Toxicology in the 21st Century

Tox21 General Meeting on May 11th, 2023

Cross-Partner Project 8:

Automation of Reference Chemical Generation

CPP8 Team

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EPA | NIH National Center for Advancing Translational Sciences | FDA U.S. FOOD & DRUG ADMINISTRATION | NTP National Toxicology Program, U.S. Department of Health and Human Services

Disclaimer

The views expressed are those of the authors and do not necessarily represent the views or the policies of the U.S. EPA.

Purpose is to Identify Reference Chemicals

Develop an automated process to develop lists of positive and negative *in vitro* reference chemicals for target genes and/or cell processes

Use case study targets to generate reference chemicals

Use literature mining and ToxCast/Tox21 data [initially no new experiments]

A reference chemical is one that gives consistent results (active vs inactive) across multiple different assays that measure activity against a target or molecular mechanism (Judson et al, 2019)

Proposed Methods and Goals

Quality Control:
Will start by filtering the assay data to remove cytotoxic doses

Positive reference chemicals:
Will need to be consistently positive in the target assays at concentrations below the burst and will also check whether any of the chemicals have other assays with >10 fold greater potency (e.g. 10-fold lower BMD) and remove those chemicals

Negative reference chemicals:
Will need to be have a lack of evidence in the literature, plus consistently negative for the target assays while having positive activity in off-target assays

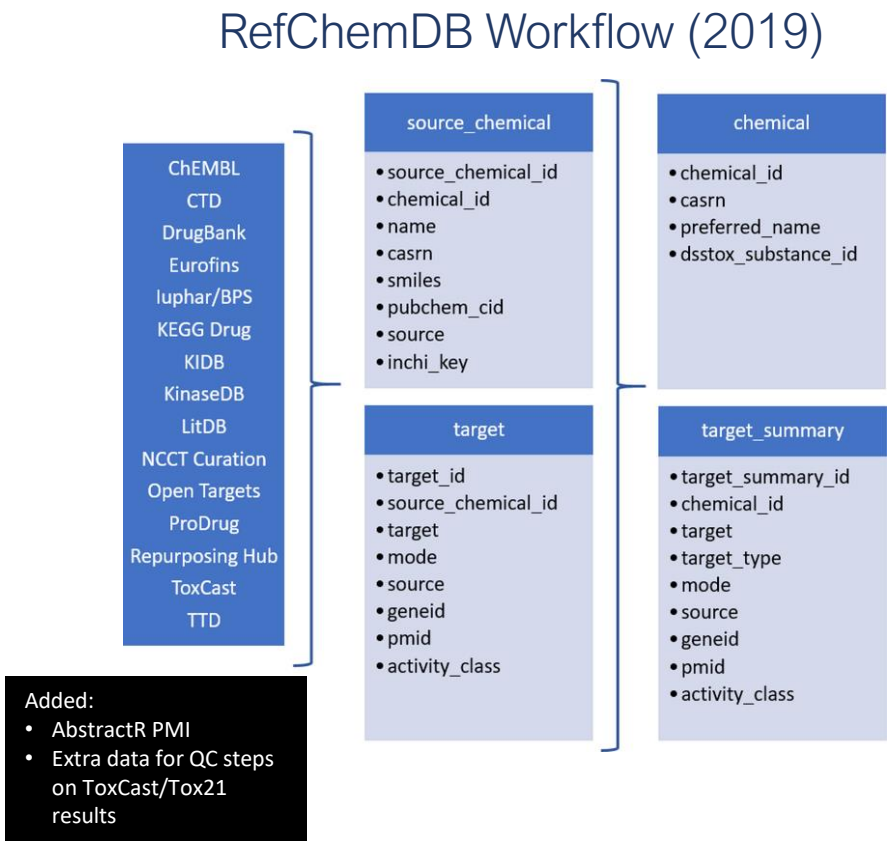
Results Goal:
Generate at least 2+ reference chemicals per target, but 10+ reference chemicals per target would be best, with a range of potencies

Assessment of method performance:
Will compare to expert-panel selected reference chemicals (ER/AR)

Database and Literature Mining Compilation

Richard Judson refreshed RefChemDB

- RefChemDB is a database matching chemicals to molecular targets
- Added new literature from 2019-2023
- Added AbstractR pairwise mutual information (PMI) for gene-based results in addition to LitDB, which searches for protein MeSH terms
- Added additional data for applying QC steps, such as excluding cytotoxic doses



Judson, R. S., Thomas, R. S., Baker, N., Simha, A., Howey, X. M., Marable, C., Kleinstreuer, N. C., & Houck, K. A. (2019). Workflow for defining reference chemicals for assessing performance of in vitro assays. *ALTEX*, *36*(2), 261–276. https://doi.org/10.14573/altex.1809281

Literature Mining Update

Nancy Baker and Bryant Chambers updated the literature mining, conducting nearly 8 million searches each, using LitDB and AbstractR, respectively

Grouping	#Chemicals	#Targets	#Searches
All unique chemicals in RefchemDB and all targets	4,409	1,777	**7,834,793**

Candidate Targets for Case Studies

For a candidate target to be included as a case study, we required there to be at least 3 orthogonal assays

Gene Targets List	Gene Symbol(s)	Assay Sources
Androgen Receptor	AR	ACEA, ATG, NVS, OT, TOX21, UPITT
Estrogen Receptors	ESR1/2	ACEA, ATG, NVS, OT, TOX21
Glucocorticoid Receptor	NR3C1	ATG, NVS, TOX21
Peroxisome Proliferator Activated Receptor Gamma	PPARG	ATG, NVS, OT, TOX21
Progesterone Receptor	PGR	ATG, NVS, TOX21
Retinoic Acid Receptors	RARA/B/G	ATG, NVS, TOX21
Thyroid Hormone Receptor	TRA/B	ATG, NVS, TOX21
Tumor Protein P53	TP53	APR, ATG, TOX21

Cell Processes List	Assay Sources
Mitochondria Toxicity	APR, NCCT, NVS, TOX21
Stress Pathways	APR, ATG, TOX21

Assay Abbreviations ACEA: ACEA Biosciences, ATG: Attagene, NVS: NovaScreen, OT: Oddessy Theta, TOX21: Tox21, UPITT: University of Pittsburg, NCCT: National Center for Computational Toxicology (now CCTE)

Previously Proposed Methods

Two workflows to compare:

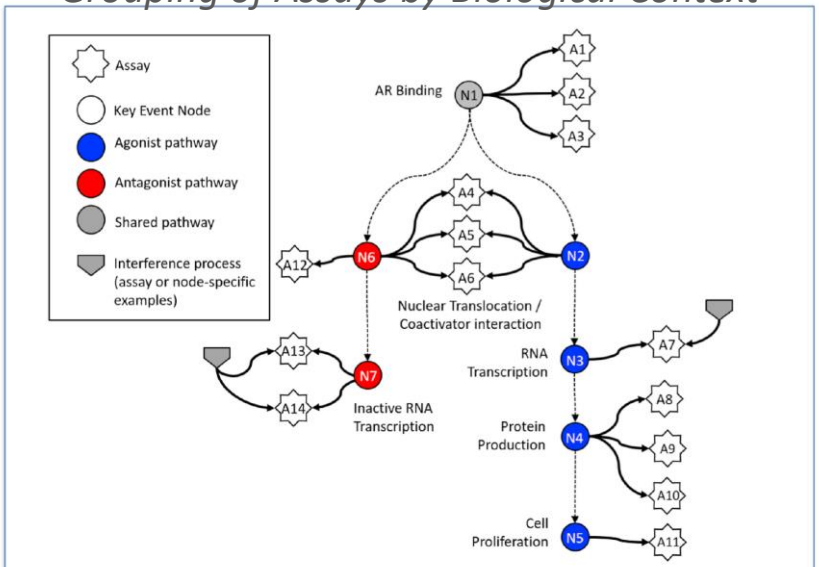
- Majority rule
  If the majority of assays are positive for the target (around a similar concentration), then the chemical is deemed a reference chemical

- Biological context
  Use assays for different parts of the biological pathways and ensure that a chemical is affecting the pathway (around a similar concentration) for it to be deemed a reference chemical

Majority rule testing workflow

Some sources have multiple assays for the target

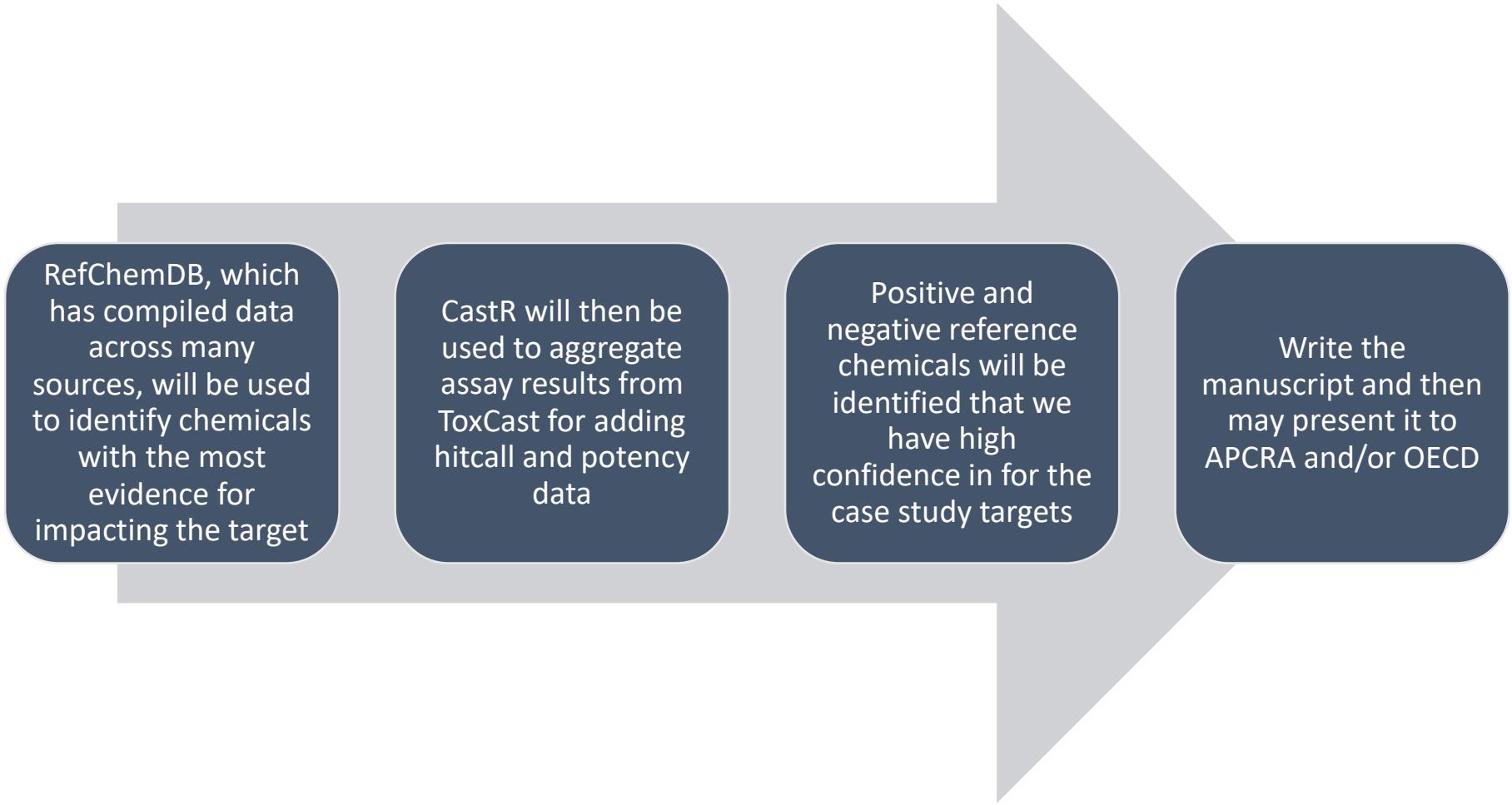
Grouping of Assays by Biological Context



Judson, Richard, et al. "Selecting a minimal set of androgen receptor assays for screening chemicals." *Regulatory Toxicology and Pharmacology* 117 (2020): 104764.

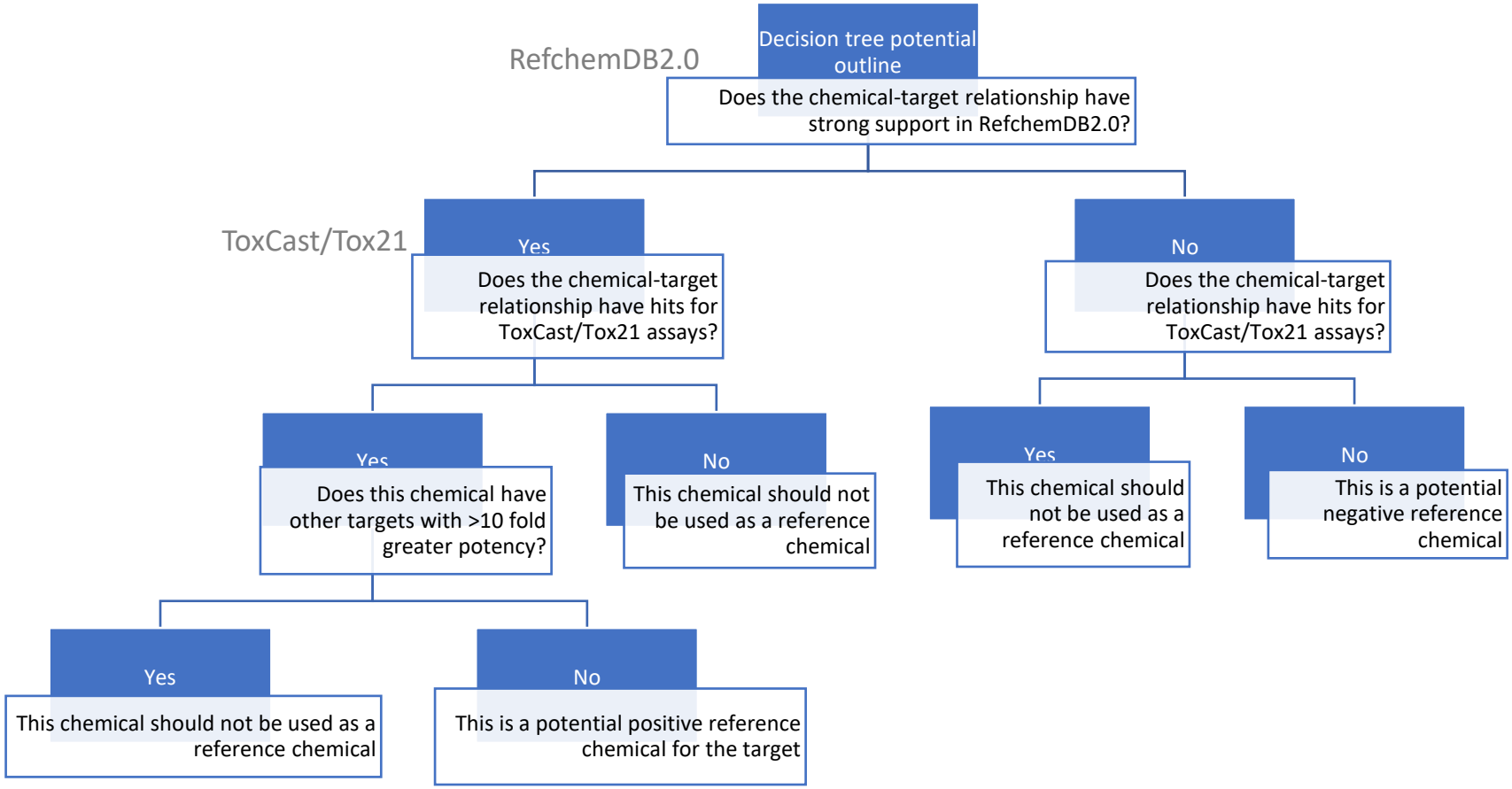
**Similar concentration refers to concentrations within 10-fold*

CPP8 Summary: Big Picture Workflow



New Approach: Decision Tree

First: remove cytotoxic doses, use exemplar data only, and limit to targets with at least 3 of the relevant types of assays



Preliminary Results: Example Results for Estrogen Receptor Top Hits

Using CastR built by Bryant Chambers to extract ToxCast data

Summary data is across all assays for ER

Provides median potency, the percent of target-specific assays that were positive, and calculates a p-value for how specific the chemical activity is for that particular target vs all other assays

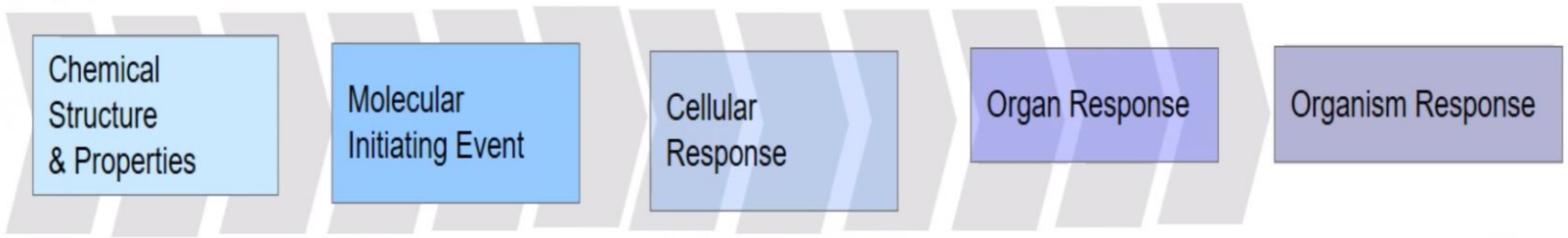
chemical	super.cat	assays.hit	assays.total	not.cat.assays.hit	not.cat.assays.total	significance.to.all.assays	median.ac.50
Bisphenol A	ER	112	136	654	3845	2.33E-59	0.659413962
Estrone	ER	88	114	199	1879	3.84E-56	0.022707746
Ethylparaben	ER	54	102	29	1621	2.61E-52	30.58596786
Norethindrone	ER	85	103	243	1685	4.26E-49	1.535036526
17beta-Estradiol	ER	93	112	342	1925	3.31E-47	0.009
Genistein	ER	90	107	342	1916	1.35E-46	0.70119284
7,4'-Dihydroxyisoflavone	ER	61	77	146	1621	8.64E-45	2.125401947
17alpha-Estradiol	ER	88	102	358	1672	3.34E-41	0.012339876
5alpha-Dihydrotestosterone	ER	77	106	242	1737	6.37E-39	3.938525163
Fulvestrant	ER	72	106	222	1870	7.08E-38	0.087271176
Butylparaben	ER	63	89	211	1874	1.58E-36	14.86009619
Diethylstilbestrol	ER	95	109	547	1978	2.48E-36	0.008559529
17beta-Trenbolone	ER	76	98	335	1859	5.10E-35	3.908675247
meso-Hexestrol	ER	90	102	492	1681	2.73E-33	0.014409248
17alpha-Ethinylestradiol	ER	94	119	539	2168	5.25E-33	0.011975351
2,2',4,4'-Tetrahydroxybenzophenone	ER	57	77	210	1691	7.00E-33	1.902006025
Bisphenol B	ER	81	97	435	1653	6.24E-30	0.272178228
Bisphenol AF	ER	89	103	612	1772	4.68E-26	0.211599046
4-(2-Methylbutan-2-yl)cyclohexanol	ER	29	63	33	1348	7.59E-26	23.58209659
Levonorgestrel	ER	47	114	131	1865	3.73E-22	0.789662411
Methoxychlor	ER	46	70	245	1725	1.87E-21	6.34708373
4-Heptylphenol	ER	50	92	177	1393	1.33E-19	10.3223518
Tris(2-ethylhexyl) trimellitate	ER	17	40	13	1105	3.31E-19	39.01568634
4-Cumylphenol	ER	62	78	497	1672	1.17E-18	3.413075938
4-(1,1,3,3-Tetramethylbutyl)phenol	ER	62	78	489	1636	1.63E-18	3.358757183

Working on updating the code so that the exemplar results are used in the case of replicates

Potential Application: Adverse Outcome Pathways (AOPs) Validation

- Screen chemicals that disrupt any part of AOP pathways to generate reference chemicals for particular adverse outcomes
- Compare list of reference chemicals with zebrafish toxicity screen results and other curated findings, such as from CPP13 for RAR AOPs

Adverse Outcome Pathway (AOP) Framework (OECD 2014)



Example AOPs for developmental defects from RAR pathway perturbation

REGION	MIE	KE1	KE2	KE3	KE4	KE5	AO
Headfold	Inhibition of CYP26A1 enzymatic activity	Local increase in endogenous ATRA levels	Hyperactivation of the RAR/RXR heterodimer	Repression of *Fgf8* limits FGF8 signaling	Mis-specification of CNC cell fate and behavior	Maxillary arch dysplasia alters palatal outgrowth	Cleft palate
Paraxial Mesoderm	Reduction in RDH/RALDH2 activity	Local decrease in endogenous ATRA levels	Hypoactivation of the RAR/RXR heterodimer	Overextension of FGF8 signaling	Disruption of the periodic somitic wavefront	Altered somite number, shape, and alignment	Hemivertebra
Limb-bud	Hyperactivation of the RAR/RXR heterodimer	Underextension FGF8 signaling from the AER	Dysregulation of *Meis1/2* and *Hox* gene expression	Proximalization of the limb-bud mesenchyme	Mis-specification of precartilage blastema	Delayed or aberrant differentiation	Phocomelia

Knudsen, Thomas B., Jocylin D. Pierro, and Nancy C. Baker. "Retinoid signaling in skeletal development: Scoping the system for predictive toxicology." *Reproductive Toxicology* 99 (2021): 109-130.

Timeline and Deliverables

Timeframe	Project progress goal
May 2021 – Dec 2021	**Selection of targets** (genes/cell processes) Determine methods and start **data analysis**
Jan 2022 – June 2022	**Data analysis**
July 2022 – Dec 2022	Write manuscript on gene/cell processes targets
Jan 2023 – June 2023	**Submit code package and manuscript for gene/cell processes targets**
June 2023 – Dec 2023	Conclude CPP
TBD	Present work to APRCA and/or OECD

Current target delivery date:
Dec 2023

Big Picture – Benefits

Increases the speed of generating new reference chemicals by automating the process and removing the need for upfront manual curation

The list of assays and reference chemicals could be used to **validate new assays or AOPs**

Improves chemical screening abilities when assessing chemical toxicity

Comments?

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Extra slides for Q&A

CPP8 StRAP4 Proposal

Product Title: Non-StRAP: Automation of Reference Chemical Generation

Primary output: CSS.401.2

Partner Needs: Building confidence in New Approach Methodologies (NAMs)

Brief Description and Research Use
This product is an ongoing Tox21 cross-partner project (CPP#8)

Overview: Developing new *in vitro* assays for screening chemical activity is critical for the advancement of New Approach Methodologies (NAMs) with the goal of reducing animal testing. Reference chemicals are needed to validate the performance of new *in vitro* assays for screening which gene protein products a chemical impacts. Knowledge about which protein pathways are impacted by a chemical then helps to predict what adverse outcomes may occur following *in vivo* exposure to the chemical. Previously, it has required teams of experts to compile reference chemical lists after many hours of research and deliberation. This process is time-consuming and is unreasonable to do for every gene target of interest. Thus, we propose to create an automated process for generating reference chemicals.

Method: This project will use ToxCast and Tox21 data that has already been generated as the primary data source. Automated literature mining techniques will also be applied for supplementing the *in vitro* assay results with what has been reported in the literature about chemical-gene interactions. Both active (positive) and inactive (negative) reference chemicals will be identified for a set of gene targets and cell processes of interest. Targets must have at least three orthogonal assays to be included. Androgen receptor and estrogen receptor will be used to evaluate how the automated method compares to hand-curated reference chemical lists for those targets. Other targets that will be examined include glucocorticoid receptor, peroxisome proliferator activated receptor gamma, progesterone receptor, retinoic acid receptor, thyroid hormone receptor, and tumor protein P53. Cell processes such as cell stress pathways and mitochondria toxicity may also be examined. After generating reference chemical lists, another goal could be to identify a minimal set of *in vitro* assays to run when screening new chemicals for their activity. Future partners we may contact include OECD and APCRA.

Importance: This work will improve the ability of researchers to quickly query the most up-to-date scientific literature and *in vitro* ToxCast assay results to identify reliable reference chemicals in an automated manner. This will advance chemical screening abilities and will increase confidence in NAMs.

Subproducts
Sub-product type: Code package
Potential Title: R coding functions for querying and analyzing ToxCast data
Brief Description | 1-2 sentences: R Coding package release for querying and analyzing ToxCast data, including for identifying reference chemicals. FY22 Q4 – FY23 Q3

Sub-product type: Journal Article peer-reviewed with dataset
Potential Title: Automation of Reference Chemical Generation
Brief Description | 1-2 sentences: Will describe methods for researchers to use the R package and literature mining tools (such as Abstract Sifter and/or AbstractR) in order to automatically generate reference chemical lists. Manuscript will include the reference chemical lists we generate. FY23 Q4 – FY24 Q1

Team members
- Laura Taylor [primary contact]
- Richard Judson
- Bryant Chambers
- Menghang Xia
- Nicole Kleinstreuer
- Kamel Mansouri
- Ruili Huang

Budget
None needed

Timeline
Start: Q2 FY22
End: Q1 FY24

Other Gene Targets that were considered

Excluded because there are only two assay source groups
[minimum of three sources were necessary to be included in our list]

Candidate Target List	Gene Symbol(s)	Assay Sources
Acetylcholinesterase	AChE	NVS, TOX21
Cytochrome P450 Enzymes	CYP19A1 + CYP3A4	NVS, TOX21
G-Protein Coupled Receptor	GPCR	ERF, NVS
Transforming Growth Factor Beta 1	TGFB1	ATG, BSK

Assay Abbreviations ATG: Attagene, BSK: BSK Associates, ERF: Eurofins, NVS: NovaScreen, TOX21: Tox21

Androgen Receptor Reference Chemicals

Expert Curated List

in vitro *left image*
Kleinstreuer et al 2017

https://doi.org/10.1021/acs.chemrestox.6b00347

in vivo *right image*
Kleinstreuer et al 2018

https://doi.org/10.1016/j.reprotox.2018.08.017

Table 2. AR Pathway In Vitro Reference Chemicals

CASRN	chemical name	agonist potency category	antagonist potency category	in ToxCast 10/2015 release
52806-53-8	hydroxyflutamide	NA	strong	yes
90357-06-5	bicalutamide	NA	strong	yes
122-14-5	fenitrothion	NA	strong	yes
84371-65-3	mifepristone	NA	strong/moderate	yes
52-01-7	spironolactone	NA	strong/moderate	yes
63612-50-0	nilutamide	negative	moderate	yes
427-51-0	cyproterone acetate	weak	moderate	yes
80-05-7	bisphenol A	NA	moderate/weak	yes
330-55-2	linuron	NA	moderate/weak	yes
50471-44-8	vinclozolin	NA	moderate/weak	yes
13311-84-7	flutamide	negative	moderate/weak	yes
67747-09-5	prochloraz	negative	moderate/weak	yes
140-66-9	4-*tert*-octylphenol	NA	weak	yes
72-43-5	methoxychlor	NA	weak	yes
72-55-9	*p,p'*-DDE	NA	weak	yes
60207-90-1	propiconazole	NA	weak	yes
17924-92-4	zearalenone	NA	weak	yes
789-02-6	*o,p'*-DDT	negative	weak	yes
32809-16-8	procymidone	NA	very weak	yes
60168-88-9	fenarimol	negative	very weak	yes
58-18-4	methyl testosterone	strong	negative	yes
58-22-0	testosterone	strong	negative	propionate form
63-05-8	4-androstenedione	moderate	negative	yes
1912-24-9	atrazine	negative	negative	yes
52918-63-5	deltamethrin	negative	negative	yes
486-66-8	daidzein	NA	negative	yes
16752-77-5	methomyl	NA	negative	yes
122-34-9	simazine	NA	negative	yes
10161-33-8	17b-trenbolone	strong	NA	yes
797-63-7	levonorgestrel	strong	NA	yes
965-93-5	methyltrienolone (R1881)	strong	NA	no
68-22-4	norethindrone	strong	NA	yes
51-98-9	norethindrone acetate	strong	NA	no
76-43-7	fluoxymestrone	strong/moderate	NA	no
434-22-0	19-nortestosterone	moderate	NA	no
521-18-6	5a-dihydrotestosterone	moderate	NA	yes
10418-03-8	stanozolol	moderate	NA	no
71-58-9	medroxyprogesterone acetate	moderate/weak	NA	no
68-23-5	norethynodrel	moderate/weak	NA	no
57-91-0	17a-estradiol	negative	NA	yes
68359-37-5	b-cyfluthrin	negative	NA	yes
52315-07-8	b-cypermethrin	negative	NA	yes
17804-35-2	benomyl	negative	NA	yes
85-68-7	butylbenzyl phthalate	negative	NA	yes
10605-21-7	carbendazim	negative	NA	yes
51630-58-1	fenvalerate	negative	NA	yes
98319-26-7	finasteride	negative	NA	yes
129453-61-8	ICI 182,780	negative	NA	yes
36734-19-7	iprodione	negative	NA	yes
50-29-3	*p,p'*-DDT	negative	NA	yes
52645-53-1	permethrin	negative	NA	yes
501-36-0	resveratrol	negative	NA	no
10540-29-1	tamoxifen	negative	NA	yes
7696-12-0	tetramethrin	negative	NA	yes

Table 1

Summary of comparison between 39 chemicals with reproducible in vivo effects (green = androgenic, red = anti-androgenic, grey = negative) and results of ToxCast AR pathway model (green = agonist, red = antagonist, yellow = inconclusive, grey = negative). Abbreviations: *p,p'*-DDE = *p,p'*-dichlorodipheny chloroethylene, AUC = area under the curve (AR pathway model score), CI = confidence intervals, NA = no activity (in antagonist confirmation assa LOEL = lowest observed effect level, NOEL = no observed effect level, EAD = equivalent administered dose, ACC = activity concentration at cutoff, HCT = hig concentration tested.

Chemicals	CAS	In Vivo Reference Classification	AUC Agonist	AUC Antagonist	Antagonist Confidence Score	Confirmation Assay Flag	Overall AR Model Call	Concordance with In Vivo	HB LOEL range or NOEL (mg/kg/day)	EAD from range of in vitro ACC or HCT (mg/kg/day)
testosterone propionate	57-85-2	Androgenic	1.53	0	1	NA	Agonist	1	0.0125 – 0.25	1.6E-03 – 2
17-methyl testosterone	58-18-4	Androgenic	1.55	0	1	NA	Agonist	1	0.5 – 100	1.8E-06 – 0.4
trenbolone	10161-33-8	Androgenic	1.59	0	1	NA	Agonist	1	8 – 200	1.4E-07 – 0.8
flutamide	13311-84-7	Anti-androgenic	0	0.547	5	True antagonist shift (Hit/Hit)	Antagonist	1	0.1 – 100	0.06 – 26.1
linuron	330-55-2	Anti-androgenic	0	0.3	5	True antagonist shift (No hit/Hit)	Antagonist	1	10 – 100	0.9 – 41.3
vinclozolin	50471-44-8	Anti-androgenic	0	0.416	6	True antagonist shift (Hit/Hit)	Antagonist	1	10 – 100	1.5 – 129.8
procymidone	32809-16-8	Anti-androgenic	0	0.316	6	True antagonist shift (Hit/Hit)	Antagonist	1	3 – 100	0.7 – 57.2
p,p'-DDE	72-55-9	Anti-androgenic	0	0.251	4	True antagonist shift (No hit/Hit)	Antagonist	1	5 – 200	2.6 – 5
prochloraz	67747-09-5	Anti-androgenic	0	0.295	5	True antagonist shift (Hit/Hit)	Antagonist	1	50 – 250	0.6 – 12
finasteride	98319-26-7	Anti-androgenic	0	0	3	True antagonist shift (No hit/Hit)	Inconclusive	NA	0.008 – 25	43.6 – 787.9
propargite	2312-35-8	Anti-androgenic	0	0.187	1	FLAG: Wrong direction shift (Hit/Hit)	Inconclusive	NA	15 – 15	1.8 – 71
fenarimol	60168-88-9	Anti-androgenic	0	0.0446	4	True antagonist shift (Hit/Hit)	Inconclusive	NA	200 – 200	1.4 – 5.6
benfluralin	1861-40-1	Anti-androgenic	0	0.00012	0	NA	Inconclusive	NA	750 – 750	4.6 – 9
permethrin	52645-53-1	Anti-androgenic	0	0	0	NA	Negative	0	10 – 50	*13.2*
bis(2-ethylhexyl) phthalate (DEHP)	117-81-7	Anti-androgenic	0	0	0	NA	Negative	0	100 – 200	142.7 – 142.7
noflurazon	27314-13-2	Anti-androgenic	0	0	0	NA	Negative	0	1000 – 1000	*26.4*
ethoprop	13194-48-4	Anti-androgenic	0	0	0	NA	Negative	0	16 – 16	*333.3*
cyfluthrin	68359-37-5	Anti-androgenic	0	0	0	NA	Negative	0	18 – 50	*30.4*
iprodione	36734-19-7	Anti-androgenic	0	0	0	NA	Negative	0	200 – 200	*0.8*
pronamide	23950-58-5	Anti-androgenic	0	0	0	NA	Negative	0	200 – 200	7.7 – 7.7
trifluralin	1582-09-8	Anti-androgenic	0	0	0	NA	Negative	0	450 – 450	*10.3*
dibutyl phthalate (DBP)	84-74-2	Anti-androgenic	0	0	0	NA	Negative	0	500 – 1000	*67.4*
metolachlor	51218-45-2	Anti-androgenic	0	0	0	NA	Negative	0	500 – 500	238.6 – 427
carbofuran	1563-66-2	Negative	0	0	0	NA	Negative	1	*0.3*	*441*
oxamyl	23135-22-0	Negative	0	0	0	NA	Negative	1	*0.5*	*410.1*
esfenvalerate	66230-04-4	Negative	0	0	0	NA	Negative	1	*9*	*26.2*
2,4-dinitrophenol	51-28-5	Negative	0	0	1	FLAG: Antagonist shift, but CI overlap	Negative	1	*10*	1.2 – 1.3
chlorpyrifos	2921-88-2	Negative	0	0	0	NA	Negative	1	*12*	10.4 – 12.1
metribuzin	21087-64-9	Negative	0	0	0	NA	Negative	1	*120*	*418.5*
metalaxl	57837-19-1	Negative	0	0	1	NA	Negative	1	*375*	0.05 – 0.05
3-amino-1,2,4-triazole	61-82-5	Negative	0	0	0	NA	Negative	1	*1000*	641.9
flutolanil	66332-96-5	Negative	0	0	0	NA	Negative	1	*1000*	21.8 – 21.8
pentachloronitrobenzene (PCNB)	82-68-8	Negative	0	0	1	FLAG: Antagonist shift, but CI overlap	Negative	1	*1000*	0.3 – 4.1
methomyl	16752-77-5	Negative	0	0	3	True antagonist shift (No hit/Hit)	Inconclusive	NA	*1*	4.9E-04 – 0.7
abamectin	71751-41-2	Negative	0	0.388	1	FLAG: Wrong direction shift (Hit/Hit)	Inconclusive	NA	*5*	2.4 – 18.7
tetrachlorvinfos	22248-79-9	Negative	0	0.0315	3	FLAG: Antagonist shift, but CI overlap	Inconclusive	NA	*350*	17.9 – 70.2
folpet	133-07-3	Negative	0	0.141	1	FLAG: Wrong direction shift (Hit/Hit)	Inconclusive	NA	*800*	7.8 – 65.2
mgk-264	113-48-4	Negative	0	0.0935	2	FLAG: Antagonist shift, but CI overlap	Inconclusive	NA	*850*	2 – 7.6
chlorothalonil	1897-45-6	Negative	0	0.481	1	FLAG: Wrong direction shift (Hit/Hit)	Inconclusive	NA	*1000*	3.4 – 311.9

Estrogen Receptor Reference Chemicals

Expert Curated List

Browne et al 2015

https://doi.org/10.1021/acs.est.5b02641

Table 2. In Vitro Estrogen Receptor (ER) Agonist Reference Chemicals

CASRN	chemical name	agonist potency[a]	ToxCast ER model score
57−63−6	17α-Ethinyl estradiol	strong	1
84−16−2	*meso*-Hexestrol	strong	0.99
56−53−1	Diethylstilbestrol (DES)	strong	0.94
50−28−2	17β-Estradiol	strong	0.94
57−91−0	17α-Estradiol	moderate	1.06
53−16−7	Estrone	moderate	0.81
140−66−9	4-*tert*-Octylphenol	moderate	0.39
446−72−0	Genistein	weak	0.54
77−40−7	Bisphenol B	weak	0.49
80−05−7	Bisphenol A	weak	0.45
486−66−8	Daidzein	weak	0.44
521−18−6	5α-Dihydrotestosterone	weak	0.40
789−02−6	*o,p′*-DDT	weak	0.39
599−64−4	4-Cumylphenol	weak	0.38
143−50−0	Kepone	weak	0.17
58−18−4	17α-Methyltestosterone	very weak	0.50
520−36−5	Apigenin	very weak	0.31
72−43−5	Methoxychlor	very weak	0.25
520−18−3	Kaempferol	very weak	0.25
85−68−7	Butylbenzyl phthalate	very weak	0.18
480−40−0	Chrysin	very weak	0.13
60168−88−9	Fenarimol	very weak	0.11
104−40−5	*p-n*-Nonylphenol	very weak	0.1
120−47−8	Ethylparaben	very weak	0.1
72−55−9	*p,p′*-DDE	very weak	0.1
84−74−2	Di-*n*-butyl phthalate	very weak	0.03
115−32−2	Dicofol	very weak	0
117−81−7	Diethylhexyl phthalate	very weak	0
52−86−8	Haloperidol	inactive	0.01
52−01−7	Spironolactone	inactive	0
50−22−6	Corticosterone	inactive	0
13311−84−7	Flutamide	inactive	0
1912−24−9	Atrazine	inactive	0
32809−16−8	Procymidone	inactive	0
330−55−2	Linuron	inactive	0
50−55−5	Reserpine	inactive	0
52806−53−8	Hydroxyflutamide	inactive	0
57−30−7	Phenobarbital Sodium	inactive	0
65277−42−1	Ketoconazole	inactive	0
66−81−9	Cycloheximide	inactive	0

[a]Reference chemical potency, determined by concentration required to elicit 50% of the maximal response (AC_{50}), in low throughput in vitro ER assays.[28,29] Strong = AC_{50} < 0.0001 μM, moderate = AC_{50} < 0.1 μM, weak = AC_{50} < 1 μM, very weak = all other activities, and inactive = no detected activity.[29]

Table 3. In Vivo Estrogen Receptor (ER) Agonist Reference Chemicals with at Least Two Independent Active or Inactive Guideline-Like Uterotrophic Studies[a,14]

CASRN	name	active	inactive	bioactivity	ToxCast ER model score
57−91−0	17α-Estradiol	2	0	active	1.06
57−63−6	Ethinyl Estradiol	59	0	active	1
56−53−1	Diethylstilbestrol (DES)	8	1	active	0.94
50−28−2	Estradiol	25	0	active	0.94
474−86−2	Equilin	2	0	active	0.82
53−16−7	Estrone	9	0	active	0.81
50−27−1	Estriol	4	0	active	0.79
72−33−3	Mestranol	3	0	active	0.74
17924−92−4	Zearalenone	4	0	active	0.71
1478−61−1	Bisphenol AF	4	0	active	0.55
446−72−0	Genistein	27	1	active	0.54
68−22−4	Norethindrone	2	0	active	0.52
58−18−4	Methyltestosterone	3	0	active	0.50
77−40−7	Bisphenol B	2	0	active	0.49
80−05−7	Bisphenol A	37	6	active	0.45
104−43−8	4-Dodecylphenol	3	0	active	0.41
521−18−6	Dihydrotestosterone	3	0	active	0.4
131−55−5	Benzophenone-2	6	0	active	0.40
140−66−9	4-(1,1,3,3-Tetramethylbutyl)phenol	3	1	active	0.39
789−02−6	*o,p′*-DDT	15	1	active	0.39
599−64−4	*p*-Cumylphenol	2	0	active	0.38
5153−25−3	Benzoic acid, 4-hydroxy-, 2-ethylhexyl ester	2	0	active	0.37
80−46−6	4-(1,1-Dimethylpropyl)phenol	4	0	active	0.28
131−56−6	2,4-Dihydroxybenzophenone	3	0	active	0.27
80−09−1	Bisphenol S	2	0	active	0.26
72−43−5	Methoxychlor	18	1	active	0.25
94−26−8	Butylparaben	8	2	active	0.25
98−54−4	*p-tert*-Butylphenol	2	0	active	0.16
104−40−5	Nonylphenol	5	4	active	0.10
556−67−2	Octamethylcyclotetrasiloxane	3	0	active	0
520−18−3	Kaempferol	0	3	inactive	0.25
84−74−2	Dibutyl phthalate	0	2	inactive	0.03
84−61−7	Dicyclohexyl phthalate	0	2	inactive	0.02
84−75−3	Dihexyl phthalate	0	2	inactive	0.01
51630−58−1	Fenvalerate	0	2	inactive	0.01
103−23−1	Bis(2-ethylhexyl)hexanedioate	0	2	inactive	0
117−81−7	Bis(2-ethylhexyl)phthalate	0	2	inactive	0
1461−22−9	Tributylchlorostannane	0	2	inactive	0
1912−24−9	Atrazine	0	2	inactive	0
61−82−5	Amitrole	0	2	inactive	0
84−66−2	Diethyl phthalate	0	2	inactive	0
87−86−5	Pentachlorophenol	0	2	inactive	0
99−96−7	4-Hydroxybenzoic acid	0	2	inactive	0

[a]The numbers of guideline-like active and inactive study results are reported for each chemical.

Judson, Richard, et al. "Selecting a minimal set of androgen receptor assays for screening chemicals." *Regulatory Toxicology and Pharmacology* 117 (2020): 104764.

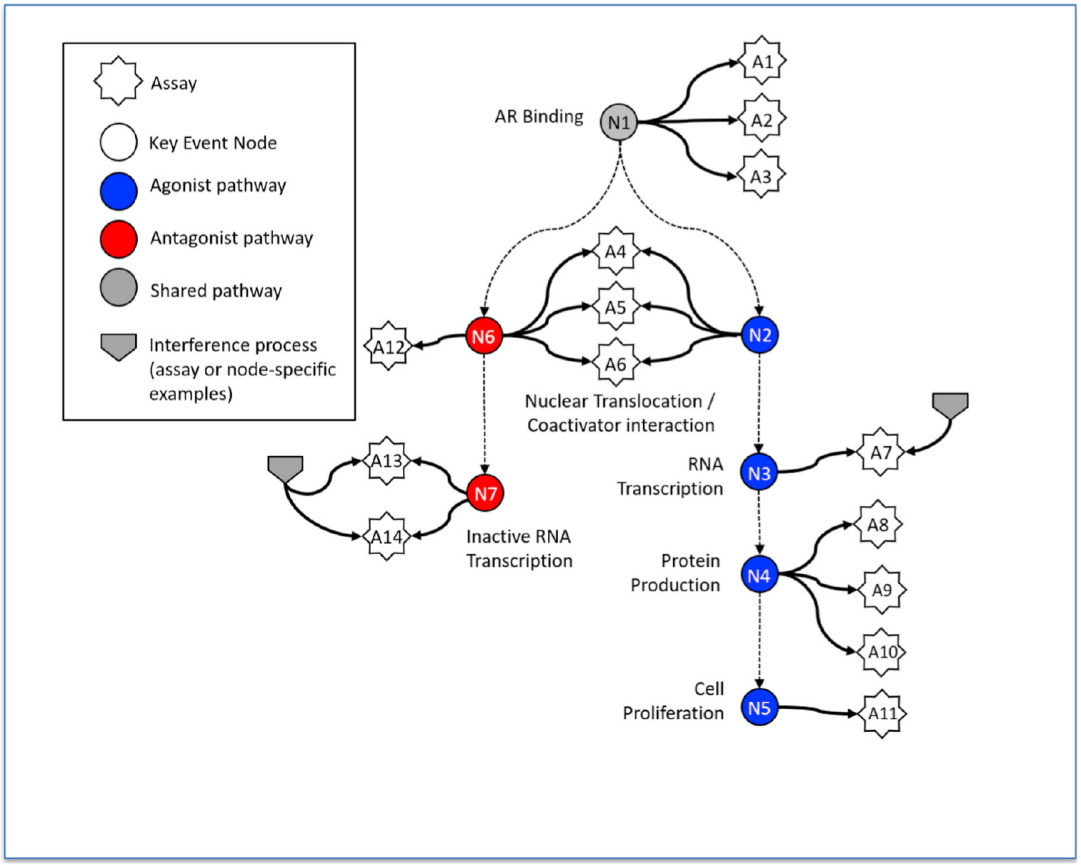

Fig. 1. Diagram indicating the nodes in the AR signaling pathway associated with the assays. White stars indicate assays and correspond to the rows of Table 1. N1–N7 nodes correspond to processes in the underlying biological pathway, where blue is agonism, red is antagonism, and grey is common to both agonism and antagonism. Two representative assay interference processes are indicated, one affecting a single assay (A7) and another affecting a pair of related assays (A13 and A14). All single assays and groups of assays have their corresponding assay interference processes represented in the underlying mathematical model. See Kleinstreuer et al., 2017 for further details (Kleinstreuer et al., 2017). The strengths of these interference processes are indicated by the corresponding AUC values in Supplemental File S2. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)