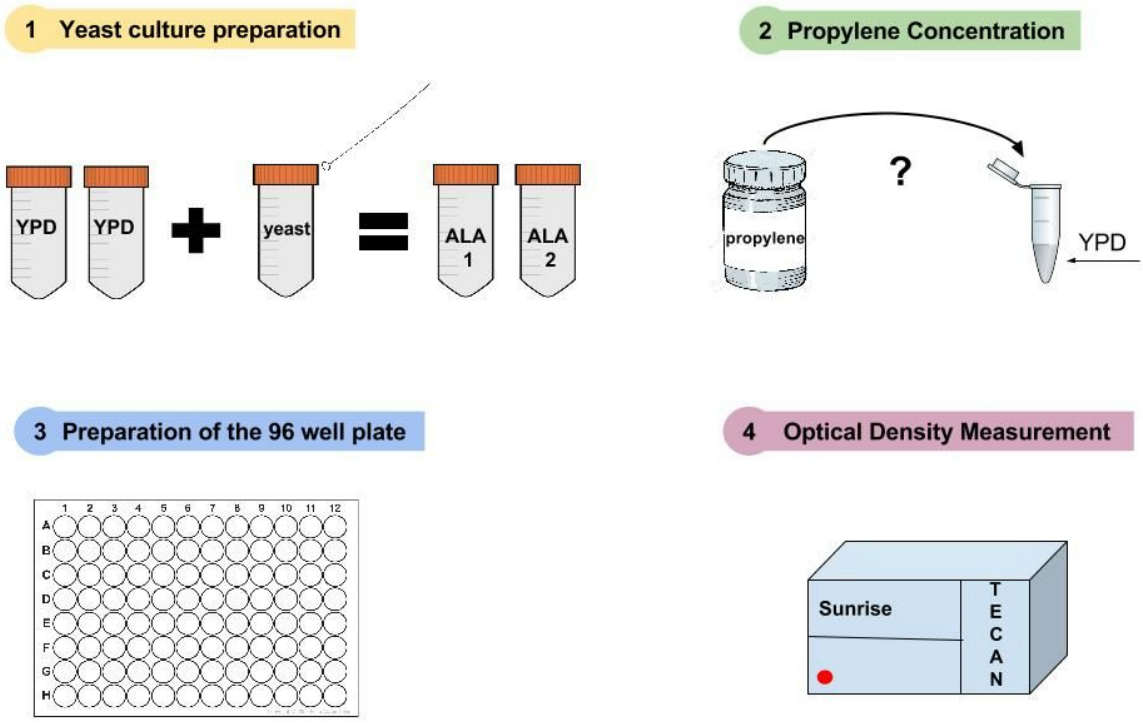
Biological experiment

We want to observe the impact of the propylene glycol which is the main component of the liquid in e-cigarette on an organism to see if it is toxic. So we choose to study yeast because it is an organism which is relatively similar to humans. Indeed, the 2 organisms are eukaryotes. We put yeasts in culture with different concentration of propylene glycol to look if there is an impact on their growth.



Materials:

- yeasts solution
- YPD
- propylene glycol (8euros)
- falcons
- pipettes
- tips
- hood (to have sterile conditions)
- 96 well plate
- oil
- spectrophotometer (TECAN)

Protocol:

I) The first step is the preparation of yeast culture:

1) Put 2mL ml of YPD into two 10ml falcon tube.
2) Add the yeast from yeast solution (wild-type strain) into your falcons using a loop (one strain per falcon) under the hood. We obtain two yeast solution that we called ALA1 and ALA2
3) Put them at the incubator during 12h.

II) The second step is to determine the concentration of propylene glycol that we put with yeast. The LD50 is 20g / kg and the weight of one yeast is in range 3-10 picograms (we consider that the average weight is 5 picograms). In one well, we will put 2 microliters of yeast solution. Thus, we have to calculate the number of cells that are in this volume to next, find out the total weight of the sample.

1) Put 6,6 microliters of yeast solution in counting chambers.
2) Count the number of cells in one little square
3) Multiply this number by 90 to obtain the number of yeast in 1 microliters of solution (here you can directly multiply by 180 to have the cells number in 2 microliters)
4) Multiply this number by 5 x 10^{-12} to have the weight due to the yeast in your samples.
5) Repeat step 1-6 for the second yeast solution

In our case we have 720 cells per small square for ALA2 and 9 cells per small square for ALA1. So the weights are 648 000 x 10^{-12} g for ALA2 and 8100 x 10^{-12} for ALA 1.

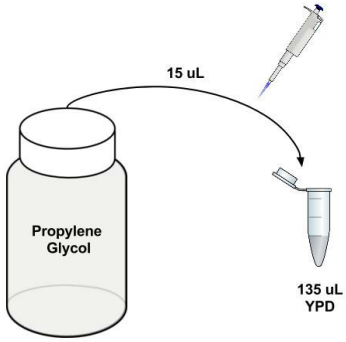
Now we have the yeast weight, we can deduce the concentration. As the density of PG is the same than water (1g/ml), the lethal dose is also equal to 20mL/ kg. By doing a rull of three, you can deduce simply the volume of PG as the LD50 dose.

LD50

volume (mL)	masse (g)
20	1000
1,2960 x 10^{-8}	648 000 x 10^{-12}
1,62 x 10^{-8}	8100 x 10^{-12}

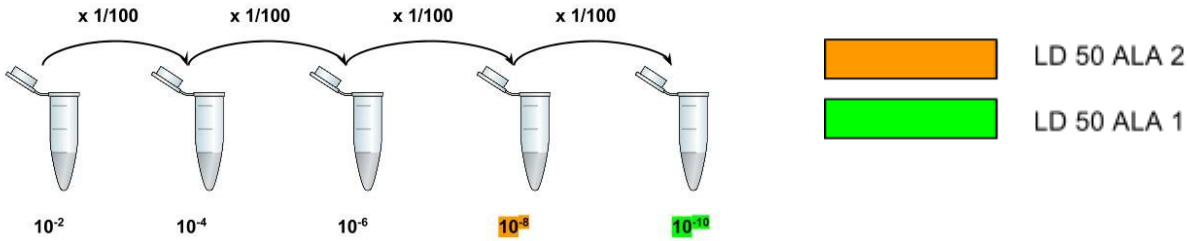
So we used 1.5 * 10^{-8} mL for ALA2 and 1.5 * 10^{-10} mL for ALA1

6) Take 15 microliters from the PG bottle using a P20 and put it into 135 microliters of YPD in an ependorf using a P1000



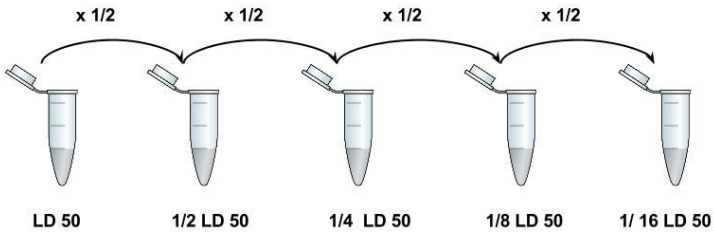
LD 50 preparation – step 1

7) Do 4 serial dilutions by hundred from the solution prepared before (do the dilution in YPD and not water !!) to obtain the LD50 concentration for ALA2 and do 5 serial dilutions for ALA1



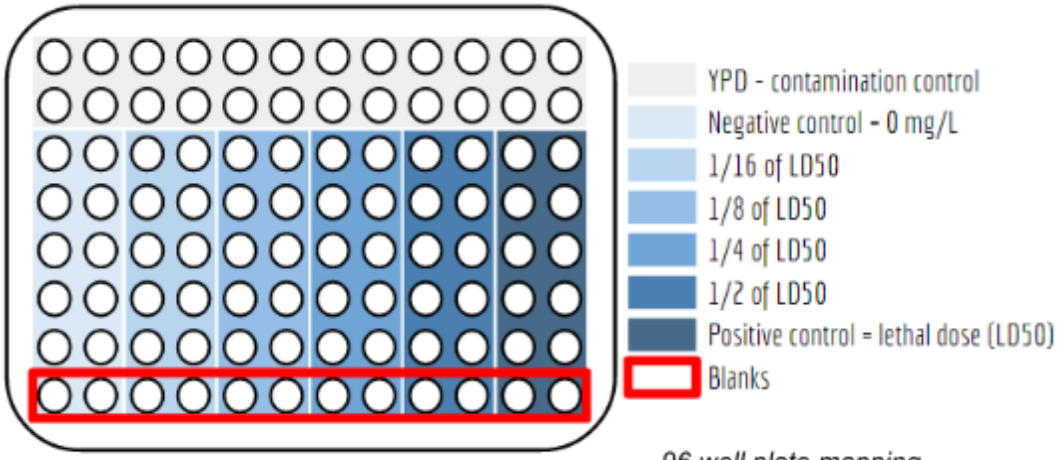
LD 50 preparation – step 2

8) Prepare the concentration by doing 4 serial dilution of two from the LD 50 (ALA2 and ALA1)



Concentration preparation

III) The last step is the preparation of the 96 well plate (refer to the picture below):



96 well plate mapping

1) Put 100 microliters of YPD using a P200 in the first two lines

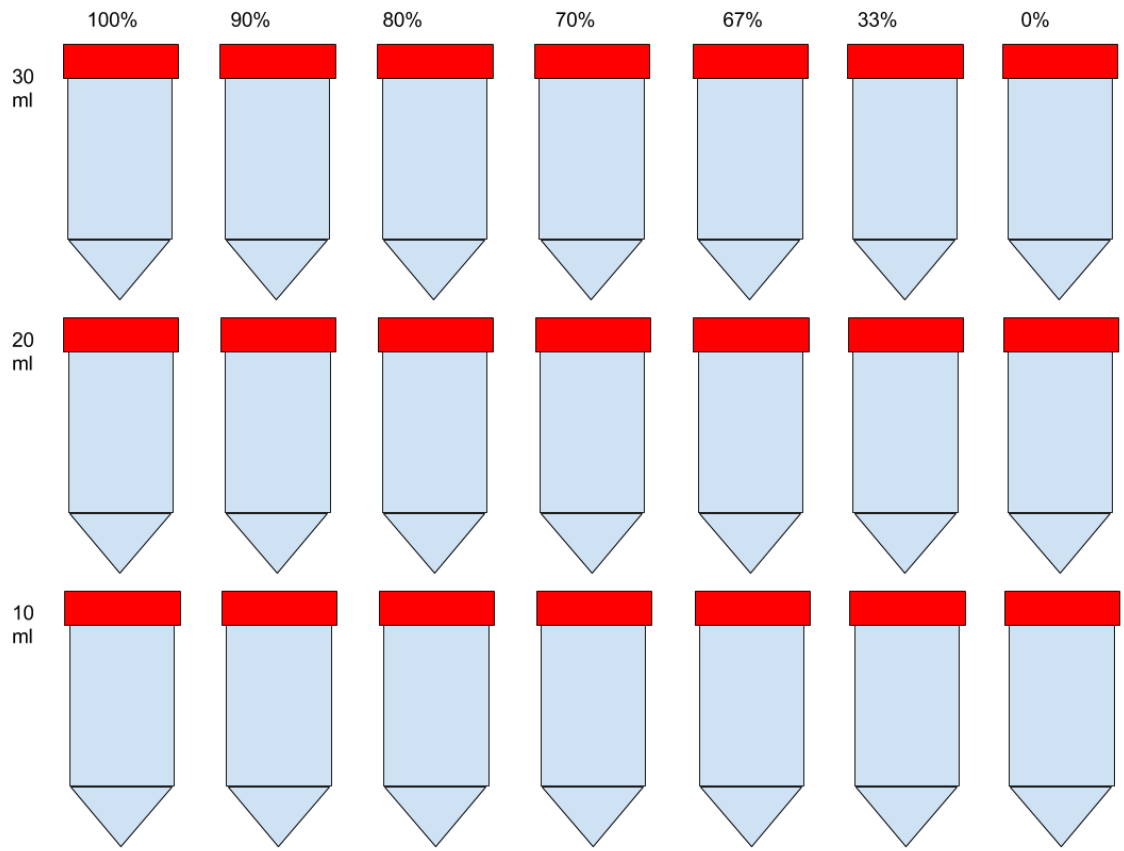
2) Put 98 microliters of PG diluted solutions (refer to the picture above) and 2 microliters of yeast in each well except for the last lines where you replace the yeast by 2 microliters of YPD.

3) Put 30 microliters of oil in each well to avoid dessication

IV) Measure the optical density of each well using a TECAN spectrophotometer. Take measure every 30 minutes during 12h.

Electronic experiment

With this experiment, we want to evaluate also the toxicity of the propylene glycol. So we know that the propylene glycol is a liquid which absorbs the air humidity. Thus, we go to measure the air humdity with a DHT 22 captor at different volumes of propylene glycol and with or not YPD.

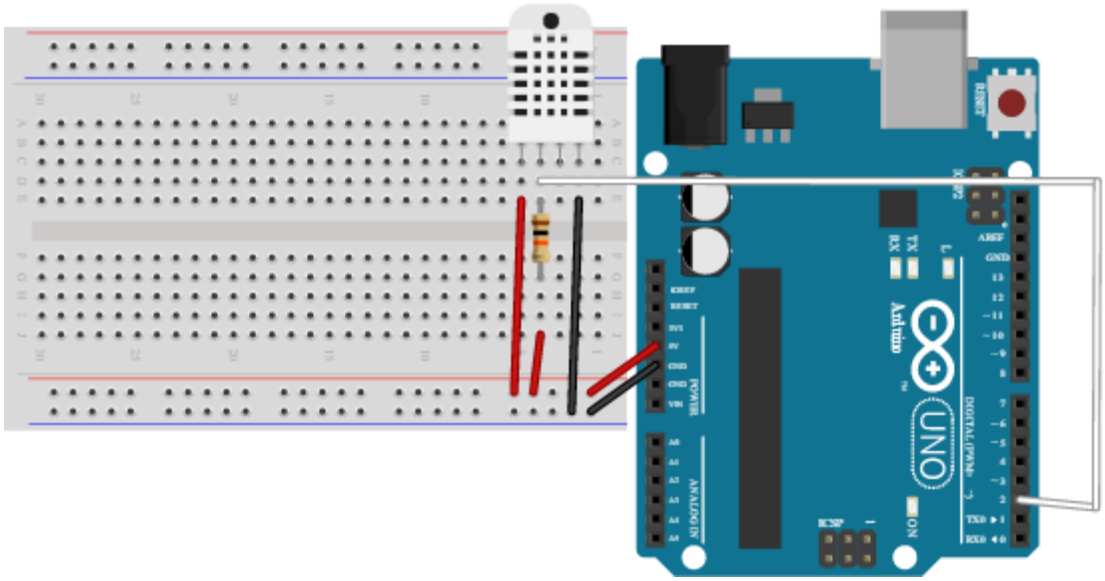


Materials:

- 1 humidity captor DHT 22 (4euros)
- propylene glycol (8euros)
- YPD
- falcons
- 1 arduino
- thread of connections
- parafilm
- scotch
- A needle or one pulls cork

Protocol:

1) Realize the montage to can use the humidity captor. We have 4 connections on the humidity captor. In this order, connect the 5V, digital 2, nothing and the ground.



2) If you can make welding, it is better for the measures with the captor.
3) Use this link to download the librairy to use the captor.
4) Take a falcon and drill the cork three times to pass each connections. We put on the cork parafilm and scotch to have a better insulation.
5) Put you solution in the falcon.
6) Put the humidity captor inside the falcon.
7) Close the falcon.
8) Measure the humidity all the 2 secondes during 10min.