

**MOST PROBABLE NUMBER (MPN)
CALCULATOR**

Version 2.0

**User and System Installation and
Administration Manual**

**Version 4.0
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MPN Calculator User Manual

Acknowledgements

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1.0 INTRODUCTION

Mr. Albert J. Klee (Risk Reduction Engineering Laboratory, United States Environmental Protection Agency, Cincinnati, OH 45268, USA) developed and published a FORTRAN console program to determine Most Probable Number (MPN) estimates in 1993 (see reference 8.3). The program was developed for bacteriologists and named “mpn.exe.” It computes the MPN value estimate based upon a probability algorithm, the Spearman-Kärber estimates, the Salama correction for bias, the approximate confidence limits of Cornish & Fisher, and the exact confidence limits of Loyer & Hamilton. Data is input using the keyboard or an input text file. The program contained a series of default values based upon typical bacteriological analyses, but allowed the user to change the default values. These included defaults for the Confidence Level (95%), the Number of Dilutions (3), the Number of Tubes per Dilution (5), and the Size of the Initial Volume (10 mL). Users can also toggle the default setting of “10-Fold Serial Dilutions” to “Non-Standard Dilutions.” For 10-fold dilutions, data is input as the number of positive tubes per dilution level. For non-standard dilutions, users input both the number of positive tubes per dilution level and the amount of sample represented at each dilution level.

Following EPA’s publication of an Information Collection Rule in the mid 1990s requiring large public drinking water utilities to monitor their water for contaminants, including viruses, Mr. Klee developed a modified program for virologists, named “mpnv.exe.” This version computes only the MPN and the confidence limits (Cornish & Fisher, or Loyer & Hamilton). It contains default values for the number of dilutions (1), the number of replicates per dilution (20), and the inoculum volume (1 mL). Users can choose the default setting of “5-Fold Serial Dilutions” or change the default to “Non-Standard Dilutions.” For 5-fold dilutions, data is input as the number of positive replicates per dilution level. For non-standard dilutions, users input both the number of positive replicates per dilution level and the amount of sample represented at each dilution level.

The MPN and MPNV programs were developed to run using the DOS operating system. They run efficiently in MS-DOS command prompt window only under Windows operating systems through Windows XP. Version 2.0 of the MPN Calculator was developed to run on XP and later operating systems. There are two versions available: one for 32 bit (x86) based operating systems and one for 64 bit systems. There are no versions available that run using the Mac operating system.

The new MPN Calculator is an easy-to-use stand alone Windows application built by Avineon, Inc. for the EPA. The calculator was built using Microsoft .NET (dot NET) version 3.5 SP1 (C#) and Windows Presentation Foundation technologies. The new calculator not only combines the main functionalities of both “mpn.exe” and “mpnv.exe”, it allows users to save computed results to 3 different file formats (TEXT, WORD and EXCEL) and to automatically record all results in a local database for later retrieval. The algorithms for computing the Most Probable Numbers (MPN) continue to be based on the Mr. Klee’s “mpn.exe” FORTRAN code. The graphical user interface (GUI) was redesigned (based on the old FORTRAN program’s user interface).

The current version (2.0) of the EPA-MPN Calculator corrects a problem associated with the calculation of MPN values associated with certain abnormal results (see section 3.2 for a definition of abnormal results).

The following sections contain instructions on how to install, configure and use the new MPN Calculator.

2.0 INSTALLATION

2.1 Installation Requirements

Installation of the EPA-MPN program requires administrative rights. Users may need to have the software installed by Information Technology personnel with these rights. The program requires the prior installation of the latest versions of Microsoft .NET Framework and SQL Server express appropriate for the installed operating system. Both of these can be downloaded freely and installed by an individual with administrative rights by following the directions on the Microsoft websites. The download site for .NET Framework is <http://msdn.microsoft.com/en-us/vstudio/aa496123.aspx> and for the SQL Server Express is <http://www.microsoft.com/en-us/download/details.aspx?id=29062>. After installing these files, download and save the x86 version of the EPA-MPN-Setup.msi installation file for 32 bit versions of Windows 7 by clicking on the following link, <http://www.epa.gov/nerlcwww/documents/x86-EPA-MPN-Setup.msi> or the version for 64 bit versions of Window 7 and Windows 8 by clicking of the following link, <http://www.epa.gov/nerlcwww/documents/64bit-EPA-MPN-Setup.msi>. After the download is complete, double click on the downloaded installation file and follow the file's installation instructions.

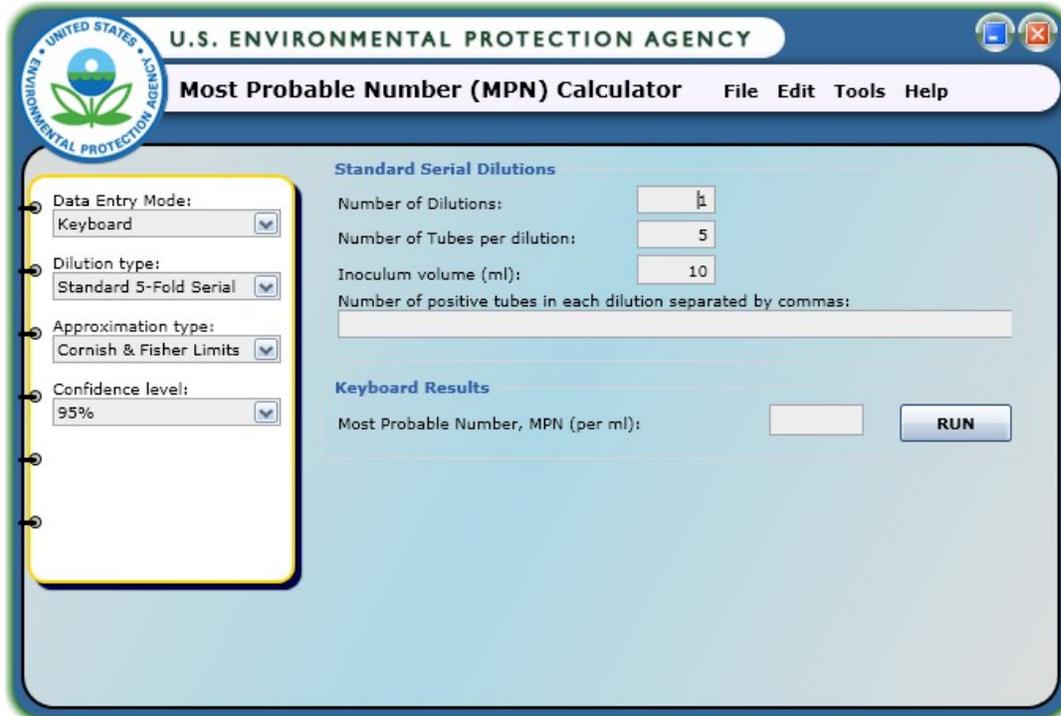
2.2 Installation Problems

Please notify Dr. Shay Fout at fout.shay@epa.gov concerning problems with the installation or use of the program or with any suggestions for its improvement. Note: some computers running early Microsoft Windows version or non-Microsoft platforms may not run files with an "msi" extension. Should this problem occur, an executable file and instructions for installing the MPN program are available upon request.

3.0 DEFAULT BEHAVIOR CONFIGURATIONS

3.1 Using the MPN Calculator for bacterial and viral MPN calculations

The default opening screen is as shown:



The MPN Calculator has added functionality over the older program, but some of the terminology of the original programs is retained. In the screen shot above the term “Number of Tubes per dilution” is equivalent to the term “Number of Replicates per Dilution” used by the MPNV program. The term “Inoculum volume (ml)” is equivalent to the term “Size of the Initial Volume” used in the MPN program. The program defaults as installed are 1 dilution (i.e., an undiluted sample), 5 tubes per dilution (i.e., 5 replicates), an inoculum volume of 10 mL, and standard 5-fold serial dilutions. The MPN and MPNV programs used standard 10-fold and standard 5-fold dilutions, respectively. All other dilutions were non-standard and required the user to input both the number of positive samples and their volumes. The MPN Calculator allows the user to select standard 2-fold, 5-fold, or 10-fold dilution defaults. When these defaults are selected, only the number of positive tubes (replicates) per dilution has to be entered.

Bacterial and viral MPN analyses often use different approaches for making and using dilutions. These differences were taken into account when the original MPN and MPNV programs and the new MPN Calculator were developed. Dilutions based upon the requirement to measure the bacterial content in 100 mL of water are used when performing bacterial analysis of drinking water,. Ten-fold dilutions typically are done either by directly assaying volumes of 10, 1.0, and 0.1 mL (or another dilution series depending on the likely bacterial content of the sample) or by filtering each volume separately and then assaying the resulting filter. In each case, the ten-fold lesser amounts represent serial 10-fold dilutions. To use the MPN Calculator to determine the MPN of an analyses using 5 tubes each with 10, 1.0, and 0.1 mL volumes, the user would change the Calculator's default settings to 3 dilutions and standard 10-fold dilutions. The default for the number of tubes per dilution and the inoculum volume would remain at 5 and 10 mL, respectively. After entering the positive tubes per dilution and clicking "RUN," the program will show the MPN value per mL and the 95% Cornish and Fisher confidence limits. Note that the MPN value is on a per mL basis. This value must be multiplied by 100 to obtain the MPN/100

mL value. As an example, in the case of a 3 dilution assay (10, 1, 0.1 mL) using 5 tubes per dilution and resulting in 5 (10 mL dilution), 1 (1 mL dilution), and 0 (0.1 mL dilution) positive tubes per dilution, the MPN calculator gives a value of 0.33 MPN per mL with 95% confidence values of 0.11 to 0.94 per mL or 33 (11-94) MPN per 100 mL. For this combination Table 9221.IV in Standard Methods (2005) gives a value of 30 (10-120) MPN per 100 mL. One of the default settings in the MPN Calculator is the number of decimal places to use in MPN calculations. For MPN values greater than 1, the default should be set to "0," because the decimal values are not significant figures (see Section 1050 in Standard Methods for a discussion of significant figures). Due to the wide confidence limits, even the second 3 in 33 is not significant; therefore, Standard Methods rounds the result to 30.

Viral analyses are performed by preparing the dilutions and then using the same volume from each dilution to inoculate the appropriate set of replicates. This approach is often also used with bacterial analyses from surface waters and biosolids, but is necessary for virus assays because the inoculum volume affects the adsorption efficiency of virus to cells in a tissue culture flask. The use of different inoculum volumes would therefore result in an uncontrolled and unknown variable. To prepare a 10-fold three dilution series for a virus assay, one might take 2 mL of an undiluted sample and add it to 18 mL of a diluent to prepare a 1:10 dilution. After mixing, 2 mL of the 1:10 dilution would be added to another 18 mL of the diluent to prepare a 1:100 dilution. If an inoculum volume of 1 mL were used, then 1 mL of the undiluted sample would be inoculated into each of 5 replicate tubes (e.g., tissue culture flasks), 1 mL of the 1:10 dilution would be inoculated into each of 5 additional replicate tubes, and 1 mL of the 1:100 dilution would also be inoculated into each of 5 additional replicates. The program defaults would be set to 3 dilutions, 5 tubes per dilution, 1 mL inoculum volume, and standard 10-fold dilutions.

3.2 Limitations on MPN Calculator Usage

The MPN Calculator gives MPN values equivalent to those in Standard Methods (2005) for all combinations given in Table 9221.IV. The calculator, however, uses an algorithm for the calculation of confidence limits that results in similar, but not equivalent, values from those in Standard Methods.

MPN data are not directly comparable unless consistent rules for usage are followed. Calculations should be based upon the highest dilution at which all replicates are positive and the next two to three higher dilutions. The number of dilutions used should always be consistent.

Experiments can result in abnormal results. Abnormal results include any values where the number of positive replicates increase with dilution. The occurrence of abnormal results in more than 1% of the data sets suggests technical issues or problems under which the statistics used to develop an MPN may not apply; thus, MPN values calculated from abnormal results should be not be used or used with caution only when there is a scientifically defensible reason for their occurrence. Technical problems which may lead to abnormal results include 1) dilutions made on clumped organisms at low concentrations, especially if the diluent results in the release of organisms from clumps, and 2) the presence of inhibitory substances that are diluted out before the target organism, e.g., when measuring virus concentration in environmental samples by PCR.

3.3 Solution Evaluation

There are two ways to compute MPNs: either manually (via the keyboard) or by loading dilution data sets from a text file (also known as an input file).

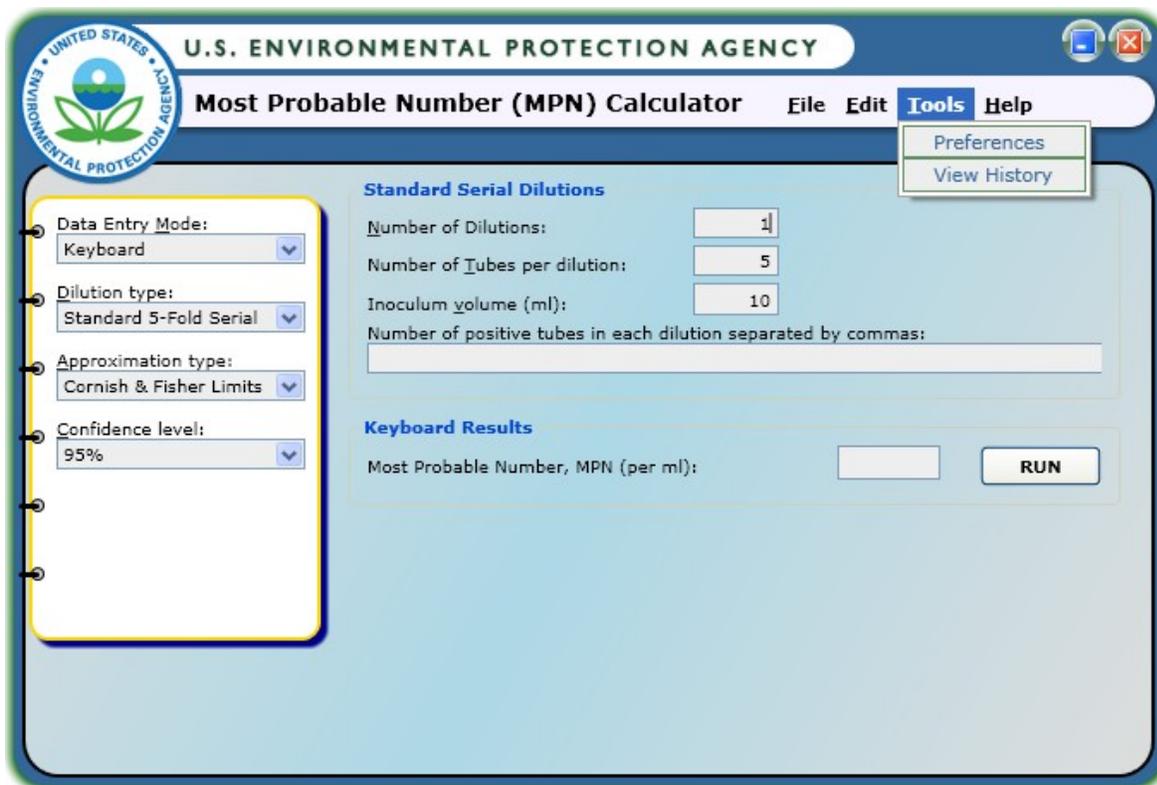
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The new input files are the exact same as the input files used by the two previous FORTRAN programs “mpn.exe”, and “mpnv.exe”. Since these FORTRAN programs were built for a specific type of dilution (10-Fold Serial Dilution for “mpn.exe”, and 5-Fold Serial Dilution for “mpnv.exe”), they did not contain information about the type of dilution. For example, when loading an input file through “mpnv.exe”, the program automatically applies “5-Fold Serial Dilution” for any standard dilution data sets encountered in the file.

Because the new input files are the same as the old and do not include the specific type of dilution, the default for the dilution type must be set in the MPN Calculator before use. To configure the default dilution type for file input:

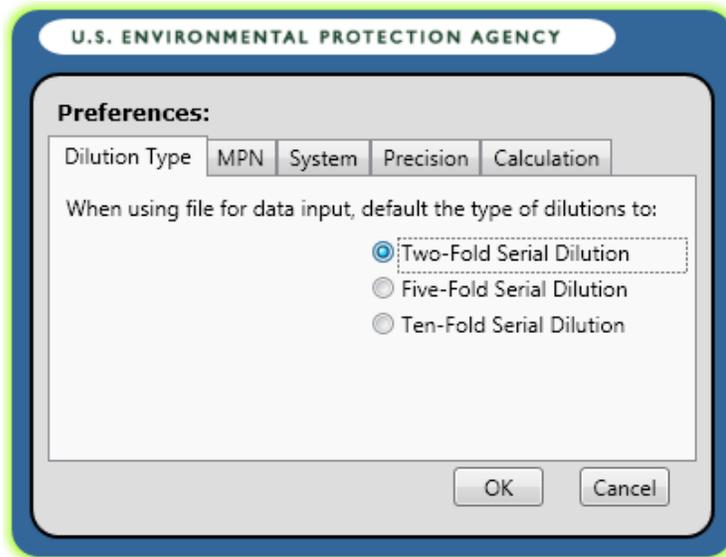
- Launch the MPN Calculator.

On the menu bar in the main window, click on “Tools” and then select “Preferences”.



The “Preferences” window will appear.

- Click on the “Dilution Type” tab.



- Check the radio button corresponding to the desired dilution type and then click on the “OK” button to save the setting and return to the main window (see screen capture below).

In the above example, “Two-Fold Serial Dilution” was checked; however, whichever dilution type is selected, that dilution type will be applied to all standard dilution data sets found in any input file.

Note:

- To keep the default the same as the “mpn.exe” program, check the “Ten-Fold Serial Dilution” radio button.
- To keep the default the same as the “mpnv.exe” program, check the “Five-Fold Serial Dilution” radio button.

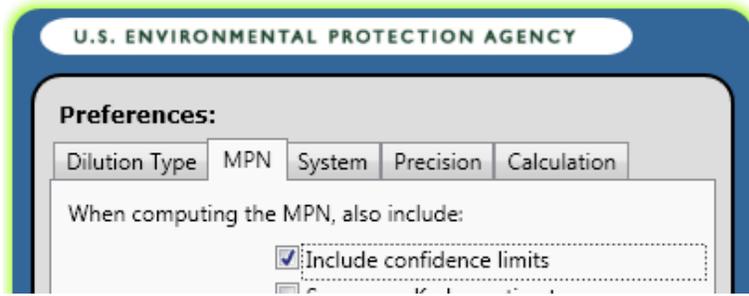
3.4 Including Other Values to MPN Calculations

The “mpn.exe” program also computes confidence limits, Spearman-Kärber estimates, and bias corrections. The “mpnv.exe” program on the other hand only adds the confidence limits to the MPN values.

The new MPN Calculator can be configured to compute any number of these values in addition to the MPNs. To configure the application to add these values when computing the MPN, follow the below steps:

- Launch the MPN Calculator.
- On the menu bar in the main window, click on “Tools”, and then select “Preferences”. The “Preferences” window will appear.
- Click on the “MPN” tab.
- Check the desired checkbox(es), then click on the “OK” button to save the setting and return to the main window (see screen capture below).

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In the above example, “Include confidence limits”, and “Bias correction” checkboxes were checked. This setting instructs the program to include confidence limits and bias corrections when computing MPN values.

Note:

- To keep the default the same as the “mpn.exe” program, check all three checkboxes.
- To keep the default the same as the “mpnv.exe” program, check the “Include confidence limits” checkbox.

Note: when using input files to compute MPNs, checking or un-checking the “Include confidence limits” has no effect on the end results. Instructions taken from the input files take precedence.

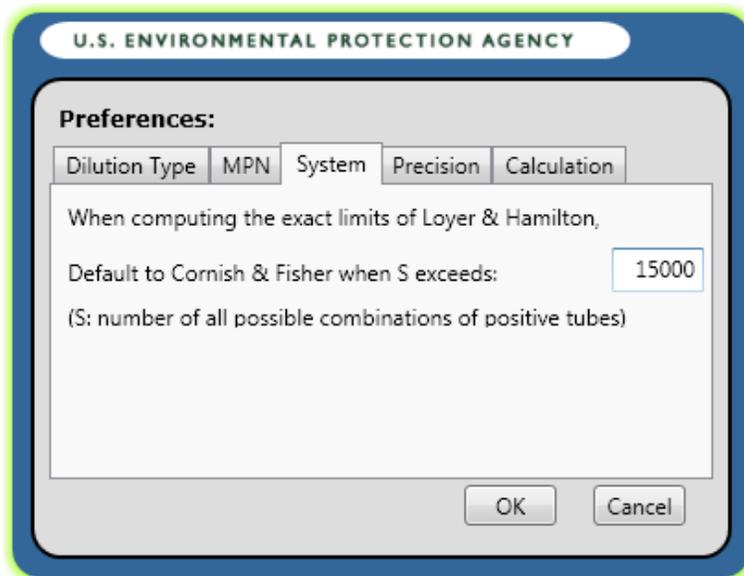
3.5 Exact Limit Calculation

In the “mpn.exe” or “mpnv.exe” programs, the exact limits of Loyer & Hamilton can only be computed if the total number of positive tubes combinations does not exceed 4,096. This number represents the size of an array of double precision numbers that is used for every exact limit calculation. For example, suppose there is a standard serial dilution with 4 dilutions and 8 tubes per dilution. In this case, the total number of positive tubes combinations is $9 \times 9 \times 9 \times 9 = 6561$ (there are 9 possible results in each dilution). Even though both FORTRAN programs are configured to compute the Loyer & Hamilton’s limits, they both “ignore the setting” and the approximate limits of Cornish & Fisher are calculated instead. This 4,096 limit was a threshold set with the limitation of computing power in the early 1990s.

In the new MPN Calculator, the number of positive tubes combination is configurable. The default value is set to 54,096. This value means that for each exact limit calculation, the program may use an array of double precision numbers of the size up to 54,096 elements. To decrease or increase this number:

- Launch the MPN Calculator.
- On the menu bar in the main window, click on “Tools”, and then select “Preferences”. The “Preferences” window will appear.
- Click on the “System” tab

- Type in the desired number in the textbox, then click on the “OK” button to save the setting and return to the main window (see screen capture below).

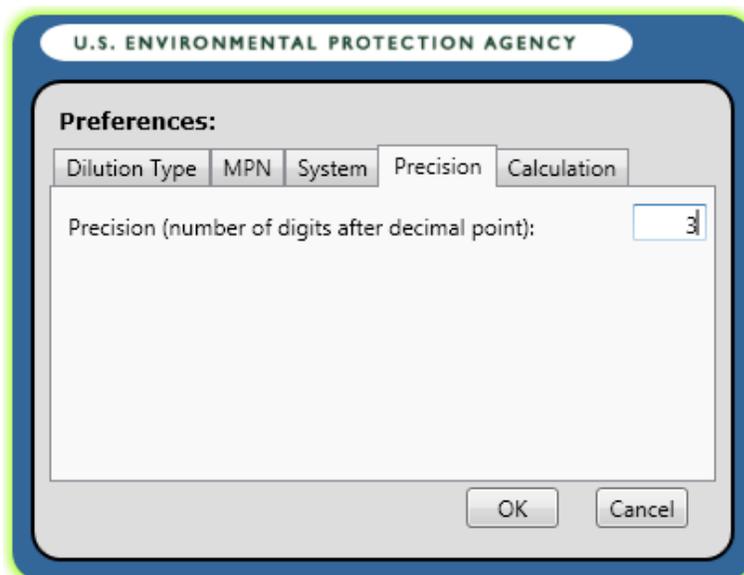


In the above example, the threshold is set to 15,000. With this setting, the program will switch to the approximate limits of Cornish & Fisher if, for a given series of dilutions, the total number of positive tubes combinations exceeds 15,000, even if the exact limit of Loyer & Hamilton is requested.

3.6 Results Precision

The new MPN calculator can be configured to truncate all computed results to a certain number of digits after the decimal point: the default is 2. To change this number:

- Launch the MPN Calculator
- On the menu bar in the main window, click on “Tools”, and then select “Preferences”. The “Preferences” window will appear.
- Click on the “Precision” tab.
- Type in the desired number in the textbox, then click on the “OK” button to save the setting and return to the main window (see screen capture below).

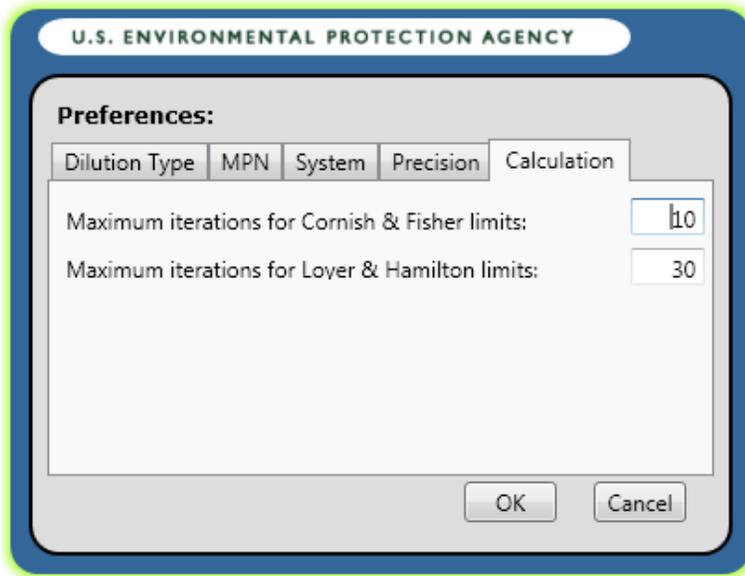


In the above example, the number of digits after the decimal point is set to 3.

3.7 Maximum Number of Iterations

In the “mpn.exe” or the “mpnv.exe” program, to be successful, a search for the approximate limits must reach its target within 10 iterations. Beyond this number, both programs consider that the algorithm does not converge, and the search for the approximate limits is considered to have failed. In the same way, to be successful, a search for the exact limits must reach its target within 30 iterations. Beyond this threshold, the algorithm is not convergent. These threshold numbers were hard-coded in both FORTRAN programs. In the new MPN calculator, these numbers are configurable. To configure these numbers:

- Launch the MPN Calculator.
- On the menu bar in the main window, click on “Tools”, and then select “Preferences”. The “Preferences” window will appear.
- Click on the “Calculation” tab.
- Type in the desired number in the textboxes, then click on the “OK” button to save the setting and return to the main window (see screen capture below).



In the above example, calculations of Cornish & Fisher limits are limited to 10 iterations (the default value), and calculations of Loyer & Hamilton limits are limited to 30 iterations (the default value). On most modern computers these defaults can be set to much higher values (e.g., 50 and 100, respectively).

4.0 KEYBOARD DATA ENTRY MODE

Keyboard data entry mode allows computing one MPN value at a time only, by entering dilution data manually (via keyboard). To compute multiple MPN values at a time, see the “File Data Entry Mode” section. As in “mpn.exe” or “mpnv.exe”, users can run the entered data with two types of dilutions:

- A standard serial dilution (which can be either “Standard 2-Fold”, “Standard 5-Fold”, or “Standard 10-Fold”), or
- A Non-Standard dilution.

4.1 Standard Serial Dilutions

To calculate MPNs using standard serial dilutions, follow the below steps:

- Launch the new MPN Calculator.
- In the main window, click on the “Data Entry Mode:” dropdown list and select the “Keyboard” option.
- Click on the “Dilution type:” dropdown list and select one of the three standard serial dilution options:
 - “Standard 2-Fold Serial”
 - “Standard 5-Fold Serial”
 - “Standard 10-Fold Serial”.
- Click on the “Approximation type:” dropdown list and select one of the two available options: “Cornish & Fisher Limits” or “Loyer & Hamilton Limits”.
- Click on the “Confidence level:” dropdown list and select one of the four available options:

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- “99%”,
- “95%”,
- “90%”, or
- “50%”.
- In the “Standard Serial Dilutions” group box, type in:
 - The number of dilutions in the “Number of Dilutions:” textbox.
 - The number of tubes in the “Number of Tubes per dilution:” textbox.
 - The initial inoculum volume (in ml) in the “Inoculum volume (ml):” textbox.
 - The comma-separated list of observed positive tubes of the series of dilutions in the “Number of positive tubes in each dilution separated by commas:” textbox.
Note: Press the “Tab” key or “Shift” + “Tab” keys to move forward or backward respectively between textboxes
- Click on the “RUN” button for results (see example screen below).

Standard Serial Dilutions	
Number of Dilutions:	3
Number of Tubes per dilution:	5
Inoculum volume (ml):	10
Number of positive tubes in each dilution separated by commas:	5,3,0

Keyboard Results	
Most Probable Number, MPN (per ml):	0.39
SPEARMAN-KARBER Estimate:	0.59
MPN Corrected for Bias (Salama):	0.33
Lower 95% Confidence Limit (Loyer & Hamilton):	0.1
Upper 95% Confidence Limit (Loyer & Hamilton):	1.01

In the example screen above, the program computed the MPN value, the Spearman-Karber estimate, the bias correction, and the Loyer & Hamilton Confidence Limits for a Standard 5-Fold Serial dilution with:

- 3 as the number of dilutions in the series,
- 5 as the number of tubes per dilution,
- 10ml as the initial inoculum volume,
- And 5, 3, 0 as the number of observed positive tubes (5 observed positive tubes in the first dilution, 3 in the second and 0 in the third).

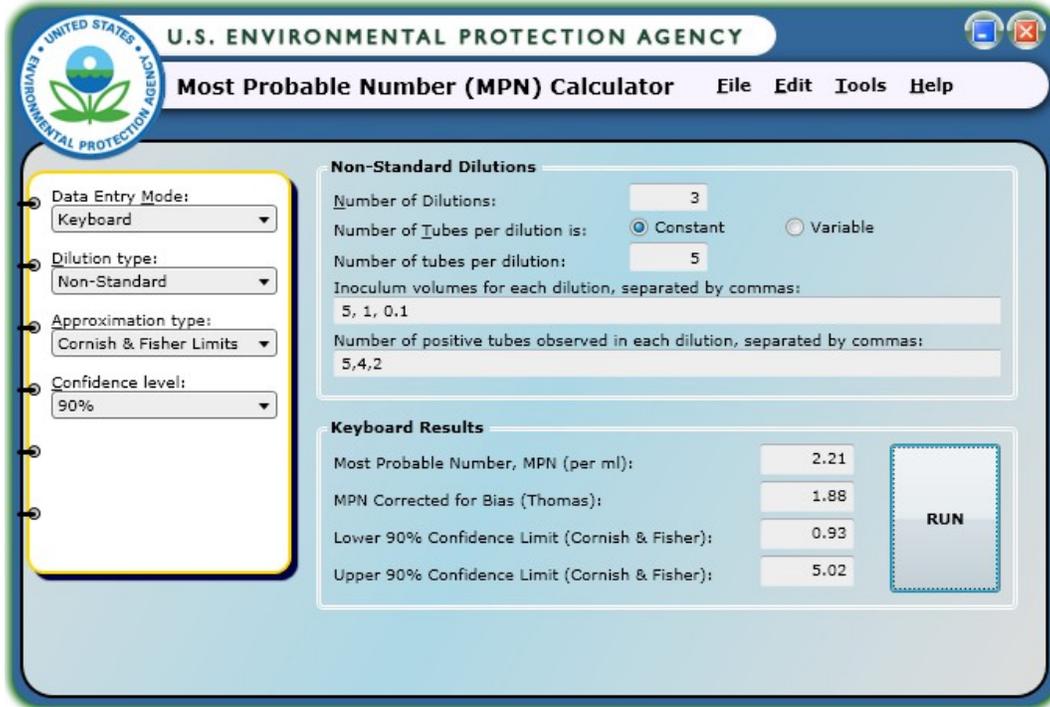
(See section 2 for configuring the program to compute other values on top of the MPN).

4.2 Other (Non-Standard) Dilutions

With non-standard dilutions, the number of tubes per dilution can either be constant or variable.

4.2.1 Constant Number of Tubes Per Dilution

- Launch the new MPN Calculator.
- In the main window, click on the “Data Entry Mode:” dropdown list and select the “Keyboard” option.
- Click on the “Dilution type:” dropdown list and select “Non-Standard”.
- Click on the “Approximation type:” dropdown list and select one of the two available options: “Cornish & Fisher Limits” or “Loyer & Hamilton Limits”.
- Click on the “Confidence level:” dropdown list and select one of the four available options:
 - “99%”,
 - “95%”,
 - “90%”, or
 - “50%”.
- In the “Non-Standard Dilutions” group box, do the following:
 - Type in the number of dilutions in the “Number of Dilutions:” textbox.
 - Check the “Constant” radio button in the “Number of Tubes per dilution is:” radio button list.
 - Type in the number of tubes per dilution in the “Number of Tubes per dilution:” textbox
 - Type in the comma-separated list of inoculum volumes (in ml) in the “Inoculum volumes for each dilution, separated by commas:” textbox.
 - Type in the comma-separated list of observed positive tubes of the series of dilutions in the “Number of positive tubes in each dilution separated by commas:” textbox.
Note: Press the “Tab” key or “Shift” + “Tab” keys to move forward or backward respectively between textboxes.
Note: The number of elements (separated by commas) in the last 2 textboxes must match the number specified in the “Number of Dilutions:” textbox.
- Click on the “RUN” button for results (see example screen below).



In the example screen above, the program computed the MPN value, the bias correction, and the Cornish & Fisher Confidence Limits for a Non-Standard dilution with constant number of tube per dilution with:

- 3 as the number of dilutions.
- The number of tubes per dilution being constant and equal to 5.
- The inoculum volumes in each successive dilution being 5ml in the first dilution, 1ml in the second dilution, and 0.1ml in the third.
- The numbers of observed positive tubes being 5 in the first dilution, 4 in the second and 2 in the third.

Note: See section 2 for configuring the program to compute other values on top of the MPN.

4.2.2 Variable Number of Tubes Per Dilution

- Launch the new MPN Calculator.
- In the main window, click on the “Data Entry Mode:” dropdown list and select the “Keyboard” option.
- Click on the “Dilution type:” dropdown list and select “Non-Standard”.
- Click on the “Approximation type:” dropdown list and select one of the two available options: “Cornish & Fisher Limits” or “Loyer & Hamilton Limits”.
- Click on the “Confidence level:” dropdown list and select one of the four available options:
 - “99%”,
 - “95%”,
 - “90%”, or
 - “50%”
- In the “Non-Standard Dilutions” group box:
 - Type in the number of dilutions in the “Number of Dilutions:” textbox.

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- Check the “Variable” radio button in the “Number of Tubes per dilution is:” radio button list.
- Type in the comma-separated list of number of tubes per dilution in the “Number of Tubes for each dilution, separated by commas:” textbox.
- Type in the comma-separated list of inoculum volumes (in ml) in the “Inoculum volumes for each dilution, separated by commas:” textbox.
- Type in the comma-separated list of observed positive tubes of the series of dilutions in the “Number of positive tubes in each dilution separated by commas:” textbox.

Note: Press the “Tab” key or “Shift” + “Tab” keys to move forward or backward respectively between textboxes.

Note: The number of elements (separated by commas) in the last 3 textboxes must match the number specified in the “Number of Dilutions:” textbox.

- Click on the “RUN” button for results (see example screen below).

The screenshot shows the 'Most Probable Number (MPN) Calculator' window from the U.S. Environmental Protection Agency. The window has a menu bar with 'File', 'Edit', 'Tools', and 'Help'. On the left, there is a sidebar with four dropdown menus: 'Data Entry Mode' (Keyboard), 'Dilution type' (Non-Standard), 'Approximation type' (Cornish & Fisher Limits), and 'Confidence level' (90%). The main area is titled 'Non-Standard Dilutions' and contains several input fields: 'Number of Dilutions' (3), 'Number of Tubes per dilution is' (radio buttons for Constant and Variable, with Variable selected), 'Number of tubes for each dilution, separated by commas' (6,4,2), 'Inoculum volumes for each dilution, separated by commas' (10,1,0.1), and 'Number of positive tubes observed in each dilution, separated by commas' (6,3,1). Below these fields is a 'Keyboard Results' section with a table of values: Most Probable Number, MPN (per ml): 1.8; MPN Corrected for Bias (Thomas): 1.41; Lower 90% Confidence Limit (Cornish & Fisher): 0.56; Upper 90% Confidence Limit (Cornish & Fisher): 3.86. A 'RUN' button is located to the right of the results table.

In the example screen above, the program computed the MPN value, the bias correction, and the Cornish & Fisher Confidence Limits for a Non-Standard dilution with variable number of tubes per dilution, with:

- 3 as the number of dilutions.
- The number of tubes per dilution being 6 in the first dilution, 4 in the second dilution, and 2 in the third.
- The inoculum volumes in each successive dilution being 10ml in the first dilution, 1ml in the second dilution, and 0.1ml in the third.
- And the numbers of observed positive tubes being 6 in the first dilution, 3 in the second and 1 in the third.

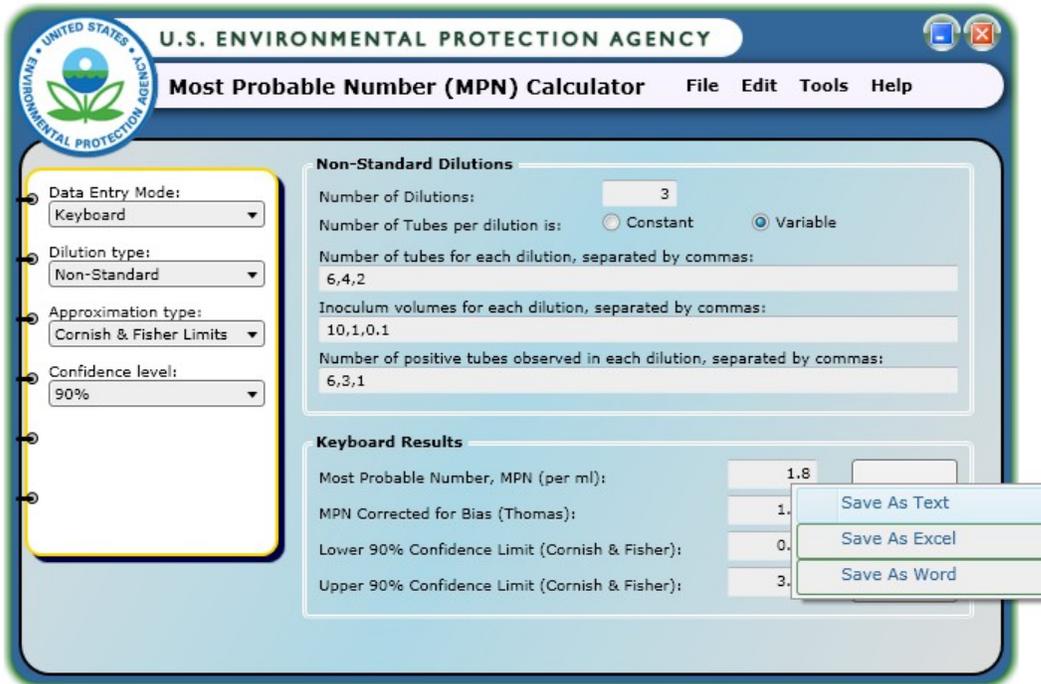
Note: See section 2 for configuring the program to compute other values on top of the MPN).

4.3 Saving Results

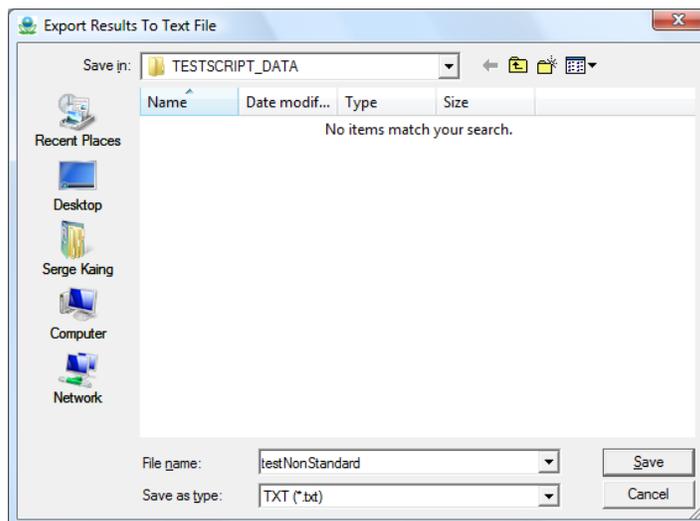
Results can be saved in one of the following formats: Excel spreadsheet, Word document, or Text file.

4.3.1 Save Results as a Text File

- Right-click on the result textbox.
- In the pop-up context menu, select “Save As Text”.



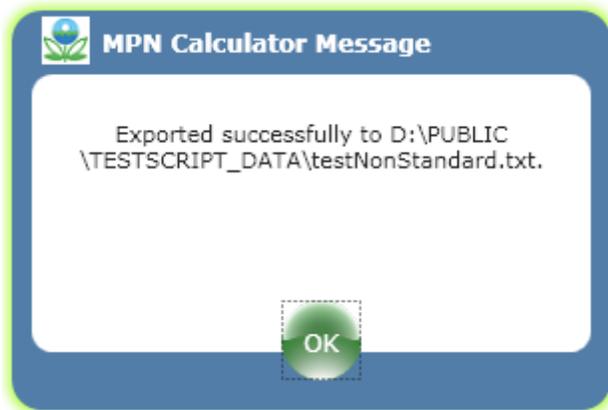
- In the “Export Results To Text File “ dialog box, navigate to the location where the results will be saved, then type in the text file name.



- Click on the “Save” button.

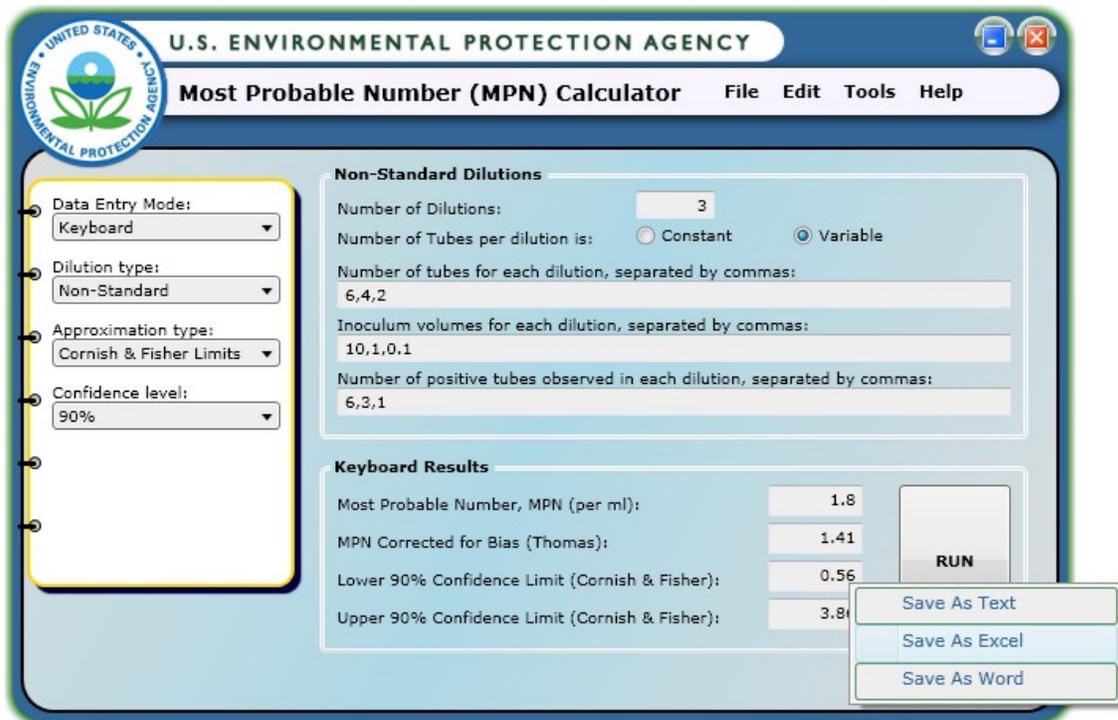
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- In the “MPN Calculator Message” dialog box, click on the “OK” button to finish.

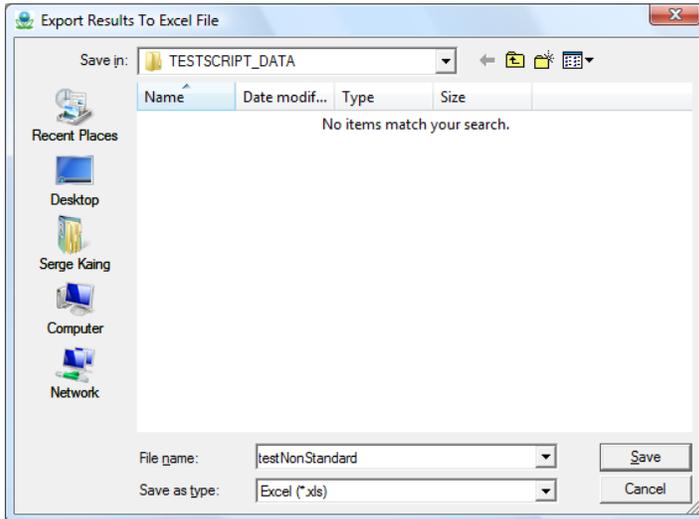


4.3.2 Save Results as an Excel File

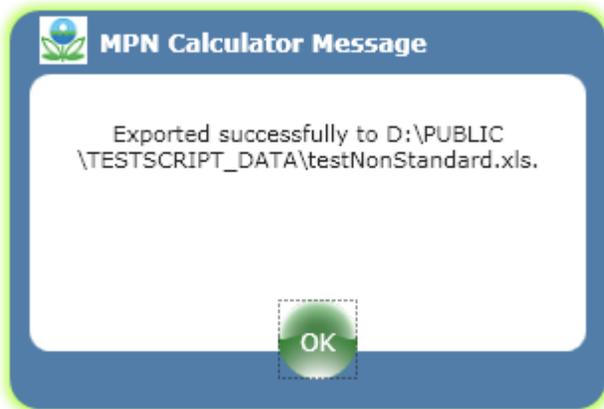
- Right-click on the result textbox.
- In the pop-up context menu, select “Save As Excel”.



- In the “Export Results To Excel File “ dialog box, navigate to the location where the results will be saved, then type in the text file name.



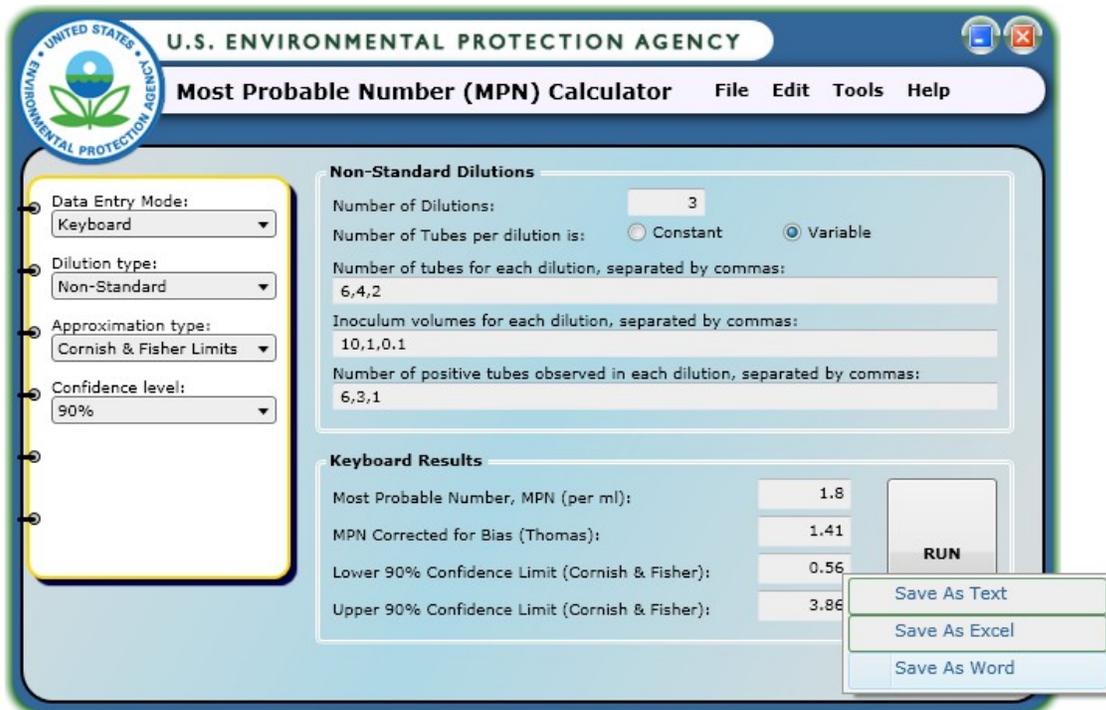
- Click on the “Save” button.
- In the “MPN Calculator Message” dialog box, click on the “OK” button to finish.



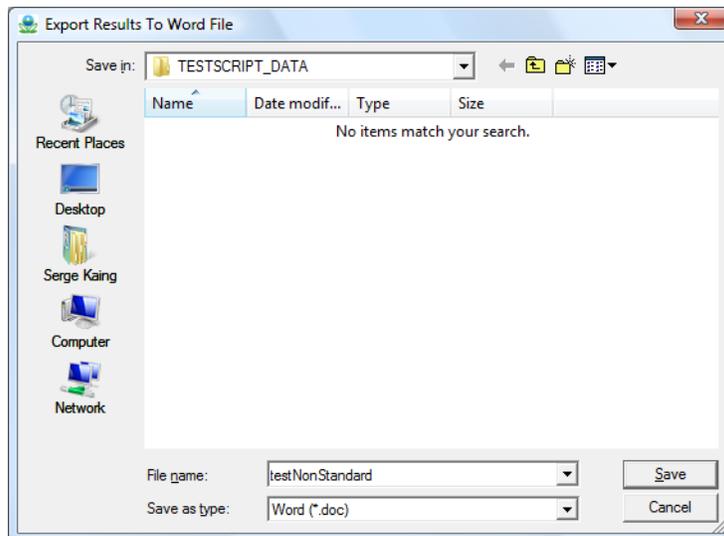
4.3.3 Save Results as a Word File

- Right-click on any result textbox.
- In the pop-up context menu, select “Save As Word”.

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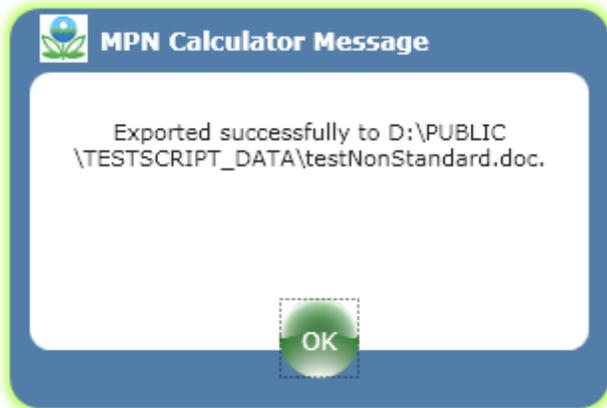


- In the “Export Results To Word File“ dialog box, navigate to the location where the results will be saved, then type in the text file name.



- Click on the “Save” button.

- In the “MPN Calculator Message” dialog box, click on the “OK” button to finish.



5.0 FILE DATA ENTRY MODE

5.1 Input File Specifications

The MPN Calculator accepts 3 types of input file formats: “STANDARD”, “SHORT/MAX”, and “SHORT/MIN”.

5.1.1 STANDARD Format Specifications

Any line that starts with a semicolon is a COMMENT line (e.g. “;”). All blank lines are ignored. When LINE is mentioned in the following discussion, it is meant to exclude BLANK or COMMENT lines.

- The first line must contain the string value “STANDARD”.
- The second line contains two pieces of information:
 - One of the following three-character codes for the confidence level desired:
 - “99%”,
 - “95%”,
 - “90%”, or
 - “50%”.

-AND-

- One of the following two-character codes for the types of limits desired (Cornish & Fisher or Loyer & Hamilton):
 - “CF”, or
 - “LH”

The following lines in the file then contain information for the individual dilution data sets.

- The first line of a dilution data set always contains the following two pieces of information (separated by a comma or one or more spaces):
 - The number of dilutions, and
 - The number of tubes per dilution (e.g. either a number or the string value “VARIABLE”).
- If standard dilutions (2-fold, 5-fold, 10-fold dilutions) are to be calculated for the data set, then the inoculum volume must be entered on the 1st line following the number of tubes per dilution.

- If standard dilutions (2-fold, 5-fold, 10-fold dilutions) are used, the second line contains the number of positive tubes (separated by a comma or one or more spaces) observed in the successive dilutions.
- If non-standard dilutions are to be calculated for the data set, then the second and following lines contain the data for each of the dilutions (one dilution data set to a line) (e.g. the inoculum volume, the number of tubes per dilution if this was a variable, and the number of positive tubes separated by a comma or one or more spaces).

5.1.2 STANDARD Input File Example

The following is a sample “STANDARD” MPN input file containing three dilution data sets. Cornish & Fisher limits at the 95% confidence level are specified for all the sets.

```

STANDARD

95%    CF
;BLUE RIDGE – TUESDAY RUN
3      10    1
10     6     1

;SPECIAL ANALYSIS FOR T.A. BROWN
3      8
1      10
.1     2
.01    0
    
```

The first dilution data set is a standard serial dilution with 3 dilutions, 10 tubes per dilution, and an inoculum volume of 1ml. The number of observed positive tubes in each of the 3 dilutions is 10 for the first dilution, 6 for the second dilution and 1 for the third dilution.

Note: This is a standard serial dilution because an inoculum volume of 1ml is present in the first line of the dilution data set. However, the dilution type (2-Fold, 5-Fold, 10-Fold) is unknown. To see how this is determined by the program, see section 4.3.

The second dilution data set is a non-standard dilution with 3 10-fold dilutions and 8 tubes per dilution. The number of observed positive tubes in each of the 3 dilutions is 10 for the first dilution, 2 for the second dilution, and 0 for the third. It is a non-standard dilution because the inoculum volume was not specified in the first line of the dilution data set. It is a 10-fold dilution because the inoculum volumes decreased by a factor of 10.

The third dilution data set is also a non-standard dilution with 3 dilutions, but with a variable number of tubes per dilution.

- First dilution: inoculum volume = .1ml, number of tubes per dilution = 10, and number of observed positive tubes = 10
- Second dilution: inoculum volume = 0.03ml, number of tubes per dilution = 5, and number of observed positive tubes = 4

- Third dilution: inoculum volume = 0.0045ml, number of tubes per dilution = 6, and number of observed positive tubes = 0

5.1.3 SHORT/MAX Format Specifications

Any line that starts with a semicolon is a COMMENT line (e.g. “;”). All blank lines are ignored. When LINE is mentioned in the following discussion, it is meant to exclude BLANK or COMMENT lines.

- The first line must contain the string value “SHORT/MAX”.
- The second line contains two pieces of information:
 - One of the following three-character codes for the confidence level desired:
 - “99%”,
 - “95%”,
 - “90%”, or
 - “50%”
 - AND-
 - One of the following two-character codes for the types of limits desired (Cornish & Fisher or Loyer & Hamilton):
 - “CF”, or
 - “LH”

The following lines in the file then contain information for the individual dilution data sets.

- The first line of a dilution data set contains the following three pieces of information (separated by a comma or one or more spaces):
 - The number of dilutions,
 - The number of tubes per dilution, and
 - The initial inoculum volume in ml.
- The following lines then contain the data for each of the dilutions, one dilution data set to a line (e.g. the inoculum volume, the number of tubes per dilution if this was a variable and the number of positive tubes separated by a comma or one or more spaces).

Note: The number of lines (e.g. the number of dilution data sets) must match the number of dilutions specified in the first line of the dilution data sets.

5.1.4 SHORT/MAX Input File Example

If the number of dilutions, the number of tubes per dilution, and the initial inoculum volume are constant over all dilution data sets, the input files can be simplified by specifying the “SHORT/MAX” format. The following is a “SHORT/MAX” data file with 4 dilution data sets:

```

SHORT/MAX

95%   CF

3 5 1

5,3,0
5,4,2
5,0,0
5,4,3
    
```

The first dilution data set is a standard serial dilution with 3 dilutions, 5 tubes per dilution, and an initial inoculum volume of 1ml. The observed numbers of positive tubes are 5 for the first dilution, 3 for the second, 0 for the third dilution.

For the second dilution data set, the observed numbers of positive tubes are 5 for the first dilution, 4 for the second, and 2 for the third dilution.

For the third dilution data set, the observed numbers of positive tubes are 5 for the first dilution, 0 for the second and third dilution.

For the fourth dilution data set, the observed numbers of positive tubes are 5 for the first dilution, 4 for the second, and 3 for the third dilution.

Note: “SHORT/MAX” notation is only for standard serial dilutions because the number of dilutions, the number of tubes per dilution, and the initial inoculum volume must be present. However, the dilution type (2-Fold, 5-Fold, 10-Fold) is unknown. To see how this is determined by the program, see section 4.3.

5.1.5 SHORT/MAX Input File Example

Any line that starts with a semicolon is a COMMENT line. All blank lines are ignored and when LINE is mentioned in the following discussion, it is meant to exclude BLANK or COMMENT lines.

- The first line must contain the string value “SHORT/MIN”.
- The second line contains two pieces of information:
 - One of the following three-character codes for the confidence level desired:
 - “99%”,
 - “95%”,
 - “90%”, or
 - “50%”.

-AND-

- One of the following two-character codes for the types of limits desired (Cornish & Fisher or Loyer & Hamilton):
 - “CF”, or
 - “LH”.

The following lines in the file then contain information for the individual dilution data sets.

- The first line of a dilution data set contains the following three pieces of information (separated by a comma or one or more spaces):
 - The number of dilutions,
 - The number of tubes per dilution, and
 - The initial inoculum volume in ml.

The following lines contain the data for each of the dilutions, one dilution data set to a line (e.g. the inoculum volume, the number of tubes per dilution if this was a variable and the number of positive tubes separated by a comma or one or more spaces).

Note: The number of lines (e.g. the number of dilution data sets) must match the number of dilutions specified in the first line of the dilution data sets.

5.1.6 SHORT/MIN Input File Example

Contents of SHORT/MIN input files are exactly the same as those of SHORT/MAX input files except at the very first line, where the string value “SHORT/MIN” replaces “SHORT/MAX”: below is an example.

```
SHORT/MIN

95%   CF

3 5 1

5,3,0
5,4,2
5,0,0
5,4,3
```

5.2 Output Specifications

The “SHORT/MIN” input file format instructs the program to compute ONLY the MPN values (regardless of any other settings) for each of the dilution data sets found in the input file.

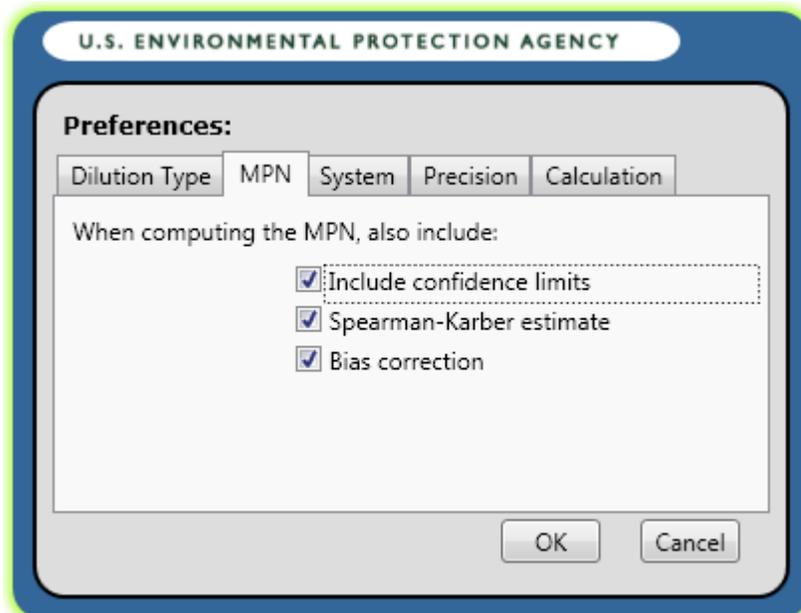
The output of “STANDARD” and “SHORT/MAX” formats are identical. These types of input file formats instruct the program to compute the MPN values for each of the dilution data sets present in the input file, as with the “SHORT/MIN” format. Additionally, the program can compute 3 other types of values depending on the settings in the “Preferences” window.

- The Spearman-Kärber estimate
- The bias correction
- The confidence limits (Cornish & Fisher, or Loyer & Hamilton).

To instruct the program to additionally compute one or all three of these values, follow the below steps:

- Launch the MPN Calculator.

- On the menu bar, click on “Tools”, and then select “Preferences”. The “Preferences” window will appear.
- On the “MPN” tab, check the desired value(s) to be included in the calculation.

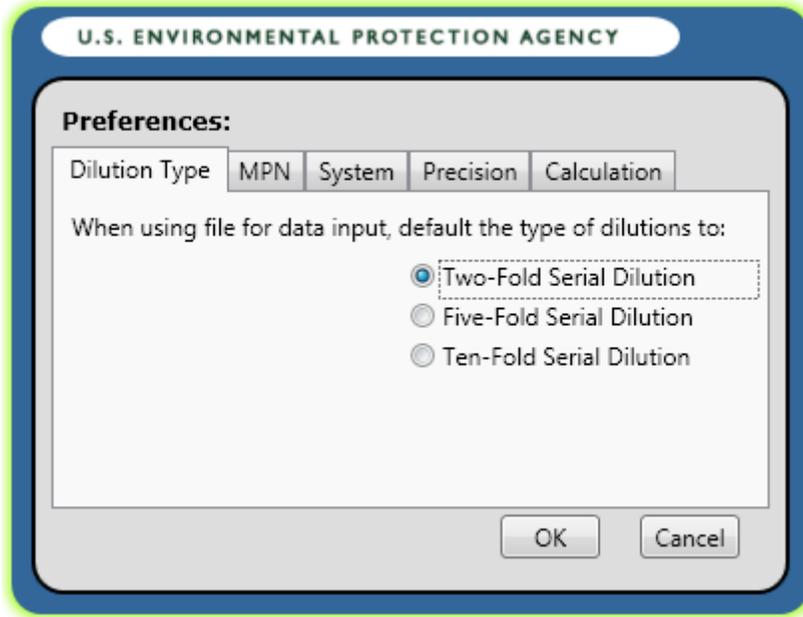


- Click on the “OK” button to save your setting, and then close the “Preferences” window. In the example above, all 3 values were checked. This setting instructs the program to compute the Spearman-Karber estimate, the bias correction and the confidence limits in addition to the MPN values for each of the dilution data sets found in any input file.

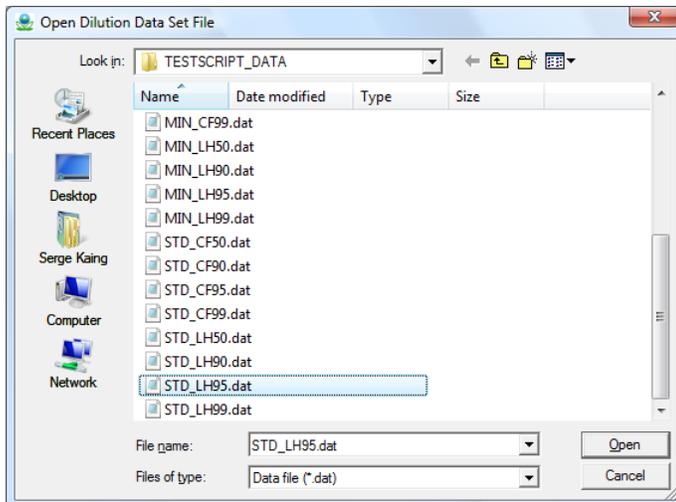
5.3 Running with Input Files

Input files do not contain information that tells the program the type of standard serial dilution (2-Fold, 5Fold, or 10-Fold dilution) to apply to a dilution data set when needed, so the following steps are needed to run the calculator with input files:

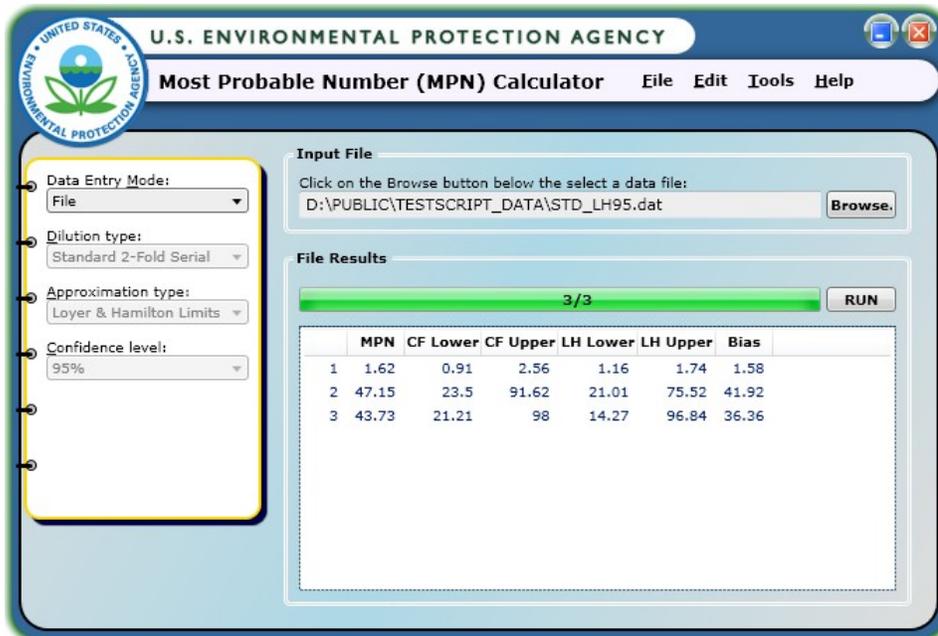
- Make sure that your data file complies with the specifications mentioned above.
- Launch the MPN Calculator.
- On the menu bar, click on “Tools”, and then select “Preferences”. The “Preferences” window will appear.
- On the “Dilution Type” tab, check the desired dilution type: “Two-Fold Serial Dilution”, “Five-Fold Serial Dilution” or “Ten-Fold Serial Dilution”.



- Click on the “OK” button to save your setting, and then close the “Preferences” window. In the example above, the “Two-Fold Serial Dilution” radio button was checked. This setting instructs the program to apply 2-Fold Dilution Type to all standard dilution data sets found in any input file.
- In the main window, click on the “Data Entry Mode:” dropdown list and select the “File” option.
- In the “Input File” box of the main window, click on the “Browse” button.
- In the “Open Dilution Data Set” dialog box, select the file to open.
- Click the “Open” button



- In the “File Results” box, click on the “RUN” button to start computing MPN values.
- The results are displayed in the “File Results” group box (see screen capture below).



In the example result screen above, we can conclude that:

- The file named “STD_LH95.dat” was selected as the input file
- The program is configured to treat all standard serial dilution data sets contained in the file as those of “Standard 2-Fold Serial” dilutions.
- The input file instructs the program to compute exact limits of Loyer & Hamilton at 95% confidence interval.
- There are 3 dilution data sets in the input file.

5.4 Saving Results

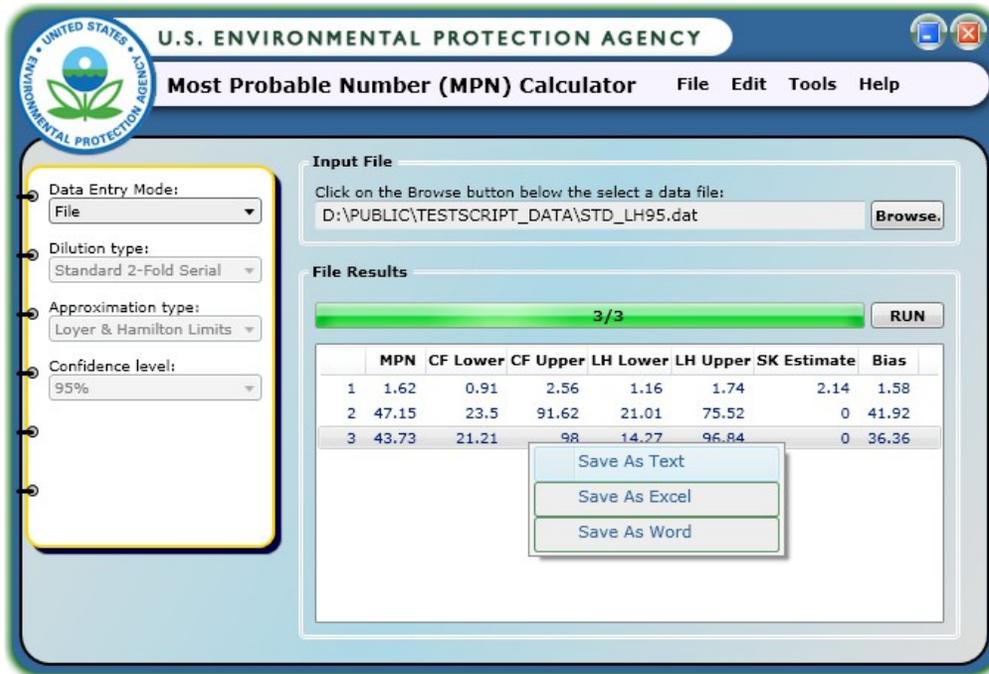
Results from file inputs can also be saved into an Excel spreadsheet, a Word document, or Text file.

5.4.1 Save Results as a Text File

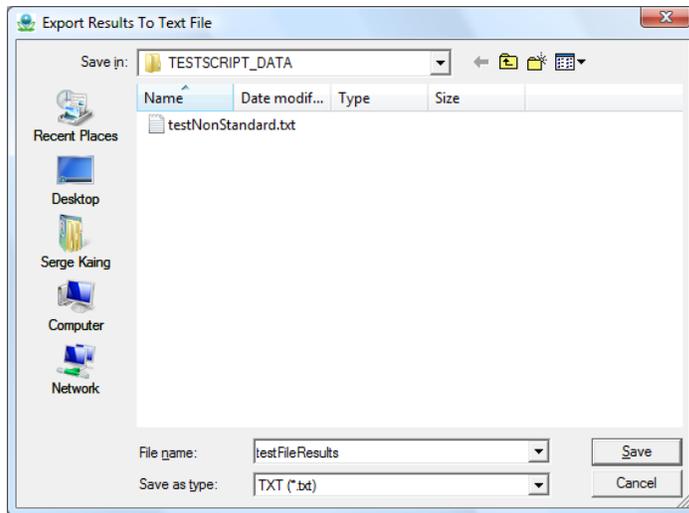
The following describes the steps needed to save the results into a Text file.

- Follow the steps described in section 4.3 to load an input file and compute MPNs and other values.
- In the “File Results” box, right-click on the result list.
- Select “Save As Text”.

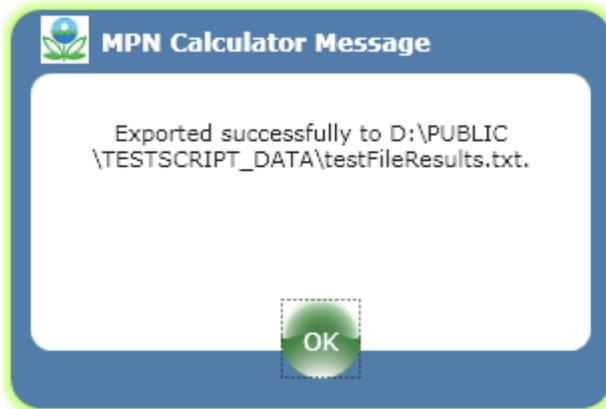
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- In the “Export Results To Text File” dialog box, choose the desired location to save the file, and then type in the text file name.



- Click on the “Save” button.
- In the “MPN Calculator Message” dialog box, click the “OK” button to finish.



NOTE:

- To save results to a Word document, repeat the steps described above, but select “Save As Word” in the popup context menu.
- To save results to an Excel spreadsheet, repeat the steps described above, but select “Save As Excel” in the popup context menu.

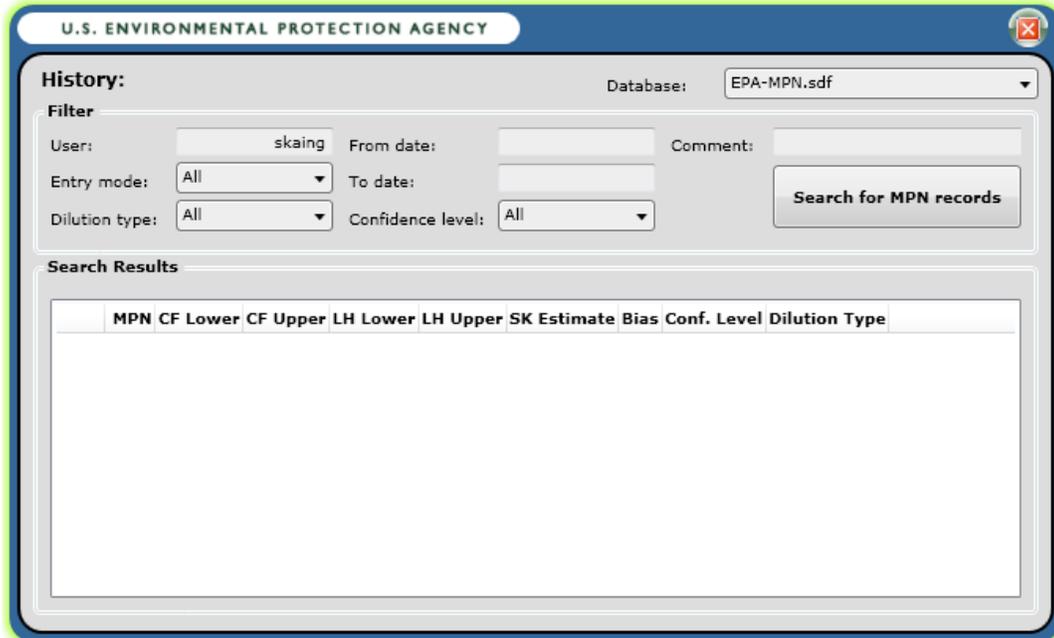
6.0 VIEW HISTORY

By default, all dilution data sets and their corresponding results are saved locally in a SQL Server Compact Edition database. The following pieces of information are saved:

- Dilution Data Set:
 - Data entry mode: either Keyboard or Input File
 - User name: login name of the user who entered the dilution data
 - Dilution type: 2-Fold Serial, 5-Fold Serial, 10-Fold Serial, or Other (non-standard)
 - Number of dilutions
 - Number of tubes per dilution: a comma-separated list of integers
 - Inoculum volumes: a comma-separated list of double precision numbers
 - Observed positive tubes: a comma-separated list of integers
 - Confidence Level: 99%, 95%, 90% or 50%
 - Approximation type: approximate (Cornish & Fisher), or exact (Loyer & Hamilton)
 - Comment (comment of a given dilution data set, if any, only from input file)
- MPN Records
 - MPN value: computed most probable number
 - Lower X% Confidence limit (Cornish & Fisher, or Loyer & Hamilton)
 - Upper X% Confidence Limit (Cornish & Fisher, or Loyer & Hamilton)
 - Spearman-Karber estimate (if computed)
 - Bias correction (if computed)
 - Compute date

6.1 History Panel Discussion

To open the History panel, click on “Tools” on the menu bar, and then select “View History” (see screen capture below).



The History panel has 3 main sections: “Database”, “Search Results”, and “Filter”. The “Search Results” section is used to display records matching the search criteria specified in the “Filter” section. The “Database” section is used to specify which database the search operations should operate on (the default is the current database). When the History panel loads, the “Database” dropdown list is populated with all the database files that have been archived so far, including the current database. The “Filter” section contains seven (7) fields. Each field can be used by itself or can be combined with the others to form search criteria. The seven fields are as follows:

- **User:** This field is used to look for records that were computed under a given login name (the default is the current user’s login name).
- **Entry mode:** This field is used to look for records whose dilution data sets were entered via the Keyboard or Input Files (the default behavior is All e.g. Keyboard AND Files).
- **Dilution type:** This field is used to look for records with a certain type of dilution (the default is All e.g. “2-Fold Serial” AND “5-Fold Serial” AND “10-Fold Serial” AND “Other” Dilutions).
- **From date:** This field is used to look for records that were computed/saved on a given date/time. It also can be used to specify the lower bound of a date range (the default is empty).
- **To date:** This field is used to look for records that were computed/saved on a given date/time. It can also be used to specify the upper bound of a date range (the default is empty).

NOTE: see section 5.2 for details.

- **Confidence level:** This field is used to look for records with confidence limits computed with 99%, 95%, 90%, or and 50% confidence intervals (the default is All e.g. all 4 levels).
- **Comments:** This field is used to look for records whose dilution data sets came from input files and have some comments. Comments from input files are lines that start with a semi-column. See section 4 for more details).

6.2 Date and Time Formats

Accepted date formats are US date/time formats. The following formats are valid:

- “MM/DD/YYYY”,
- “MM/DD/YY”,
- “MM/DD/YY HH:MM AM”,
- “MM/DD/YYYY HH:MM PM”.

If ONLY ONE of the “From date:” or “To date:” fields has a valid date/time value, then

- If the date/time value does not have a time component then the program will search for the entire 24 hour range of the specified date.
- If the date/time value does have a time component, then the program will search from the specified time until the end of the specified date (e.g. 11:59:59 PM).

If the “From date:” date is later then the “To date:” date, the user is notified of the incoherent date/time range.

6.3 Search Examples

The search criteria and results described below are specific to the desktop on which the MPN Calculator is installed. Modify the search criteria accordingly.

- Launch the MPN Calculator.
- On the menu bar, click on “Tools”, and then select “View History”. The “History” window will appear.

6.3.1 Search for Records Computed on a Specific Date

	MPN	CF Lower	CF Upper	LH Lower	LH Upper	SK Estimate	Bias	Conf. Level	Dilution Type
1	1.62	0.72	2.91	0.83	2.3	2.14	1.58	99%	2-Fold
2	47.15	18.27	123.4	16.83	100.8	0	41.92	99%	Other
3	43.73	16.52	160.09	11.53	124.36	0	36.36	99%	Other
4	1.62	1.36	1.93	1.39	1.63	2.14	1.58	50%	2-Fold
5	47.15	38.55	60.1	36.11	47.55	0	41.92	50%	Other
6	43.73	35.71	57.41	23.39	58.27	0	36.36	50%	Other

- In the “From date:” textbox, type in a date time value. In the example screen above, the date entered is October 27th 2009, at 11 AM.

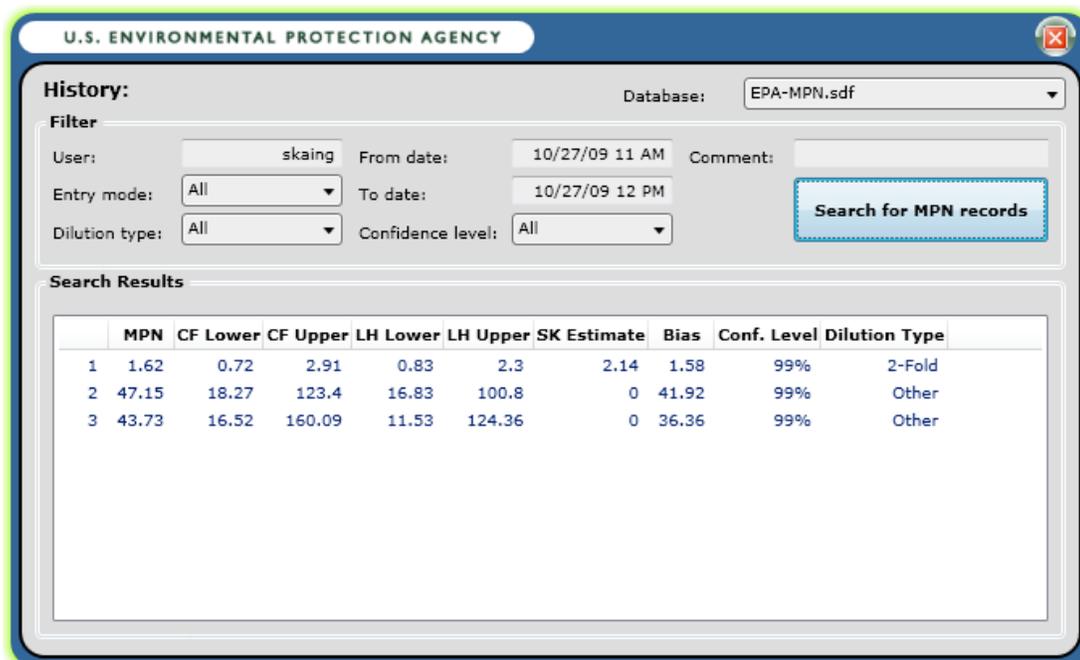
- Click on the “Search for MPN records” button.

In the example screen above, the “Search Results” section shows that there are 6 records that were computed between 11 AM and Midnight on October 27th 2009, under the login name “skaing”.

6.3.2 Search for Records Computed Between Dates

This section narrows the search criteria by a date range.

- In the “To date:” textbox, type in “10/27/09 12 PM”.
- Click on the “Search for MPN records” button.

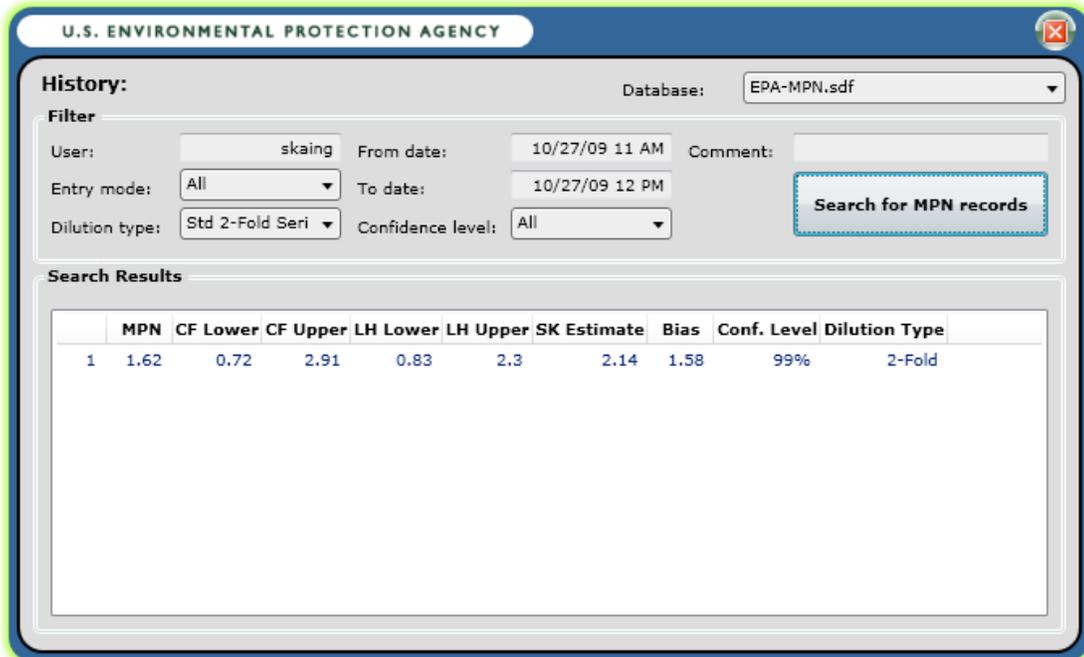


In the example screen above, the “Search Results” panel shows that there are 3 records that were computed between 11 AM and 12 PM on October 27th 2009, under the login name “skaing”.

- Next: let’s narrow down the search criteria with a specific dilution type.

6.3.3 Search for 2-Fold Serial Dilution Records

- Click on the “Dilution type:” dropdown list and select “Std 2-Fold Serial”.
- Click on the “Search for MPN records” button.



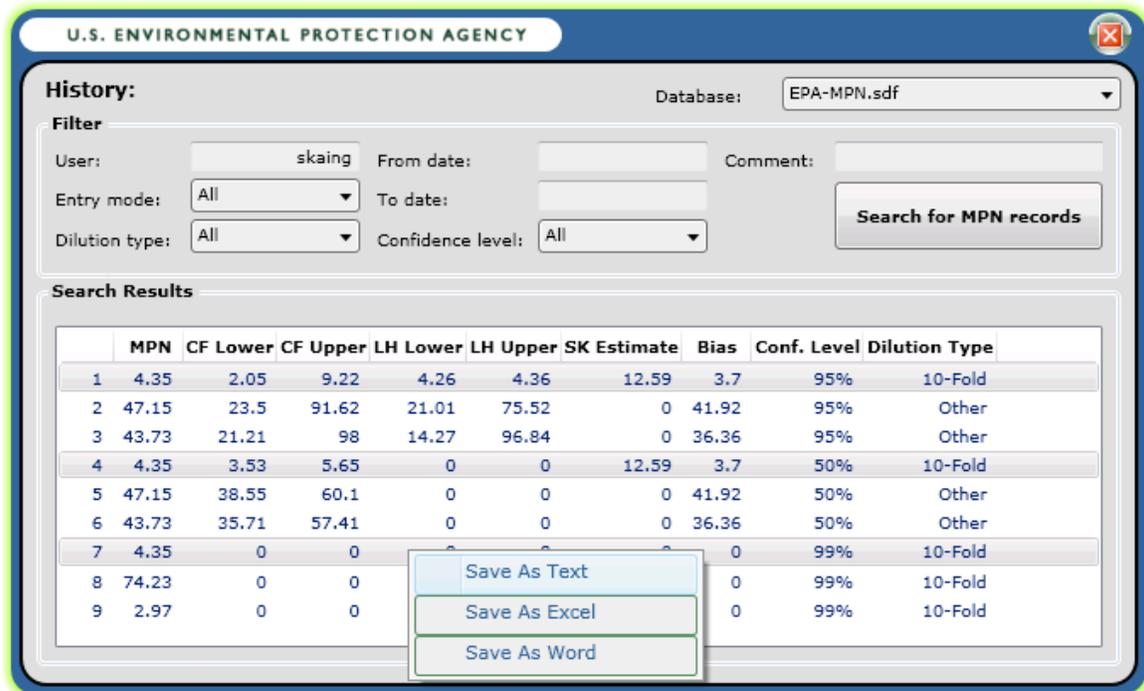
In the example screen above, the “Search Results” panel shows that there is only one record that was computed between 11 AM and 12 PM on October 27th 2009, under the login name “skaing” and with a standard 2-fold serial dilution.

6.4 Save Matched Records

Matched records can be saved as a Text file, a Word document or an Excel spreadsheet.

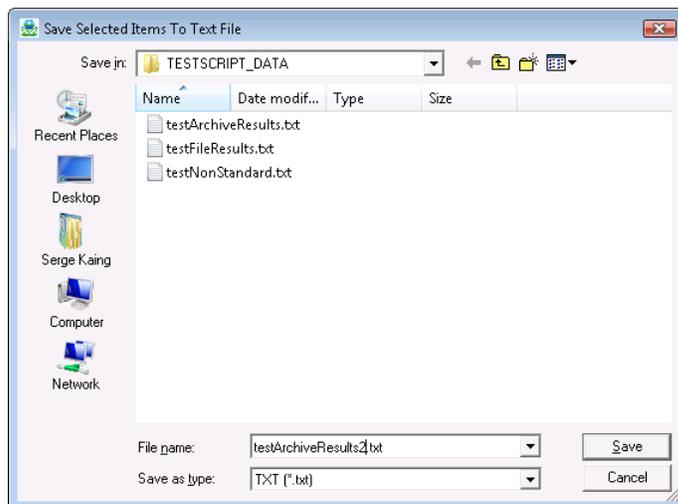
- In the “Search Results” panel, select the items to save (press and hold down the “Ctrl” key to select multiple items).
- Right-click on a selected item.
- In the popup context menu, select “Save As Text”, “Save As Word”, or “Save As Excel”.

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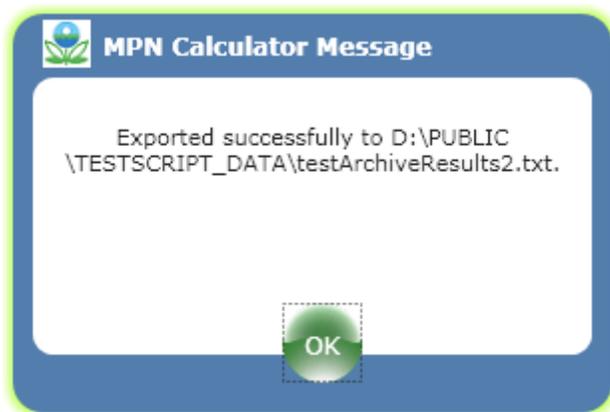


In the example screen above, 3 MPN were selected and saved as a text file.

- In the popup dialog box, choose the location to save the file to, and then type in the file name.



- Click on the “Save” button.



- In the popup dialog box, click on “OK” to finish.

7.0 RECORDS SAVING & DATABASE ARCHIVING

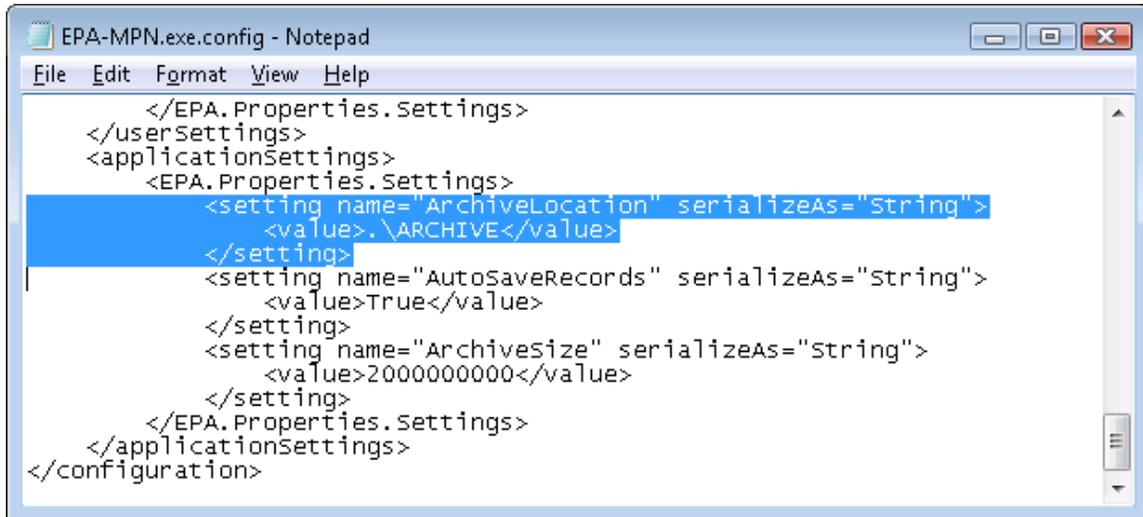
The new MPN Calculator, by default, automatically saves all computed results and the corresponding input dilution data into a local Microsoft SQL Server Compact Edition (MSSQL CE) v3.5 database file. This default behavior can be turned off by setting the value of the “AutoSaveRecords” flag to the Boolean “False” in the application’s configuration file. See further in this section the steps for locating and editing the configuration file. Since all MSSQL CE v3.5 database files are limited to a maximum size of 4GB, the Calculator also has a mechanism that automatically archives the current database file into a configurable location (the “ArchiveLocation”) once it reaches a certain threshold (the “ArchiveSize”).

The following sections describe how to configure the value of this threshold, the archive location, and how to switch on/off the automatic records saving feature. These 3 values are set in the application’s configuration file named “EPA-MPN.exe.config”, which is located in the application’s installation folder and can be edited with any text editor.

7.1 Archive Location

The MPN Calculator automatically archives its database file in the ARCHIVE folder under the application’s installation directory. To change this location, follow the below steps:

- Exit the MPN Calculator.
- With NOTEPAD.exe, open the file named “EPA-MPN.exe.config” in the application’s installation folder.
- Locate the xml tag named “<applicationSettings>”. This tag should be located at the end of the file (see screen capture below).



- Within the “<applicationSettings>” and “</applicationSettings>” tags, locate the “<setting>” tag named “ArchiveLocation”, i.e. the line that reads: <setting name="ArchiveLocation" serializeAs="String">.
- If this is the first time that the application’s configuration file is being edited, then the line below ‘<setting name="ArchiveLocation" serializeAs="String">’ should read: <value>.\ARCHIVE</value>.

The “.\ARCHIVE” represents the default location. This value means that the application will automatically archive its database file in the “ARCHIVE” folder, in the application’s installation directory.

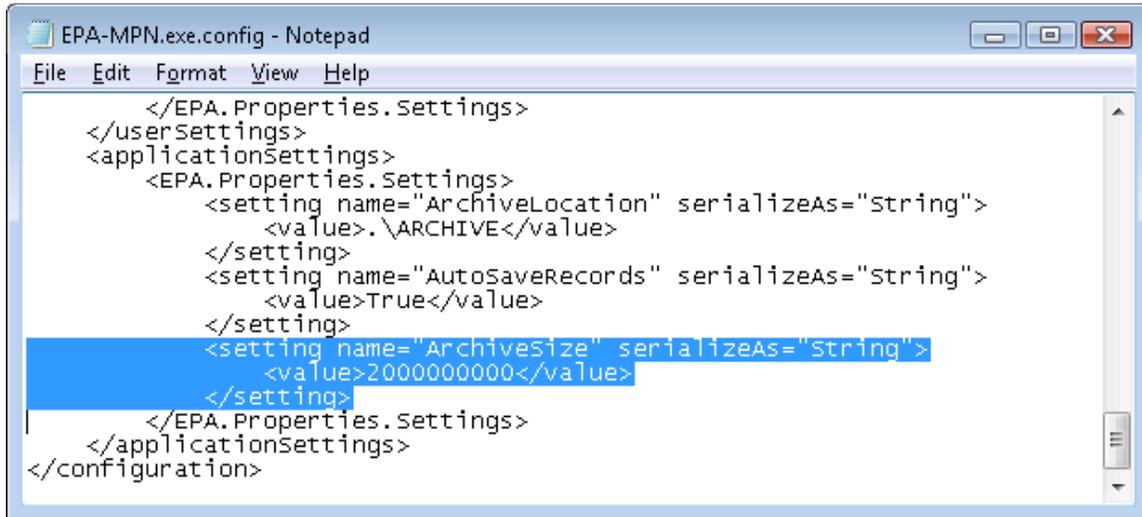
- Change the value between the “<value>” and “</value>” tags to the desired location (e.g. the value “D:\DATA\MPN-ARCHIVE”).
- Save the changes.
- Exit NOTEPAD.exe to finish.

7.2 Archive Size

The MPN Calculator automatically starts the archiving process once its database file reaches a certain threshold defined in the application’s configuration file: the default value is 2,000,000,000 bytes, i.e. 2 GB. To change this value,

- Exit the MPN Calculator.
- With NOTEPAD.exe, open the file named “EPA-MPN.exe.config” in the application’s installation folder.

- Locate the xml tag named “<applicationSettings>”. This tag should be located at the end of the file (see screen capture below).



- Within the “<applicationSettings>” and “</applicationSettings>” tags, locate the “<setting>” tag named “ArchiveSize” i.e. the line that reads: *<setting name="ArchiveSize" serializeAs="String">*
- If this is the first time that the application’s configuration file is being edited, then the line below ‘<setting name="ArchiveSize" serializeAs="String">’ should read: *<value>2000000000</value>*.

The value 2,000,000,000 represents the default threshold value in number of bytes. This means that once the application’s database file reaches 2GB, the archiving process starts. At the end of this process the application’s database file will return to its starting size (of about 200KB) and a new archived database file of about 2GB will be created in the archive location defined in 6.1 Archive Location.

- Change the value between the “<value>” and “</value>” tags to the desired value (e.g. the value “5000000” for 5MB).
- Save the changes.
- Exit NOTEPAD.exe to finish.

7.3 Auto-Save Records

By default, the MPN Calculator automatically saves all computed results and associated dilution data sets in its database file. The ArchiveLocation (see section 6.1) and the ArchiveSize (see section 6.2) do not have any impact on the application’s behavior if the automatic records saving feature is turned off because the application’s database file will not be growing. To turn this feature off, follow the below steps:

- Exit the MPN Calculator.
- With NOTEPAD.exe, open the file named “EPA-MPN.exe.config” in the application’s installation folder.
- Locate the xml tag named “<applicationSettings>”. This tag should be located at the end of the file (see screen capture below).

```

    </EPA.Properties.Settings>
  </userSettings>
  <applicationSettings>
    <EPA.Properties.Settings>
      <setting name="ArchiveLocation" serializeAs="String">
        <value>.\ARCHIVE</value>
      </setting>
      <setting name="AutoSaveRecords" serializeAs="String">
        <value>True</value>
      </setting>
      <setting name="ArchiveSize" serializeAs="String">
        <value>2000000000</value>
      </setting>
    </EPA.Properties.Settings>
  </applicationSettings>
</configuration>

```

- Within the “<applicationSettings>” and “</applicationSettings>” tags, locate the “<setting>” tag named “AutoSaveRecords” i.e. the line that reads: <setting name="AutoSaveRecords" serializeAs="String">.
- If this is the first time that the application’s configuration file is being edited, then the line below ‘<setting name="AutoSaveRecords" serializeAs="String">’ should read: <value>True</value>.
- Change the value between the “<value>” and “</value>” tags to the “False”.
- Save the changes.
- Exit NOTEPAD.exe to finish.

8.0 REFERENCES

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