

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
 - Phiifer (2021) - in vitro prediction of in vivo toxicity
 - 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)

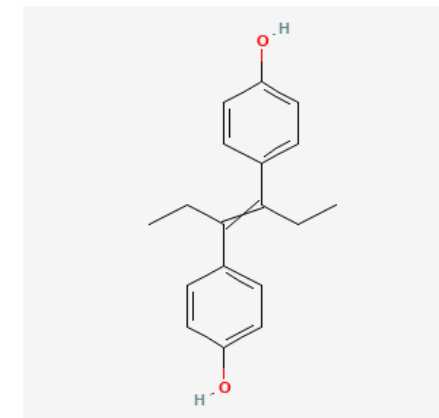
ToxCast ER Model for Bioactivity, or Pathway Model, 2015 (Browne, EPA)

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- β 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		-

EDSP continued

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2016 – CERAPP Model for **estrogen** receptor activity

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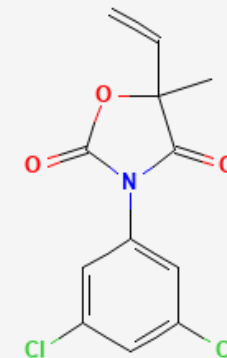
2017-2020 – COMPARA Model for **androgen** receptor activity

- Combine many QSAR models

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+**

EDSP continued

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- 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

ClC1(Cl)C(C1)C2=CC=C(Cl)C=C2C3=CC=C(Cl)C=C3ClC1(Cl)C(C1c2ccc(Cl)cc2)c3ccc(Cl)cc3ClC1(Cl)C(C1c2ccc(Cl)cc2)c3ccc(Cl)cc3

EDSP continued

2015-2016 – Pathway Models

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- Combine many QSAR models

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

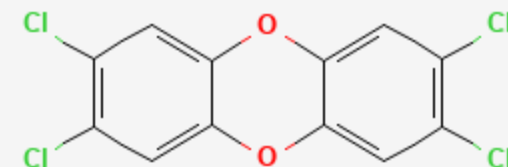
- Combine many QSAR models

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

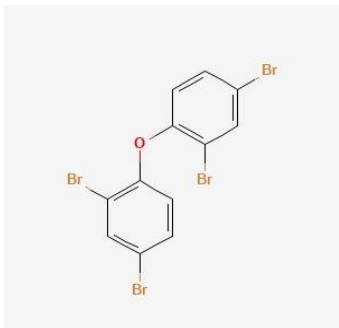
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)



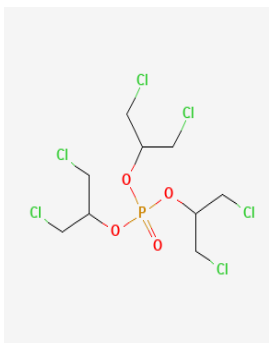
Model ↓↑	Receptor ↓↑	Agonist ↓↑	Antagonist ↓↑	Binding ↓↑
<u>COMPARA (Consensus)</u>	Androgen	0.00	1.00	1
Potential binding androgen antagonist				

How do the following chemicals perform in the models?

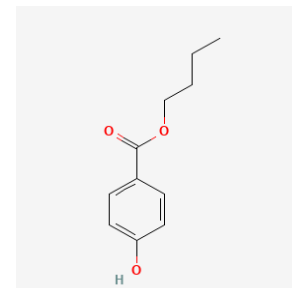
BDE-47



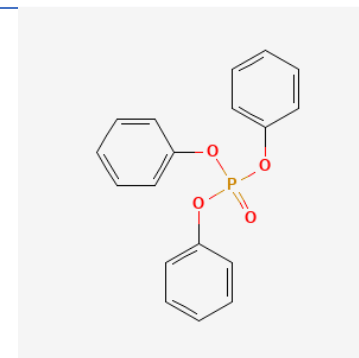
TDCPP



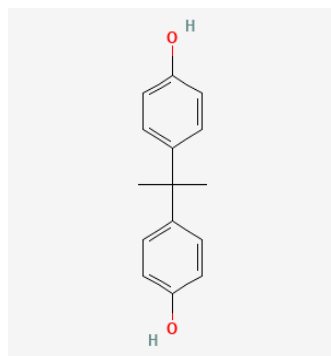
Butyl paraben



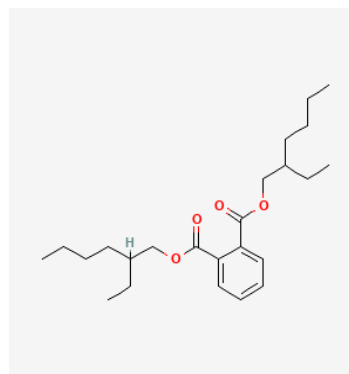
TPP



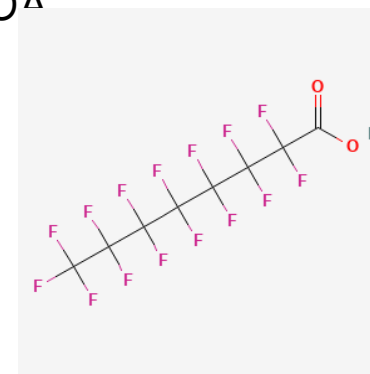
BPA



DEHP

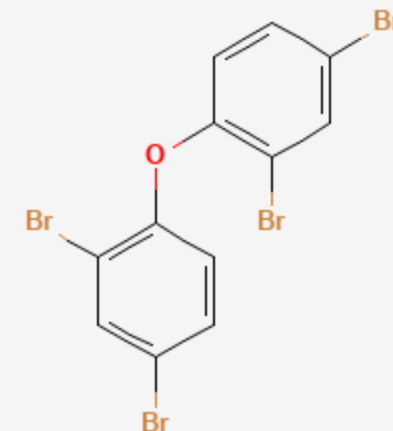


PFO^Δ



ToxCast Endocrine Model Results for BDE-47

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

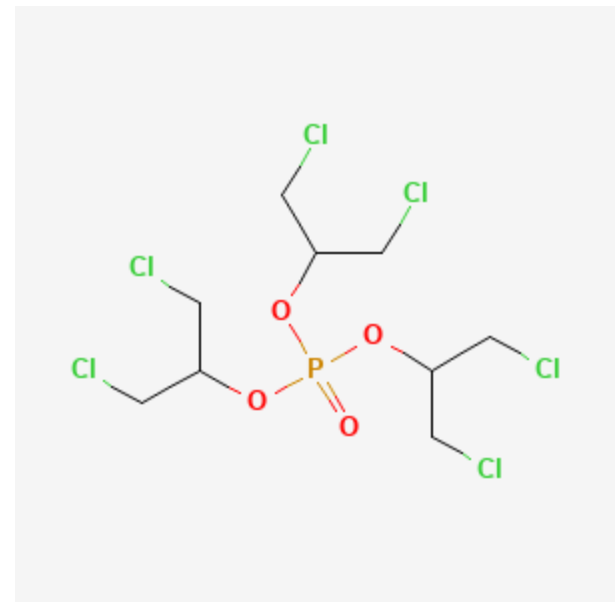


ToxCast Model Predictions

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

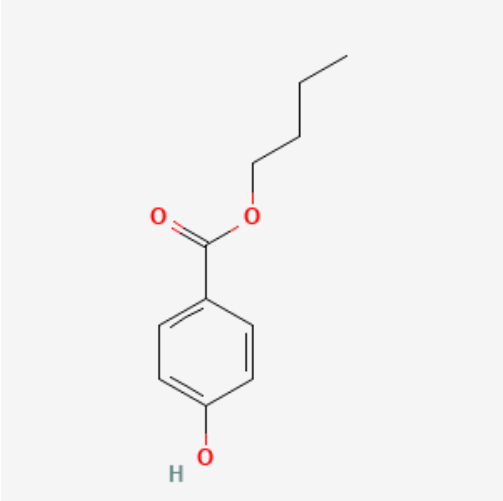
ToxCast Model Predictions for tris(1,3-dichloro-2-propyl) phosphate (TDCPP)



Model ↓↑	≡ Receptor ↓↑	≡ Agonist ↓↑	≡ Antagonist ↓↑	≡ Binding ↓↑
<u>CERAPP Potency Level (Consensus)</u>	Estrogen	0.00	0.00	0
<u>CERAPP Potency Level (From Literature)</u>	Estrogen	Inactive	Inactive	Inactive
<u>ToxCast Pathway Model (AUC)</u>	Estrogen	0.00	0.00	-
<u>ToxCast Pathway Model (AUC)</u>	Androgen	0.00	0.290	-
<u>COMPARA (Consensus)</u>	Androgen	0.00	1.00	1

Indication of androgen receptor binding antagonist

ToxCast Endocrine Model Results for butyl paraben



EXPORT

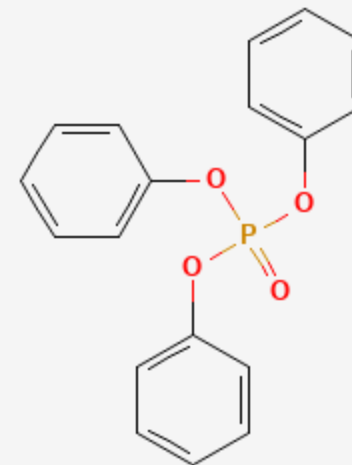
ToxCast Model Predictions

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

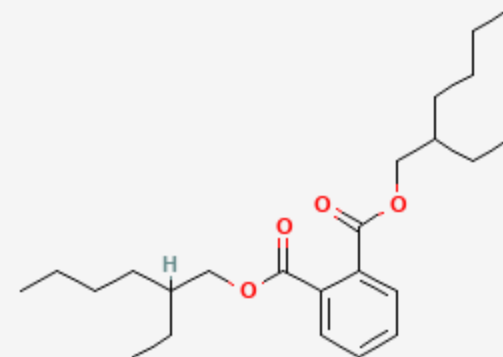
Model ↓↑	Receptor ↑	Agonist ↓↑	Antagonist ↓↑	Binding ↓↑
COMPARA (Consensus)	Androgen	0.00	1.00	1
ToxCast Pathway Model (AUC)	Androgen	0.00	0.345	-
ToxCast Pathway Model (AUC)	Estrogen	0.450	0.00	-
CERAPP Potency Level (From Literature)	Estrogen	Weak	Strong	Weak
CERAPP Potency Level (Consensus)	Estrogen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



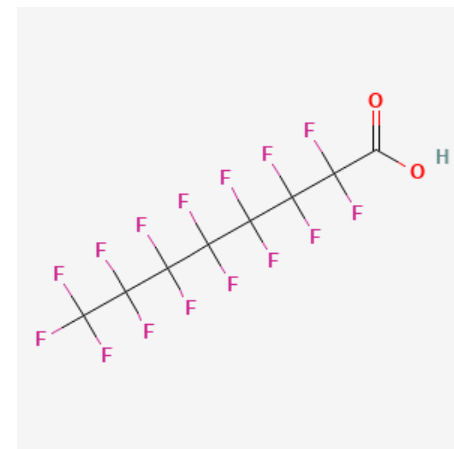
ToxCast Model Predictions

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 ↓↑
<u>COMPARA (Consensus)</u>	Androgen	0.00	0.00	0
<u>CERAPP Potency Level (Consensus)</u>	Estrogen	0.00	0.00	0
<u>CERAPP Potency Level (From Literature)</u>	Estrogen	Inactive	Inactive	Inactive
<u>ToxCast Pathway Model (AUC)</u>	Estrogen	0.00	0.00	-
<u>ToxCast Pathway Model (AUC)</u>	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 PPAR α (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.
-
- Cytotoxicity 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 perturbations) - 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 FDA's EADB – Estrogenic
Activity Database