

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:

The screenshot shows the 'BMC calculation' web interface. It features several sections for user input:

- File 1:** Input File 1 (Viability) with a 'Browse...' button and 'No file selected' status. Below it is a 'Sheet Name' field with 'Summary' entered.
- File 2 (optional):** Input File 2 with a 'Browse...' button and 'No file selected' status. Below it is a 'Sheet Name' field with 'Summary' entered.
- Compound:** 'Enter name of compound (exactly as in file)' and 'Name of control (optional) as indicated in the file:' fields.
- Axis labels (optional):** 'x axis label' and 'y axis label' fields.
- BMC options:** 'Bench mark response' section with checkboxes for BMR05, BMR10, BMR15, BMR20, BMR25, BMR30, and BMR50. 'own BMR: [in %]' field. 'Confidence Level' dropdown set to '95%'. 'BMC Interval Method' dropdown set to 'delta'. A checkbox for 'BMC values in logM' is checked.
- Graphical options:** 'Data curves' section with 'Color 1' (gray30), 'Color 2' (darkorchid1), 'Point Type 1' (24), and 'Point Type 2' (19) dropdowns. 'Legend' section with 'show legend' checked, 'Legend position' (topright), and 'Legend 1' (Viability), 'Legend 2' (NA) fields.
- Axes and Lines:** 'maximum of y axis' (150), 'steps of y axis' (25), 'horizontal lines at' (checkboxes for 100, 90, 75, 50), 'horizontal line color' (gray80), 'vertical lines at' (empty field), 'vertical line color' (gray80).
- Size of text:** 'Title' (3), 'Axis' (3), 'Size of points' (3), 'Line widths of axes' (6), 'of curves' (8).

A 'Submit' button is located at the bottom center of the form.

Figure 1: Screenshot of the BMC program

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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.

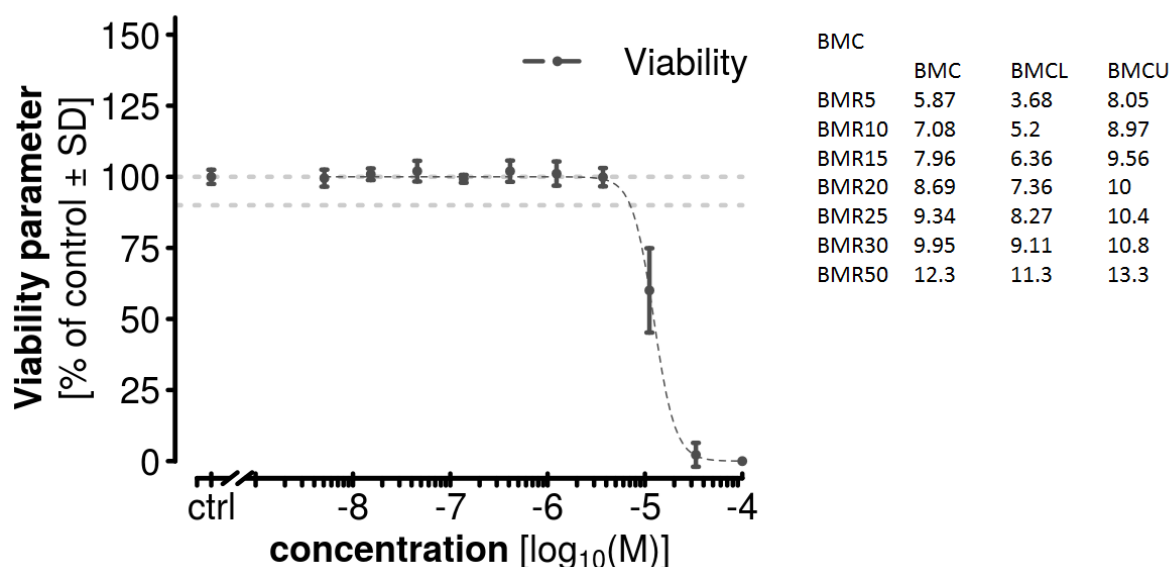


Figure 2: Concentration-response curve with lowest concentrations nicely at 100%.

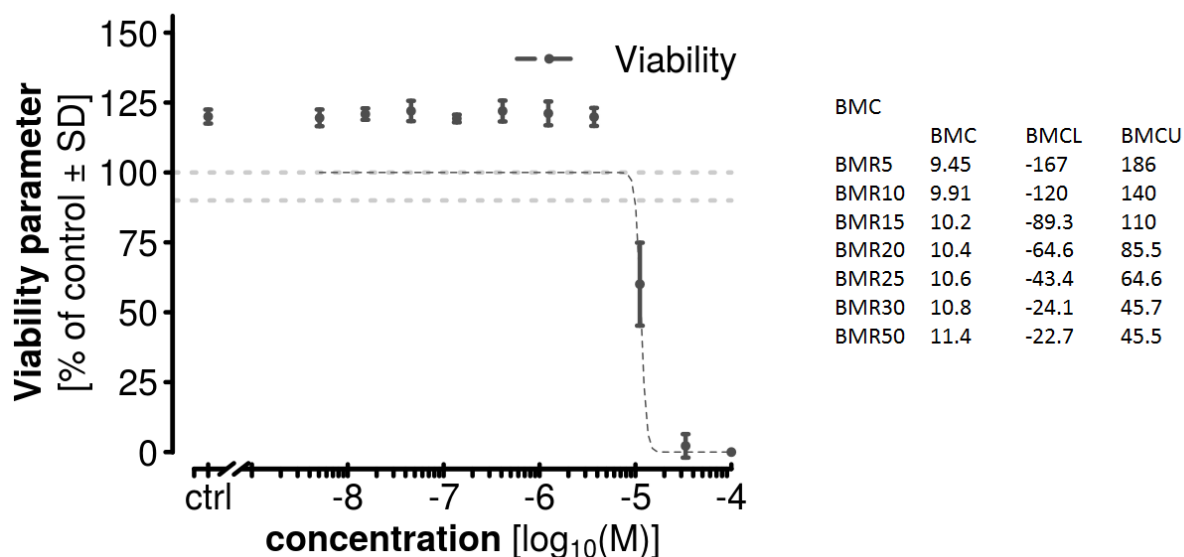


Figure 3: Concentration-response curve with lowest concentrations higher than 100%. The data is the same than in Figure 2, but shifted about 20%. While the BMCs are still comparable, the BMCLs are completely out of range.

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 μ M). 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.

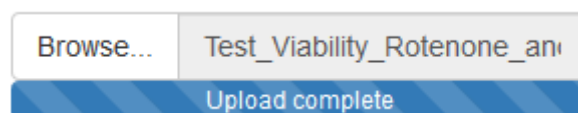


Figure 6: The blue bar indicates that the upload is completed.

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.

BMC calculation
by Johanna Hyttel
using packages 'shiny', 'drc' and 'XLConnect'

File 1
Input File 1 (Viability)
Browse... No file selected

Sheet Name
Summary

File 2 (optional)
Input File 2
Browse... No file selected

Sheet Name
Summary

Compound
Enter name of compound (exactly as in file)
Name of control (optional) as indicated in the file:
desired label on the plot:

BMC options
Bench mark response
☐ BMR05
☒ BMR10
☐ BMR15
☐ BMR20
☒ BMR25
☐ BMR30
☒ BMR50
own BMR: [in %]
Confidence Level
95%
BMC interval Method
delta

Compound
Enter name of compound (exactly as in file)
Name of control (optional) as indicated in the file:
desired label on the plot:

es
Size of text
Title
3
Axis
3
Legend
3
Size of points
3
Size of points
3
Line widths
of axes
6
of curves
8

Submit

Your compound is:

Graph

BMC of File 1
BMC of File 2

Coefficients of fit curves
File1 File2

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

BMC calculation
by Johanna Nyfeler
using packages 'shiny', 'drc' and 'XLConnect'
Manual and example data file: <http://invitrotox.uni-konstanz.de/>

File 1
Input File 1 (Viability)
Browse... No file selected

Sheet Name
Summary

File 2 (optional)
Input File 2
Browse... No file selected

Sheet Name
Summary

Compound
Enter name of compound (exactly as in file)

Name of control (optional)
as indicated in the file:

desired label on the plot:

Axis labels (optional)
x axis label

y axis label

BMC options
Bench mark response
☐ BMR05
☒ BMR10
☐ BMR15
☐ BMR20
☒ BMR25
☐ BMR30
☒ BMR50

own BMR: [in %]

Confidence Level
95%

BMC Interval Method
delta
☒ BMC values in logM

Submit

BMC options
Bench mark response
☐ BMR05
☒ BMR10
☐ BMR15
☐ BMR20
☒ BMR25
☐ BMR30
☒ BMR50

own BMR: [in %]

Confidence Level
95%

BMC Interval Method
delta

☒ BMC values in logM

Axes and Lines
maximum of y axis
150
steps of y axis
25
horizontal lines at
☒ 100
☒ 90
☐ 75
☐ 50
horizontal line color
gray80
vertical lines at

vertical line color
gray80

Size of text
Title
3
Axis
3
Legend
3
Size of points
3
Line widths of axes
6
of curves
8

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 choose 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 $\log_{10}(\text{M})$. To change between these options, tick or untick "BMC values in log(M)" at the bottom of the BMC options section. After (un)tick, 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.

Graphical options

Data curves

Color 1

gray30

Color 2

darkorchid1

Point Type 1

24

Point Type 2

19

Axes and Lines

x axis label

y axis label

maximum of y axis

150

steps of y axis

25

horizontal lines at

☒ 100
☒ 90
☐ 75
☐ 50

horizontal line color

gray80

Size of text

Title

3

Axis

3

Legend

3

Size of points

3

Line widths of axes

6

of curves

8

Legend

☒ show legend

Legend position

topright

Legend 1

Viability

Legend 2

NA

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

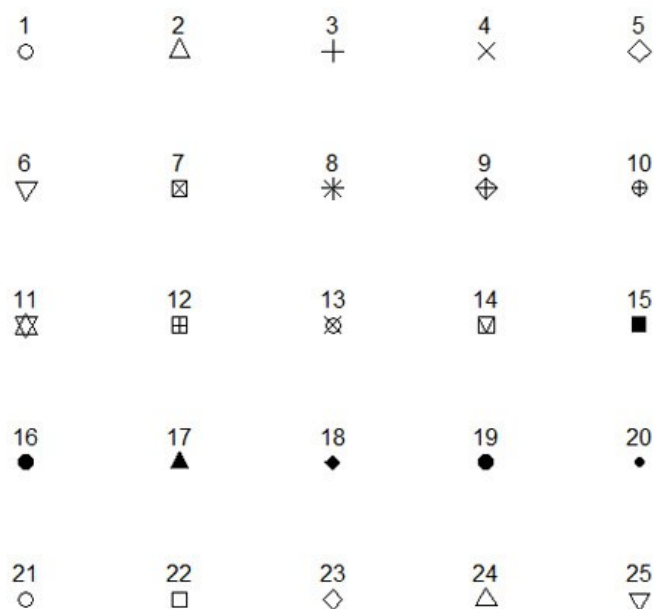


Figure 10: List of symbol codes in R. The symbols with which the data points are represented are coded by numbers. Number 19 (filled circles) and number 24 (open triangles) are set as default.

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 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 \pm 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

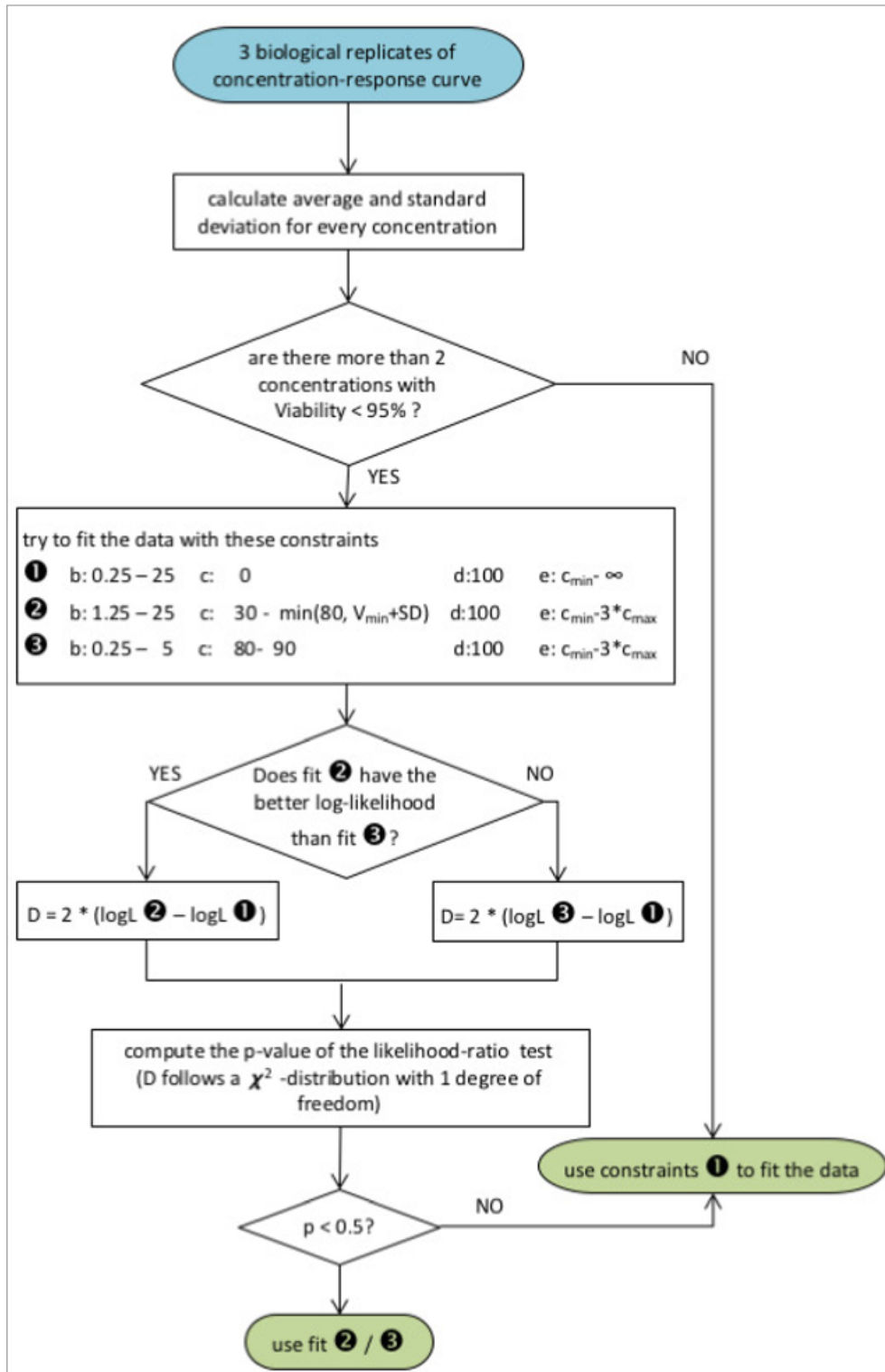


Figure 12: Flow diagram representing the choice of fits for the first readout (viability).

Second readout

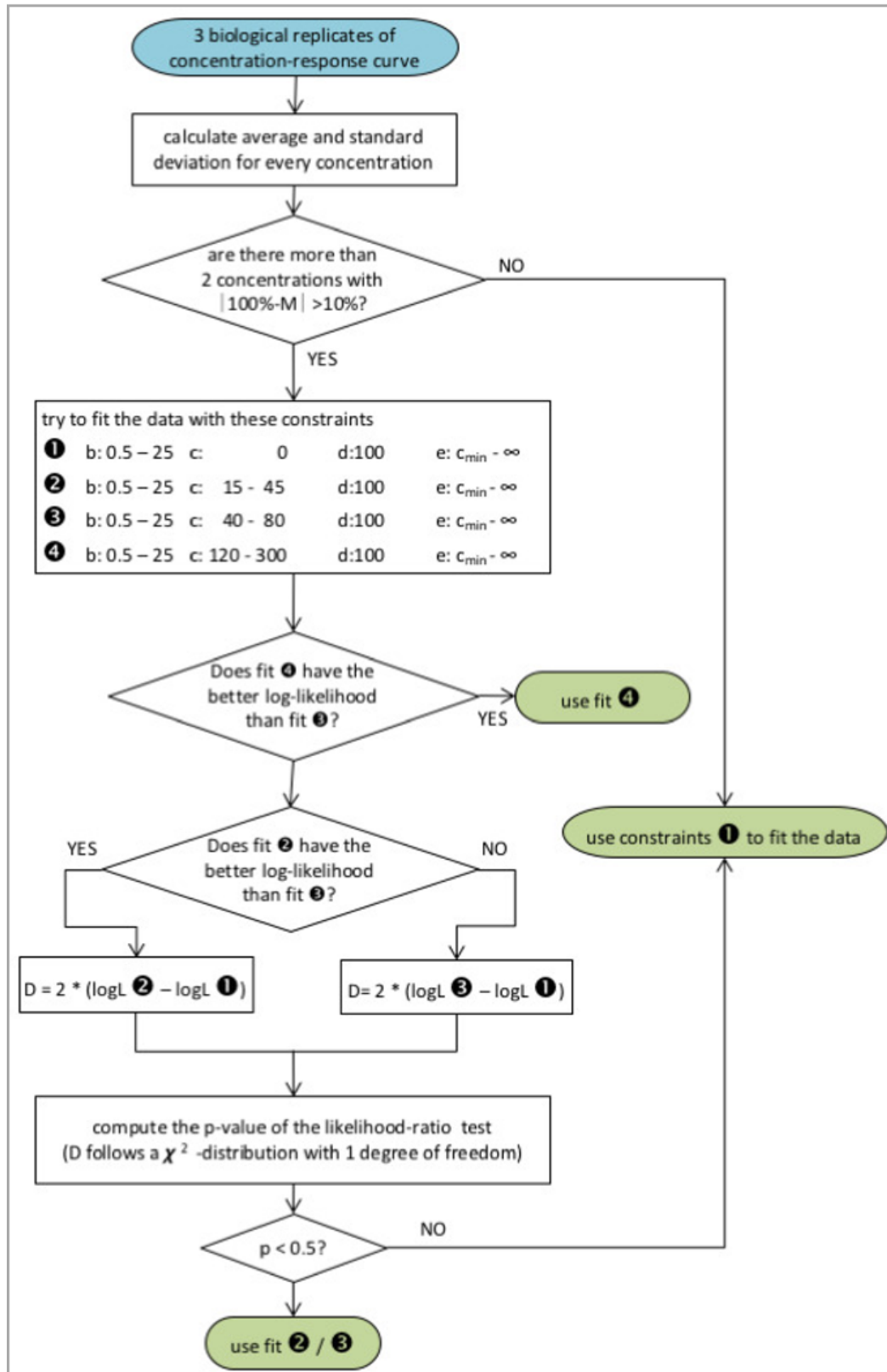


Figure 13: Flow diagram representing the choice of fits for the second readout.

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).

BMC of File 1

	BMC	BMCL	BMCU
BMR10	-7.46	-7.7	-7.31
BMR25	-7.2	-7.43	-7.06
BMR50	NA	NA	NA

BMC of File 2

	BMC	BMCL	BMCU
BMR10	-7.88	-8.13	-7.73
BMR25	-7.75	-7.85	-7.66
BMR50	-7.6	-7.65	-7.55

Coefficients of fit curves

	File1	File2
slope	3.64	3.63
lower asymptote	68.7	15
upper asymptote	100	100
inflection point	-7.37	-7.64


 Download the tables

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 and questions

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.

The program calculates the BMR values, but doesn't return the plot.

Most likely it is, because you have entered "0" as concentration. As the concentration are getting logarithmized, zero obviously doesn't work. If you want to include the readout values without any compound treatment in your plot, we recommend to label it as control. Also the the question below.

Which concentration should the control be labeled with?

If you want to include the control (and its variation) in the plot, just fill in the according response in column E. Columns C and D ("Test.Conc" and "unit") can be left empty. If you give it the concentration 0 μ M/mM/nM/etc., the program won't return a plot. The concentrations are being logarithmized, and logarithmizing zero does not work. Therefore, avoid to enter zero in column C.

Is the control/no treatment considered in the curve fit?

No, it is not considered for fitting the curve to the data. It can only be included in the plot to depict the base variance of the method without any exposure or compound treatment.

Does the standard deviation (SD), the number of replicates (N) or the p-value in columns F, G and H influence the curve fit?

No, these values of the single experiments do not have any influence on the curve fit. Actually these columns could stay empty. They are there for historical reasons, and we decided to keep them for better overview.

How many replicates do I need to get a curve fit?

Actually only one data point per concentration is enough to get some output. So basically, you can upload just one response value per concentration. In that case, no variance (standard deviation) is shown in the plot of course. Also the BMCL and BMCU values might be very far from the calculated BMC. You can increase the quality of the BMR data (BMC and according BMCL/BMCU) by putting in more data points per concentration. The more data is available, the better the confidence interval can be estimated.

Does the appearance of the x-axis (logarithmized or not) influence the curve fit?

No, if the concentrations on the x-axis are in the original unit or in $[\log_{10}(M)]$ does not influence the curve fitting. It is just the depiction on the plot.

Can I enter the concentrations already logarithmized?

No. The program will logarithmize the values given there. So if you enter pre-logarithmized values there, the concentrations of the summary data and the plot won't be correct. Please enter the concentrations in their molar unit (μM /mM/nM, etc).

white	coral4	deepskyblue	gray28	gray68	gray40	gray100	lightpink2	mistyrose2	plum	slategray2
aliceblue	cornflowerblue	deepskyblue1	gray29	gray69	gray41	honeydew	lightpink3	mistyrose3	plum1	slategray3
antiquewhite	cornsilk	deepskyblue2	gray30	gray70	gray42	honeydew1	lightpink4	mistyrose4	plum2	slategray4
antiquewhite1	cornsilk1	deepskyblue3	gray31	gray71	gray43	honeydew2	lightsalmon	moccasin	plum3	slategray
antiquewhite2	cornsilk2	deepskyblue4	gray32	gray72	gray44	honeydew3	lightsalmon1	navajowhite	plum4	snow
antiquewhite3	cornsilk3	dinggray	gray33	gray73	gray45	honeydew4	lightsalmon2	navajowhite1	powderblue	snow1
antiquewhite4	cornsilk4	dinggray	gray34	gray74	gray46	honeydew5	lightsalmon3	navajowhite2	purple	snow2
aquamarine	cyan	dodgerblue	gray35	gray75	gray47	honeydew6	lightsalmon4	navajowhite3	purple1	snow3
aquamarine1	cyan1	dodgerblue1	gray36	gray76	gray48	honeydew7	lightsalmon5	navajowhite4	purple2	snow4
aquamarine2	cyan2	dodgerblue2	gray37	gray77	gray49	honeydew8	lightsalmon6	navajowhite5	purple3	springgreen
aquamarine3	cyan3	dodgerblue3	gray38	gray78	gray50	honeydew9	lightsalmon7	navajowhite6	purple4	springgreen1
aquamarine4	cyan4	dodgerblue4	gray39	gray79	gray51	honeydew10	lightsalmon8	navajowhite7	purple5	springgreen2
azure	darkblue	firebrick	gray40	gray80	gray52	indianred1	lightsalmon9	navajowhite8	red	springgreen3
azure1	darkcyan	firebrick1	gray41	gray81	gray53	indianred2	lightsalmon10	navajowhite9	red1	springgreen4
azure2	darkgoldenrod	firebrick2	gray42	gray82	gray54	indianred3	lightsalmon11	navajowhite10	red2	steelblue
azure3	darkgoldenrod1	firebrick3	gray43	gray83	gray55	indianred4	lightsalmon12	navajowhite11	red3	steelblue1
azure4	darkgoldenrod2	firebrick4	gray44	gray84	gray56	ivory	lightsalmon13	navajowhite12	rosybrown	steelblue2
beige	darkgoldenrod3	forestgreen	gray45	gray85	gray57	ivory1	lightsalmon14	navajowhite13	rosybrown1	steelblue3
bisque	darkgoldenrod4	forestgreen	gray46	gray86	gray58	ivory2	lightsalmon15	navajowhite14	rosybrown2	steelblue4
bisque1	darkgray	gainsboro	gray47	gray87	gray59	ivory3	lightsalmon16	navajowhite15	rosybrown3	tan
bisque2	darkgreen	ghostwhite	gray48	gray88	gray60	ivory4	lightsalmon17	navajowhite16	royalblue	tan1
bisque3	darkgray	gold	gray49	gray89	gray61	khaki	lightsalmon18	navajowhite17	royalblue1	tan2
bisque4	darkkhaki	gold1	gray50	gray90	gray62	khaki1	lightsalmon19	navajowhite18	royalblue2	tan3
black	darkmagenta	gold2	gray51	gray91	gray63	khaki2	lightsalmon20	navajowhite19	royalblue3	thistle
blanchedalmond	darkolivegreen	gold3	gray52	gray92	gray64	khaki3	lightsalmon21	navajowhite20	royalblue4	thistle1
blue	darkolivegreen1	goldenrod	gray53	gray93	gray65	khaki4	lightsalmon22	navajowhite21	saddlebrown	thistle2
blue1	darkolivegreen2	goldenrod1	gray54	gray94	gray66	lavender	lightsalmon23	navajowhite22	salmon	thistle3
blue2	darkolivegreen3	goldenrod2	gray55	gray95	gray67	lavenderblush	lightsalmon24	navajowhite23	salmon1	thistle4
blue3	darkolivegreen4	goldenrod3	gray56	gray96	gray68	lavenderblush1	lightsalmon25	navajowhite24	salmon2	tomato
blue4	darkorange	goldenrod4	gray57	gray97	gray69	lavenderblush2	lightsalmon26	navajowhite25	salmon3	tomato1
blueviolet	darkorange1	gray	gray58	gray98	gray70	lavenderblush3	lightsalmon27	navajowhite26	salmon4	tomato2
brown	darkorange2	gray	gray59	gray99	gray71	lavenderblush4	lightsalmon28	navajowhite27	sandybrown	tomato3
brown1	darkorange3	gray0	gray60	gray100	gray72	lavenderblush5	lightsalmon29	navajowhite28	seagreen	tomato4
brown2	darkorange4	gray1	gray61	gray101	gray73	lavenderblush6	lightsalmon30	navajowhite29	seagreen1	turquoise
brown3	darkorchid	gray2	gray62	gray102	gray74	lavenderblush7	lightsalmon31	navajowhite30	seagreen2	turquoise1
brown4	darkorchid1	gray3	gray63	gray103	gray75	lavenderblush8	lightsalmon32	navajowhite31	seagreen3	turquoise2
burlwood	darkorchid2	gray4	gray64	gray104	gray76	lavenderblush9	lightsalmon33	navajowhite32	seagreen4	turquoise3
burlwood1	darkorchid3	gray5	gray65	gray105	gray77	lavenderblush10	lightsalmon34	navajowhite33	seashell	turquoise4
burlwood2	darkorchid4	gray6	gray66	gray106	gray78	lavenderblush11	lightsalmon35	navajowhite34	seashell1	violet
burlwood3	darkred	gray7	gray67	gray107	gray79	lavenderblush12	lightsalmon36	navajowhite35	seashell2	violet1
burlwood4	darksalmon	gray8	gray68	gray108	gray80	lavenderblush13	lightsalmon37	navajowhite36	seashell3	violet2
cadetblue	darkseagreen	gray9	gray69	gray109	gray81	lavenderblush14	lightsalmon38	navajowhite37	seashell4	violet3
cadetblue1	darkseagreen1	gray10	gray70	gray110	gray82	lavenderblush15	lightsalmon39	navajowhite38	sienna	violet4
cadetblue2	darkseagreen2	gray11	gray71	gray111	gray83	lavenderblush16	lightsalmon40	navajowhite39	sienna1	wheat
cadetblue3	darkseagreen3	gray12	gray72	gray112	gray84	lavenderblush17	lightsalmon41	navajowhite40	sienna2	wheat1
cadetblue4	darkseagreen4	gray13	gray73	gray113	gray85	lavenderblush18	lightsalmon42	navajowhite41	sienna3	wheat2
chartreuse	darkslateblue	gray14	gray74	gray114	gray86	lavenderblush19	lightsalmon43	navajowhite42	skyblue	wheat3
chartreuse1	darkslategray	gray15	gray75	gray115	gray87	lavenderblush20	lightsalmon44	navajowhite43	skyblue1	wheat4
chartreuse2	darkslategray1	gray16	gray76	gray116	gray88	lavenderblush21	lightsalmon45	navajowhite44	skyblue2	wheat5
chartreuse3	darkslategray2	gray17	gray77	gray117	gray89	lavenderblush22	lightsalmon46	navajowhite45	skyblue3	whitesmoke
chartreuse4	darkslategray3	gray18	gray78	gray118	gray90	lavenderblush23	lightsalmon47	navajowhite46	skyblue4	yellow
chocolate	darkslategray4	gray19	gray79	gray119	gray91	lavenderblush24	lightsalmon48	navajowhite47	skyblue5	yellow1
chocolate1	darkslategray5	gray20	gray80	gray120	gray92	lavenderblush25	lightsalmon49	navajowhite48	skyblue6	yellow2
chocolate2	darkslategray6	gray21	gray81	gray121	gray93	lavenderblush26	lightsalmon50	navajowhite49	skyblue7	yellow3
chocolate3	darkslategray7	gray22	gray82	gray122	gray94	lavenderblush27	lightsalmon51	navajowhite50	skyblue8	yellow4
chocolate4	darkslategray8	gray23	gray83	gray123	gray95	lavenderblush28	lightsalmon52	navajowhite51	skyblue9	yellowgreen
coral	deeppink	gray24	gray84	gray124	gray96	lavenderblush29	lightsalmon53	navajowhite52	skyblue10	
coral1	deeppink1	gray25	gray85	gray125	gray97	lavenderblush30	lightsalmon54	navajowhite53	skyblue11	
coral2	deeppink2	gray26	gray86	gray126	gray98	lavenderblush31	lightsalmon55	navajowhite54	skyblue12	
coral3	deeppink3	gray27	gray87	gray127	gray99	lavenderblush32	lightsalmon56	navajowhite55	skyblue13	

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