# How to use the BMC program

## Executive Summary

The program uses concentration-response data from *in vitro* toxicity test methods (with up to two endpoints) to generate log-logistic curve fits. These are based on the established R package drc. The program uses smart, data-guided constraint settings and calculates benchmark concentrations (BMC) and their confidence interval (BMCL and BMCU) for freely selectable benchmark responses (BMR). A graphical user interface (shiny app) allows simple choices of data display and visual control options.

## Glossary and Abbreviations

| | |
|---|---|
| BMC | benchmark concentration |
| BMCL | benchmark concentration lower limit |
| BMCU | benchmark concentration upper limit |
| BMR | benchmark response |
| CI | confidence interval |
| file | the Excel file itself, the whole file including all tabs |
| File1 | data for viability |
| File2 | data for second readout |
| Fit | the different forms the model can have. Can be achieved through setting different parameters to different values |
| LL.4 | 4-parameter log logistic function |
| Model | the basic formula that describes the curve, in our case LL.4 |
| NA | neurite area, a second functional readout often assessed in our lab |
| sheet | tab within the Excel file |

## Access

access through this link:

http://invitrotox.uni-konstanz.de/BMC/

This is the upper part of the web interface, that you face when opening the link:

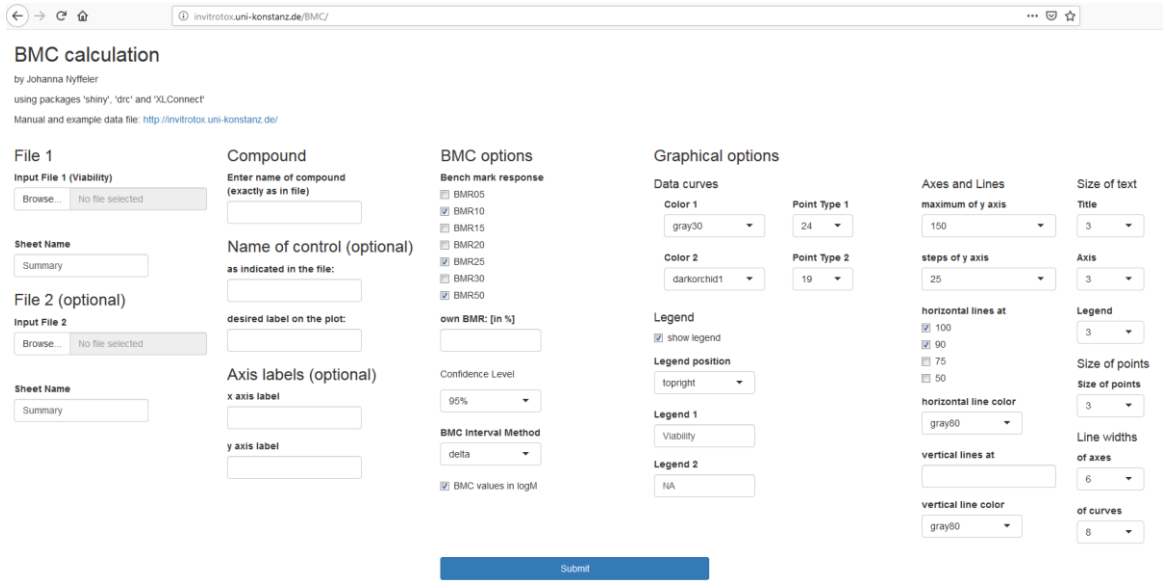



*Figure 1: Screenshot of the BMC program*

## CONTENT:

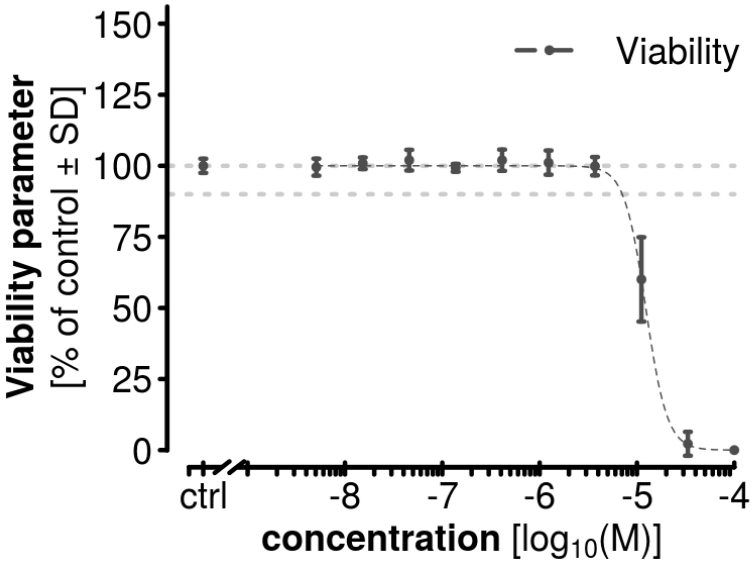


**FIGURES:**

# I. BASIC DATA REQUIREMENTS AND LIMITATIONS

Concerning the **structure** of the data, the data always has to be normalized to 100 %. The program does not normalize data to a control on its own, and this has to be done beforehand.

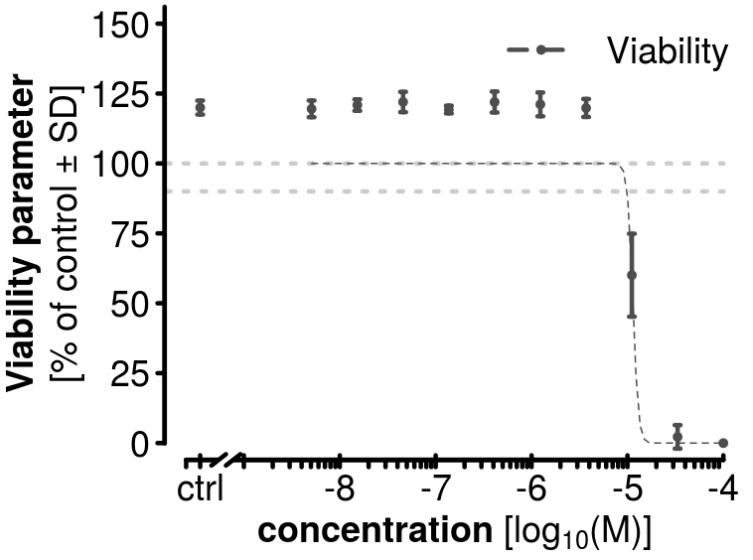
Also the lowest concentrations should be close to 100%, otherwise the calculated BMCLs are out of range. Figure 2 and Figure 3 depict the effect of data that strongly deviates from 100% on the uncertainty.




BMC

| | BMC | BMCL | BMCU |
|---|---|---|---|
| BMR5 | 5.87 | 3.68 | 8.05 |
| BMR10 | 7.08 | 5.2 | 8.97 |
| BMR15 | 7.96 | 6.36 | 9.56 |
| BMR20 | 8.69 | 7.36 | 10 |
| BMR25 | 9.34 | 8.27 | 10.4 |
| BMR30 | 9.95 | 9.11 | 10.8 |
| BMR50 | 12.3 | 11.3 | 13.3 |

*Figure 2: Concentration-response curve with lowest concentrations nicely at 100%.*




BMC

| | BMC | BMCL | BMCU |
|---|---|---|---|
| BMR5 | 9.45 | -167 | 186 |
| BMR10 | 9.91 | -120 | 140 |
| BMR15 | 10.2 | -89.3 | 110 |
| BMR20 | 10.4 | -64.6 | 85.5 |
| BMR25 | 10.6 | -43.4 | 64.6 |
| BMR30 | 10.8 | -24.1 | 45.7 |
| BMR50 | 11.4 | -22.7 | 45.5 |

*Figure 3: Concentration-response curve with lowest concentrations higher than 100%. The data is the same than in Figure 2, but shifted about 20%. While the BMCs are still comparable, the BMCLs are completely out of range.*

# II. INPUT

## 1. Data upload

After bringing the data to the right format, it can be uploaded and submitted to the program, which is done on the left side of the page.

The data upload is carried out with **Excel sheets**. If you have two different readouts, you need to upload an Excel with at least two different sheets (one sheet for each readout, named differently), or separate the data to two completely different excel files. The two readouts can NOT be combined within the same sheet.

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| 3 | | | | | | | | |
| 4 | Nr | Chem.Name | Test.Conc | unit | response | SD | N | p |
| 5 | 1 | Compound A | 100 | uM | 0.00 | 0.00341 | 3 | |
| 6 | 2 | Compound A | 33.3333 | uM | 0.00 | 0 | 3 | |
| 7 | 3 | Compound A | 11.1111 | uM | 0.01 | 0.01317 | 3 | |
| 8 | 4 | Compound A | 3.7037 | uM | 0.05 | 0.03525 | 3 | |
| 9 | 5 | Compound A | 1.2346 | uM | 0.09 | 0.00893 | 3 | |
| 10 | 6 | Compound A | 0.4115 | uM | 3.16 | 1.27523 | 3 | |
| 11 | 7 | Compound A | 0.1372 | uM | 103.17 | 9.66925 | 3 | |
| 12 | 8 | Compound A | 0.0457 | uM | 105.91 | 7.65634 | 3 | |
| 13 | 9 | Compound A | 0.0152 | uM | 108.01 | 4.19641 | 3 | |
| 14 | 10 | Compound A | 0.0051 | uM | 103.55 | 5.60209 | 3 | |
| 15 | 11 | Compound A | 100 | uM | 0.06 | 0.04297 | 3 | |
| 16 | 12 | Compound A | 33.3333 | uM | 0.03 | 0.01135 | 3 | |
| 17 | 13 | Compound A | 11.1111 | uM | 0.06 | 0.01272 | 3 | |
| 18 | 14 | Compound A | 3.7037 | uM | 0.15 | 0.03683 | 3 | |
| 19 | 15 | Compound A | 1.2346 | uM | 0.85 | 0.28484 | 3 | |
| 20 | 16 | Compound A | 0.4115 | uM | 29.46 | 6.93298 | 3 | |
| 21 | 17 | Compound A | 0.1372 | uM | 94.53 | 10.7442 | 3 | |
| 22 | 18 | Compound A | 0.0457 | uM | 96.05 | 29.3479 | 3 | |
| 23 | 19 | Compound A | 0.0152 | uM | 100.78 | 14.2962 | 3 | |
| 24 | 20 | Compound A | 0.0051 | uM | 102.94 | 13.4085 | 3 | |
| 25 | 21 | Compound A | 100 | uM | 0.02 | 0.03511 | 3 | |
| 26 | 22 | Chemical B | 50 | uM | 35.927 | 5.916 | 3 | |
| 27 | 23 | Chemical B | 16.6667 | uM | 36.722 | 9.367 | 3 | |
| 28 | 24 | Chemical B | 5.5556 | uM | 34.590 | 4.837 | 3 | |
| 29 | 25 | Chemical B | 1.8519 | uM | 36.132 | 1.429 | 3 | |
| 30 | 26 | Chemical B | 0.6173 | uM | 38.053 | 5.590 | 3 | |
| 31 | 27 | Chemical B | 0.2058 | uM | 33.695 | 3.868 | 3 | |
| 32 | 28 | Chemical B | 0.0686 | uM | 31.525 | 4.975 | 3 | |
| 33 | 29 | Chemical B | 0.0229 | uM | 41.292 | 3.071 | 3 | |
| 34 | 30 | Chemical B | 0.0076 | uM | 69.025 | 19.023 | 3 | |
| 35 | 31 | Chemical B | 0.0025 | uM | 95.624 | 6.536 | 3 | |
| 36 | 32 | Chemical B | 50 | uM | 39.837 | 5.292 | 3 | |
| 37 | 33 | Chemical B | 16.6667 | uM | 35.563 | 3.258 | 3 | |
| 38 | 34 | Chemical B | 5.5556 | uM | 28.082 | 4.183 | 3 | |
| 39 | 35 | Chemical B | 1.8519 | uM | 28.068 | 5.605 | 3 | |
| 40 | 36 | Chemical B | 0.6173 | uM | 29.664 | 4.114 | 3 | |
| 41 | 37 | Chemical B | 0.2058 | uM | 22.990 | 7.215 | 3 | |
| 42 | 38 | Chemical B | 0.0686 | uM | 22.464 | 4.066 | 3 | |
| 43 | 39 | Chemical B | 0.0229 | uM | 32.948 | 2.426 | 3 | |
| 44 | 40 | Chemical B | 0.0076 | uM | 69.839 | 11.960 | 3 | |
| 45 | 41 | Chemical B | 0.0025 | uM | 103.257 | 10.335 | 3 | |
| 46 | 42 | DMSO | | | 100.000 | 14.034 | 5 | |

Summary

*Figure 4: Example of an Excel sheet structure for data upload. The column names are fixed and cannot be changed. Also the column headers have to be in line 4. If any of the parameters are listed in the wrong column, you will receive an error message and the program won't run.*
*column A: number of data point, can be left out*
***column B: chemical or compound name***
***column C: concentration***
***column D: unit of the concentration given***
***column E: response. In our case the mean of the technical replicates within one biological replicate***
*column F: the standard deviation of the technical replicates within one biological replicate*
*column G: N = number of replicates*

Figure 4 shows a screenshot of an example Excel sheet for data upload. In this example, the compound A has been tested in two different biological replicates (so there are 2 different lines per compound concentration) at 10 different concentrations each (100 – 0.005 µM). The mean of the technical replicates is given in column E, and the standard deviation of the technical replicates in column F.

Generally, the column headers have to be in line 4. The four columns B-E (that are shaded in dark grey) are mandatory. Their order can be freely chosen, so the compound name could also be listed in column F, for example. However, the name of the columns mustn't be changed. The columns that are shaded in light grey are optional and do not need to be filled. They are just for better data overview of the user. Also, additional columns can be introduced if needed/wanted. The program will ignore them.

The **order** of compounds and their respective values is not important, so the according biological replicates of one compound do not necessarily need to be listed directly underneath each other, like in this example. However it is essential that the associated data points are labelled in the same way within the list, e.g. the compound name is spelled exactly the same way for all replicates, and the concentrations are given in the same unit. Otherwise the algorithm is not able to match the different data points and will return an error message.

All Excel sheets containing data always need to have this structure of columns, no matter for which readout.

As mentioned earlier, the data of the two different readouts need to be in two different sheets, either in the same Excel file or two separate Excel files. This means that for example, within one Excel file one sheet is for viability, and another for the second readout, or there are two different Excel files, one for viability and one for second readout. However, the algorithm does not care which other sheets you have included within a file, also the example here contains several other sheets. So if you want you, can include other sheets for plate layout, calculations, etc. you are free to do so. It won't impair the program.

You can elongate the list discretionarily, so if you want to analyze the data of e.g. 80 compounds, you can list all 80 compounds within one file. Also you can include solvent controls (e.g. DMSO) in the list. The control values can be shown in the plot later if desired. However, their variance is not taken into account for curve fit modeling.

Once your data is in the required excel sheet format, you can upload them on the left side of the web interface:

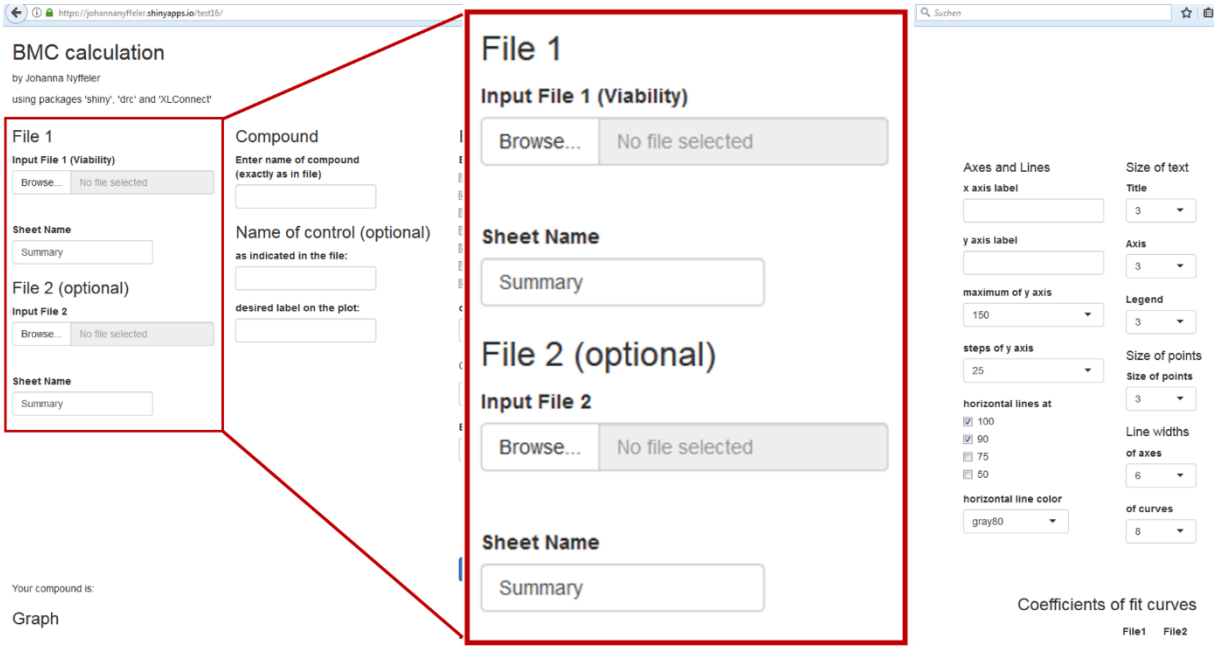



*Figure 5: The fields for uploading data on the left side of the web interface. If you have the data of two different readouts in two different Excel files, you can upload the two different files here (viability data at File 1, the second, functional readout at File 2). If you have your data in the same file, please upload the same file twice. In both cases, you have to specify the sheet name in the corresponding field.*

If you have the data of two different readouts in two different Excel files, you can upload the two different files here (viability data at File 1, the second, functional readout at File 2). If you have your data in the same file, please upload the same file twice. On both cases you have to specify the sheet name in the field “Sheet Name”. Please mind to enter the exactly same spelling, otherwise the upload will fail. The sheet name ‘Summary’ which appears in this field is a default option.

When the upload is completed, a blue bar will appear underneath the “Input File” field.

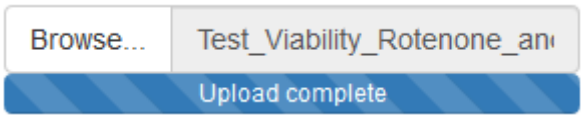



*Figure 6: The blue bar indicates that the upload is completed.*

## 2. Specifying which data to be analyzed

Upon uploading the data, it needs to be specified which data within the uploaded Excel sheet should be analyzed and plotted.

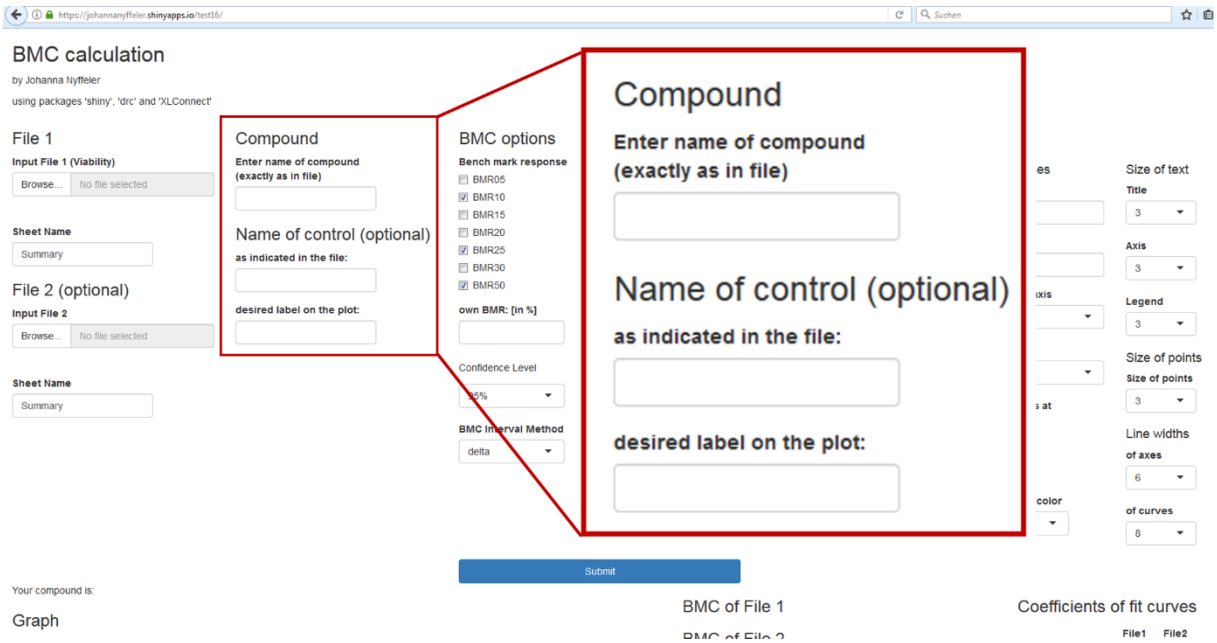



*Figure 7: The fields in the web interface to specify which data within the uploaded Excel sheets should be analyzed.*

Even if there is data from only one compound within the Excel file(s), you need to enter the name of the compound whose data is supposed to be analyzed in the upper field. Otherwise the program will not know what to do. As indicated also in the web interface, the compound name that is to be entered here needs to be spelled exactly like it is spelled in the uploaded Excel sheet.

If there is data of a **solvent control** included in the Excel files, and its variance should be included in the plot, you can enter the name of the control in the middle field. Again, it should be spelled exactly as it is within the Excel files. In the lowest field you can indicate how the control should be labelled within the plot (e.g. ctrl, DMSO, untreated, etc.). If the control data should not appear in the plot, these fields can be left empty. The control data will not be included in the curve fit modeling, no matter if something has been entered into the two lowest fields or not (see III. Curve fit modeling for more information).

## 3. Defining the benchmark options

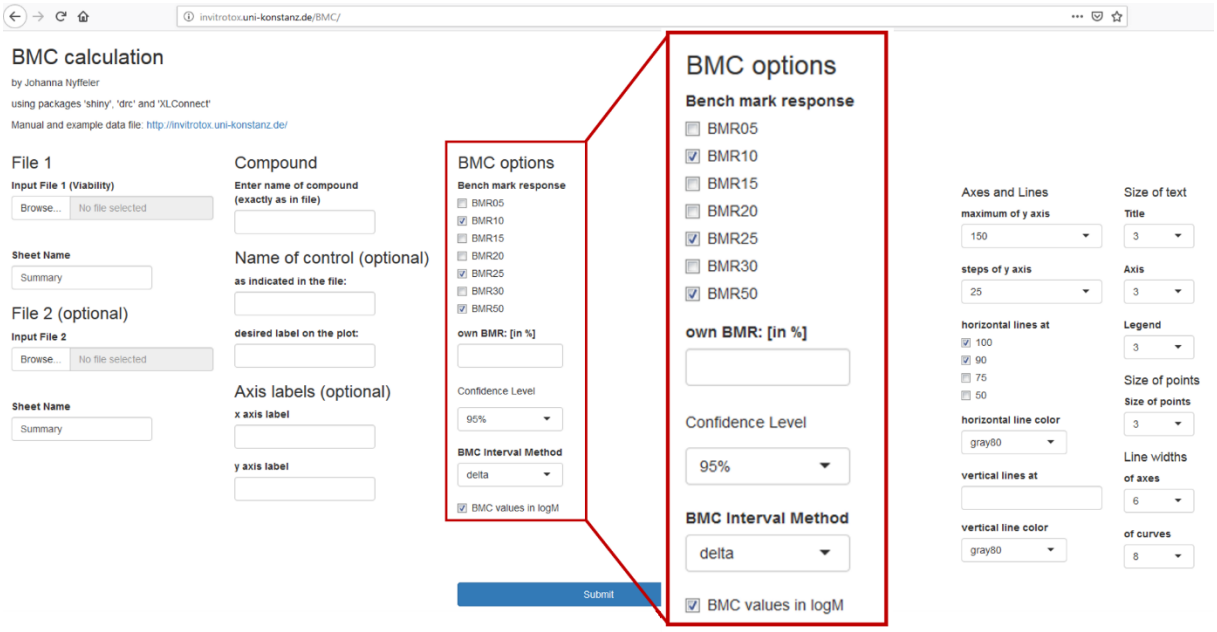



*Figure 8: The fields in the middle of the web interface to specify the desired benchmark options.*

In a next step, the options for the benchmark calculations are set. You can chose either from pre-defined benchmark responses (5, 10, 15, 20, 25, 30 or 50 %), or enter the desired benchmark response manually. This is useful when the benchmark response is e.g. defined by the standard deviation or multiples of it.

Concerning the confidence interval (CI) calculations for BMCL assessment, three different levels are for choice: 90%, 95% and 99%. Besides the confidence level, also the method for CI calculations can be chosen: either the method 'delta' or 'tfls'. There is only a little difference between the results of both methods, however we advise to choose the method 'delta'.

The resulting BMC values and the x-axis of the plot can be either given in the unit originally used in the Excel file (e.g. µM), or in or in $\log_{10}$(M). To change between these options, tick or untick "BMC values in log(M)" at the bottom of the BMC options section. After (un)ticking, the program has to re-run to implement the change. Therefore, press the submit button.

## 4. Defining the graphical options for plotting

The right side of the web interface gives various options to design the graphical appearance of the plot (Figure 9). There are options to design the data curves, the legends, the axes and the data points.

The **data curves** can be designed with respect to their colours, as well as to the symbols that represent the given data points. The names of the colours for the curves is pre-defined by R. Figure 14 in the appendix gives an overview of the colours and their respective codes in R. The symbols of the data points are also R-coded. Figure 10 shows the available symbols, and which number to enter to use them in the plot.

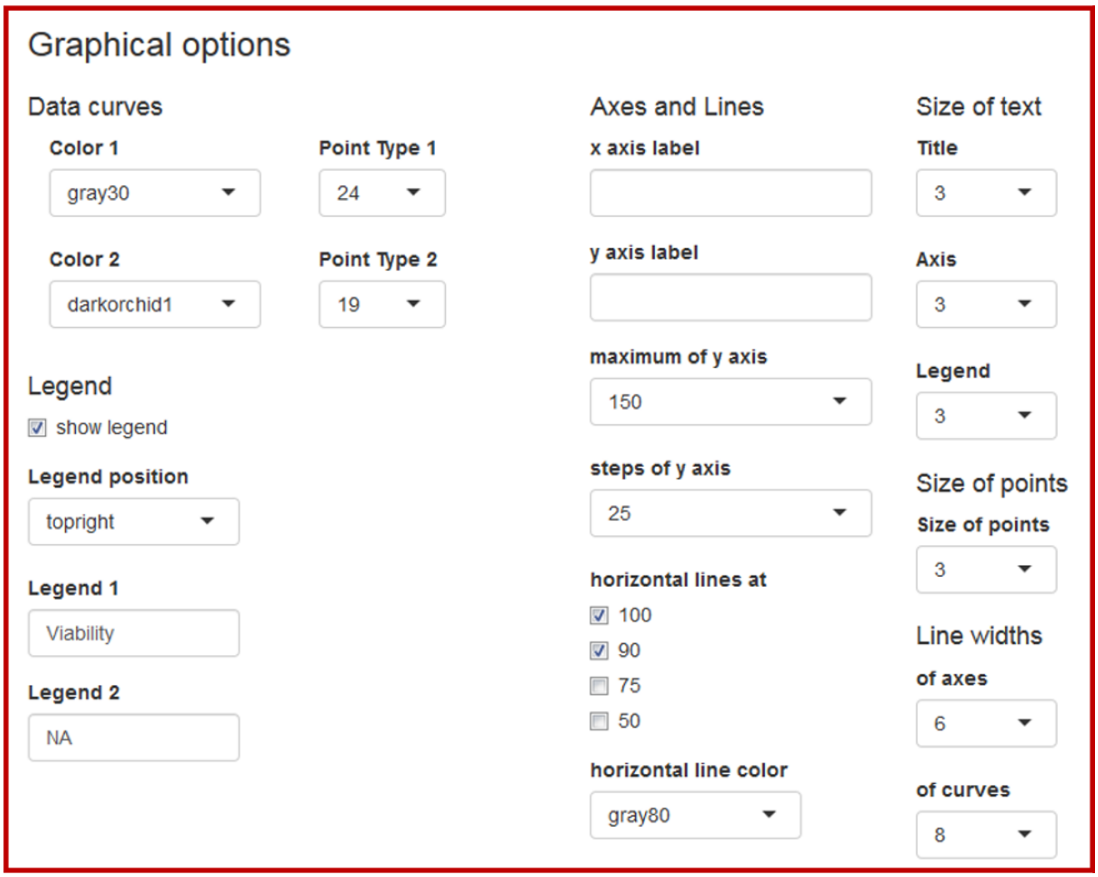



*Figure 9: Options for designing the plot graphics on the right side of the web interface.*

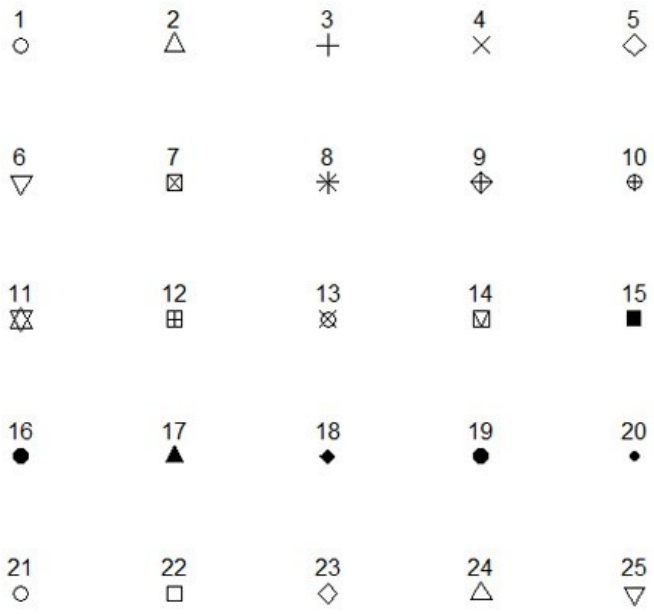



*Figure 10: List of symbol codes in R. The symbols with which the data points are represented are coded by numbers. Number 19 (filled circles) and number 24 (open triangles) are set as default.*

In the **legend** section it can be chosen if the legend should be shown at all, and if yes where within the plot (top right, top left, bottom right, bottom left). The legend labels can be entered in the two fields, whereas “Legend 1” refers to File 1 (viability) and “Legend 2” refers to File 2 (second readout, optional).

The legend labels 'Viability' and 'NA' for neurite area are the default option, it can be entered whatever you want. However, the label must exclude special characters or inferior/superior characters.

The **axes** can be designed with respect to labels, the maximum and major tick interval of the y-axis, as well as horizontal lines and their colours.
The range of the **x-axis** is automatically determined by the concentration range entered. The x-axis is always shown in logarithmic scale. The concentrations can either be displayed in the original unit given in the Excel sheet or in $\log_{10}$(M), depending on what was chosen in the BMC options section (see above).

The maximum of the **y-axis** can be can be chosen from 100-250. The steps/ticks of the y-axis can be chosen to be either, 10, 20, 25 or 50. The label of the y-axis is "Viability parameter [% of control ± SD]" by default.

Eventually, the size of texts and data point symbols can be chosen, as well as the width / thickness of the lines of axes and curves.

After customizing all the settings, clicking the "Submit"-button will start calculations and create the graphical output.

## III. CURVE FIT AND SETTING OF CONSTRAINTS

The curve fit is always based on a four-parameter log-logistic function:

$$f(x) = c + \frac{d - c}{1 + \exp(b(\log(x) - \log(e)))}$$

*Figure 11: Four-parameter log-logistic function used for curve fit modeling of concentration-response curves.*

b: slope around e
c: lower limit
d: upper limit
e: inflexion point

According to the data, the best model fit is chosen. Figures 12 and 13 describe the decisions taken by the program, for viability and the second readout accordingly.

# Viability

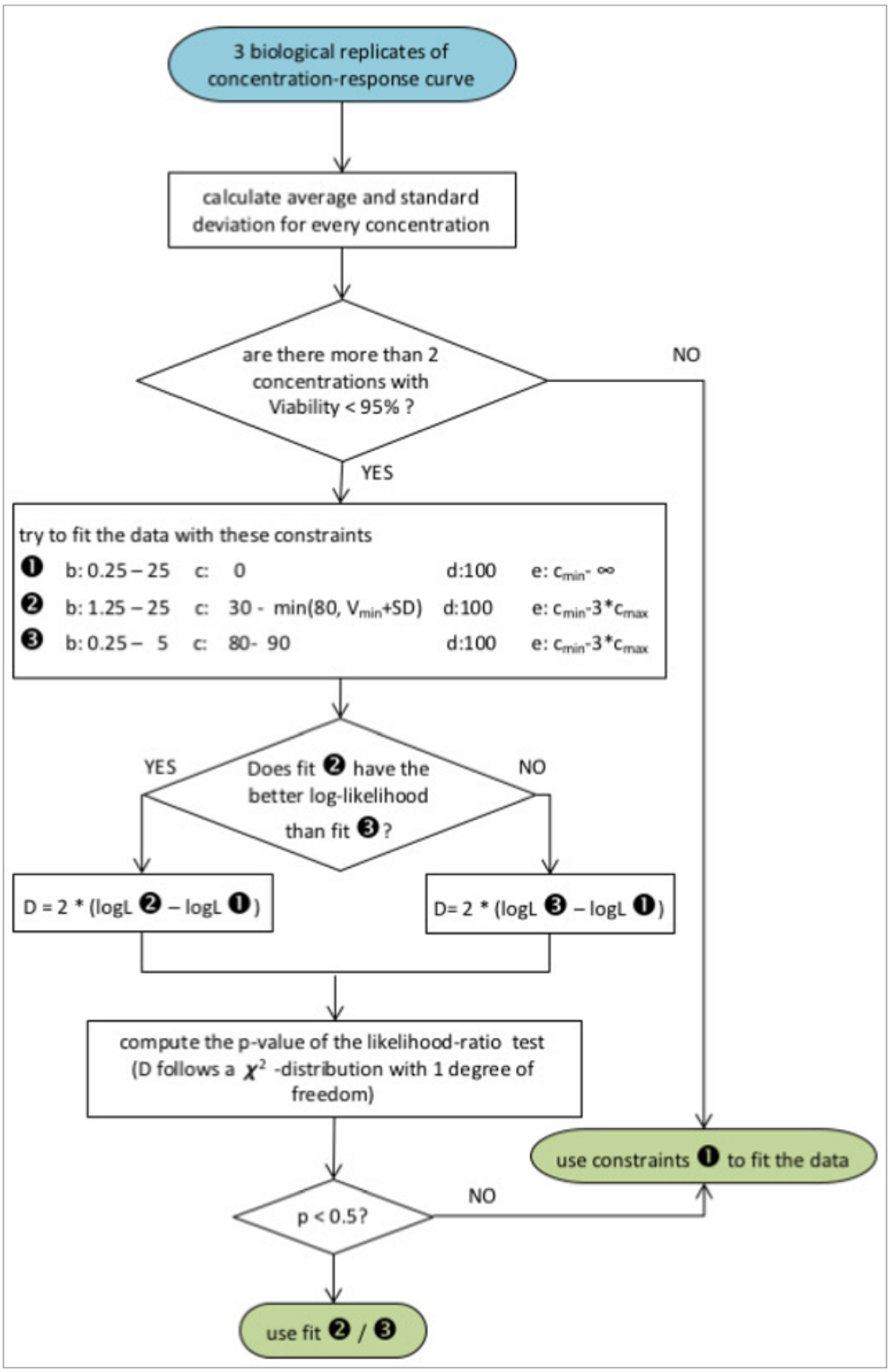



*Figure 12: Flow diagram representing the choice of fits for the first readout (viability).*

# Second readout

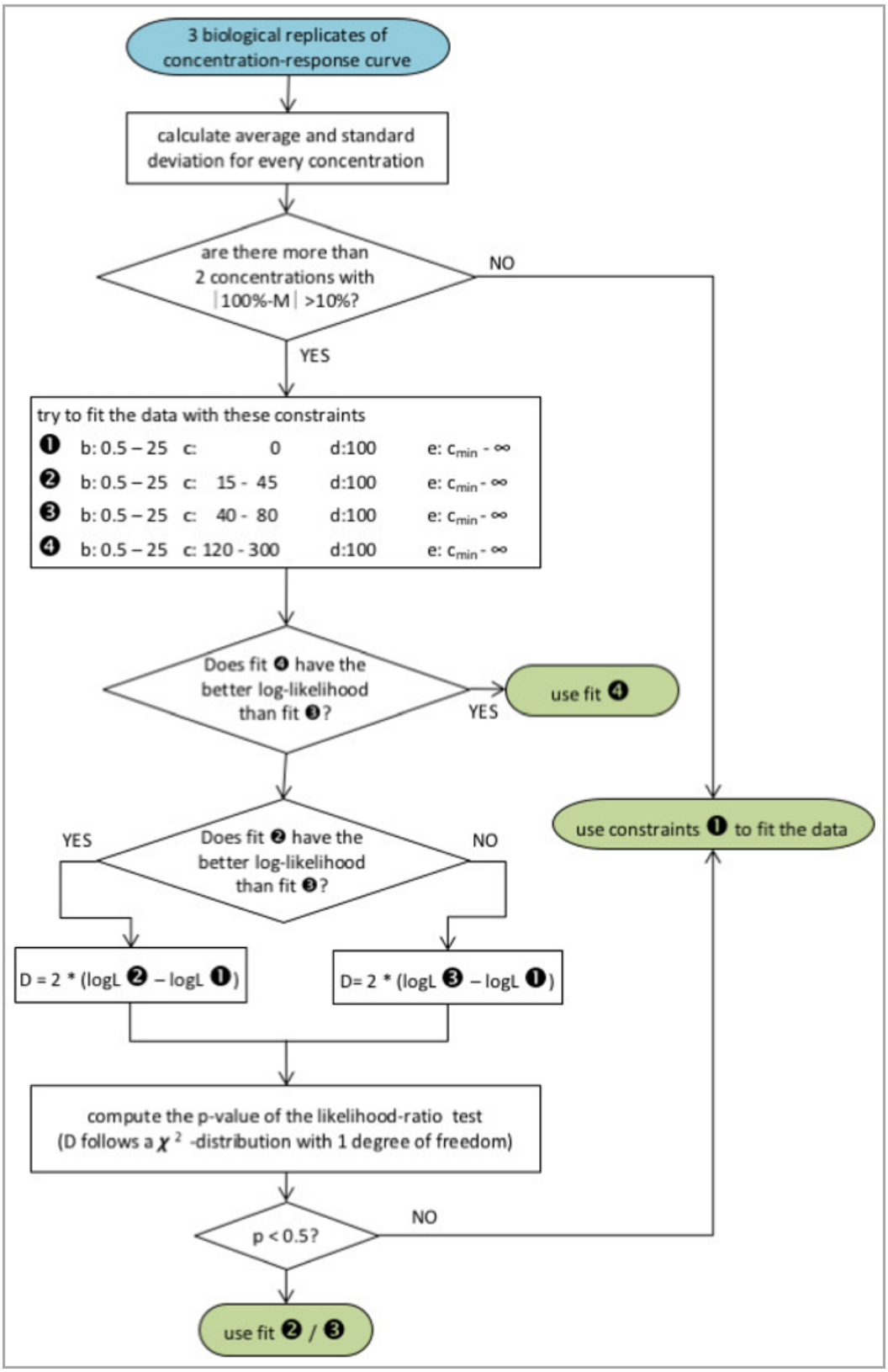



*Figure 13: Flow diagram representing the choice of fits for the second readout.*

# IV. OUTPUT

## 5. Graphical output

The plot produced by the program can be extracted by copy-pasting. The file format is PNG, and the resolution is 600 x 800 pixel.

## 6. BMC and BMCL values

After a successful run, the benchmark concentrations and their according confidence intervals are given next to the plot. Depending on the previously chosen settings, the concentrations are given in the original unit or in $\log_{10}$(M). The coefficients of the curve fits (of both endpoints) are also given to ensure transparency.

All the derived BMC and BMCL & BMCU values, as well as the curve fit coefficients can be downloaded as an Excel file (click the "Download the tables" button on the right).

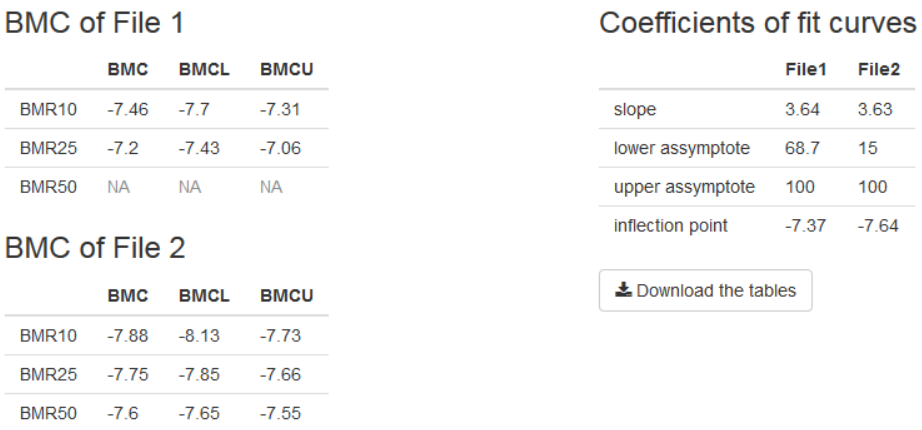



*Figure 14: exemplary output after concentration-response curve fitting and calculation of BMCs and their according confidence interval (BMCU and BMCL). The values here are given in $\log_{10}$(M). If the desired BMR is outside of the tested concentration range, the program returns "NA" or "NaN". Values are not extrapolated. The coefficients of the applied curve fits are given on the right. All the data can be downloaded as an Excel file.*

# V. APPENDIX

## 7. Trouble shooting

**I have uploaded an Excel file in the fields of "File 2", but the program gives me an error message.**

It's mandatory to upload a file under "File 1 (Viability)", otherwise the program won't run. If you only upload only one file at "File 2", but no data at "File 1", you will get an error. So if you want to analyze and plot the data of only one readout (usually viability), you have to upload it as "File 1". We assume that if there is a functional, second endpoint, there is always cell viability assessed in parallel.

**I have uploaded the Excel sheets and entered the compound name to be analyzed, but I get an error message.**

Please check if a) your Excel file is formatted correctly, meaning all columns contain the parameter they should, or if b) if you have any spelling differences between the compound name in the Excel file and the compound name as you entered it in the web interface.

**My data doesn't reach up to 100%, and the curve fit is bad.**

The curve fit is forced to 100% by a constraint within the formula. So if you encounter this problem, you might either re-test your desired compound at lower concentrations, so that you reach the no effect level and your test system is as healthy as the control, or you renormalize your data to adapt the data points of the lowest concentrations to a level close to 100%.

**How about the concentration, how is it entered in the Excel sheet and how does it appear in the plot?**

The concentration is entered in the Excel file in absolute concentrations, without any transformation. So 100 mM or 45 nM are entered like this, with the value in one column and the unit in another. For displaying the data in the plot the program first converts the concentration to molar (M) and then transforms them to logarithm ($log_{10}$). The x-axis of the plot is logarithmic.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| white | coral4 | deepskyblue | gray28 | gray88 | grey40 | grey100 | lightpink2 | mistyrose2 | plum | slategray2 |
| aliceblue | cornflowerblue | deepskyblue1 | gray29 | gray89 | grey41 | honeydew | lightpink3 | mistyrose3 | plum1 | slategray3 |
| antiquewhite | cornsilk | deepskyblue2 | gray30 | gray90 | grey42 | honeydew1 | lightpink4 | mistyrose4 | plum2 | slategray4 |
| antiquewhite1 | cornsilk1 | deepskyblue3 | gray31 | gray91 | grey43 | honeydew2 | lightsalmon | moccasin | plum3 | slategrey |
| antiquewhite2 | cornsilk2 | deepskyblue4 | gray32 | gray92 | grey44 | honeydew3 | lightsalmon1 | navajowhite | plum4 | snow |
| antiquewhite3 | cornsilk3 | dimgray | gray33 | gray93 | grey45 | honeydew4 | lightsalmon2 | navajowhite1 | powderblue | snow1 |
| antiquewhite4 | cornsilk4 | dimgrey | gray34 | gray94 | grey46 | hotpink | lightsalmon3 | navajowhite2 | purple | snow2 |
| aquamarine | cyan | dodgerblue | gray35 | gray95 | grey47 | hotpink1 | lightsalmon4 | navajowhite3 | purple1 | snow3 |
| aquamarine1 | cyan1 | dodgerblue1 | gray36 | gray96 | grey48 | hotpink2 | lightseagreen | navajowhite4 | purple2 | snow4 |
| aquamarine2 | cyan2 | dodgerblue2 | gray37 | gray97 | grey49 | hotpink3 | lightskyblue | navy | purple3 | springgreen |
| aquamarine3 | cyan3 | dodgerblue3 | gray38 | gray98 | grey50 | hotpink4 | lightskyblue1 | navyblue | purple4 | springgreen1 |
| aquamarine4 | cyan4 | dodgerblue4 | gray39 | gray99 | grey51 | indianred | lightskyblue2 | oldlace | red | springgreen2 |
| azure | darkblue | firebrick | gray40 | gray100 | grey52 | indianred1 | lightskyblue3 | olivedrab | red1 | springgreen3 |
| azure1 | darkcyan | firebrick1 | gray41 | green | grey53 | indianred2 | lightskyblue4 | olivedrab1 | red2 | springgreen4 |
| azure2 | darkgoldenrod | firebrick2 | gray42 | green1 | grey54 | indianred3 | lightslateblue | olivedrab2 | red3 | steelblue |
| azure3 | darkgoldenrod1 | firebrick3 | gray43 | green2 | grey55 | indianred4 | lightslategray | olivedrab3 | red4 | steelblue1 |
| azure4 | darkgoldenrod2 | firebrick4 | gray44 | green3 | grey56 | ivory | lightslategrey | olivedrab4 | rosybrown | steelblue2 |
| beige | darkgoldenrod3 | floralwhite | gray45 | green4 | grey57 | ivory1 | lightsteelblue | orange | rosybrown1 | steelblue3 |
| bisque | darkgoldenrod4 | forestgreen | gray46 | greenyellow | grey58 | ivory2 | lightsteelblue1 | orange1 | rosybrown2 | steelblue4 |
| bisque1 | darkgray | gainsboro | gray47 | grey | grey59 | ivory3 | lightsteelblue2 | orange2 | rosybrown3 | tan |
| bisque2 | darkgreen | ghostwhite | gray48 | grey0 | grey60 | ivory4 | lightsteelblue3 | orange3 | rosybrown4 | tan1 |
| bisque3 | darkgrey | gold | gray49 | grey1 | grey61 | khaki | lightsteelblue4 | orange4 | royalblue | tan2 |
| bisque4 | darkkhaki | gold1 | gray50 | grey2 | grey62 | khaki1 | lightyellow | orangered | royalblue1 | tan3 |
| black | darkmagenta | gold2 | gray51 | grey3 | grey63 | khaki2 | lightyellow1 | orangered1 | royalblue2 | tan4 |
| blanchedalmond | darkolivegreen | gold3 | gray52 | grey4 | grey64 | khaki3 | lightyellow2 | orangered2 | royalblue3 | thistle |
| blue | darkolivegreen1 | gold4 | gray53 | grey5 | grey65 | khaki4 | lightyellow3 | orangered3 | royalblue4 | thistle1 |
| blue1 | darkolivegreen2 | goldenrod | gray54 | grey6 | grey66 | lavender | lightyellow4 | orangered4 | saddlebrown | thistle2 |
| blue2 | darkolivegreen3 | goldenrod1 | gray55 | grey7 | grey67 | lavenderblush | limegreen | orchid | salmon | thistle3 |
| blue3 | darkolivegreen4 | goldenrod2 | gray56 | grey8 | grey68 | lavenderblush1 | linen | orchid1 | salmon1 | thistle4 |
| blue4 | darkorange | goldenrod3 | gray57 | grey9 | grey69 | lavenderblush2 | magenta | orchid2 | salmon2 | tomato |
| blueviolet | darkorange1 | goldenrod4 | gray58 | grey10 | grey70 | lavenderblush3 | magenta1 | orchid3 | salmon3 | tomato1 |
| brown | darkorange2 | gray | gray59 | grey11 | grey71 | lavenderblush4 | magenta2 | orchid4 | salmon4 | tomato2 |
| brown1 | darkorange3 | gray0 | gray60 | grey12 | grey72 | lawngreen | magenta3 | palegoldenrod | sandybrown | tomato3 |
| brown2 | darkorange4 | gray1 | gray61 | grey13 | grey73 | lemonchiffon | magenta4 | palegreen | seagreen | tomato4 |
| brown3 | darkorchid | gray2 | gray62 | grey14 | grey74 | lemonchiffon1 | maroon | palegreen1 | seagreen1 | turquoise |
| brown4 | darkorchid1 | gray3 | gray63 | grey15 | grey75 | lemonchiffon2 | maroon1 | palegreen2 | seagreen2 | turquoise1 |
| burlywood | darkorchid2 | gray4 | gray64 | grey16 | grey76 | lemonchiffon3 | maroon2 | palegreen3 | seagreen3 | turquoise2 |
| burlywood1 | darkorchid3 | gray5 | gray65 | grey17 | grey77 | lemonchiffon4 | maroon3 | palegreen4 | seagreen4 | turquoise3 |
| burlywood2 | darkorchid4 | gray6 | gray66 | grey18 | grey78 | lightblue | maroon4 | paleturquoise | seashell | turquoise4 |
| burlywood3 | darkred | gray7 | gray67 | grey19 | grey79 | lightblue1 | mediumaquamarine | paleturquoise1 | seashell1 | violet |
| burlywood4 | darksalmon | gray8 | gray68 | grey20 | grey80 | lightblue2 | mediumblue | paleturquoise2 | seashell2 | violetred |
| cadetblue | darkseagreen | gray9 | gray69 | grey21 | grey81 | lightblue3 | mediumorchid | paleturquoise3 | seashell3 | violetred1 |
| cadetblue1 | darkseagreen1 | gray10 | gray70 | grey22 | grey82 | lightblue4 | mediumorchid1 | paleturquoise4 | seashell4 | violetred2 |
| cadetblue2 | darkseagreen2 | gray11 | gray71 | grey23 | grey83 | lightcoral | mediumorchid2 | palevioletred | sienna | violetred3 |
| cadetblue3 | darkseagreen3 | gray12 | gray72 | grey24 | grey84 | lightcyan | mediumorchid3 | palevioletred1 | sienna1 | violetred4 |
| cadetblue4 | darkseagreen4 | gray13 | gray73 | grey25 | grey85 | lightcyan1 | mediumorchid4 | palevioletred2 | sienna2 | wheat |
| chartreuse | darkslateblue | gray14 | gray74 | grey26 | grey86 | lightcyan2 | mediumpurple | palevioletred3 | sienna3 | wheat1 |
| chartreuse1 | darkslategray | gray15 | gray75 | grey27 | grey87 | lightcyan3 | mediumpurple1 | palevioletred4 | sienna4 | wheat2 |
| chartreuse2 | darkslategray1 | gray16 | gray76 | grey28 | grey88 | lightcyan4 | mediumpurple2 | papayawhip | skyblue | wheat3 |
| chartreuse3 | darkslategray2 | gray17 | gray77 | grey29 | grey89 | lightgoldenrod | mediumpurple3 | peachpuff | skyblue1 | wheat4 |
| chartreuse4 | darkslategray3 | gray18 | gray78 | grey30 | grey90 | lightgoldenrod1 | mediumpurple4 | peachpuff1 | skyblue2 | whitesmoke |
| chocolate | darkslategray4 | gray19 | gray79 | grey31 | grey91 | lightgoldenrod2 | mediumseagreen | peachpuff2 | skyblue3 | yellow |
| chocolate1 | darkslategrey | gray20 | gray80 | grey32 | grey92 | lightgoldenrod3 | mediumslateblue | peachpuff3 | skyblue4 | yellow1 |
| chocolate2 | darkturquoise | gray21 | gray81 | grey33 | grey93 | lightgoldenrod4 | mediumspringgreen | peachpuff4 | slateblue | yellow2 |
| chocolate3 | darkviolet | gray22 | gray82 | grey34 | grey94 | lightgoldenrodyellow | mediumturquoise | peru | slateblue1 | yellow3 |
| chocolate4 | deeppink | gray23 | gray83 | grey35 | grey95 | lightgray | mediumvioletred | pink | slateblue2 | yellow4 |
| coral | deeppink1 | gray24 | gray84 | grey36 | grey96 | lightgreen | midnightblue | pink1 | slateblue3 | yellowgreen |
| coral1 | deeppink2 | gray25 | gray85 | grey37 | grey97 | lightgrey | mintcream | pink2 | slateblue4 | |
| coral2 | deeppink3 | gray26 | gray86 | grey38 | grey98 | lightpink | mistyrose | pink3 | slategray | |
| coral3 | deeppink4 | gray27 | gray87 | grey39 | grey99 | lightpink1 | mistyrose1 | pink4 | slategray1 | |

*Figure 15: Table with all the colours coded in R. However, currently only a small choice is available within the program.*