

National Institute of **Environmental Health Sciences**

Division of Translational Toxicology

Integration of Technological Interference into Curated HTS Data V. Hull¹, A. Borrel¹, A.L. Karmaus^{1*}, D.G. Allen¹, N. Kleinstreuer² ¹Inotiv, RTP, NC, USA; ²NIH/NIEHS/DTT/NICEATM, RTP, NC, USA

Introduction

- Highly curated, robust data are essential for building confidence in new approach methodologies. However, large quantities of data generated by high-throughput screening (HTS) assays such as the U.S. Environmental Protection Agency's (EPA's) Tox21/ToxCast programs can be difficult to interpret.
- To increase confidence in HTS bioactivity calls, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) has derived curated HTS (cHTS) data from EPA's invitrodb v3.5 (Feshuk et al. 2022).
 - This data set incorporates curation flags for chemical quality control, curve fit, and assay performance, as well as additional levels of expert review.
 - cHTS data are available through NICEATM's Integrated Chemical Environment (ICE; https://ice.ntp.niehs.nih.gov/).
- Technological interference refers to an assay response that is driven by the interaction of chemicals with the assay technology rather than by true bioactivity.
 - Chemical structure can result in false signals for fluorescence through quenching or autofluorescence, and for luminescence through luciferase inhibition.
 - Many assays in Tox21/ToxCast use either luminescence or fluorescence readouts which are susceptible to this type of interference.
- This poster presents the approach to integrate technological interference into the ICE cHTS curation pipeline and ultimately into ICE Tools.
 - The ICE interference flags will allow users to make more informed decisions when interpreting bioactivity calls.

Summary

- Technological interference is an important consideration in the interpretation of fluorescence and luminescence assays.
- Flags identifying such interference are being incorporated into the ICE cHTS pipeline to highlight potential limitations in data interpretation.
- We searched EPA's invitrodb for luminescence and fluorescence assay detection technologies in Tox21 and ToxCast data. Tox21 interference-specific assays and InterPred predictions were used to determine interferent chemicals in these assays.
- We created eight new warning flags for potentially problematic chemical/assay endpoint pairs and incorporated them into the cHTS dataset. These flags allows users to more critically interpret bioactivity calls that could be false positives.
- Warning flags for technological interference will soon appear in ICE cHTS dataset downloads and the ICE Curve Surfer tool.

Methods: Sourcing Interferent Chemicals

Methods: Workflow for Flag Generation

- To identify potential interferent chemicals, we used data from Tox21 assays specifically designed to test luciferase and auto-fluorescence interference (Figure 1; Borrel et al. 2020a).
- These assays were used to test chemicals in the Tox21 10k library for luciferase inhibition and autofluorescence. The autofluorescence assays evaluated red, blue, and green fluorescence across HepG2 and HEK293 cell lines.
- InterPred is an open-access quantitative structure-activity relationship (QSAR) modeling workflow developed to predict luciferase inhibition and autofluorescence under blue, green, and red wavelengths.
- For ~500 ToxCast chemicals that were not tested in the Tox21 interference assays, we used the InterPred tool to predict luciferase and fluorescence interference (Figure 2; Borrel et al. 2020b; https://sandbox.ntp.niehs.nih.gov/interferences/).

	Calendar & Events News & Media	Get Involved				
Netional Toxicale and Program	Support		Chemic	cals	Luciferase	
U.S. Department of Health and Human Services	Q Search the NTP Website	SEARCH	ID	SMILES	M-Lucifera	SD
			1	COc1cnccc10	0.09	0.03
Therefore the compute interferences Help			2	Oc1ccnc(O)c1	0.09	0.04
			3	Oc1cccnc1	0.15	0.04
InterPred: Prediction of Chemical-Assay Interference This work represents one of the largest screening efforts to date specifically intended to identify and characterize chemical-assay interference via luciferase inhibition and autofluorescence, and to interrogate the influence of cell types and culture conditions.			e probabil	ity of a	0.15	0.05
				c(0)c1	0.16	0.06
			chemical being luciferase interferent.			0.03
The resulting predictive models can be used to predict interference potential of new chemicals, and to provide insight into structural features that may influence activity and inform		dinform	Chemicals are			0.04
molecular design and assay selection.			considered to be likely			0.08
Please cite Research publication: High-Throughput Screening to Predict Chemical-Assay Interference, Scientific Reports approbability > 0.50.					0.58	0.07
					0.52	0.05
				² 0.50.	0.08	0.02
Webserver publication: InterPred: a webtool to predict chemical autofluorescence and lumin		ence,	13	Cn1c(=S)[nH]c2ccccc21	0.32	0.04
<u>NAR webserver</u>			14	CCCCOC(=0)c1ccccc10	0.61	0.07
* This website is free and open to all users and there is no login requirement.			15	O=[N+]([O-])c1ccc2[nH]c(S)nc2	0.29	0.05

Figure 2: Left panel shows home page of the InterPred tool. Right panel shows InterPred output, which provides chemical structure in Simplified Molecular-Input Line-Entry System (SMILES) format ("SMILES"), the chemical's probability of being interferent ("M-Luciferase"), the standard deviation of probabilities from multiple random forest models ("SD"), and whether or not a chemical is present in the Tox21 library ("Included").

Results: Integrating Interference Flags into ICE Tools

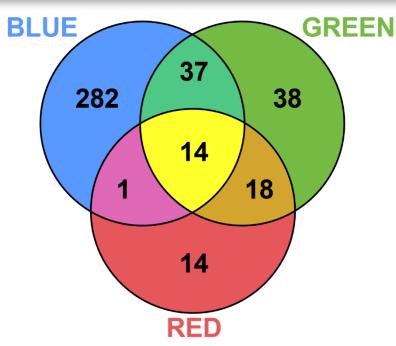


Figure 1: Venn Diagram of interferent chemicals by fluorescence color. Adapted from Borrel et al. 2020a.

	Chemicals		Luciferase	Luciferase	
	ID SMILES		M-Lucifera	SD	Included
	1	COc1cnccc1O	0.09	0.03	0
	2	Oc1ccnc(O)c1	0.09	0.04	0
	3	Oc1cccnc1	0.15	0.04	0
n	robability of a mical being ase interferent. emicals are ered to be likely interferent if ability > 0.50.		0.15	0.05	0
			0.16	0.06	0
			0.1	0.03	0
			0.07	0.04	0
			0.46	0.08	0
			0.58	0.07	0
			0.52	0.05	0
aD	mty -	0.50.	0.08	0.02	0
	13	Cn1c(=S)[nH]c2ccccc21	0.32	0.04	0
				0.07	-

Step 1: Retrieved Tox21 and ToxCast data

• Data from EPA's invitrodb v3.5 (Feshuk et al. 2022) using EPA's tcpl v.2.1.0 R package (Filer et al. 2017).

Step 2: Identified assay endpoints that used luciferase and fluorescence detection technologies

- Reviewed "detection technologies" descriptions within invitrodb v3.5 to identify assays that used luminescence and fluorescence to determine readouts.
- Reviewed additional methods descriptions within invitrodb v3.5, assay information from the National Institutes of Health Tripod website (https://tripod.nih.gov/tox/), and relevant references to determine which assays used luciferase and the wavelength/color of fluorescence. Assays that used fluorescence other than green, red, or blue were excluded from flagging.

Step 3: Determined which chemicals were tested in assay endpoints from Step 2

• Used tcpl to pull chemical lists from invitrodb v3.5.

Step 4: Identified which chemicals from Step 3 were potentially interferent

- Cross-referenced chemical lists with chemicals identified as luciferase-inhibiting or autofluorescent in the Tox21 interference assays.
- Generated InterPred predictions for ToxCast chemicals that were not tested in the Tox21 interference assays.

Step 5: Assigned interference flags to chemical-assay endpoint pairs

• If a chemical was interferent for a technology and the assay endpoint used that technology, a flag was created and incorporated into the cHTS data.

Results: Number of Interferent Assays and Chemicals

• Future ICE Curve Surfer Tool results will integrate interference flags (Figure 3). This tool allows users to explore cHTS concentrationresponse curves, under which text fields provide users details pertaining to the results, including interference warning flag(s).

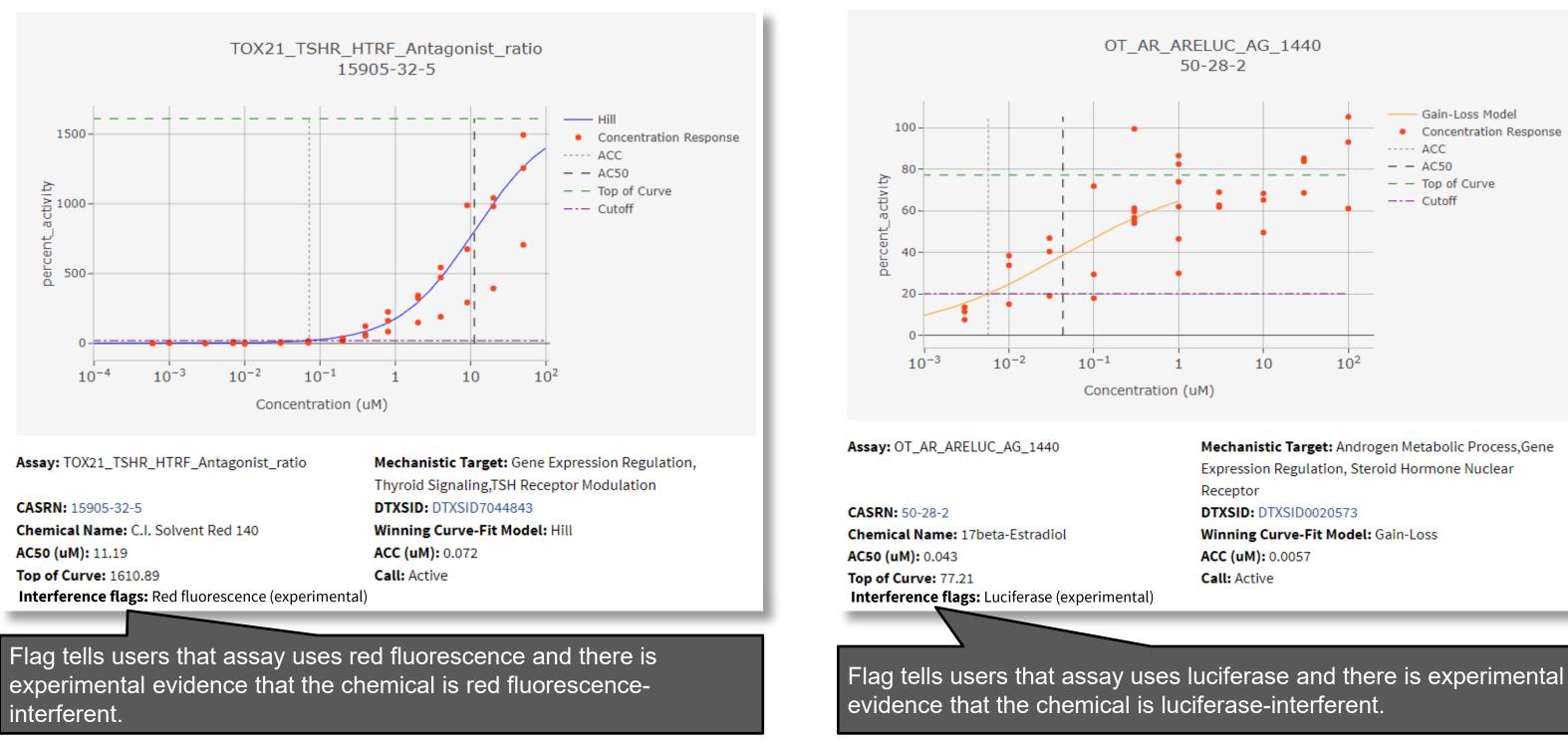


Figure 3: Example of how interference flags will be incorporated into future updates of the ICE Curve Surfer tool.

Detection Technology Type	Number of Assay Endpoints	Number of Interferent Chemicals	Number of Positive Calls
Luciferase	120	567	10,669
Blue fluorescence only	301	330	1,375
Green fluorescence only	277	109	865
Red fluorescence only	24	45	42
Blue and green fluorescence	68	55	260
Red and green fluorescence	17	35	36

Blue fluorescence was the most prevalent detection technology and had the most interferent chemicals. Red fluorescence was the least prevalent technology and had the fewest interferent chemicals.

We created eight interference flags:

- Blue fluorescence interferent (experimental)
- Blue fluorescence interferent (predicted)
- Green fluorescence interferent (experimental)
- Green fluorescence interferent (predicted)
- Red fluorescence interferent (experimental)
- Red fluorescence interferent (predicted) 6.
- Luciferase interferent (experimental)
- 8. Luciferase interferent (predicted)
- Interference flags will be provided as warning flags and do not affect bioactivity calls.
 - This differs from the ICE QC-Omit and Flag-Omit flags, which override the bioactivity calls and recommend that the assay endpoint-chemical pair be omitted.



 Gain-Loss Mode Concentration Response

- ACC

- - AC50

--- Cutoff

ICE Data Sets	Data Sets					
Data Sets	ICE contains data sets curated for targeted toxicity endpoints by <u>NICEATM</u> , <u>ICCVAM</u> , and their partner organizations. ICE also contains other data sets that may be useful in evaluating or developing new approaches for assessing chemical safety.					
Acute Lethality	Click zip file icon to					
Cancer	contains information on the download ays and chemicals included in each data set can be					
Cardiotoxicity	selected to query data in Sealer or the sealer o					
DART	Data in ICE are mostly organized at the contains of regulatory interest and assay type. ICE also includes a data set drawn from 2.1.0 at time of recase.					
Endocrine						
Irritation-Corrosion	CHTS 🕼 Data generated by the Tox21 high-through screening program, including data from the					
Sensitization	EPA ToxCast program, and curater by NICEATM. All cHTS data in ICE were retrieved from invitrodb v3.4 (October 2021), as analyzed using the ToxCast Pipeline (tcpl, version 2.0.2)					
снтѕ	processing algorithm. Learn more					
	Data SetsAcute LethalityCancerCardiotoxicityDARTEndocrineIrritation-CorrosionSensitization					

DTXSID7025635	TOX21_DT40_657	Technological interference	luciferase (experimental)	invitroDBv3.5	https://doi.org/10.23645/epacomptox.6062623.v8
DTXSID6045953	TOX21_PXR_viability	Technological interference	blue (experimental)	invitroDBv3.5	https://doi.org/10.23645/epacomptox.6062623.v8
6 DTXSID7032424	BSK_KF3CT_TIMP2_up	Technological interference	blue (experimental)	invitroDBv3.5	https://doi.org/10.23645/epacomptox.6062623.v8
1 DTXSID6041682	CCTE_Harrill_HTTr_Viability_MCF7_DMEM_6hr	Technological interference	red (experimental)	invitroDBv3.5	https://doi.org/10.23645/epacomptox.6062623.v8
4 DTXSID5044782	ATG_AP_2_CIS_up	Technological interference	green (experimental)	invitroDBv3.5	https://doi.org/10.23645/epacomptox.6062623.v8
4 DTXSID7020425	TOX21_p53_BLA_p1_ratio	Technological interference	blue;green(experimental)	invitroDBv3.5	https://doi.org/10.23645/epacomptox.6062623.v8
4 DTXSID4032372	BSK_hDFCGF_PAI1_down	Technological interference	blue (experimental)	invitroDBv3.5	https://doi.org/10.23645/epacomptox.6062623.v8
2 DTXSID0025234	TOX21_MMP_ratio_up	Technological interference	red;green(experimental)	invitroDBv3.5	https://doi.org/10.23645/epacomptox.6062623.v8
2 DTXSID3032541	TOX21_RT_HEK293_GLO_00hr_viability	Technological interference	luciferase (experimental)	invitroDBv3.5	https://doi.org/10.23645/epacomptox.6062623.v8
	0 DTXSID6045953 16 DTXSID7032424 11 DTXSID6041682 14 DTXSID5044782 14 DTXSID7020425 14 DTXSID4032372 15 DTXSID0025234	DTXSID6045953TOX21_PXR_viabilityDTXSID6045953TOX21_PXR_viabilityDTXSID7032424BSK_KF3CT_TIMP2_upDTXSID6041682CCTE_Harrill_HTTr_Viability_MCF7_DMEM_6hrDTXSID5044782ATG_AP_2_CIS_upDTXSID7020425TOX21_p53_BLA_p1_ratioDTXSID4032372BSK_hDFCGF_PAI1_downDTXSID0025234TOX21_MMP_ratio_up	0DTXSID6045953TOX21_PXR_viabilityTechnological interference16DTXSID7032424BSK_KF3CT_TIMP2_upTechnological interference17DTXSID6041682CCTE_Harrill_HTTr_Viability_MCF7_DMEM_6hrTechnological interference18DTXSID5044782ATG_AP_2_CIS_upTechnological interference14DTXSID7020425TOX21_p53_BLA_p1_ratioTechnological interference14DTXSID4032372BSK_hDFCGF_PAI1_downTechnological interference14DTXSID4032372TOX21_MMP_ratio_upTechnological interference	DTXSID6045953TOX21_PXR_viabilityTechnological interferenceblue (experimental)DTXSID7032424BSK_KF3CT_TIMP2_upTechnological interferenceblue (experimental)DTXSID6041682CCTE_Harrill_HTTr_Viability_MCF7_DMEM_6hrTechnological interferencered (experimental)DTXSID5044782ATG_AP_2_CIS_upTechnological interferencegreen (experimental)DTXSID7020425TOX21_p53_BLA_p1_ratioTechnological interferenceblue;green(experimental)DTXSID4032372BSK_hDFCGF_PAI1_downTechnological interferenceblue;green(experimental)DTXSID0025234TOX21_MMP_ratio_upTechnological interferenceblue (experimental)	DDTXSID6045953TOX21_PXR_viabilityTechnological interferenceblue (experimental)invitroDBv3.5DTXSID7032424BSK_KF3CT_TIMP2_upTechnological interferenceblue (experimental)invitroDBv3.5DTXSID6041682CCTE_Harrill_HTTr_Viability_MCF7_DMEM_6hrTechnological interferencered (experimental)invitroDBv3.5DTXSID5044782ATG_AP_2_CIS_upTechnological interferencegreen (experimental)invitroDBv3.5DTXSID7020425TOX21_p53_BLA_p1_ratioTechnological interferenceblue;green(experimental)invitroDBv3.5DTXSID4032372BSK_hDFCGF_PAI1_downTechnological interferenceblue (experimental)invitroDBv3.5DTXSID0025234TOX21_MMP_ratio_upTechnological interferencered;green(experimental)invitroDBv3.5

Response shows the type of interference flag, shown here with color-coding. Assays can have more than one flag if more than one fluorescence color is used.

Figure 4: Assay technological interference information in cHTS download file, planned for a future release of ICE.

References

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