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ToxCast Continued…

Endocrine Disruption Screening Models

June 2026

Previously on ToxCast…

Oct 2025 – "Overview"

- Origin of ToxCast – usefulness to SAB discussions
  - Potential useful endpoints – endocrine disruption, dev, geno, neuro
  - Potential target organs – heart, thyroid, liver
  - Specific cell functions – interactions with nuclear receptors
- What can be learned from a single chemical? (TBBPA)
- What can be learned from a group of chemicals? (aryl phosphate esters)

Jan 2026 – "Continued"

- Considered 4 recent studies on using ToxCast data
  - Jeong (2022) - review article
  - Phifer (2021) - in vitro prediction of in vivo toxcity
  - Schaupp (2023) - eco hazard
  - Krishna (2021) - cardiotoxicity

Mar 2026 and June 2026 – ED models

Comptox.epa.gov – search by CAS

Bioactivity – tab on left

ToxCast: Models

Bioactivity - ToxCast: Models

EXPORT

ToxCast Model Predictions

Model	Receptor	Agonist	Antagonist	Binding
CERAPP Potency Level (Consensus)	Estrogen			
CERAPP Potency Level (From Literature)	Estrogen			
ToxCast Pathway Model (AUC)	Estrogen			X
COMPARA (Consensus)	Androgen			
ToxCast Pathway Model (AUC)	Androgen			X

ToxCast Endocrine Disruptor Screening Program (EDSP)

- 1996: Congress amended the Federal Food, Drug and Cosmetic Act and the Federal Insecticide, Fungicide and Rodenticide Act through passage of the **Food Quality Protection Act (FQPA)**
- Required EPA to develop a **screening program** to identify chemicals that may have estrogenic, androgenic or thyroid hormone effects - "pesticides and other substances"
- 1999: created the EDSP
- Estimated 10,000 chemicals to be screened
- Selected 67 chemicals for initial screening, mostly pesticide active ingredients and high production volume chemicals used as pesticide inert ingredients "List 1"
- Two-tiered in vitro and in vivo screening assays to determine the potential of a chemical to interact with the estrogen, androgen or thyroid hormone systems (initially ER agonist only)
  - Tier 1 = 5 in vitro and 6 in vivo screening assays (~completed for List 1)
  - Tier 2 = in vivo multigenerational tests with apical end points
  - Regulatory guideline studies
- 2007 National Research Council "Toxicity Testing in the 21st Century" which became known as Tox21 – need to move to more rapid HT assays and computational models

EDSP continued

- In 2010 "List 2" chemicals (109) added pesticides and priority drinking water chemicals (e.g., halogenated organic chems, dioxins, FRs, PCBs)
- 2012-2014: Use of computational toxicology and high throughput methods discussed

2015-2016 – Pathway Models

Pathway Model for estrogen receptor activity

- 1800 chemicals tested using HTS assays + computational model for the estrogen receptor pathway

Pathway Model for **androgen** receptor activity

- 1855 chemicals, androgen pathway much less defined

2016 – CERAPP Model for estrogen receptor activity

- Combine many QSAR models

2017-2020 – COMPARA Model for **androgen** receptor activity

- Combine many QSAR models

- "It is important not to equate a determination of a chemical's bioactivity with a determination that a chemical causes endocrine disruption." (EPA NAMs paper 2022)

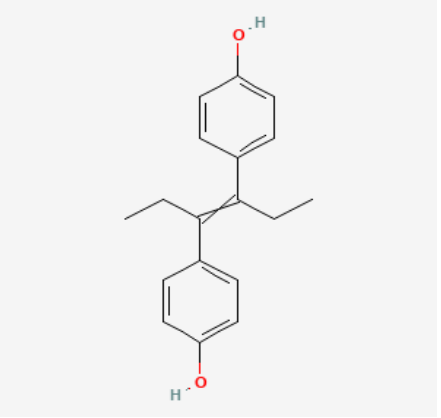
ToxCast ER Model for Bioactivity, or Pathway Model, 2015 (Browne, EPA)
estrogen agonist

- Alternative to initial guideline studies – HTS + computational methods (in order to discriminate actual bioactivity from interference and cytotoxicity)
- Used 18 estrogen receptor assays with varying key events in the AOP, ER agonist only, combines data from the resulting 18x14 conc/response curves, area under the curve
- Training & Validation: 40 in vitro agonist chems, 28 in vivo active chems, 103 guideline-like uterotrophic chems from literature (442 studies), results of EDSP Tier 1 assays
- Score based on AC50; range from 0=no activity to 1=bioactivity similar to 17-b estradiol
- Used to predict 1812 chemicals – EPA accepted this model as alternative for 2 (of 5) in vitro and 1 (of 6) in vivo Tier I tests.
- Accuracy: 86-93% for reference chemicals, 81-100% for Tier 1 assay outcomes and literature studies depending on how "inconclusive" results are incorporated
- Initial model was agonist only, antagonist added later, Judson et.al, 2015
- Of the 1812 chemicals evaluated 111 were strongly ER active in either agonist/antagonist/both

ToxCast ER Model for Bioactivity, or Pathway Model, 2015 (Browne, EPA)

estrogen agonist

Results for diethylstilbestrol (DES)



ToxCast Model Predictions

Model	Receptor	Agonist	Antagonist	Binding
ToxCast Pathway Model (AUC)	Estrogen	0.943		-

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EDSP continued

2015-2016 – Pathway Models

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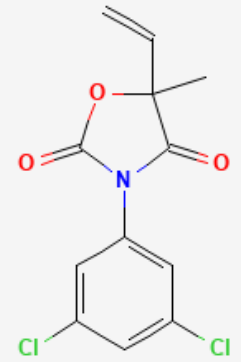
ToxCast ER Model for Bioactivity, or Pathway Model, 2016 (Kleinstreuer, NIEHS) **androgen agonist or antagonist**

- 11 assays
  - Several points in the AR pathway: receptor binding, coregulator recruitment, gene transcription, and protein production
  - Multiple cell types
- Reference chemicals for AR pathway much less defined than for ER
- Training & Validation: 158 reference chemicals from a combination of test methods/validation efforts with reliable in vitro results
- Computational network model: AR agonist, AR antagonist, false positive (specific assay interference), true negative
- Outcomes could be: no assays activated, all agonist or antagonist assays activated, specific assays across technologies activated, technology-specific activation
- Model used to predict 1855 chemicals – 220 AR agonist or antagonist; 174 weak AR activity
- Model "over 95% accuracy" in predicting AR agonism and antagonism for reference chemicals

ToxCast ER Model for Bioactivity, or Pathway Model, 2016 (Kleinstreuer, NIEHS)

androgen agonist or antagonist



Results for Vinclozolin (fungicide)

ToxCast Model Predictions

Model	Receptor	Agonist	Antagonist	Binding
ToxCast Pathway Model (AUC)	Androgen	0.00	0.416	-

Most inactive chemicals cluster near **~0.0–0.1**
Weak or borderline actives often fall in **~0.1–0.3**
Strong reference antagonists tend to be **~0.3–1.0+**

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EDSP continued

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2016 – CERAPP Model for estrogen receptor activity

- Combine many QSAR models

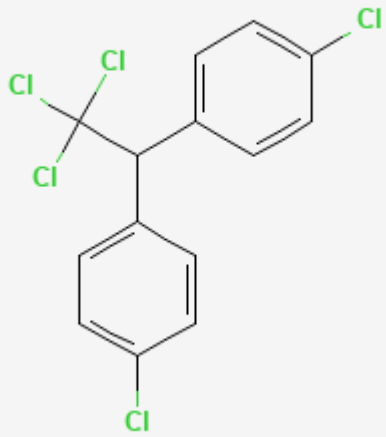
2020 – COMPARA Model for **androgen** receptor activity

- Combine many QSAR models

Collaborative Estrogen Receptor Activity Prediction Project (CERAPP), 2016 Mansouri NIEHS

- Built on previous work to develop 48 QSAR models for ER binding, agonist and antagonist activity
- QSAR models: 40 categorical (Y/N), 8 continuous (# prediction of potency)
- Combine all QSAR models (weighted based on accuracy) to prioritize chemicals based on estrogen receptor activity – overcome single model limitations
- 18 ER-related assays: binding, reporter gene/transactivation, cell proliferation
- Steps: Structure curation – apply the training set (1677 chemicals) – test on validation set (7500 chemicals with 85-93% accuracy)
- Outputs: Receptor (androgen or estrogen), agonist and antagonist (scores 0 or 1), binding (1 or 0 or sometimes "-" meaning 'no valid prediction')
- 32,464 chemical structures, found 4,000 chemicals with evidence of ER activity

ToxCast Endocrine Model Results for DDT



1-chloro-4-(2,2,2-trichloro-1-(4-chlorophenyl)ethyl)benzene

Model	Receptor	Agonist	Antagonist	Binding
CERAPP Potency Level (Consensus)	Estrogen	1.00	1.00	1
CERAPP Potency Level (From Literature)	Estrogen	VeryWeak	Moderate	VeryWeak

EDSP continued

2015-2016 – Pathway Models

Pathway Model for estrogen receptor activity

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Pathway Model for **androgen** receptor activity

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2016 – CERAPP Model for estrogen receptor activity

- Combine many QSAR models

2017-2020 – COMPARA Model for **androgen** receptor activity

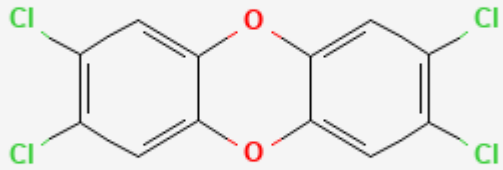
- Combine many QSAR models

Collaborative Modeling Project for Androgen Receptor Activity (CoMPARA) Mansouri, NIEHS 2020

- Combine 91 QSAR models to prioritize chemicals based on androgen receptor activity – overcome single model limitations
- Generally followed the CERAPP process
- Separate models for binding – antagonist - agonist
- Training set: 11 AR-related assays run on 1855 chemicals used in Pathways model
- Validation set: between 4000-5000 for each of the 3 endpoints
- Strong bias toward 'inactives' was a problem – added 15 chems from literature
- Prediction set: ~50,000 chemical structures
- Outputs: Receptor (androgen or estrogen), agonist and antagonist (scores 0 or 1), binding (1 or 0 or sometimes "-" meaning 'no valid prediction')
- Approx 10% were determined to be potential binders to the AR in agonist or antagonist modes
- For reference chemicals: 95.2% accuracy for androgen agonist; 97.5% accuracy for androgen antagonist
- In evaluation set, prediction accuracy approx 80%

Collaborative Modeling Project for Androgen Receptor Activity (CoMPARA) Mansouri, NIEHS 202

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

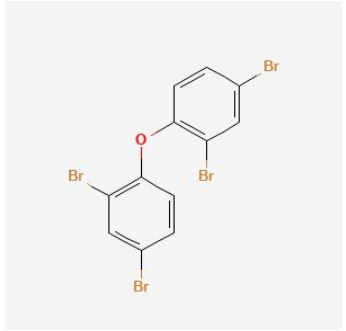


Model	Receptor	Agonist	Antagonist	Binding
COMPARA (Consensus)	Androgen	0.00	1.00	1

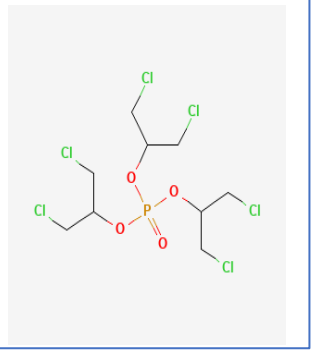
Potential binding androgen antagonist

How do the following chemicals perform in the models?

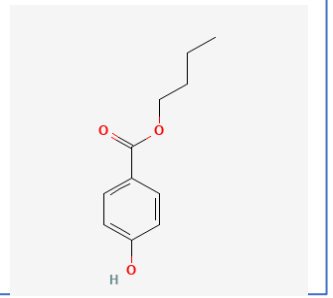
BDE-47



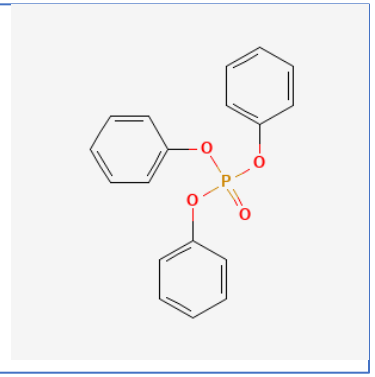
TDCPP



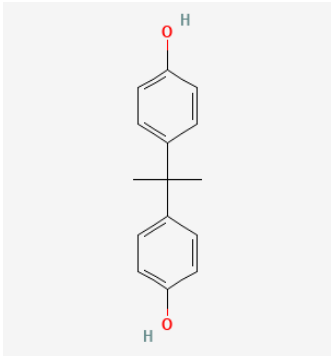
Butyl paraben



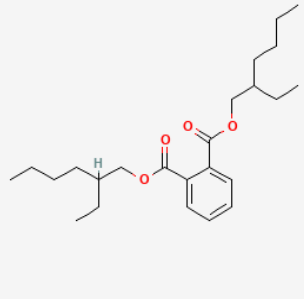
TPP



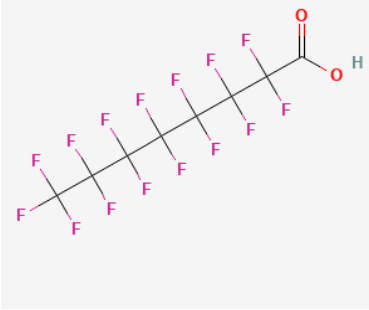
BPA



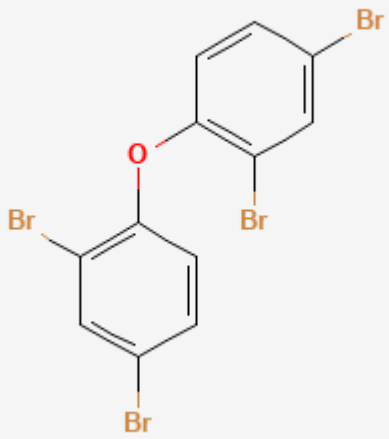
DEHP



PFOA



ToxCast Endocrine Model Results for BDE-47



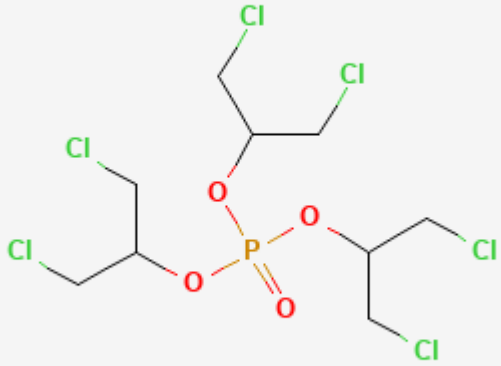
2,2',4,4' - tetrabromodiphenyl ether

ToxCast Model Predictions

Model	Receptor	Agonist	Antagonist	Binding
COMPARA (Consensus)	Androgen	0.00	1.00	1
CERAPP Potency Level (From Literature)	Estrogen	Inactive	Inactive	Inactive
CERAPP Potency Level (Consensus)	Estrogen	0.00	0.00	0

Indication of androgen receptor binding antagonist

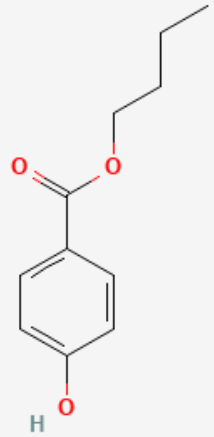
ToxCast Model Predictions for tris(1,3-dichloro-2-propyl) phosphate (TDCPP)



Model	Receptor	Agonist	Antagonist	Binding
CERAPP Potency Level (Consensus)	Estrogen	0.00	0.00	0
CERAPP Potency Level (From Literature)	Estrogen	Inactive	Inactive	Inactive
ToxCast Pathway Model (AUC)	Estrogen	0.00	0.00	-
ToxCast Pathway Model (AUC)	Androgen	0.00	0.290	-
COMPARA (Consensus)	Androgen	0.00	1.00	1

Indication of androgen receptor binding antagonist

ToxCast Endocrine Model Results for butyl paraben



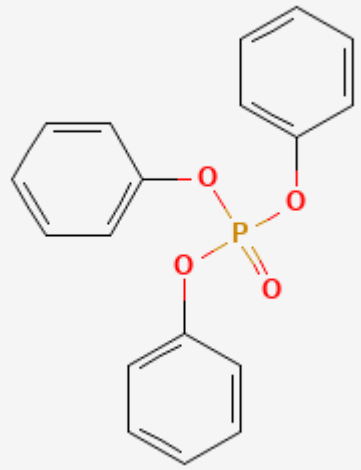
EXPORT

ToxCast Model Predictions

Model	Receptor	Agonist	Antagonist	Binding
ToxCast Pathway Model (AUC)	Androgen	0.00	0.00	-
COMPARA (Consensus)	Androgen	0.00	0.00	0
ToxCast Pathway Model (AUC)	Estrogen	0.251	5.16e-7	-
CERAPP Potency Level (From Literature)	Estrogen	Weak	Strong	Weak
CERAPP Potency Level (Consensus)	Estrogen	1.00	1.00	1

"biologically relevant" estrogenic endocrine disruptor

ToxCast Endocrine Model Results for TPP



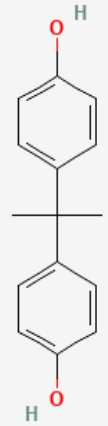
Triphenyl phosphate

ToxCast Model Predictions

Model	Receptor	Agonist	Antagonist	Binding
COMPARA (Consensus)	Androgen	0.00	0.00	0
ToxCast Pathway Model (AUC)	Androgen	0.00	3.79e-2	-
ToxCast Pathway Model (AUC)	Estrogen	6.41e-2	3.14e-2	-
CERAPP Potency Level (From Literature)	Estrogen	Inactive	VeryWeak	Weak
CERAPP Potency Level (Consensus)	Estrogen	0.00	1.00	1

weak estrogen receptor interaction and possible androgen antagonist activity

ToxCast Model Predictions for Bisphenol-A



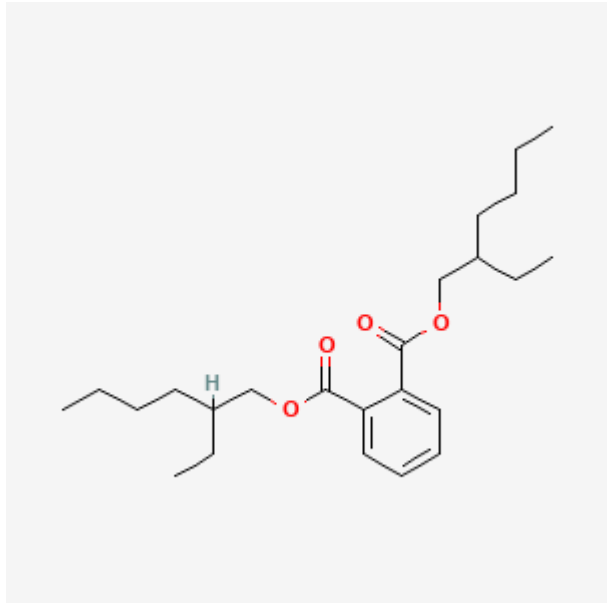
EXPORT

ToxCast Model Predictions

Model	Receptor	Agonist	Antagonist	Binding
COMPARA (Consensus)	Androgen	0.00	1.00	1
ToxCast Pathway Model (AUC)	Androgen	0.00	0.345	-
ToxCast Pathway Model (AUC)	Estrogen	0.450	0.00	-
CERAPP Potency Level (From Literature)	Estrogen	Weak	Strong	Weak
CERAPP Potency Level (Consensus)	Estrogen	1.00	1.00	1

ChatGPT summary - The chemical is likely a receptor-binding endocrine disruptor with strong antiandrogenic properties and moderate/selective estrogenic activity.

ToxCast Endocrine Model Results for DEHP



Known for causing anti-androgenic health issues

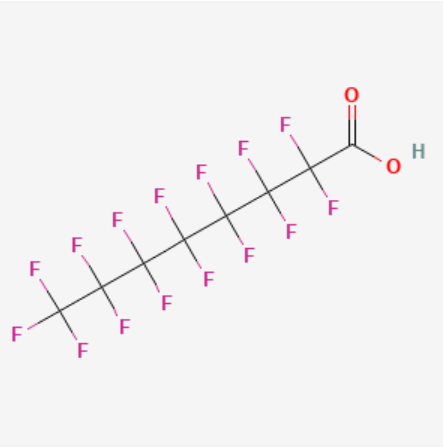
ToxCast Model Predictions

Model	Receptor	Agonist	Antagonist	Binding
COMPARA (Consensus)	Androgen	0.00	0.00	0
ToxCast Pathway Model (AUC)	Androgen	0.00	0.00	-
CERAPP Potency Level (Consensus)	Estrogen	0.00	0.00	0
ToxCast Pathway Model (AUC)	Estrogen	0.00	0.00	-
CERAPP Potency Level (From Literature)	Estrogen	Inactive	Inactive	Inactive

What's the problem with DEHP?

- Different pathway – models look for action on the receptor (direct receptor binding) phthalates act by blocking testosterone from getting to the receptor (models miss the upstream action)
- Models miss action by the metabolites
- Structurally different than the classic ED chemicals used to train the models (fungicides, phenolic compounds, steroid like)

ToxCast Endocrine Model Results for PFOA



Model	Receptor	Agonist	Antagonist	Binding
COMPARA (Consensus)	Androgen	0.00	0.00	0
CERAPP Potency Level (Consensus)	Estrogen	0.00	0.00	0
CERAPP Potency Level (From Literature)	Estrogen	Inactive	Inactive	Inactive
ToxCast Pathway Model (AUC)	Estrogen	0.00	0.00	-
ToxCast Pathway Model (AUC)	Androgen	0.00	0.00	-

What's the problem with PFOA and the models in general?

- Like DEHP it does not act through direct binding. It acts indirectly through many possible routes such as activating PPARα (peroxisome proliferator-activated receptor alpha) which regulates many metabolic pathways
- Like DEHP, PFOA is structurally different from classic ER chems used to train the models.

- Cyctotoxicity safety filter might mask actual ED activity for chemicals that are highly cytotoxic (not necessarily true for DEHP or PFOA)
- Models are known to underperform for:
  - PFAS compounds
  - phthalates
  - metabolism-mediated disruptors
  - steroidogenesis disruptors
  - thyroid-active compounds
- Solutions? AOPs would help but slow going. Steroidogenesis assay H295R (testosterone pertubations) - included in ToxCast now but not included in models.

What do the models add to the current ED sources?

ECHA Endocrine Disruptor Assessment List:

- 70 confirmed ED
- 18 inconclusive
- 12 not ED
- 11 postponed
- 70 under development

European Commission – Endocrine Disruptor Lists

- 96 "List I" confirmed ED
- 129 "List II" under evaluation
- 11 unique "List III" considered to be ED

TEDX List of Potential Endocrine Disruptors

- 1482 chemicals
- Not updated since 2019

EPA's EDSP

- *67 Tier 1 – based on exposure potential*
- *107 Tier 2 – pesticides, PFCs, pharmaceuticals*
- *10,046 Universe of Chemicals (pesticide active ingredients, HPV chemicals, drinking water contaminants)*

Published studies

ToxCast Assay Data – 11 estrogen assays, 8 androgen assays, steroidogenesis assay H295R, plus newer assays?

Also FDAs EADB – Estrogenic Activity Database