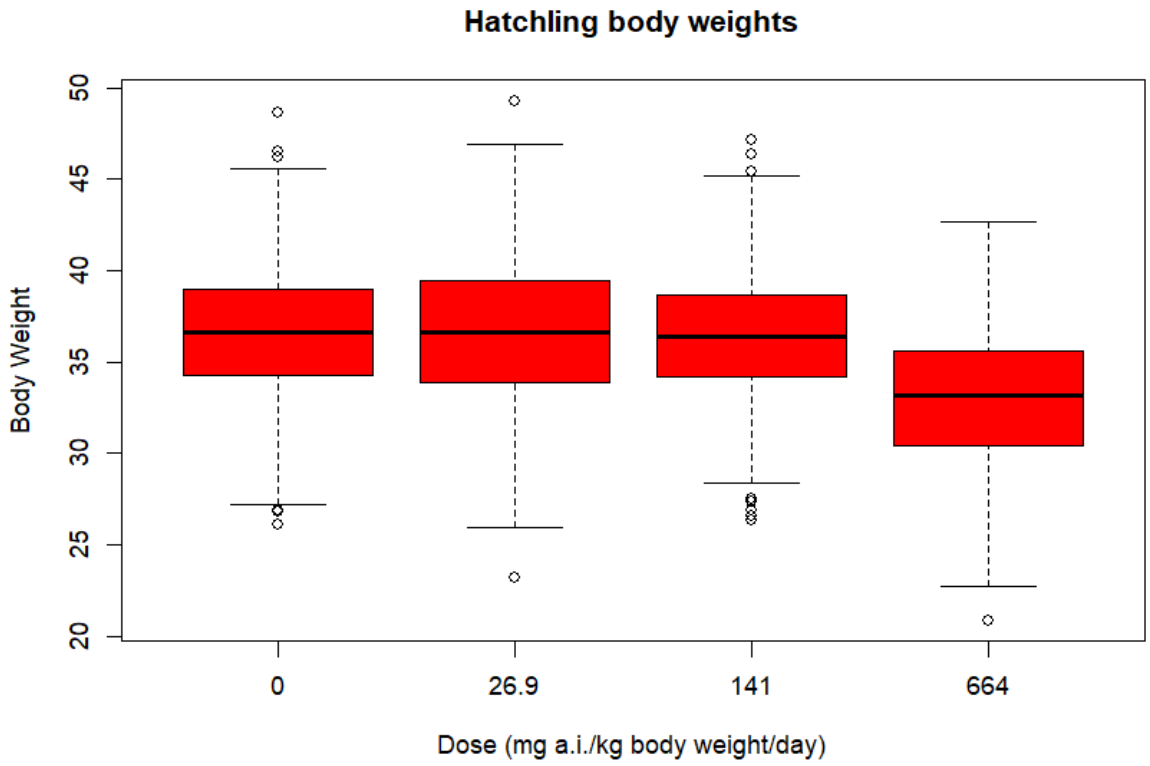
Example 1: Hatchling body weight

A study was conducted to assess environmental safety of substance **X**, several endpoints were studied, and information was recorded for all endpoints considering a dosing scheme of 0, 200, 1000 and 5000 ppm active ingredients (which corresponds to 0, 26.9, 141 and 664 mg a.i./kg body weight/day) on Mallard ducks. The individual body weights of surviving hatchlings will be used in this example as well as the summary statistics at each of the doses tested. The summary statistics of the hatchling body weights for each dose are provided in the table below:

Dose (mg a.i./kg body weight/day)	Hatchling Body Weight		Nweight
	Mean	Standard deviation	
0	36.614	3.733	741
26.9	36.548	4.024	644
141	36.46	3.279	602
664	32.875	3.877	319

A box plot of the data that will be used can be seen below:



An ANOVA model was fitted to compare the different dose groups (results shown below) and the results indicate that there is a difference in weights for the dose groups tested.

```
             Df Sum Sq Mean Sq F value Pr(>F)
Dose          3   3712    1237    89.1 <2e-16 ***
Residuals  2302  31966      14
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

To correct for multiple testing a Dunnett correction was used, and the results (see below) show a significant decrease in weight for the highest dose group tested (highlighted in red) with respect to the control group indicating possible adversity.

```
         Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Dunnett Contrasts


Fit: aov(formula = Weight ~ Dose, data = IndividualData)

Linear Hypotheses:
              Estimate Std. Error t value Pr(>|t|)
26.9 - 0 == 0   -0.066      0.201   -0.33     0.98
141 - 0 == 0    -0.154      0.204   -0.75     0.81
664 - 0 == 0    -3.739      0.249  -14.98   <1e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)
```

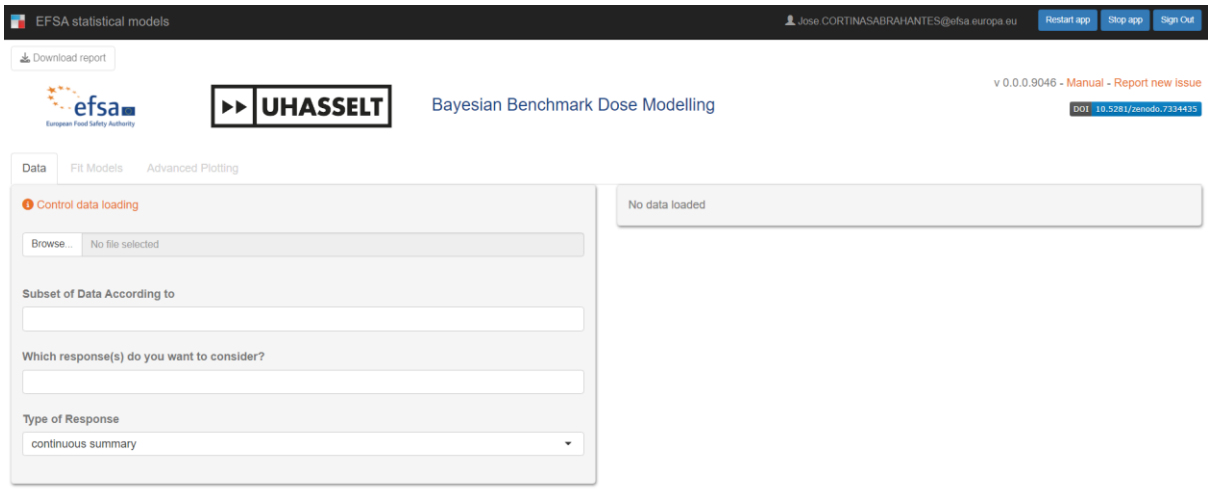
The purpose of this exercise is to fit a dose response curve to the reported data (summarize and individual data) and to estimate the BMD and its credible interval (90, 5, 50 and 95th percentiles should be estimated from the posterior distribution) for a benchmark response (BMR) of 10% relative decrease of body weight with respect to the background body weight (body weight expected in the control group), in line with the Commission Regulation No 283/2013 (here) and EFSA Risk assessment for Birds and Mammals (2023). The question of interest is to estimate the BMD and construct its credible interval for the endpoint hatchling body weights considering a BMR of 10%.

Options to be used:

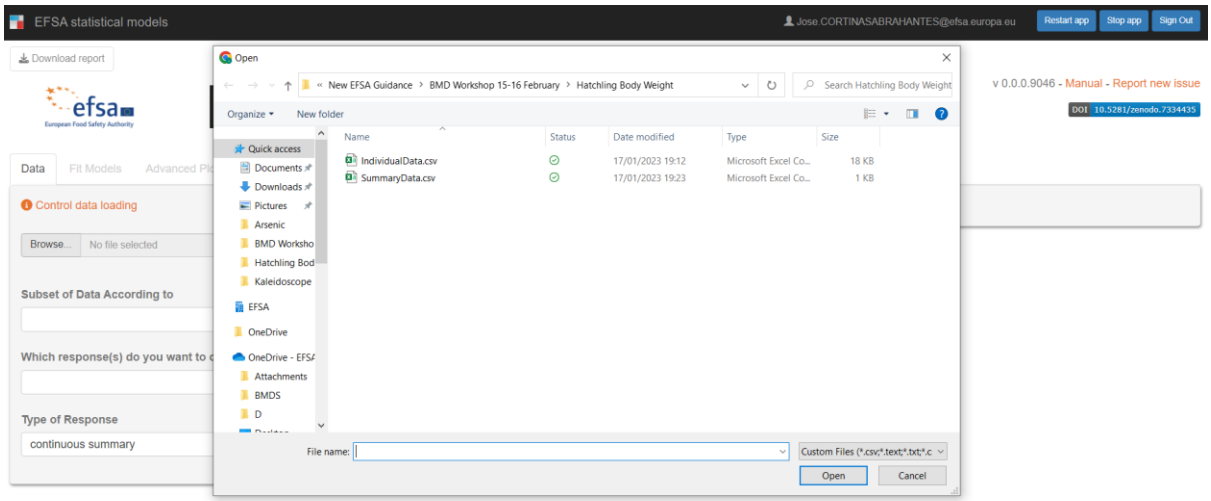
a. Bridge sampling method and do not perform a sensitivity analysis
b. Bridge sampling method and perform a sensitivity analysis
c. Bridge sampling method without performing sensitivity analysis for individual data

Answer: Summary dataset

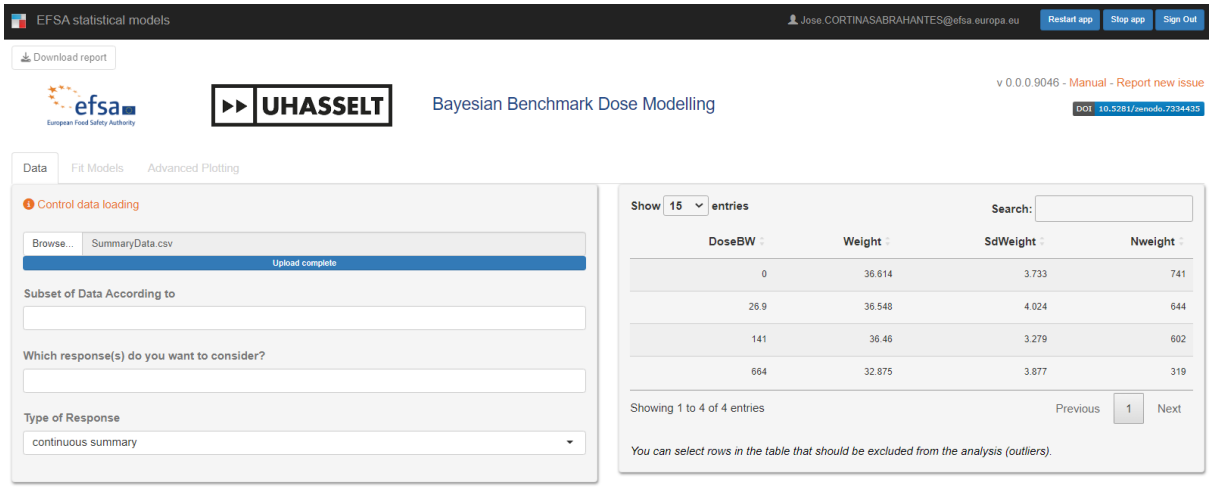
- The first thing to do after registration in the R4EU environment would be to open the application https://r4eu.efsa.europa.eu/app/bmdbayesian. The following window should be displayed in your web browser.



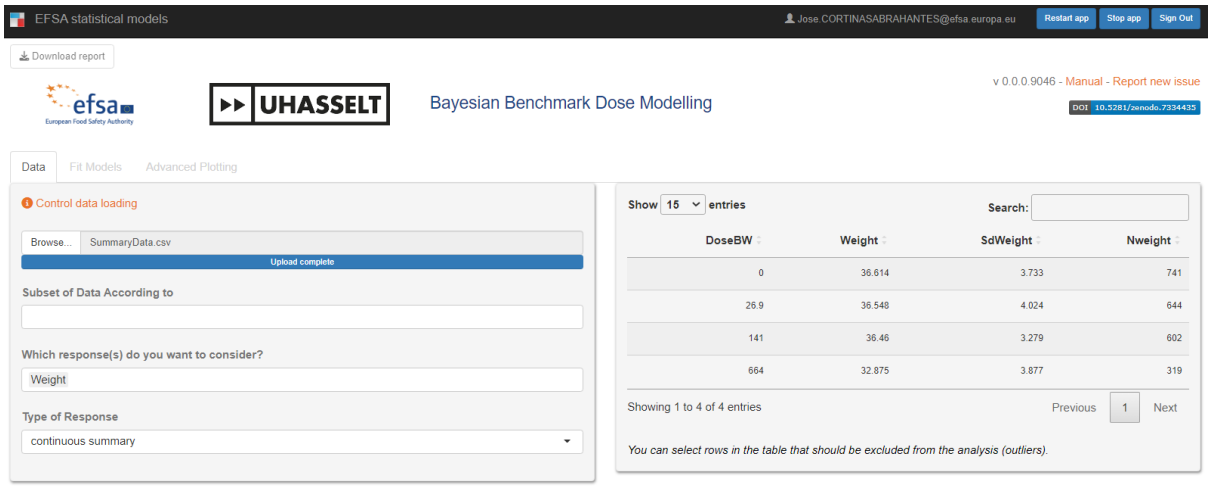
- The data should be uploaded in the web application and for this the user should click on the browser button, where the following window will open. The user should navigate to the folder in which the data has been placed. Subsequently the file should be selected and the button open should be clicked.



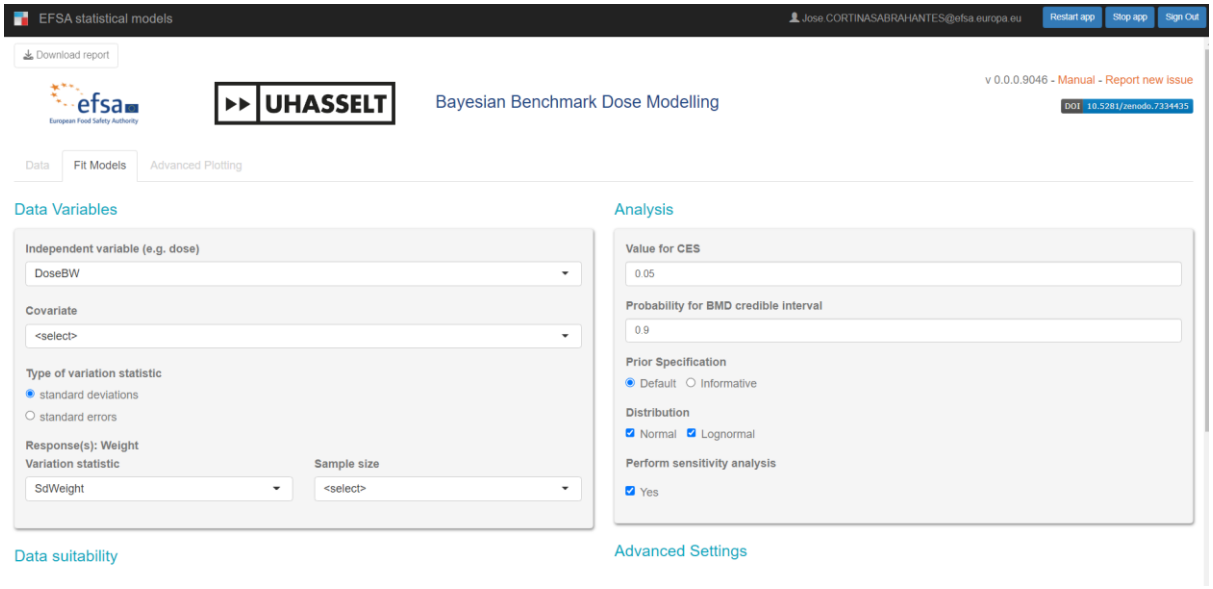
- Once the data is opened the application will show the data on the right side of the window as it is shown below



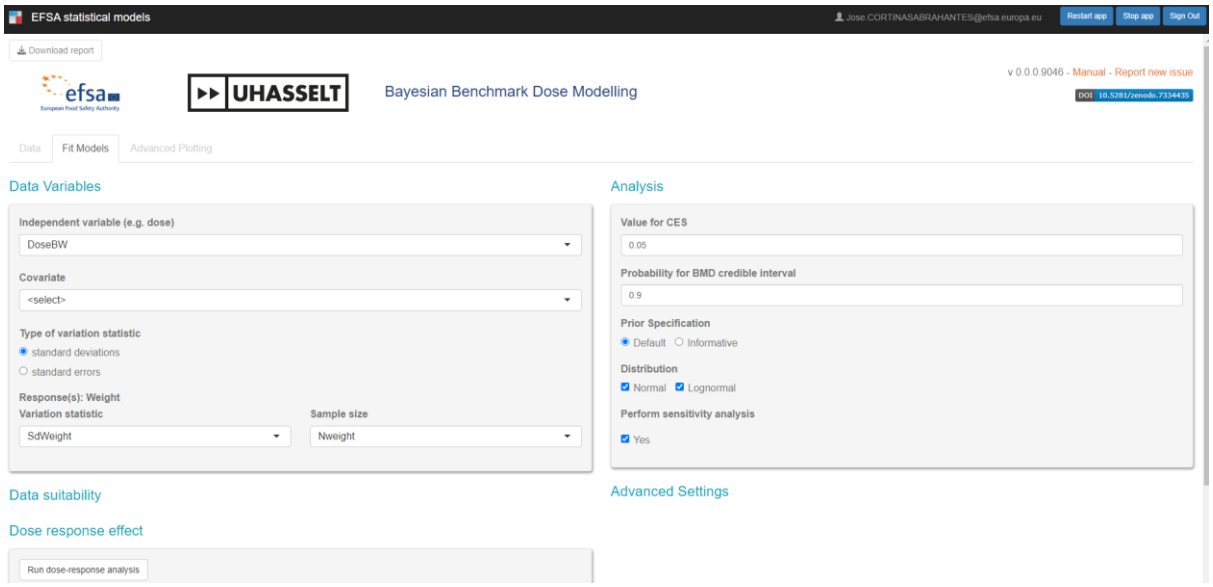
- The next step will be to select the column containing the response for the data uploaded that corresponds to the endpoint measured that we would like to analyse (under the question Which response(s) do …).



- Once the endpoint has been selected, then the type of response that will be analysed should be selected, the choices are quantal, continuous summary or continuous individual. For this specific data the choice is continuous summary, which is the default option of WEB application (meaning that nothing needs to be done in this case).
- Once this is done the next thing to do is to move to the Fit Models tab, where the following window will appear.

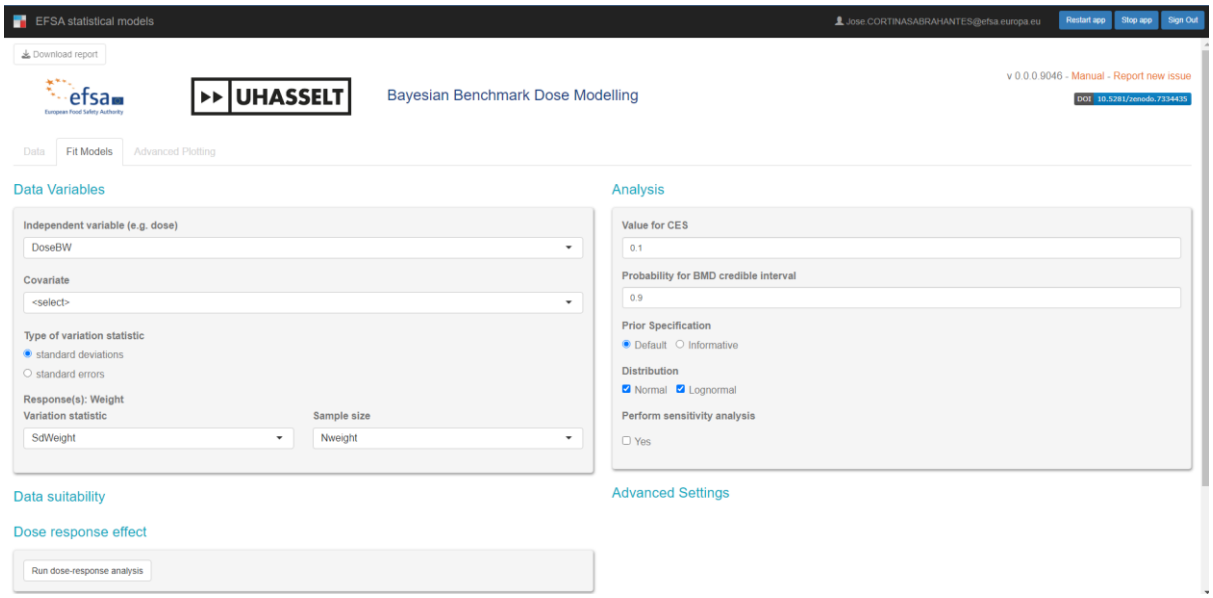


- You can see that some variables are already prefilled, and it is because the application recognises if the variable name contains the string dose in the column names of the data uploaded it will place it as the selection for the independent variable. In case it is not the right column, the appropriate column should be selected. Similarly, the variation statistic and sample size should be selected in order to be able to perform the analysis (see below).

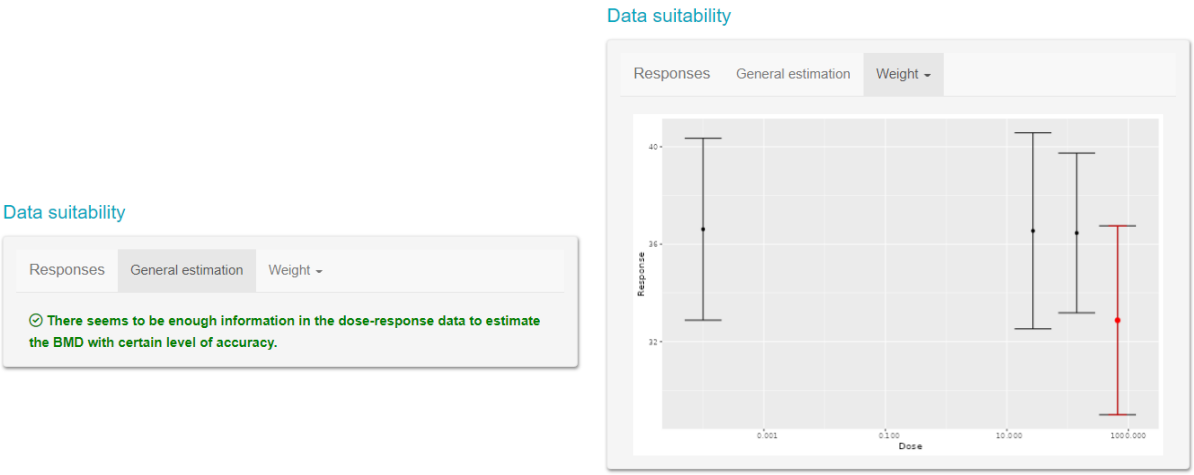


- On the right-hand side of the screen other options are given to the user, the critical effect size (CES) or also called BMR, which in our case should be 0.1, the credible interval of interest, the default value is the one proposed in the EFSA BMD guidance, 90% credible interval. As well the possibility to specify informative priors for the background response, the expected maximum response, and the BMD, also two options are given to the technical

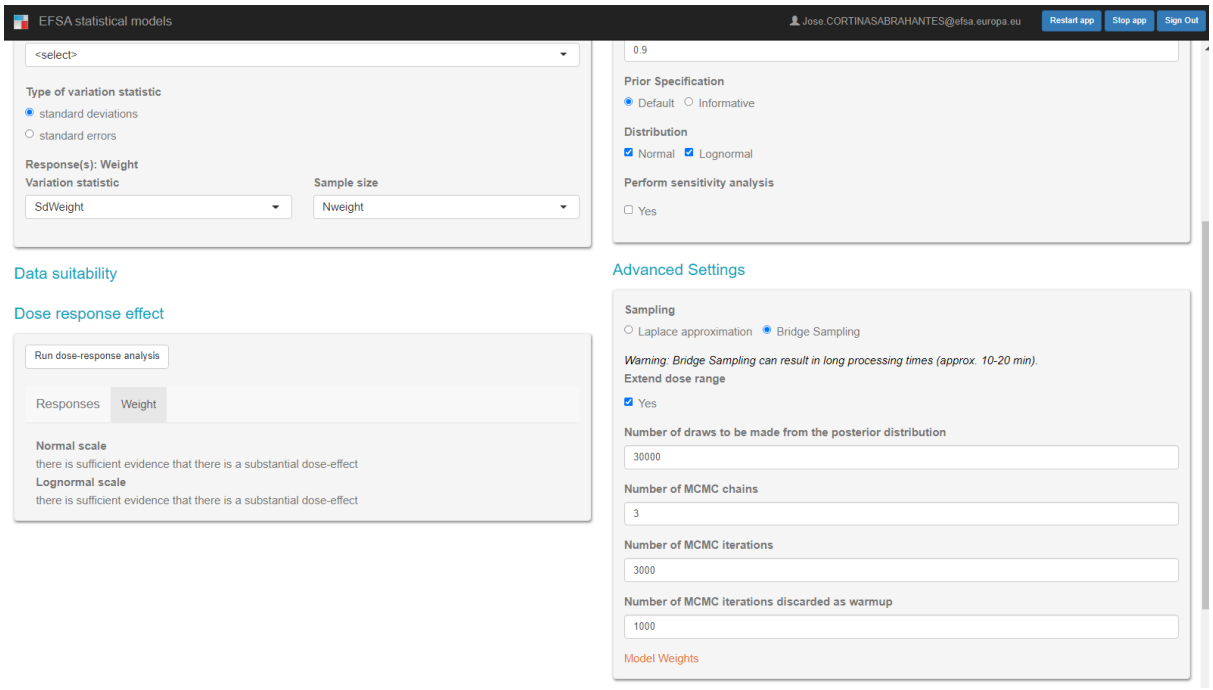
parameter d that has been mentioned yesterday. The choices of distributions that can be used when fitting the models, the default is to have both selected and the possibility to perform a sensitivity analysis in case that homoscedasticity assumptions are not satisfied, by performing the analysis considering the observed minimum variance for all dose groups as well as the maximum one to explore the effect on the resulting credible intervals. Other advanced settings can be specified, and these were also shown yesterday in the presentation of the WEB application. For this specific exercise the CES used is 0.1 and no sensitivity analysis will be performed (see screenshot below).



- Once the options have been selected, for this example the advanced setting “Bridge Sampling” option is ticked, as it is considered the best fitting procedure to be used, but it can take a longer time for specific datasets.
- The next step is to investigate the data suitability for BMD estimation, in other words, to know if there is sufficient information to estimate the BMD with a certain level of accuracy. The following window shows that for this data enough information is present to estimate the BMD with a level of accuracy that could be considered acceptable. It is important to highlight that an alert regarding inadequate level of information in the dose response data to estimate the BMD does not prevent you from going further and perform the BMD analysis, it is just to flag beforehand the amount of information that your data contain to construct a dose-response curve.

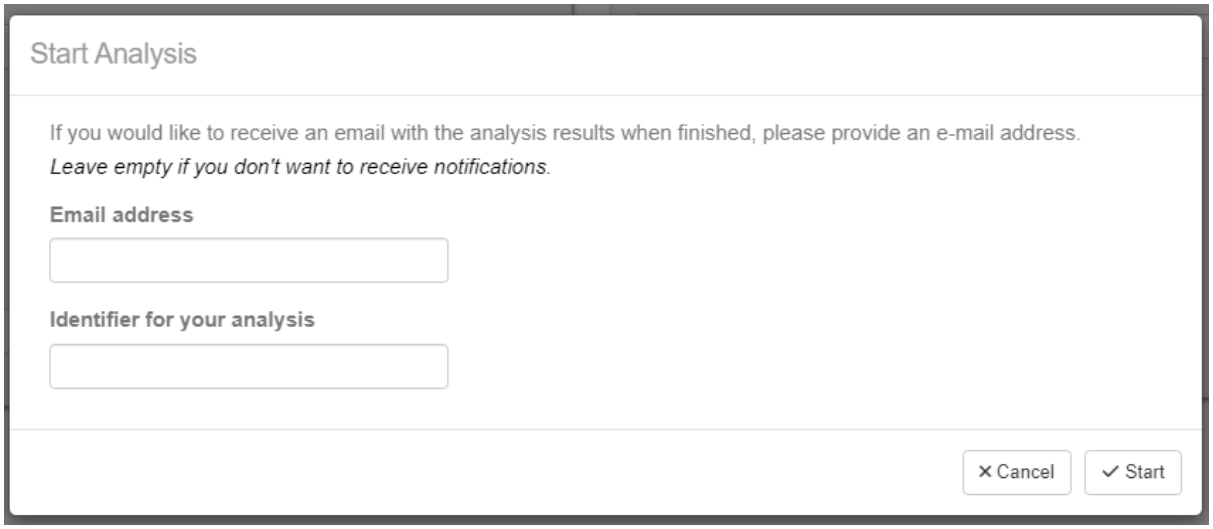


- Then the next step is to investigate if a dose response effect can be identified in the data at hand. Once clicked, the resulting window shows the result for both distributional assumptions (clearly indicating for this data that there is sufficient evidence of a substantial dose-effect).

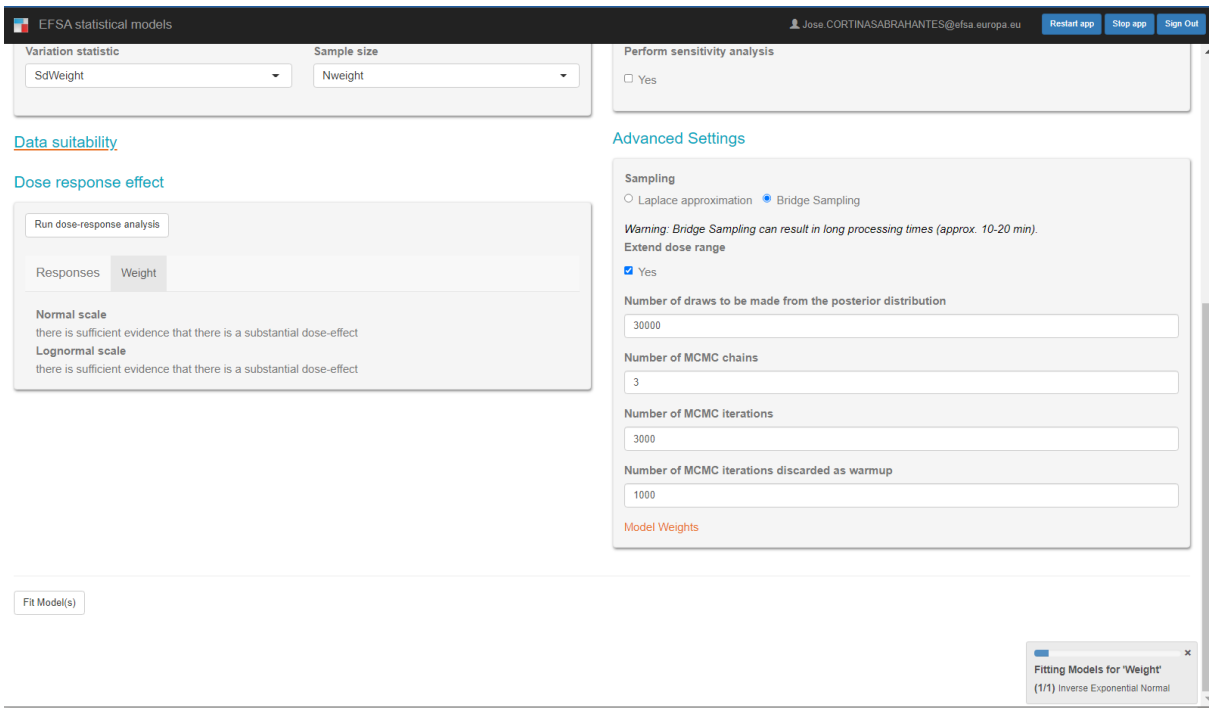


- Once this is done, the models can be fitted, as you probably notice, a new button Fit Model(s) have now appeared and once is clicked then the following popup window will appear, where you can fill in your email address and a name for your analysis, which you will received the report of the analysis in your email inbox once finished the analysis performed, if you leave it in blank, then you will need to download the report later on when the analysis has been finished. It should be highlighted that the options in terms of number of draws, MCMC chains, and the rest of the options, has

been set in order to ensure stable estimation of the posterior distribution, of course the larger the number of draws and MCMC iterations the better the estimation of the posterior distribution, but the default values shown to provide stable results across different simulation scenarios.

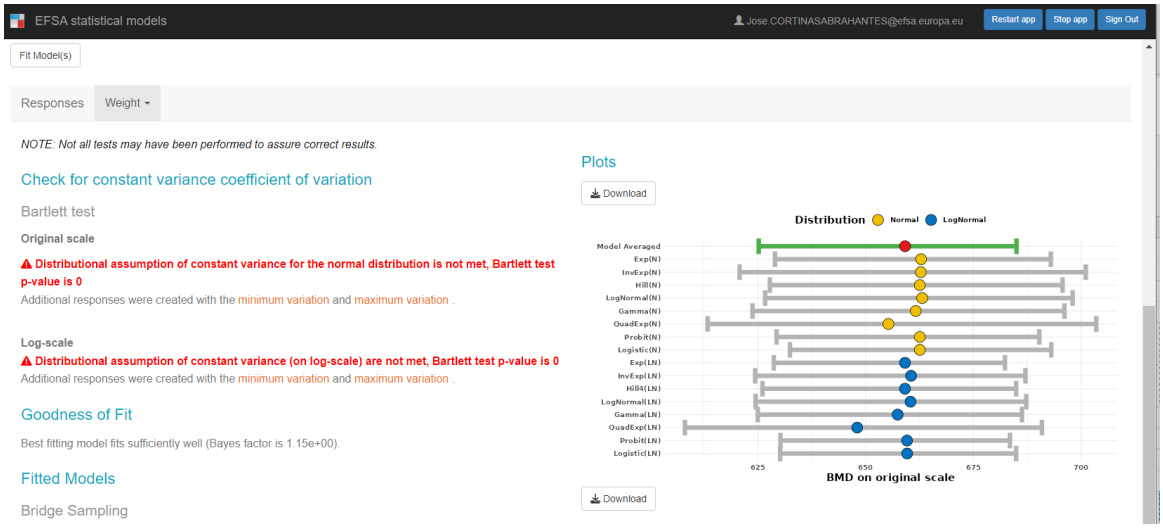


- Once you click on Start then the model will be run and the following window will appear, clearly indicating the model that is being fitted and providing a progress bar to allow the user to know at which point of the analysis the application is.

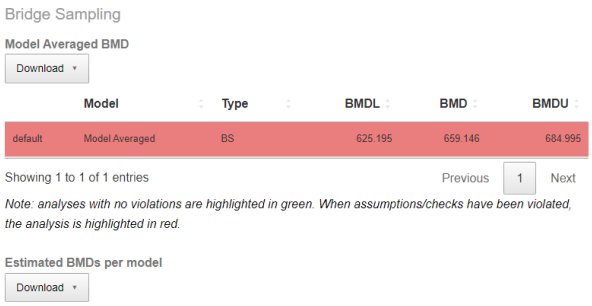


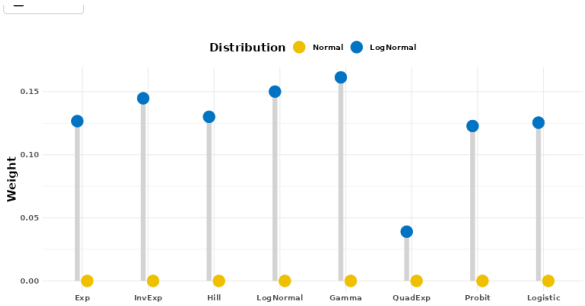
- The resulting outputs of the models fitted are presented here below.
  - Left hand-side: assumptions are checked about homoscedasticity (constancy of variance) for the normal distributional assumption and

constancy of coefficient of variation for the log normal distributional assumption, as well the best fitting model is checked against the saturated model to assess if any of the models is fitting well the data. The test results provide insights in relation to the assumptions of homoscedasticity, which indicates that a sensitivity analysis should be conducted, using the smallest and largest variance observed. Simulations showed that the estimations are fairly robust to violations of homoscedasticity. The sensitivity analysis should provide enough insights on the effect when estimating the lower bound of the credible interval. On the right hand-side the plot with all credible intervals for all models and the model averaged one are shown.



- The table providing the model averaged credible interval for the BMD is providing, highlighting violations on the assumptions of homoscedasticity and constant coefficient of variations for the distributions assumed. The right hand-side shows the plot of the weights of each of the 16 models fitted.





- The table with model specific credible intervals and weights for all models is also provided

Estimated BMDs per model

Download

	Model	BMDL	BMD	BMDU	Model Weights	Converged
1	E4_N	628.964	662.851	692.958	0	1
2	IE4_N	620.756	662.785	701.058	0	1
3	H4_N	627.78	662.567	695.682	0	1
4	LN4_N	626.678	663.115	698.025	0	1
5	G4_N	623.75	661.625	696.13	0	1
6	QE4_N	613.342	655.289	703.494	0	1
7	P4_N	629.331	662.565	690.28	0	0
8	L4_N	632.444	662.582	693.061	0	1
9	E4_LN	628.694	659.124	682.32	0.127	1
10	IE4_LN	624.37	660.544	687.071	0.145	1

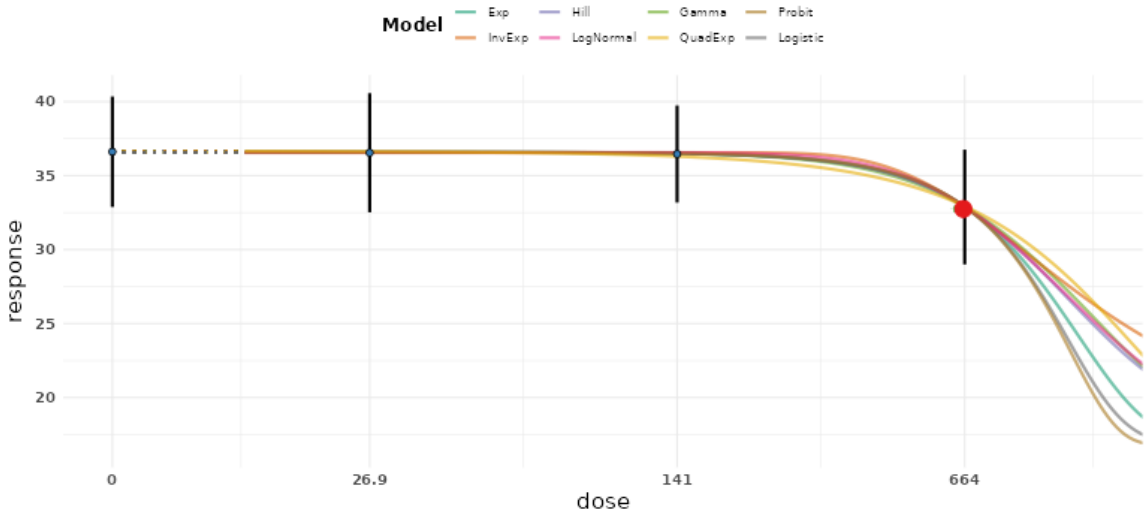
Showing 1 to 10 of 16 entries

Previous 1 2 Next

Note: Numeric values are rounded to 3 decimals.

- The different model fitted for each distributional assumption as well as all models together with the model averaging result and the posterior distribution is shown below

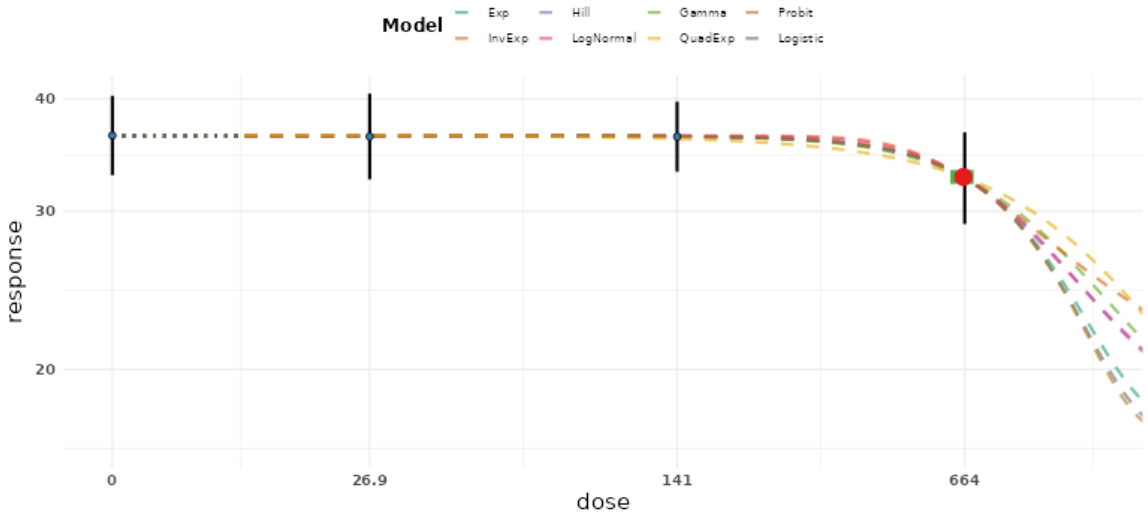
Normal distribution



data and vertical bars based on arithmetic sample means and standard deviations

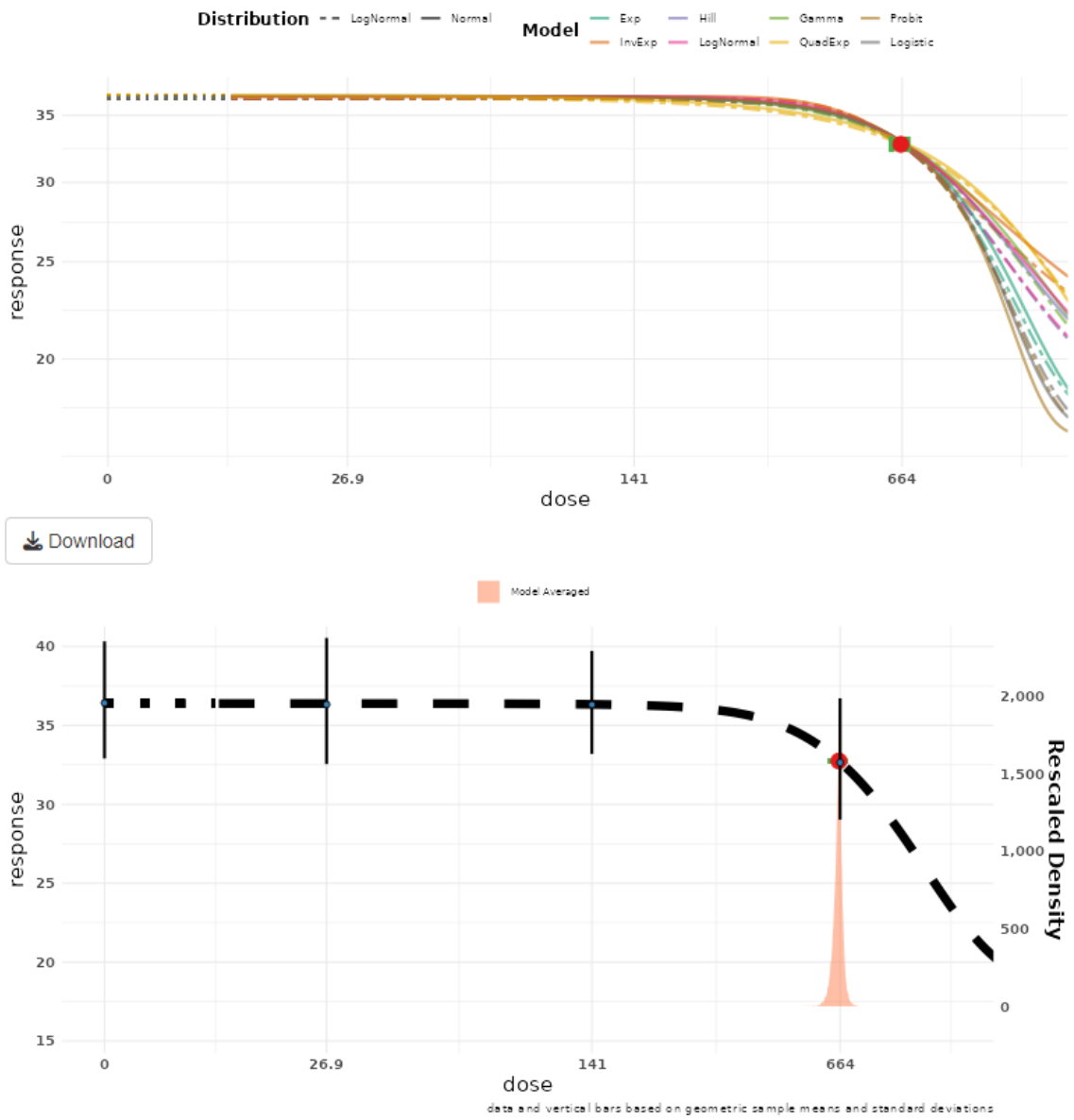
Download

LogNormal distribution



data and vertical bars based on geometric sample means and standard deviations

Download



- EFSA's Scientific Committee Guidance on the use of the BMD approach in risk assessment recommends using the $BMDL_{10}$ of the averaged model as reference point which will be 625.2 mg a.i./kg body weight/day. If instead a biological/scientifically based decision is taken to select a different reference point for this substance, this should be justified. In this specific case, the Birds and mammals' guidance (here) clearly stipulate that the value to be used should be the BMD_{10}, given a study conducted with different endpoints and species, which clearly identifies the BMD_{10} as the estimate of interest in this setting. In this case a BMD_{10} of 659.1 should be selected as the reference point.

- For completeness, the results using the sensitivity analysis were also run and the results are reported below. The lowest BMD_{10} obtained from the sensitivity analysis is 659.1 mg a.i./kg body weight/day, which is rather stable for all analysis performed (659 – 662 mg a.i./kg body weight/day).

Bridge Sampling

Model Averaged BMD

Download

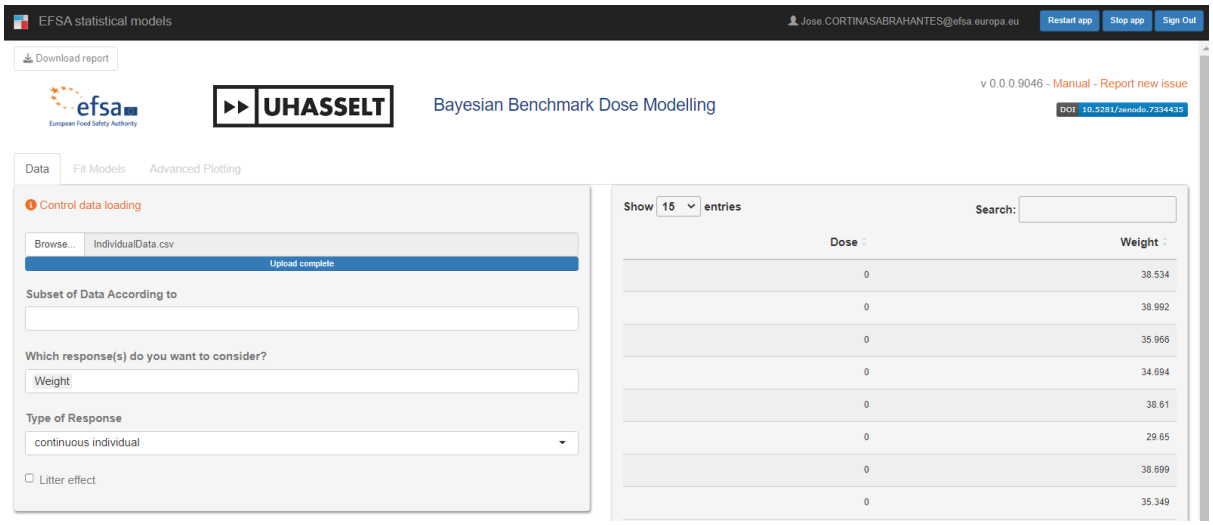
	Model	Type	BMDL	BMD	BMDU
default	Model Averaged	BS	625.195	659.146	684.995
N_min	Model Averaged	BS	630.112	662.31	691.667
N_max	Model Averaged	BS	622.264	662.438	700.504
LN_min	Model Averaged	BS	629.585	659.197	681.001
LN_max	Model Averaged	BS	620.922	660.118	691.188

Showing 1 to 5 of 5 entries Previous 1 Next

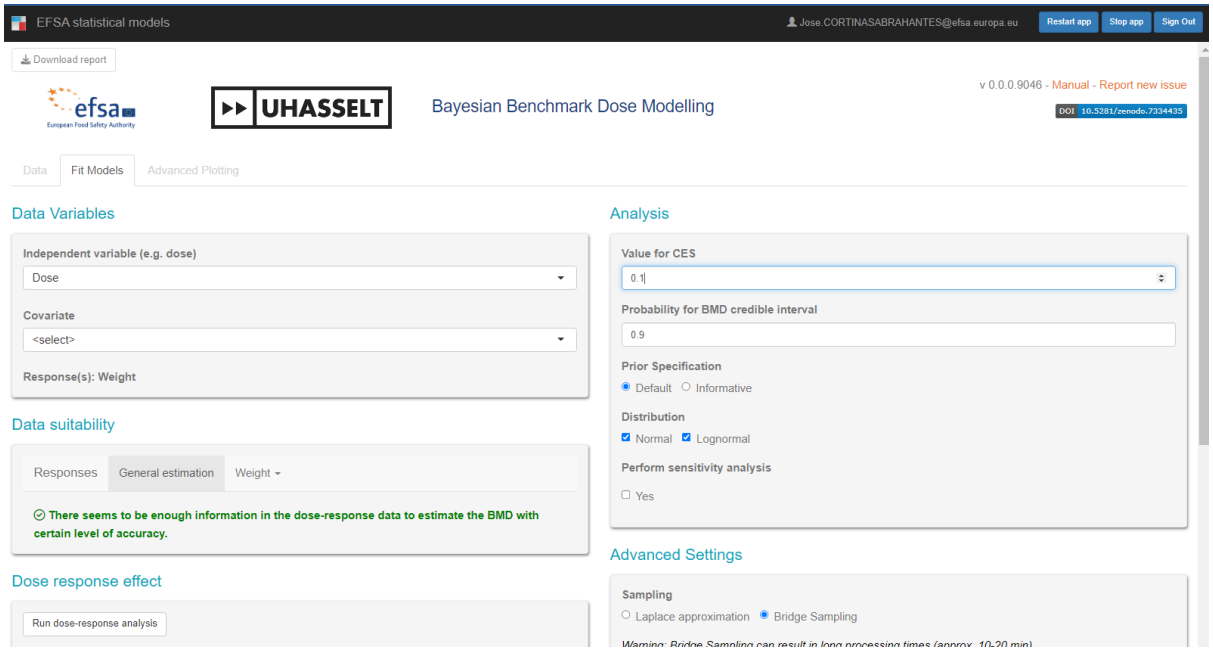
Note: analyses with no violations are highlighted in green. When assumptions/checks have been violated, the analysis is highlighted in red.

Answer: Individual dataset

- Similarly, the individual data is uploaded, the response variable is selected and as well the type of response which will be analysed (in this case continuous individual), see screenshot below

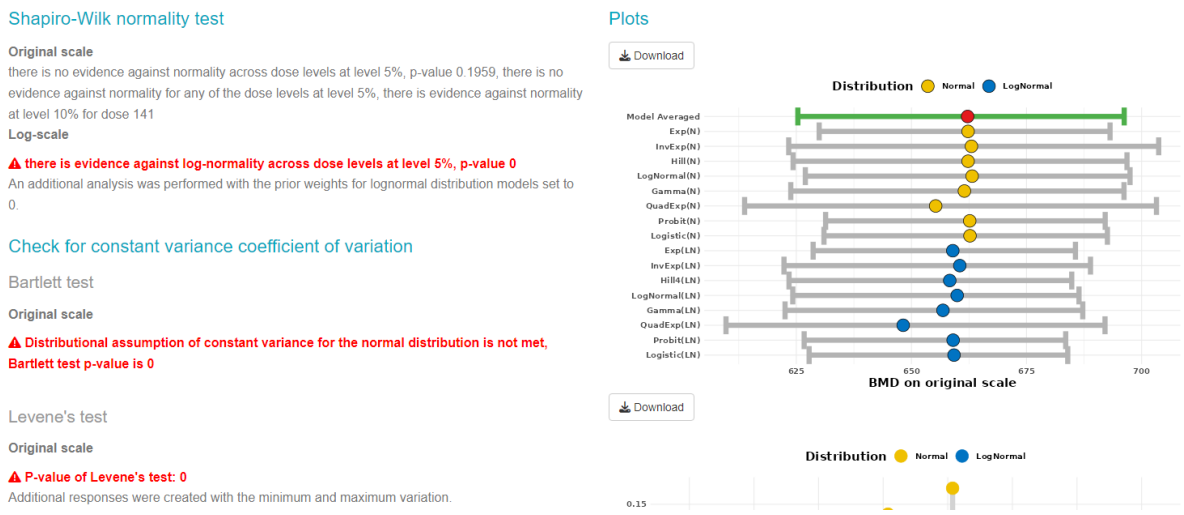


- The options to run the analysis were kept the same, notice that in this case there is no need to select the column containing neither the variation statistic, nor the sample size, as individual data is provided (see below).

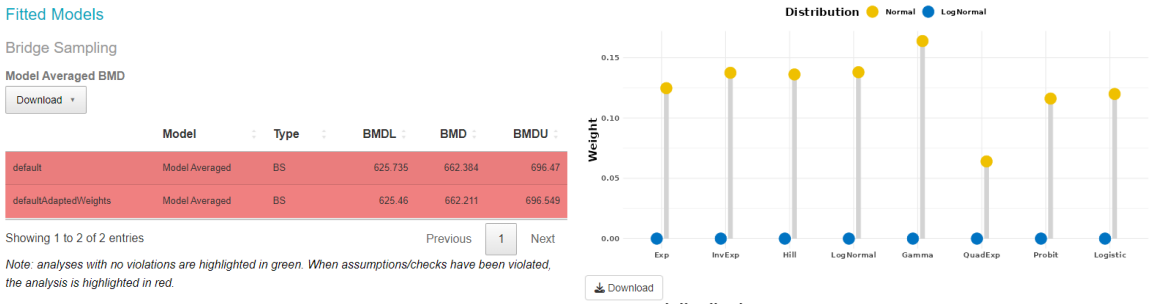


- The resulting outputs of the models fitted are presented as for the case in which summary data was uploaded
  - Left hand-side assumptions about normality or log normality, given that individual data is uploaded, are checked. Also, assumptions about homoscedasticity (constancy of variance) for the normal distributional

assumption and constancy of coefficient of variation for the log normal distributional assumption, as well the best fitting model is checked against the saturated model to assess if any of the models is fitting well the data. On the right hand-side, the plot with all credible intervals for all models and the model averaged one are shown. It is important to highlight here that, as individual data is provided, the distributional assumptions can be formally tested. The Shapiro-Wilk test for the data of this example provide no evidence against normality at 5%, while there is clear evidence against log normality.



- The table providing the model averaged credible interval for the BMD is highlighting violations on the assumptions of homoscedasticity and constant coefficient of variations for the distributions assumed. The right hand-side shows the plot of the weights of each of the 16 models fitted. Also, here it is evident, that the normal models got a much higher weights in comparison to the log normal models, which is the opposite to what was encountered when summary data was provided. This is to illustrate the importance of providing the most detailed information possible to the model, because some of the assumptions made can be statistically tested.



- The table with model specific credible intervals and weights for all models is also provided

Estimated BMDs per model

Download

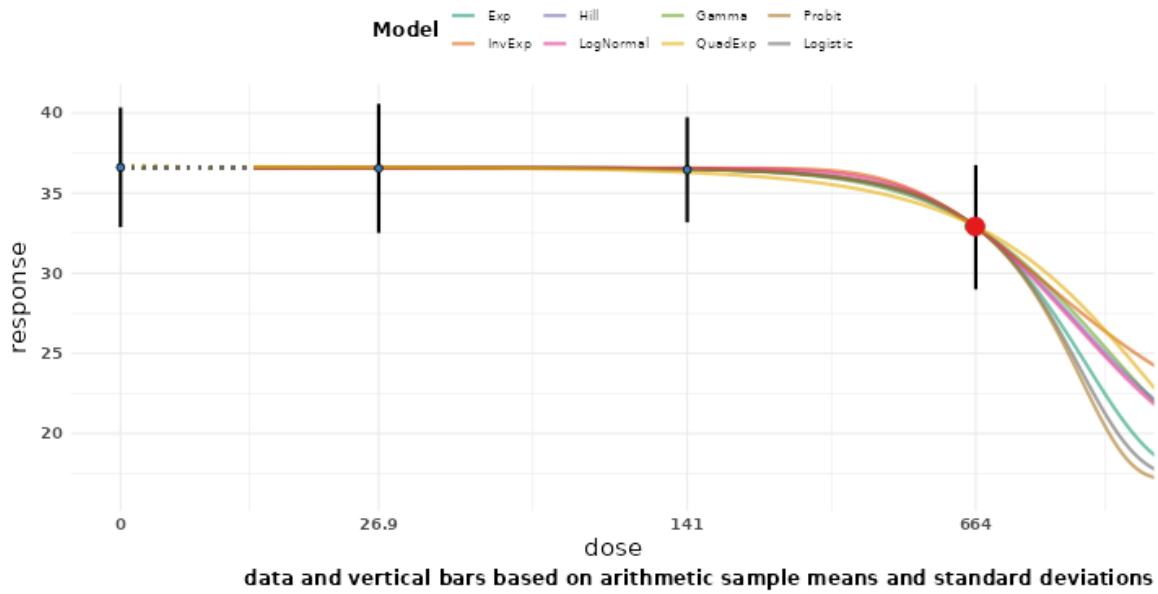
	Model	BMDL	BMD	BMDU	Model Weights	Converged
1	E4_N	627.475	662.102	694.569	0.125	1
2	IE4_N	625.411	664.017	705.841	0.137	1
3	H4_N	625.631	662.34	696.905	0.136	1
4	LN4_N	628.493	663.367	695.224	0.138	1
5	G4_N	622.614	661.307	698.84	0.164	1
6	QE4_N	613.308	654.73	701.65	0.064	1
7	P4_N	630.908	662.379	691.905	0.116	1
8	L4_N	630.421	662.572	691.525	0.12	1
9	E4_LN	624.624	658.654	683.227	0	1
10	IE4_LN	622.213	660.541	689.491	0	1

Showing 1 to 10 of 16 entries Previous 1 2 Next

Note: Numeric values are rounded to 3 decimals.

- The different model fitted for each distributional assumption as well as all models together with the model averaging result and the posterior distribution is shown below

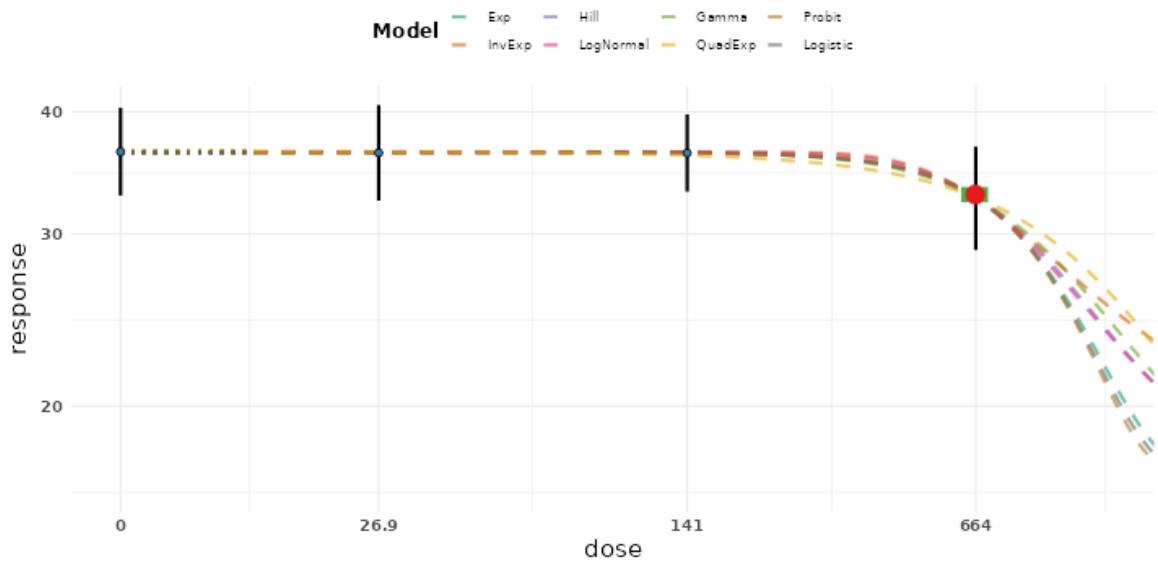
Normal distribution



data and vertical bars based on arithmetic sample means and standard deviations

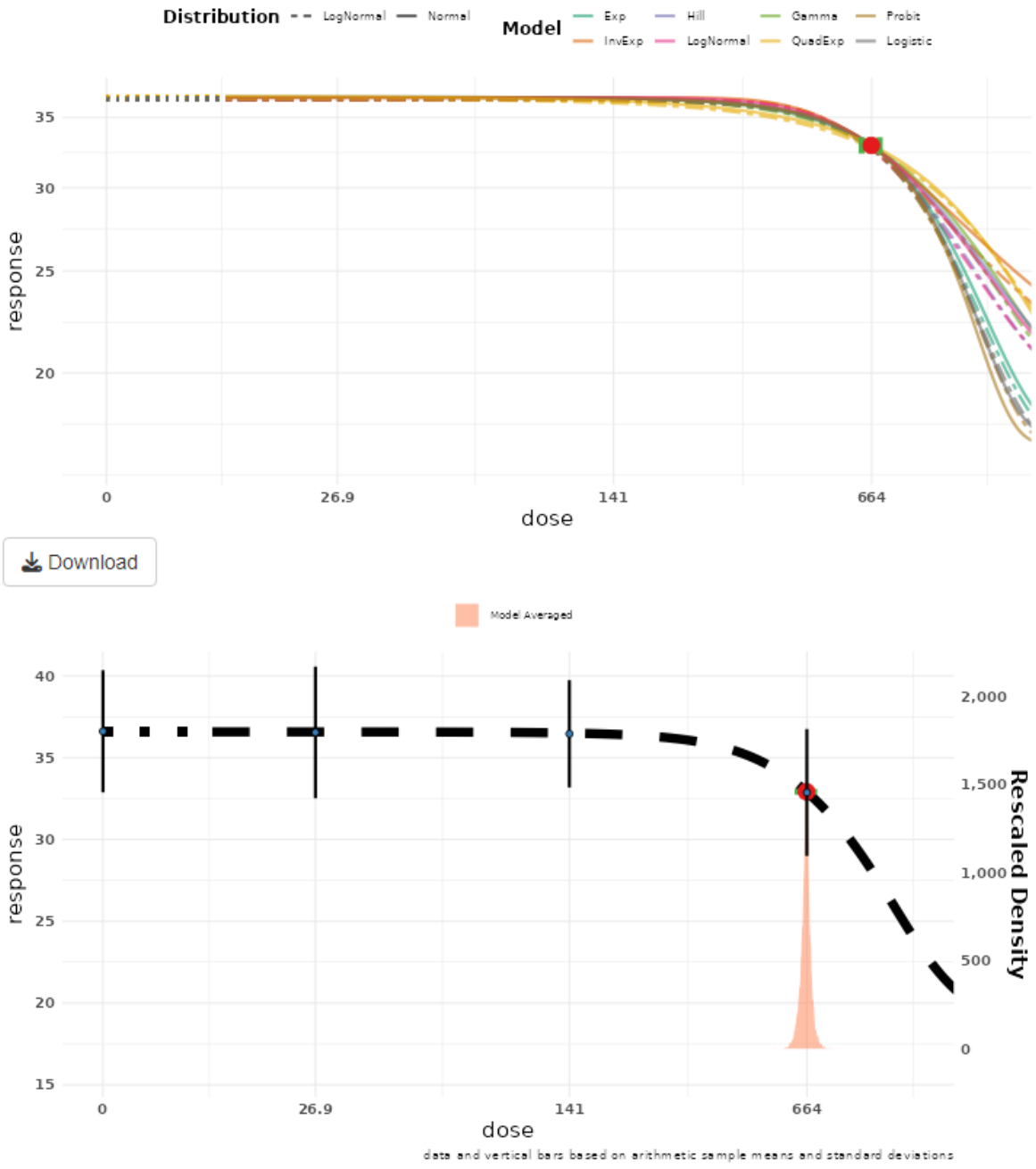
Download

LogNormal distribution



data and vertical bars based on geometric sample means and standard deviations

Download



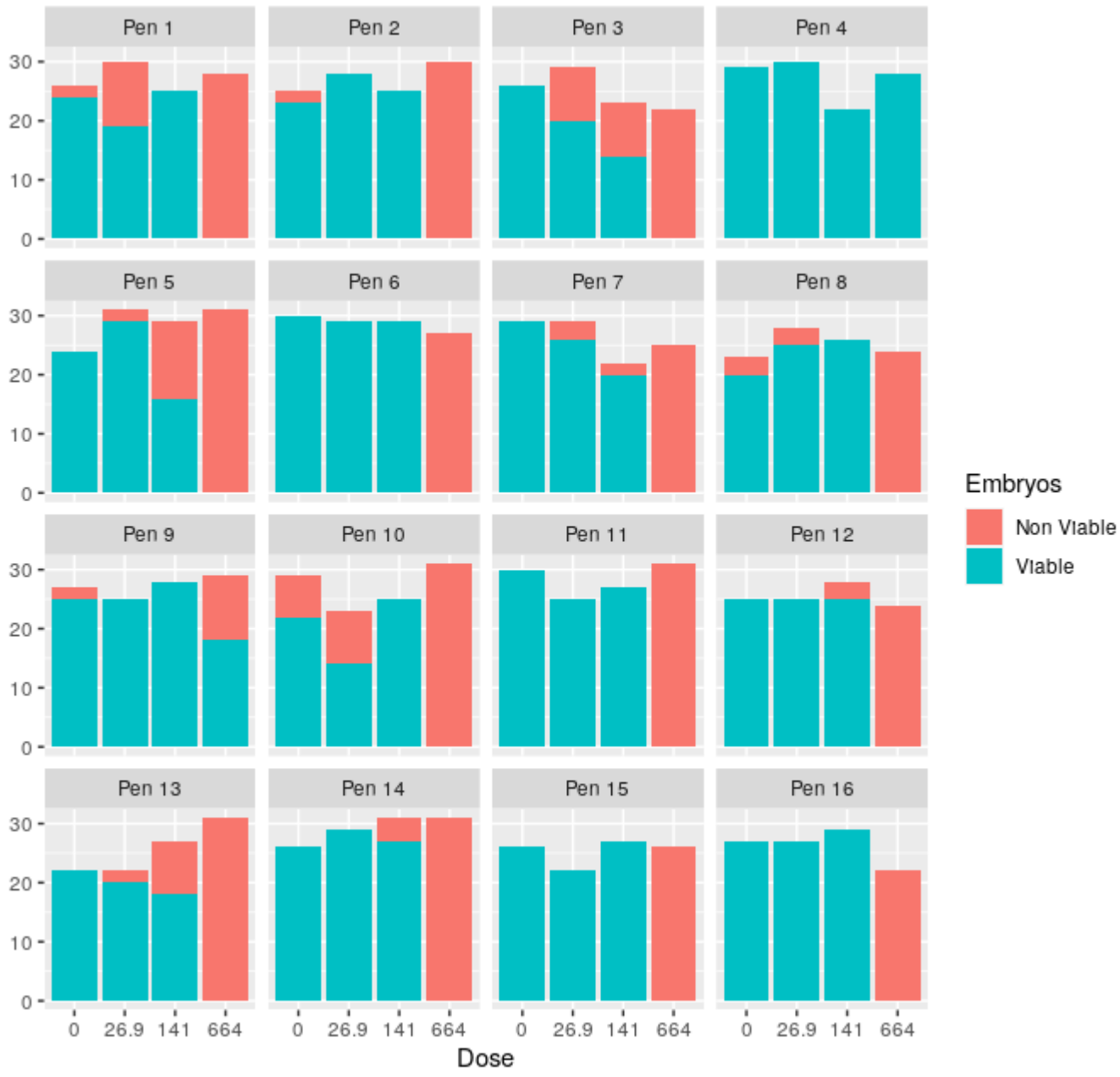
- The BMD_{10} obtained from this analysis indicates that a dose of 662.4 mg a.i./kg body weight/day is the reference point for this substance. It is important to highlight that the values obtained for both datasets are very similar, indicating little impact on the estimation procedure, but individual data would allow to specify the appropriate distribution when analysing the data.

Example 2: Three-weeks nonviable embryos

A study was conducted to assess environmental safety of substance **Y**, several endpoints were studied, and information was recorded for all endpoints considering a dosing scheme of 0, 200, 1000 and 5000 ppm a.i (which corresponds to 0, 26.9, 141 and 664 mg a.i./kg body weight/day) on Mallard ducks. The number of three-weeks nonviable embryos from the eggs set will be used in this example. The dataset for each dose for the first 5 Pens is provided below:

Dose (mg a.i./kg body weight/day)	Live Three-Week Viable Embryos		
	Pen	Nonviable embryos	Eggs set
0	1	2	26
0	2	2	25
0	3	0	26
0	4	0	29
0	5	0	24
26.9	1	11	30
26.9	2	0	28
26.9	3	9	29
26.9	4	0	30
26.9	5	2	31
141	1	0	25
141	2	0	25
141	3	9	23
141	4	0	22
141	5	13	29
664	1	28	28
664	2	30	30
664	3	22	22
664	4	0	28
664	5	31	31

A bar plot of the data that will be used for all 16 Pens can be seen below:



A generalized linear mixed model was fitted considering pen as a clustering factor to compare the different dose groups (results shown below) and the results indicate that there is a difference in the probability of observing three-weeks nonviable embryos for the dose groups tested.

```
Analysis of Deviance Table (Type III Wald chisquare tests)

Response: NonViable
              Chisq Df Pr(>Chisq)
(Intercept)  32.924  1  9.583e-09 ***
Dose        127.676  3  < 2.2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

To correct for multiple testing a Dunnett correction was used, and the results (see below) show a significant increase in the probability of observing three-weeks nonviable embryos for all dose groups tested (highlighted in red) with respect to the control group indicating possible adversity.

```
         Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Dunnett Contrasts


Fit: glmer(formula = NonViable ~ Dose + (1|Pen), data=data, family = binomial)

Linear Hypotheses:
              Estimate Std. Error z value Pr(>|z|)
26.9 - 0 == 0   0.9705     0.3180   3.052  0.00645 **
141 - 0 == 0    1.0687     0.3177   3.364  0.00203 **
664 - 0 == 0    9.6612     0.8555  11.293  < 0.001 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)
```

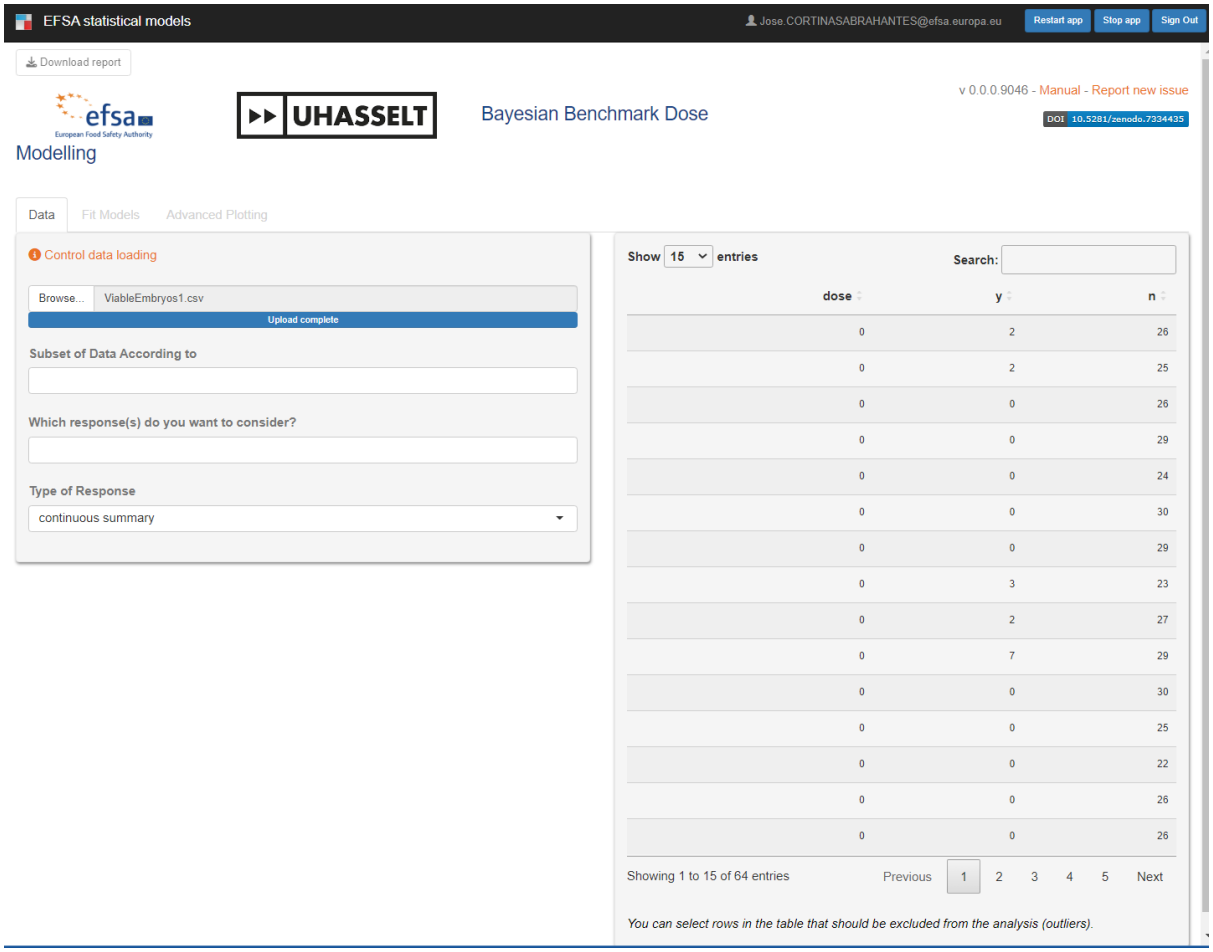
The purpose of this exercise is to fit a dose response curve to the reported data and to estimate the BMD and its 90th credible interval (5, 50 and 95th percentiles should be estimated from the posterior distribution) for a benchmark response (BMR) of 10% relative increase with respect to the background probability of observing three-weeks nonviable embryos, which is the default value mentioned in the legislation as well as the default value for quantal responses considered in the BMD guidance. The question of interest is to estimate the BMD and its credible interval for the endpoint the number of three-weeks nonviable embryos from the eggs set considering a BMR of 10%.

Options to be used:

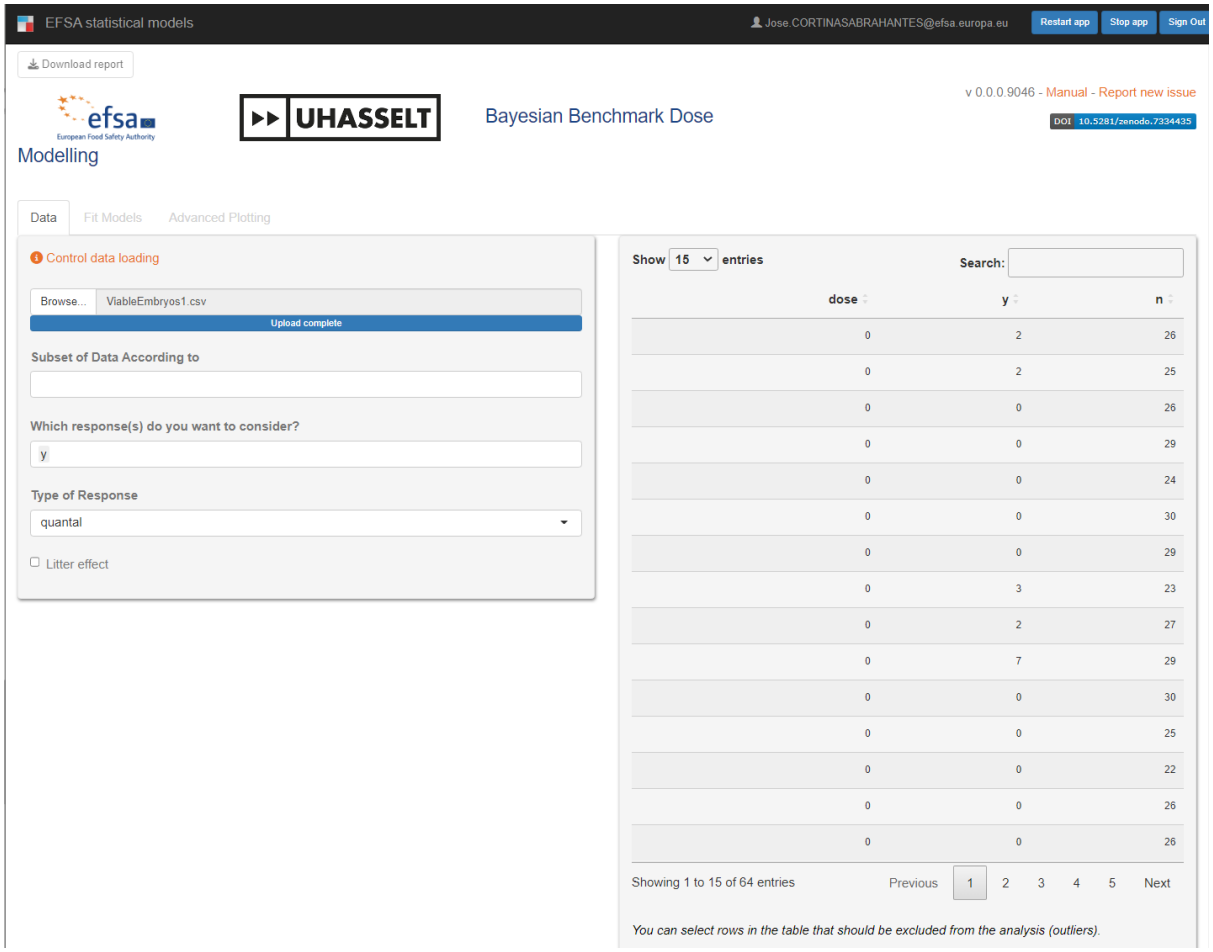
a. Default options (Laplace method) and litter effect

Answer: Three-weeks nonviable embryos

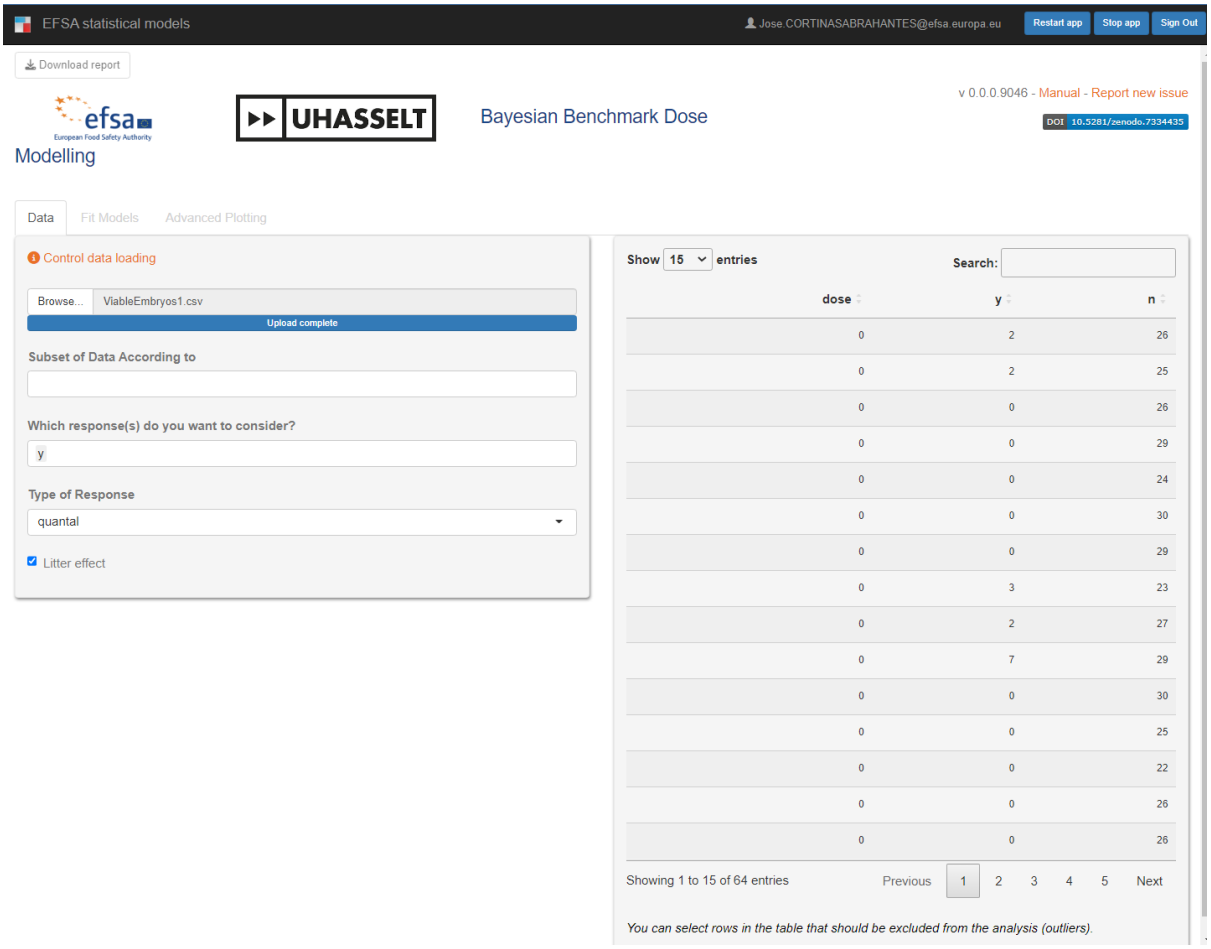
- The data should be uploaded in the web application similarly to the previous example and for this the user should click on the browser button, where the following window will open. The user should navigate to the specific folder in which the data has been placed. Subsequently the file should be selected and the button open should be clicked. Once the data is opened, the application will show the data on the right side of the window as it is shown below



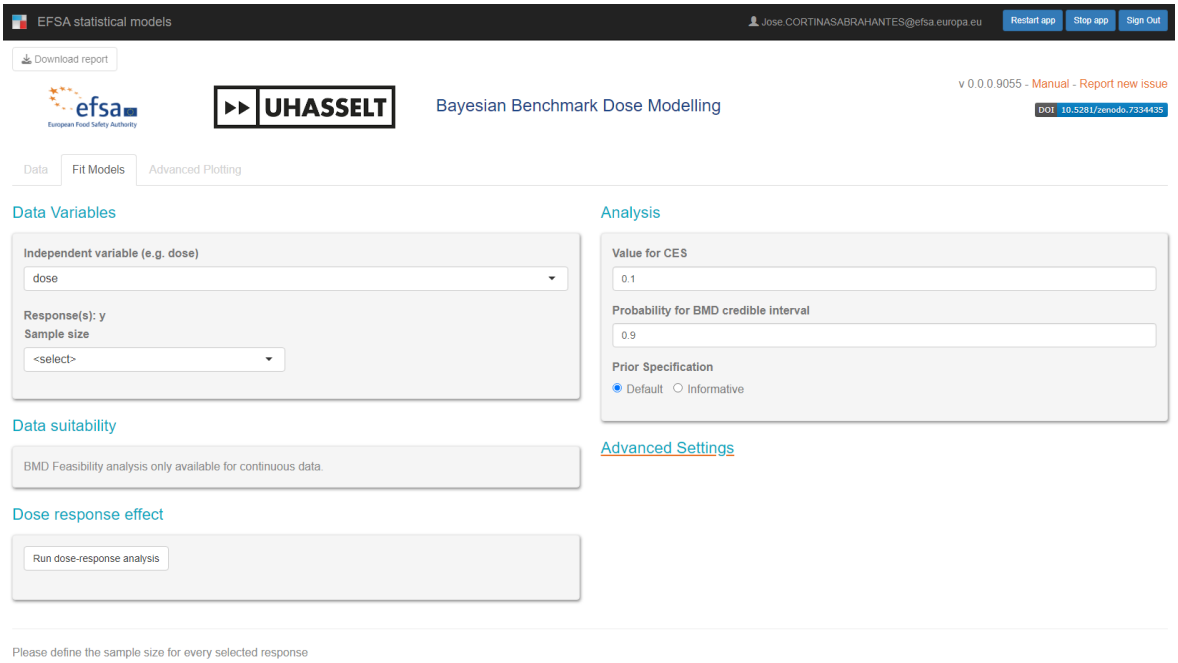
- The next step will be to select the column in the data uploaded that corresponds to the endpoint measured that we would like to analyse. Once the endpoint has been selected, then the type of response that will be analysed should be selected, the choices are quantal, continuous summary or continuous individual. For this specific data, the choice is quantal considering that the data of interest is reflecting the incidence of three-weeks nonviable embryos for each dose and Pen. Note that there are several lines in the data containing the same dose, each line is referring to each of the Pens



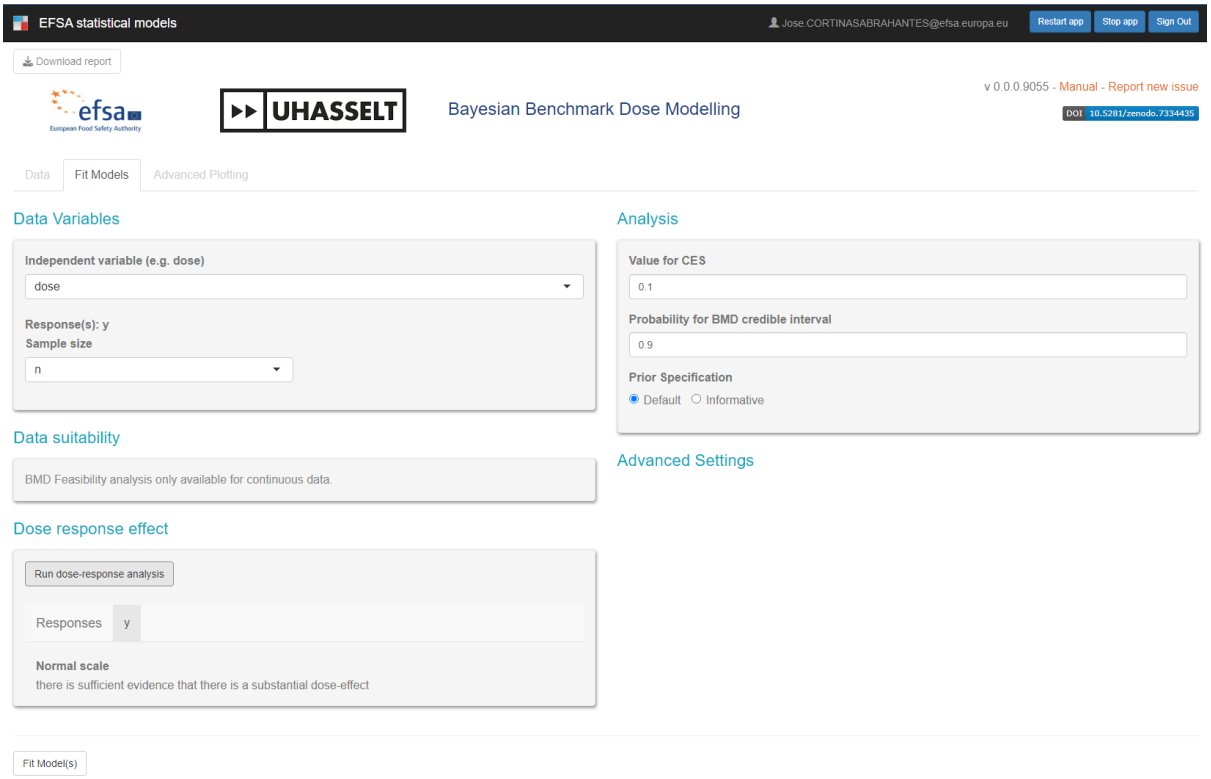
- It can be seen now that below the type of response a new option has appeared, giving the possibility to consider litter effect in the model. In this specific, the eggs sets are coming from 16 different pens, and the likelihood of three-weeks nonviable embryos within a Pen might be correlated, and for this the option litter effect should be marked.



- Once this is done the next thing to do is to move to the Fit Models tab, where the following window will appear. You can notice that this window is now tailored for this type of endpoint, no selection for the variation statistic is displayed.

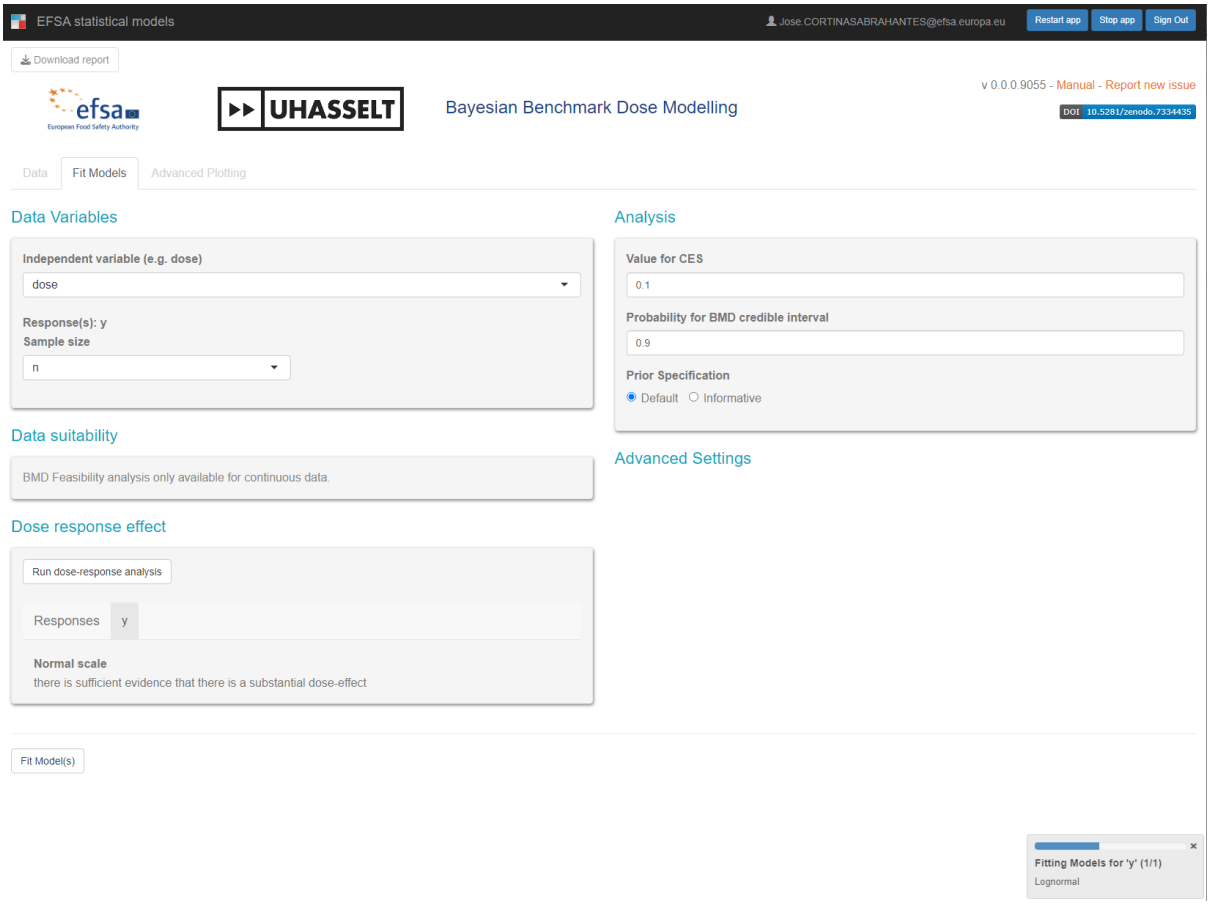


- Once the column in the dataset containing the sample size is selected, a dose response effect can be investigated. This example indicates sufficient evidence of a dose response effect.



- Now the models can be fitted, notice that the BMR for this type of endpoint is already set at 10% (CES = 0.1). In this case we will use the default option of Laplace method to estimate the model parameters, previously the Bridge

sampling method was used, thus no need to show Advance setting in this case.



- Once all models are fitted, the results are shown as for the previous dataset.
- Left hand-side, notice that there is no need to check assumptions about normality or log normality neither about homoscedasticity, but the best fitting model is still checked against the FULL model to assess if any of the models is fitting well the data. On the right hand-side the plot with all credible intervals for all models and the model averaged one are shown. The table providing the model averaged credible interval for the BMD is provided. Clearly, the quadratic exponential model is showing a different behaviour with respect to the other 7 models fitted and its effect will be evaluated in the next output.

Goodness of Fit

Best fitting model fits sufficiently well (Bayes factor is 1.36e-70).

Fitted Models

Full Laplace

Model Averaged BMD

Download

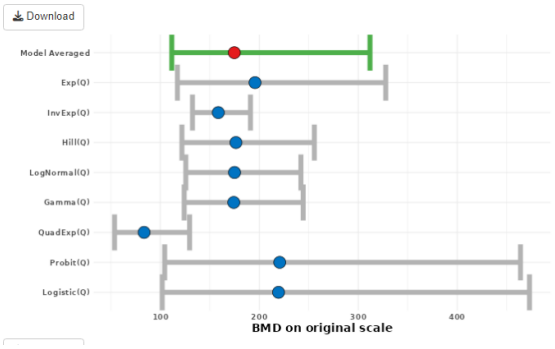
	Model	Type	BMDL	BMD	BMDU
BMDL	Model Averaged	LP	111.528	174.736	311.813

Showing 1 to 1 of 1 entries Previous 1 Next

Estimated BMDs per model

Download

Plots

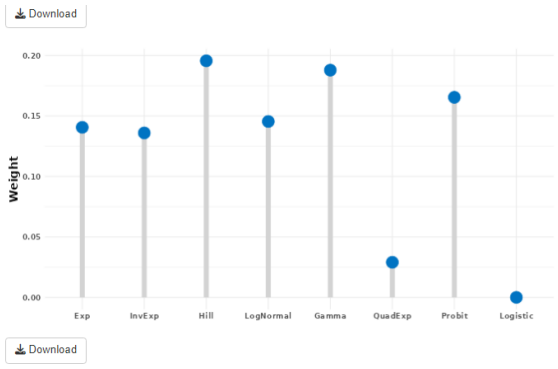


- The table with model specific credible intervals and weights for all models is also provided as well as the plot with the weights for each of the 8 models fitted. It should be highlighted that the Logit model clearly is disregarded from the model averaging and the quadratic exponential provides little contribution to the model averaging results.

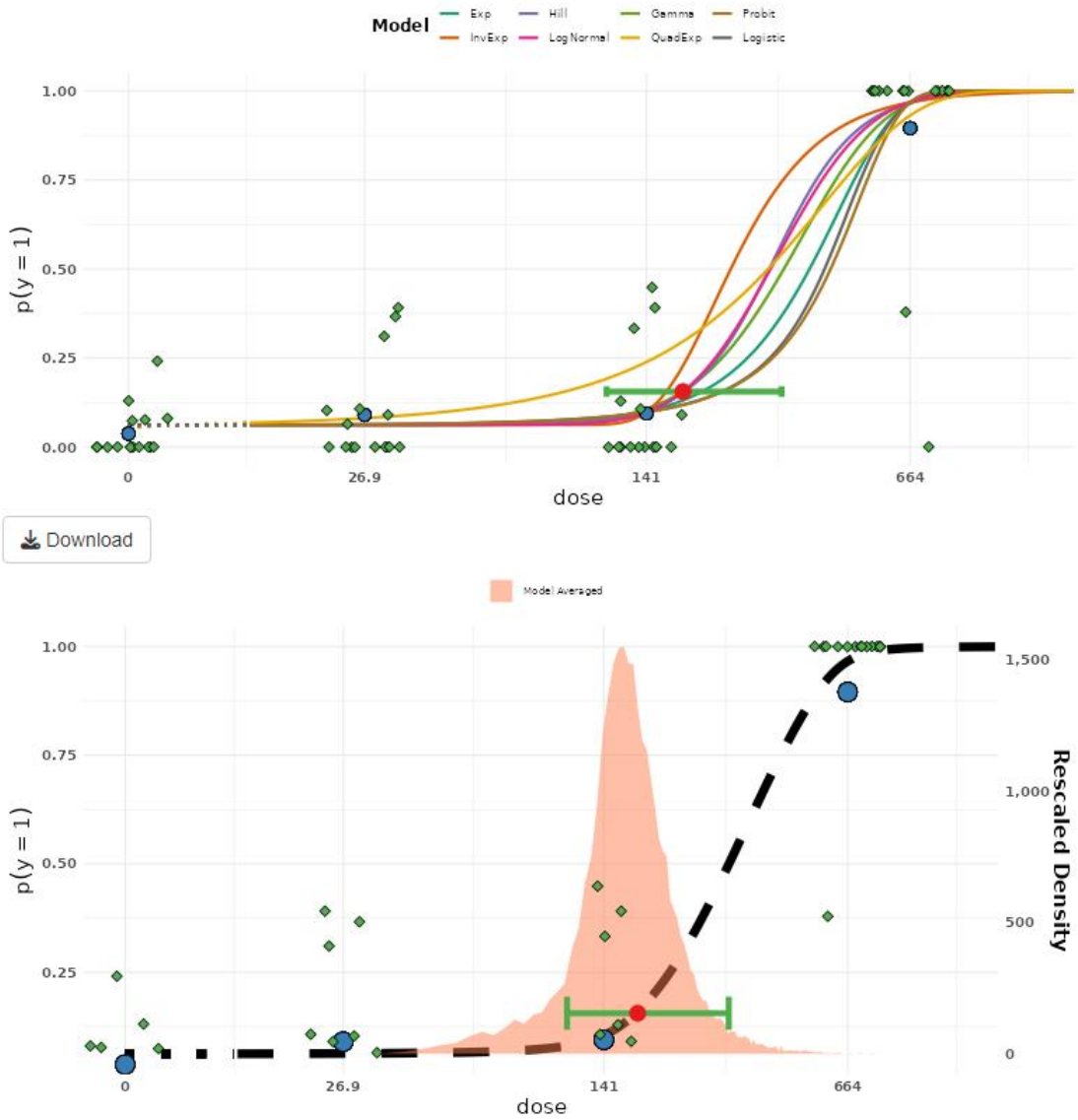
	Model	BMDL	BMD	BMDU	Model Weights
1	E4_Q	117.231	195.648	327.722	0.141
2	IE4_Q	132.444	158.54	191.047	0.136
3	H4_Q	121.783	176.359	255.576	0.196
4	LN4_Q	125.697	174.951	242.023	0.145
5	G4_Q	124.08	174.165	244.295	0.188
6	QE4_Q	53.694	83.56	129.532	0.029
7	P4_Q	104.344	220.568	463.887	0.165
8	L4_Q	101.895	219.405	472.972	0

Showing 1 to 8 of 8 entries Previous 1 Next

Note: Numeric values are rounded to 3 decimals.



- The different models fitted as well as the model averaging result and the posterior distribution is shown below. Notice that the blue dots represent the crude average of the incidence of three-weeks nonviable embryos, and the green rhombus represent the incidence observed in each Pen.



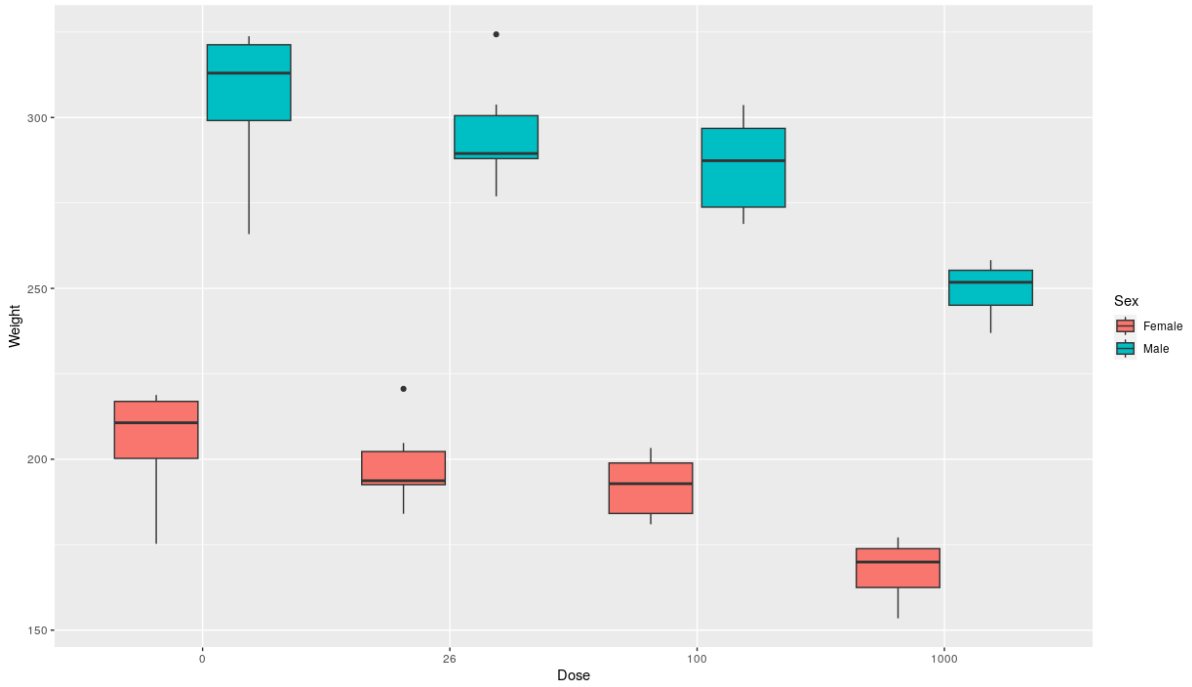
- The BMD_{10} obtained from this analysis indicates that a dose of 174.7 mg a.i./kg body weight/day is the value to use when setting a reference point for substance **Y**.

Example 3: Rat body weight

A 28-day oral rat study on substance **Z** was conducted to assess its repeated-dose toxicity, several endpoints were studied, and information was recorded for all endpoints considering a dosing scheme of 0, 26, 100 and 1000 mg/kg bw per day on Wistar rats. The summary body weights of each of the doses tested will be used for this analysis. The summary of the rats' body weights for each dose are provided below:

		Rats Body Weight		
Dose (unit)	Sex	Mean	Standard deviation	N
0	Male	305.7	22.2	6
26	Male	295.4	16.6	6
100	Male	286.1	14.5	6
1000	Male	249.7	8.1	6
0	Female	205.2	16.7	6
26	Female	198.2	12.8	6
100	Female	192.0	9.3	6
1000	Female	167.6	9.0	6

A box plot of the data that will be used can be seen below:



An ANOVA model was fitted to compare the different dose groups also considering the interaction with the Sex (results shown below) and the results indicate that there is a no interaction effect (highlighted in red), meaning that a model containing the main effects only can be used instead.

```
            Df Sum Sq Mean Sq F value   Pr(>F)
Dose         3  14921    4974  24.050 4.63e-09 ***
Sex          1 104749  104749 506.500  < 2e-16 ***
Dose:Sex     3    577     192   0.931    0.435
Residuals   40   8272     207
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

When the model containing the main effects was used, the results indicate that there is a difference in weights for the dose groups tested.

```
            Df Sum Sq Mean Sq F value   Pr(>F)
Dose         3  14921    4974   24.17 2.54e-09 ***
Sex          1 104749  104749  508.96  < 2e-16 ***
Residuals   43   8850     206
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

To correct for multiple testing a Dunnett correction was used, and the results (see below) show a significant decrease in weight for the two highest dose group tested (highlighted in red) with respect to the control group indicating possible adversity.

```
         Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Dunnett Contrasts


Fit: aov(formula = Weight ~ Dose + Sex, data = IndividualData)

Linear Hypotheses:
               Estimate Std. Error t value Pr(>|t|)
26 - 0 == 0     -8.674      5.857  -1.481   0.3274
100 - 0 == 0   -16.403      5.857  -2.801   0.0207 *
1000 - 0 == 0  -46.808      5.857  -7.992   <0.001 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)
```

The purpose of this exercise is to fit a dose response curve to the reported data and to estimate the BMD and its credible interval (90, 5, 50 and 95th percentiles should be estimated from the posterior distribution) for a benchmark response

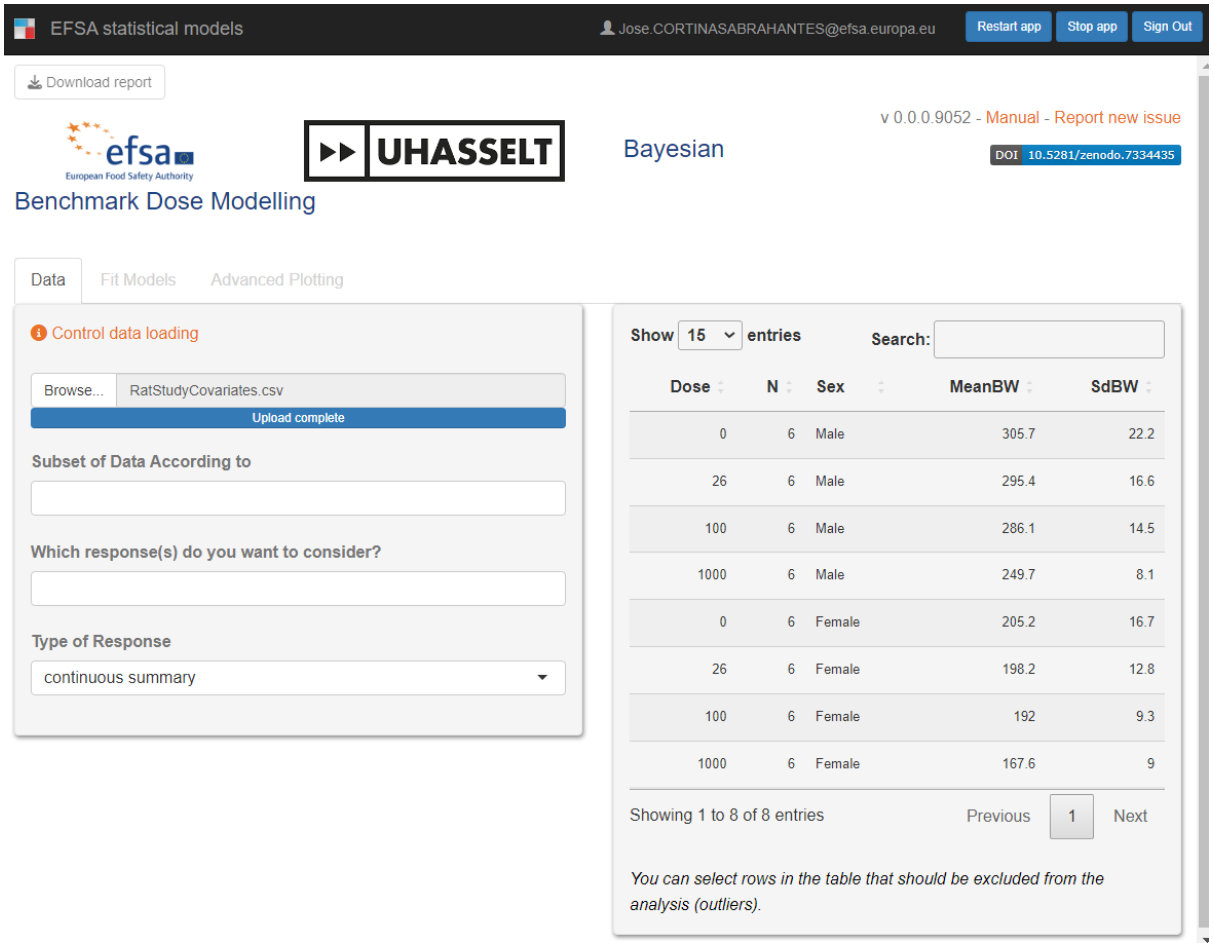
(BMR) of 10% relative decrease of body weight with respect to the background body weight (body weight expected in the control group), which was justified considering the biological relevance of the effects and the variability observed in the parameters (variability observed is greater than 5 % relative change of the mean levels) used in a similar assessment performed by EFSA (https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2022.7582). The question of interest will then be to estimate the BMD and its credible interval for the endpoint rats body weights considering a BMR of 10% taking also into account the effect of sex.

Options to be used:

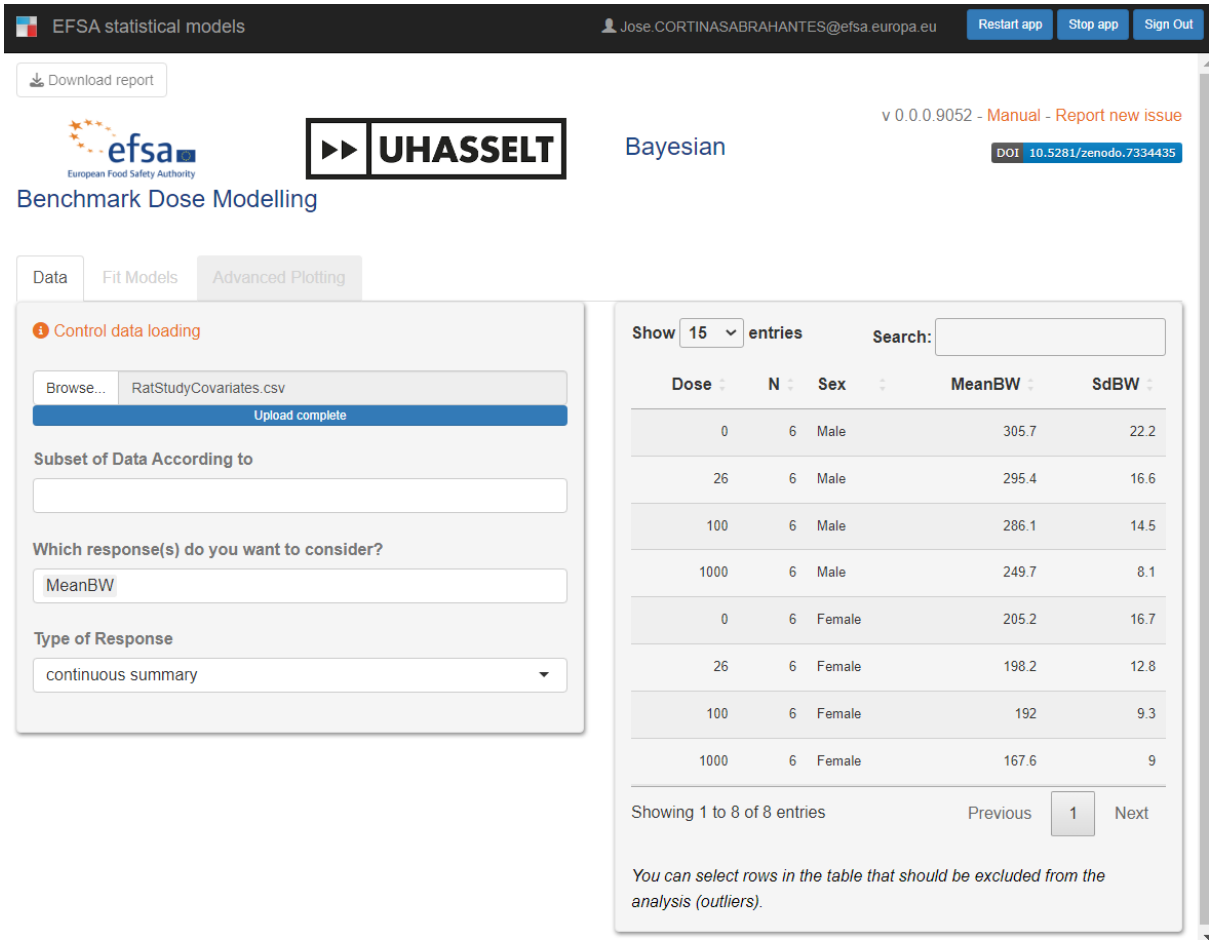
a. Default options (Laplace method) and covariates

Answer: Rat body weights

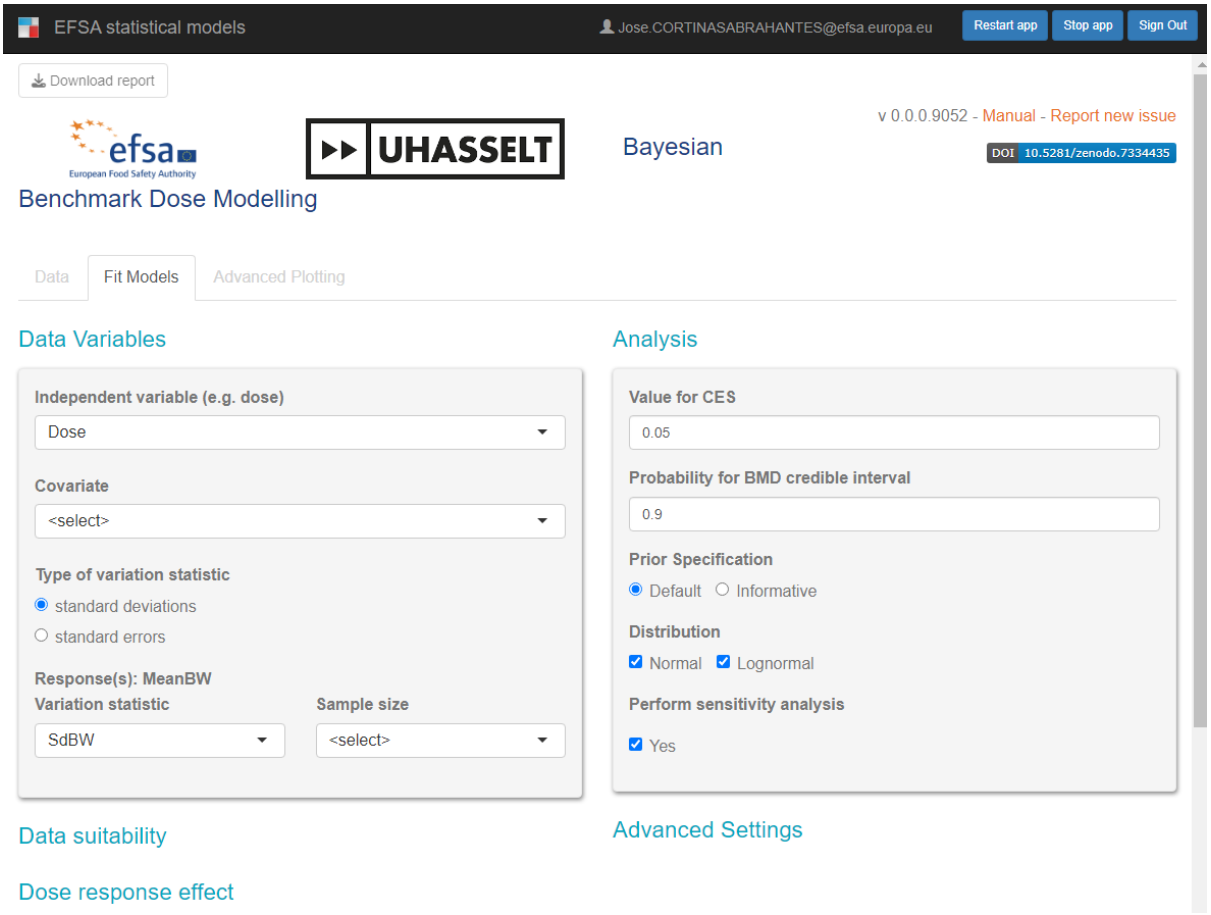
- The data should be uploaded in the web application similarly to the previous example and for this the user should click on the browser button, where the following window will be open. The user should navigate to the specific folder in which the data has been placed. Subsequently the file should be selected and the button open should be clicked. Once the data is opened the application will show the data on the right side of the window as it is shown below



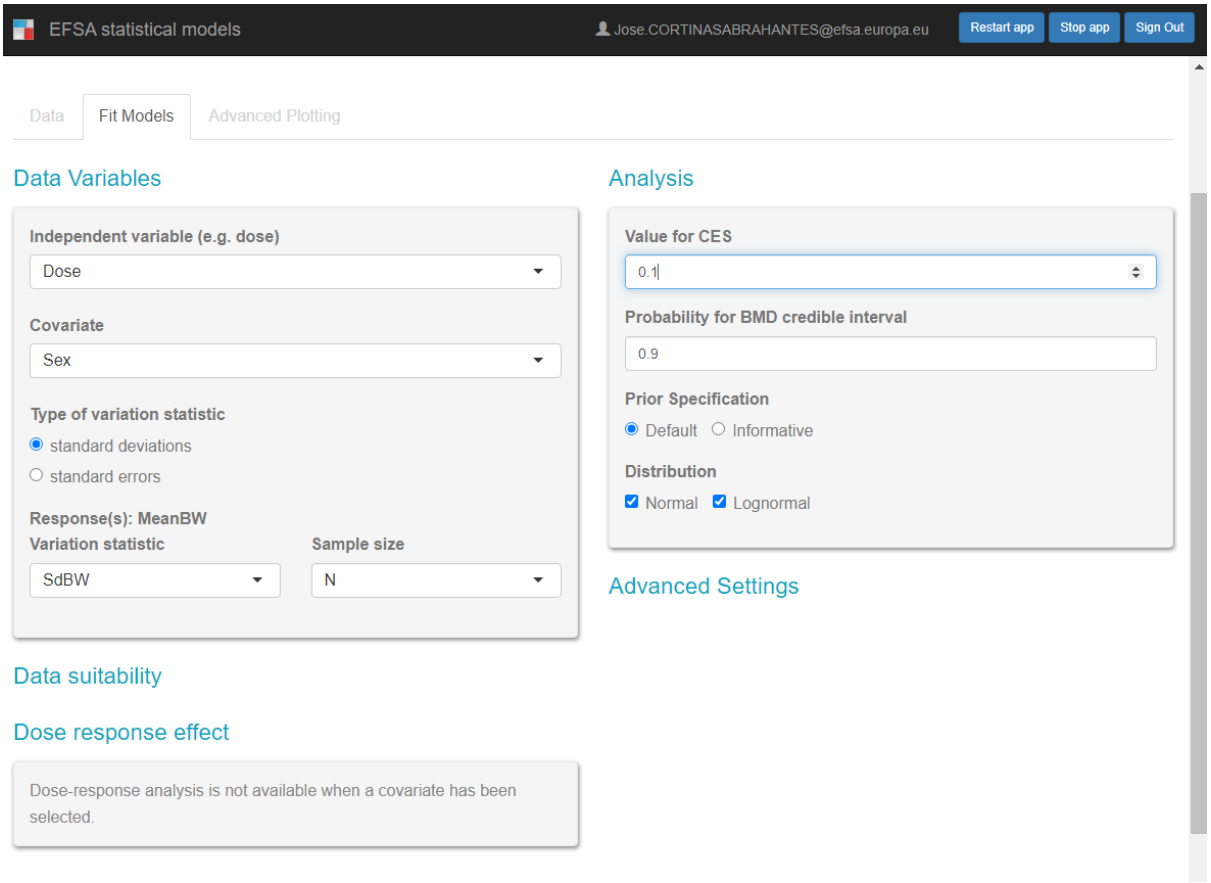
- The next step will be to select the column in the data uploaded that corresponds to the endpoint measured that we would like to analyse. Once the endpoint has been selected, then the type of response that will be analysed should be selected, the choices are quantal, continuous summary or continuous individual. For this specific data the default option is the correct. Notice that there is a new column in the table containing the covariate of interest (Sex), and this will be used later to perform the BMD analysis.



- Once this is done the next thing to do is to move to the Fit Models tab, where the following window will appear. You can notice that this window is the same as what it was shown for exercise 1. Note that the CES is set to be 0.05, but we should use 0.1 instead according to the justification provided in the exercise.



- Now in this case the BMD analysis should account for potential differences between the two sexes and the covariate option should be used, selecting the appropriate column in the table containing the covariate information, also the sample size should be provided.



- In this case we will use the default option of Laplace method to estimate the model parameters. In general, the recommended option to use for the final analysis would be to use the Bridge sampling method, in general results of both methods are similar, but the Bridge sampling could be computer intensive, that is why the Laplace option is good for explorative purposes. Once all models are fitted, the results are shown as for the previous dataset.

- For analysis with covariates, the results provided by the tool are the model averaging result for each covariate level, the table with the different models fitted, their respective credible intervals, final weights, and weights from the selection within each model considering the parameters to be covariate dependent, showing the results for each covariate level. Also, the 16 best sub models fitted are graphically presented showing the data and the curve that represents the dose-response relationship.

Fitted Models

Model Averaged BMD

Download

	BMDL	BMD	BMDU
Male	125.994	299.636	707.076
Female	123.571	308.233	789.556

Showing 1 to 2 of 2 entries Previous 1 Next

- The table with model specific credible intervals and overall weights for all models is also provided as well as the weight of the best sub model for each of the 16 models fitted.

Estimated BMDs per model

Download

	Model	Weight	Submodel	Submodel Weight	Sex	BMDL	BMD	BMDU
1	E4_N	0.032	a_sigma2	1	Male	142.522	294.499	608.798
2	E4_N	0.032	a_sigma2	1	Female	142.522	294.499	608.798
3	IE4_N	0.011	a_sigma2	0.897	Male	112.531	235.575	493.995
4	IE4_N	0.011	a_sigma2	0.897	Female	112.531	235.575	493.995
5	H4_N	0.064	all	0.619	Male	115.714	306.517	796.602
6	H4_N	0.064	all	0.619	Female	105.355	324.513	999.27
7	LN4_N	0.015	a_sigma2	0.941	Male	128.477	264.64	550.891
8	LN4_N	0.015	a_sigma2	0.941	Female	128.477	264.64	550.891
9	G4_N	0.029	a_sigma2	0.664	Male	154.382	312.53	621.489
10	G4_N	0.029	a_sigma2	0.664	Female	154.382	312.53	621.489

Showing 1 to 10 of 32 entries Previous 1 2 3 4 Next

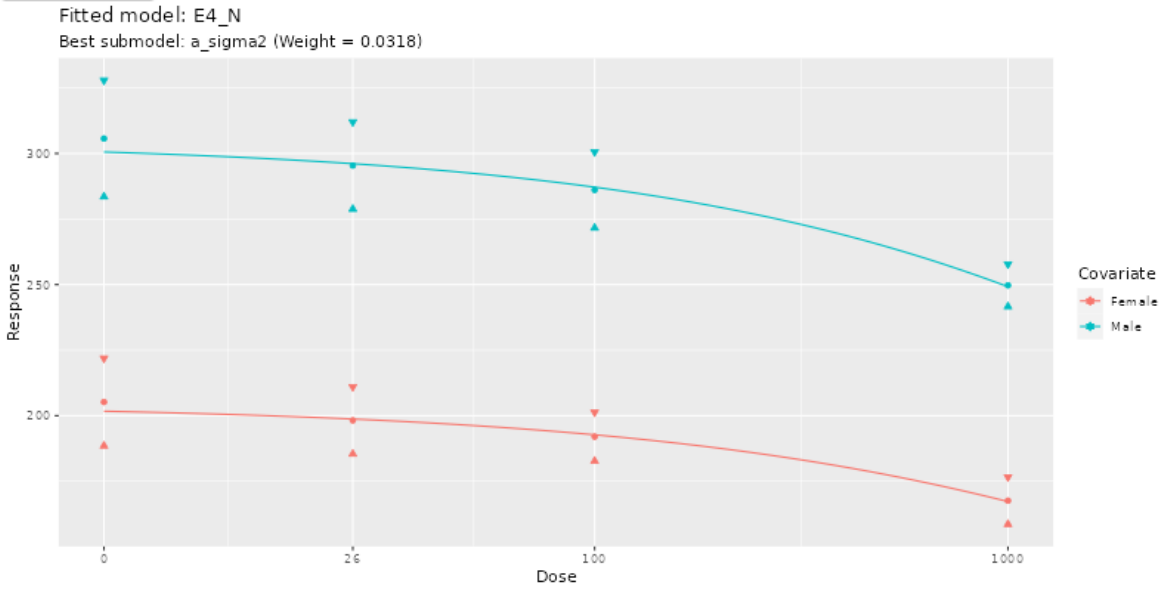
Note: Numeric values are rounded to 3 decimals.

- The different model fitted are presented, showing the data and the fitted curve for each of the covariate level, notice that only the best sub model

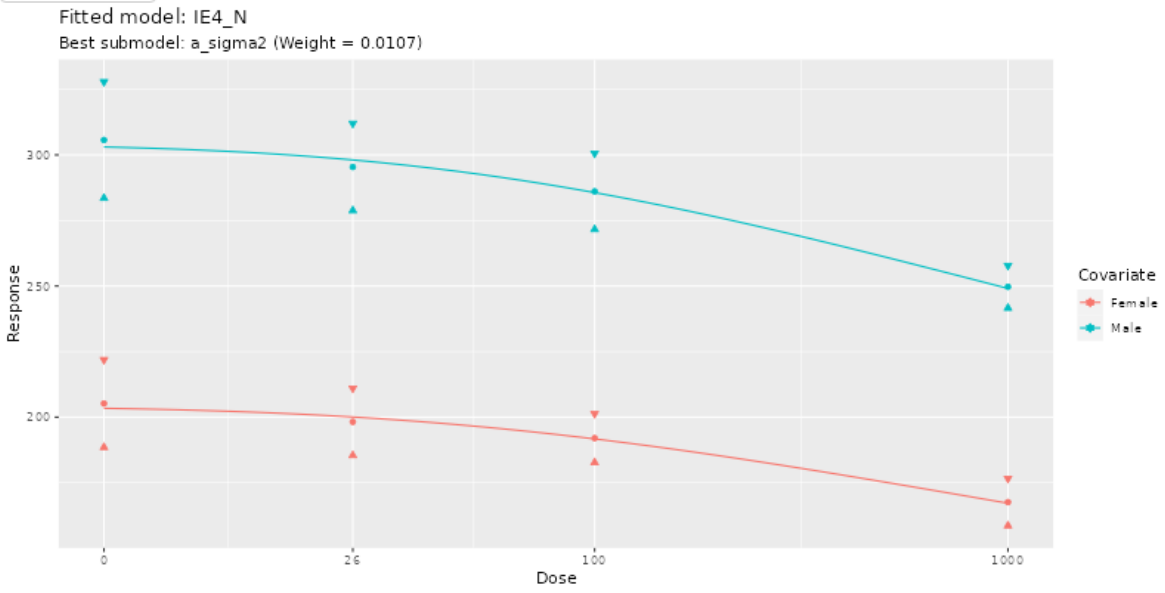
of the set of sub models fitted are shown. It is also important to highlight that for the log normal models, the variation around the geometric mean seems not to be shown, but the data that are displayed are the geometric mean and geometric standard deviation (GSD), which in this case the GSDs are rather small compared to the scale of the geometric means, with a maximum value being less than 1.06.

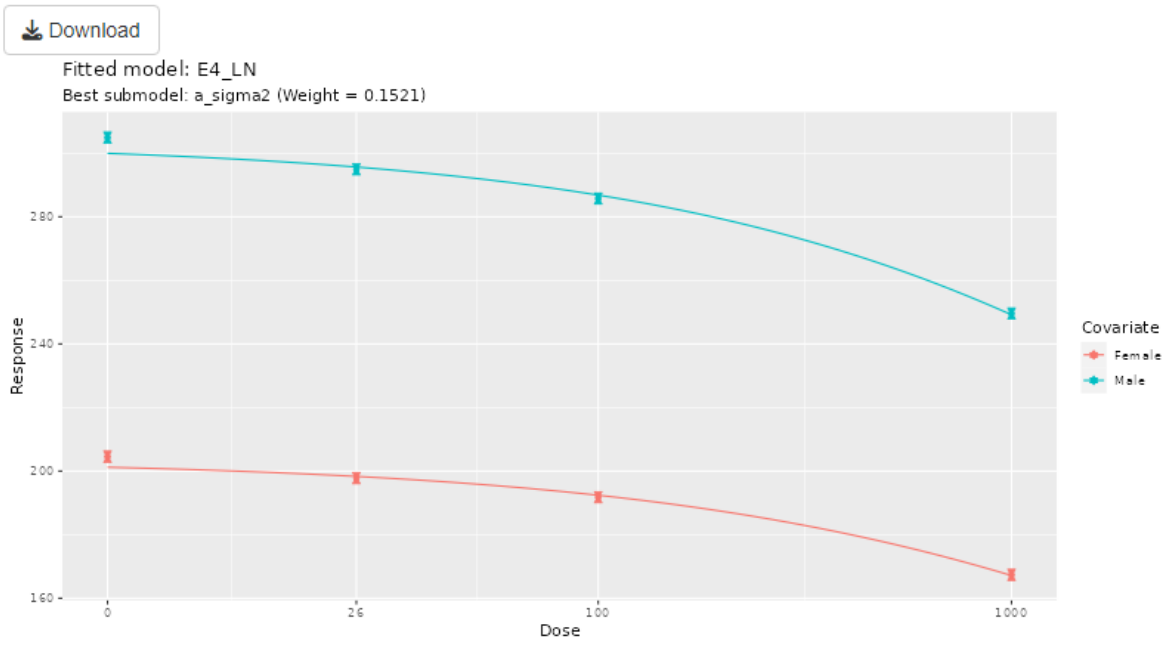
Plots

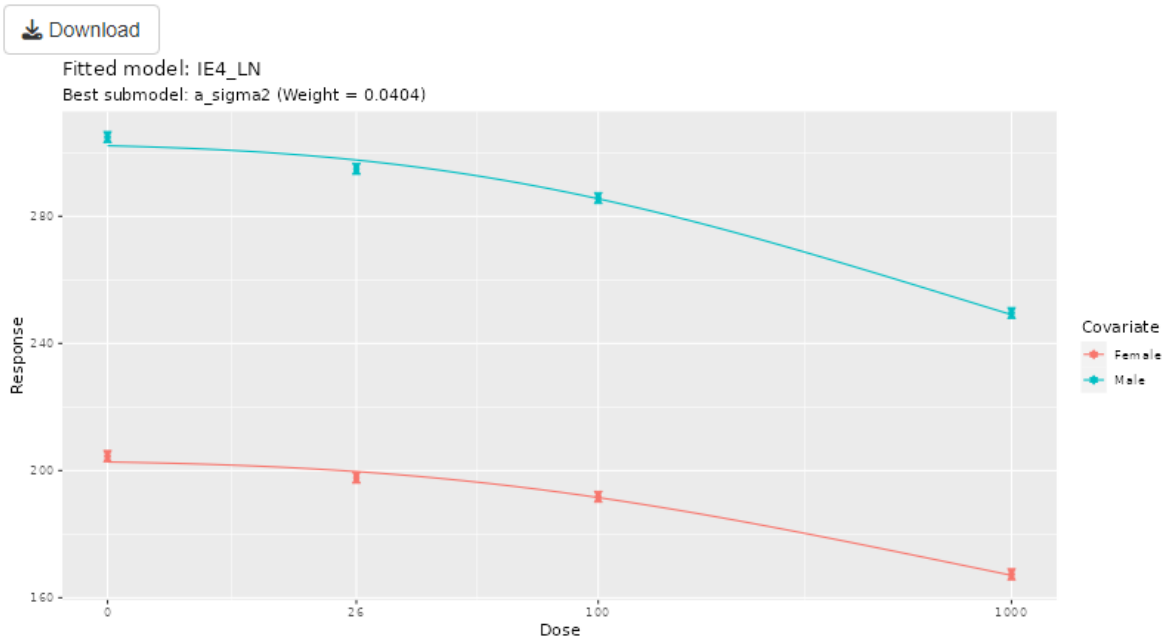
Download



Download







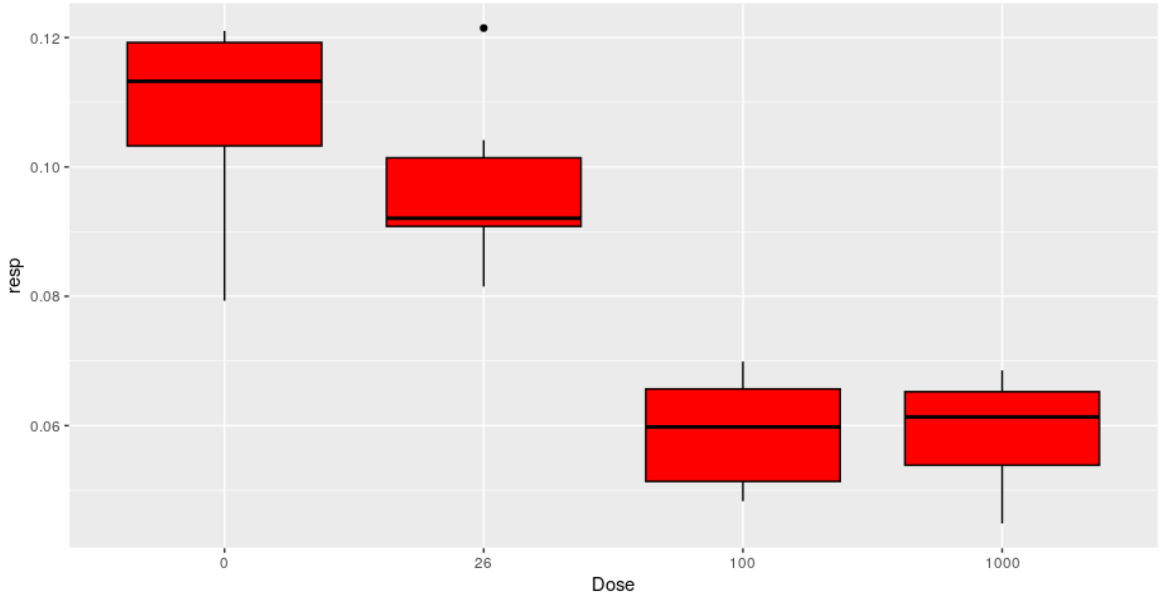
- The $BMDL_{10}$ obtained from this analysis indicates that similar lower bounds are estimated for both sexes, being 126 for male rats and 123.6 for female rats. These values can now be used for identifying a reference point for substance **Z**.

Example 4: Female ovary weight

In the same 28-day oral rat study on substance **Z** conducted to assess its repeated-dose toxicity, female ovary weights were measured, considering the same dosing scheme of 0, 26, 100 and 1000 mg/kg bw per day on Wistar rats. The summary of female ovary weights of each of the doses tested will be used for this analysis and it is provided below:

	Female ovary weight		
Dose (unit)	Mean	Standard deviation	N
0	0.108	0.016	6
26	0.097	0.014	6
100	0.059	0.009	6
1000	0.059	0.009	6

A box plot of the data that will be used can be seen below:



An ANOVA model was fitted to compare the different dose groups (results shown below) and the results indicate that there is a difference in female ovary weights for the dose groups tested.

```
            Df  Sum Sq  Mean Sq F value   Pr(>F)
Dose         3 0.01172 0.003906   25.44 4.96e-07 ***
Residuals   20 0.00307 0.000154
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

To correct for multiple testing a Dunnett correction was used, and the results (see below) show a significant decrease in female ovary weight for the two highest dose group tested (highlighted in red) with respect to the control group indicating possible adversity.

```
         Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Dunnett Contrasts


Fit: aov(formula = resp ~ Dose, data = IndividualData)

Linear Hypotheses:
               Estimate Std. Error t value Pr(>|t|)
26 - 0 == 0   -0.011000   0.007153  -1.538    0.312
100 - 0 == 0  -0.049000   0.007153  -6.850   <0.001 ***
1000 - 0 == 0 -0.049000   0.007153  -6.850   <0.001 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)
```

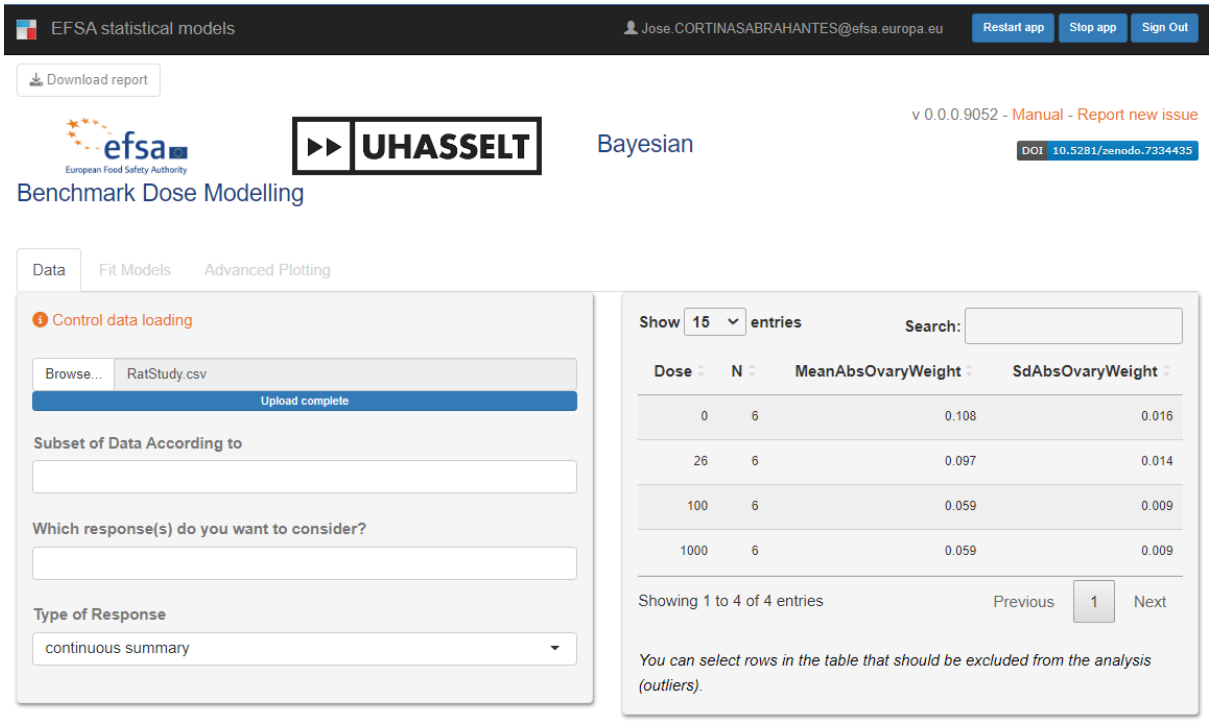
The purpose of this exercise is to fit a dose response curve to the reported data and to estimate the BMD and its credible interval (90, 5, 50 and 95th percentiles should be estimated from the posterior distribution) for a benchmark response (BMR) of 10% relative decrease of female ovary weight with respect to the background ovary weight (ovary weight expected in the control group), with similar justification as before based on the biological relevance of the effects and the variability observed for this endpoint (variability observed is greater than 5 % relative change of the mean levels) as the assessment done by EFSA (https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2022.7582). The question of interest will then be to estimate the BMD and its credible interval for the endpoint rats body weights considering a BMR of 10%.

Options to be used:

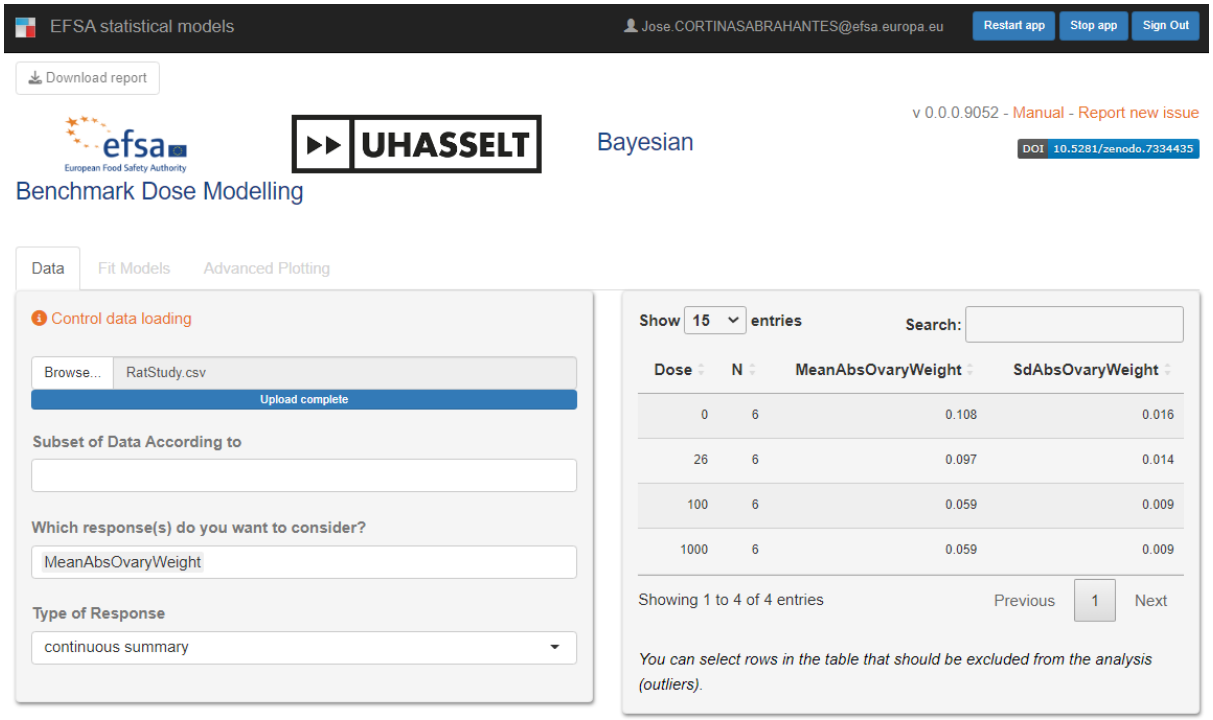
a. Default options (Laplace method) and performing sensitivity analysis

Answer: Female Rat ovary weights

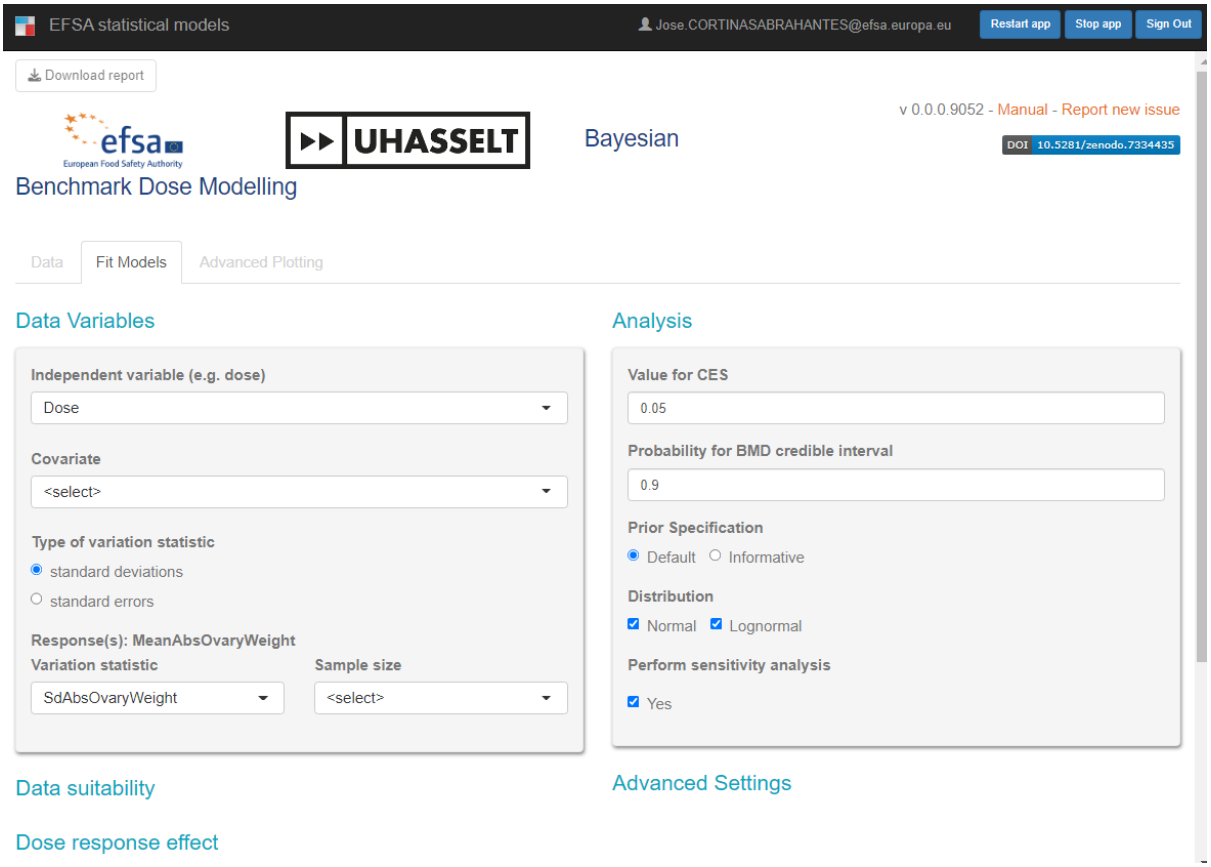
- The data should be uploaded in the web application similarly to the previous example and for this the user should click on the browser button, where the following window will be open. The user should navigate to the specific folder in which the data has been placed. Subsequently the file should be selected and the button open should be clicked. Once the data is opened the application will show the data on the right side of the window as it is shown below



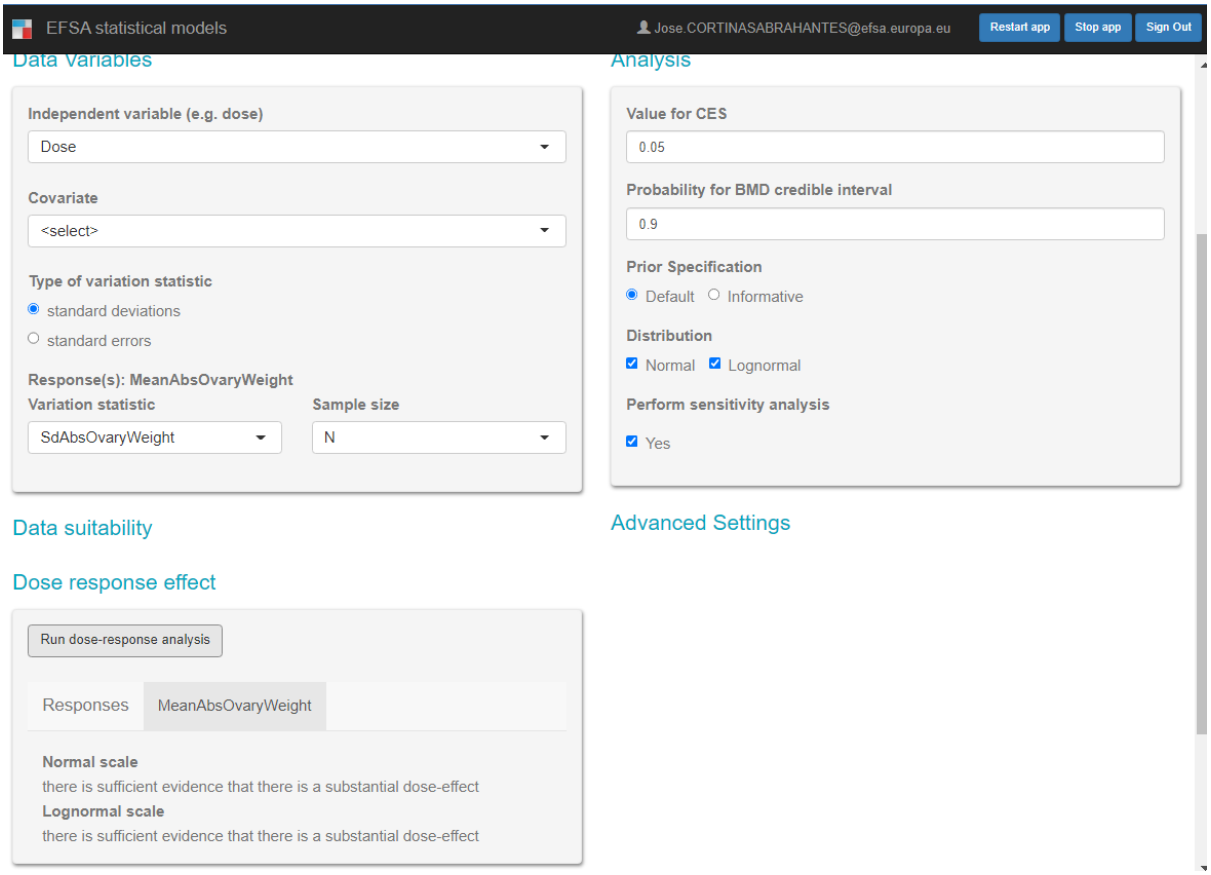
- The next step will be to select the column in the data uploaded that corresponds to the endpoint measured that we would like to analyse. Once the endpoint has been selected, then the type of response that will be analysed should be selected, the choices are quantal, continuous summary or continuous individual. For this specific data the choice should be continuous summary considering that the data of interest is measuring female ovary weights, a continuous parameter.



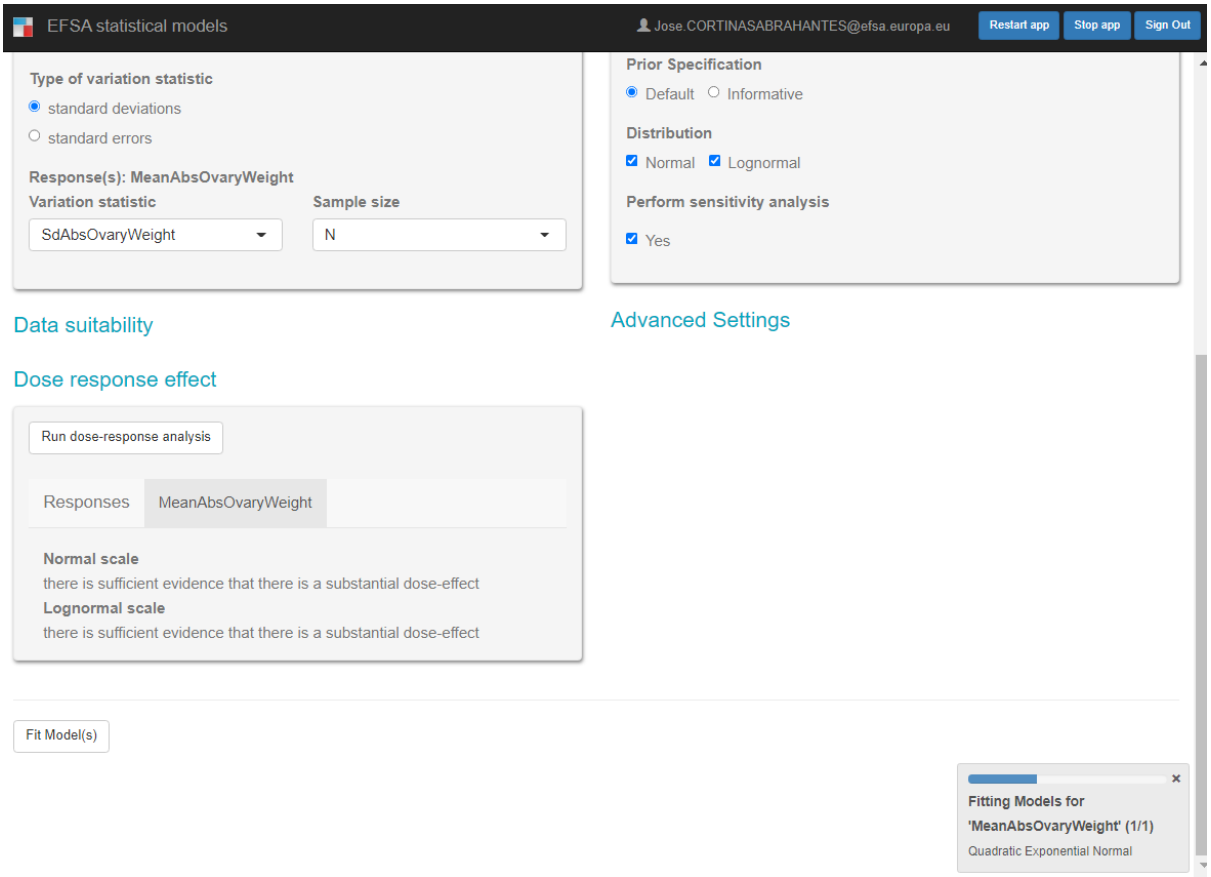
- Once this is done the next thing to do is to move to the Fit Models tab, where the following window will appear. You can notice that this window is the same as it was shown for the first exercise.



- Once the column in the dataset containing the sample size is selected then a dose response effect should be investigated, this here indicates sufficient evidence of dose effect for both distributional assumptions.



- Now the models can be fitted, notice that the BMR for this type of endpoint is set at 5% (CES = 0.05), but we have indicated in the question that the BMR should be set to be 10% instead. In this case we will use the default option of Laplace method to estimate the model parameters, previously Bridge sampling method was used, thus not need to expand the Advance setting option in this case. For illustration purposes, we will use the default option method without changing any of the advanced setting options.



- Once all models are fitted, the results are shown as for the previous dataset.
  - Left hand-side assumptions are checked about homoscedasticity (constancy of variance) for the normal distributional assumption and constancy of coefficient of variation for the log normal distributional assumption, as well the best fitting model is checked against the saturated model to assess if any of the models is fitting well the data. For this specific exercise assumptions of homoscedasticity are fulfilled for both distributional assumptions and there is at least one model from the suit of 16 candidates that fits sufficiently well the data at hand. On the right hand-side the plot with all credible intervals for all models and the model averaged one are shown, indicating that the quadratic exponential model provides different evidence with respect to the other 14 models which are more aligned.

Responses | MeanAbsOvaryWeight

NOTE: Not all tests may have been performed to assure correct results.

Check for constant variance coefficient of variation

Bartlett test

Original scale

Distributional assumption of constant variance are met, Bartlett test p-value is 0.4914

Log-scale

Distributional assumption of constant variance (on log-scale) are met, Bartlett test p-value is 0.9993

Goodness of Fit

Best fitting model fits sufficiently well (Bayes factor is 1.00e+00).

Fitted Models

Full Laplace

Model Averaged BMD

Download

Plots

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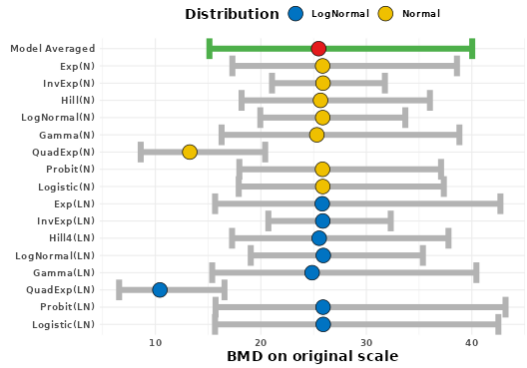

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- The table providing the model averaged credible interval for the BMD is shown below, highlighting that no violations on the assumptions of homoscedasticity and constant coefficient of variations for the distributions assumed. The right hand-side shows the plot of the weights of each of the 16 models fitted, indicating that models considering the lognormal assumptions contributed more to the model averaging results than those for the Normal distributional assumptions. Also, in general the Quadratic exponential models contributed less than any other model, being the exponential, Probit and Logistic the one with largest contribution.

Model Averaged BMD

Download

	Model	Type	BMDL	BMD	BMDU
default	Model Averaged	LP	15.122	25.475	40.032

Showing 1 to 1 of 1 entries Previous 1 Next

Note: analyses with no violations are highlighted in green. When assumptions/checks have been violated, the analysis is highlighted in red.

Estimated BMDs per model

Download

	Model	BMDL	BMD	BMDU	Model Weights
1	E4_N	17.301	25.853	38.585	0.024

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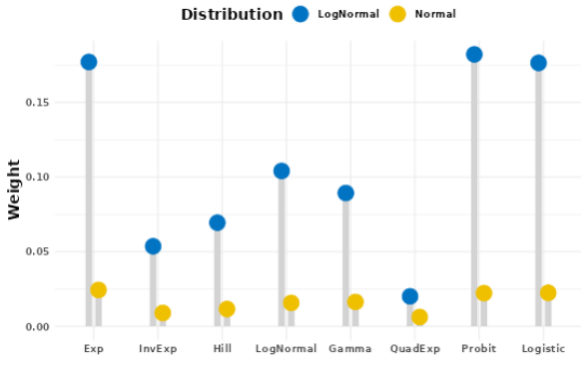


- The table with model specific credible intervals and weights for all models is also provided

Estimated BMDs per model

Download

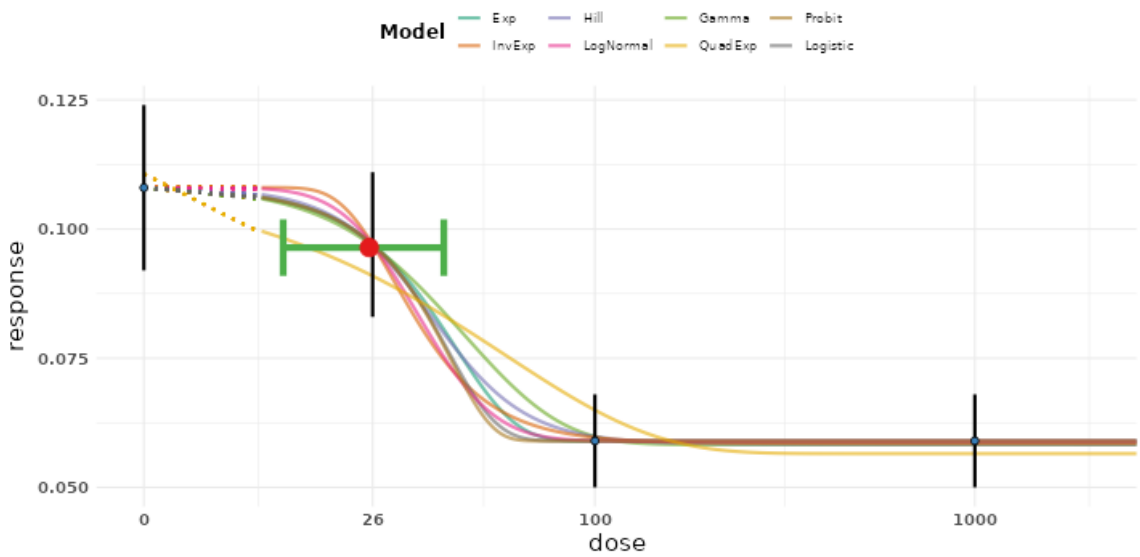
	Model	BMDL	BMD	BMDU	Model Weights
1	E4_N	17.301	25.853	38.585	0.024
2	IE4_N	21.055	25.892	31.755	0.009
3	H4_N	18.171	25.652	36.023	0.012
4	LN4_N	19.945	25.861	33.692	0.016
5	G4_N	16.273	25.305	38.82	0.016
6	QE4_N	8.61	13.259	20.41	0.006
7	P4_N	17.961	25.844	37.08	0.022
8	L4_N	17.9	25.866	37.343	0.022
9	E4_LN	15.66	25.819	42.707	0.177
10	IE4_LN	20.711	25.866	32.309	0.054

Showing 1 to 10 of 16 entries Previous 1 2 Next

Note: Numeric values are rounded to 3 decimals.

- The different model fitted for each distributional assumption as well as all models together with the model averaging result and the posterior distribution is shown below

Normal distribution



data and vertical bars based on arithmetic sample means and standard deviations

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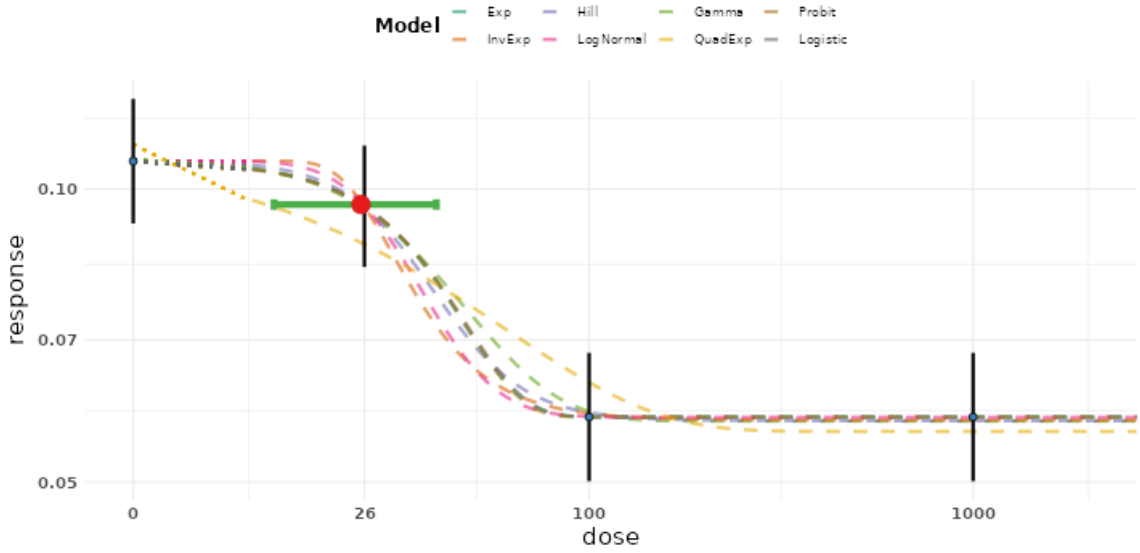
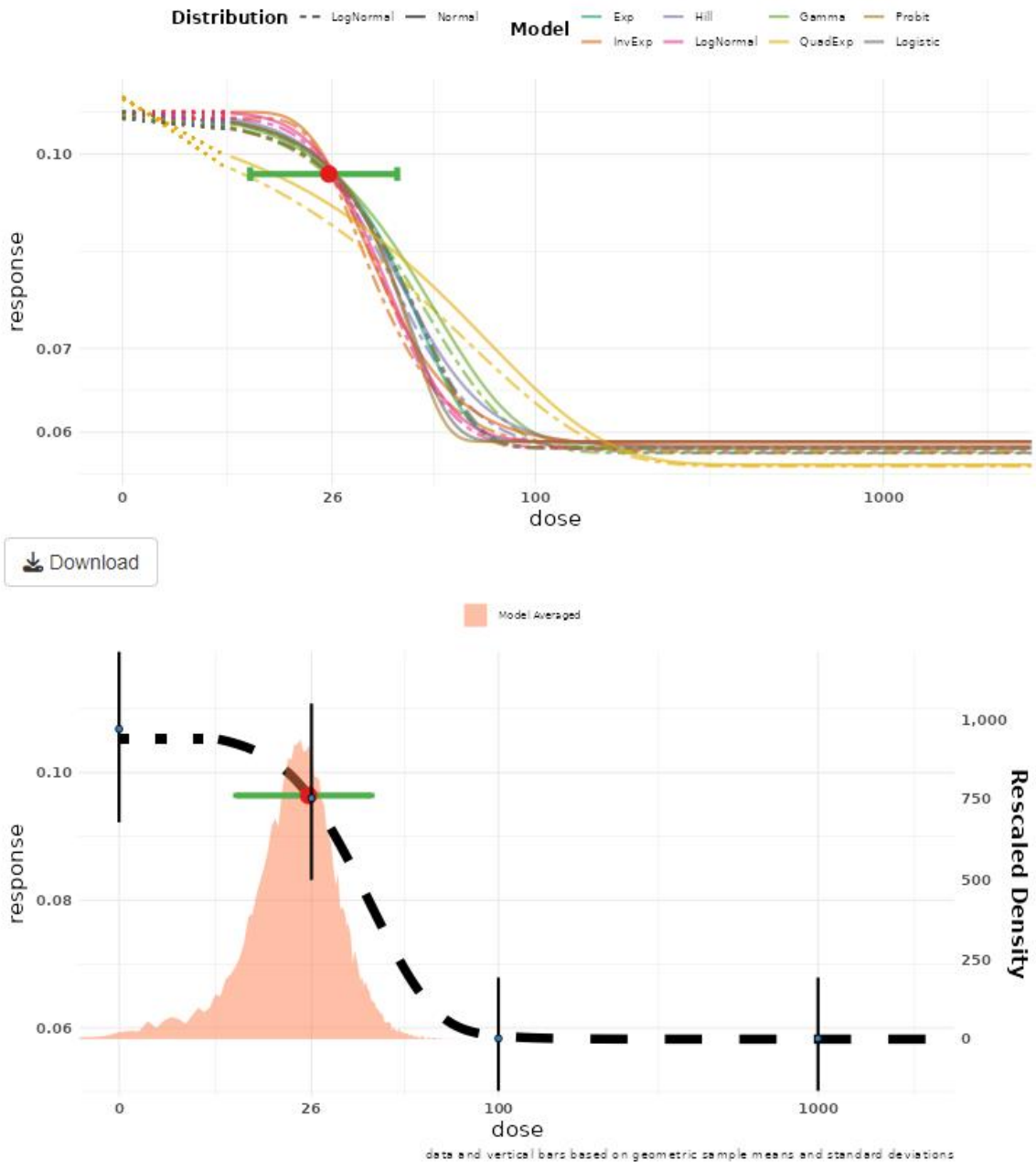
LogNormal distribution



data and vertical bars based on geometric sample means and standard deviations

Download



- The $BMDL_{10}$ obtained from this analysis indicates that it is at a dose of 15.1 mg/kg bw per day.

Example 5: Female ovary weight

Considering the same data as for Example 4, summary of female ovary weights of each of the doses tested will be used for this analysis and it is provided below:

	Female ovary weight		
Dose (unit)	Mean	Standard deviation	N
0	0.108	0.016	6
26	0.097	0.014	6
100	0.059	0.009	6
1000	0.059	0.009	6

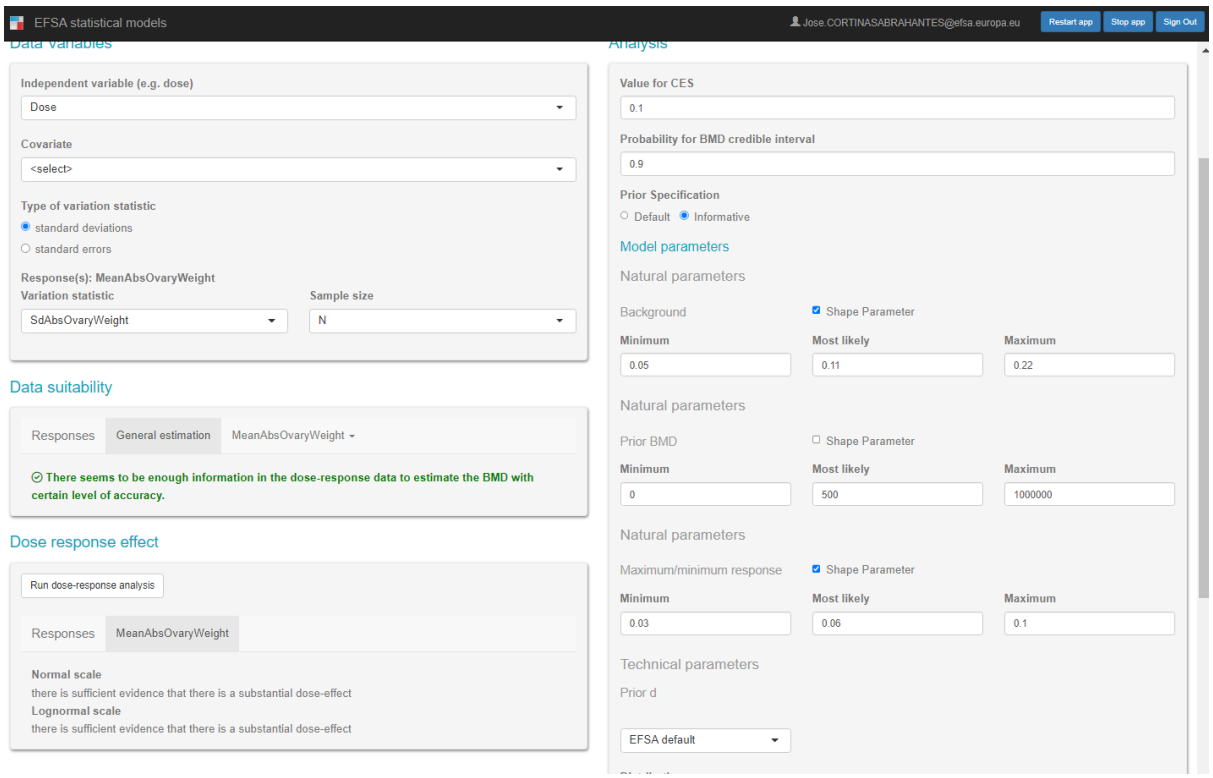
The purpose of this exercise is again to fit a dose response curve to the reported data and to estimate the BMD and its credible interval (90, 5, 50 and 95th percentiles should be estimated from the posterior distribution) for a benchmark response (BMR) of 10% relative decrease of female ovary weight with respect to the background ovary weight (ovary weight expected in the control group). Now the idea is to incorporate additional information to the analysis, considering previous BMD assessment on the same endpoints (https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2Fj.efsa.2022.7582&file=efs27582-sup-0006-Annex_F.pdf). The question of interest will then be to estimate the BMD and its credible interval for the endpoint female ovary weights considering a BMR of 10%, also using informative priors for the background for which it was estimated to be 0.106, with a minimum value being 0.09 and a maximum of 0.12. Also, based on an expert knowledge elicitation conducted to gather information on the minimum response expected for ovary weight, it was concluded the minimum weight is expected to be between 0.02 and 0.06, with a most likely value being 0.05. From the analysis performed by EFSA, the model average BMD confidence interval obtained was 0.01 to 206, which can be used as prior for the analysis of this endpoint for Substance **Z.**

Options to be used:

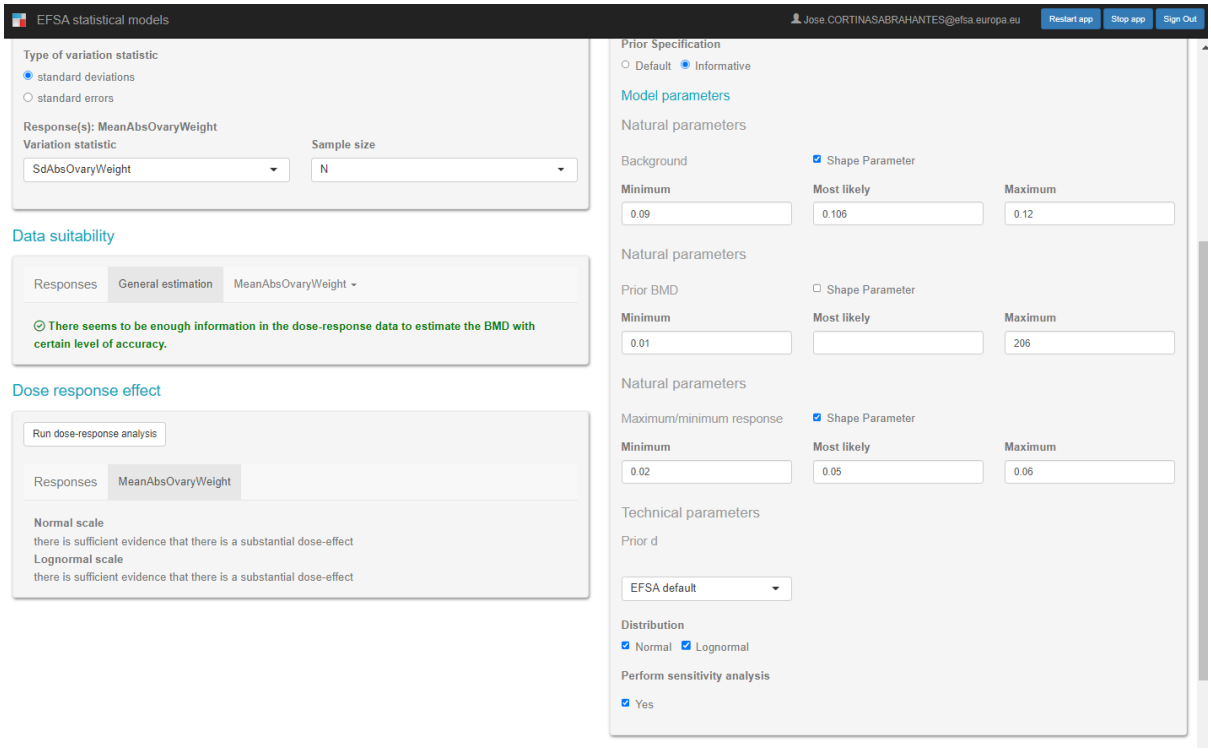
a. Default options (Laplace method), select informative priors and input information provided above for each parameter

Answer: Female Rat ovary weights

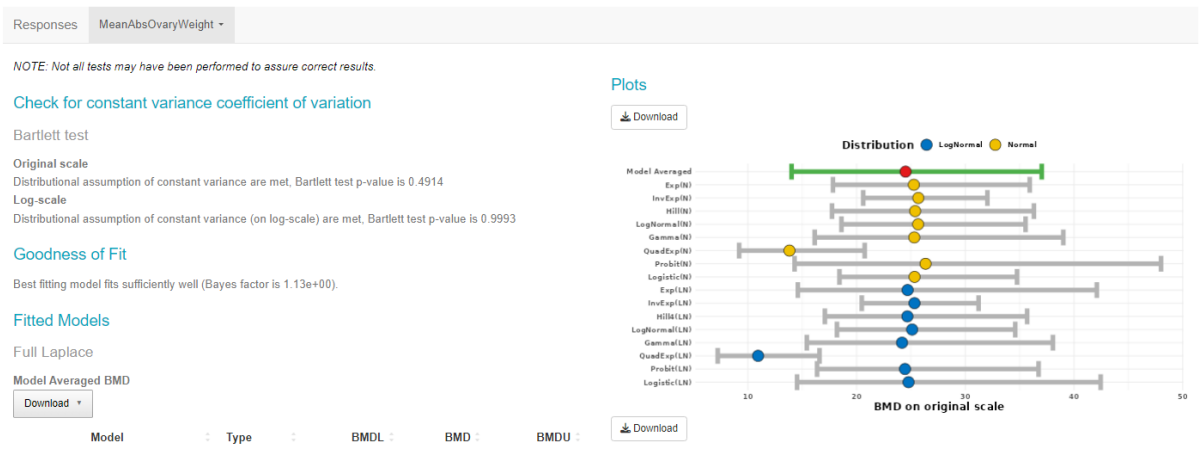
- Building on the analysis performed earlier, we can now click on the Informative prior option and the following window is then opened. The weakly informative priors for the natural parameters in the model used as default are then shown. The default weakly informative prior for the background uses the observed mean response as the most likely value, and the minimum and maximum value are calculated based on a factor of 2 of the observed background response value. For the BMD parameter, the default weakly informative prior is set to be between 0 dose and the maximum dose tested squared, while the most likely value is set to be the midpoint of range of dose tested. For the minimum response in this case that is a decreasing dose-response, the default weakly informative prior is defined based on the observed minimum response as the most likely value, and as well here a factor of 2 is used to define the range. For the technical parameter d, which defines the curvature of the dose response, two options are available (EFSA default or EPA/BMDS default), the EFSA default based on a lognormal distribution in which the probability of being below one is around 0.15, while the other option is based on the US-EPA default, which restrict further the probability of getting values for d below one to 0.05.



- The next step will be to input for each natural parameter the information provided in the Exercise 5 based on available information as well as the expert knowledge elicitation conducted. The screenshot below shows the informative prior distribution for each parameter introduce in the WEB application.



- Now the models can be fitted, and the results are shown below
  - Left hand-side assumptions are checked about homoscedasticity, but as the data has not changed, the results from the previous analysis are still valid here. There is at least one model from the suit of 16 candidates that fits sufficiently well the data at hand. On the right hand-side the plot with all credible intervals for all models and the model averaged one are shown providing similar insights.



- The table providing the model averaged credible interval for the BMD is shown below, highlighting again no violations on the assumptions of homoscedasticity and constant coefficient of variations for the distributions assumed. The right hand-side shows the plot of the weights of each of the 16 models fitted, indicating again that models considering the lognormal assumptions contributed more to the model averaging than those for the Normal distributional assumptions. Also, in general the Quadratic exponential models contributed less than any other model, being only the Probit model with largest contribution.

Model Averaged BMD

Download

	Model	Type	BMDL	BMD	BMDU
default	Model Averaged	LP	14.015	24.504	37.031

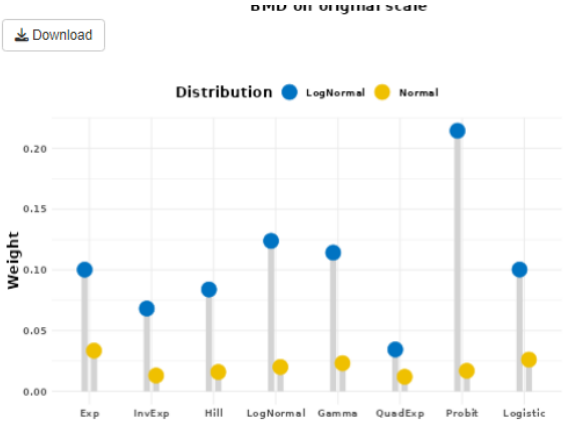
Showing 1 to 1 of 1 entries Previous 1 Next

Note: analyses with no violations are highlighted in green. When assumptions/checks have been violated, the analysis is highlighted in red.

Estimated BMDs per model

Download

	Model	BMDL	BMD	BMDU	Model Weights
1	E4_N	17.823	25.264	35.924	0.033



- The table with model specific credible intervals and weights for all models is also provided, showing now larger contribution of the normal models compared to the analysis with default priors.

Estimated BMDs per model

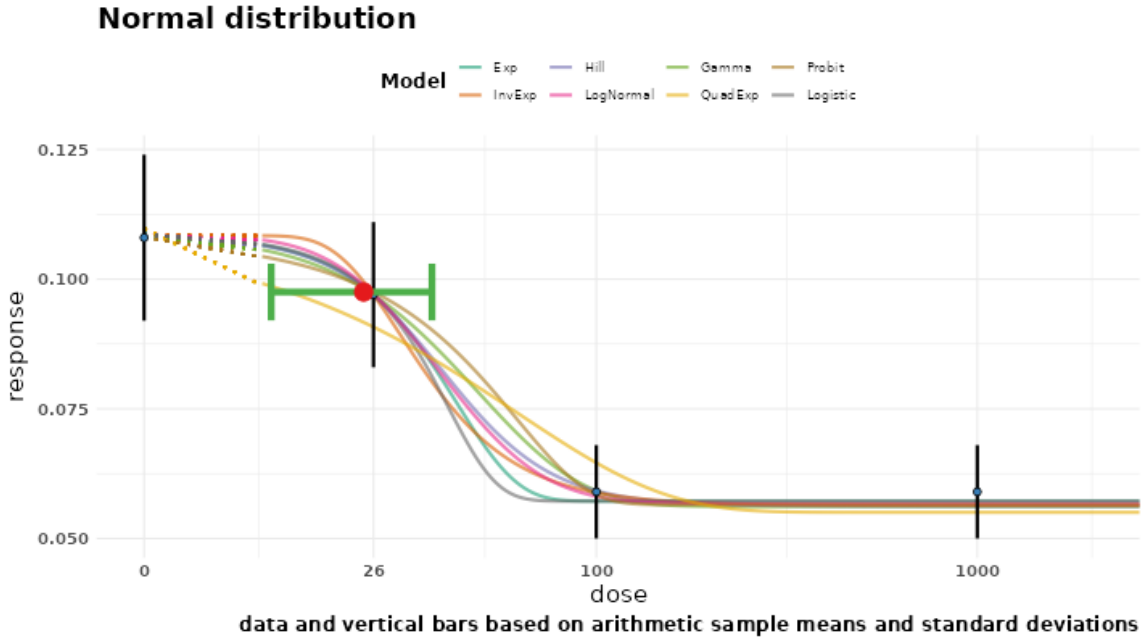
Download

	Model	BMDL	BMD	BMDU	Model Weights
1	E4_N	17.823	25.264	35.924	0.033
2	IE4_N	20.606	25.673	32.029	0.013
3	H4_N	17.737	25.381	36.309	0.016
4	LN4_N	18.602	25.655	35.542	0.02
5	G4_N	16.143	25.303	39.015	0.023
6	QE4_N	9.194	13.819	20.744	0.012
7	P4_N	14.291	26.341	48.004	0.017
8	L4_N	18.422	25.329	34.758	0.026
9	E4_LN	14.593	24.688	42.097	0.1
10	IE4_LN	20.475	25.322	31.219	0.068

Showing 1 to 10 of 16 entries Previous 1 2 Next

Note: Numeric values are rounded to 3 decimals.

- The different model fitted for each distributional assumption as well as all models together with the model averaging result and the posterior distribution is shown below



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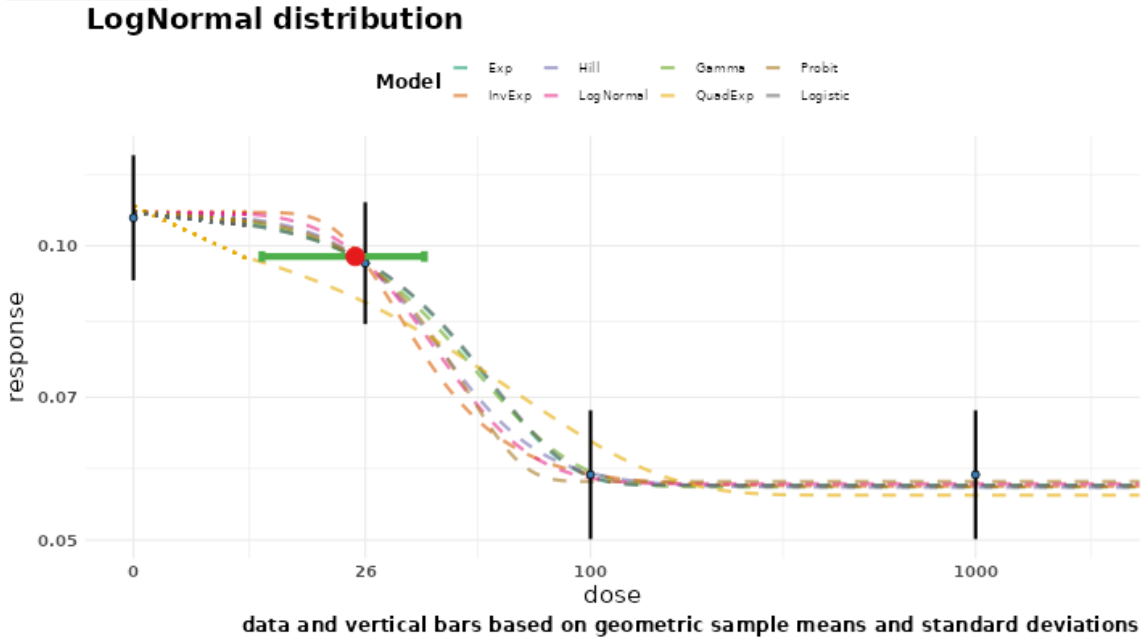

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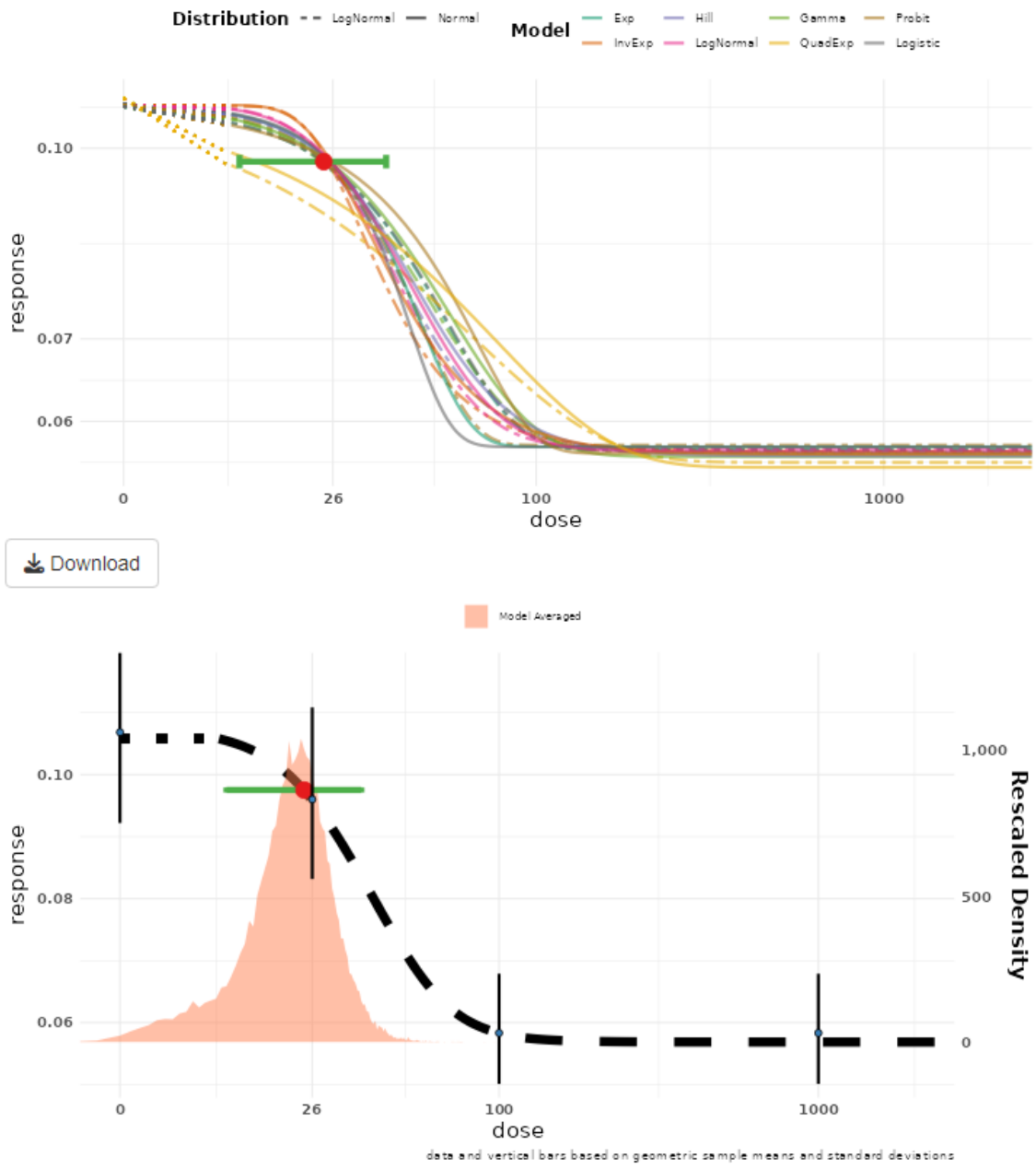

- The $BMDL_{10}$ obtained from this analysis indicates that it is at a dose of 14 mg/kg bw per day, slightly lower and more precise than when using the default prior distributions but showing a shift towards the lower dose ranges.