

MDRA

Version 1.0

WebApp for Dose-Response Analysis of Multiple Experiments

User's guide

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The web application MDRA performs the dose-response analysis from multiple experiments.

- The four-parameter log-logistic (4pLL) model is used to fit dose-response data from each individual experiment. The 4pLL model function is given by

$$response = f(dose) = \phi^{(c)} + \frac{\phi^{(d)} - \phi^{(c)}}{1 + \exp\{\phi^{(b)}[\log(dose) - \log(\phi^{(e)})]\}}$$

The parameters $\phi^{(c)}$ and $\phi^{(d)}$ correspond to the lower and upper horizontal limits for mean response, respectively. The parameter $\phi^{(e)}$ is the effective concentration EC50. The parameter $\phi^{(b)}$ determines the slope of the curve. The natural logarithm ('log') is used in this model function.

- The DORES (**d**ose-**r**esponse **s**creening) plot provides visualization of multiple dose-response experimental data sets in a single plot. It displays information from studies involving a large number of experiments: which concentration levels and ranges are chosen for each experiment; whether all experiments exhibit complete and biologically relevant dose-response relationships; and what the individual ECx estimates and their confidence intervals are. For details about the DORES plot, see Jiang, Xiaoqi and Annette Kopp-Schneider. Statistical strategies for averaging EC50 from multiple dose-response experiments. *Archives of Toxicology*, (2014).
- The meta-analysis approach is applied to average ECx estimates from multiple dose-response experiments. For details, see: Jiang, Xiaoqi, and Annette Kopp-Schneider. Summarizing EC50 estimates from multiple dose-response experiments: A comparison of a meta-analysis strategy to a mixed-effects model approach. *Biometrical Journal*, 56(3): 493-512 (2014).
- The 4pLL model is fitted using the R package drc (Ritz, C., Streibig, J.C., 2005. Bioassay analysis using R. *Journal of Statistical Software*. 12: 1–22.). The R package nlme (Pinheiro, J. C. and Bates, D. M. (2000). The R package metafor (Viechtbauer W., 2010. Conducting meta-analyses in r with the metafor package. *Journal of Statistical Software* 36:1-48.) is used for computing the heterogeneity estimators in meta-analysis.




1. Getting started with MDRA

Enter <http://biostatistics.dkfz.de/mdra/> in a browser window to access the WebApp (Web Application) MDRA. The browsers Chrome and Firefox are recommended.

To follow this user's guide, please first enter the webpage 'About' and download the exemplary data file "Example_DataSet.csv".

2. Data Upload

Only the CSV-File format is supported for uploading your data to the WebApp MDRA. CSV-Files can be easily created from Excel data by *saving as type* "(*.csv)".

- (1) Click on the  button at the top of the left sidebar to choose the data file that you want to upload.
- (2) Tick the  Header item to use the first row of the file for column titles. Make sure the column titles in the first row of your data do not contain any special characters or spaces. Short column titles are recommended. If there are no column titles shown in the first row of your CSV data, please untick the  Header item and then your data will be imported with column titles V1, V2, V3...
- (3) Select the column delimiter and decimal point appropriate for your data file.

Column delimiter:	Decimal point:
<input checked="" type="radio"/> Comma	<input type="radio"/> Dot
<input type="radio"/> Semicolon	<input checked="" type="radio"/> Comma
<input type="radio"/> Tab	

In the exemplary data file "Example_DataSet.csv", both the column delimiter and decimal point are commas.

On the preview page 'Data Set' –at the top of the webpage- you can see how your data is affected by changing the above items.

Before going to the next step, make sure that your data set has been correctly imported by MDRA. The numeric variables must be displayed with a dot ('.') as a decimal point or in scientific (e-) notation, whether the decimal point in your CSV-File is a dot or comma.

A correctly imported data set should look like this:

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	Chemical	LabID	ExperimentID	Unit	Concentration	Response
1	Sodium_valproate	C	5	M	1e-04	0.981404
2	Sodium_valproate	C	5	M	1e-04	1.03256332906318
3	Sodium_valproate	C	5	M	1e-05	1.00942187693706
4	Sodium_valproate	C	5	M	1e-04	0.979999173519567
5	Sodium_valproate	C	5	M	1e-04	0.987768089590479
6	Sodium_valproate	C	5	M	1e-05	0.949336749452457
7	Sodium_valproate	E	7	M	0	0.991691341700312
8	Sodium_valproate	E	7	M	0	0.860742663385681
...

3. Selecting variables for multiple experimental data for dose-response analysis

In the drop-down boxes, you will see all column titles from your data set, which can be selected as variable names for analyses in the next step.

*Select variable 'dose'

Concentration

...

Chemical

LabID

ExperimentID

Unit

Concentration

Response

*Select variable 'response'

...

Select additional identifier (e.g. Lab IDs)

...

... is optional, if this information is available.

☐ IC ☐ ED ☐ ID

To perform a dose-response analysis from multiple experimental data, the variables 'dose' and 'response' and an identifier for 'experiment' (IDs) are required fields. An additional identifier is optional, e.g., it can be used when experiments are carried out in different laboratories and this information is available. An additional identifier can also be used for any other potential factors that may influence experimental results, such as technicians and experiment dates.

In the exemplary data, the variables “Concentration” and “Response” have been selected for dose-response analysis, and the variables “ExperimentID” and “PartnerID” act as identifiers for ‘experiment’ and ‘lab’.

The screenshot shows a web form with four dropdown menus arranged in a 2x2 grid. The top-left dropdown is labeled '*Select variable 'dose'' and has 'Concentration' selected. The top-right dropdown is labeled '*Select variable 'response'' and has 'Response' selected. The bottom-left dropdown is labeled '*Select identifier for 'experiment'' and has 'ExperimentID' selected. The bottom-right dropdown is labeled 'Select additional identifier (e.g. Lab IDs)' and has 'LabID' selected. Below the dropdowns, a note states: '* = Required Fields. The additional identifier is optional, if this information is available.'

4. Choice of conditions for dose-response analysis

- (1) Choose the name of a measure used to describe the potency or toxicity of a chemical

Choose the name of a measure of toxicity (potency): ☒ EC ☐ IC ☐ ED ☐ ID

- EC: effective concentration (increasing curve)
- IC: inhibition concentration (decreasing curve)
- ED: effective dose (increasing curve)
- ID: inhibition dose (decreasing curve)

Note that the four options do not result in different estimated values. The selected name is used in results tables and plots. These four terms can be defined in two ways: relative and absolute. The relative EC_x (IC_x, ED_x or ID_x) refers to the concentration or dose that causes x% change of the maximal effect, which is the most common definition. We only used this relative definition in the dose-response analysis.

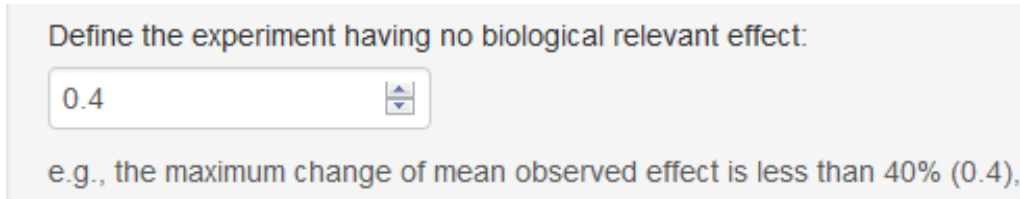
- (2) Choose the quantile of the measure.

For example, enter “50” in the input box to calculate EC₅₀ for the exemplary data:

The screenshot shows a web form with a label 'The quantile of EC/IC/ED/ID:' above a text input box containing the number '50'. To the right of the input box is a small up/down arrow icon. Below the input box, a note states: 'e.g., enter 50 for estimation of EC50/IC50/ED50/ID50.'

- (3) Define the criteria for the experiment having no biologically relevant effect. The lack of relevance can be quantitatively assessed upon inspection of the difference

between the lower and upper limits $\phi^{(c)}$ and $\phi^{(d)}$. Specifically, for a predefined percentage $r\%$, if $|(\phi^{(d)} - \phi^{(c)}) / \phi^{(d)}| < r\%$, then the observed dose-response relationship may be considered as not biologically relevant. In the exemplary data set, our choice was $r=40$, so we then entered 0.4 in the input box.

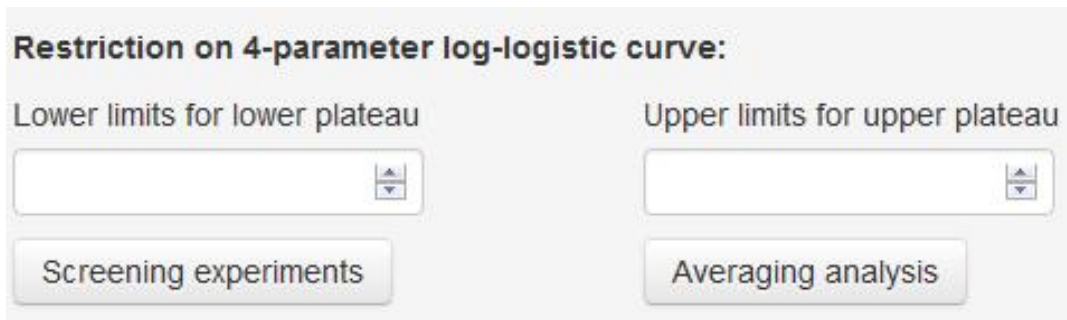


Define the experiment having no biological relevant effect:

0.4

e.g., the maximum change of mean observed effect is less than 40% (0.4).

- (4) Restrictions on the limits for lower $\phi^{(c)}$ and upper $\phi^{(d)}$ plateaus of a 4-parameter log-logistic curve can be chosen, e.g., the lower limit could be restricted to a positive value (i.e., enter 0 for lower limit for lower plateau). Note that the model parameters $\phi^{(c)}$ and $\phi^{(d)}$ are estimated, even when their limit values are restricted.



Restriction on 4-parameter log-logistic curve:

Lower limits for lower plateau

Upper limits for upper plateau

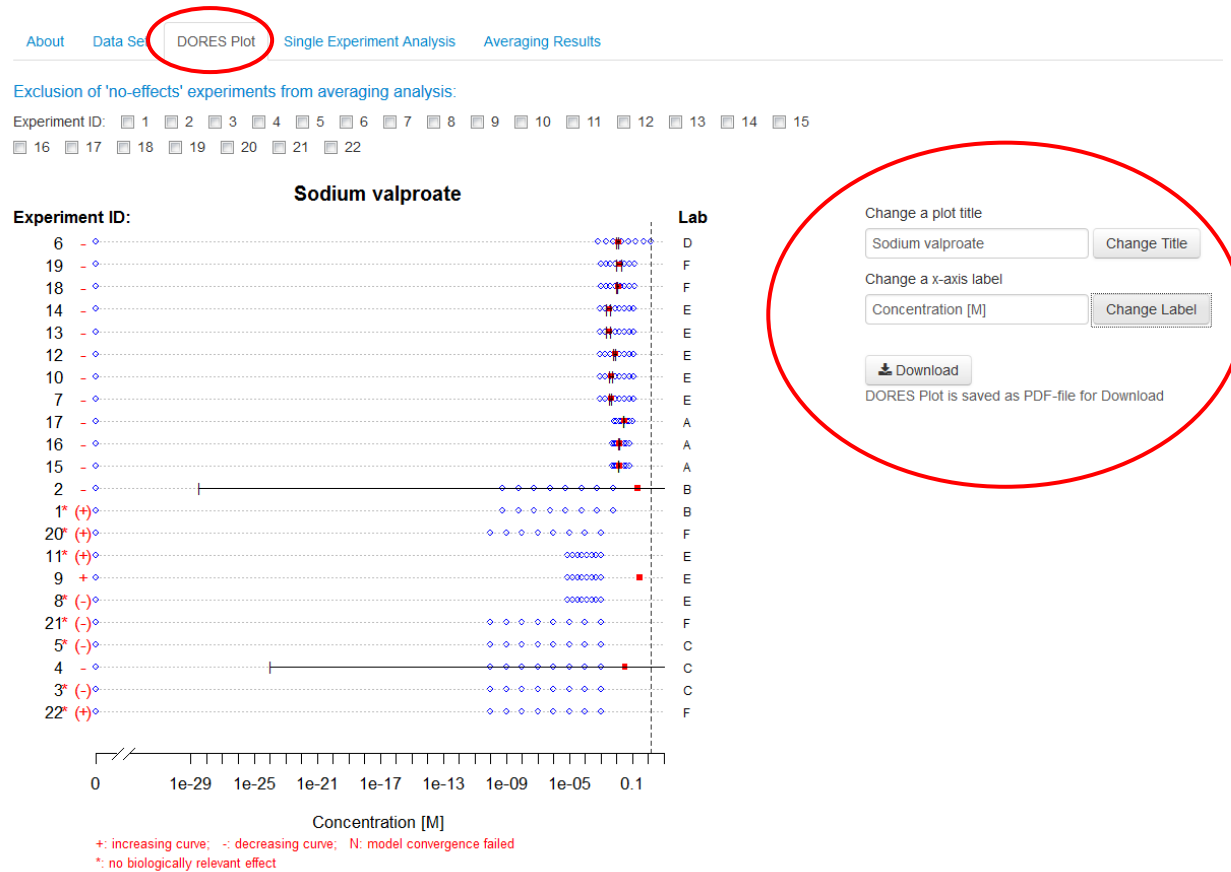
Screening experiments

Averaging analysis

If restrictions are not given (default setting), the 4-parameter log-logistic curve is allowed to run from negative infinity to positive infinity or the opposite. In case response values are normalized between 0 and 100%, lower limits for the lower plateau could be restricted to 0 or upper limits for the upper plateau could be restricted to 1. This may improve the estimation of individual ECx values, but only when the highest concentration (dose) level does not cover the lower or upper plateau of the dose-response curve

5. Screening dose-response experimental data

Click on the **Screening experiments** button at the end of the right sidebar, choose the webpage 'DORES plot', and you will get an overview of all your dose-response data from multiple experiments. The title and x-axis label can be changed in the corresponding input boxes to the right of the DORES plot. Click on the **Download** button or right mouse click the button and choose "Save Link as" to download the DORES plot.



The resulting table displayed in the webpage 'Single Experiment Analysis' provides this information about the fitted curve and estimated measure of toxicity for each single experiment:

- the four estimated parameters of the log-logistic curve, i.e., 'Hill Slope', 'lower asymptote', 'upper asymptote' and 'natural logEC50' (natural logarithm of EC50);
- estimated measure of toxicity (e.g. EC50) and its 95% confidence interval.

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Results Table Download

The following result table is saved as CSV-file for Download

Single Curve Download

The dose-response curve plotted for each single experimental data is saved as PDF-file for Download

Experiment ID	b (=Hill Slope)	c (=lower asymptote)	d (=upper asymptote)	e (=natural logEC50)	EC50	Lower 95%-CL	Upper 95%-CL
1	-7.98e+00	8.90e-01	9.84e-01	-1.32e+01	1.85e-06	4.95e-23	6.93e+10
2	1.32e-01	1.91e-01	1.00e+00	-1.50e+00	2.22e-01	3.64e-29	1.36e+27
3	5.29e+00	8.85e-01	1.02e+00	-1.87e+01	7.54e-09	1.75e-10	3.24e-07
4	5.84e-01	-2.30e-03	1.01e+00	-3.40e+00	3.35e-02	1.08e-24	1.04e+21
5	4.23e-05	1.03e+00	1.00e+00	-5.04e+01	1.25e-22	0.00e+00	Inf
6	2.32e+00	4.57e-03	9.76e-01	-4.37e+00	1.26e-02	1.05e-02	1.52e-02
7	1.69e+00	-3.63e-02	9.75e-01	-5.42e+00	4.41e-03	3.96e-03	4.91e-03
8	8.44e-01	6.64e-01	9.98e-01	-7.95e+00	3.52e-04	1.93e-05	6.45e-03
9	-5.41e-01	1.01e+00	3.25e+00	-1.30e+00	2.74e-01	NA	NA
10	2.25e+00	-7.35e-03	9.39e-01	-5.36e+00	4.71e-03	3.84e-03	5.78e-03
11	-5.26e-05	1.00e+00	7.50e-01	-3.10e+00	4.49e-02	0.00e+00	Inf
12	2.96e+00	1.39e-03	1.04e+00	-4.79e+00	8.33e-03	6.91e-03	1.00e-02
13	1.56e+00	-1.30e-02	9.95e-01	-5.73e+00	3.23e-03	2.46e-03	4.24e-03
14	1.85e+00	2.18e-03	9.67e-01	-5.65e+00	3.52e-03	2.62e-03	4.72e-03
15	6.45e+00	1.79e-03	9.60e-01	-4.21e+00	1.49e-02	1.42e-02	1.56e-02
16	4.03e+00	-2.69e-02	9.55e-01	-4.18e+00	1.54e-02	1.45e-02	1.63e-02
17	2.97e+01	2.51e-02	9.85e-01	-3.48e+00	3.10e-02	2.98e-02	3.22e-02
18	2.60e+00	3.75e-03	9.64e-01	-4.40e+00	1.23e-02	1.13e-02	1.34e-02
19	1.39e+01	-1.46e-02	1.19e+00	-4.17e+00	1.54e-02	1.12e-02	2.13e-02
20	-5.81e+00	9.33e-01	1.04e+00	-1.36e+01	1.29e-06	2.22e-09	7.55e-04
21	4.13e-05	1.10e+00	1.00e+00	-3.16e-02	9.69e-01	0.00e+00	Inf
22	-4.39e-06	1.00e+00	6.42e-01	1.04e+01	3.34e+04	NA	NA

In this step, the input of conditions for dose-response analysis can still be changed. After inputs have been changed, click on the **Screening experiments** button again, and the DORES plot and resulting table will be updated. For example, enter 0 and 1 in the input box of the lower and upper limits for plateaus.


Restriction on 4-parameter log-logistic curve:

Lower limits for lower plateau Upper limits for upper plateau

Experiment ID	b (=Hill Slope)	c (=lower asymptote)	d (=upper asymptote)	e (=natural logEC50)	EC50	Lower 95%-CL	Upper 95%-CL
1	-1.16e-05	1.00e+00	8.19e-01	5.62e+00	2.77e+02	0.00e+00	Inf
2	1.36e-01	2.11e-01	1.00e+00	-1.82e+00	1.62e-01	1.89e-27	1.39e+25
3	5.40e+00	8.85e-01	1.00e+00	-1.87e+01	7.91e-09	1.86e-10	3.36e-07
4	8.83e-01	0.00e+00	1.00e+00	-4.44e+00	1.18e-02	NA	NA
5	2.44e-05	1.03e+00	1.00e+00	-5.04e+01	1.29e-22	NA	NA
6	2.32e+00	4.57e-03	9.76e-01	-4.37e+00	1.26e-02	1.05e-02	1.52e-02
7	1.87e+00	0.00e+00	9.68e-01	-5.47e+00	4.23e-03	3.79e-03	4.72e-03
8	8.44e-01	6.64e-01	9.98e-01	-7.95e+00	3.53e-04	1.92e-05	6.46e-03
9	-3.47e-01	1.04e+00	1.00e+00	2.40e+01	2.63e+10	0.00e+00	Inf
10	2.31e+00	0.00e+00	9.37e-01	-5.36e+00	4.68e-03	3.82e-03	5.73e-03
11	-7.76e-05	1.00e+00	7.50e-01	-4.42e+00	1.20e-02	0.00e+00	Inf
12	3.22e+00	5.50e-03	1.00e+00	-4.76e+00	8.56e-03	7.08e-03	1.04e-02
13	1.60e+00	0.00e+00	9.94e-01	-5.76e+00	3.17e-03	2.42e-03	4.15e-03
14	1.85e+00	2.18e-03	9.67e-01	-5.65e+00	3.52e-03	2.62e-03	4.72e-03
15	6.45e+00	1.79e-03	9.60e-01	-4.21e+00	1.49e-02	1.42e-02	1.56e-02
16	4.42e+00	0.00e+00	9.48e-01	-4.19e+00	1.52e-02	1.44e-02	1.61e-02
17	2.53e+02	1.06e-02	9.85e-01	-3.47e+00	3.12e-02	3.11e-02	3.12e-02
18	2.60e+00	3.74e-03	9.64e-01	-4.40e+00	1.23e-02	1.13e-02	1.34e-02
19	3.00e+01	9.10e-03	1.00e+00	-4.13e+00	1.61e-02	1.11e-02	2.34e-02
20	-6.09e+00	9.33e-01	1.00e+00	-1.37e+01	1.15e-06	1.77e-09	7.43e-04
21	1.89e-05	1.10e+00	1.00e+00	-3.16e-02	9.69e-01	0.00e+00	Inf
22	-2.41e-05	1.00e+00	6.44e-01	1.07e+01	4.30e+04	0.00e+00	Inf




Results Table Download

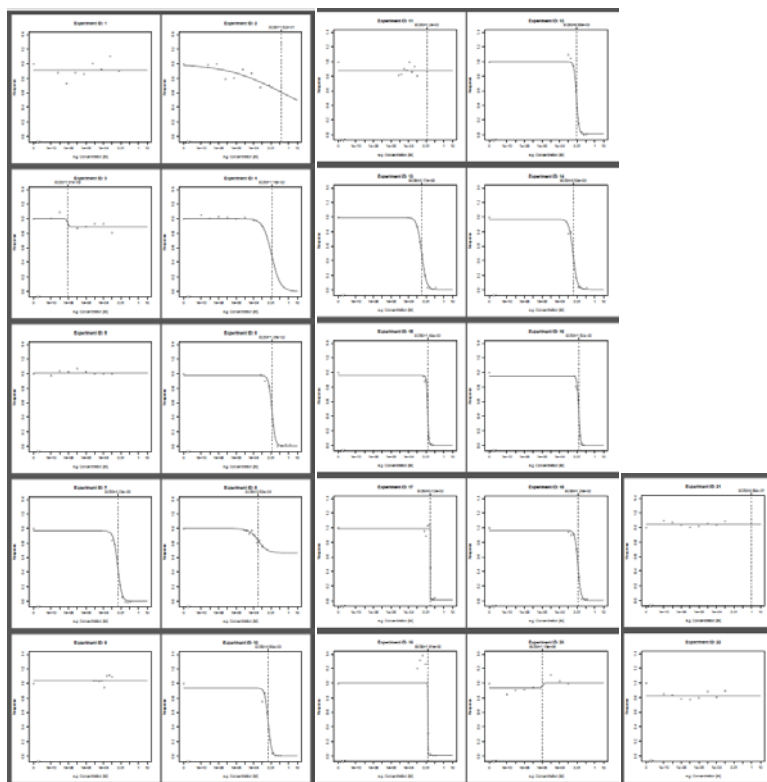
Click on the  Results Table Download button or right mouse click the button and choose “Save Link as” to download the resulting table saved in a CSV-File.

If you want to see a fitted log-logistic curve for each experiment data, click on the



Single Curve Download

 Single Curve Download button or right mouse click the button and choose “Save Link as” to download plots saved in a PDF-File.



6. Averaging dose-response data from multiple experiments

Experimental data sets not containing useful information for further averaging analysis should be excluded, i.e., data sets for which no EC50 estimate could be obtained or where EC50 estimates have very large confidence intervals.

Under the webpage 'DORES plot', all the experiment IDs are listed at top of this webpage:

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Exclusion of 'no-effects' experiments from averaging analysis:

Experiment ID: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 ☐ 11 ☐ 12 ☐ 13 ☐ 14 ☐ 15

☐ 16 ☐ 17 ☐ 18 ☐ 19 ☐ 20 ☐ 21 ☐ 22

The elements of experiment ID are extracted from the identifier for 'experiment' that you select in the step 'Uploading data'.

For the exemplary data, the experiment IDs: 1,2,3,4,5,8,9,11,20,21,22 are excluded from the averaging analysis. Thus, tick these elements in the check box list and click on the

Screening experiments

button again, and the DORES plot will be updated.

The screenshot displays the 'Data Set' tab of the WebAPP-MDRA interface. On the left, the 'Uploading Files' section shows a file named 'Example_DataSet.csv' uploaded successfully. Below this, various settings for data parsing are visible, including column delimiters (Comma, Semicolon, Tab) and decimal points (Dot, Comma). The 'Select variable' dropdowns are set to 'Concentration' for 'dose' and 'Response' for 'response'. The 'ExperimentID' is selected as the identifier. The 'Restriction on 4-parameter log-logistic curve' is set to 'Screening experiments'. A red circle highlights the 'Screening experiments' button.

On the right, the 'Single Experiment Analysis' tab is active, showing a forest plot titled 'Sodium valproate'. The plot displays the concentration [M] on a logarithmic scale (0 to 10) for various experiments. The legend indicates: '+' for increasing curve, '-' for decreasing curve, and 'N' for model convergence failed. A red circle highlights the 'Exclusion of 'no-effects' experiments from averaging analysis:' section, which lists Experiment IDs 1 through 22, with checkboxes indicating their status.

Finally, click on the **Averaging analysis** button at the end of the left sidebar and choose the webpage 'Averaging Results' to average dose-response data from multiple experiments. The results of the averaging analysis are displayed in a forest plot. The title can be changed in the input box to the right of the forest plot. Click on the **Download** button or right mouse click the button and choose "Save Link as" to download the forest plot.

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Forest plot

