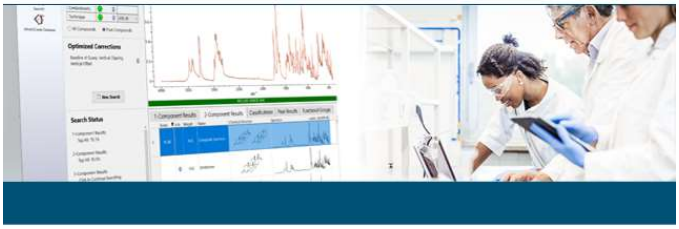
KnowItAll for MS and GC/MS

Quick Reference Guide



KnowItAll
Analytical Edition

WILEY

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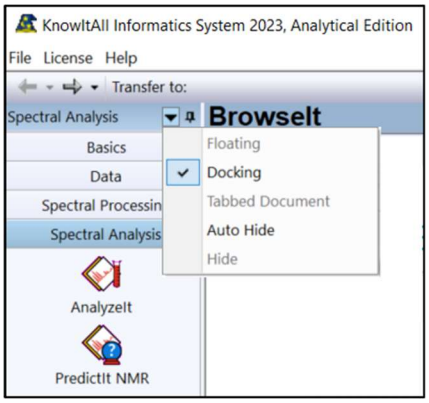
Prepared By
O. David Sparkman

How to Use KnowItAll Mass Spectrometry Applications

Before Using KnowItAll, Review the Random Notes at End of This QRG

KnowItAll for MS and GC/MS

Opening Display
Upper Left Corner (Depends on closing)



Selected Item (**Spectral Analysis**) on top and bottom of display (Other three options appear between the two displays of **Spectral Analysis**.).

Icons pertain to lower and top item on the Top part of the page. The Primary Functions for selection will move between the top and bottom of this column on the Desktop based on what is selected, i.e., when **Basics** is selected, **Data** , **Spectral Processing** , and **Spectral Analysis** will appear on the bottom and only **Basics** will appear at the top.

Right side of Desktop only changes when an icon under a selected Primary Function is selected.

Spectral Analysis does not have selections used for MS or GC/MS Data.

Basic button has **ChemWindow** (for drawing Structures), **ReportIt** (for drawing chemical reactions,) and **BrowseIt** (returns to opening promotional screen,).

Data selection has two selections recommended for GC/MS and MS, **MineIt/Create Databases** ( ) and **SearchIt** ( ) for individual mass spectra.

Spectral Processing is the primary selection for GC/MS and MS with the options of **ProcessIt** ( ) and **MS Expert** ( ). **MS Expert** is not explained in the Quick Reference Guide.

Important Notice

The first time the **MineIt** window is displayed, select **View** from the Main Menu Bar. Make sure that there is a check mark (✓) next to the **Implicit Hydrogens** selection.

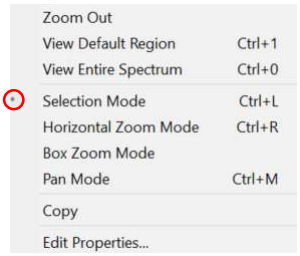
GC/MS Data Analysis ProcessIt window

After selecting **Spectral Processing**, put the Mouse pointer on the **ProcessIt** icon ( ) and Click the **left Mouse button** (**LMB**). In the upper left corner of the **ProcessIt** window is a button labeled **Open Data File** (Open Data File). Put the Mouse pointer on this button and single Click the **LMB**. This causes a **File Section** dialog box to be displayed. Navigate to the name of the desired GC/MS data file. Put the Mouse pointer on the name of the GC/MS data file to be viewed and Click the **LMB** once. A preview of the RTIC chromatogram will appear in the lower part of the dialog box. Put the Mouse pointer on the button labeled **Open** and Click the **LMB** once to open the file in the **ProcessIt** window.

KnowItAll recognizes most instrument manufactures GC/MS formats. If not, export the data file in the AIA format (*.CDF; netCDF file).

The display changes to four widows. The vertical window on the right is the Text Information contained in the data file. This window can optionally be closed. Just Click on the **X** box in the upper left corner. To re-open, select **View** in the Main Menu Bar. On the **View** menu highlight **Properties Table** and Click the **LMB**.

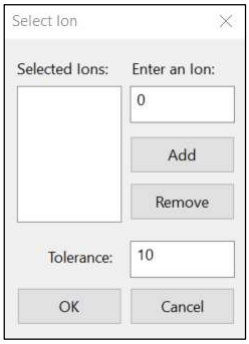
The three horizontal windows on the left side of the **Properties Table** are: **Top**, reconstructed total ion current (**RTIC**) chromatogram; **Middle**, the mass spectrum of wherever the Mouse pointer is located on the RTIC chromatogram; and **Bottom**, the selected spectrum.



The Mouse Pointer will have different images depending on the selected function. The functions can be assigned by using the **Right Mouse Button** (**RMB**) menu that can be displayed when the pointer is in one of the three horizontal windows. The selected function is designated by the • symbol on the left edge. There are also buttons above the **Top** window to change the Mouse pointer to some of its functions. Passing the Mouse pointer over these buttons will show (Tool tips) that indicate what option can be selected. Only some of the **RMB** menu selections can be assigned by click on one of these buttons.

Mass Chromatograms (Extracted Ion Chromatograms, EIC)

Mass Chromatograms (EIC) can be displayed along with RTIC chromatograms. Currently, this is limited to single *m/z* values. Feature versions of KnowItAll will also allow for sums and ranges of *m/z* values.



Click on **Process** in the Main Menu. From the **Process** menu select **Selected Ion…** This results in the display of a dialog box. Enter values, one at a time, in the box under the label **Enter an Ion:** and then click on the **Add** button. The box will clear, and the number will appear in the window with the **Selected Ions:** label, on the left side of the box dialog. **Do Not** press the <Enter> key after entering the number. This will close the dialog box and the entered number will not be retained. Add additional numbers to the list by repeating the above process. Once all numbers have been added, click on the **OK** button. If you want to cancel the display of any EIC, redisplay the dialog box, highlight the value(s) in the list and click on the **Remove** button.

By default, the RTIC and EIC(s) will be presented in a stacked display in the Chromatogram window with the RTIC Chromatogram on the bottom. The intensities of the peaks in the EIC(s) will be relative to the most intense peak in the RTIC chromatogram. The display can be changed to an overlay by selecting the **Overlay** button ( ) in the button bar just under the **ProcessIt** label. To return the the stacked display, select **Stacked** button (second to right of the **Overlay** button – look for Tool Tips).

By default, the Active Chromatogram is the RTIC chromatogram. By placing the Mouse pointer, when it is in the Select Mode ( ), on one of the lines of a EIC and clicking the left Mouse button, will make that chromatogram the Active. That chromatogram's line color will turn to that of the Active and the previous Active (the RTIC in this discussion) will change to the color of the first spectrum in the **Edit Properties** dialog box's **Color and Font** tab. Just to the right of the Stack button, there are four buttons related to the scaling of multiple chromatogram displays; **Scale Independently** ( ); **Scale To Active** ( ); **Scale To Largest** ( ); and **No Autoscale** ( ). These buttons are grayed unless the Mouse pointer in the select mode ( ).

Selecting Spectra to be Searched

When the Mouse pointer is a normal arrow (the **Select Mode**), placing it on a specific position on the RTIC chromatogram will cause that spectrum to be locked into the **Bottom** window when the **left Mouse button** (**LMB**) is Clicked once.

Averaged spectra can be selected for searching. There is a Green-colored Bar labeled **AVERAGE RANGE BAR** at the bottom of the RTIC chromatogram window, just under the **abscissa** label. Put the Mouse pointer in this **Bar**; click and hold down the **LMB** and drag to the right or left to paint a region of the chromatogram. All spectra in the region will be averaged. The region can be redefined by putting the Mouse pointer on either the right or left edge of the painted region in the **Bar** and dragging to the right or left while holding down the **LMB**. When the button is released, the adjusted margin of that side of the region will be moved. The entire range can be move to a different part of the chromatogram by putting the Mouse pointer on the center part of the region in the Bar, holding the **LMB** down and moving the Pointer to the right or left. When the button is released, the new region will be painted. Multiple, non-contiguous groups of spectra can be averaged. The average spectra ranges are painted a light green.

When the Mouse pointer is in the **Select** mode, a single range of spectra can be selected as an averaged spectrum by holding down the **LMB** and dragging the pointer over the region (to the left or right) that is to be averaged in the RTIC chromatogram. If this process is repeated, the previous averaged is removed.

Background subtraction: Searching of a background subtracted spectra is possible. The range or ranges of the background spectra are selected by putting the Mouse Pointer in the dark red **BACKGROUND RANGE BAR** at the top of the RTIC chromatogram. Selections are accomplished in the same ways as Averages are selected and the overall behavior is the same.

At the right end of the **AVERAGE** & **BACKGROUND** Bars are Trashcan 🗑 icons. Putting the Mouse pointer on the range in these bars, holding down the LMB, and dragging to the 🗑 deletes the range. Click on the 🗑 deletes all the range.

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CAUTION

It is possible to zoom in on a portion of the RTIC chromatogram using the **Horizontal Zoom Mode** or **Box Zoom Mode** selections from the **RMB** menu. If this is done, it may cause a previously selected Average Spectrum or Background Range to no longer be visible. Be sure to carefully review the **Title Box** in the lower Spectrum window to see if multiple averages have been selected for either.

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The spectrum in the **Bottom** window can be sent to the **SearchIt** window by putting the Mouse pointer on the **SearchIt** button in the button bar just below the Main Menu Bar and Clicking the **LMB**.

When the spectrum is sent to the **SearchIt** window, the **ProcessIt** window is closed and the **SearchIt** window, with the spectrum, is displayed. There will be a tab at the bottom of the the display with label **Search #1** in the **SearchIt** window.

The displayed spectrum can be searched by putting the Mouse pointer on the **Search** button in the lower right of the **Search** window and Clicking the **LMB** once OR the **ProcessIt** window can be re-displayed by putting the Mouse pointer on the arrow icon (⇦) pointing to the left on the left side of the top button bar and Clicking the **LMB** once. Another spectrum can then be selected and sent to **SearchIt** window.

When the display is returned to the **ProcessIt** window, the previously select background range(s) and averaged spectra range(s) are still displayed. Putting the Mouse pointer in the RTIC chromatogram window and Clicking the **LMB** once, with the Mouse pointer in the **Select** mode, will clear all the averaged range(s). The background range(s) can be removed by putting the Mouse pointer in the highlight section(s) of the dark red **BACKGROUND RANGE BAR**, Clicking the **RMB**, and selecting the **Yes** button in the displayed dialog box.

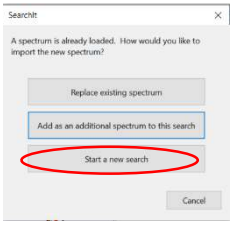
As many spectra as desired from a single data file can be queued by repeating the above processes process.

NOTE: The displayed spectrum in the bottom window of the ProcessIt window can be sent to the NIST MS Search Program by selecting **Edit** from the Main Menu Bar and then selecting **Transfer to NIST MS Search**.

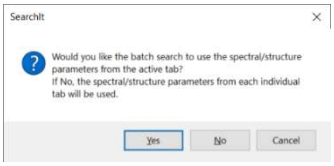
Selection of Additional Spectra

Once the **ProcessIt** window is re-displayed, select another spectrum on the RTIC chromatogram in the same way as the first spectrum was select. Then Click the **LMB** with the pointer on the **SearchIt** button in the top button bar.

This time, when the **SearchIt** window is display, the box to the right is displayed.

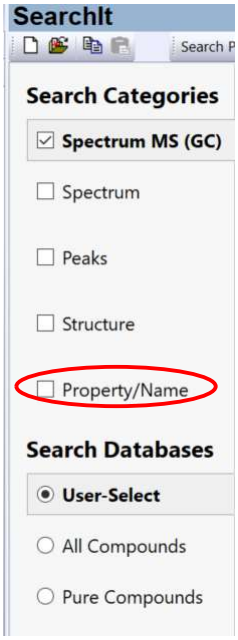


Select the **Start a new search** button. The spectrum will be displayed and there will now be two tabs at the bottom labeled **Search #1** and **Search #2**. The previous spectrum can be displayed by putting the Mouse pointer on the tab labeled **Search #1** and clicking the **LMB**. To search all the spectra in the queue, select **Edit** from the Main Menu Bar to display the **Edit** dropdown menu and select **Do Batch Search**.



The dialog box shown on the right is displayed. Although not likely, it is possible that different sets of libraries will be associated with each spectrum. If the **Yes** is selected, all the spectra in the queue will be searched using the libraries associated with the currently displayed spectrum. If **No** is selected, then each individual spectrum will be searched with its associated libraries.

After all the spectra are searched, the **SearchIt** window will close and the **MineIt** window will be displayed with the **Hit List** for the last searched spectrum displayed, and there will be separate tabs for the **Hit List** of each of the other spectra at the bottom of the **MineIt** window. Click on any tab to display the Hit List for the searched spectrum, the searched spectrum, and the selected **Hit List** spectrum.



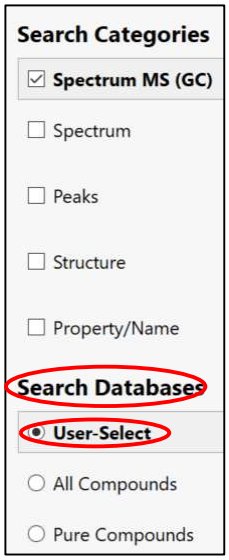
IMPORTANT NOTE

Under the **Search Categories** section of the **SearchIt** window is **Property/Name** label next to a check box. It is **VERY** important that this check box is **NOT** selected when the spectrum is to be searched. Any selections in the **Properties/Name** section of the **SearchIt** window can have a negative effect on the results of the spectrum search.

All options other than the **Spectrum MS (GC)** should **NOT** be selected.

Selecting Libraries to Search

On the left side of the **SearchIt** window is a section labeled **Search Databases** (midway down the column). There is a selected radio button to the left of a **User-select** label. Put the Mouse pointer on this label (not the radio button) and Cick the **LMB**. A window is displayed. The top part has a list of all available libraries for all techniques. At the top of this display is a drop-down list box next to the **Limit to spectral technique** label, where the desired technique can be selected and only libraries for that technique can be displayed in the upper part. The lower part of this window shows the selected libraries to be searched for the selected **Search # X** tab.



Libraries can be added (highlight the desired library names in the upper area) and then Click on the **Add** button or deleted (highlight the name of the library to be removed in the lower area) and then Click on the **Remove** button.

Once this window is no longer needed, Click in the the **Spectrum MS (GC)** label at the top of the column to display the bar graph spectrum. This caused the spectrum in the **SearchIt** window to be displayed and the spectrum will reappear. The search of the spectrum displayed (selected tab) in the **SearchIt** window by Clicking on the **Search** button on the lower right of the **SearchIt** window or the spectra in each tab can be searched by doing a **Batch Search** from the Main Menu's **Edit** menu.

After the spectrum (or a batch of spectra is (are) search the **SearchIt** window closes, and a tab appears at the bottom of the **MineIt** window with the label **Hit List Search #X**. Each Hit List is display by Clicking on the individual tab. The **MineIt** widow is divided into three sections. The vertical window (optionally displayed) is the Structure (if present) and the meta data for the library spectrum. The upper horizontal window contains the selected Hit List spectrum and the searched spectrum. The lower horizontal window is a display of the all the Hits, with a scroll bar.

MS Library Search Settings

The default Library Search settings will perform what is often referred to as a Normal Identity Search and should be used when it is possible that there is a spectrum of the same compound that produced the spectrum being searched in one or more of the selected libraries.

The *m/z* value of each peak in the searched spectrum and its relative intensity is considered as is each peak in the library spectrum along with its relative intensity. By default, this Hit List is ordered based on the best match (**HQI**, **hit quality index**) regardless of which library the matched spectrum is in.

The spectrum submitted for search can represent a **Single** compound, or a **Mixture** of 2 or 3 compounds. The type of spectrum is selectable in the drop-down list box under the **Number of compounds:** label. The default is **1 (Single)**. If it is believed that the spectrum is a mixture of two compounds, set this to **2** (**Mixture)** and if the spectrum is believed to represent three compounds set this to **3 (Mixture)**. When multiple matches are found in the searched libraries, the Hit List spectrum will be of a mixture and multiple structures and names will appear.

This Search uses the **Search Method Dot Product (Cosine)** and the check boxes next to the labels **Adaptive Search** and **Reverse Search** are **NOT** selected. The drop-down list box below the **Search Method** label has three other selections. These can be ignored. Unless your level of mass spectrometry knowledge is high, this Default Search should be used most often. This is the same as the NIST Mass Spectra Search Program's **Normal Identity Search** when using the default Limit Settings. The other three search types are explained in explained in more detailed **KnowItAll** documentation.

If it is believed that there is no spectrum of the compound that produced the spectrum being searched, the **Adaptive Search** should be used. The **Adaptive Search** finds compounds that are similar to the submitted spectrum but have additional or missing fragments. This search is initiated by placing a check (✓) mark in the box next to the **Adaptive Search** label on top of the **SearchIt** window with the spectrum displayed.

Selecting the check box next to the **Adaptive Search** label results in a text entry box preceded by the label **Molecular m/z:** being displayed. The **Adaptive Search** requires that the nominal mass of the analyte be specified. If it is not, the software will try to assign this value. It is easy to do this when a molecular ion peak is present in the spectrum; however, if it is not, a soft ionization technique like CI or FI or derivatization should be used. If no value is entered, the search will be much slower, and results will possibly be poorer.

The results of the **Adaptive Search** are displayed in **MineIt** window in the same way as an Identity Search, sorted by the **HQI** (**Hit Quality Index**) and the delta-mass (**Δm** [u]) value will be shown. The **HQI** reported is a special value determined by adding and subtracting peaks and suggested replacement fragments are described. This approach essentially extends the dynamic range of reference libraries by "hinting" at likely structures not found in the reference library. For example, an unknown compound with a methyl group on an aromatic ring would yield a very high adaptive HQI for a reference spectrum of a methoxy group of an aromatic ring. The delta mass (**Δm** [u]) of 16 for oxygen would be noted in the Hit List results for the methoxy library spectrum. In addition to the (**Δm** [u]) column in the Hit List item, there is a **Δm info** labeled column with the ⓘ symbol. Put the Mouse pointer on this symbol and Click the **LMB** once. This cause the **Adaptive Corrections** window to be displayed with three spectra stacked on top of one another (Query, Corrected Reference [Hybrid], and Uncorrected Reference [Library]). There are dashed lines from the abscissa of the Corrected Spectrum to the abscissa of the Uncorrected Spectrum to show what alterations were made to the Uncorrected Spectrum to produce the Corrected spectrum. A suggested structure can be drawn and associated with with the Queried spectrum to use with NIST's Mass Spec Interpreter to see if the two make sense relative to one another.

The **Reverse Search** option might be selected when doing a Normal Identity Search (the **Adaptive Search** box would not be checked). This is used when there is a possibility that the searched spectrum represents a mixture of two or more components. The Standard Identity Search is setup as described above. The difference is that the **Reverse Search** selection is checked. In **MineIt**, the Hit List is ordered according to the highest **Reverse Hit Quality Index** (**R.HQI**) value. If the searched spectrum represents more than a single compound, the best matches for each of the possible components will be at the top. The **R.HQI** is the

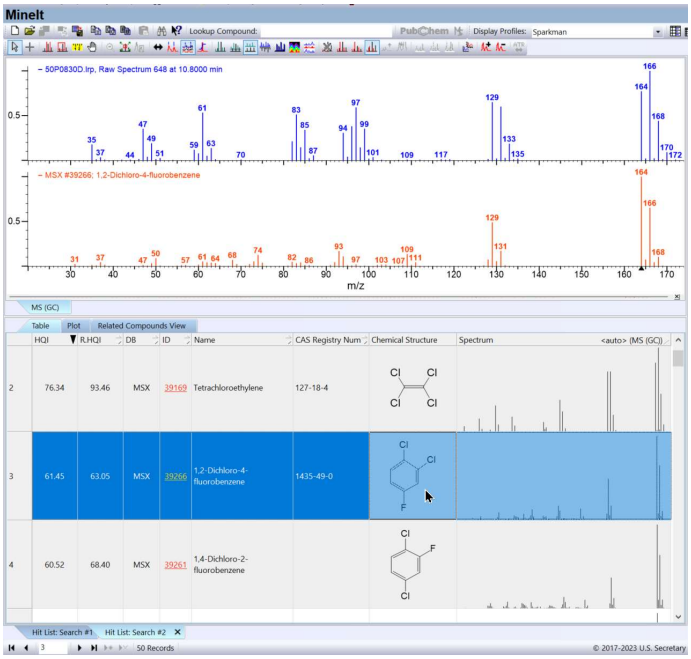
same as the **HQI** except all peaks in the sample spectrum that are not in the library are ignored when the value is calculated. This way the same spectrum can have two very high **R.HQI** values, one for each of two compounds present.

Hit List Size Limit

On the lower left of the **SearchIt** window is the following display:

You can use the Up/Down arrows to change the value in the box just to the right of the **Hit List Size Limit** Label or you can highlight the number and change it by typing a new value. For practical reasons, this should be between 50 and 100 when searching spectra. The **All Hits** check box should **NEVER** be selected. Selecting this can greatly prolong the search and adds no value.

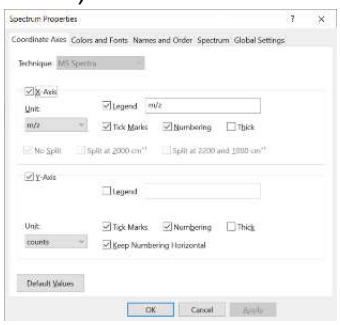
NOTE: The displayed spectrum in the bottom spectrum window of the **ProcessIt** window or the upper spectrum (that of Sample Spectrum) of the **MineIt** window can be sent to the NIST MS Search Program by selecting **Edit** from the Main Menu Bar and then selecting **Transfer to NIST MS Search**.



MineIt window showing partial Hit List

Customizing Window Displays

The three **KnowItAll** windows worked with in GC/MS and MS Data Analysis are **ProcessIt**, **SearchIt**, and **MineIt**. The window displays can be customized in several ways. In the **ProcessIt** window put the Mouse pointer on the RTIC chromatogram or one of the graphic spectrum sub-windows, Click the Right Mouse button to show the **RMB** menu, and select **Edit Properties** (last selection on the **RMB** menu) This is a tabbed dialog box as shown to the left. Examine all the options, make your choice, and see what they can do for you. The **Colors and Fonts** tab has a drop-down list box just below the **Item** label, The selected item in this list will cause the contents of this tab to change, i.e., the ability to change a font size or type is only available if **Peak** or **Axis numbering** or a similar item that involves the displayed font is selected in the list.



All selections are saved, even when exiting **KnowItAll**, until changed.

The **ProcessIt** window has a button that turns the labeling of the *m/z* values of mass spectral peaks on and off in the button bar just to the right of the button with the + symbol. **MineIt** has two such buttons: one to label the **Active Spectrum** and the other button to label all spectra. They are to the right of the ↔ button. **MineIt** has buttons to change the view options of the **Hit List** spectrum and the sample spectrum. These can also be selected by choosing **View** from the Main Menu Bar and selecting the **Display Mode** from the **View** menu to display a second level menu.

In the **MineIt** window, the **Display Properties** dialog box only pertains to the top half of the display that has the searched spectrum and the selected **Hit List** spectrum.

The **Hit List** display has separate tools used for customizing the display. The height of the rows and width of the columns can be adjusted. Columns can be added and deleted as well as edited.

To **Adjust Row Height**, put the Mouse pointer on the horizontal line between any two rows in the column with the Hit # (far left). The Mouse pointer

changes to a ↕. Hold down the **LMB** and pull down to make the row wider. Push up to make the row narrower. The height of all rows changes the same amount.

To **Adjust Column Width**, put the Mouse pointer on the vertical line to the left of the the column's width that you want to change, in the row that has the names of the columns. The Mouse pointer changes to a ↔. Hold down the **LMB** and pull the pointer to the right to increase the width of the column on the left. Pushing to the left while holding down the **LMB** will decrease the column's width.

Additional columns can be added. Placing the Mouse pointer any place in the **Hit List** and Clicking the **RMB**, will result in the display of a **RMB** menu. The first item is **Add Column**. This causes a **Choose Single Column** dialog box to be displayed that has a drop-down list box with several items and a scroll bar so that all can be displayed. Highlight the item and Click on the **OK** button. The column with that header will be added next to the last column on the right.

The second item is **Insert Column**. If the **RMB** menu is displayed when the Mouse pointer is on a specific column, the inserted item's column will be placed to the left of that column.

The other three items on this **RBM** menu are **Delete Column** (place Mouse pointer on Row to be deleted and Click **LMB**), **Delete Row**, and Edit Column, which allows you to change the column header.

Unlike other display changes, **Hit List** display changes are NOT AUTOMATICALLY saved and even are not used when switching between **Hit List** in the **MineIt** window. The changes are saved to a Display Profile: 

(right edge of upper button bar of the **MineIt** window). The two buttons are for **Add a New Profile** (left) and **Save a Current Profile** (right). The dropdown arrow allows you to select a profile. Highlight the name of the desired **Profile** and it will be applied to the current **Hit List** when you Click the **LMB**. If you select the tab for another **Hit List**, you will have to re-select the **Profile** to have in applied. When the new **Hit List** is selected, the list box will read **<no profile>**. The Profiles are permanently saved and cannot be deleted. They can be edited.

Importing Spectra in a Text Format

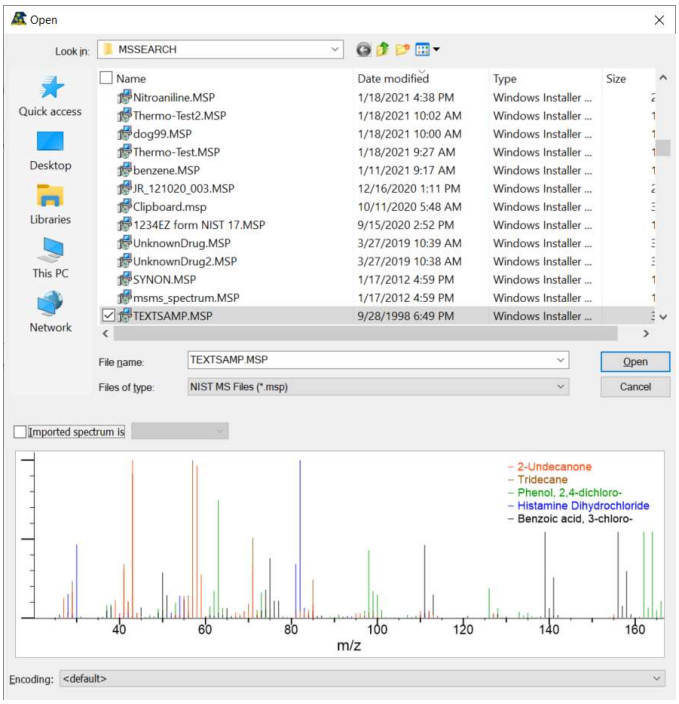
Spectra do not have to be imported from chromatographic data. They can be imported from a text file as long as they are in the format described by NIST (the NIST MSP format). The extension of the file is not relevant. It can be TXT, MSP, or any other three-character string.

Such spectra are imported when the **SearchIt** window is displayed. If **KnowItAll** is started from its

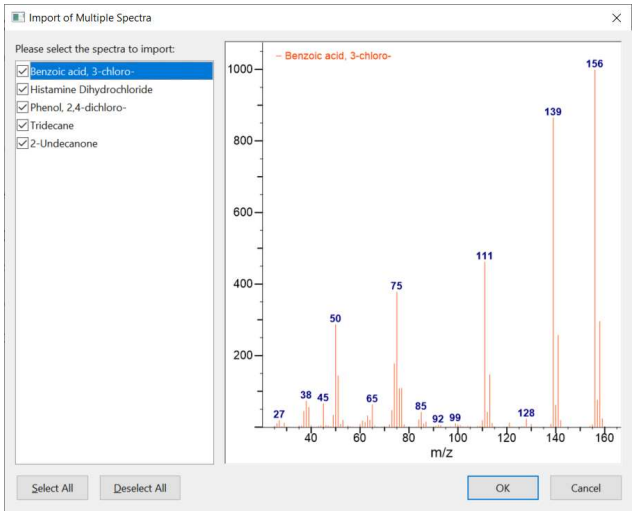
icon (not already running), select the button labeled **Data** in the left vertical panel. Then select the **SearchIt** icon. This results in the display of the **SearchIt** window with the **Library Selection** window overlaid. If the desired library is shown in the bottom of this window, go to the next step. If not, make the appropriate edits to the **Selected for Searching** window.

Next Step: On the far left of the upper button bar are two buttons. The Mouse-over label for the first is **New Search** and for the second is **Open Spectrum or Structures**. Select the second button.

This causes the **Open** file dialog box to be displayed (shown below).



Navigate to the location of the text file to be imported and highlight the file name by placing the Mouse pointer over it and Clicking the **LMB ONCE**. The preview window at the bottom of the **Open** file dialog box will show a spectrum which is a composite of all the spectra in the file and the name of each spectrum will also be displayed in a column. If this is the file you want, Click on the **Open** button (shown at bottom of previous page).



NOTE

The **Open** file dialog box can also be displayed by putting the Mouse pointer on the **Spectrum** label in the vertical panel to the left of the **Library Selection** window; however, when the display shown above appears, only a single spectrum can be imported to the **SearchIt** spectrum window at one time. Therefore, the **Open** file dialog box should only be displayed using the icon.

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A new window with two panes is opened on the upper left of the display (see above). The left pane has a list of all the names, with a check box (selected by default), of all the spectra in the file. The pane on the right shows a bar-graph of the selected spectrum (1st spectrum, by default). Putting the Mouse pointer on any name (not the check box) and Clicking the **LMB ONCE**, results in the spectrum for that name being displayed in the right pane. If the pointer is put on the the check box, and the **LMB** is Clicked once, the spectrum for that name is displayed and the check is removed for the box.

The Microsoft Windows multiple item-select commands of **LMB** Clicked while holding the <Shift> or <Ctrl> Keys can be used to select or deselect groups of spectra.

The window has **Select All** and **Deselect All** buttons as well as an **OK** and a **Cancel** button.

After making sure that the check boxes next to the names of the spectra to be import have been selected, Click the **OK** button. This results in the **Library Selection** window closing, each spectrum with a check box being imported, and a tab with the spectrum's name appearing at the bottom of display, which shows the bar-graph of the last spectrum imported. The bar-graph of any imported spectrum can be displayed by Clicking on the desired tab.

All the imported spectra or one spectrum at a time can be searched. To search a single spectrum, display it by Clicking on the appropriate name tab and then Clicking on the **Search** button. To search all the spectra represented by the various tabs, select **Edit** from the Main Menu Bar, and Click on the **Do Batch Search** selection on the **Edit** menu. A dialog box is displayed. Normally you would Click on the **Yes** button.

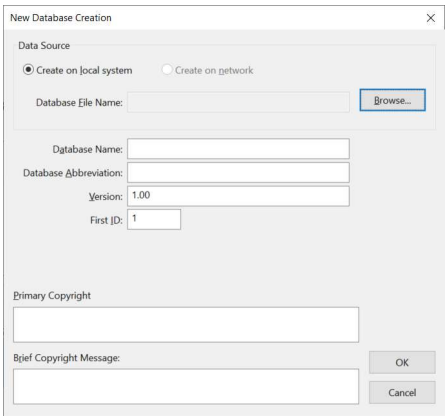
When one or all searches are complete, the **SearchIt** window will close and the **MineIt** window will be displayed with a **Hit List** for the last searched spectrum being displayed. If multiple spectra were searched, the display of each **Hit List** can be selected one at time by Clicking on the tabs at the bottom of the display.

You may want to apply a **Profile** for the **Hit List** appearance (see information above).

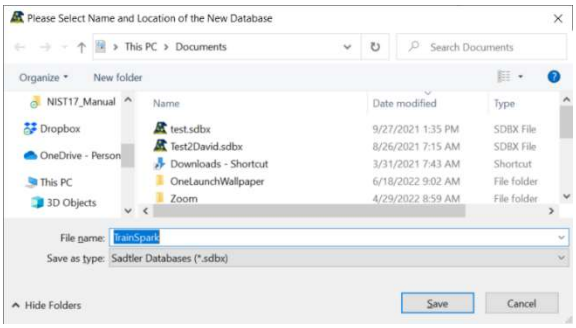
How To Create User-Databases

User-databases are primarily created from *.MSP or *.SDF files. *.MSP files only contain text. *.SDF files can contain text and structures.

When the **MineIt** window is displayed, Click the **New Database** icon (first icon on the button bar displayed under the **MineIt** label). This results in the display of the **New Database Creation** dialog box (shown below).



Click the radio button next to the **Create on local system** label. Next, Click the **Browse** button. This results in the display of the **Please Select Name and Location of the New Database** dialog box (shown below).



The mass spectral database being created will be created in the \This PC >Documents folder, by default. Type the name in the **File Name** text entry area at the bottom of the dialog box. The default extension of **sdbx** will automatically appended when you Click the **Save** button. The **Select Name and Location** dialog box is closed, and the **New Database Creation** dialog box comes into focus.

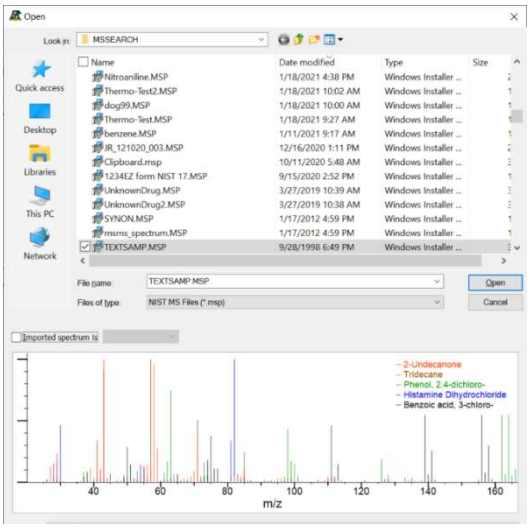
The default **Database Name** in the **New Database Creation** dialog box is the same as was entered into the dialog box displayed by Clicking on the **Browse** button. Some other name can be entered, if desired. You should enter a short code (suggestion of no more than 3 to 5 characters) in the **Database Abbreviation** field. This field may not contain numbers or symbols. After you are finished with this **New Database Creation** dialog box, Click on the **OK** button.

This results in a blank use-database in the **MineIt** view divided into three parts if the **Structure/Properties** window display is selected.

Select **File** from the Main Menu, then select **Import** from the **File** menu, or use the Hot Key **<Ctrl> + I**. This will cause the **Open** dialog box with the preview pane to be displayed (shown below). You can select a *.MSP file or a *.SDF file.

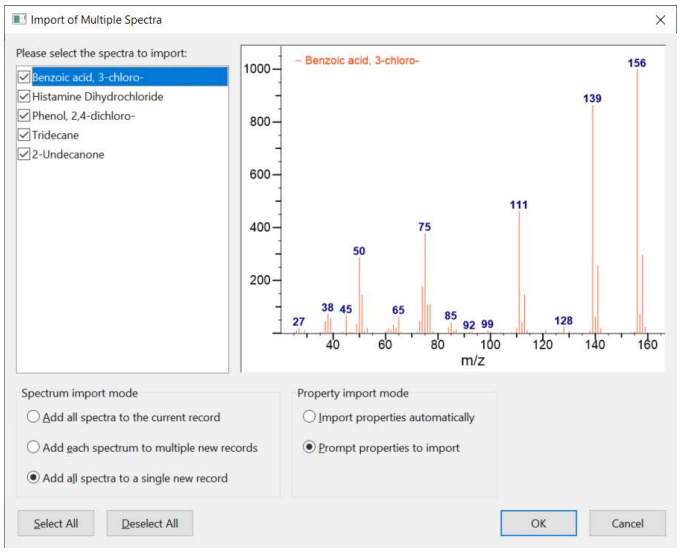
*.MSP file have a preview view as shown at the top of the next column. There is no Preview for *.SDF files. Select a *.MSP file.

Once you are satisfied that you have selected the correct file to import, Click on the **Open** button. This results in one of two other displays. If the file contains multiple spectra, the Input of Multiple Spectra dialog box in the middle of the next column is displayed.



File Open dialog box looking for *.MSP files

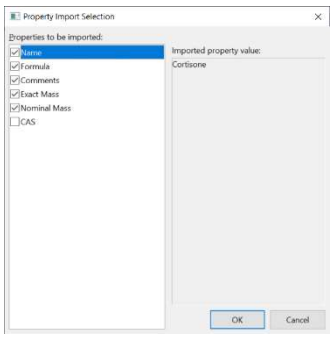
The
The
check next to each the compound name means that spectrum will be imported.



If you do not want some of the spectra to be imported, de-select that spectrum in the **Spectrum to Import** window.

There are three radio buttons under the **Spectrum import mode** label. The Add all spectra to a single new record is selected by default. Change this to **Add each spectrum to multiple new records**.

There are two radio buttons under the **Property import mode** label. If you know that there are field headers in the imported data that match Properties in KnowItAll, select **Import Properties automatically**. If you are unsure, select the other choice. Selecting

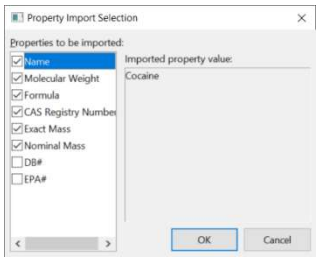


Prompt Properties to impot will result in the display of the **Properties Import Selection** dialog box when you Click the **OK** button in this dialog box (shown at bottom of previous column).

As seen in the **Properties Import** dialog box, the KnowItAll properties that correspond to those in the data being imported have check marks. Those that are not, don't. Selecting one that is not checked, results in the opening of the **Properties Field Definition** dialog box (not shown). There are two choices: **Link to existing field** or **Define as new field**. It is best to look for an existing field. Click the down arrow on the right side of the displayed list box. This displays a long list of Properties. In this case, we are looking for correspond to a CAS registry number. Type "CAS" This will take you to the **CAS Registry Number** property. This causes the **Properties Import Selection** dialog box to be displayed and now there is a check make in CAS.

This selection of a KnowItAll field being paired with a filed from the imported data is saved. The next time a file with a **CAS** field header is used, it will automatically be matched the KnowItAll Property **CAS Registry Number**.

If the *.MSP file contains a single spectrum, the dialog box below is displayed.



If values for the selected fields are in the spectrum file being imported, they will be imported with the spectrum. If there are un-selected fields and you select them, the **Properties Field Definition** dialog box (not shown) is displayed. This is delt with as described for *.MSP files containing multiple spectra. Clicking the **OK** button results in the spectrum being added as a record to the bottom of the database.

If other meta data needs to be associated with record, this can be accomplished when you Click the **Add** button at the bottom of **Structure/Properties** window. This causes the **Property Field Definition** dialog box to be displayed. Select the meta data category and complete the information. Once edits are complete, Click on the **OK** button

Structures are added via the link to ChemWindow. Double-click on the **Structure** window. Structures are added one at a time.

Meta data and structures can be added from the **PubChem** Web site. Highlight the record to be updated in the lower part of the **MineIt** window and Click on the PubChem button  in the Button Bar just below the **MineIt** label bar This will find the compound in the PubChem database on the Internet and allow the importation of the meta data and the structure to the record in the user-database. The button next to the PubChem button (just to the right) sends the selected spectrum/structure record to the NIST MS Interpreter program.

If another spectrum is being added from a single-spectrum file, **How would you like to import spectrum?** dialog box is displayed. The **Add as a new record** button is usually selected. After you Click this button follow the directions for adding a spectrum to a new database. The spectrum is added to the bottom of the database.

The spectrum of a selected record can be sent from a user-database to the NIST Mass Spectral Search Program by Clicking on **Edit** in the Main Menu of **MineIt** and selecting the last item on the **Edit** menu, **Transfer to NIST MS Search**. The MS Search is brought into focus and if the NIST Autosearch is active, the spectrum is searched. To return to the **MineIt** display, Click on the Switch to Caller button in the button ba  of MS Search.

The structure (if one is associated with the record) is not transferred with the spectrum to MS Search. To associate the structure with the spectrum in MS Search, use the **MineIt** Main Menu **Edit** selection, **Copy Structure**. This copies the structure to the Windows Clipboard. Use the <Alt> <Tab> command to switch the focus back to MS Search. In MS Search, select the **Librarian** tab at the bottom of the display. Highlight the name of the just transferred spectrum. Click on the **Edit Spectrum** button in the button bar. 

This will display a dialog box that has a button labeled **From Clipboard**. Click that button and the structure you put on the Clipboard in **MineIt** will associated with the spectrum. Click on the **Replace** button in this dialog box and the spectrum/structure pair will be in the **Spec List** of MS Search.

One CAUTION: If the structure in KnowItAll has a phenyl ring with a circle rather than alternating double and single bonds, the circle will not be present in the imported structure in MS Search. The structure is imported as a cyclohexyl ring. Such structures should be edited in the ChemWindow function of **KnowItAll** before the association is made in MS Search.

User-Libraries and SDF Files

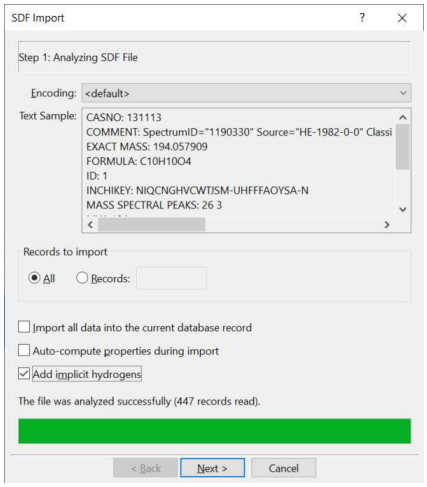
The terms user-databases and user libraries are somewhat synonymous. NIST user-libraries can be copied into KnowItAll user-databases. The NIST user-libraries can, and often do, have structures. It is important to remember, spectra can have structures displayed by one of two routes. The first, is to have a structure associated with the user-library spectrum. The second is to assign a Chemical Abstracts Services registry number (CASrn) to the user-library spectrum that is the same as an NIST library spectrum. If the user-library, with that CASrn, does not have an associated structure, the structure associated with the NIST spectrum having that CASrn will be displayed. Such displays will be referred to as pseudo-structure displays.

To create a KnowItAll user-database from an NIST MS Search user-library, the user-library must be converted to a *.SDF file. This is done using the NIST Lib2NIST program which can be found on the NIST Database Start menu. The Lib2NIST.exe file is in the C:\NIST20\MSSearch\ folder. NIST user-libraries are all located in this same folder. The user-libraries will have a specific icon ( ) in the screen used to input the name of the user-library to be converted. The NIST user-libraries are folders with almost 20 or more different files.

Important Note: Pseudo-structures in NIST user-libraries will not be transferred as SDF structures. These entries will have "No structure" in the SDF file.

Once the NIST user-library has been converted to a a *.SDF (a single file with the same name as the NIST user-library folder in the and located in the C:\NIST20\MSSearch\ folder), follow the instructions above to create a blank KnowItAll user-database. Use the same name as the NIST user-library.

The next step is to select the the *.SDF file for import by using the **Import** selection from the **File** menu of the Main Menu in the **MineIt** display.



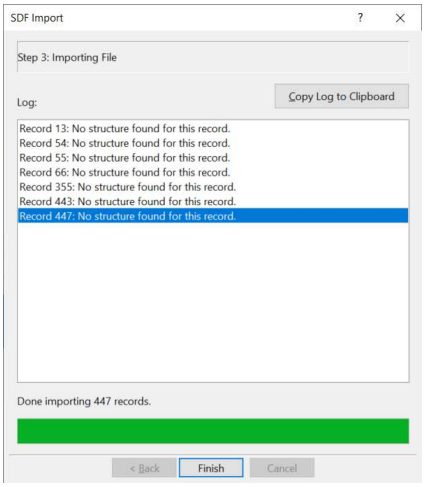
The above **SDF Import Step 1** dialog box will be displayed when the *.sdf file is opened. Make there is a check the box next to the **Add implicit hydrogens** label.

To continue the process, Click on the **Next>** button. This takes you to the **Step 2** dialog box (not shown) which is matching the fields containing meta data in the *.SDF file with those for the Know-It-All user-database. As you highlight an **SDF file** field, examples of the field contents are displayed in the lower part of the dialog box. You can Click on the **Suggest** button to see if there is a match. If there is no match or the match does not look like a good match, Click the down arrow next to the list box on the right side of the dialog box. Look for what might be a match by typing a few of the first letters of the field list in the **SDF file** window. After finding a match, Click on it in the list to save it. Sometimes the selection will result in a check mark being in the box net to the **Store as a numeric value** label when it is not a numeric value like in the case of **Comment**, **Formula**, and **Name**. If this happens, make sure to un-check this box.

In the **SDF file** list, there is a **MASS SPECTRAL PEAKS**. The suggested shows **Gas Chromatogram**. This is NOT what you want. You want **MS Peaks List** (this is the list of *m/z* intensity pairs) and is very important. **Nominal Mass** should be selected for the **MW** SDF file field.

DON'T FORGET to Click on the **SUGGEST** button before searching the list for a corresponding filed.

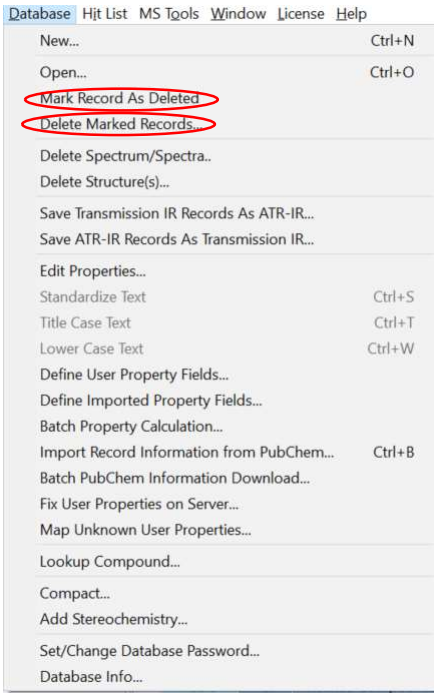
Once all the desired fields are matched (it is not necessary to match all fields in the **SDF file**), Click the **NEXT>** button. This imports the data into the KnowItAll user-database. A log will be display showing information such as which spectra did not have structures associated. See dialog box top of next column.



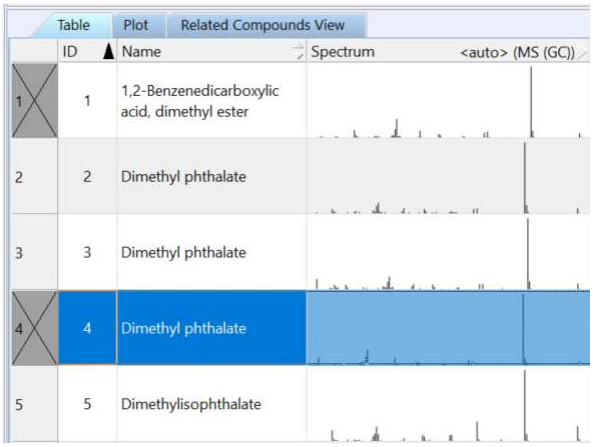
When the **Finish** button is Clicked, you will be prompted to reindex the nascent user-database. It is a good idea to do this.

User-Database Maintenance

KnowItAll has a lot of tools to assist in the maintenance of User-Database. Many of these are found on the **MineIt** Main Menu **Database** selection.

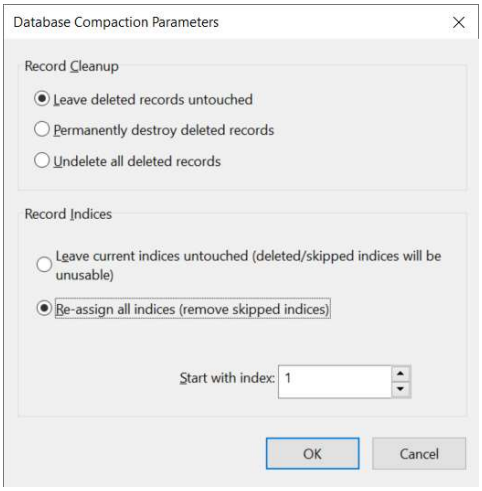


Both Records and Field Contents can be deleted. To delete records, highlight the record(s) to be deleted. Select **Mark Record As Deleted** from the **Database** Menu. This puts a check by this selection in the menu and an X make on a dark background in the user-databse Record No. Column.



The next step is to select the **Delete Marked Records** from the **Database** menu. You will be asked if this is what you want to do. Click on the **Yes** button.

The edited user-database can be reindexed by selecting **Compact** from the **Database** menu which results in the Database Compaction Parameters dialog box being displayed.



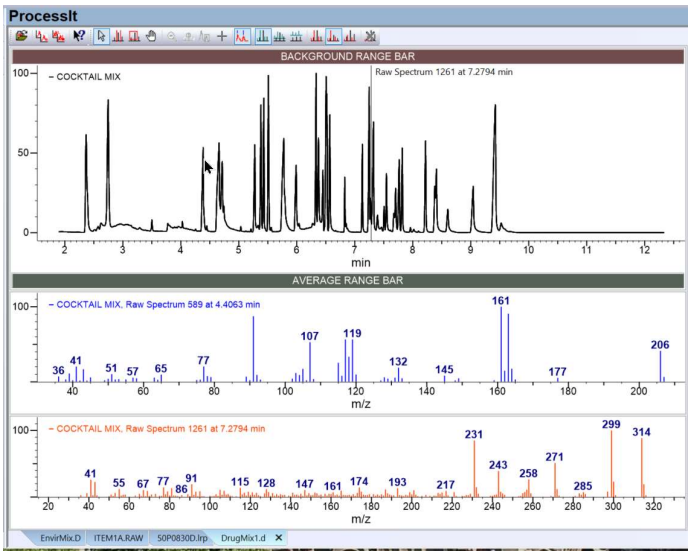
The logical option might be to re-index stating at 1; however, if the Database number is used for evidentiary purpose, you may want to reconsider this.

Random Notes

More detailed training for KnowItAll can be found at: https://sciencesolutions.wiley.com/knowitall-software-training-course/ You should Bookmark this URL.

One of the Most powerful facts about KnowItAll is the fact that multiple files of the same type (GC/MS from nearly all vendors) and different types (IR, Raman, NMR, etc. From the same and different vendors) can be used at the same time.

The default folder for KnowItAll temples and sample data files is: C:\Users\Public\Public Documents\Wiley\KnowItAll\. Sample files are found in \Samples\

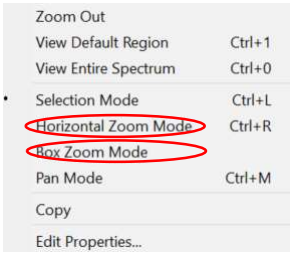


The **ProcessIt** window with four separte GC/MS Data Files open. Each one can be brought into focus by Clicking on the tab.

Right Mouse Button menu & Button Bar

Many of the selections in the Button Bar are found

on the Right Mouse Button (RMB) menu.



The RMB menu when displayed in the RIC chorogram window of the **ProcessIt** window, has two selections that allow for Zooming: **Horizontal Zoom Mode** and **Box Zoom Mode**. The **Horizontal Zoom Mode** expands the abscissa (X-axis) and the Box Zoom expands both the x-axis and the y-axis (ordinate). There are four buttons used to control the display of overlayed EICs and the RTICC. These will be grayed when the Mouse pointer is in the **Box Zoom Mode**. They can made active by selecting another mode for the Mouse pointer (Horizontal Zoom, Select, UnZoom, or Pan).

When the chromatographic display is Zoomed, the **Pam Mode** for the Mouse pointer is very useful. It allows a new section of the chromatogram display to be brought into focus. Click on the **Pan Mode** ( ) button. The Mouse Pointer now has this image. Hold down the left Mouse button and move the pointer from right to left or left to right to bring a different region into focus.

Pasting Structures from NIST MS Search into ChemWindow

When a structure is put on the Windows Clipboard using MS Search (MS Search's RMB menu command **Copy Structure to Clipboard** command), the **Past** selection in ChemWindow is grayed on its RMB menu and on the **Edit** menu selected from its Main Menu. However, if you select **Edit** from the Main Menu of ChemWindow and select **Paste Special** from the **Edit** menu and then select **MOL text** (Alt-Shift-Ctrl-P) from the **Paste Special** menu the structure is copied to the ChemWindow desktop. This works for NIST structures and NIST user-library structures.

If the structure has a phenyl ring with a circle, it is replaced with a ring with alternating double and single bonds.

If you do not have a Structure Drawing Program installed, when you install KnowItAll, ChemWindow will become the default Structure Program. Clicking on **Default Structure Editor** in the **Send To** menu of the NIST MS Search Program's RMB menu will send the structure to ChemWindow from MS Search.

More information on the use of KnowItAll for GC/MS can be found on James Little's personal Web page:

https://littlemsandsailing.wordpress.com/2022/09/01/wiley-knowitall-mass-spec-training/