ICE Curve Surfer Tool

Introduction

The ICE Curve Surfer tool allows you to view and interact with concentration–response curves from the ICE curated high-throughput screening (cHTS) data. This tool takes a chemical list and/or assay group selection as input to return a set of concentration–response curves that can be filtered based on chemical, assay name, assay system, response units, mechanistic target, bioactivity call, technological interference, and curve characteristics such as AC50 and top-of-curve. Both graphics and data can be exported for other applications outside of ICE.

Located throughout the tool are green information buttons to help you better understand key features and results. When you hover over a button, brief explanatory text will appear. When you click a button, more details will appear in a text box that can be resized and relocated on the screen.

The “Help” button on the left side of the display opens a text box with a brief description of the tool and links to the webpage and downloadable user guides. Below this, clicking on the "Help Video" button will provide access to videos demonstrating how to build a query and evaluate results.

If you encounter a problem using a tool, click the “Report an Issue” button below the “Help” button to generate an email to ICE Support. Click the “Cite ICE” button to view example citation formats for referencing ICE in your research publication that utilizes ICE data or outputs from ICE tools.

Building a Curve Surfer Query

Figure 1 shows the default ICE Curve Surfer tool Input view. You can toggle between the Input view and Results view by clicking tabs on the left side of the screen. The tool window defaults to the Input view when it is first opened.

To run a Curve Surfer query, you must specify at least one chemical. Queries can include one or more assay groups.

- Specifying only chemicals and leaving the data input field blank will return all available data for those chemicals for all assays within cHTS.
- Specifying both chemicals and assays will return available data only from specified assays for the specified chemicals.

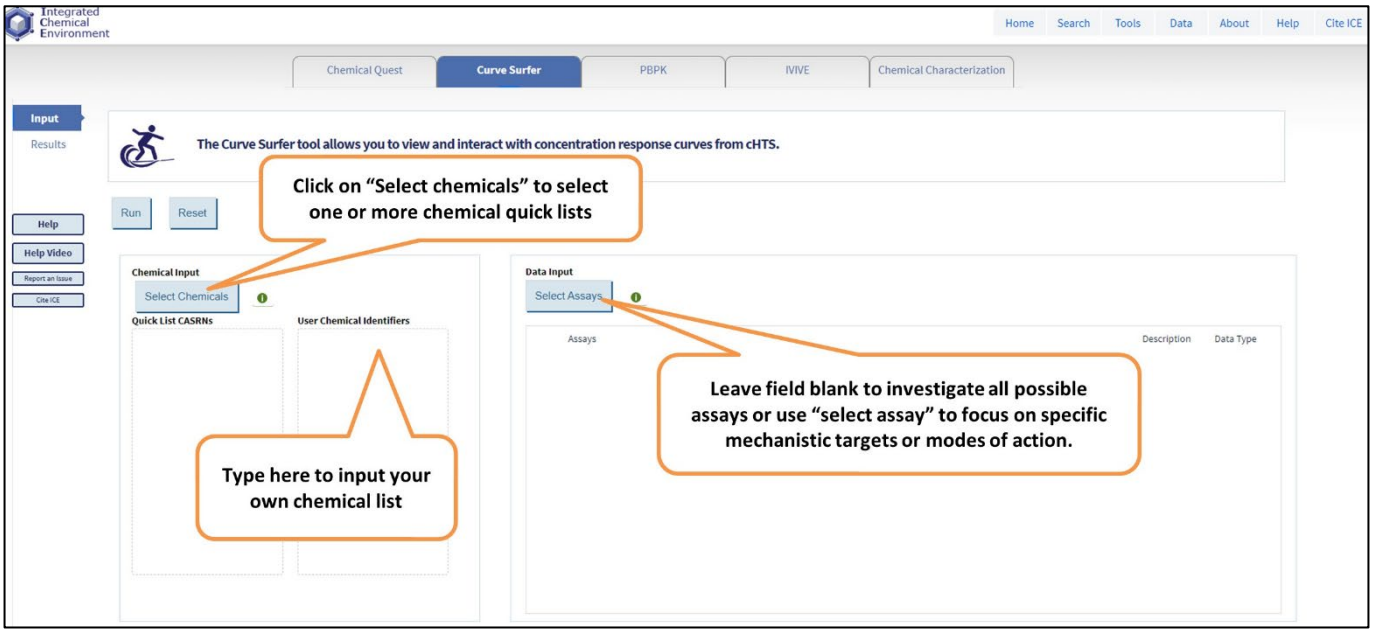

Figure 1. ICE Curve Surfer tool Input view.

Chemical Input

The Chemical Input field on the left side of the Input view (**Figure 2**) has two text boxes into which you can enter chemicals. Use one or both input methods to enter chemicals. Curve Surfer visualizations can be generated for all chemicals that have cHTS data in ICE. However, because Curve Surfer returns one concentration–response curve for every chemical–assay combination in a query, outputs can become very large. Therefore, we suggest limiting the number of chemicals included in a query to approximately 100. For queries involving more than that, we recommend using ICE REST API functionality to obtain Curve Surfer results. Information about how to use the ICE REST API is available on ICE REST API User Guide page.

- To populate the left-hand text box, select one or more ICE Chemical Quick Lists by clicking the "Select Chemicals" button. In the dialog box that opens, select the checkboxes to choose one or more chemical lists. Click "Finished" when you are done. Chemical Abstracts Service Registry Numbers (CASRNs) from the selected ICE Chemical Quick Lists will populate the Quick List CASRNs text box.
- While in the dialog box, you can download ICE Chemical Quick Lists by clicking on the download icons to the right of the list names.
- Each download provides the list of chemicals along with additional metadata relevant to the Chemical Quick List.

- To populate the right-hand text box, enter your own list of chemical identifiers, one per row, in the "User Chemical Identifiers" text box. You can use any combination of the following identifiers:
    - CASRNs.
    - Chemical names.
    - Distributed Structure-Searchable Toxicity Substance Identifiers (DTXSIDs).
    - Simplified molecular-input line-entry system (SMILES) strings.
    - Hashed International Chemicals Identifiers (InChIKeys).

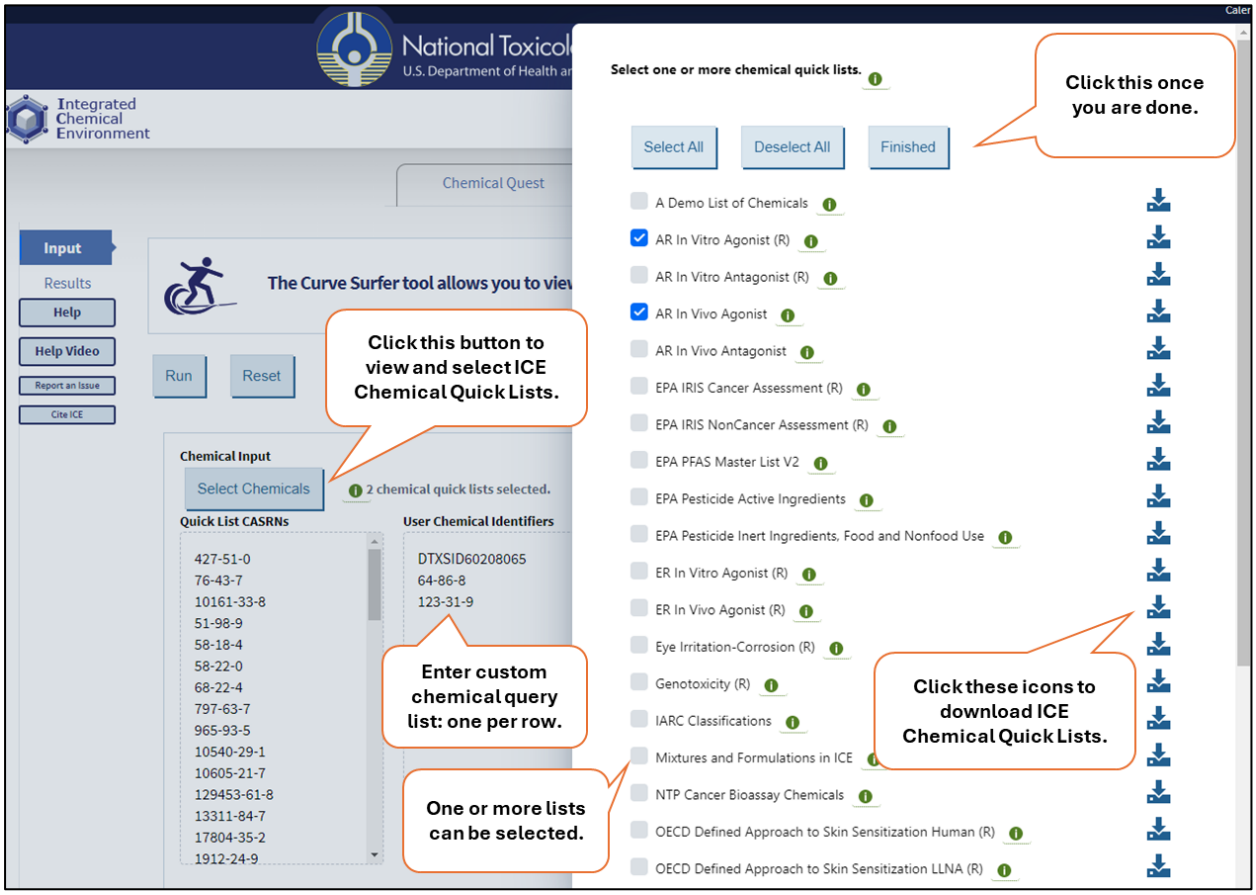

Figure 2. Input chemicals by typing chemical identifiers into the text box (right background) and/or selecting ICE Chemical Quick Lists (left background). Dialog box for selecting Chemical Quick Lists is in the foreground.

Data Input

Select ICE assay data for your query in the "Data Input" field on the right (**Figure 3**). Click the "Select Assays" button, which will open a dialog box offering two options for selecting assays. Assay selection is limited to ICE cHTS data (discussed in **Appendix 1**).

- Under the “cHTS” tab is a list of high-throughput assays that are organized by mechanistic target terms. Under the “Mode of Action” tab assays are organized by mode of action relevant to toxicity endpoints of regulatory concern.

You can add assays from both the “cHTS” and “Mode of Action” tabs to your query; clicking on one tab will not change or remove your selections on the other tab.

Within each category, assays are organized in a parent-child hierarchical relationship. Expanding the parent term using the arrowheads will reveal additional child terms. Selecting a parent term will automatically select all child assays under that term.

When you are finished selecting assays, click on "Finished". The assay categories you have selected will be displayed in the “Data Input” box. To add more assays, you will need to click “Select Assays” again. To remove assays from the query, deselect the checkbox to the left of the assay groups. The assay will be removed from the Data Input box.

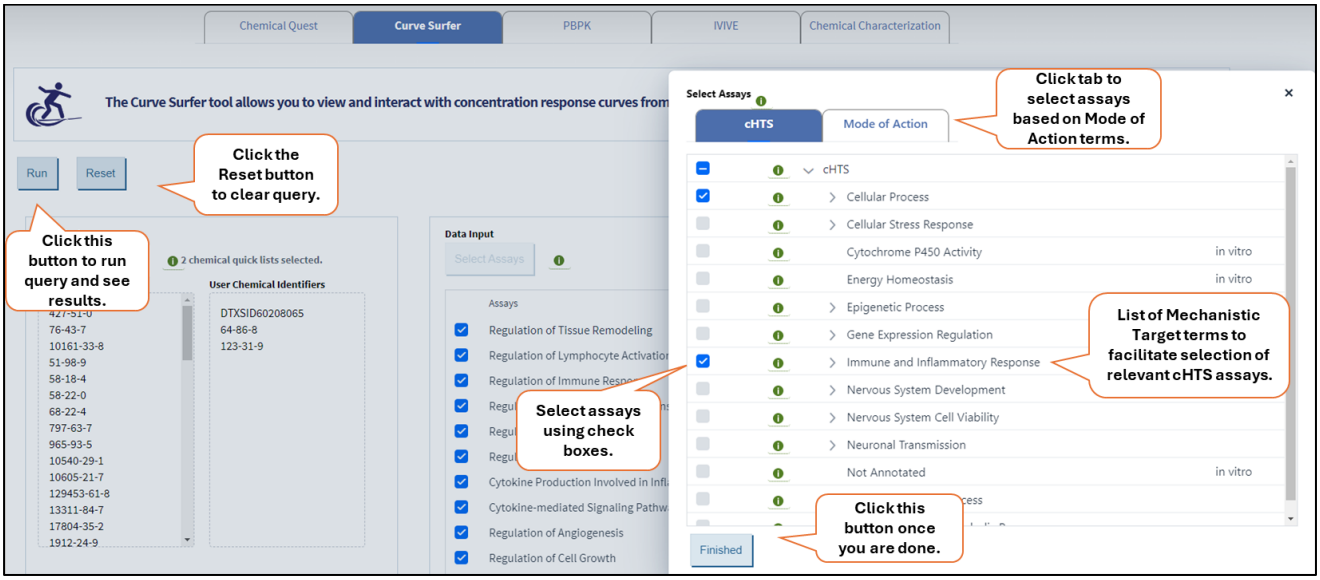

Figure 3. Specify assay groupings to query.

Run Query

Click on "Run" to run your query. To reset your query parameters, select "Reset".

View and Sort Curve Surfer Results

The window will switch to “Results” view when the query is run (**Figure 4**). Click “Input” in the top left to return to Input view to review or change your query parameters and rerun your query.

Each graph displayed in Results view shows one chemical–assay concentration–response curve. The Results view will show 10 curves per page. You can view the page count and select or browse pages by clicking the arrows or dropdown list under “Select Page.”

More information about interaction with individual curves is provided in “Interacting with Plots” section

below.

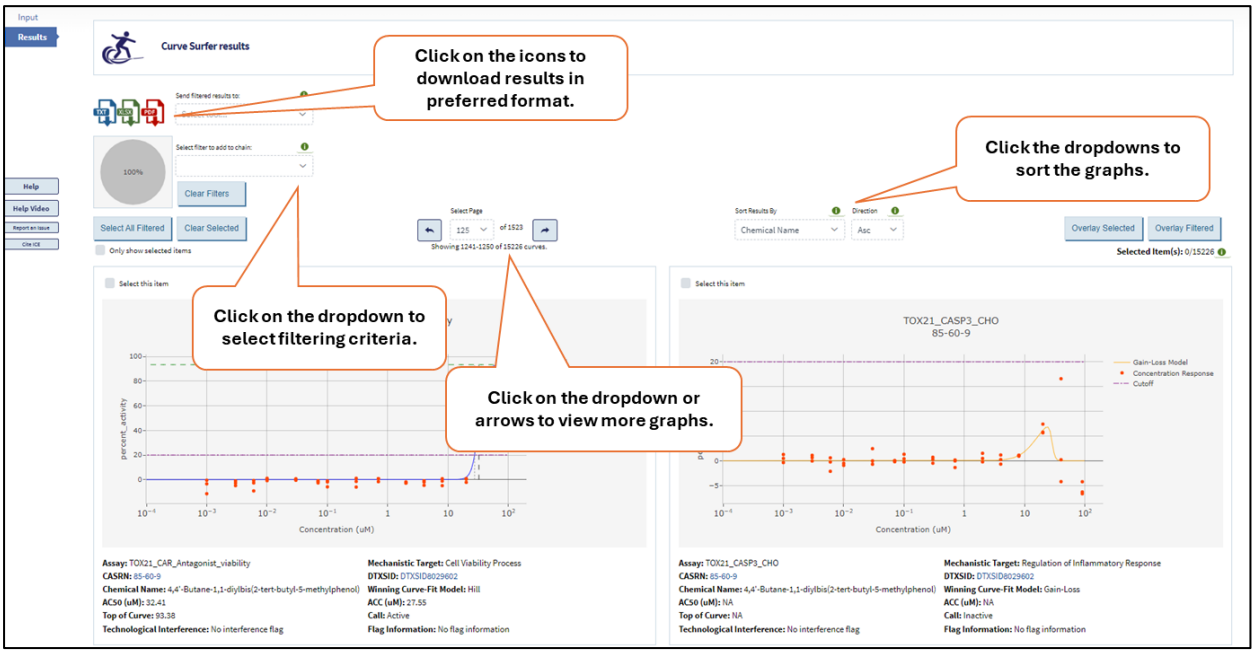

Figure 4. ICE Curve Surfer tool Results view.

Sorting and Filtering Curve Surfer Results

Click the "Sort Results By" dropdown list to choose from seven sorting criteria, and "Direction" to display curves in ascending ("Asc") or descending ("Desc") order.

In addition to sorting, you can construct filter chains to narrow down results to view. To add a filter, click the "Select Filter to add to chain" dropdown and choose a filter type from the list. When you select a filter, a dialog box will open. Details on how to use each filter can be found by clicking the green information buttons icons within each dialog box.

- Text-based filters (**Figure 5A**) will display options with selectable checkboxes and show statistics including the number and percentage of results that fall within each option. Text-based filters also have a text box at the top of the list of options. Type in this text box to view available options containing that text string. Select the checkboxes to select item(s) of interest.

- Numerical filters (**Figure 5B**) allow you to use sliders or text boxes to set the desired range.

After the desired parameters have been added to the filter, click "Close" on the bottom right to apply the filter.

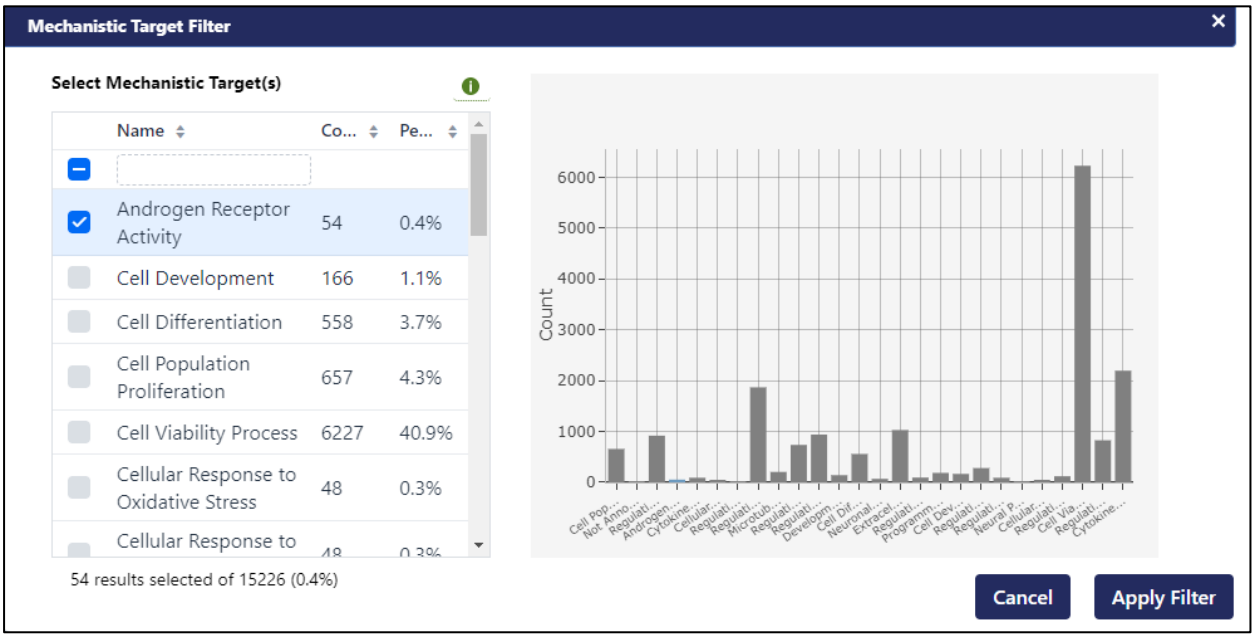

Figure 5A. Text-based filter example with checkbox selection.

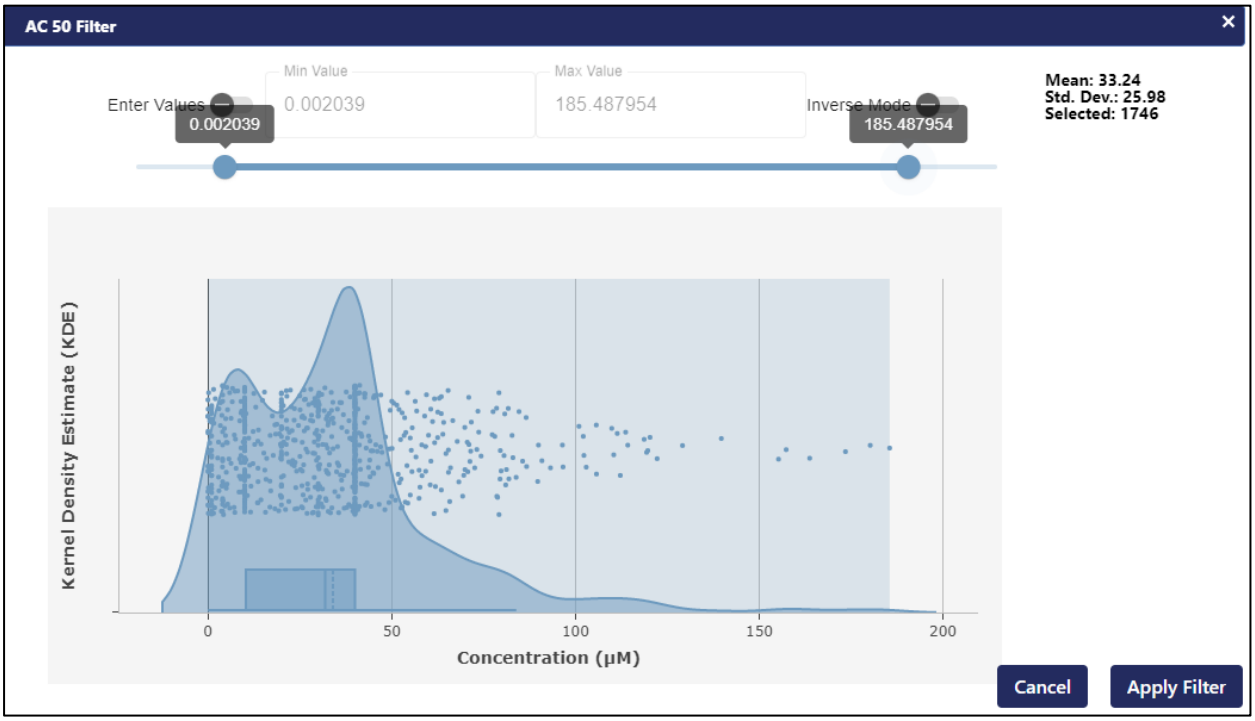

Figure 5B. Numerical range filter example with range slider selection.

The available filters are:

- Mechanistic Target: Assay groupings based on biological processes to facilitate assay interpretation. For more information on mechanistic targets, visit the ICE Data Sets page and click “cHTS” on the left menu.

- Call: Active, Inactive, Flag-Omit, or QC-Omit.
    - Flag-Omit: data omitted due to concentration–response curve filter.
    - QC-Omit: data omitted due to chemical QC filter.
- The reason for the Flag-Omit or QC-Omit are given in the "Flag Information" filter option on the "Flag Information" field on the results card. Detailed explanations for these curation criteria are available on the ICE Data Sets page under "cHTS".
- Assay Text: Filter by complete or partial assay names to display treemap distributions that show the count and percentage for each assay, as well as results for search text matches.
- Assay: Filter by assay names of interest to obtain the count and percentage for each assay, along with the percentage of assay records for the selected assays.
- CASRN: Filter based on Chemical Abstracts Services Registry Number chemical identifier.
- Chemical Name: Filter based on complete chemical name text.
- DTXSID: Filter based on DSSTox Substance Identifier chemical identifier.
- SMARTS: Filter chemicals with the same substructures.
- Assay System: Filter results based on the cell type (or cell-free media, where applicable) used in the assay. More information about assays can be found on the U.S. Environmental Protection Agency (EPA)CompTox Chemicals Dashboard.
- Assay Response Unit: Filter results based on the response units of the assay readout, including percent activity, fold change, log2 fold change, and log10 fold change.
- AC50: Filter curves based on a custom range of AC50 values (the concentration at which a half-maximal response is generated). This filter is a range filter and displays the data density on a violin plot. Move the sliders to select a range or enter the upper and lower bounds directly.
- Top-of-Curve: Filter curves according to the maximum response value reached in the assay. This is a range filter works like the AC50 filter.
- Technological Interference: Potential interference for the chemical–assay pair due to luciferase inhibition or autofluorescence for red, blue, or green fluorescence. Flags are based on experimental evidence from Tox21 assays that tested luciferase and autofluorescence interference (Borrel et al. 2020a) or predicted using the NIEHS InterPred tool (Borrel et al. 2020b).
    - There are eight technological interference flags: Blue (experimental) and Blue (predicted) for blue fluorescence, Red (experimental) and Red (predicted) for red fluorescence, Green (experimental) and Green (predicted) for green fluorescence, and Luciferase (Experimental) and Luciferase (predicted).
    - Detailed explanations for these curation criteria are available on the ICE Data Sets page under "cHTS".

- Flag Information: Detailed explanations of Flag-Omit and QC-Omit flags for assay-chemical pairs. Additional information regarding these flagging criteria are available on the ICE Data Sets page under “cHTS”.

When a filter is added to the chain it will appear as a colored box with the filter name and information on how many curves passed the filter (**Figure 6**). The red text in the filter box states how many curves did not pass the filter and the ratio displays the number of results that passed the filter over the total number of results. Each filter added to the chain considers only data that passed the previous filter, and the final filter in the chain will include only curves that passed all the applied filters. The pie chart to the left shows the number of chemicals meeting the criteria in each layer of filters.

Edit filters in the chain by clicking on the pen icon on the bottom right of the filter box, which will reopen the dialog box. To re-order filters, click and drag a filter box to the desired location in the chain. To remove a filter, click the ‘x’ in the top right corner of the filter box. Click the “Clear Filters” button to remove all filters from the chain.

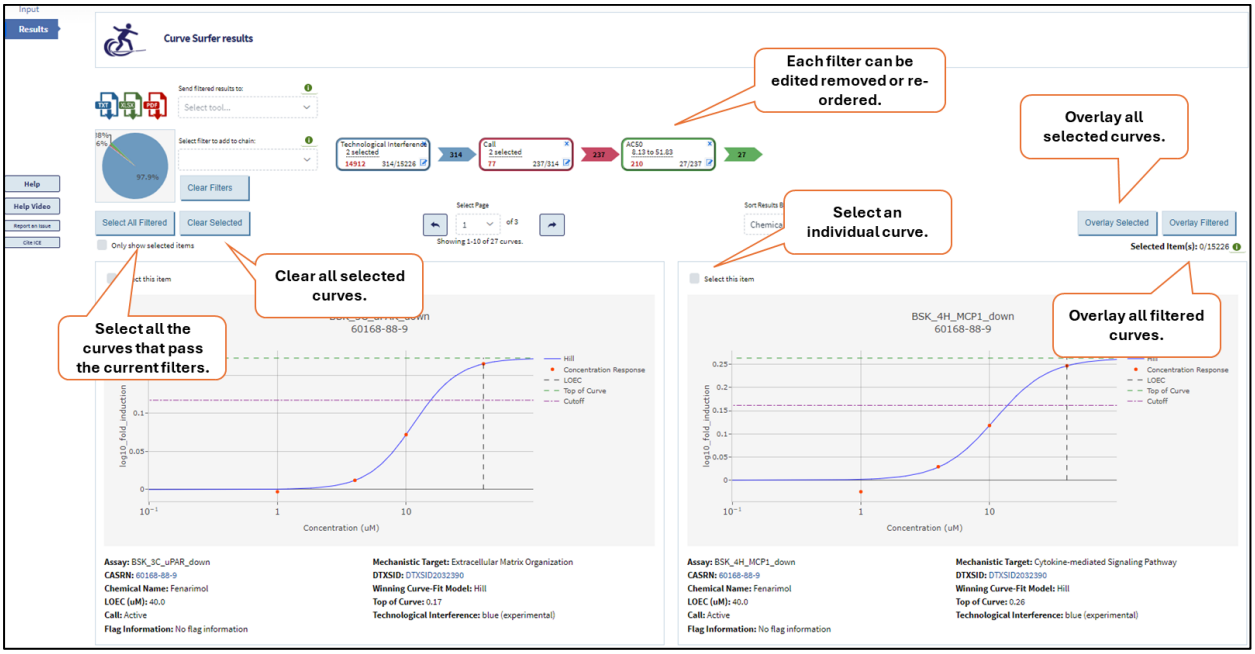

Figure 6. Filtering, selecting, and overlaying results.

Selecting and Overlaying Multiple Curves

You can overlay multiple curves to compare them more directly. Curve Surfer allows up to 50 curves to be overlayed on a single plot. There are several ways to select the curves you want to overlay (**Figure 6**):

- Overlaying all Filtering Results: Click the “Overlay Filtered” button to overlay all curves that have passed any filters you have applied.

- Custom Selection: You can also select curves to overlay by clicking the “Select this item” checkbox in the top left corner of each curve. Click the “Overlay Selected” button will display anoverlay of these curves, ignoring any filters you may have applied to the data set.

- Combine Filter Results and Custom Selection:
- Apply any desired
- Select all curves that pass the filter chain by clicking the “Select All Filtered” button. Like a shopping cart on a retail site, selected curves are saved until the “Clear All Selected” button is clicked, even when you navigate away from the selected results. If no filters are applied, the “Select All Filtered” button will select all assays.
- Click the “Overlay Selected” button to display an overlay of all selected curves, which now includes all curves that passed your filter.
- Add Curves to Your Overlay:
- Change any filters: the curve selections will reflect the changed filters.
- Select additional curves individually and/or apply a new set of filters to your results and click “Select All Filtered.”
    - Click “Overlay Selected” to add the new set of selected curves to your overlay.

Once you click “Overlay Filtered” or “Overlay Selected,” a dialog box will open (**Figure 7**). At the top of the plot there are checkboxes that allow you to view or hide each call (Active, Inactive, Flag-Omit or QC-Omit). Each call is shown on the plot using a different line color and type (e.g., solid vs. dashed). The dropdown list allows you to select which response unit to view. Only assays that have the selected response units will appear on the graph. To place curves with different response units on one graph select “scaled response” as the response unit. Scaled response is calculated for each data point in a curve as the response divided by the curve’s concentration cutoff. The dropdown menu next to “Select AC50 or ACC” allows you to toggle between displaying AC50 or ACC (activity concentration at cutoff) curves.

Hover over a curve to view additional information about that curve. To remove a curve from the plot, click on its listing in the legend; click again to restore it to the plot.

Checking the “3-D” check box in the top left corner will open the current plot in an interactive 3-D format (Figure 8); uncheck the check box to return to the 2-D display.

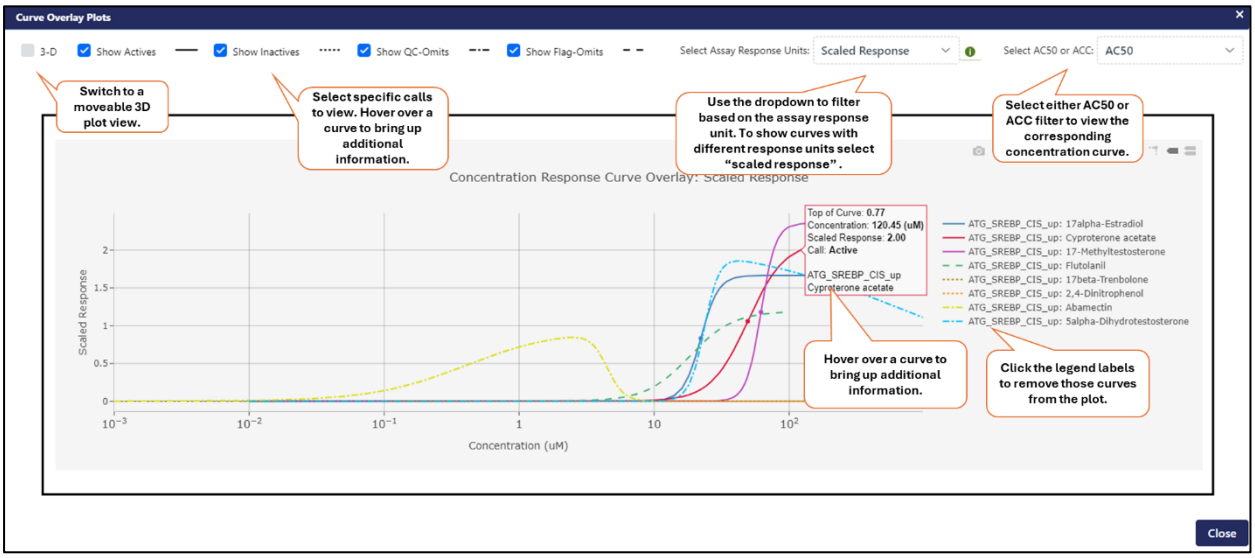

Figure 7. Overlay plot.

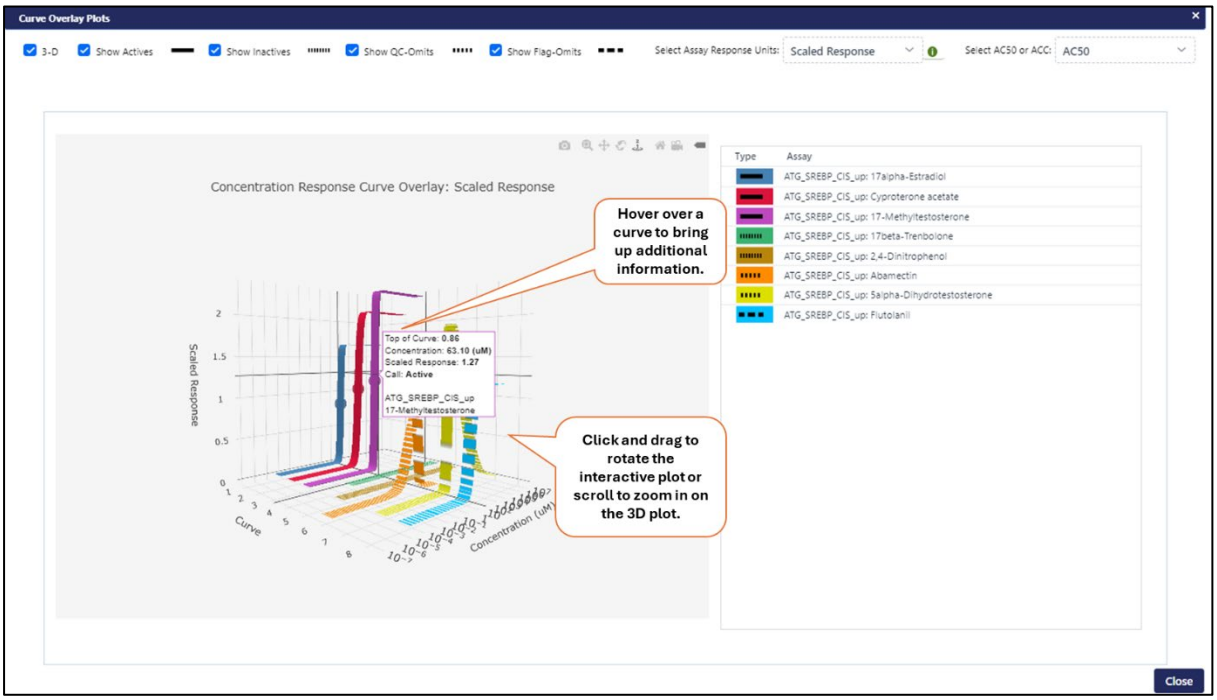

Figure 8. 3-D overlay plot.

Interacting with Plots

Hover over a curve to bring up dialog boxes that provide values for the graph features.

- Orange dots indicate individual concentration-response data points
- For concentration–response curves with Active calls:

- The solid curved line represents the winning model for the curve. In **Figure 9**, the left graph has a yellow line representing a gain–loss model, while the right graph has a blue line representing a Hill model winning curve. The best fit model type is also identified in the metadata below the curve.
    - The horizontal green dashed line denotes the top-of-curve value.
    - The vertical gray dotted line denotes the ACC.
    - The vertical black dashed line denotes the AC50.
- The horizontal purple dot-dash line indicates the assay cutoff, a threshold value used to determine if a response is expected to be active.
    - For “BSK” (BioSeek) assays, a lowest observed effect concentration (LOEC) is provided instead of an AC50 or ACC and will be denoted by a vertical gray dashed line.
- For concentration–response curves with Inactive, Flag-Omit, and QC-Omit, not all the features mentioned above will appear. This is explained in more detail below and in the "Curve/assay-based curation" section of the cHTS data sets page.

Hover over the plot area (**Figure 9**) to display a menu of tools in the top right corner that can be used to adjust the graph display. For more information about interactive graph visualization options, consult the Interactive Graphs User Guide.

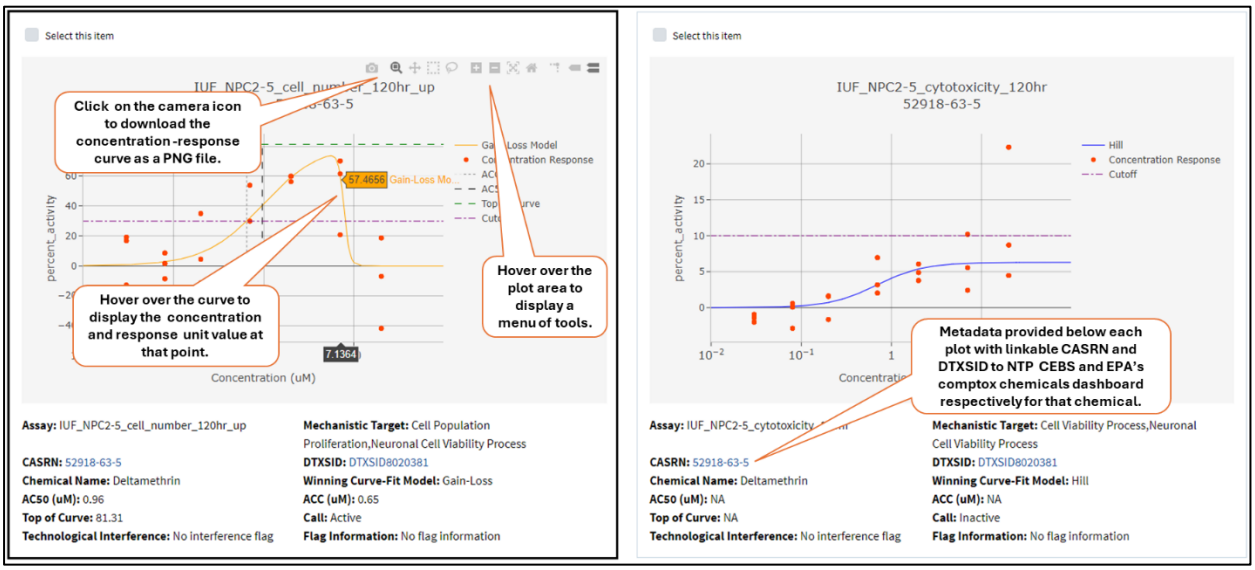

Figure 9. ICE interactive curve plots.

For inactive curves or curves that NICEATM has tagged as Inactive, Flag-Omit, and QC-Omit, not all the features mentioned above will appear. This is a result of the NICEATM curation process for cHTS data which bolsters confidence in hit calls. The curation is described on the Data Sets page under “cHTS Data”. If a chemical–assay pair has a call value of QC-Omit or Flag-Omit it will not show any summary metrics (e.g., AC50, ACC, or top-of-curve value) as they are filtered out as part of the curation process. However, curves for assays tagged with QC-Omit and Flag-Omit calls can be viewed in Curve Surfer to

enable user review of the data.

Metadata are provided below each plot. For all plots, metadata includes the name of the assay, chemical identifiers, the assay's mechanistic target, and the winning curve-fit model. If curves have an active call, then AC50, ACC and top-of-curve are given. If assays have an inactive call or have been tagged as "Flag-Omit" or "QC-Omit" assays then AC50, ACC, and top-of-curve are marked as NA. If assays have been tagged as "Flag-Omit" or "QC-Omit", detailed information on the flag(s) associated with each assay-chemical pair is provided as "Flag Information". If assay and chemical pairs have potential technological interference then the curve is tagged as having luciferase inhibition or red, blue, or green autofluorescence. More information on these technological interference flags can be found at the cHTS data sets page.

- Click on a chemical's CASRN to be directed to the available test article page about that chemical in the NTP Chemical Effects in Biological Systems (CEBS) database.
- Click on a chemical's DTXSID to be directed to the EPA CompTox Chemicals Dashboard entry for that chemical, which provides the chemical structure and other physicochemical and experimental properties.

Download Results

Query results can be downloaded using the download icons at the top of the page (**Figure 4**). Sorts and filters applied to the display will be applied to the downloads. Use the "Clear Filter" or "Clear Selected" buttons to clear any filters or individual selections.

- Clicking the "TXT" icon will export your results to a tab-delimited text file. Data in this file are organized as one row per chemical–assay combination. They include the m4id (a unique ID for each curve in EPA's invitrodb database, which is the source data for this dataset), identifiers for the assay and chemical, identification of the assay response units, information about the mechanistic target in ICE for assay annotation, applicable summary information about the curve, and concentration–response information.
- The concentration–response information is formatted as a colon delimited string with each data point described as "point number:concentration:response value."
    - Each data point is enclosed in parentheses; data points in a series are separated by commas.
    - Multiple assays run at the same concentration will have the same point number and concentration but may have different response values.
    - The unit used for the response value is stated in the "response units" column.
- Clicking the "XLSX" icon will export your results to an Excel file. This file includes the same data in the same formatting as the tab-delimited text file described above.
        - Clicking the "PDF" icon will export your results to a PDF file that will capture each graph and assorted data exactly as it appears on the webpage. The file will show one graph per page andmay take some time to generate.

Using Results to Query Other ICE Tools

Click the “Send filtered results to” dropdown list (**Figure 10**) next to the download icons to send Curve Surfer query results to other ICE tools or to copy chemical identifiers to the clipboard. Filters and individual selections applied to results will define what is sent to other tools. For details on theuse and outputs of these tools, refer to their user guides.

- Click "Search" to query and retrieve all data in ICE for the selected chemicals.
- Click “Chemical Quest” to send chemicals to the Chemical Quest tool to query ICE for chemicals that are structurally similar to these chemicals.
- Click “PBPK” to send chemicals to the ICE Physiologically Based Pharmacokinetics (PBPK) tool. This tool generates predictions of tissue-specific chemical concentration profiles following a dosing event.
- Click "IVIVE" to send chemicals and cHTS assays to the In Vitro to In Vivo Extrapolation (IVIVE) tool. This tool estimates the daily equivalent administered dose (EAD) that would result in the plasmaconcentration of a chemical equal to the activity concentration in a given in vitro assay.
- Click "Chem Characterization" to send chemicals to the Chemical Characterization tool. This tool allows you to view physicochemical properties, absorption, distribution, metabolism, and excretion (ADME) properties, and chemical use categories of a set of chemicals.

You can also copy the CASRNs, DTXSIDs, SMILES strings, or quantitative structure–activity relationship (QSAR)-ready SMILES to the clipboard.

Figure 10. Send filtered results to other ICE tools.

Accessing Curve Surfer from Other ICE Tools

- The ICE Search tool provides a link to Curve Surfer in Search results. The dialog box that opens when you view the detailed chemical information includes a Curve Surfer tab at the top (**Figure 11**). Click on this tab to display all available curves for the chemical. "View Details" button for detailed chemical information is located in the Data Summary table as well as the individual substance distribution table.

- In the IVIVE tool results table (**Figure 12**), a curve icon appears on the far left of each row. This icon will bring up the Curve Surfer graph for the relevant assay results.

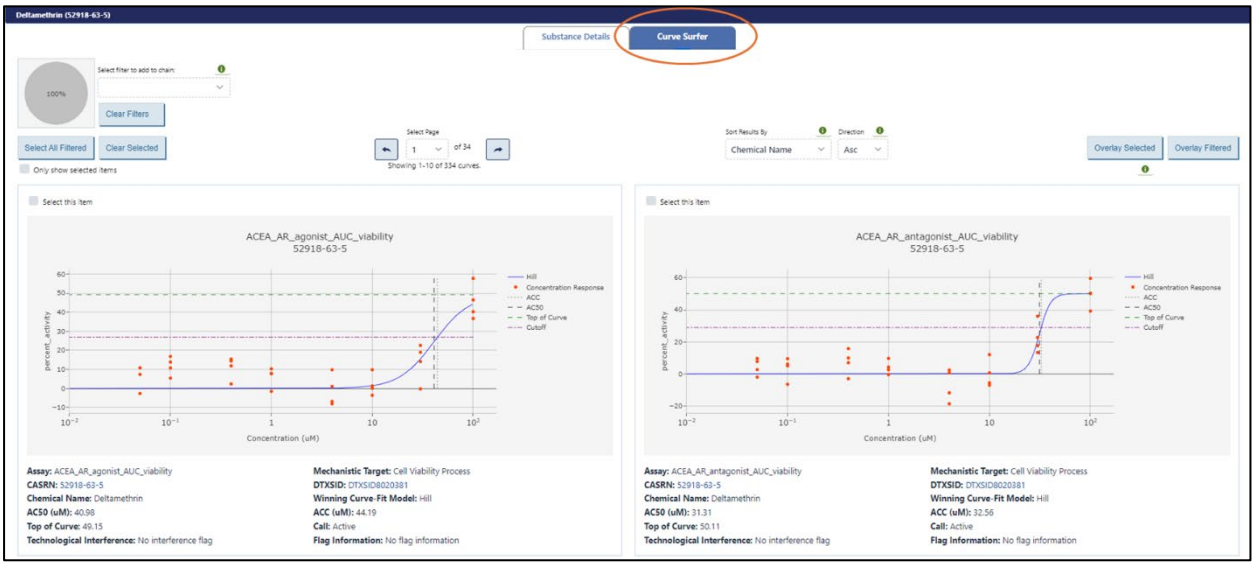

Figure 11. ICE interactive curve plots view in Search results.

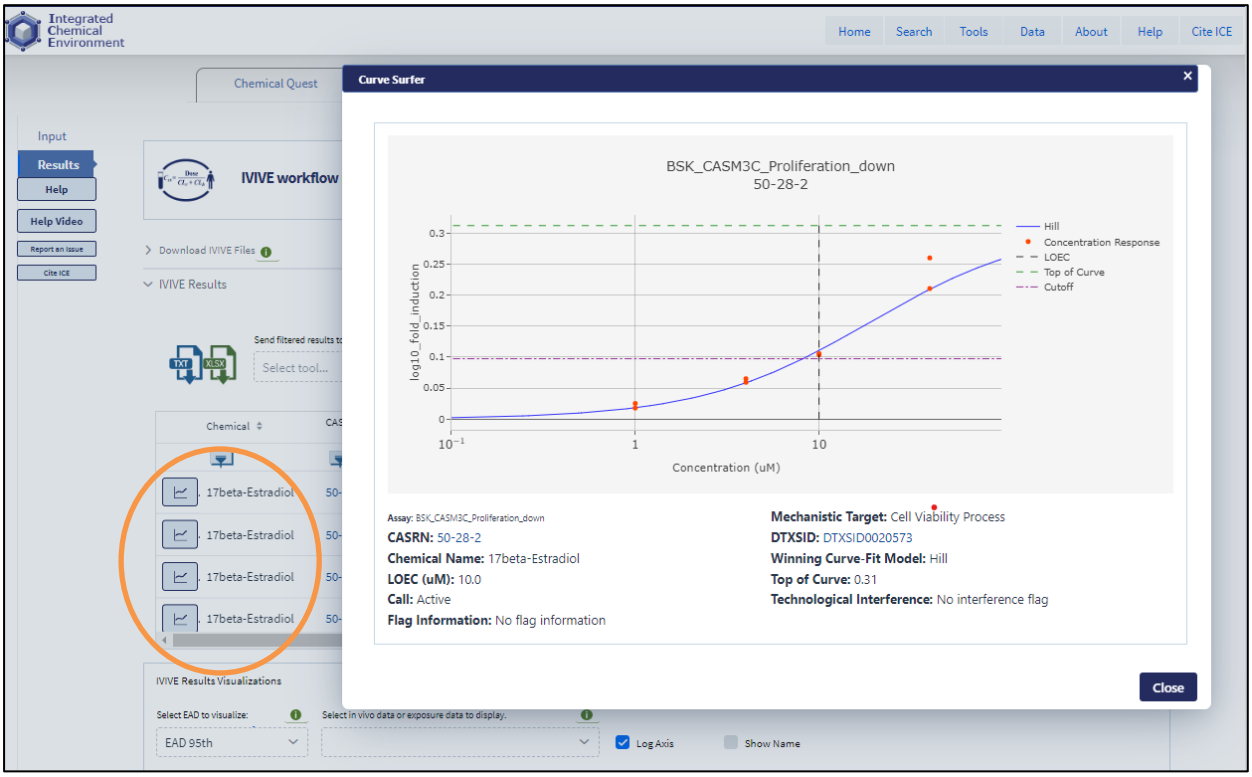

Figure 12. ICE interactive concentration–response curve view in IVIVE tool results.

Appendix 1: ICE cHTS Data

cHTS data in ICE were derived from bioactivity data from the U.S. government's interagency Tox21 collaboration and the EPA's ToxCast program. These data were generated using quantitative high-throughput screening assays and analyzed using the EPA's tcpl package. For inclusion in the ICE cHTS data, these data go through an additional curation, described briefly below and in detail in the ICE Data Sets section.

cHTS data in ICE are annotated to mechanistic targets that facilitate interpretation with regards to biochemical pathways and processes. Annotation to mechanistic targets considers:

- Annotation information provided in the invitrodb v3.5 annotation files. Examples of fields used include "intended_target_family" and "biological_process_target."
- Additional information on the assay or platform available in publications or through the Tox21 and ToxCast programs.
- Terminology used in the Open Biological and Biomedical Ontology (OBO) Foundry (referred to as "mechanistic targets" in ICE).

Terms from the curated annotation and assay information are mapped to terms from the Open Biological and Biomedical Ontology (OBO) Foundry to create connections to widely used and established terminology with controlled identifiers. This allows the annotations found in ICE to be accessed more broadly and the ICE data linked to other resources and terms, including toxicological endpoints of regulatory interest.

NICEATM continues the cHTS mapping process with expert review to annotate the mechanistic targets to terms derived from modes of action relevant to regulated toxicity endpoints as defined by published literature using terms like " Cellular Stress Response " and "epigenetic process." These terms are curated to harmonize similar descriptors and ensure consistency and appropriateness of the annotation details.

Appendix 2: References and Other Resources

Borrel A, Huang R, Sakamuru S, Xia M, Simeonov A, Mansouri K, Houck KA, Judson RS Kleinstreuer NC. 2020. High-throughput screening to predict chemical-assay interference. Scientific Reports. 10:3986. https://doi.org/10.1038/s41598-020-60747-3

Borrel A, Mansouri K, Nolte S, Saddler T, Conway M, Schmitt C, Kleinstreuer NC. 2020. InterPred: a webtool to predict chemical autofluorescence and luminescence interference. Nucleic Acids Research. 48:W586–W590. https://doi.org/10.1093/nar/gkaa378

Filer DL, Kothiya P, Setzer RW, Judson RS, Martin MT. 2017. tcpl: The ToxCast pipeline for high-throughput screening data. Bioinformatics. 33:618–620. https://doi.org/10.1093/bioinformatics/btw680

National Center for Computational Toxicology. 2018. ToxCast Database (invitroDB). U.S. Environmental Protection Agency. Dataset. https://doi.org/10.23645/epacomptox.6062623.v5

Appendix 3: Abbreviations

This list includes both abbreviations used within this User Guide and abbreviations used in the ICE Curve Surfer tool interface.

AC50: concentration that causes a half-maximal response

ACC: activity concentration at cutoff

ADME: absorption, distribution, metabolism, and excretion

BSK: BioSeek

CASRN: Chemical Abstracts Service Registry Number

CEBS: Chemical Effects in Biological Systems (National Toxicology Program database)

cHTS: curated high-throughput screening

DTXSID: Distributed Structure-Searchable Toxicity substance identifier (EPA chemicals database)

EPA: U.S. Environmental Protection Agency

ICE: Integrated Chemical Environment

InChIKeys: Hashed International Chemicals Identifiers

invitrodb: repository for ToxCast data (EPA)

IVIVE: in vitro to in vivo extrapolation

LOEC: lowest observed effect concentration

m4id: unique ID for each curve serving as an identifier in invitrodb

NICEATM: National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

OBO: Open Biological and Biomedical Ontology

PBPK: physiologically based pharmacokinetics

QSAR: quantitative structure–activity relationship

SMILES: simplified molecular-input line-entry system

Tox21: Toxicology in the 21st Century (high-throughput screening program, interagency federal collaboration)

ToxCast: Toxicity Forecaster (high-throughput screening program, EPA)