

LC/MS Unknown Identifications Using MSMS Libraries

Part I: Overview of Software and User-Customized Configurations

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Kingsport, TN

- Retired* Research Fellow, Eastman Chem. Co.
- 42 years experience unknown identification
- Now Consultant, MS Interpretation Services
- Specialties¹ EI GC-MS, LC-MS/MS, Chemical Ionization,² Accurate Mass, Derivatization,^{3,4} MS library management, SciFinder⁶, ChempSpider⁶, Surfactant ID,⁷ NMR, GC-IR, organic synthesis, matrix ionization effects,⁵ etc.



Eastman Chemical Company, Main Site, Kingsport, TN
50 Manufacturing Sites Worldwide, ~14,500 Employees

* https://en.wikipedia.org/wiki/Eastman_Chemical_Company



>50 Mass Specs Networked
Worldwide

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LC/MS Unknown Identifications Using MSMS Libraries

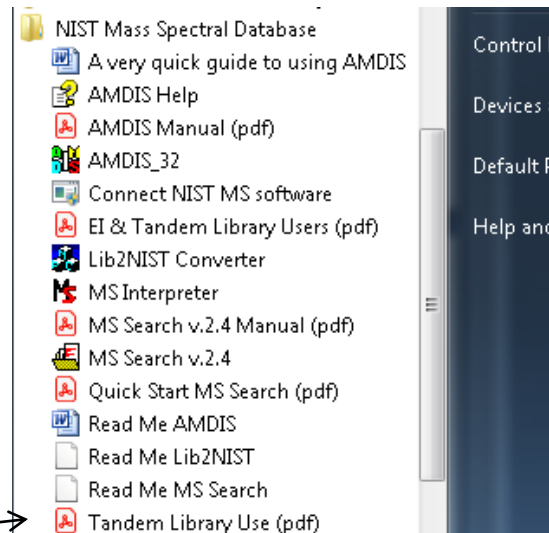
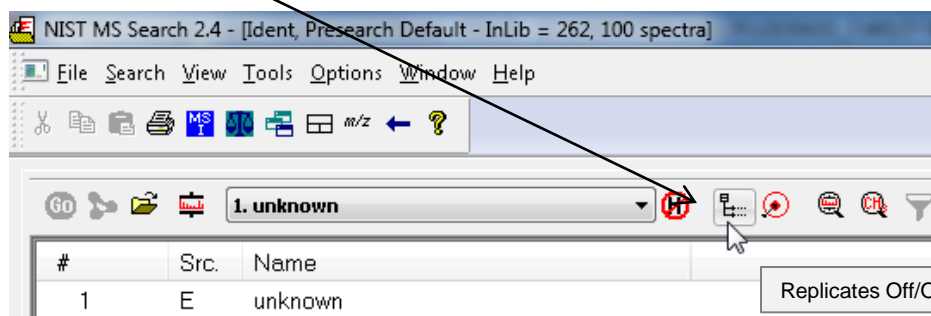
- Part I: Overview of Software and User Customized Configurations
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- Part VII: Using and Creating Other MSMS Libraries
- Part VIII: Identification of Unknowns with "Spectraless" Libraries

NIST Mass Spectrometry Software and MSMS (Tandem) Libraries

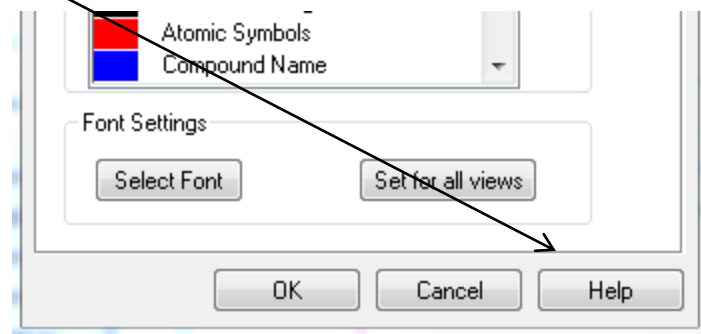
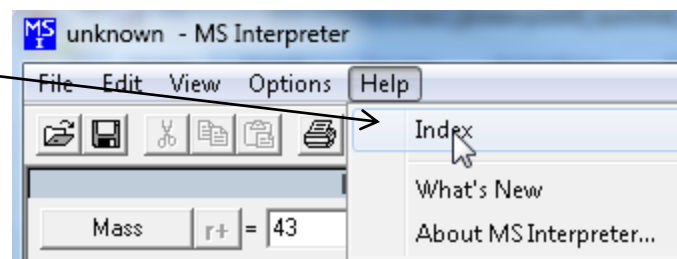
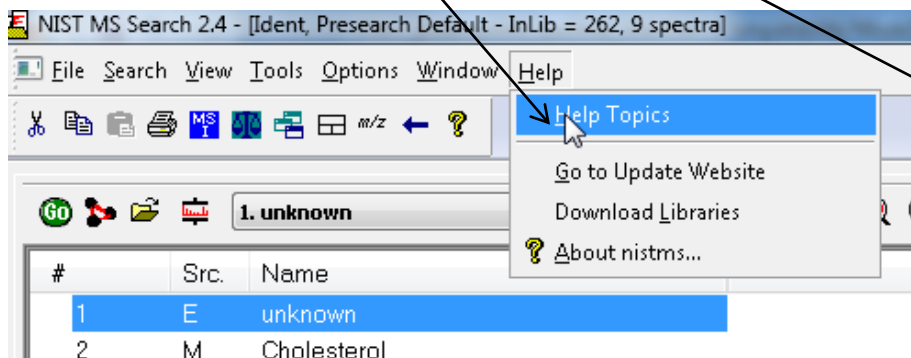
- Free software program for searching and processing MSMS (tandem) libraries
- Aggressive NIST in-house program for obtaining new spectra of purchased compounds
- Supplied with purchased NIST MSMS libraries
- Developed by NIST for curating and searching MSMS libraries
- Import data from variety of instrument manufacturers
- Searches by spectrum, structure, name, CAS No., peaks, MW, MF, etc.
- MS Interpreter^{10,17-19} for correlating molecular substructures to fragment ions
- Structure export and import using vendor drawing packages
- Searches other libraries including user, Wiley, MoNA, etc.

Help Files for NIST Search

- "Hover" over Program Icon with mouse and function description displayed



- **Detailed** documentation for NIST Search⁸
- MS Interpreter included in NIST manual⁸
- Windows Program Group
- NIST Tandem Search **Quick Start Guide**⁹
- "In program" assistance for both programs
- **Help button** at corner of all windows



NIST Software in General is “Windows Compliant”

- left click (LMB)** to select an item, **double LMB** on that item to perform operation
- right click (RMB)** in area or item to see operations that can be performed or to change properties of window
- LMB** on first item and last item to select group **while** holding **shift key**
- LMB** to select/deselect individual items **while** holding **Ctrl button**
- use up and down arrows **on keyboard** to step between entries
- some NIST windows such as librarian have no delete button to delete ions, **must** use delete key on keyboard!
- control a** (select all), **control x** (delete selected), **control c** (copy); **control v** (paste)
- control k** copies entries into windows in tab-separated text format, e.g., **paste** into Excel
- F1 MS Search help
- F9 send spectrum to MS Interpreter

Tip 3: **LMB** and **drag** to rearrange order of column headers

#	Lib.	Name	▼ Match	Prob. (%)	RI	B. Match	Syn	DBs
1	R	Undecane	955	44.8	1100	955	4	8
2	M	Undecane	945	44.8	1100	945	4	8
3	R	Undecane	944	44.8	1100	958	4	8
4	w1	Undecane	937	44.8	-	955	11	0
5	w1	Undecane	933	44.8	-	950	11	0
6	w1	Undecane	932	44.8	-	939	11	0

- LMB** on column of interest
- Can sort in lower value first or higher

Tip 1: When reviewing search results, use up and down arrows on keyboard to quickly step through results!

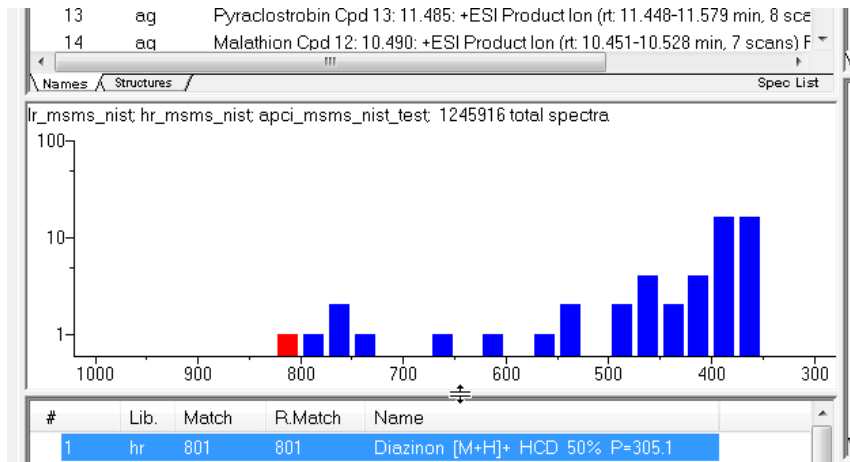


Tip 2: When viewing structures in MS Interpreter, use left and right arrows on keyboard to quickly review results!



Customizing the Windows

- place cursor over any bar between (**top or side**) windows and then **LMB** and drag to change the size of a window or make window so small it essentially disappears
- *E.g.*, I prefer to minimize the middle window bar graph and only display libraries searched



The screenshot shows a software interface displaying a list of search results. The results are shown in a table format with columns for #, Lib., Match, R.Match, and Name. The results are sorted by match score, with Diazinon [M+H]⁺ HCD 50% P=305.1 being the top result.

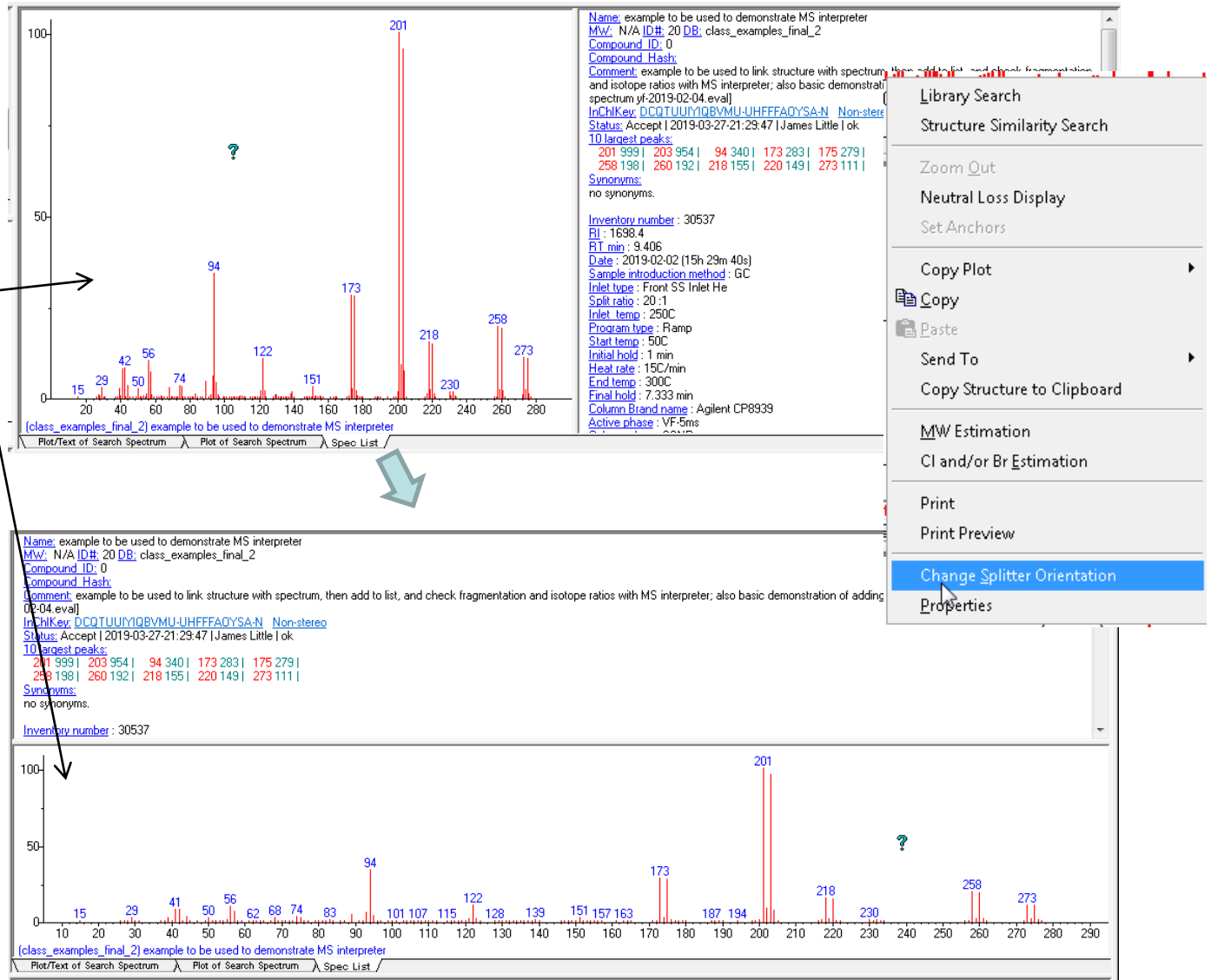
9	A			Test_126 699 (3.923) Cm (697:700-(709:714+687:692))
12	ag			Diazinon (Dimpylate) Cpd 14: 11.566: +ESI Product Ion (rt: 11.528-11.622 min, 8 scans) F
13	ag			Pyraclostrobin Cpd 13: 11.485: +ESI Product Ion (rt: 11.448-11.579 min, 8 scans) F
14	aq			Malathion Cpd 12: 10.490: +ESI Product Ion (rt: 10.451-10.528 min, 7 scans) F

#	Lib.	Match	R.Match	Name
1	hr	801	801	Diazinon [M+H] ⁺ HCD 50% P=305.1
2	hr	786	800	Diazinon [M+H] ⁺ HCD 60% P=305.1
3	hr	774	788	Diazinon [M+H] ⁺ HCD 45% P=305.1

Customizing the Windows (continued)

-**RMB** in display windows then **LMB** to “Change Splitter Orientation”

Tip: All mass spectra in windows can be “Boxed” to expand by **LMB** and dragging to expand/enlarge; **restore** to original by **RMB** in spectrum region and selecting “Zoom Out”



Customizing the Windows (continued)

- Removing *unnecessary items* in displays
- E.g. Removing *m/z* intensity list

1) RMB anywhere in window

Name: Diazinon (Dimpylate) Cpd 14: 11.566: +ESI Product Ion (rt: 11.528-11.622 min, 6 scans) Frag=125.0/CID@22.3 (305.1090[z=1]->**) Pesticides_TestMix_pos_TMSMS.d
Subtract
Instrument type: Q-TOF MS
Collision energy: 22.31 V
Precursor m/z: 305.1090
MW: N/A **ID#:** 1 **DB:** agilent_pesticide_targeted_msms
Spectrum type: ms2
Ionization: ESI
10 m/z Values and Intensities:
70.0655 43.80 | 80.9734 18.65 | 84.0448 103.56 | 96.9510 435.01 |
100.0217 134.80 | 109.0050 15.98 | 124.9814 47.19 | 153.1021 548.98 |
169.0793 999.00 | 305.1082 41.97 |
Synonyms:
no synonyms.

2) LMB properties

Library Search Properties

Spec List Text Info Comp. Result Histogram
Hits List Spec List Plot of Hit Unknown Plot Spec List Plot

Hit Text Info Unknown Text Info

Display

Compound Information m/z Intensity List
 Ten Largest Peaks Synonyms
 GC Retention Indices

All First 2

Arrange peaks by

Rows Columns

Wrap text Noise level % 0

Color Settings

■ Titles
■ Plain text
■ Masses

Font Settings

Select Font Set for all views

OK Cancel Help

3) Deselect "m/z intensity list" then LMB OK

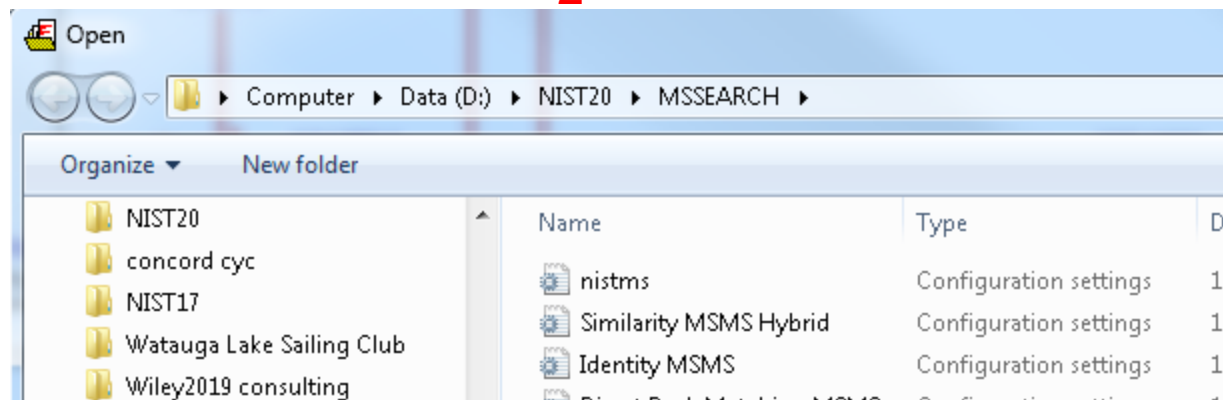
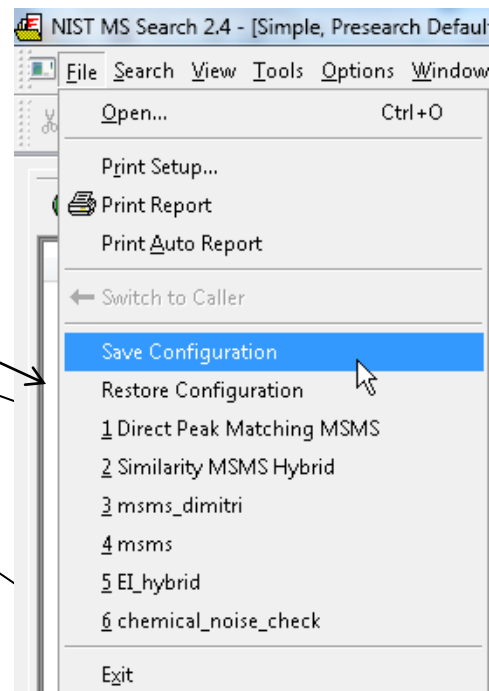
4) *m/z* intensity pairs removed from display

Name: Diazinon (Dimpylate) Cpd 14: 11.566: +ESI Product Ion (rt: 11.528-11.622 min, 6 scans) Frag=125.0/CID@22.3 (305.1090[z=1]->**) Pesticides_TestMix_pos_TMSMS.d
Subtract
Instrument type: Q-TOF MS
Collision energy: 22.31 V
Precursor m/z: 305.1090
MW: N/A **ID#:** 1 **DB:** agilent_pesticide_targeted_msms
Spectrum type: ms2
Ionization: ESI
Synonyms:
no synonyms.

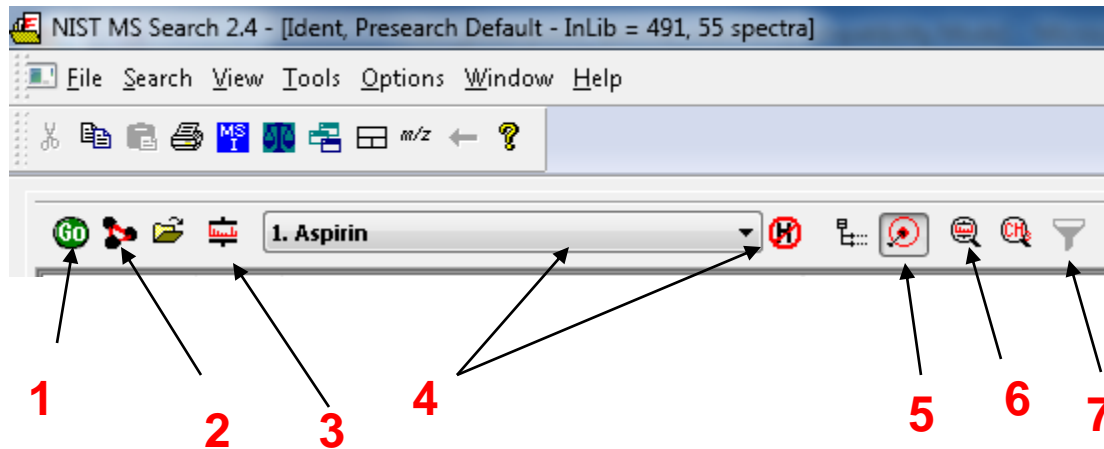
Saving and Restoring User Configurations

-Restore and Save user Configurations

- Most used listed (1) or select from a file listing (2)



Main Functions on Toolbar



1. **LMB** to start search or **double LMB** on entry in spec list window
2. **LMB** to do structure search
3. **Critical** user settings for structure and spectra searches
4. Search results stored and ability to clear list
5. Only show best hit of entry with same CAS number, minimizes looking at redundant entries in search window
6. View search options used on **last** search performed
7. Filter settings to remove spectra from search list by type of ion, polarity, type of instrument, etc.

View of Search Options *Employed*

1. **LMB** the “View Hit List Search Options”
2. See details of the last search performed
3. Also, an abbreviated description of the last search noted at **top** of NIST search window
4. Furthermore, at the **bottom** of the screen, *respectively* displayed, are the types of searches that **will** be performed and the type of results **currently** displayed
5. List of libraries searched with total # spectra

The image shows a screenshot of the NIST MS Search 2.4 software interface. The main window displays the search results for the query "1. unknown MS interpreter no structure". The interface is annotated with red numbers 1 through 5, corresponding to the list of actions provided in the text above.

1. A red arrow points to the "View Hit List Search Options" button in the main window's toolbar.

2. A red arrow points to the "Hit List Search Options" dialog box, which is open and shows the search type "Identity: Normal" and search options.

3. A red arrow points to the search query "1. unknown MS interpreter no structure" displayed in the main window's search bar.

4. Two red arrows point to the "Type of Search: Ident" and "Displayed: Ident" fields in the main window's status bar.

5. A red arrow points to the "Names / Structures" section of the search results, which shows the total number of spectra searched (1245916) and the top result: Diazinon [M+H]⁺ HCD 50% F.

The "Hit List Search Options" dialog box contains the following information:

- Search Type: Identity: Normal
- Search Options: Presearch default, Limits: Minimum m/z equals to 10. Minimum abundance 1. No constraints
- Library Involved: mainlib, replib, w12lq, w12main, w12rep

The search results table is as follows:

#	Lib.	Match	R.Match	Name
1	hr	801	801	Diazinon [M+H] ⁺ HCD 50% F

Main Window in Lib Search View

The screenshot displays the NIST MS Search 2.4 interface. The main window title is "NIST MS Search 2.4 - [Ident, Presearch Default - InLib = 489, 72 spectra]". The search criteria are "Atrazine Cpd 8: 8.295: +ESI Product Ion (rt: 8.257-8.351 min, 6 scans) Frag=125.0V CID@17.0 (216.1015)z...".

1. Spec list window: A table listing search results with columns for #, Src, and Name. The first entry is "Atrazine Cpd 8: 8.295: +ESI Product Ion (rt: 8.257-8.351 min, 6 scans) Frag=125.0V CID@17.0 (216.1015)z...".

2. Histogram: A bar chart showing the distribution of search results across a range of m/z values from 1000 to 400.

3. Hits list: A table listing hits with columns for #, Lib, Mat., R.Met., and Name. The first hit is "Atrazine [M+H]⁺ HCD 60% P=216.1".

4. Unknown spectra and info: A plot of the search spectrum showing peaks at m/z 68.0251, 79.0061, 104.0010, 132.0319, 174.0536, and 216.1010. The right panel shows metadata for "Atrazine Cpd 8: 8.295: +ESI Product Ion (rt: 8.257-8.351 min, 6 scans) Frag=125.0V CID@17.0 (216.1015)z...".

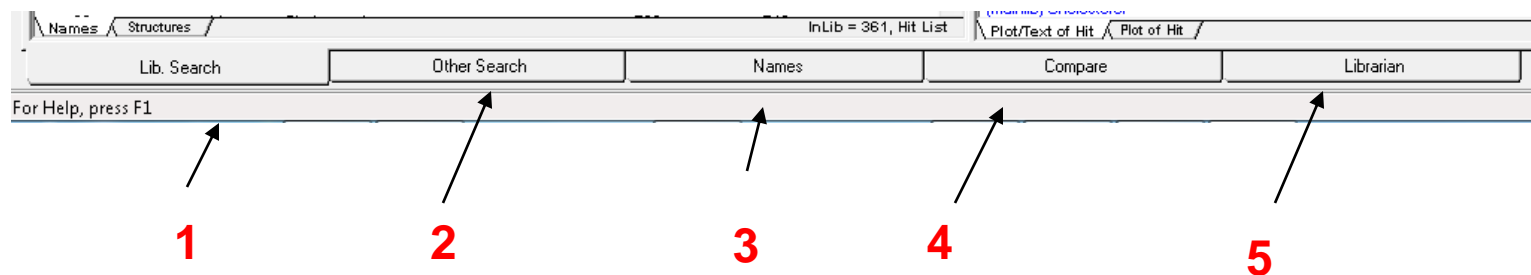
5. Comparison of hit result: A plot comparing the search spectrum (red) with a reference spectrum (blue) for "Atrazine Cpd 8: 8.295: +ESI Product Ion (rt: 8.257-8.351 min) mir". The right panel shows metadata for "Atrazine".

6. Spectrum of hit and other associated information: A plot of the hit spectrum for "Atrazine [M+H]⁺ HCD 60% P=216.1" showing peaks at m/z 96.0555, 132.0323, 174.0541, and 216.1011. The right panel shows the chemical structure of Atrazine and its associated metadata.

7. Bottom navigation bar: A bar with buttons for "Lib. Search", "Other Search", "Names", and "Compare".

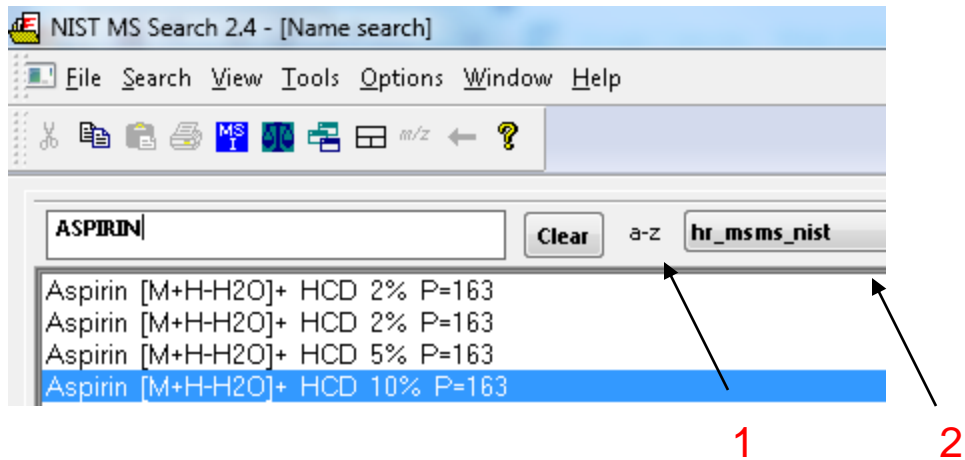
1. Spec list window for import of spectra and structures from other programs
2. Histogram, Statistics on search
3. Hits list, step through by **LMB** then **up** and **down arrows on keyboard**
4. Unknown spectra and info
5. Comparison of unknown to selected hit result, **many different display options** with tabs at bottom left of window
6. Spectrum of hit and other associated information
7. Accessing other windows and associated functions **Other Search, Names, Compare, Librarian**

Tabs for Other Functions Accessed at *Bottom* of Main Library Page (Detailed Discussions in *Future Sessions* for Tabs 4 and 5)



1. **Lib Search**-main window for searching spectra and structures
2. **Other Search**-search by CAS, MW, ID no., partial name, MF, etc.
3. **Names**-search by name, e.g. see *aspirin on next slide*
4. **Compare**-Window used to compare spectra, also can display best hits from search
5. **Librarian**-window used to edit spectra, correlate spectra with structure, create user libraries, type in spectra manually, etc.

Names Search Tab



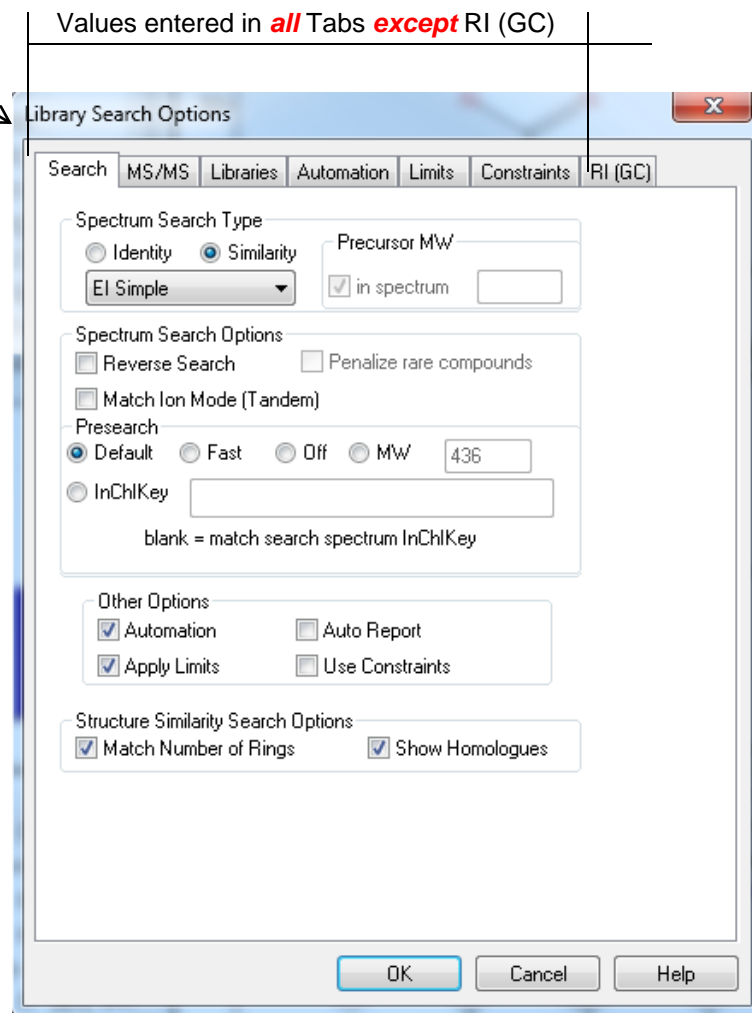
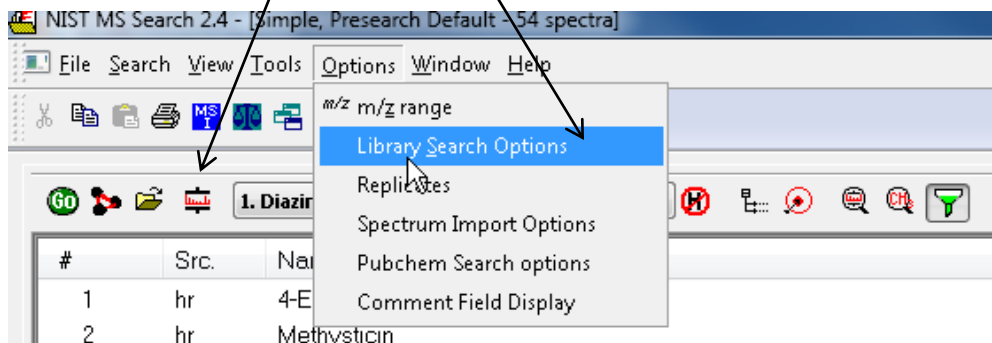
1. Toggle: letters only *or* letters/numbers
2. Search **one library** at a time

Setting Up Search Parameters for MSMS Searches

Critical Step

- Critical part of process is user setting up search options
- Use “Library Search Options”
- Values selected in *all* “tabs” on submenu *except* RI (GC)
- Actual values discussed in *Part II* of the series

Two ways to access, *icon*
on toolbar or *menu*



InChIKey Field Link to PubChem on Web

Name: Pentabromophenyl ether
Formula: C₁₂Br₁₀O
MW: 950 Exact Mass: 949.17829 CAS#: 1163-19-5 NIST#: 376366 ID#: 267338 DB: mainlib
Other DBs: Fine, TSCA, RTECS, EPA, NIH, EINECS
Compound ID: 0
Compound Hash:
Contributor: R.A. Hites, Indiana Univ., Bloomington, IN
Related CAS#: 145538-74-5; 109945-70-2
InChIKey: [WHHGLZMJPXIBIX-UHFFFAOYSA-N](#) Non-stereo
10 largest peaks:

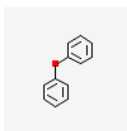
-InChIKey is ASCII “hashed” representation of structure* and is searchable

-Double **LMB** on **any** InChIKey in NIST software and you will be taken to PubChem Web Page on internet

NCBI Resources How To

PubChem Compound PubChem Compound "USIUUVZYUHIAEV-UHFFFAOYSA-N"[InChIKey]
Create alert Limits Advanced

Summary ▾



[DIPHENYL ETHER; Diphenyl oxide; 101-84-8 ...](#)

MW: 170.210 g/mol MF: C₁₂H₁₀O
IUPAC name: phenoxybenzene
Create Date: 2005-03-26

CID: 7583

[Summary](#) [Similar Compounds](#) [Same Parent, Connectivity](#) [Mixture/Component Compounds](#)

Selecting PubChem Options:

NIST MS Search 2.4 - [Ident, Presearch Default - InLib = 617, 51 spectra]

File Search View Tools Options Window Help

m/z m/z range
Library Search Options
Replicates
Spectrum Import Options
Pubchem Search options
SOS options

#	Src.	Name
1	M	Diph

* https://en.wikipedia.org/wiki/International_Chemical_Identifier

Sending data to PubChem

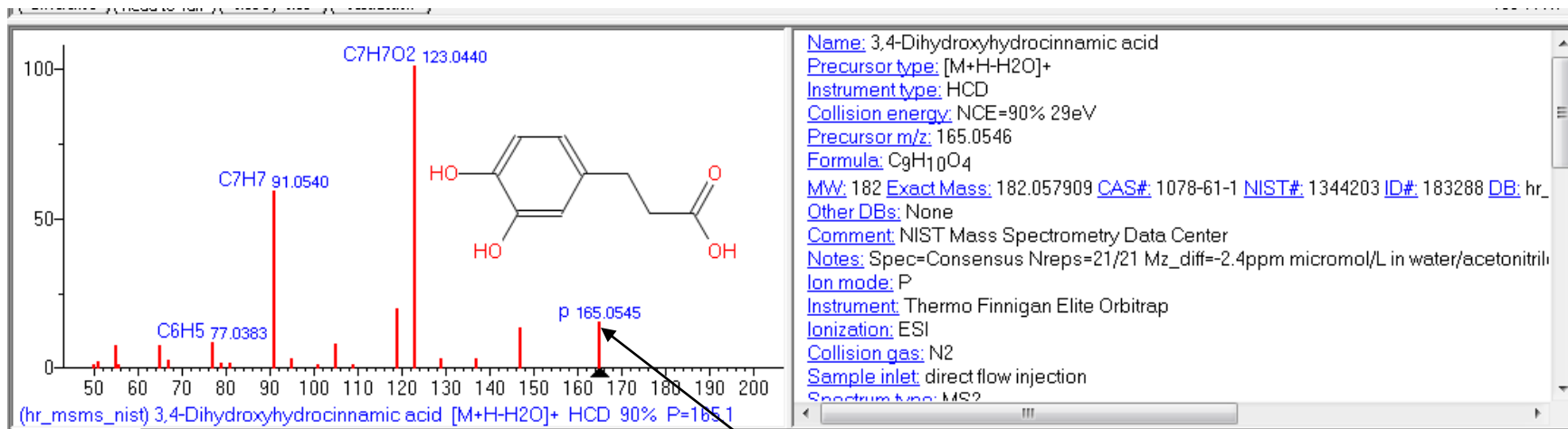
The structural data may be sent to pubchem (<https://pubchem.ncbi.nlm.nih.gov>) to find matching structures.

How should the program proceed?

Ask each time
 Always allow data to be sent
 Never send data

OK Cancel

Molecular Formulae Can be Shown in High Resolutions (HR) MSMS



Library Search Properties

Hit Text Info		Unknown Text Info	
Spec List Text Info		Comp. Result	Histogram
Hits List	Spec List	Plot of Hit	Unknown Plot
Hits List	Spec List	Plot of Hit	Unknown Plot

Options

- Label peaks greater than (%) 0
- m/z Label
- Formula/y-b
- Loss/other
- Wrap legend
- Peak Width 2

Show

- Molecular Weight
- Formula
- CAS Number
- Structure on Plot

Color Settings

- Scale (plot)
- Peaks (plot)
- Labels (plot)

Font Settings

Select Font Set for all views

OK Cancel Help

Select to show molecular formulae
"p" is for precursor

Live Demo on YouTube

Part I: Overview of Software and User Customized Configurations

-Live demo summarizes basic functions used in MSMS searches and saving configurations

The screenshot displays the NIST MS Search 2.4 interface. The top window shows search results for 'Diazinon (Dimpylate) Cpd 14: 11.566'. Below this, a mass spectrum plot shows relative intensity versus m/z, with major peaks at 96.9510, 153.1021, and 169.0793. A second window shows a zoomed-in mass spectrum of the same compound, with peaks at 84.0448, 96.9510, 109.0050, 124.9814, 153.1021, and 169.0793. A third window shows a list of search results for Diazinon, including library matches and their respective retention times and collision energies. The bottom window shows a chemical structure of Diazinon, with a callout box highlighting a peak at m/z 169.0793.

#	Src	Name
1	hr	4-Ethoxy-7-methoxycoumarin
2	hr	Methysticin
3	hr	4-Hydroxydiclofenac
4	hr	4-Hydroxydiclofenac
5	hr	5-(2-Chlorobenzyl)-1,3-thiazol-2-amine
6	hr	5-(2,3-Dichlorobenzyl)-1,3-thiazol-2-amine
7	hr	N-(2-Chlorobenzyl)-2-(1H-indol-3-yl)ethanamine
8	A	Test_1041147 (6.789) Cm (1146.1149)
9	A	Test_126639 (3.923) Cm (697.700-709.714+687.692)
12	ag	Diazinon (Dimpylate) Cpd 14: 11.566 +ESI Product Ion (rt: 11.528-11.622 min, 6 scans) Frag=125.0V CID...
13	ag	Pyraclostrobin Cpd 13: 11.485 +ESI Product Ion (rt: 11.448-11.579 min, 8 scans) Frag=125.0V CID@27.3 (...)
14	ag	Melathion Cpd 12: 10.490 +ESI Product Ion (rt: 10.451-10.528 min, 7 scans) Frag=125.0V CID@23.9 (331...)
15	ag	Malinapate Cpd 11: 10.072 +ESI Product Ion (rt: 10.020-10.162 min, 12 scans) Frag=125.0V CID@15.3 (188...

#	Lib.	Match	R.Match	Name
1	hr	801	801	Diazinon [M+H] ⁺ HCD 50% P=305.1
2	hr	786	800	Diazinon [M+H] ⁺ HCD 60% P=305.1
3	hr	774	780	Diazinon [M+H] ⁺ HCD 45% P=305.1
4	lr	756	872	Diazinon [M+H] ⁺ QQQ 18V P=305.1
5	hr	734	753	Diazinon [M+H] ⁺ HCD 40% P=305.1
6	hr	654	749	Diazinon [M+H] ⁺ HCD 35% P=305.1
7	hr	618	629	Diazinon [M+H] ⁺ HCD 75% P=305.1
8	hr	558	773	Diazinon [M+H] ⁺ IT-FT 35% P=305.1
9	hr	536	866	2,5-Furazinedicarboxylic acid [M+H] ⁺ H...
10	hr	536	619	Diazinon [M+H] ⁺ HCD 30% P=305.1
11	hr	495	799	3-(2-Oxocyclohexyl)propanoic acid [M...
12	hr	485	783	3-(2-Oxocyclohexyl)propanoic acid [M...
13	hr	464	750	2,5-Furazinedicarboxylic acid [M+H] ⁺ H...
14	hr	463	739	3-(2-Oxocyclohexyl)propanoic acid [M...
15	hr	463	738	2-(Ethylsulfonyl)benzoic acid [M+H]2O...

Tip: All mass spectra in windows can be “Boxed” to expand by **LMB** and dragging to expand/enlarge; **restore** to original by **RMB** in spectrum region and selecting “Zoom Out”

Presentation References (*Internet Links*)

1. [James Little Mass Spectral Resource Website](#)
2. [Chemical Ionization for MW Determination](#)
3. [Trimethylsilyl Derivatives for GC-MS](#)
4. [Methyl Ester Derivatives for GC-MS](#)
5. [Lipid Matrix Ionization Effects in LC-MS](#)
6. [SciFinder/ChemSpider and Accurate Mass LC-MS Data for Unknown ID's](#)
7. [Surfactant Identification](#)
8. [NIST Search Software Detailed Manual](#)
9. [NIST Tandem Quick Start Guide](#)

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