User Manual for the Danish (Q)SAR Database

23 November 2015

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Permission is granted to use information from the database as is. The database is an expert tool where the final assessment of properties is not dictated by the (Q)SAR estimates, but by the user's own scientific judgment. Aside from the fact that models are never perfect, the (Q)SAR field is under rapid development and models are regularly updated and improved. It is also impossible to provide the detailed information accompanying each individual prediction that is available to those who do not own licences to the software platforms. The structural information in the database stems from many sources and in some cases it may be wrong. The structures are also in some cases abbreviated in that possible anions and cations have been removed. This can have important toxicological significance (e.g. for Heavy Metal salts).

All access to the database should happen through the provided client-side software and without any use of automated workflow or scripting.

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Background

The Danish QSAR database has been freely available on the internet since 2004. It is a tool that allows industry, research, authorities and others to search for hazard information on chemical substances, especially those with little or no testing data. The information provided may be useful to identify chemical substances of potential concern.

With the EU chemicals legislations, e.g. the REACH regulation, there is increased focus on the use of alternatives to animal testing. The QSAR database is used for a wide variety of tasks such as screening for potentially harmful substances and for assessment of specific substances e.g. in relation to dossier evaluation under REACH. The results in the database have also been used to generate the Danish Advisory Self-classification List and to screen for potential PBTs.

Besides direct replacement of experimental tests in some cases, QSAR predictions can help prioritize further *in vitro* and *in vivo* testing of chemicals. In cases where animal testing is still needed, QSAR predictions of mechanistic properties for the chemical can contribute in optimizing the experimental design. In this way, QSARs can reduce the need for later animal testing. It is anticipated that the use of QSAR predictions, and hence the need for good tools will grow in the future.

The new version of the QSAR database has been rebuilt from scratch, and is an updated, extended and improved version of the previous 2004 version of the online QSAR predictions database. It contains an improved, user-friendly interface, new functionalities and updated predictions for a considerably larger substance structure set than the previous database. The new database is a dynamic system, which will be updated continuously in terms of functionalities and content.

Introduction

The new Danish QSAR database is a repository of model estimates for more than 600,000 substances. The QSAR models include endpoints for physico-chemical properties, environmental fate, bioaccumulation, eco-toxicity, absorption, metabolism and toxicity. As far as possible all organic single constituent substances that were pre-registered under REACH (around 72,000) are included in the structure set. In addition, chemical structures from other relevant databases are included leading to the new structure set of more than 600,000 unique chemical structures.

When possible, the endpoints have been modelled in the three software systems Leadscope, CASE Ultra and SciQSAR. All DTU in-house models and a number of commercial models have with the kind permission from MultiCASE® been modelled in two or three systems. The structure set has been predicted in the different systems and an overall battery prediction is made. With the battery approach it is in many cases possible to reduce “noise” from the individual model estimates and thereby improve accuracy and/or broaden the applicability domain.

All applied DTU QSAR models are documented in QMRFs (QSAR Model Reporting Format). Permissions to publish predictions for more than 600,000 substances were kindly provided by MultiCASE Inc., Leadscope Inc., Scimatics, ACD/Labs, and US EPA. The published predictions

are abbreviated predictions (simple yes/no) and do not include detailed information about specific alerts identified. Applicability domain calls are however available.

Main features at a glance

- Estimates for more than 600,000 chemicals in over 200 QSAR models.
- Contains experimental training set data for DTU models, for which data are public.
- Search on substance ID and affiliation.
- Structure search on 2D structures as substructure or exact match.
- Search on all contained QSAR predictions and training set data.
- Combination of search results to make complex AND, OR and NOT algorithms.
- Download of QSAR predictions in an RTF format document compatible with Microsoft Word and OpenOffice.
- Sorting on chemical similarity to facilitate read-across groupings.

Launching the Danish QSAR Database

Type in the following link in the address bar of the web browser: http://qsar.food.dtu.dk

Figure 1: Opening screen for the Danish QSAR Database.

To begin searching, click the ***Search*** button, and the screen shown in Figure 2 should appear. Click the button marked ***I agree*** to enter the database.

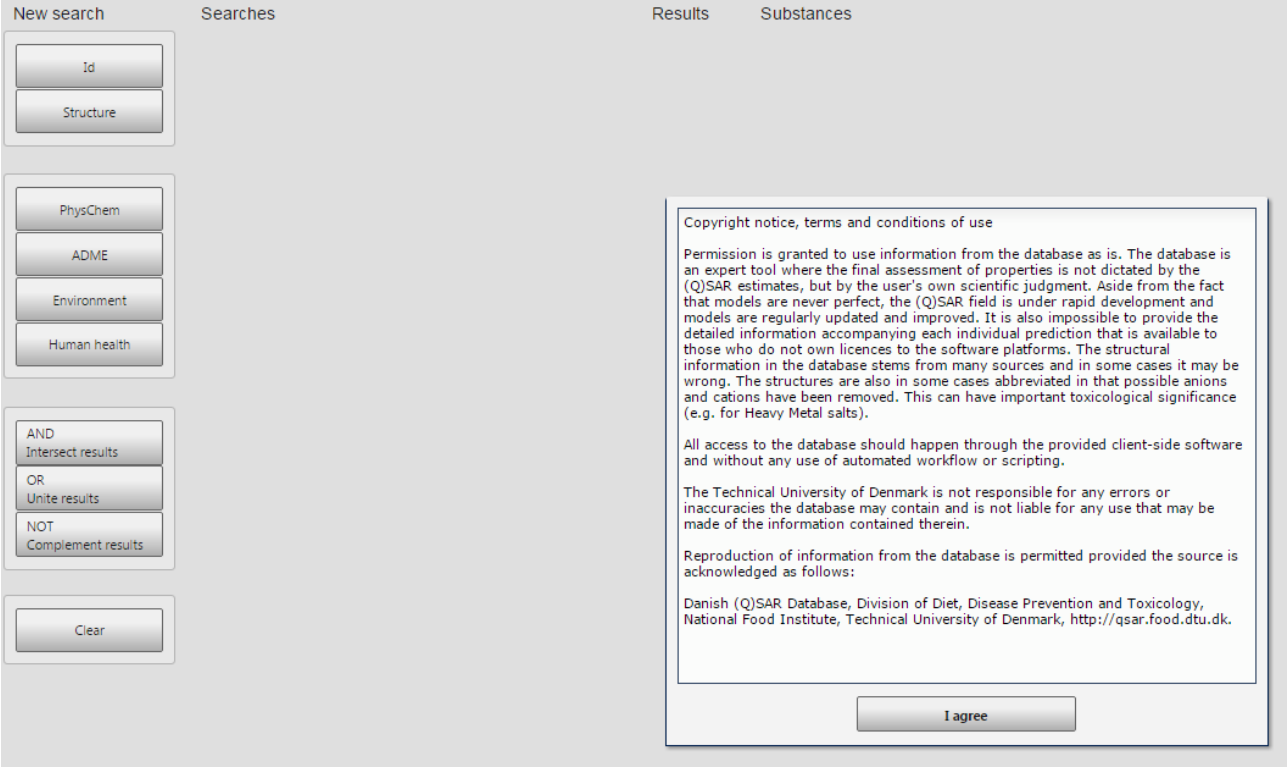


Figure 2. Main search screen with disclaimer box.

Main search screen

In the left part of this screen a number of buttons and the headline "New Search" is shown. There are three basic search options in the interface window: **ID**, **Structure** and **Model endpoint** (divided into PhysChem, ADME, Environment and Human health). These are explained in more detail below.

Each search can be combined with others in order to form more complex search queries. The combined searches are performed using the three buttons ***AND***, ***OR*** and ***NOT*** and are described in more detail below in the section: **Combining searches**.

The **Clear** button is used to clear the previous searches from the screen.

Searching by identification data

The ID search button is designed for queries by **Single ID**, **ID List** or **Affiliation** (see Figure 3).

When choosing Single ID, a number of options are possible: **Registry Number**, **EC Number**, **PubChem CID** and **Chemical name**. To start a Single ID search, type in the query in the white box and click the ***Search*** button. The Registry number can be typed both with or without hyphens. The structures matching your search will be listed in a browser window similar to the window shown in Figure 8 and give the possibility to download a report containing the prediction results of the

resulting substance. The search section and the result window are further described below in the sections **Searches and Results** and **Results window with substances.**

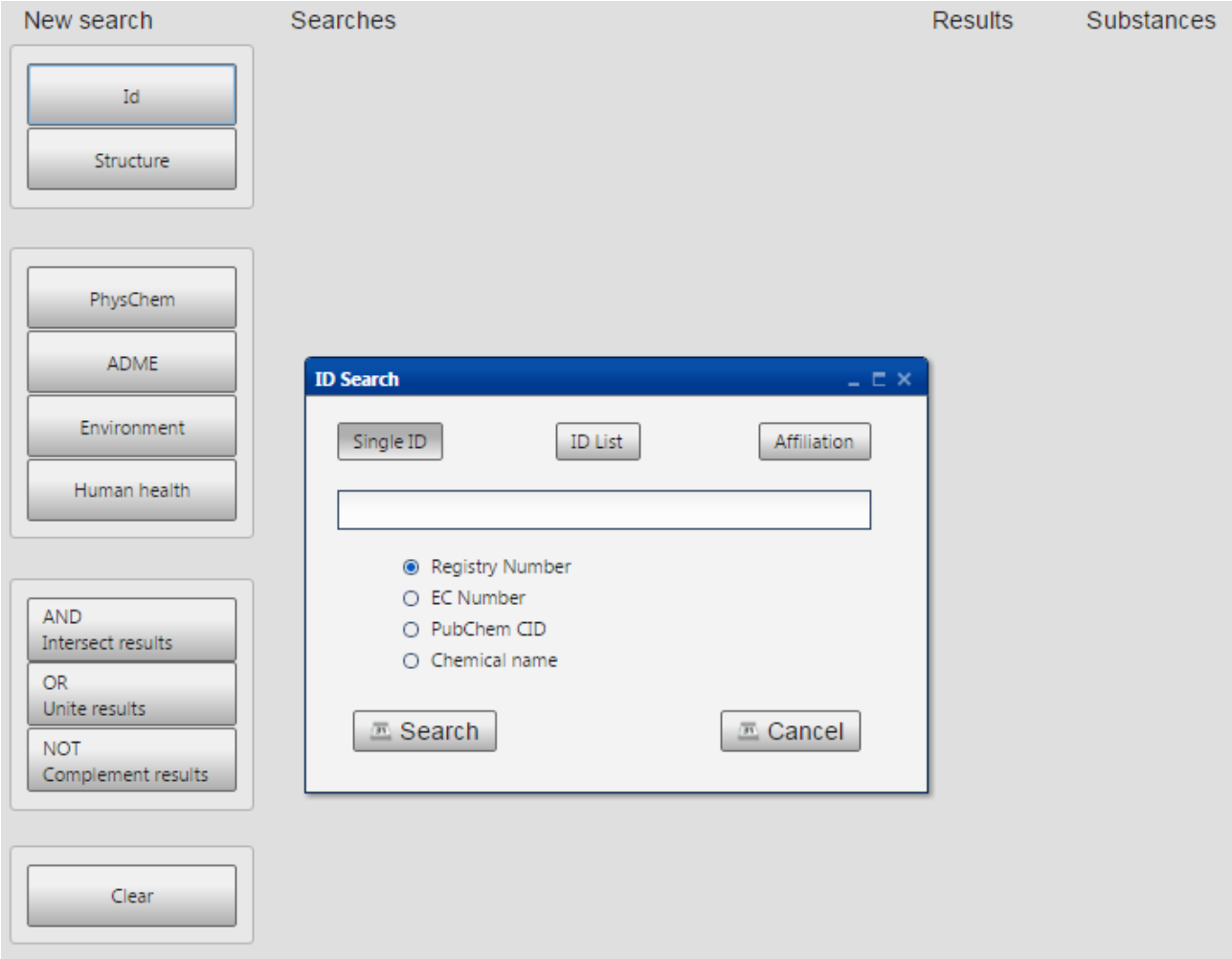


Figure 3. The ID search box.

When choosing ID list (in the ID Search box), two options are possible: **Registry numbers** and **PubChem CIDs**. To start an ID list search, type or paste in the query in the white box and click the ***Search*** button.

Choosing Affiliation gives two options for retrieving database structures: **REACH Pre-registration list** and **PubChem**. To retrieve the structures, choose the database of interest and click the ***Search*** button.

Search example is given in Appendix 1.

Searching by structure

The **Structure** section offers a 2D fragment editor, where it is possible to build structure fragments to search for. Click the ***Structure*** button to the left on the main search screen to open the edit box (see Figure 4). Structures can be drawn and searched for by using the tools in the box. It also contains other functionalities such as SMILES pasting and name lookup.

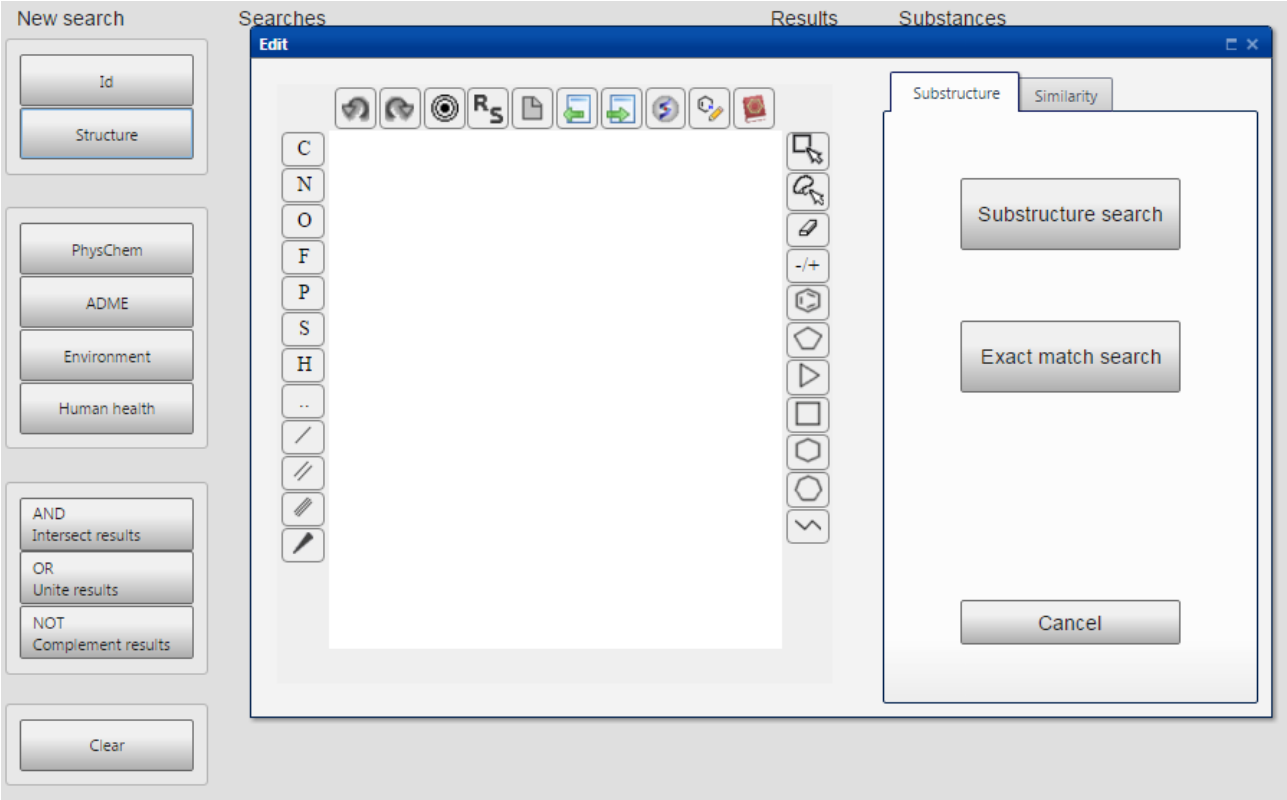


Figure 4. The Structure search interface.

Building a fragment: To add an item, click on the corresponding button and then click on the blank canvas. Add atoms / fragments / bonds one by one.

To start a structure search, select either **Substructure** or **Similarity** to the right on the screen. When choosing **Substructure**, two options are possible: Select ***Substructure search*** to search for the built fragment as a subfragment within the database, or ***Exact match search*** to search for the exact same structure. When choosing **Similarity**, either all analogs in the database or a user-defined number of closest analogs will be displayed. The search will generate a browser window similar to the window shown in Figure 8 and give the possibility to download a report containing the prediction results. The results window is further described below in the section: **Results window with substances.**

Editor operations:

Undo: Undoes the last operation.

Redo: Repeats the last undone operation.

Center: Moves the fragment to the center of the canvas.

Toggle R/S labels: Marks R/S isomeri.

Clear: Clears the Edit window.

Import: Imports MOL or SMILES file.

Export: Displays MOL or SMILES information for current structure.

About: Displays version number etc. of the fragment editor.

2D cleanup/depiction: Corrects bond angles etc.

Chemical dictionary search: Name lookup in PubChem dictionary.

Search examples are given in Appendix 2.

Searching by model endpoint

The ***PhysChem***, ***Environment***, ***ADME*** and ***Human health*** buttons to the left on the main screen can be used to search for specific model endpoints. Each of the four categories covers a number of different endpoints. To start a search by model endpoint, click the category button of interest, e.g. ***Human health***. This will generate a drop-down menu with a list of subcategories as shown in Figure 5. Figure 5 shows an example query to search for prediction results in the model for Bacterial Reverse Mutation Test (Ames test in S. typhimurium (in vitro)), which is found in the genotoxicity subcategory.

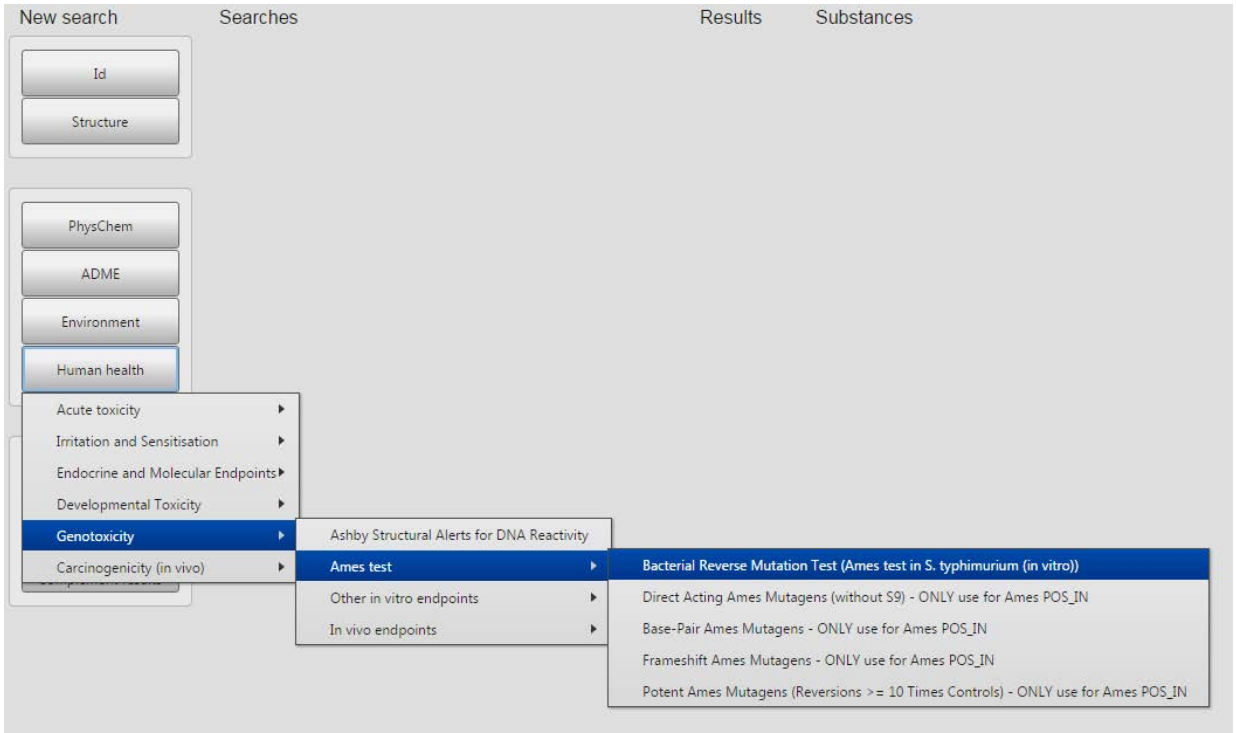


Figure 5. An example query to search for prediction results in Ames test. As shown, a number of submodels are available.

When the model of interest is chosen, a dialog box appears (Figure 6). Select the heading **Search** at the top of the dialog box to start a search. The menu in the dialog box depends on whether the selected model is made in one or more software systems. The selected model in Figure 6 is made in three systems, CASE Ultra, Leadscope and SciQSAR. Based on predictions from the three systems, a fourth and overall battery prediction is made. These four predictions (three predictions from the individual systems and the battery prediction) can be selected individually. The battery prediction approach is further described below in the section: **Battery algorithm**. It is also possible to select and search for experimental results from the training set.

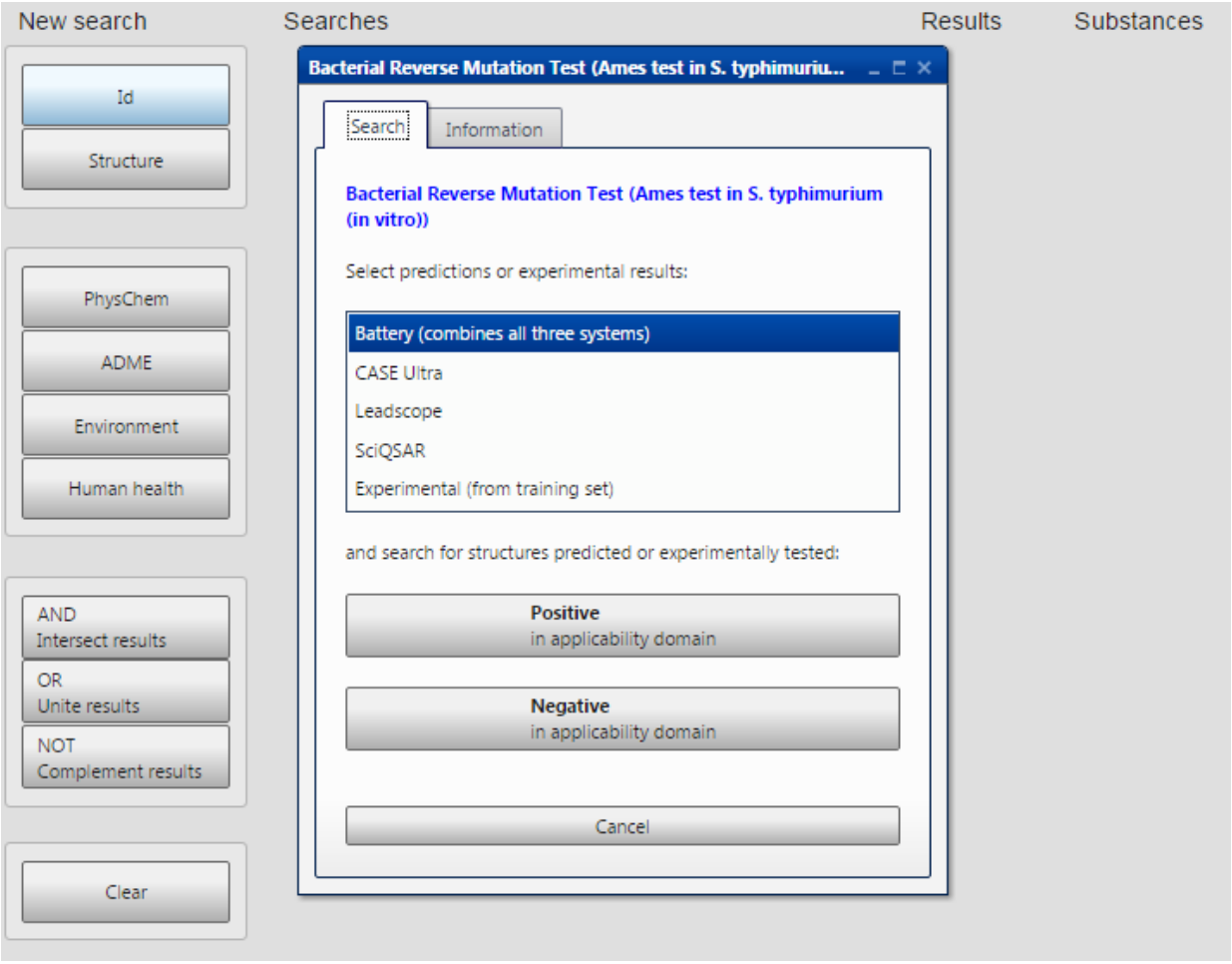


Figure 6. Dialog box from query shown in Figure 5.

Select the relevant results type (predictions/experimental) in the dialog box and then click either the ***positive*** or ***negative*** button to start the search. Only the predictions within applicability domain will be searched and displayed.

The search will generate the browser window shown in Figure 8 and give the possibility to download a report containing the prediction results in the rtf.file format. The results window is further described below in the section: **Results window with substances.**

Information about the selected model can be found by selecting the **information** tab at the top of the dialog box. A list of options will appear enabling you to download QMRFs of the relevant model versions.

Search examples are given in Appendix 3.

Combining searches

Combinations of searches are also possible. These are performed using the two buttons to the left on the main screen, ***AND*** and ***OR.*** Using the ***OR*** button will display all substances from two or more searches, whereas the ***AND*** button will only display the intersection of the individual searches. The individual queries are made as described in the previous text so that they appear under “Searches” on the main search screen.

To combine searches, click the search definition buttons for the searches of interest that appear under the field “Searches”. This will highlight the text in the selected buttons, which change color to green. Then select either the ***AND*** or ***OR*** button to start the search. The results of the combination search are displayed to the right under “Searches”, “Results” and “Substances”. The example in figure 7 shows the result of a combination of searches for AR antagonism and PXR binding using the ***AND*** button. The result window is further described below in the section: **Results window with substances.**

The ***NOT*** button to the left on the main search screen is for inverting a search. Click the search definition button of interest (only one) under the field “Searches” and select ***NOT***. Inverted searches, as well as results of AND and OR searches, can in turn be combined with other individual or combined ones to form more complex combined searches.

Search example is given in Appendix 4.

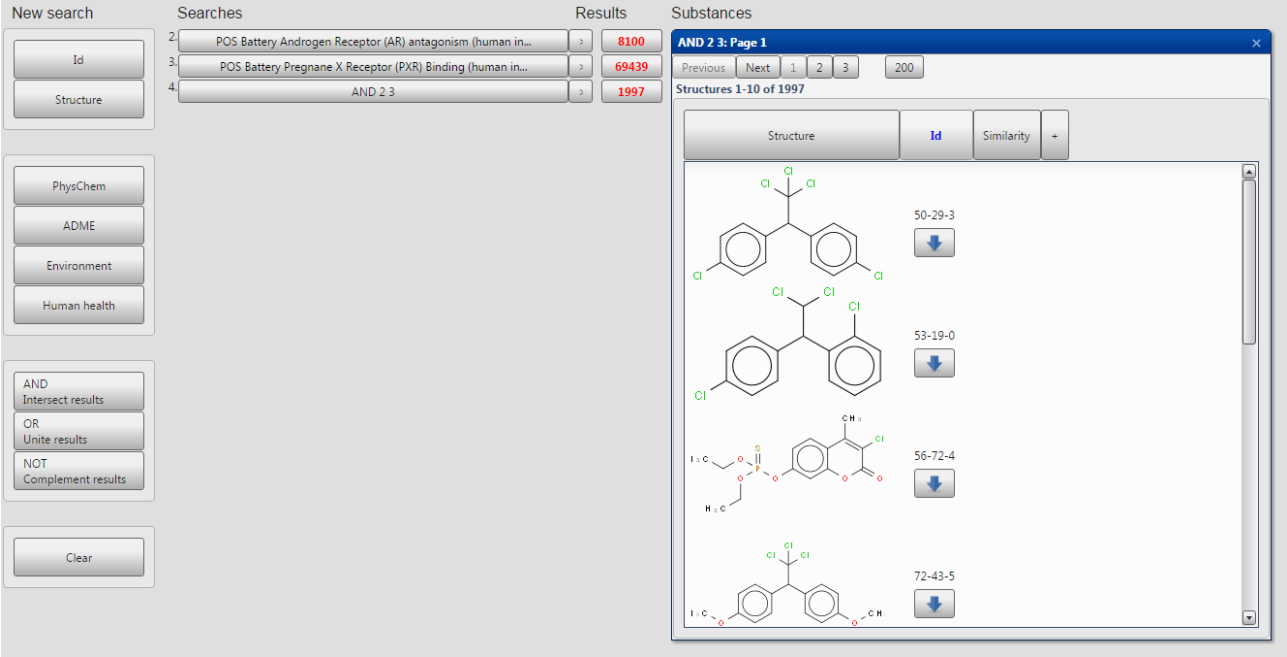


Figure 7. An example where searches for AR antagonism and PXR binding are combined by using the ***AND*** button.

Searches and Results sections

Every time you perform a search, several new screen elements will appear. A search definition button will be added to the Searches section (Figure 7). It can be used for combining searches, which is described in the section **"Combining searches"**.

Another button in the Results section will display the number of structures resulting from the search. The actual structures will be listed in a browser window similar to the window shown in Figure 8 (described below in the section **Results window with substances).**

The Searches and Results sections will keep track of all searches you have performed. You can clear them by clicking the Clear button, or delete individual searches using the small '>' button next to the search definition. You can revisit previous search results at any time by clicking the button displaying the number of structures in the Results section. The relevant searches are not executed again but instead retrieved quickly from a repository of searches.

Results window with substances

The searches described in the previous sections will generate a results window and give the possibility to download a report containing database results for selected substances. One report per substance will be generated.

The example in Figure 8 shows the result of a Model endpoint search of the Bacterial Reverse Mutation Test. The results of the search are displayed to the right under "Results" and "Substances".

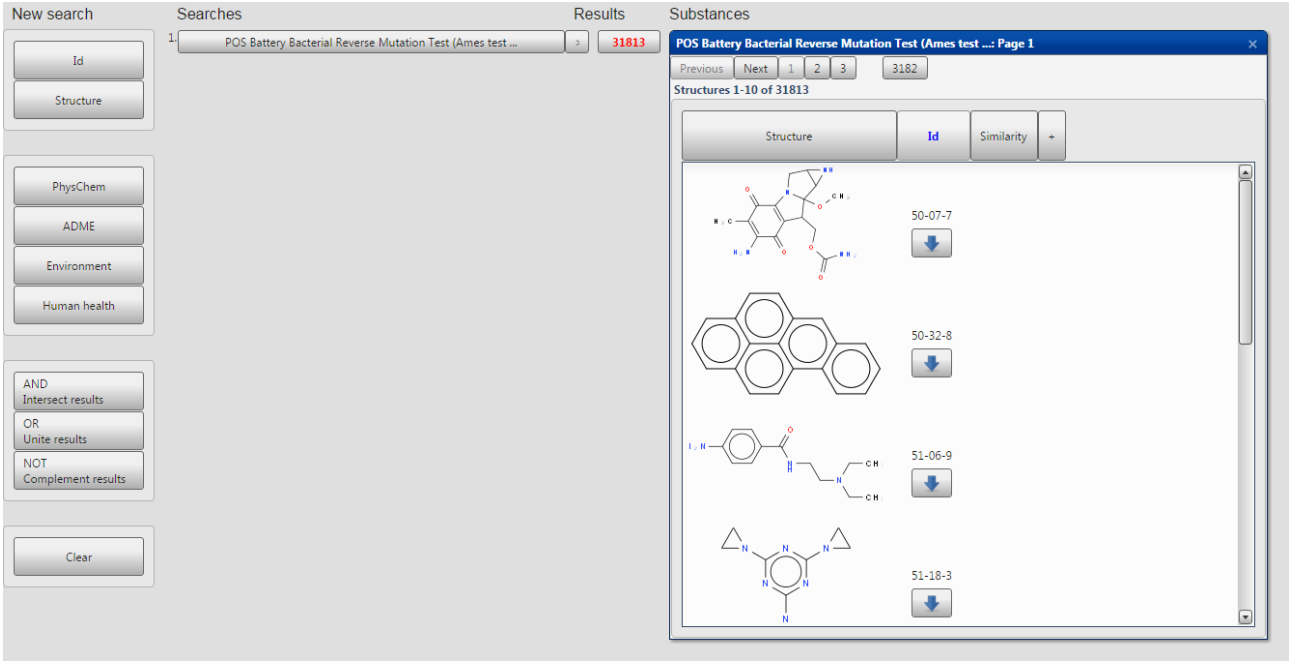


Figure 8. An example of a results window from a Model endpoint search of the Bacterial Reverse Mutation Test.

The window under "Substances" shows the resulting structures. When there are more than 10 structures, they are shown in pages with 10 structures per page. Use the top button row (Previous, Next, First, Current, Last etc.) to navigation through the result pages. All result pages are directly accessible the moment the search is executed, so you can e.g. view any page directly without having to go first through the preceding ones.

To download a single substance report, click the  button in the id column next to the substance of interest. This will provide an .RTF file containing all predictions as well as training set data when available. The .RTF document format is supported by Microsoft Word, OpenOffice and other viewers/editors.

Clicking the ***Similarity*** button will open the 2D fragment editor, where it is possible to search for substances similar to a query substance within the current result set. The current result set will be ordered by decreasing similarity to the query substance.

To revert back to the Id order, click the Id button above the structure list.

Clicking the + button next to ***Similarity*** opens a dialog box, where you can select any database property (experimental or predicted in any model and predictive system) and display its values in the result window. You can select up to eight properties to display. The extra information will be displayed in new columns and refresh as you navigate through result pages.

The Substances window can be resized and moved and scroll bars will automatically appear if necessary.

Technical requirements and notes

All operations with the Danish (Q)SAR database are performed in a web browser. There is no need to download or install any software. Likewise, there is no need to install any browser plugin or add-on (the previous version of the web site used Java).

The system is can be accessed from both personal computers and mobile devices. The minimum screen resolution for using the system is 640x480 pixels. For convenience, higher resolution display settings can be recommended (preferably 1280 or more pixels on the horizontal axis).

The client-side software is implemented entirely in JavaScript and is compatible with all major browsers and operating systems without the need for third-party software. Depending on the security settings of your browser, you may need to enable JavaScript in order to use the website.

The system has been tested with the following browser versions: Google Chrome 46.0, Microsoft Internet Explorer 11, Opera 33.0, Mozilla Firefox 37.0.2.

Battery algorithm

Some of the models are made in two or three of the following independent systems: CASE Ultra (CU), Leadscope Predictive Data Miner (LS) and SciQSAR (SQ). The systems are described in Appendix 5. Based on predictions from each of the applied systems, a battery prediction is made using a so-called battery algorithm. The battery approach can give more reliable predictions and can also expand the applicability domain, which was shown in a previous pilot project including 32 different models and the three systems mentioned above (not published).

For a given effect, QSAR predictions are made in each of the independent QSAR model systems and combined into a battery prediction by using the criteria shown in Table 1. The first column shows the total number of predictions (positive/negative) in domain. The next two columns show the number of positive and negative predictions, respectively. The final battery prediction based on the individual predictions is shown in the fourth column.

Table 1. Battery algorithm.

Total POS/NEG in domain	POS in domain	NEG in domain	Battery prediction[a]	Remarks
3	3	0	POS_IN	
3	0	3	NEG_IN	
3	2	1	POS_IN	
3	1	2	INC_OUT or (see remark) NEG_IN	EXCEPT when CU and LS are both NEG_IN, in this case the battery call is NEG_IN
2	2	0	POS_IN	
2	1	1	INC_OUT	

2	0	2	NEG_IN	
1	1	0	POS_OUT	
1	0	1	NEG_OUT	
0	0	0	INC_OUT	If minimum one prediction (out of domain)
0	0	0	-	None predicted

[a] POS, positive; NEG, negative; INC, inconclusive; IN, inside applicability domain; OUT, outside applicability domain. [b] Less weight is put on an SQ POS compared to LS or CU POS in cases where LS and CU agree on a NEG in AD prediction, because SQ in many cases has lower specificity than LS and CU.

Table 2. Training set numbers and cross validation results. See QMRFs for more information.

Endpoint	N in training set	Software	Cross validation result (%)[a]
Not ready biodegradability (POS=Not Ready)	735	CASE Ultra	Sens=68.9, Spec=87.8, Conc=77.2
		Leadscope	Sens=87.3, Spec=85.2, Conc=86.4
		SciQSAR	Sens=63.0, Spec=92.7, Conc=77.8
Fathead minnow 96h LC50 (mg/L)	565	CASE Ultra	No robust model
		Leadscope	R^2=0.75, Q^2=0.73
		SciQSAR	R^2=0.74, Q^2=0.72
Daphnia magna 48h EC50 (mg/L)	626	CASE Ultra	No robust model
		Leadscope	R^2=0.67, Q^2=0.64
		SciQSAR	R^2=0.65, Q^2=0.63
Pseudokirchneriella s. 72h EC50 (mg/L)	531	CASE Ultra	No robust model
		Leadscope	R^2=0.74, Q^2=0.71
		SciQSAR	R^2=0.64, Q^2=0.60
Cytochrome P450 2D6 (CYP2D6) substrates (human clinical data)	746	CASE Ultra	Sens=43.9, Spec=87.0, Conc=74.1
		Leadscope	Sens=60.0, Spec=89.4, Conc=80.1
		SciQSAR	Sens=59.5, Spec=79.8, Conc=73.1
Cytochrome P450 2C9 (CYP2C9) substrates (human clinical data)	736	CASE Ultra	Sens=30.6, Spec=83.6, Conc=68.8
		Leadscope	Sens=30.0, Spec=89.6, Conc=75.4
		SciQSAR	Sens=26.3, Spec=91.5, Conc=74.7
Rat oral	6464	ACDLabs	Ext. validation, RI>0.5, Q^2=0.64
Rat intraperitoneal	3751	ACDLabs	Ext. validation, RI>0.5, Q^2=0.56
Mouse oral	14,678	ACDLabs	Ext. validation, RI>0.5, Q^2=0.55
Mouse intraperitoneal	27,004	ACDLabs	Ext. validation, RI>0.5, Q^2=0.61
Mouse intravenous	14,972	ACDLabs	Ext. validation, RI>0.5, Q^2=0.66
Mouse subcutaneous	6432	ACDLabs	Ext. validation, RI>0.5, Q^2=0.57
Maximum recommended daily dose (MRDD) in humans ≤ 2.69 mg/kg-2bw/d	1222	CASE Ultra	Sens=69.4, Spec=92.5, Conc=82.5
		Leadscope	Sens=78.6, Spec=82.5, Conc=80.7
		SciQSAR	Sens=73.1, Spec=77.3, Conc=75.3
Severe skin irritation in rabbit	836	CASE Ultra	Sens=63.4, Spec=86.7, Conc=75.8
		Leadscope	Sens=79.5, Spec=81.7, Conc=80.6
		SciQSAR	Sens=77.3, Spec=71.3, Conc=74.3

Allergic contact dermatitis in guinea pig and human	1032	CASE Ultra	Sens=76.7, Spec=93.9, Conc=89.3
		Leadscope	Sens=75.0, Spec=96.3, Conc=90.8
		SciQSAR	Sens=61.6, Spec=96.8, Conc=85.8
Respiratory sensitisation in humans	80	CASE Ultra	Sens=68.2, Spec=96.3, Conc=86.4
		Leadscope	Sens=91.7, Spec=95.5, Conc=93.9
		SciQSAR	Sens=80.0, Spec=87.5, Conc=83.8
Estrogen Receptor α binding (human in vitro) ALL	802	CASE Ultra	Sens=60.9, Spec=95.2, Conc=85.7
		Leadscope	Sens=75.2, Spec=90.1, Conc=84.7
		SciQSAR	Sens=67.3, Spec=89.0, Conc=81.3
Estrogen Receptor α binding (human in vitro) Balanced	595	CASE Ultra	Sens=81.7, Spec=89.2, Conc=85.4
		Leadscope	Sens=83.7, Spec=89.0, Conc=86.3
		SciQSAR	Sens=76.1, Spec=83.3 Conc=79.8
Estrogen Receptor α activation (human in vitro)	481	CASE Ultra	Sens=73.7, Spec=86.6, Conc=80.9
		Leadscope	Sens=73.1, Spec=86.6, Conc=80.7
		SciQSAR	Sens=77.9, Spec=80.8, Conc=79.6
Androgen Receptor antagonism (human in vitro)	874	CASE Ultra	Sens=57.4, Spec=87.2, Conc=78.3
		Leadscope	Sens=51.7, Spec=91.2, Conc=80.4
		SciQSAR	Sens=56.3 Spec=91.1, Conc=81.9
Thyroid receptor α binding – log(IC_{50} in μM) (human in vitro)	118	CASE Ultra	Q^2=0.59
		Leadscope	R^2=0.83, Q^2=0.68
		SciQSAR	R^2=0.64, Q^2=0.57
Thyroid receptor β binding – log(IC_{50} in μM) (human in vitro)	130	CASE Ultra	Q^2=0.61
		Leadscope	R^2=0.83, Q^2=0.64
		SciQSAR	R^2=0.65, Q^2=0.58
Pregnane X receptor binding (human in vitro)	631	CASE Ultra	Sens=72.4, Spec=83.9, Conc=78.5
		Leadscope	Sens=80.4, Spec=80.4, Conc=80.4
		SciQSAR	Sens=79.9, Spec=82.7, Conc=81.4
Teratogenic potential in Humans	323	CASE Ultra	Sens=65.0, Spec=85.1, Conc=76.4
		Leadscope	Sens=72.0, Spec=85.5, Conc=80.1
		SciQSAR	Sens=64.6, Spec=92.7, Conc=81.4
Ashby structural alerts	782	CASE Ultra	Sens=89.7, Spec=95.1, Conc=91.9
		Leadscope	Sens=87.5, Spec=90.7, Conc=88.5
		SciQSAR	Sens=81.7, Spec=80.6, Conc=81.1

Bacterial reverse mutation test (Ames test in S. typhimurium in vitro)	4102	CASE Ultra	Sens=83.9, Spec=89.1, Conc=86.4
		Leadscope	Sens=84.3, Spec=85.7, Conc=84.9
		SciQSAR	Sens=79.3, Spec=79.1, Conc=79.2
Direct acting Ames mutagens (without S9) – ONLY use for Ames POS_IN	388	CASE Ultra	Sens=63.5, Spec=90.4, Conc=79.5
		Leadscope	Sens=66.9, Spec=78.9, Conc=74.0
		SciQSAR	Sens=56.5, Spec=72.9, Conc=68.6
Base pair Ames mutagens - ONLY use for Ames POS_IN	204	CASE Ultra	Sens=52.8, Spec=88.4, Conc=71.9
		Leadscope	Sens=70.2, Spec=66.4, Conc=68.4
		SciQSAR	Sens=68.6, Spec=67.7, Conc=68.1
Frame shift Ames mutagens - ONLY use for Ames POS_IN	309	CASE Ultra	Sens=73.5, Spec=84.1, Conc=78.9
		Leadscope	Sens=74.4, Spec=78.6, Conc=76.6
		SciQSAR	Sens=68.3, Spec=78.2, Conc=73.8
Potent Ames mutagens, reversions ≥ 10 times controls - ONLY use for Ames POS_IN	187	CASE Ultra	Sens=73.7, Spec=87.7, Conc=81.2
		Leadscope	Sens=68.9, Spec=70.0, Conc=69.8
		SciQSAR	Sens=75.0, Spec=74.7, Conc=74.9
Chromosome aberrations in CHO cells (*in vitro*)	233	CASE Ultra	Sens=40.4, Spec=94.5, Conc=74.4
		Leadscope	Sens=54.1, Spec=79.3, Conc=68.8
		SciQSAR	Sens=50.5, Spec=84.3, Conc=70.3
Chromosome aberrations in CHL cells (*in vitro*)	600	CASE Ultra	Sens=63.3, Spec=86.7, Conc=76.4
		Leadscope	Sens=74.6, Spec=75.2, Conc=74.9
		SciQSAR	Sens=73.0, Spec=72.8, Conc=72.9
Mutations in thymidine kinase locus in mouse lymphoma cells (*in vitro*)	555	CASE Ultra	Sens=76.5, Spec=86.3, Conc=81.2
		Leadscope	Sens=85.1, Spec=83.8, Conc=84.4
		SciQSAR	Sens=79.1, Spec=80.5, Conc=79.8
Mutations in HGPRT locus in CHO cells (*in vitro*)	239	CASE Ultra	Sens=75.4, Spec=84.5, Conc=78.9
		Leadscope	Sens=81.7, Spec=78.4, Conc=80.5
		SciQSAR	Sens=80.0, Spec=73.0, Conc=76.5
Unscheduled DNA synthesis (UDS) in rat hepatocytes (*in vitro)*	415	CASE Ultra	Sens=60.6, Spec=87.0, Conc=74.1
		Leadscope	Sens=74.1, Spec=70.1, Conc=72.4
		SciQSAR	Sens=69.6, Spec=72.5, Conc=71.1
Syrian hamster embryo (SHE) cell transformation (*in vitro)*	363	CASE Ultra	Sens=50.8, Spec=86.9, Conc=74.0
		Leadscope	Sens=71.6, Spec=76.5, Conc=74.5
		SciQSAR	Sens=76.1, Spec=66.5, Conc=71.3

Sex-linked recessive lethal (SLRL) test in Drosophila m. (*in vivo*)	367	CASE Ultra	Sens=75.4, Spec=92.0, Conc=83.6
		Leadscope	Sens=79.1, Spec=80.3, Conc=79.6
		SciQSAR	Sens=74.2, Spec=78.3, Conc=76.2
Micronucleus test in mouse erythrocytes (*in vivo*)	357	CASE Ultra	Sens=31.2, Spec=95.2, Conc=75.7
		Leadscope	Sens=64.1, Spec=77.6, Conc=72.3
		SciQSAR	Sens=52.1, Spec=83.3, Conc=69.7
Dominant lethal mutations in rodents (*in vivo*)	191	CASE Ultra	Sens=42.4, Spec=92.7, Conc=73.7
		Leadscope	Sens=61.5, Spec=80.4, Conc=71.8
		SciQSAR	Sens=57.7, Spec=81.4, Conc=71.7
Sister chromatid exchange in mouse bone marrow cells (*in vivo*)	265	CASE Ultra	Sens=91.8, Spec=94.8, Conc=93.9
		Leadscope	Sens=88.6, Spec=95.9, Conc=94.0
		SciQSAR	Sens=76.7, Spec=93.2, Conc=86.8
Comet assay in mouse (*in vivo*)	286	CASE Ultra	Sens=60.1, Spec=93.1, Conc=82.9
		Leadscope	Sens=86.6, Spec=80.8, Conc=83.1
		SciQSAR	Sens=82.4, Spec=82.0, Conc=82.2
FDA RCA cancer male rat (*in vivo*)	1324	CASE Ultra	Sens=34.2, Spec=95.0, Conc=63.9
		Leadscope	Sens=62.6, Spec=74.7, Conc=69.2
FDA RCA cancer female rat (*in vivo*)	1321	CASE Ultra	Sens=44.4, Spec=93.3, Conc=71.6
		Leadscope	Sens=57.7, Spec=83.6, Conc=72.7
FDA RCA cancer rat (*in vivo*)	1379	CASE Ultra	Sens=41.7, Spec=94.0, Conc=66.9
		Leadscope	Sens=57.1, Spec=82.3, Conc=71.2
FDA RCA cancer male mouse (*in vivo*)	1197	CASE Ultra	Sens=38.4, Spec=86.1, Conc=66.1
		Leadscope	Sens=58.6, Spec=81.4, Conc=71.9
FDA RCA cancer female mouse (*in vivo*)	1208	CASE Ultra	Sens=41.5, Spec=85.9, Conc=65.6
		Leadscope	Sens=59.2, Spec=80.6, Conc=71.3
FDA RCA cancer mouse (*in vivo*)	1221	CASE Ultra	Sens=43.1, Spec=86.9, Conc=66.9
		Leadscope	Sens=56.5, Spec=83.9, Conc=72.7
FDA RCA cancer rodent (*in vivo*)	1530	CASE Ultra	Sens=51.4, Spec=88.3, Conc=68.2
		Leadscope	Sens=65.9, Spec=76.2, Conc=71.3
Liver specific cancer in rat or mouse (*in vivo*)	320	CASE Ultra	Sens=31.1, Spec=92.0, Conc=70.9
		Leadscope	Sens=35.6, Spec=88.6, Conc=69.3
		SciQSAR	Sens=38.5, Spec=84.8, Conc=69.1

[a] Sens: sensitivity; Spec: specificity; Conc: concordance; Ext. validation: external validation; RI: reliability index.

Table 3. Software names and versions for physical-chemical and environmental models.

Predicted property	Software
Melting Point (deg C), Boiling Point (deg C), Melting Point Experimental (deg C), Boiling Point Experimental (deg C), Vapour Pressure (mm Hg), Vapour Pressure (Pa), Vapour Pressure Experimental (mm Hg), Vapour pressure Subcooled Liquid (Pa)	EPI MPBPWIN v1.43
HLC Bond Method (atm-m3/mole), HLC Group Method (atm-m3/mole), HLC Via VP/WSol (atm-m3/mole), HLC Via VP/WSol (Pa-m3/mole), Henrys Law Const. Exp db (Pa-m3/mole), Henrys Law Const. Exp (atm-m3/mole)	EPI HENRYWIN v3.20
Water solubility from Kow (mg/L), Water solubility Exp (mg/L), Water solubility Exp Ref, Log Kow, Log Kow Exp, Log Kow Exp Ref,	EPI WSKOW v1.42
Water solubility from Fragments (mg/L)	EPI WATERNT v1.01
pKa Acid, pKa Base	ACD/ ToxSuite 2.95.1 Ionization\ACD/Labs pKa
LogD	ACD/ ToxSuite 2.95.1 Ionization\ACD/Labs LogD
Log Koa, Log Kaw	EPI KOAWIN v1.10
Kp (m3/ug) Mackay-based, Kp (m3/ug) Koa-based, Phi Junge-Pankow-based, Phi Mackay-based, Phi Koa-based	EPI AEROWIN v1.00
Koc from MCI (L/kg), Log Koc from MCI, Koc from Kow (L/kg), Log Koc from Kow	EPI KOCWIN v2.00
Mass Amount (%), Half-Life (hr), Emissions (kg/hr)	EPI Level III Fugacity Model (EPI Suite v4.11)
Sewage Treatment Plant (STP) overall chemical mass balance using 10,000 hr (%)	EPI STPWIN (EPI Suite v4.11)

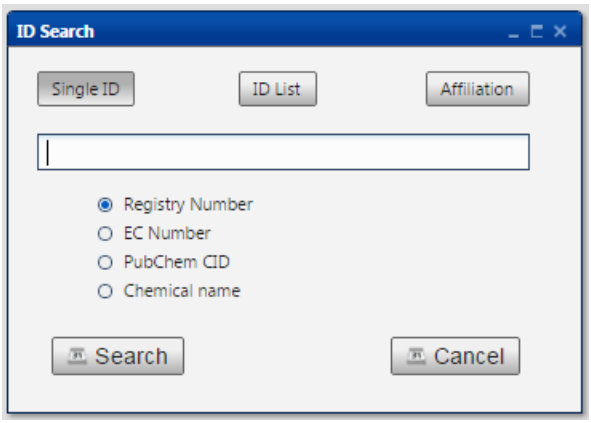
Predicted property	Software
Half-Life (d), Half-Life (hr), Overall Rate Const. (OH: E-12 cm3/molecule-sec and OZ: E-17 cm3/molecule-sec)	EPI AOPWIN v1.92
Biowin1 (linear model) Probability of Rapid Biodegradation, Biowin2 (non-linear model) Probability of Rapid Biodegradation, Biowin3 Expert Survey Ultimate Biodegradation, Biowin3 Expert Survey Ultimate Timeframe, Biowin4 Expert Survey Primary Biodegradation, Biowin4 Exp. Survey Primary Timeframe, Biowin5 (MITI linear model) Biodegradation Probability, Biowin6 (MITI non-linear model) Biodegradation Probability, Biowin7 (Anaerobic Linear) Biodegradation Probability, Petroleum Hydrocarbon Biodegradation Half-Life (days)	EPI BIOWIN v4.10
BCF (L/kg wet-wt), Log BCF (L/kg wet-wt), Whole Body Primary Biotransformation Fish Half-Life (days), BCF Arnot-Gobas (upper trophic) Including Biotransformation (L/kg wet-wt), BCF Arnot-Gobas (upper trophic) Zero Biotransformation (L/kg wet-wt), BAF Arnot-Gobas (upper trophic) Including Biotransformation (L/kg wet-wt), BAF Arnot-Gobas (upper trophic) Zero Biotransformation (L/kg wet-wt)	EPI BCFBAF v3.01
LC50 (Fish) or EC50 (Daphnid and Algae) for Most Toxic Class (mg/L), Max. Log Kow for Most Toxic Class, Most Toxic Class	EPI ECOSAR v1.11
Lipinski's Rule-of-five (bioavailability), Absorption from gastrointestinal tract for 1 mg dose (%), Absorption from gastrointestinal tract for 1000 mg dose (%), Log brain/blood partition coefficient	Equations from literature
Dermal absorption (mg/cm2/event)	EPI DERMWIN v2.02
Acute toxicity in rodents: Rat Oral, Rat Intraperitoneal, Mouse Oral, Mouse Intraperitoneal, Mouse Intravenous, Mouse Subcutaneous	ACD/ ToxSuite 2.95.1

Appendix 1: Searching by identification number

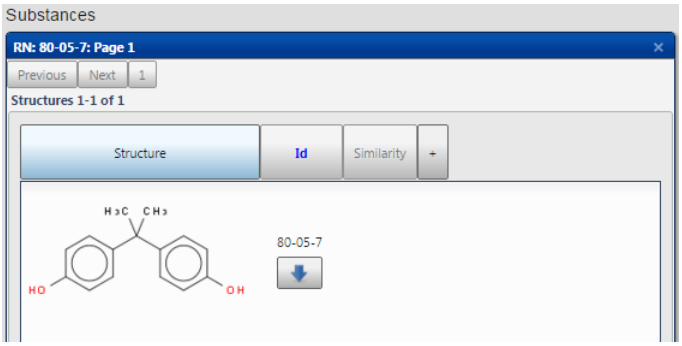
Example 1: Single ID query.

Search for registry number 80-05-7.

Start by clicking the ***Id*** button to the right on the main search screen. The ID Search box will appear on the screen:



Select Single ID and Registry number from the list in the ID Search box. Once you've done this, type in the registry number with or without hyphens in the blank field. To start the search, click the ***Search*** button. You will now see the result to the right on the screen under "Substances".

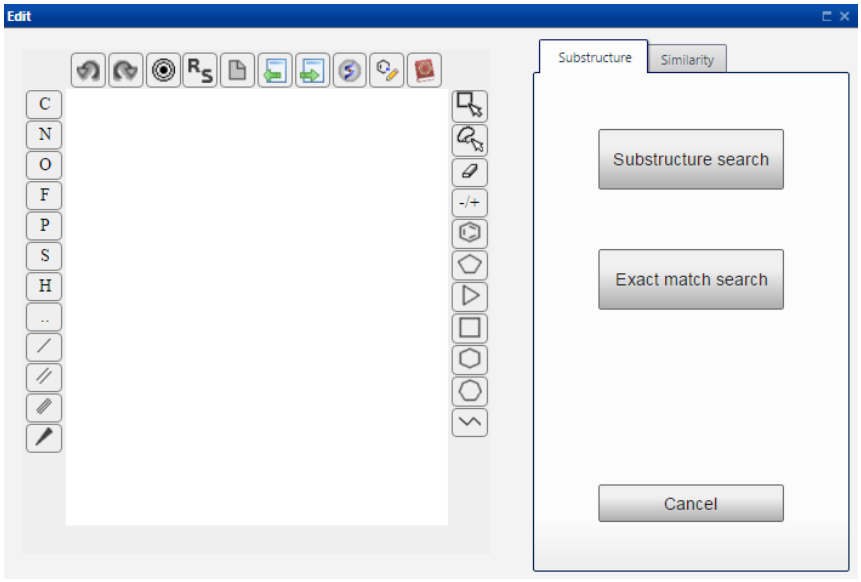


Appendix 2: Searching by structure and similarity

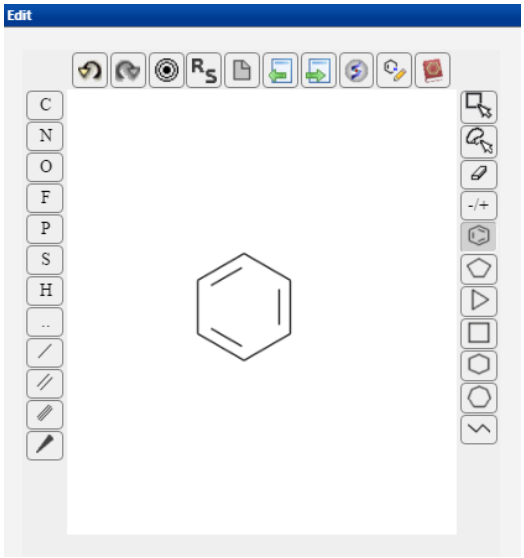
Example 1: Substructure search.

Search for molecules containing a fluorobenzene fragment.

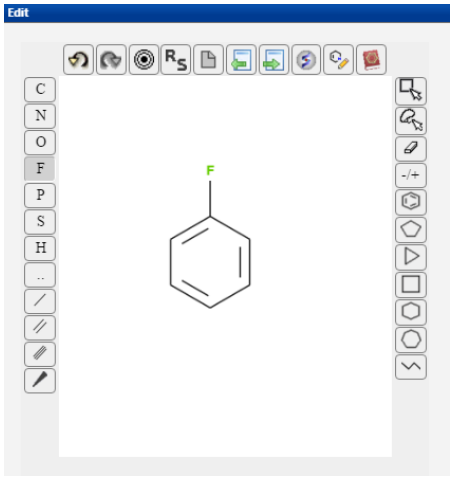
It is possible to search for molecules which contain specific molecular fragments. Start by clicking the ***Structure*** button to the left on the main search screen. This will open the edit box shown below:



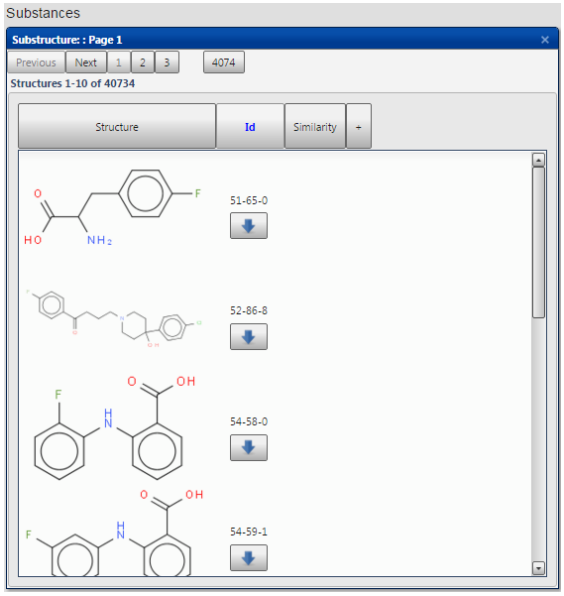
In this example we will use the drawing tools in the edit box to create the fluorobenzene fragment. Alternatively, the fragment can be imported as a SMILES/MOL file or from the chemical dictionary. When drawing a fragment, the different atoms, bonds etc. are added one by one. Start by adding the benzene part of the molecule: left-click on the ***benzene*** button and then click on the blank canvas. The canvas will look like this:



Now click on the single bond button and then click on one of the atoms in the benzene ring on the canvas. Then add the Fluor (F) atom. The fluorobenzene fragment is now completed:



To start the search, click the ***Substructure search*** button under the **Substructure** heading to the right in the edit box. The molecules that contain the fluorobenzene fragment will be shown to the right on the screen:



The total number of molecules containing the fluorobenzene fragment are shown under the heading "Results". The functionalities of the results window are described in detail in the section "Results window with substances" in the manual.

Example 2: Exact match search.

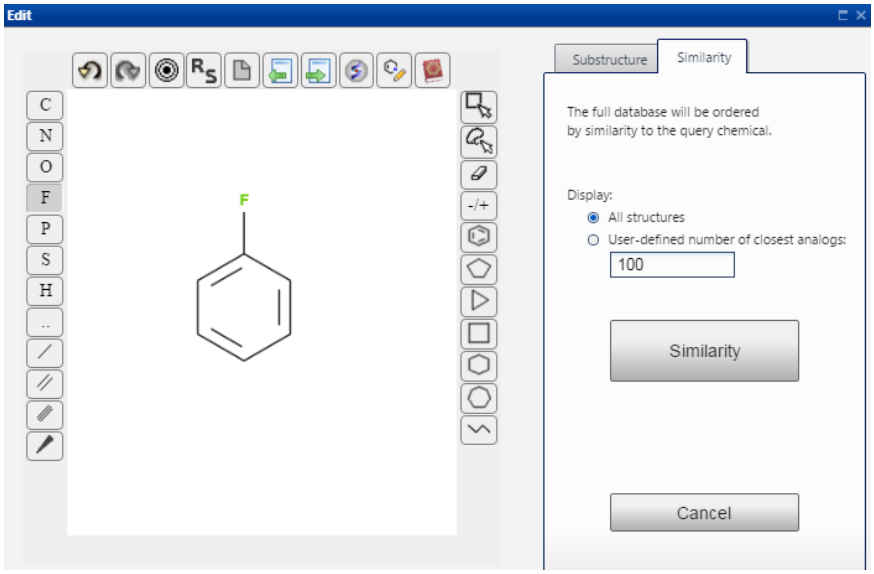
Find molecules exactly matching the fluorobenzene structure.

Start by drawing the fluorobenzene structure as described in example 1. To start the search, click the ***Exact match search*** button under the **Substructure** heading to the right in the edit box. You will now see the search result in the window under "Substances" to the right on the screen.

Example 3: Similarity search.

Find the most similar chemicals to fluorobenzene.

Start by drawing the fluorobenzene structure as described in example 1. Then click the heading **Similarity** to the right in the edit box. Before you start the search you need to select if all structures or a user-defined number (e.g. 100) should be displayed. In this example the 100 closest analogs is selected:

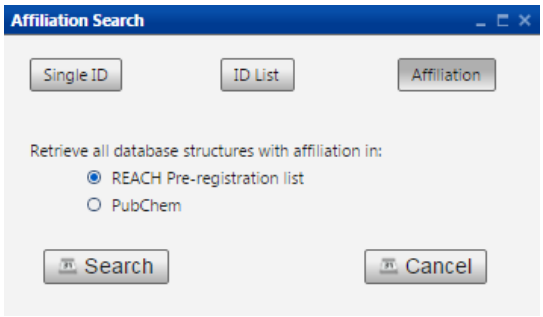


To start the search, click the ***Similarity*** button. The resulting substances will be ordered by similarity to the query chemical.

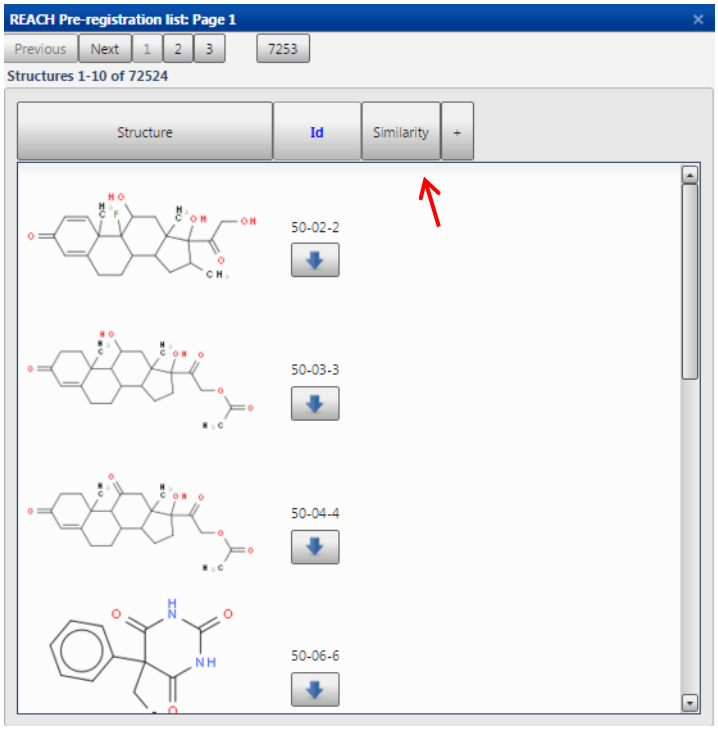
Example 4: Similarity search.

Find the REACH chemicals that are most similar to fluorobenzene.

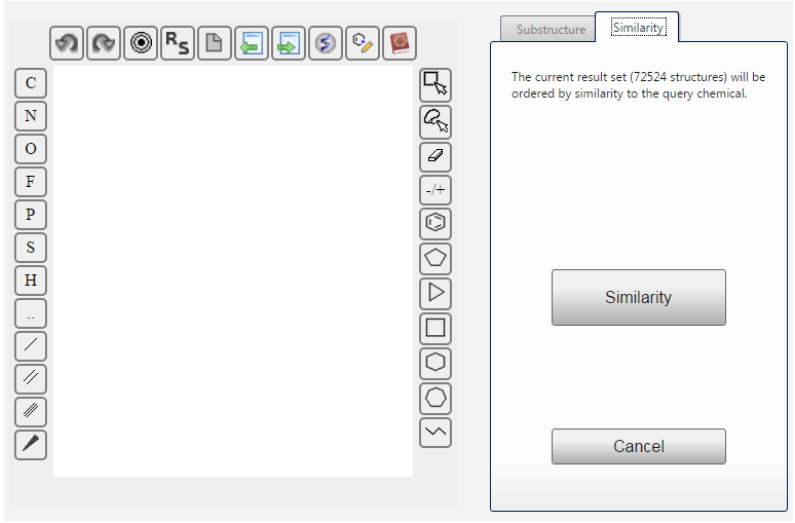
Start by searching the REACH chemicals in the database: Click the ***ID*** button to the left on the main search screen and then click the heading **Affiliation** in the ID search box. The box should now look like this:



Select "REACH pre-registration list" and then click ***Search***. This will generate a results window showing all REACH pre-registered chemicals:



Now you can search for similarity within the REACH pre-registration list. Click the ***Similarity*** button marked with a red arrow in the results window above. At this point, a new box will open:



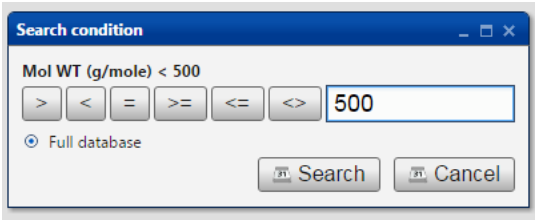
Draw the fluorobenzene structure (described in example 1) on the blank canvas and click the ***Similarity*** button to start the search. The REACH substances will now be ordered by similarity to the query chemical.

Appendix 3: Searching by model endpoint

Example 1: Simple model endpoint search.

Search for molecules with molecular weight < 500 g/mole.

Click the ***PhysChem*** button to the left on the main search screen and then select Mol WT (g/mole) from the drop-down menu:

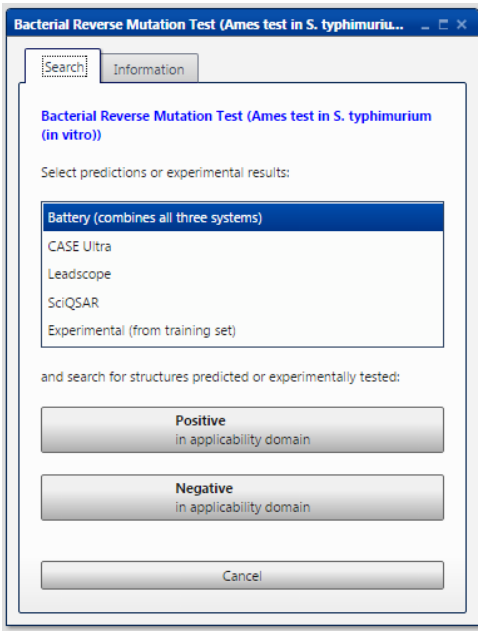


Type in 500 in the blank field in the box and click the ***Search*** button. The substances of interest will appear in the results window.

Example 2: Simple model endpoint search.

Search for molecules that are positive for Ames test.

Click the ***Human health*** button to the left on the main search screen and then select "Genotoxicity - Ames test - Bacterial Reverse Mutation Test" from the drop-down menu. The screen should now look like this:



The Ames test model is made in each of the three systems CASE Ultra, Leadscope and SCIQSAR and in this example we will select the overall battery prediction. Once the 'Battery' is selected, click the ***Positive*** button to start searching for molecules that are positive for Ames test. The results will appear on the screen.

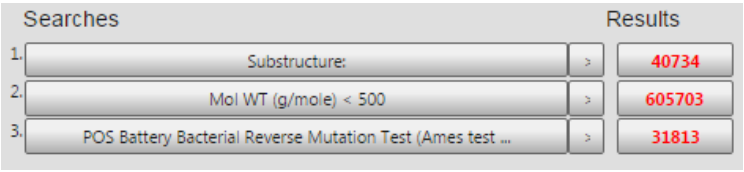
Appendix 4: Combining searches

Example 1: Complex search containing fragments and model endpoints.

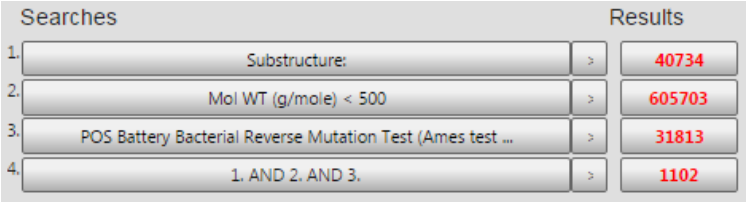
Search for molecules that have:

a fluorobenzene fragment
molecular weight < 500 g/mole
a positive Ames test

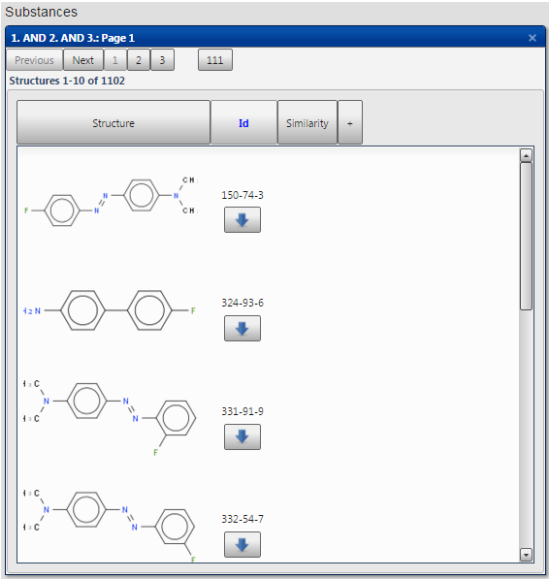
We have already made searches for each of the three criteria in the previous examples. When the three individual searches are made one by one, the screen should look like this:



To search for molecules that meet all three criteria, left-click on the three search strings. This will change the color to green. Now click on the ***AND*** button to the right on the main search screen to start the search. At this point, you should see this on the screen:



The molecules that meet all three criteria will be shown in the window under “Substances” to the right on the screen:



Appendix 5: Software systems used for modeling

Case Ultra

CASE Ultra divides each substance into fragments containing 2–10 interconnected atoms non-hydrogen atoms. These fragments are labelled with the experimental value of the parent substance as active or inactive. If a fragment is over-represented (p>95%) in the group of active or inactive substances the fragment is assumed to be relevant for the modelled activity. If the fragment is not significantly overrepresented in active or inactive substances it will not be considered important. A fragment with a statistical correlation to active or inactive substances is called a biophore or a biophobe, respectively. When all fragments have been examined for their importance to activity, a hierarchical selection takes place, starting with the biophore with the best statistical significant result. Substances containing this substructure are set aside and the next biophore is found in the same manner. This is repeated until either the entire training set is used or there are no more statistically significant fragments. The whole procedure is then performed for biophobes in an identical way. Each group of substances containing a biophore or biophobe is then analysed to find modulators that either enhance or decrease the probability of the fragment being a biophore/biophobe. The modulators can be structural fragments or chemical properties (e.g. activating fragments, deactivating fragments, $\log K_{ow}$, molecular orbital energies).

Leadscope

When data are imported into Leadscope, the substances are classified by structure into categories using a library of approximately 27,000 structural features. The structural features are substructures such as functional groups, heterocycles and pharmacophores. The program also calculates a number of physico-chemical descriptors such as logP, molecular weight and the number of hydrogen bond acceptors and donors. When a model is developed, a sub-set of the structural features and the physico-chemical descriptors are used. Features and descriptors can be chosen either manually or automatically.

SciQSAR

The software operates with various molecular descriptors, e.g. physical-chemical, electrotopological state (E-state) and hydrogen electrotopological (HE-state) indices, connectivity indices and other descriptors. The E-state is a value calculated for each atom or hydride group (e.g. CH_3, NH, OH, N and Cl) in a molecule. It is large for electronegative atoms, especially for those with a few skeletal connections and is smaller for less electronegative atoms and atoms with several σ bonds. The HE-state is calculated for each hydrogen connected to non-hydrogen atoms (e.g. OH, NH, NH_2, CH, CH_3) in a molecule. This index shows the polarity of the hydrogen where a highly polar hydride group gives a greater value (e.g. OH) than a less polar group (e.g. CH_3). The electrotopological state indexes (E-state and HE-state) are related to an attribute called electron accessibility. This attribute comes from the hypothesis that atoms with higher electron density that are accessible for contact with other molecules have a higher potential for interaction. The molecular connectivity descriptors, χ, are calculated for the whole molecule. This group of descriptors provides information on skeletal variation, including degree of branching and types of branching.