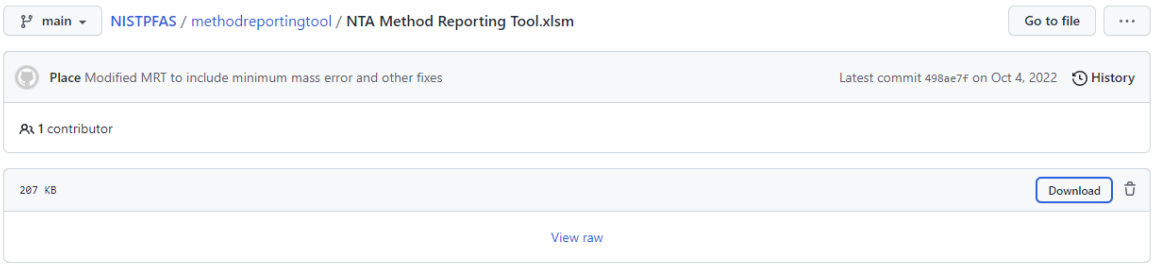
Using the Non-Targeted Analysis Method Reporting Tool

Introduction

The Non-Targeted Analysis Method Reporting Tool (NTA-MRT) is a tool designed to collect and share method and compound identification information about a single sample. The NTA-MRT is a macro-enabled Excel Workbook that has controlled vocabulary for most input values.

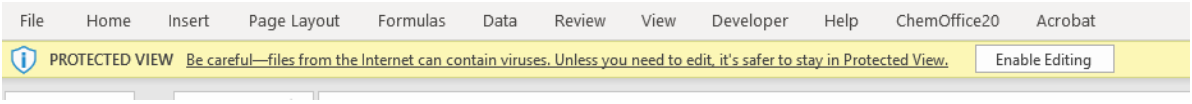
Downloading the Non-Targeted Analysis Method Reporting Tool

To download the NTA-MRT, go to: https://github.com/usnistgov/NISTPFAS and select the `methodreportingtool` folder, select `NTA Method Reporting Tool.xlsm` file and click the **Download** button.



Once downloaded you can immediately open the file in Microsoft Excel.

Upon first downloading and opening the file, you may get a `Protected View` warning. You should click **Enable Editing**.



In addition, you may see a `Security Warning` that the 'Macros have been disabled.' You must select **Enable Content** before you can use the NTA-MRT.

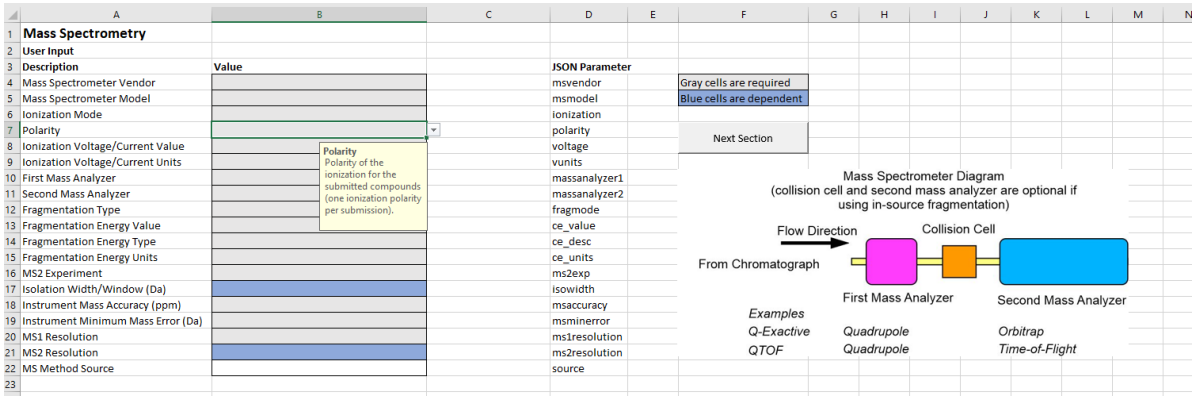
Completing the Method Input Sheets

Upon opening the NTA-MRT, you should have the *Run* sheet open, this has the general instructions for completing the NTA-MRT.

In total, there are five input sheets. The first four (Sample, Chromatography, Mass Spectrometry, QC Method) are considered the *Method Input* sheets and follow a similar format.

To complete the *Method Input* sheets, you can either select the link for the individual sheets on the *Run* sheet or you can click through the tabs at the bottom.

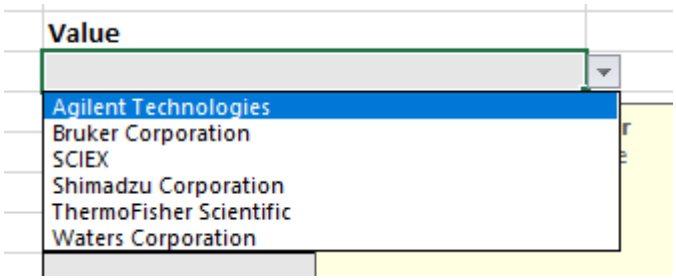
The Mass Spectrometry sheet will be demonstrated. The empty *Mass Spectrometry* input sheet looks like the following:



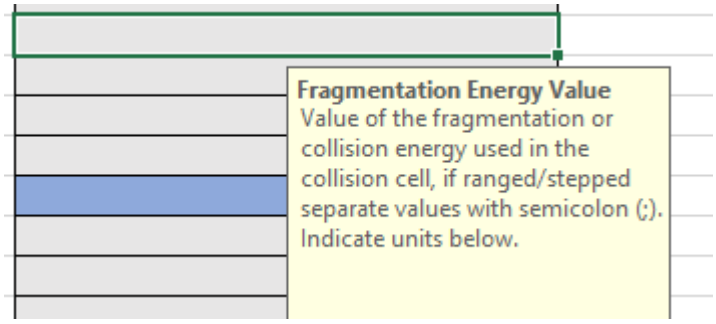
On each *Method Input* sheet, the grey cells are required, white cells are optional, and blue cells are only required in some circumstances. For example, if you use a second mass analyzer, the MS2 Resolution value is required. If you do not have a second mass analyzer, the MS2 Resolution value is not required.

If you select a cell (like above), an informational pop-up message will appear to define the required information needed for the input.

Some input values will have drop-down menus that indicate that there are only specific values allowed.



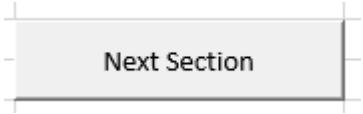
Other input values are open field and the informational pop-up message will explain the type of information that should be input.



Once finished, you can return to the *Run* sheet to see if the data is complete under the **Complete?** column,

Sheet	Complete?
Sample_Input	FALSE
Chromatography_Input	FALSE
Mass Spectrometry_Input	TRUE
QC Method_Input	FALSE
Peaks_Input	FALSE

or you can click the **Next Section** button and it will check the data before moving onto the next sheet.

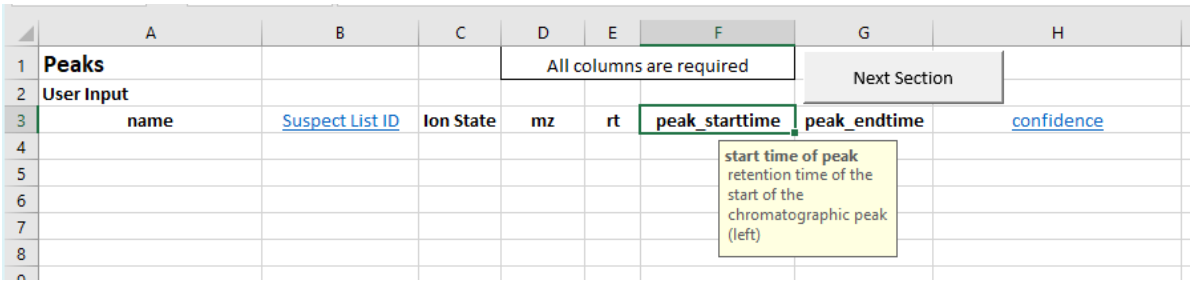


Once the *Method Input* sheets are complete, move onto the *Peaks* Input Sheet

Completing the Peaks Input Sheet

The Peaks Input sheet is for users to list all **identified compounds** they want to report in the **sample**.

The empty *Peaks Input* sheet should like the the following:



For the *Peaks Input* sheet, all values are **Required**.

If you select a column header (like above), an informational pop-up message will appear to define the required information needed for the input.

Some input values will have drop-down menus that indicate that there are only specific values allowed. Others have open input values that are defined in the informational pop-up message.

For further clarity, the specific values are defined below:

Parameter	Description
name	the user-defined name of the compound being identified. This is not restricted nor has to be correctly written, though we strongly advise against using any special characters (e.g., -*&^%$#@!{}[]‘~?<>)
Suspect List ID	The identification number from the NIST List of Possible Per- and Polyfluoroalkyl Substances (the leftmost column), located at https://data.nist.gov/od/id/mds2-2387
Ion State	The molecular ion state of the precursor ion, for example “[M+H]+” or “[M-H]-”.
mz	The **measured** precursor ion *m/z* value
rt	The retention time of the compound chromatographic peak (centroid), in minutes.
peak_starttime	The retention time of the start (left) of the compound chromatographic peak, in minutes.
peak_endtime	The retention time of the end (right) of the compound chromatographic peak, in minutes.
confidence	The PFAS Confidence of Identification (PCI) level for the compound identification. Uses the scale defined by Charbonnet et al. at https://doi.org/10.1021/acs.estlett.2c00206. It is recommended to look at the Supporting Information, which has a workflow to navigate selecting a PCI Level.

An example of the completed *Peaks Input* sheet looks as follows:

	A	B	C	D	E	F	G	H	I	J
1	**Peaks**			All columns are required			Next Section			
2	**User Input**									
3	**name**	Suspect List ID	**Ion State**	**mz**	**rt**	**peak_starttime**	**peak_endtime**	confidence		
4	PFHxA	2643	[M-H]-	312.9654	9	8.2	9.8	Level 2a - Probable by library spectrum match		
5	Perfluorooctanoic acid	2637	[M-H]-	412.9663	13	12.8	13.5	Level 1b - Indistinguishable from reference standard		
6	PFOS	3041	[M-H]-	498.9302	17	16.5	17.1	Level 3b - Fragmentation-based candidate		
7										
8										

Once complete, you can select **Next Section** or return to the *Run* sheet.

Reporting Fragment Annotations for Identified Peaks

This is an optional, but encouraged step.

The next step is to annotate the MS/MS fragments for each individual identified compound. The annotations should be done using your own workflows (or the MSMatch tool), but can be reported using the NTA-MRT.

To create the annotation tables, return to the *Run* sheet. Make sure that all of the input sheets from Step 1 have been complete, the sheet should look as follows:

	A	B	C	D	E	F	G	H	I	J	K	L
1	**Method Reporting Tool for Non-Targeted Analyses of Per- and Polyfluoroalkyl Substances**											
2			*Version 1.6P updated 10/3/2022 BJP*									
3	**Step 1) Fill out the data in each Input sheet**											
4		**Sheet**	**Complete?**									
5		Sample_Input	TRUE									
6		Chromatography_Input	TRUE									
7		Mass Spectrometry_Input	TRUE									
8		QC Method_Input	TRUE									
9		Peaks_Input	TRUE									
10												
11	**Step 2) Press "Create Annotation Tables" to annotate the fragments of the peaks [optional]**							Create Annotation Tables				
12		Tables Generated:	FALSE									
13												
14	**Step 3) Aggregate Date into output file**		Export JSON file output									
15												
16												
17	**Step 4) Give data to NIST**											
18		Contact pfas@nist.gov to find out how to share data.										
19												
20												
21												
22												
23	***Additional Tools***											
24		Export only the method data	Export Method									
25												
26		Import JSON file	Import JSON									
27												
28		Reset entire workbook	Reset									
29												
30												
31												
32												

To generate the tables that enable reporting of fragment annotations, click the **Create Annotation Tables** button. This will generate individual sheets that have the pattern `Ann_[Compound Name]` where `[Compound Name]` is the name of the compound entered in the *Peaks Input* sheet. For example, the peak name `Perfluoroctanoic acid` will have an annotation table named `Ann_Perfluorooctanoic acid`. If the name is long, or has special characters, the sheet name may be shortened.

Annotation tables are optional for each peak; though encouraged, it is not a requirement of submission to annotate all peaks reported in the NTA-MRT.

An empty *Annotation Table* will look follows:

	A	B	C	D	E
1	**Peak Annotation for Perfluorooctanoic acid**				
2	**User Input**				
3	**Fragment m/z**	**Fragment Elemental Formula**	**Fragment SMILES**	**Radical?**	**Fragment Citation**
4					
5					
6					
7					
8					
9					
10					

All columns except **Fragment SMILES** are required for every row in this sheet. For the specific compound, fill out a row for each annotated fragment. The values are defined below

Parameter	Description
Fragment m/z	The **measured** fragment *m/z* value
Fragment Elemental Formula	The proposed elemental formula for the specific fragment, do not include charges (+/-) or radicals.
Fragment SMILES	The proposed fragment structure (in SMILES notation) for the specific fragment, leave blank if unknown.
Radical?	Does the proposed fragment structure contain a radical electron (TRUE, FALSE, UNKNOWN). Use UNKNOWN if not known.
Fragment Citation	Reference or other citation (DOI or website) for the evidence of the fragment identification. Enter USER if it is based on user interpretation only.

A complete *Annotations Table* will look like the following:

	A	B	C	D	E
1	**Peak Annotation for Perfluorooctanoic acid**				
2	**User Input**				
3	**Fragment m/z**	**Fragment Elemental Formula**	**Fragment SMILES**	**Radical?**	**Fragment Citation**
4	118.9911	C2F5	FC-(F)C(F)(F)F	FALSE	USER
5	168.9881	C3F7	FC-(F)C(F)(F)C(F)(F)F	UNKNOWN	DOI:10.1002/rcm.3274
6	218.9855	C4F9	FC(F)(C-(F)F)C(F)(F)C(F)(F)F	FALSE	DOI:10.1002/rcm.3274
7	368.9756	C7F15	FC(F)(C(F)(F)C(F)(F)F)C(F)(F)C(	FALSE	USER

Once you have completed all of the *Annotations Tables* for the desired compounds. You can return to the *Run* sheet.

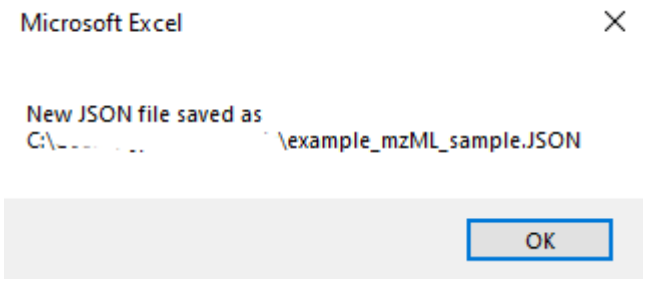
Exporting Sample JSON File

Once you have completed all of the required and optional input values, your *Run* sheet should look like the following:

	A	B	C	D	E	F	G	H	I	J	K
1	**Method Reporting Tool for Non-Targeted Analyses of Per- and Polyfluoroalkyl Substances**										
2			*Version 1.6P updated 10/3/2022 BJP*								
3	**Step 1) Fill out the data in each Input sheet**										
4		**Sheet**	**Complete?**								
5		Sample_Input	TRUE								
6		Chromatography_Input	TRUE								
7		Mass Spectrometry_Input	TRUE								
8		QC Method_Input	TRUE								
9		Peaks_Input	TRUE								
10											
11	**Step 2) Press "Create Annotation Tables" to annotate the fragments of the peaks [optional]**							Create Annotation Tables			
12		Tables Generated:	TRUE								
13											
14	**Step 3) Aggregate Date into output file**		Export JSON file output								
15											
16											
17	**Step 4) Give data to NIST**										
18		Contact pfas@nist.gov to find out how to share data.									
19											

Note: if you did not annotate any peaks, the `Tables Generated` result will say `FALSE` and you can still export the data.

The final step is to press the **Export JSON file output** button. Once the process is complete, a message will pop up that reports the location of the **Sample JSON** file. **Sample JSON** files will end with a `_sample.JSON` string.



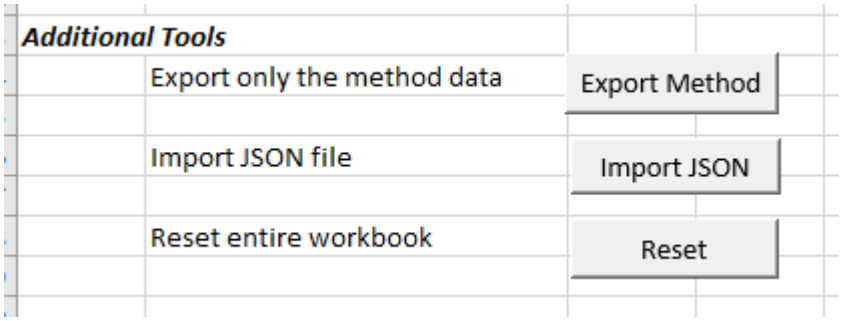
Sharing the data with NIST

To share the data with NIST, contact us at pfas@nist.gov and we can provide additional steps. It will require sharing the **Sample JSON** file and the converted **mzML** raw data file associated with the sample.

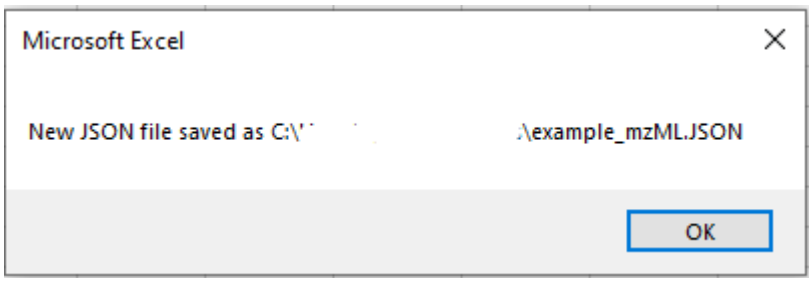
Optional: Exporting JSON Method Only files

If you are using a similar instrumental method for multiple NTA-MRT files, it may be beneficial to export only the method information into a JSON file that can be imported with each new sample.

To export just the method data (after the NTA-MRT has been completed), just select **Export Method** button on the *Run* sheet.



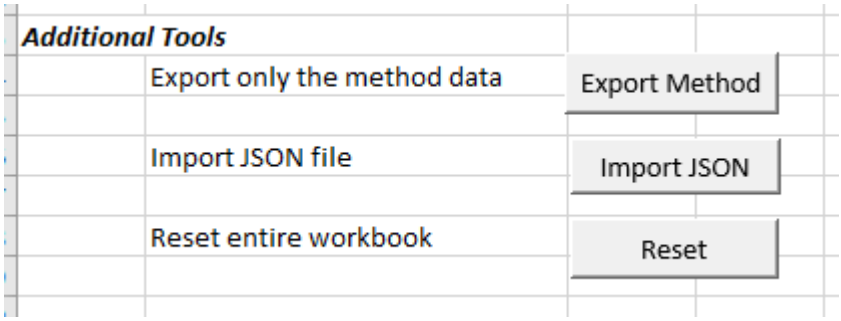
Once the process is complete, a message will pop up that reports the location of the **Method JSON** file. **Method JSON** files will end with a `_mzML.JSON` string.



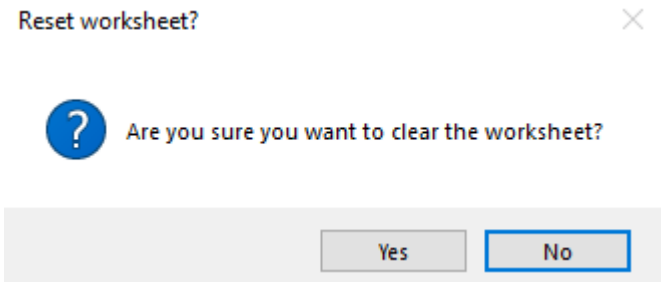
Optional: Importing JSON Sample or Method files

Warning: this step will overwrite all of the data within the NTA-MRT!

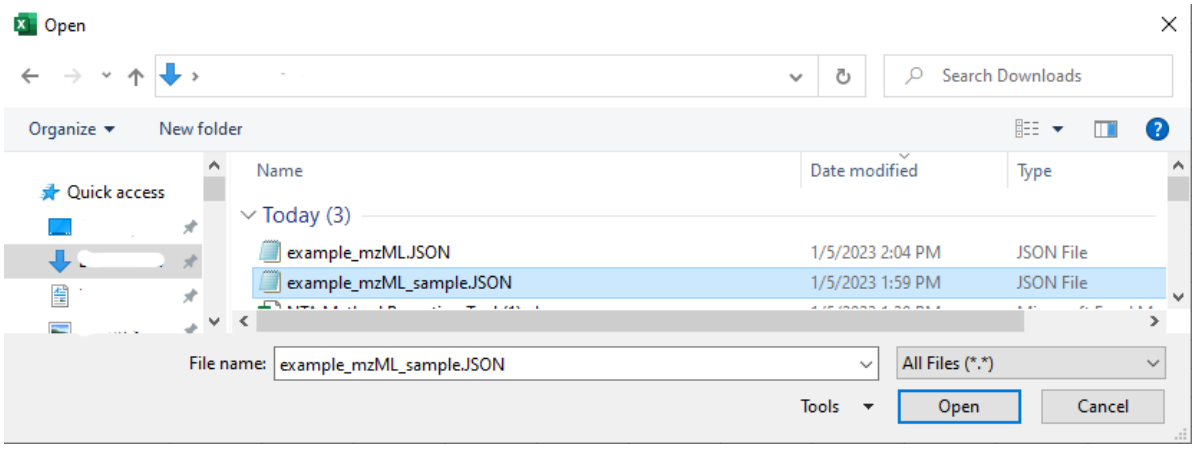
If you want to import a **Method JSON** or **Sample JSON** file, you can select the **Import JSON** button on the *Run* sheet.



A pop-up will appear asking if you would like to reset the worksheet, select **Yes**.



Then the File Explorer will open, where you can navigate to the **Method JSON** or **Sample JSON** file, select the file, and click the **Open** button.



This will populate the NTA-MRT with the data from the JSON file, you can finish completing the file and export the data again.