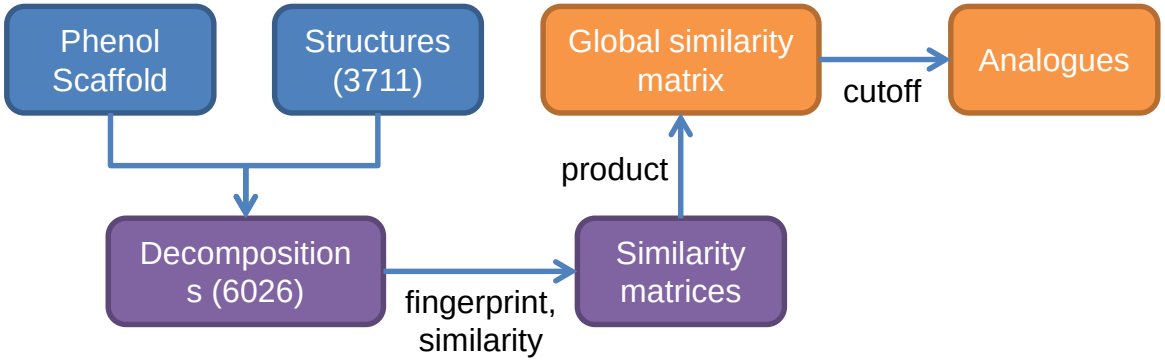
- The type of substituent(s) and position(s) relative to the hydroxyl group is hypothesized to have an impact on the estrogenic potential and potency.

- A custom similarity metric is being examined that includes substituent position and chemical identity
  - Using a phenol scaffold, we decomposed the CMP compounds and analogue source datasets into R-positions and set of fragments at each position.
  - For each R-position, we fingerprinted (Indigo) the fragments and calculated a pair-wise similarity matrix (including a penalty for different substitution patterns).
  - The global similarity matrix was taken as the product of the individual R-position similarity matrices.
  - The 10 nearest neighbors for each CMP substance were used to form the analogue set for each target.
  - The fingerprinting methods formed the preliminary basis for analogue selection/ group formation for each CMP target phenol.

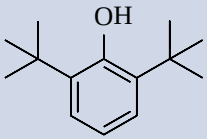
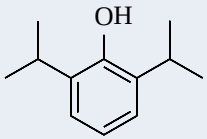
Analogue Selection – Analogue Search Methodology

Analogue Selection – Methods



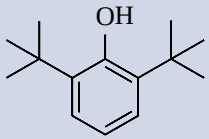
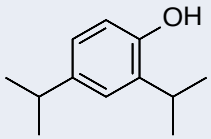


Identical substitution pattern

	R1	R2	R3	R4	R5
	*t*-butyl	H	H	H	*t*-butyl
	*i*-propyl	H	H	H	*i*-propyl
similarity	**0.75**	**1.0**	**1.0**	**1.0**	**0.75**

Global similarity = 0.75 x 1.0 x 1.0 x 1.0 x 0.75 = **0.5625**

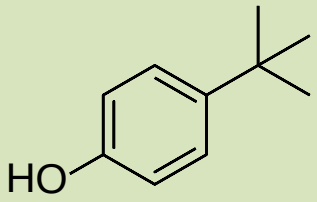
Different substitution pattern

	R1	R2	R3	R4	R5
	*t*-butyl	H	H	H	*t*-butyl
	*i*-propyl	H	*i*-propyl	H	H
similarity	**0.75**	**1.0**	**0.0**	**1.0**	**0.0**

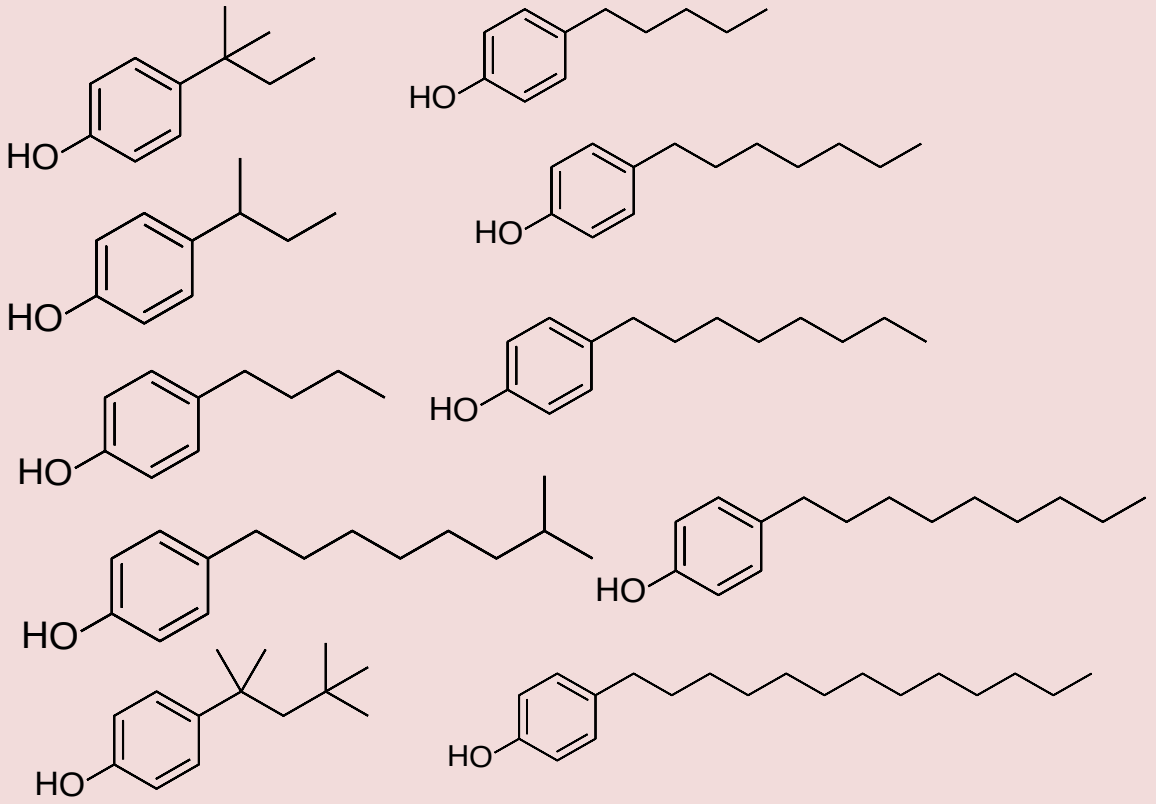
Global similarity = 0.75 x 1.0 x 0.0 x 1.0 x 0.0 = **0.0**

Analogue Selection – Example of Potential Analogues

CMP Target



Closest CERAPP analogues with *in vitro* data, based on substituent position and identity



IATA Based Hazard Identification

ER Pathway Data Collection

- Collected empirical data and modelling results related to the ER Pathway for the CMP target and potential analogues.
- Data matrices (OECD IATA Template) will be populated

- *In vivo* data
  - Uterotrophic (UT) Assay
    - Source: NICEATM UT Database of Guideline Studies (Kleinstreuer et al. 2016)
  - Female Pubertal Assay
    - Source: EPA Database of Studies (under development)

- Predictions and Alert Profilers
  - CERAPP Consensus Models
  - EPA rtER Expert System (within the OECD Toolbox)
  - Commercial Software: Derek Nexus; ACD Percepta; OASIS TIMES

IATA Based Hazard Identification

ER Pathway Data Collection

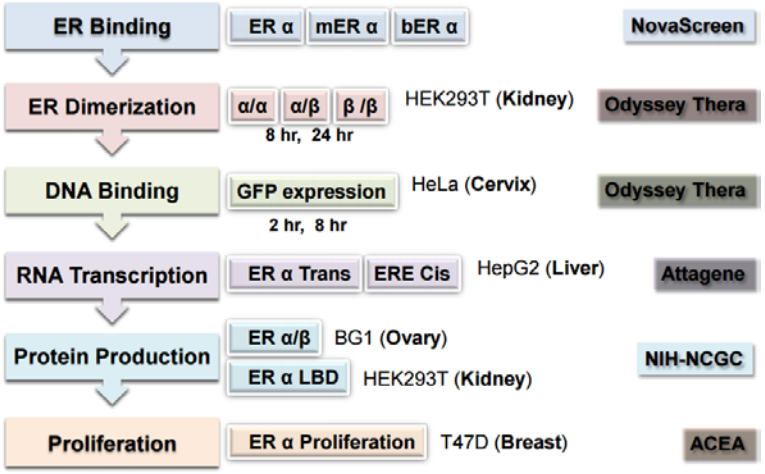
In vitro data

- ToxCast and Tox21 assays related to the ER pathway
  -Source: EPA EDSP21 Dashboard (http://actor.epa.gov/edsp21/)
- CERAPP categorization result based on literature review
- EPA ToxCast ER Pathway AUC Score (Agonist and Antagonist)

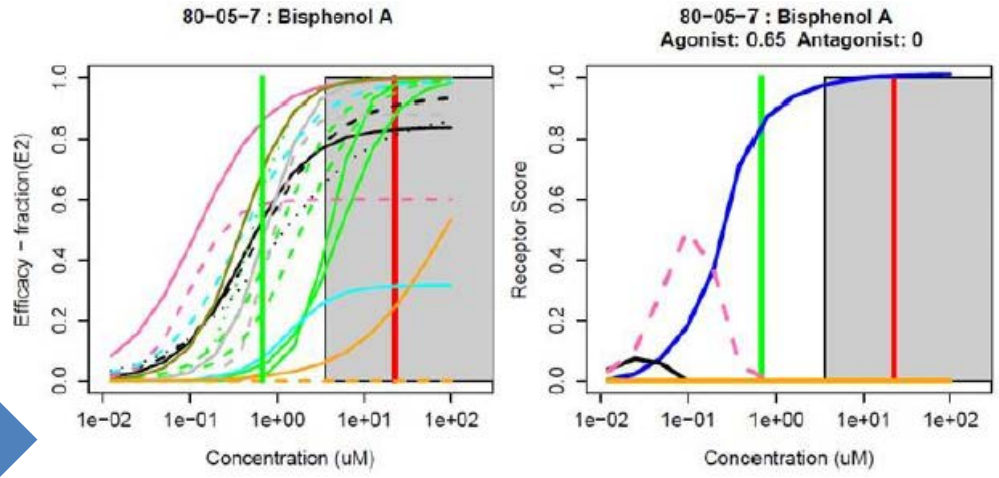
- HTS *in vitro* assays have been incorporated into the recent development of the ToxCast based Estrogen Receptor (ER) Bioactivity model for use in the US EPA EDSP Program

- The results from all the ToxCast ER assays are integrated into a computational model that can discriminate bioactivity from assay specific interference and responses related to cytotoxicity

- For each chemical, the model outputs a score (Area Under the Curve –AUC) ranging from 0 to 1 (bioactivity for 17β-estradiol))



(Casey W. 2014. Presentation NTP Board of Scientific Counselors (BSC) Meeting)

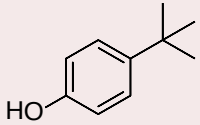
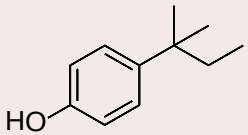
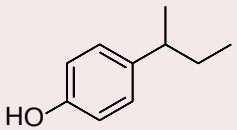
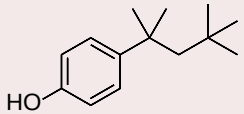


(Judson R. 2015. Presentation ICCA-LRI / EPA Workshop. New Orleans)

IATA Based Hazard Identification

Selection of Data Collected in Data Matrix

Example of active ER Pathway agonist CMP phenol and analogues
Non-Hindered Phenol (para-substituted)

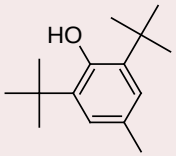
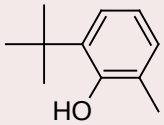
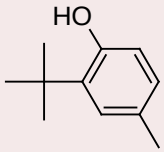
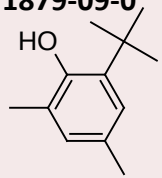
	98-54-4  **CMP Target**				…
Uterotrophic Assay NICEATM DB	**Active** LEL - 100 mg/kg/day Result: 1.3 fold increase s.c. over 3 days Crj:CD(SD) Rat (PND 20)	ND	ND	**Active** LEL - 200 mg/kg/day Result: 283% of control s.c. over 3 days SD Rat (PND 21)	…
CERAPP *in vitro* literature data	Binding: **Active** (Very Weak) Agonist: ND Antagonist: Inactive	Binding: **Active** (Weak) Agonist: ND Antagonist: Inactive	Binding: **Active** (ND) Agonist: ND Antagonist: Inactive	Binding: **Active** (Weak) Agonist: **Active** (Moderate) Antagonist: Inactive	…
ToxCast ER AUC Score	Agonist: 0.161 (**Active**) Antagonist: 0 (Inactive)	Agonist: 0.282 (**Active**) Antagonist: 0 (Inactive)	Agonist: 0.163 (**Active**) Antagonist: 0 (Inactive)	Agonist: 0.393 (**Active**) Antagonist: 0 (Inactive)	…
CERAPP Consensus QSAR	Binding: **Active** (Weak) Agonist: **Active** (Very Weak) Antagonist: Active (Strong)	Binding: **Active** (Weak) Agonist: **Active** (Weak) Antagonist: Active (Strong)	Binding: **Active** (Weak) Agonist: **Active** (Weak) Antagonist: Active (Strong)	Binding: **Active** (Weak) Agonist: **Active** (Weak) Antagonist: Active (Strong)	…
Other QSAR[a]	Binding: **Active** (Weak) (3/4) Derek Nexus: No Alert	Binding: **Active** (Weak) (3/4) Derek Nexus: No Alert	Binding: **Active** (Weak) (4/4)	Binding: **Active** (Moderate / Strong) (4/4)	…

[a] Other QSAR – Oasis TIMES, ACD Percepta, Derek Nexus; EPA rtER

IATA Based Hazard Identification

Selection of Data Collected in Data Matrix

Example of non-active ER pathway CMP phenol and analogues
phenol (di-ortho-substituted) [*Conflicting literature data on CMP phenol*]

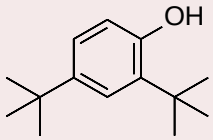
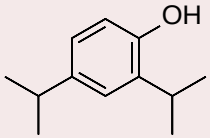
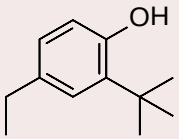
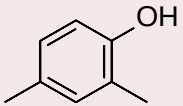
	128-37-0  **CMP Target**	**2219-82-1** 	**2409-55-4** 	**1879-09-0** 	…
Uterotrophic Assay NICEATM DB	ND	ND	ND	ND	…
CERAPP *in vitro* literature data	Binding: **Active** Agonist: ND Antagonist: Inactive	Binding: **Inactive** Agonist: **Inactive** Antagonist: **Inactive**	Binding: **Inactive** Agonist: **Inactive** Antagonist: **Inactive**	Binding: **Inactive** Agonist: ND Antagonist: ND	…
ToxCast ER AUC Score	Agonist: 0 (**Inactive**) Antagonist: 0 (**Inactive**)	Agonist: 0 (**Inactive**) Antagonist: 0 (**Inactive**)	Agonist: 0 (**Inactive**) Antagonist: 0.0164 **(inclsv.)**	Agonist: 0 (**Inactive**) Antagonist: 0 (**Inactive**)	…
CERAPP Consensus QSAR	Binding: **Inactive** Agonist: **Inactive** Antagonist: **Inactive**	Binding: **Inactive** Agonist: **Inactive** Antagonist: **Inactive**	Binding: **Inactive** Agonist: **Inactive** Antagonist: **Inactive**	Binding: **Inactive** Agonist: **Inactive** Antagonist: **Inactive**	…
Other QSAR[a]	Binding: **Inactive** (2/3) OECD TIMES: **Weakly active (due to metabolite)** EPA rtER - **Inactive** Derek Nexus – **No ER alerts**	Binding: **Inactive** (3/3) OECD TIMES: **Inactive** EPA rtER - **Inactive** Derek Nexus – **No ER alerts**	Binding: **Inactive** (2/3) OASIS TIMES - **Inactive** EPA rtER – **V weakly active** Derek Nexus – **No ER alerts**	Binding: **Inactive** (3/3) OECD TIMES - **Inactive** EPA rtER – **Inactive** Derek Nexus – **No ER alerts**	…

[a] Other QSAR – Oasis TIMES, Derek Nexus; EPA rtER

IATA Based Hazard Identification

Selection of Data Collected in Data Matrix

Example of non-active ER pathway CMP phenol and analogues
Partial Hindered Phenol (mono-ortho-substituted)

	96-76-4  **CMP Target**	**2934-05-6** 	**96-70-8** 	**105-67-9** 	…
Uterotrophic Assay NICEATM DB	**Inactive** Max Dose - 1000 mg/kg/day s.c. over 3 days Crj:CD(SD) Rat (PND 20)	ND	ND	ND	…
CERAPP *in vitro* literature data	Binding: Active (V. Weak) Agonist: **Inactive** Antagonist: **ND**	Binding: **Inactive** Agonist: **Inactive** Antagonist: **Inactive**	Binding: Active (Weak) Agonist: **ND** Antagonist: **Inactive**	Binding: Active (ND) Agonist: **ND** Antagonist: **Inactive**	…
ToxCast ER AUC Score	Agonist: 0 (**Inactive**) Antagonist: 0 (**Inactive**)	Agonist: 0 (**Inactive**) Antagonist: 0 (**Inactive**)	Agonist: 0.019 (**Inclsv.**) Antagonist: 0 (**Inactive**)	Agonist: 0 (**Inactive**) Antagonist: 0 (**Inactive**)	…
CERAPP Consensus QSAR	Binding: **Inactive** Agonist: **Inactive** Antagonist: **Inactive**	Binding: **Inactive** Agonist: **Inactive** Antagonist: **Inactive**	Binding: **Inactive** Agonist: **Inactive** Antagonist: **Inactive**	Binding: **Inactive** Agonist: **Inactive** Antagonist: **Inactive**	…
Other QSAR[a]	Binding: **Inactive** (4/4)	Binding: **Inactive** (3/4) ACD Percepta: Active	Binding: OASIS TIMES - **Inactive** Derek Nexus - **No Alert** ACD Percepta - Active EPA rtER - Active	Binding: **Inactive** (3/4) EPA rtER – Active	…

[a] Other QSAR – Oasis TIMES, ACD Percepta, Derek Nexus; EPA rtER

Comparison of *in vitro* bioactivity against effect levels derived from traditional animal studies

Data Availability for Deriving the Oral Equivalent Dose (OED) for CMP Substituted Phenols

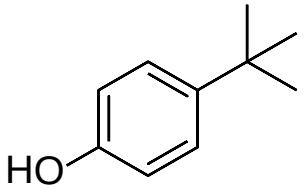
- Derivation of the OED requires:
  - HTS assay data (e.g. ToxCast data)
  - High-throughput toxicokinetics (HTTK) data
    - *in vitro* serum protein binding
    - hepatic microsomal clearance

- Three CMP substituted phenols have the required data to derive a OED

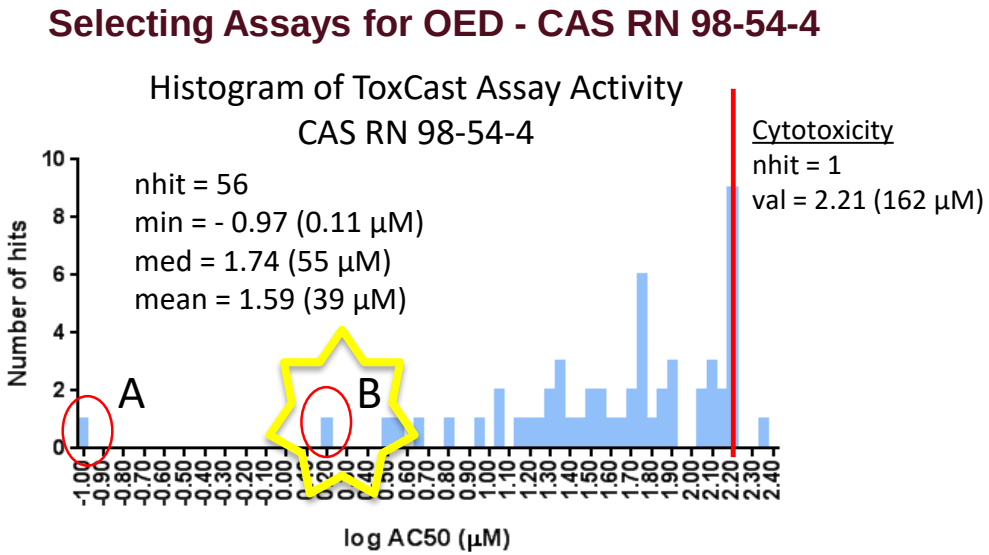
- An additional 10 have ToxCast/Tox21 HTS data but no HTTK data

- Health Canada is generating HTTK data for these additional substances
  - IVIVE analysis using the data in progress

- The following slides provide an example of the OED approach for one CMP substituted phenol
  - CAS RN 98-54-4

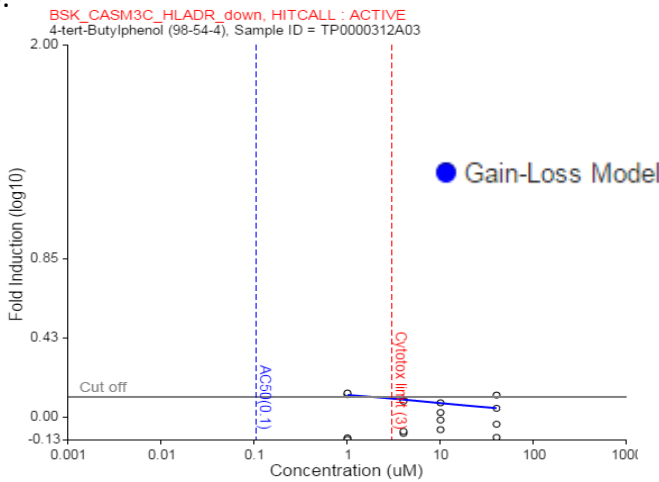


Comparison of *in vitro* bioactivity against effect levels derived from traditional animal studies



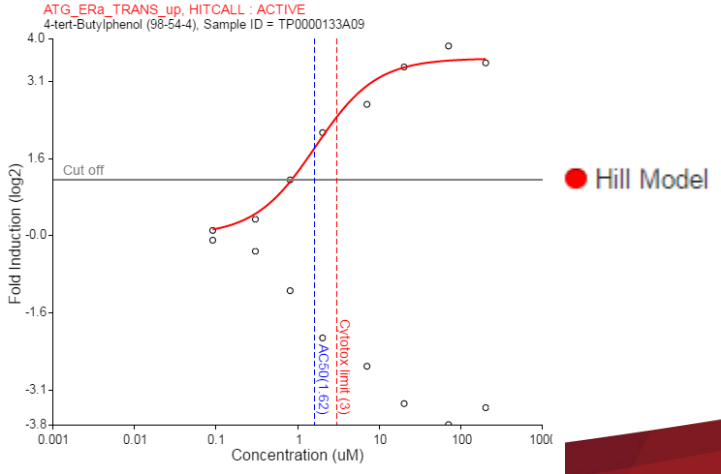
A –Assay with lowest AC50 across all activity

Considered **not suitable**: numerous flags and the result is deemed not reliable (i.e. Hit-call potentially confounded by overfitting; borderline active; only one conc above baseline, active).



B –Assay with lowest AC50 for ER pathway

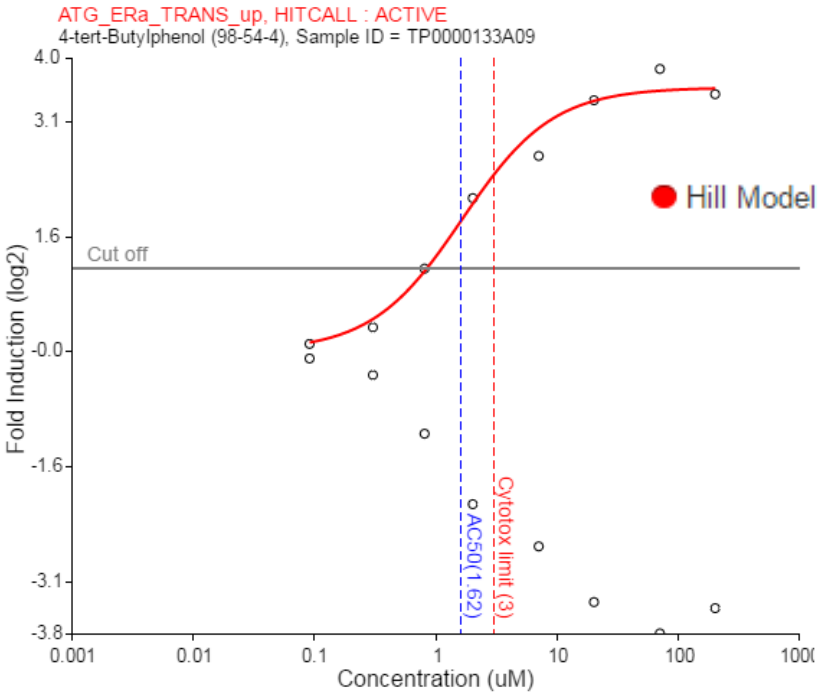
Considered **reliable**: no flags for this assay. The activity in the assay is outside the cytotoxicity region; related to the ER Pathway (effect of concern).



Comparison of *in vitro* bioactivity against effect levels derived from traditional animal studies

Selecting Assays for OED - CAS RN 98-54-4

B –Assay with lowest AC50 for ER pathway

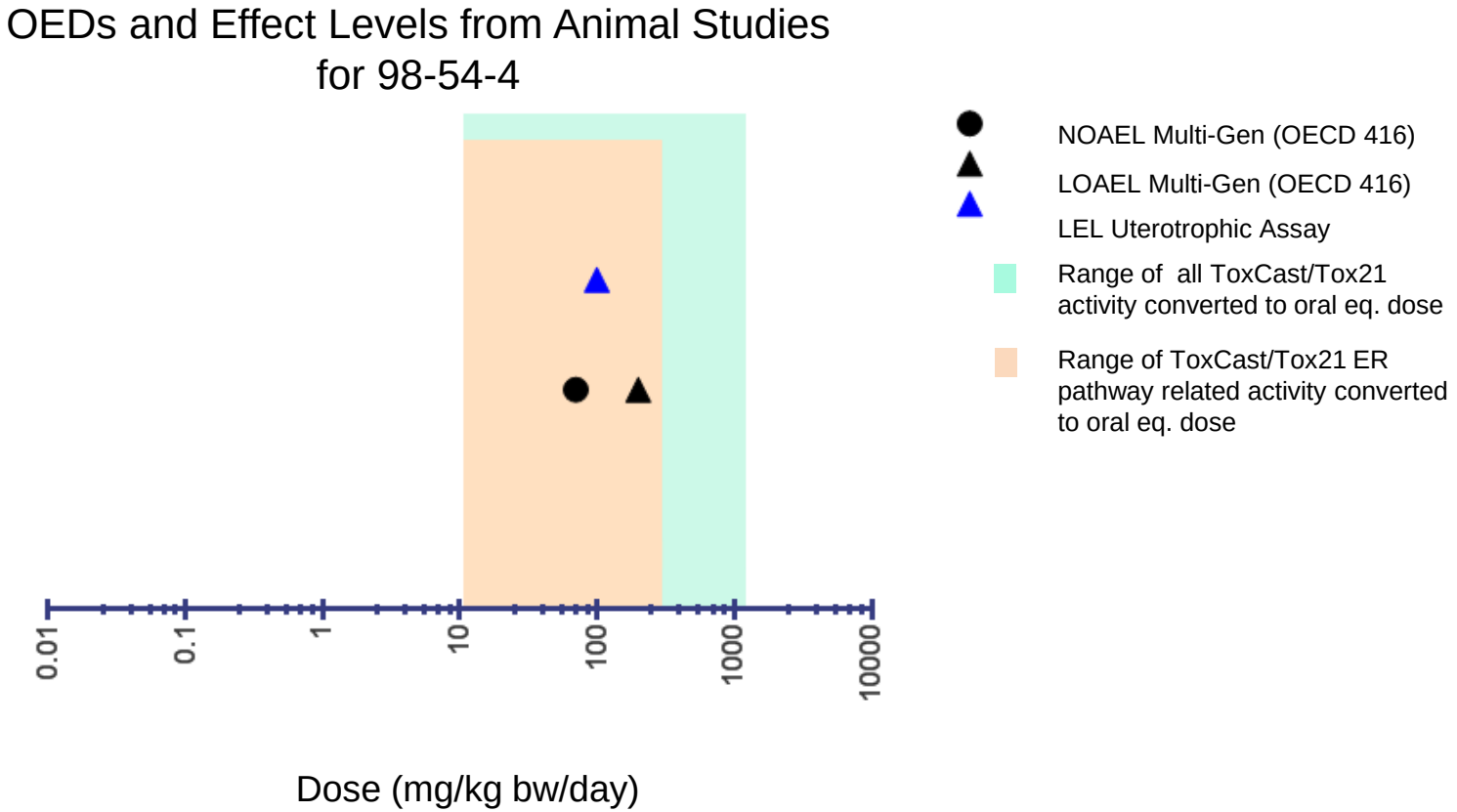


ToxCast/Tox21 AC_{50} Values Converted to Administered Dose Equivalents (ADE)		
	AC50 (µM)	ADE (mg/kg bw/day)
Lowest Activity for ER Pathway Assay	1.63[1]	11.22
Range of Activity for ER Pathway Assays	1.63 – 58.05	11.22 – 291.01

[1] Based on ER pathway assay with lowest AC50 (ATG_ERa_Trans_up)

1) Bioactivity as Points of Departure (Source: EPA ToxCast MySQL and R package – invtrodb_v2 / tcpl_1.0[1])
2) HTTK-based Conversion to Administered Dose Equivalent – (Source: Wambaugh HTTK R package[2] / Wetmore et al. 2012)

Comparison of *in vitro* bioactivity against effect levels derived from traditional animal studies



Study Type	Effect Levels (mg/kg bw/day)
Uterotrophic Assay (NICEATM DB) Immature Rat (Kleinstreuer et al. 2015)	LEL - 100 mg/kg/day Result: 1.3 fold increase s.c. over 3 days Crj:CD(SD) Rat
Sprague-Dawley rats; 2-generation study (OECD 416); oral via diet; 0, 800, 2500 and 7500 ppm corresponding to 0, 70, 200 and 600 mg/kg bw/day (EU RAR 2008)	NOAEL/LOAEL: 70/200 Increased vaginal epithelium atrophy and reduced relative weight of ovaries and adrenal glands in females at 200 mg/kg bw/day

Summary of Early Findings

IATA Based Hazard Characterization

- Integrating the analogue approach along with *in vitro* HTS data and (Q)SAR predictions show promise in facilitating an IATA-based hazard characterisation for estrogenicity of CMP phenols.
  - ToxCast ER Pathway AUC Scores agree with other tier 1 tests (e.g. Uterotrophic Assay) where examined
  - ToxCast ER Pathway AUC Scores discriminate activity between hindered and non-hindered phenols where examined
  - QSAR results for some models are mixed for CMP phenols and respective analogues and do not always agree with empirical data

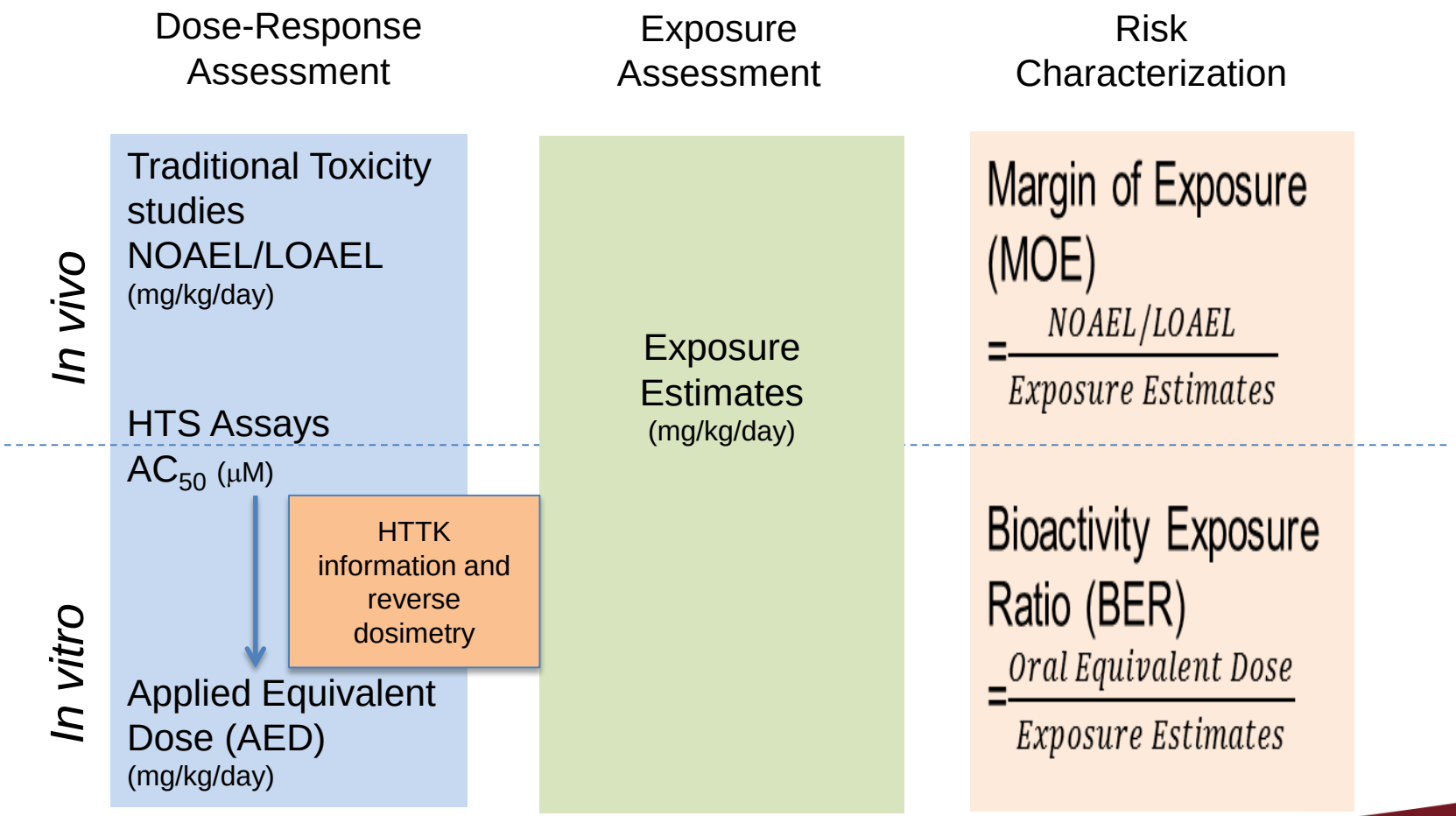
Comparison of in vitro bioactivity against effect levels derived from traditional animal studies

- Compare in vitro bioactivity-derived OEDs and PODs from traditional in vivo studies
- Early analysis suggest that OEDs based on the most sensitive assays may provide a conservative estimate of PODs from animal studies and could be useful for screening level assessments under the CMP

Annex

Exploring HTS for Priority Setting and Assessment

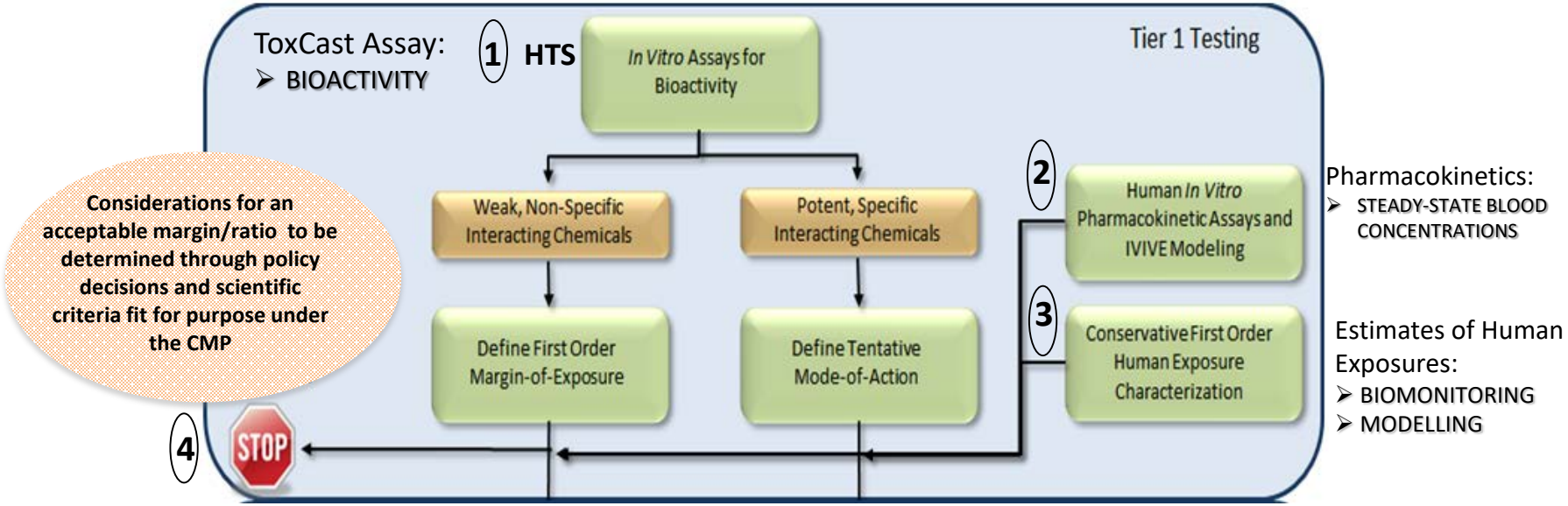
HTS data to predict potential level of concern for human health effects in priority setting or risk assessment: a Bioactivity Exposure Ratio (BER) approach



Part 1: Exploring HTS for Priority Setting and Assessment

Deriving the Bioactivity Exposure Ratio (BER)

HTS as a Basis for Tier 1 Decision-Making



Adapted from Thomas RS et al…….Yauk CL, Nong A (2013). Incorporating new technologies into toxicity testing and risk assessment: moving from 21st century vision to a data-driven framework. Toxicol Sci. 136(1):4-18.

Risk Assessment under the CMP

Risk Assessment Toolbox

Type 1 Approach

- Addresses the substance/group with a science-based policy response
- Used when regulatory assessment conclusion under s.64 of CEPA 1999 is not suitable
- Examples include: Referring to a better placed program (e.g., foods); documentation of previous action under *CEPA 1999*

Type 2 Approach

- Addresses substances using a broad-based approach, often based on low potential for exposure and conservative scenarios
- Substances do not meet criteria under s.64
- Examples include: Rapid Screening; Threshold of Toxicological Concern type approaches

Low

Level of Complexity

High

Type 3 Approach

Type 3-1

- Addresses the substance/group with a reduced amount of effort for streamlined hazard and/or exposure analysis
- Examples include: Use of international hazard characterizations; use of biomonitoring data; qualitative assessment

Type 3-2

- Substance/group requires de novo risk assessment

Type 3-3

- A complex assessment is required for the substance/group that may require cumulative assessment approaches

RM actions for those meeting s.64; additional information gathering and source attribution may be required to inform risk management