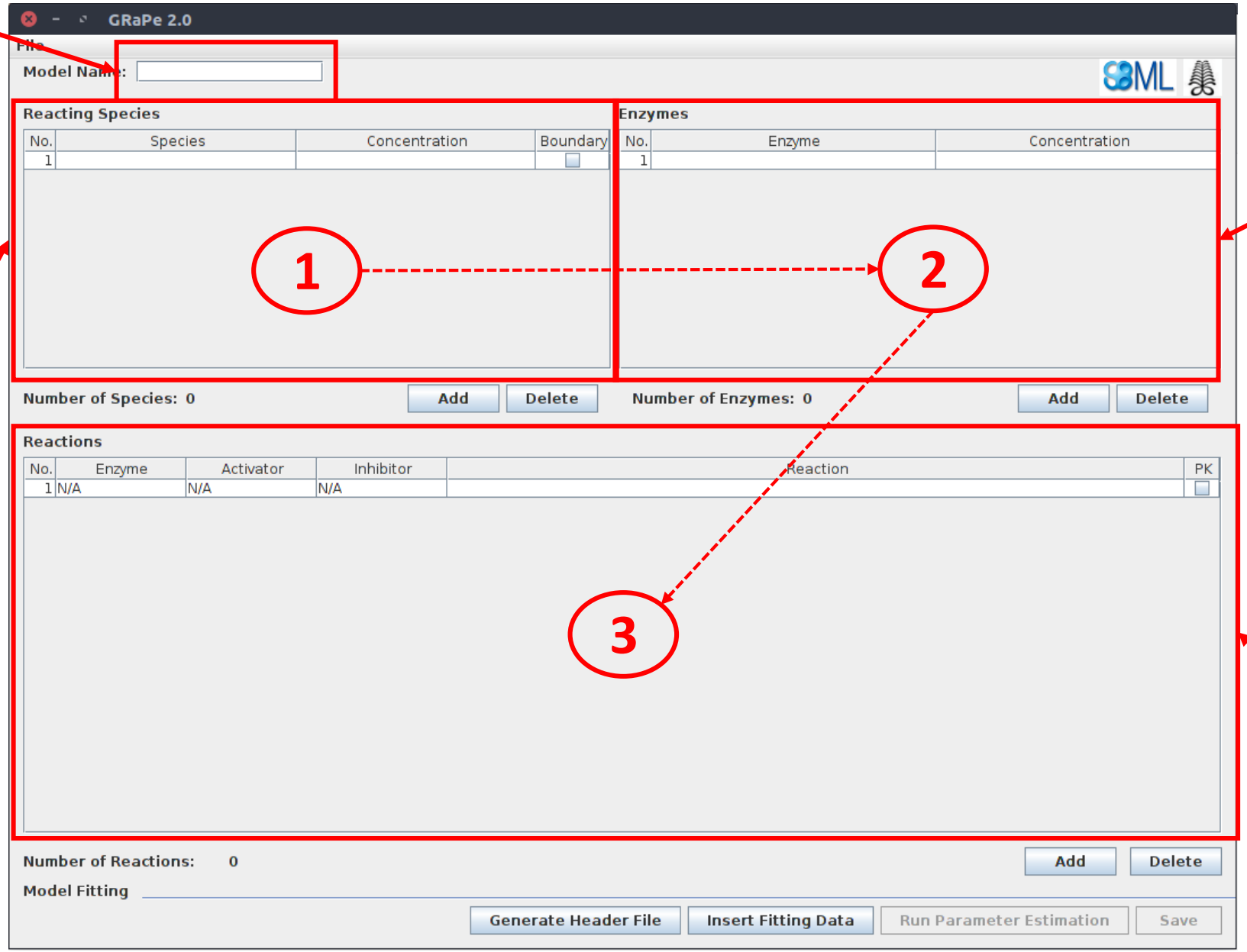
GRaPe 2.0 User Interface Crash Course

Insert Model Name here, special character (e.g. $%^&) cannot be used.



Box for inserting metabolites' information (name, initial concentration and boundary condition*) within the model

*boundary condition TRUE would make the concentration fixed and vice versa

Box for inserting enzyme information (name & concentration).

Box for inserting reaction information (enzyme that catalyses it*, activator/inhibitor, product and substrates**)

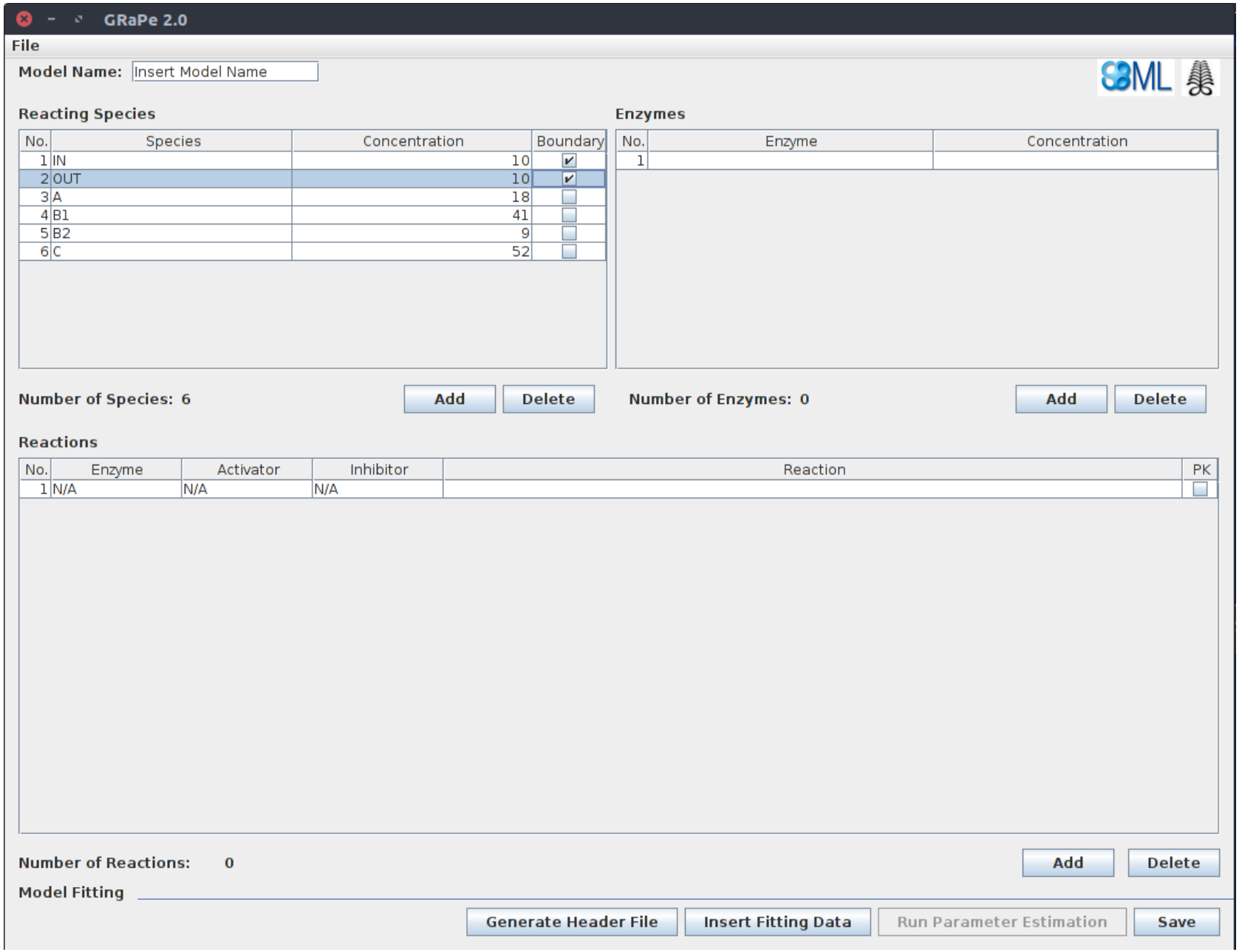
*if enzyme involved are unknown, pseudo name and concentration of 1 can be used.
**to add product and substrates users need to double click on the reaction cell for a different interface to appear.

***NUMBERS indicate ideal information entering order.
****ADD and DELETE button lets users add/delete information count for each boxes.

Example use of GRaPe 2.0

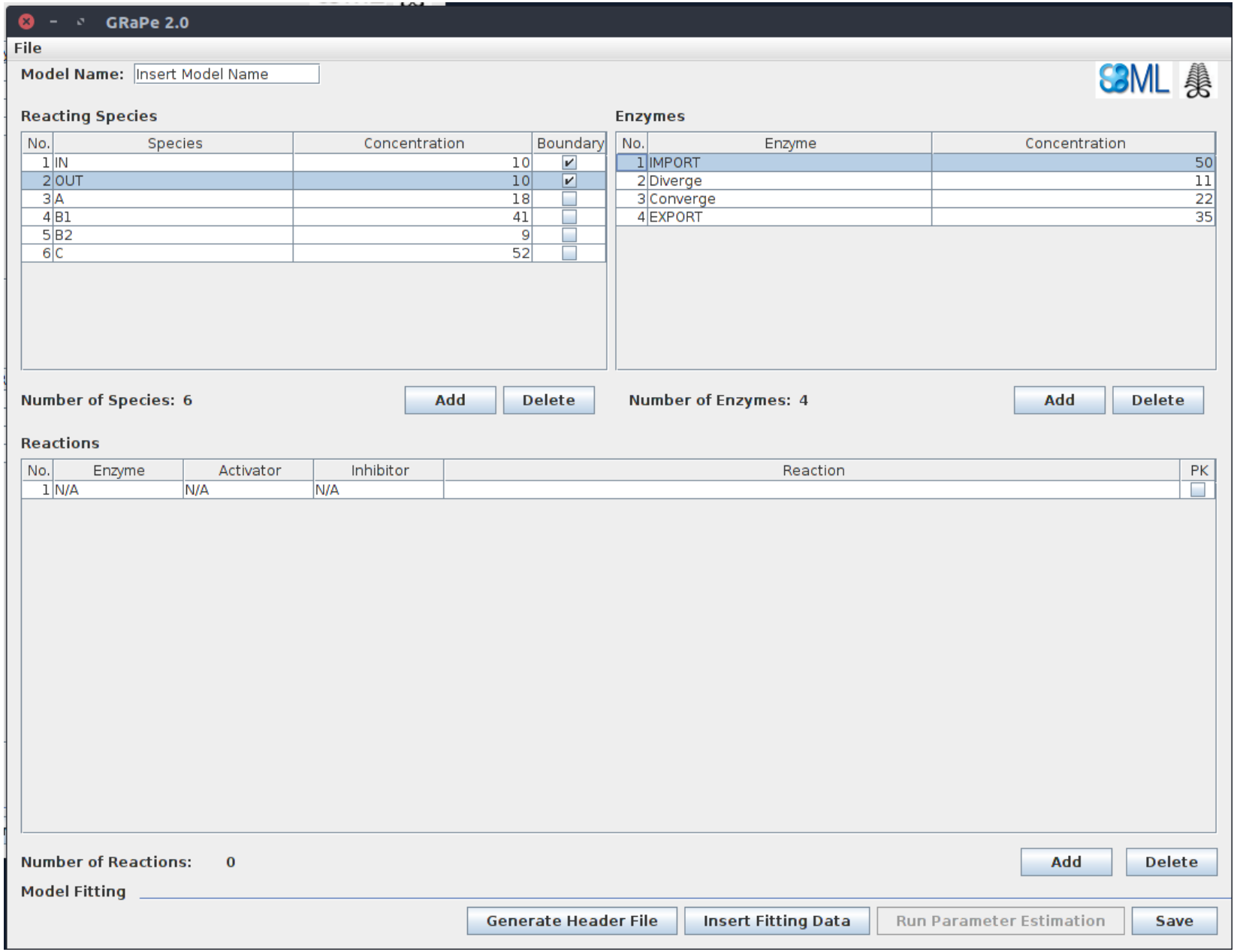
Step 1: Add all known metabolites and its initial concentration (if they are unknown, concentration of 0.1 is suggested)

*concentration unit used in this software is up to the user's choosing (not explicitly stated in software), they should however be standardised across metabolite, proteins and flux.



Step 2: Add all known enzymes and its concentration (if they are unknown, concentration of 1 is suggested)

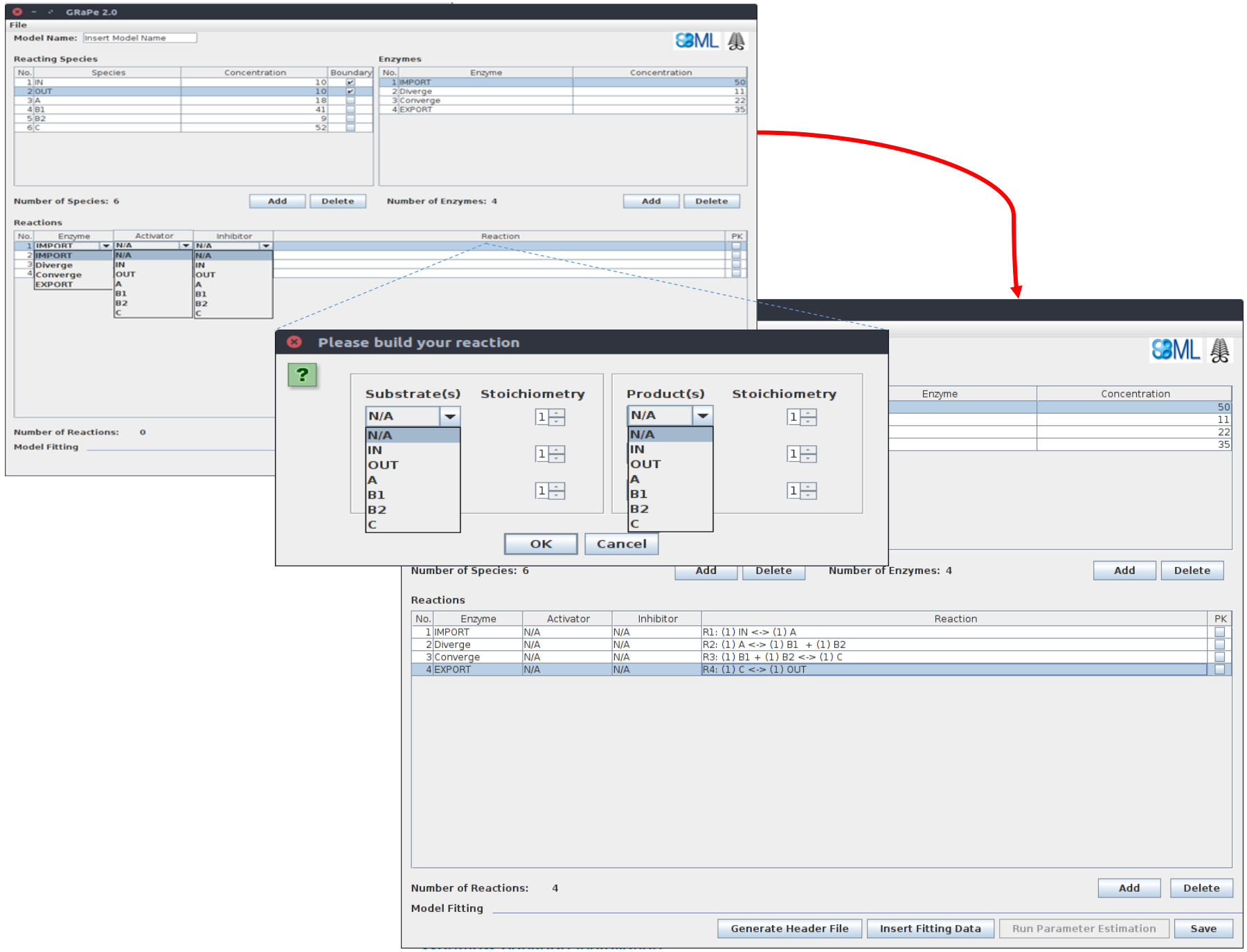
*if enzyme for an reaction is unknown OR if it is a pseudo reaction, a pseudo name can be used.



Step 3: Add reaction information. Drop down menu for enzyme/activator/inhibitor*.

*Enzyme information is compulsory, while activator and inhibitor are optional

**Double click on the reaction cell to access interface to select substrate and production, as well as their stoichiometry



Step 4: Make the fitting data file. (example used here are for steady state data, for time course data, a column would be added to indicate time point)

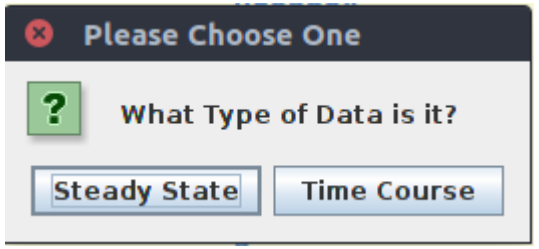
4a: click on generate header file for fitting data.

4b: move the names and IDs to a spreadsheet to edit the information much more easily.

4c: keep only IDs and expected values for metabolites/flux.

NOTE: move the data back into a text file and make sure there isn't any trailing new lines

4a:



What header file looks like in a text file.

```
Copy to Excel.
Remove the Names.
Keep only the IDs.
Paste data back into txt file to be inserted into GRaPe, make sure to avoid empty lines.

IMPORT  E1
Diverge E2
Converge        E3
EXPORT  E4
IN      S1
OUT     S2
A       S3
B1      S4
B2      S5
C       S6
IMPORT  R1
Diverge R2
Converge        R3
EXPORT  R4
```

4b:

	A	B	C
1	IMPORT	E1	
2	Diverge	E2	
3	Converge	E3	
4	EXPORT	E4	
5	IMPORT	R1	0.544777
6	Diverge	R2	0.544777
7	Converge	R3	0.544777
8	EXPORT	R4	0.544777
9	IN	S1	10
10	OUT	S2	10
11	A	S3	6.40291
12	B1	S4	32.2015
13	B2	S5	0.201489
14	C	S6	0.00152386

4c:

	A	B
1	R1	0.544777
2	R2	0.544777
3	R3	0.544777
4	R4	0.544777
5	S1	10
6	S2	10
7	S3	6.40291
8	S4	32.2015
9	S5	0.201489
10	S6	0.00152386

Step 4d: OPTIONAL

If a value is given to the steady state fitting data, GRaPe 2.0 would use the value to change the model's selected enzyme values during parameter estimation process.

This is useful for providing fitting data that has multiple conditions, which normally affect protein concentration within the system to an extent, and result in different metabolite and flux output.

As seen in the example, some information can be left out for the fitting process.

	A	B	C	D	E	F
1	E1	50	60	30	58	58
2	E2	11	15	22	15	15
3	E3	22	15	11	16	16
4	E4	35	42	29	32	32
5	R1	0.544777	0.676	0.569	N/A	0.79
6	R2	0.544777	0.676	0.569	N/A	0.79
7	R3	0.544777	0.676	0.569	N/A	0.79
8	R4	0.544777	0.676	0.569	N/A	0.79
9	S1	10	10	10	10	15
10	S2	10	10	10	10	8
11	S3	6.40291	6.27	N/A	6.27	8
12	S4	32.2015	0.2734	N/A	0.31	0.39
13	S5	0.201489	32.27	N/A	32.3	32.4
14	S6	0.00152386	0.0016	N/A	0.002	0.0023

Step 5: After inserting fitting data, user can run the parameter estimation process.

5a: Select the hyperparameters for the genetic algorithm.

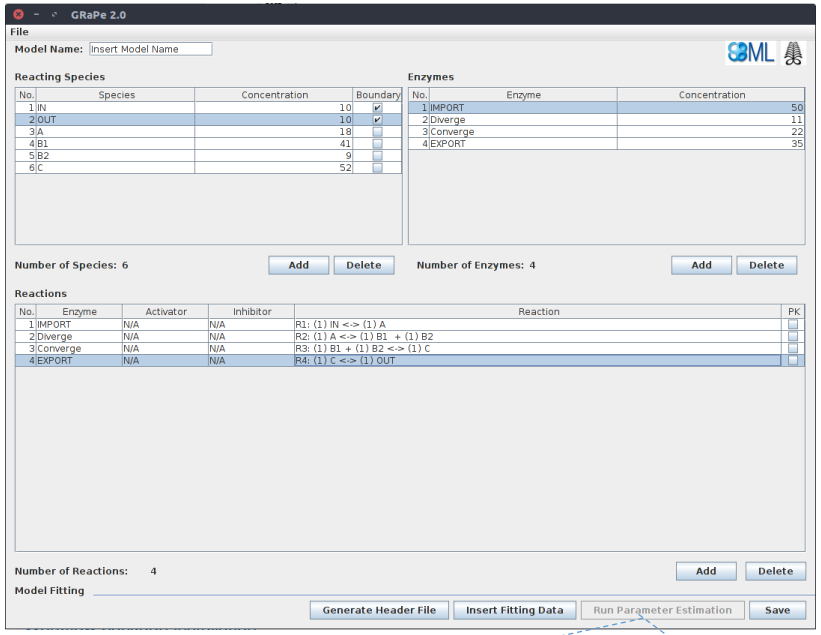
Population: Number of models to be solved (high values would lead to longer parameter estimation time)

Max Generations: Maximum number of generations to go for before stopping even if optimal parameters aren't found. (high values would lead to longer parameter estimation time)

Plateau limit: Number of generations where there is no further increase in fitness score. After reaching the given number, the process would stop.

Plague: Process of removing unfit individuals and maintaining only the initial population size at given number of generation.

*After the process is done as seen in the example where it says 'Estimation Complete!', user can click the 'Save' button to save the model with estimated parameters.



5a: