GERMINATION INHIBITORS

An investigation into the effect of a germination inhibitor on the germination of seeds.

INTRODUCTION

The necessary conditions

For a seed to germinate there are specific conditions different seeds need. In this experiment, cress seeds are to be used. Going through the basics first though, for a seed to successfully germinate there needs to be:

- An adequate supply of water
- A suitable temperature
- An appropriate partial pressure of oxygen
- A suitable supply of light

Water uptake is a crucial part of the necessary conditions because germination can only commence after the uptake of water by the seed. Water absorption is imbibition.

The optimum temperature for germination is the optimum for the enzymes involved in mobilisation of food reserves, provided that other factors are not limiting. This temperature varies from species to species (i.e. 1 - 45˚C).

Respiration makes available the energy for metabolism and growth. Germinating seeds respire very rapidly, and require oxygen for aerobic respiration. Seeds will not germinate in the total absence of oxygen. Diffusion of oxygen through the testa may be slow, however, and in the early stages of germination seeds may rely on some anaerobic respiration, at least until the testa has ruptured.

So this explains why water, oxygen and a suitable temperature are needed for germination, therefore needed for this experiment. Light is obviously the last condition that comes into place (though not in all seeds) with the cress seeds being used in this experiment.

Inhibitors

A germination inhibitor is to be used in this experiment. The hypothesis is that it will affect the germination of the seed. Inhibitors are not just used in plants; they are also used in enzymes (Enzyme inhibitors). They alter the rate of enzyme catalysed reactions.

The reactions of enzymes may be enhanced or inhibited by various substances, some formed in the cell and others absorbed from the external environment. This is similar to the water-soluble protein germination inhibitor found in tomatoes because this will also alter the rate of reaction; it will alter the rate of germination in the seeds.

Enzymes can be denatured when the temperature rises above their specific optimum temperature because the molecules vibrate so much that the bonds break and so the structure and shape is changed. So this germination inhibitor will have an optimum temperature and, like enzymes, will stop working to its particular function when this temperature is exceeded. Seeing as this particular experiment will be carried out in temperatures of 25˚C only then the inhibitor should not be 'denatured'.

For seed to germinate, the inhibitors found in the seed coat have to be overcome by leaching them out. This then enables the seed to release the germination promoting plant hormone, gibberellic acid. This acts on the aleurone layer inside the seed coat to stimulate alpha-amylase production. This enzyme then starts to break down the stored starch (water insoluble) in the endosperm of the seed embryo into simpler water soluble sugars. The embryo meristem tissues use the energy from these sugars to begin cell division and the seed then sprouts. Some inhibitors induce dormancy in the embryo therefore not allowing this to happen (this is mentioned below).

Stored food (and the mobilisation of)

Seeds need particular substances inside them for them to germinate. The stored food of seeds consists of carbohydrates, lipids and proteins. Starch forms the major food reserve of most seeds (averaging around 15 – 75%), protein is also an additional important reserve food in some seeds. Food is stored in seeds in an insoluble form, and must be hydrolysed to soluble substances early in germination, following the hydration of the seed. Stored food is hydrolysed to produce the substrates for respiration (sugars) and the building blocks for synthesis (substances such as sugars, amino acids and fatty acids). Some inhibitors alter germination in seeds by inducing dormancy in the embryo.

The Experiment

To carry out this experiment, all instructions must be carried out carefully and precise measurements given should be used.

Hypothesis: That in high concentration conditions of inhibitor then the cress seeds will germinate very slowly or none at all – showing a relationship between inhibitor concentration and germination.
Null Hypothesis: That there is no relationship between the concentration of the inhibitor and the germination of cress seeds.

The Variables

The independent variable is the one that is going to be changed, which is the Inhibitor Concentration. This is being changed by mixing it in with varying amounts of water to dilute the solution so it becomes weaker and therefore the concentration is varied.

The dependant variable is the one that is to be measured as a result, which is the cress seed height. Height is being used to determine the germination rate because it can be easily and accurately measured using a ruler and provides clear results that can be easily and accurately measured using a ruler and provides clear results that can be analysed.

The controlled variables are all the other factors which could affect the results. These are to be kept constant:

- Amount of inhibitor solution given to seeds at any given time, and the number of times this is given to the seeds. *[Must be kept constant because the inhibitor concentration is what this experiment is based on. If the solution amount on some seeds was altered then the key aspect of the experiment would be ruined and the results would be unusable. Also, if too much inhibitor solution is used then the seeds could be submerged, so not being able to get oxygen, or if not enough is used then they could be drying out – both scenarios result in useless results.]*

- The number of seeds in each pot (is indicated in the apparatus). *[If more seeds are being used in some pots then this will affect the germination of the seeds because obviously the more seeds there are the less room there is for them to grow. 10 seeds is the number to be used so that standard error can be used. 10 seeds in 10 pots will produce 100 results and if it is done another 2 times then there will be an average results for each concentration (and it will be deemed reliable).*

- The temperature. This will be fixed at 25˚C by keeping the pots in an inhibitor, therefore ensuring the temperature factor will be kept controlled. *[The temperature of the conditions around the seeds will have an effect on how the investigation will work because seeds need to be at a certain temperature for them to germinate. If the temperature is too low, germination will be slowed, if the temperature is too high, germination may be grossly accelerated.]*

- Light intensity. Again may perhaps be difficult to keep constant, unless artificial light is used. *[The amount of light may have an effect on how the investigation will work because some seeds need light to germinate; some only germinate in the dark. If the amount of light was very low, then the seeds will have little chance of germinating so the experiment would not work so well.]*

- Water amount. Obviously the seeds will need to be watered as well as exposed to the inhibitor, and the amount of water given to each seed must be the same. *[Same sort of thing as the inhibitor solution amount… if there is too much water given then the seeds could be submerged and if there is too little then they could dry out. So a suitable watering schedule should be thought of before the experiment is carried out.]*

- Number of pots. This is also indicated in the apparatus. *[If more pots are used then there will be more results than expected and this could be confusing and cause problems. Seeing as the number of pots indicated has been justified and is suitable then it's best to stick to that number.]*

- Cotton wool is being used instead of soil for reasons already stated, and this decision must be followed throughout the experiment, all the seeds must grow in

  cotton wool. *[Nutrients and other chemicals that could affect the seeds germination are found in soil, and not all of the soil in the pots would have the same amount of these nutrients so therefore some seeds would behave differently to others and it would not be due to the inhibitor concentration. This cannot afford to happen so that is why cotton wool is being used – the said substances are not found in cotton wool, and it does not take up the water quickly like sand.]*

Apparatus

- **Pots**; *30 pots are needed because 10 different concentrations are to be used so that a wide range of results are produced. Each concentration is to be repeated another 2 times to produce 3 results for each one so that the results are deemed reliable – equalling 30 pots.*
- **Cress seeds**; *300 seeds are needed because 10 seeds will be put in each pot and there will be 30 pots = 300 seeds. They are being used because they are easily accessible and germinate quite quickly therefore time is not wasted waiting for them to grow.*
- **Water**; *this is to be used to lower the concentration of the inhibitor; it will be mixed in with it to produce the varying concentrations. Details of measurements are given later on.*
- **Tomatoes;** *These are used to provide to inhibitor.*
- **Test Tubes**; *30 will be needed, because there will be 10 different concentrations but each will be done 3 times. To mix water and the inhibitor to produce the varying concentrations.*
- **Measuring cylinder (10cm^3 one);** *30 needed (same reason as test tubes), to measure out the required amount of water and inhibitor*
- **Cotton wool**; *Soil cannot be used because it contains nutrients and other substrates which will interfere with the experiment and to insure the only thing affecting the growth of the seeds is the concentration of the inhibitor then all other factors must be kept constant.*
- **Incubator**; needed to keep the same temperature conditions for all of the pots. *[T*he importance of keeping the temperature constant is mentioned above.*]*
- **A Blender**; needed for the tomatoes, to get the inhibitor provided by the tomatoes, the next best thing to centrifugation should be used, i.e. a blender.

Method

1. Make up the inhibitor solution using the table provided below and then follow the instruction given to complete that section. **Note:** After the tomato(es) have been through the blender, the liquid should be kept in a beaker and then the required amounts poured into measuring cylinders (explained in more detail by the table).
2. Place 10 seeds each in 10 of the 30 pots and then place a suitable amount of cotton wool in the pot. *[*10 seeds are to be placed in each pot, i.e. there will be 10 seeds for each concentration of inhibitor solution. *T*his is because there will be a spread of 10 results for each concentration and therefore the results will be reliable. *T*he mean can be found, to be used for standard deviation, and it will be a *very* reliable mean, rather than just the mean for one concentration which is relying on one seed alone.*]*
3. Water each of the pots occupied with seeds with 3cm^3 of water – use the measuring cylinder to measure this out accurately. *[*3c
4. Leave them all in the incubator (make sure it is at 25˚C). It may be useful to indicate they are Group 1, so the repeated ones do not get mixed up with the original pots.
5. Repeat this process for 10 more of the pots and place them in the incubator, somewhere near to the Group 1 pots but not so near that they are not distinguishable as another Group. Call these pots Group 2.
6. And repeat again for the last 10 of the 30 pots and again place them again in the incubator (**Note:** where you place all 3 groups of pots should be similar because the temperature and light intensity needs to be the same conditions for all 3; reasons for this are given in the variables section). Call these pots Group 3.
7. Now there are the 3 sets of seeds and they have been watered. Now they need to be given their separate doses of inhibitor. (See below for details).

8. The inhibitor solution doses (4.50cm^3) will be given to each pot twice a day (preferably one in the morning and then another one in the afternoon). And they will be watered at these times as well. Since there will be water in the solution
   (except for the 100% inhibitor dose) then the water required is not that much – about 5cm^3 or there about should be enough.
   Note: The amount of water given to the seeds **must** be the same. If 5cm^3 is given to one pot then 5cm^3 must be given to all the pots, if this does not happen then it will be considered as an independent variable and could alter the results therefore they will not be based on just the

inhibitor concentration. This also refers to the times the plants are watered every day – if one pot is watered two times a day they all must be watered two times a day, and same for inhibitor solution (though it is stated they must be given doses twice a day).

Seeing as the pots have been watered once already, this experiment should be done in the morning so that the next afternoon watering can be done. It will be helpful to note down the times of when you water the plants so you can then use the same time to water them on the other days.

Table for concentration amounts of inhibitor solution:

Pot	**Inhibitor amount (cm^3)**	**Water amount (cm^3)**	**Total (cm^3)**
1	4.50	0.00	4.50
2	4.00	0.50	4.50
3	3.50	1.00	4.50
4	3.00	1.50	4.50
5	2.50	2.00	4.50
6	2.00	2.50	4.50
7	1.50	3.00	4.50
8	1.00	3.50	4.50
9	0.50	4.00	4.50
10	0.00	4.50	4.50

This should be repeated 2 more times so that 3 results are ended up with so an average can be found for each concentration therefore making it more reliable than if it was just one result for each concentration (as that result could have been a fluke).

This table shows how much of what there needs to be in each solution. It shows that there is to be 10 different concentrations, including a control. The control is the pot 10, which has 100% water in it. Pot 1 is the opposite of this - it has 100% inhibitor in it. The two extremes are being used so that there is something for the other results to compare to and so it can be seen how widely the germination changes as the inhibiter strength goes from 100% to 0%.

The table is fairly self-explanatory, but details on how to mix up the solutions are given:

- Using a measuring cylinder, measure out the required amount of inhibitor and pour it into a test tube. Using another measuring cylinder measure out the required amount of water (if this is for one concentration then make sure the total amount adds up to 4.50cm^3), and then also pour that into the same test tube as the inhibitor.
- Mix the two together to make the solution and then pour it into the right pot (the number of which pot to put it in is given in the table).
- This should be done twice a day, as said above, once in the morning and then again in the early afternoon. The times that the seeds are given water and inhibitor solution should be clearly noted, and it would be wise to give each pot their doses at around the same time (otherwise several trips would have to be made and to cut down on the amount of visits; it should be done the way advised here).

Statistical Tests

Sometimes experiments produce faulty/unreliable results, perhaps through a pure fluke, or by human error, etc. And because in some cases there has only been 1 of the experiments, rather than 3 of them, the whole thing is based on one set of results that could easily have gone wrong somewhere. This is why this experiment must be repeated **at least** once, preferably twice. But even when this is done, the results cannot be deemed 95% accurate until a statistical test is carried out. Statistical tests (i.e. Chi-Squared, Spearman's Rank, Standard Error etc.) show the percentage of certainty in the results. The statistical test which should be used with this data is Standard Error because the experiment measures values of the variable (inhibitor concentration). And this is also why 10 seeds and 10 concentrations are to be used because this is enough data to carry out the statistical test.

Statistical Test

This is the section of the investigation where the statistical test is to be used; in this case the test is Chi-squared. This particular statistical test has been chosen because the experiment measures how many seeds germinated, therefore there is only two categories the results can fit into; 'Germinated'

and 'Not Germinated'. This is called 'Categorical Data', as it fits into categories. If it were measuring the length or height of seedling growth then it would be better to use standard error, but in this case it's chi-squared. So the test compares two sets of data to interpret whether the results were merely due to chance. The test provides a figure which can be interpreted to show whether this is the case.

Below is a table of 6 people's results out of a class investigation.

	Day 1						Day 2						Day 3						Day 6					
	0*	10	25	50	75	100	0	10	25	50	75	100	0	10	25	50	75	100	0	10	25	50	75	100
ah	16	3	0	0	0	0	29	11	0	0	0	0	37	29	5	0	0	0	43	33	32	0	0	0
talie	26	9	10	0	0	0	35	16	43	11	0	0	45	25	47	12	0	0	50	37	49	16	0	0
toria	28	21	0	0	0	0	42	35	9	0	0	0	46	39	13	1	0	0	47	50	16	1	0	0
th	25	13	2	0	0	0	37	37	15	0	0	0	37	39	22	0	0	0	37	39	17	0	0	0
te	20	4	2	0	0	0	36	8	9	0	0	0	39	17	12	0	0	0	40	18	12	0	0	0
na	20	8	1	0	0	0	31	16	8	1	0	0	45	26	13	2	0	0	47	45	20	2	0	0
al	135	58	15	0	0	0	210	123	84	12	0	0	249	175	112	15	0	0	264	222	146	19	0	0

* Concentration of tomato inhibitor in %.

The reason only 6 sets of results are being shown is that these are the only results being used in the test. The total of the 6 results (for specific concentrations) is given at the bottom so that the averages can be worked out. Averages are needed for the 'Group Data' Chi-squared; 2 sets of chi-squared tests are being carried out 'individual data' (my own results) and 'group data' (the average results of the 6 people). An average has to be used so that the data is around the same as the Individual Data, therefore comparisons can be made. 6 separate results are being used because 6 was thought to be enough for it to be reliable and it shows the variety of number of seeds that germinated. If just the Individual Data was used then this would not be reliable because something could have gone wrong with the experiment and the results would not answer the question of the investigation, therefore more data is used (to reduce the risk of 'flukes').

For each different inhibitor concentration the 6 different results shown used 50 seeds each, and since there are 6 people then this is based on 300 seeds for each concentration in each day.

There will be two sets of data used for chi-squared: my individual results and an average of the class extract results. And because there is 4 days that the results were collected then 2 days will be chosen to represent all the days (i.e. day 2 and day 6). This means there will be 4 chi-squared tests.

Chi-squared is a statistical test to compare experimental data with predicted results (i.e. hypothesis with null hypothesis). The test relies on comparing observed results with expected results.

The Chi-Squared Test

Observed results – In a sample of 6 Petri dishes of varying tomato inhibitor concentration (the concentrations are indicated on the class investigation table), 50 seeds are put in each Petri dish and so therefore there are 300 seeds being used. Not all of the seeds germinated and the ones that did are the "Observed results" (see the first test below).

Expected results – According to the null hypothesis there is no relationship between concentration inhibitor and seed germination, therefore all the results should be the same (the same amount of seeds should germinate in each Petri dish).

The first test is my individual results on day 2.

Individual results (Day 1)

	Tomato Inhibitor Concentration (%)					
	0	**10**	**25**	**50**	**75**	**100**
Germinated	20	8	1	0		0
Not Germinated	30	42	49	50	50	50

This is an example of how the chi-squared test is carried out; this is for individual day 1 results.

	Tomato Inhibitor Concentration (%)					
	0	**10**	**25**	**50**	**75**	**100**
Observed (O)	20	8	1	0	0	0

So now the "Germinated" values are in the table, the *Exp*ected value is needed. To find this, all of the *O*bserved data is added u*p* (20 + 8 + 1 + 0 + 0 + 0 = 29) and then divided by 6, to get the mean (29 / 6 = 4.83 = 4.8 (1d*p*)). Then this value is *p*ut into all the *conc*entrations…

Expected (E)	4.8	4.8	4.8	4.8	4.8	4.8

Now the value for *D* is needed and you get this by subtracting *E* from *O* (e.g. for the 0% concentration: 20 – 4.8 = 15.2)…

D = O – E	15.2	3.2	-3.8	-4.8	-4.8	-4.8

Now *D* needs to be squared (e.g. for 0% concentration: $15.2^{2 =}$ 23.04)…

$D^2 = (O – E)^2$	231.04	10.24	14.44	23.04	23.04	23.04

And lastly, the *D*2 value must be divided by the *E* value (e.g. for 0% concentration: 231.04 / 4.8 = 48.13

$\frac{(O – E)^2}{E}$	48.13	2.13	3.01	4.8	4.8	4.8

And then to get the final figure, everything has to be added together…

$$\mathbf{x^2 = \Sigma \frac{D^2}{E}} = 48.13 + 2.13 + 3.01 + 4.8 + 4.8 + 4.8 = \mathbf{67.67}$$

So the figure (67.67) must now be matched against a probability table. This is a theoretical table of values which gives the probability (as a portion of 1) and a value of "degrees of freedom" (*DO*F). The degrees of freedom involved here is 5 (because there are 6 groups and the *DO*F is always minus 1 of the amount of groups).

*O*n the probability table provided, it only goes up to 20.52, which is of 0.001 significance. So 67.67 must have a very low significance, therefore it is very statistically significantly different and so it is very unlikely to be due merely to chance. This means the null hypothesis is rejected and the hypothesis is correct for this set of data.

Individual results (Day 6)

	Tomato Inhibitor Concentration (%)					
	0	10	25	50	75	100
Germinated	47	45	20	2	0	0
Not Germinated	3	5	30	48	50	50

The method for the chi-squared for group data is different than the individual data because there is more than one set of results. Since there is 6 sets to be dealt with, then a mean result for each concentration is used (i.e. for 0% concentration for day 6 then add up all 6 results on table under 0% day 6 and then divide by 6.)

	Tomato Inhibitor Concentration (%)					
	0	**10**	**25**	**50**	**75**	**100**
Observed (O)	47	45	20	2	0	0
Expected (E)	19	19	19	19	19	19
D =O – E	28	26	1	-17	-19	-19
$D^2 = (O – E)^2$	784	676	1	289	361	361
$\frac{(O – E)^2}{E}$	41.2	35.6	0.05	15.2	19	19

$$\mathbf{x^2 = \Sigma \frac{D^2}{E}} = 41.2 + 35.6 + 0.05 + 15.2 + 19 + 19 = \mathbf{130.1}$$

This is an even higher number than *D*ay 1's figure, this is because a lot more seeds had germinated on the last day. So this will also not on the table so it has to be of even lower significance, and once again the null hypothesis is rejected and the hypothesis is proved correct.

Group results (Day 1)

	Tomato Inhibitor Concentration (%)					
	0	**10**	**25**	**50**	**75**	**100**

Observed (O)	22.5	9.7	2.5	0	0	0
Expected (E)	5.8	5.8	5.8	5.8	5.8	5.8
D =O – E	16.7	3.9	-1.3	-5.8	-5.8	-5.8
$D^2 = (O – E)^2$	278.9	15.21	1.69	33.64	33.64	33.64
$\frac{(O – E)^2}{E}$	48.1	2.62	0.29	5.8	5.8	5.8

$x^2 = \Sigma \frac{D^2}{E}$ = 48.1 + 2.62 + 0.29 + 5.8 + 5.8 + 5.8 = **68.40**

This result is also not on the probability table, so it too rejects the null hypothesis and accepts the hypothesis.

Group results (Day 6)

	Tomato Inhibitor Concentration (%)					
	0	**10**	**25**	**50**	**75**	**100**
Observed (O)	44	37	24	3	0	0
Expected (E)	18	18	18	18	18	18
D =O – E	26	19	6	-15	-18	-18
$D^2 = (O – E)^2$	676	361	36	225	324	324
$\frac{(O – E)^2}{E}$	37.5	20.1	2	12.5	18	18

$x^2 = \Sigma \frac{D^2}{E}$ = 37.5 + 20.1 + 2 + 12.5 + 18 + 18 = **108.1**

This figure is another high one that is not on the table. That means all of the final figures are much below 0.5, which is considered to be the probability value below which the χ^2 figure is considered to be significantly different from the expected results therefore the results are unlikely to be due merely to chance. If they are unlikely due to chance then there must be a relationship, therefore they all accept the hypothesis, so for these seeds: There is a relationship between germination inhibitor and the germination of seeds – the higher the inhibitor concentration the less seeds germinate.

Days 1 and 6 were chosen for chi-squared because they are the first and last days of the experiment and so they have the widest difference in the results (Day 1 – 67.67 and 60.48; Day 6 – 130.1 and 108.1) and show that it takes a few days for some of the seeds to start germinating (a hint at competition, see below).

Analysis of results

The experiment undertaken was designed to show how changing the concentration of tomato inhibitor affects seed germination. A graph was produced because it is easier to see the trends and patterns on this than on a table of results. Group data and Individual data is representing the results because if it were individual data only then that is just one person's results, and for an experiment to be reliable there should be at least 3. So with group data as well, the two sets of results can be compared to see the overall behaviour. And it shows that as the concentration of inhibitor is increased then the number of seeds germinating decreases. The reason for this is that the independent variable (i.e. the concentration) was increased, therefore the rate of reaction decreased (less seeds germinated).

In chemistry, changing the "rate of reaction" means changing the rate at which the chemical reaction takes place, in other words, speeding it up or slowing it down. So, in this case, the seeds were ready to germinate and the tomato juice (that contains a germination inhibitor) altered the rate of reaction of the seeds.

Germination of seeds is a complex process triggered by absorption of water (imhibition). The water activates the enzymes that catalyse the biochemical reactions of germination. And so as a result of swelling of the seed (due to absorption) may rupture the testa (seed coat). After the seed coat has ruptured then the plant starts to grow – the first organ to emerge is normally the radicle (see diagram below).

The tomato juice contains a germination inhibitor which somehow tampers with the process. The most common way is for the inhibitor to induce dormancy and therefore disallowing the embryo tissues to begin cell division. 6 days was the time limit for this experiment, so the inhibitor could well have done that and this would explain why 75% and 100% inhibitor did not allow any seeds to germinate (obviously the concentration was so high that none of the seeds managed to overcome it). There are three possible other ways in which the inhibitor altered germination:

- It could be the effect of pH germination.
- It could be an osmotic effect due to a high solute potential in the tomato juice
- It could be a chemical in the tomato juice - this would be more difficult to determine but the most likely substance would be abscisic acid.

In the experiment, the seeds were kept in Petri dishes that were placed in an incubator (kept at 30˚C constant), with access to light (the incubator had a clear plastic lid), and were watered daily. This follows the guidelines written in Skill A (The necessary requirements for germination). The reason 30˚C was used is that it was round about the optimum temperature for the enzymes in the seeds to function – and it had to be kept constant because otherwise temperature would be a factor involved and could denature the enzymes.

So while the seed is germinating, respiration makes available the energy needed for metabolism and growth. Seeds will not germinate in the total absence of oxygen (which is why the incubator had small holes in it, for which oxygen could get through to them). Diffusion of oxygen through the testa may be slow, which is why on day 1 of the individual data only 29 out of 300 seeds (6 Petri dishes x 50 seeds) had germinated – The graph shows the obvious difference between the data of day 1 and the data of day 6 (the last day). The seeds had had 6 full days to germinate and so the majority in the weaker concentrations had germinated because after the testa has ruptured (mentioned above, it is due to the water absorption) then the process is quite fast.

So the inhibitor induces some sort of dormancy, and that is why the higher concentrations stop the most seeds from germinating because there is more of it. It is like the "Collision Theory" – for a reaction to occur (i.e. the reaction between the inhibitor and the seed), the two particles must first collide so that they come into contact with each other. This will happen more often if there are more particles in a given volume (i.e. higher concentration).

On the graph, none of lines go up to 50 seeds – Individual Data Day 6 (0%) went up to 47 (47 seeds had germinated by day 6), and Group Data Day 6 (0%) went up to 44. So not all seeds managed to germinate (although one person on the group table had 50 seeds germinated, but for the group data it is averaged so that figure is put into the mean), this isn't due to those seeds not having enough time to germinate because it's fellow seeds had all grown about 3 cm by day 6. This has nothing to do with the inhibitor because this is 0% concentration so there is no inhibitor – it is just seeds and water. So this batch of seeds is the control, it is used to show how many of the seeds grow under normal conditions, so that the other concentrations of inhibitor can be compared to it and the result will be more obvious. Because, say the control germinated 47 seeds and then the 25% concentration germinated 15, you would know that it was due to the inhibitor and not because the seeds grow slowly or anything (as you have proof they don't – the control).

Ignoring the presence of the inhibitor, there is another reason why some of the seeds in the Petri dishes germinated while others in the same dish did. This is because of competition between the seeds for water and light, known as **intraspecific competition**. There are two extremes of intraspecific competition – Scramble and Contest. Scramble is when resources are shared equally between all the seeds in the Petri dish; well obviously it wasn't that type of competition because some seeds had grown more than others and some not at all. Contest is when some of the seeds in the Petri dish get a bigger share of resources (light, water, oxygen, etc.) than others, and some germinate quicker than others. So this is why most of the seeds, but not all, in the control Petri dish (no inhibitor), germinated, and it is another reason along with the increasing inhibitor concentration, that the seeds germinating went down.

Evaluating Evidence and Procedures

In every experiment there is a degree of uncertainty in the results, due to errors that could've been due to design faults, in the equipment, in the method, etc. Therefore the things that could have gone wrong should be checked to see if they did. Things gone wrong usually show up in results as 'flukes' or 'anomalies' – these are usually obvious on graphs, another reason why graphs are much better at analysing results than just a table.

- Errors

The equipment used was not 100% guaranteed to produce highly accurate results. There is always going to be something that can go wrong, and it could be due to a small fault with the equipment.

The seeds were kept in an incubator because it keeps the temperature at 30˚C constant, and if they were just placed in a classroom then the temperature would fluctuate throughout the day and night, therefore affecting germination. However, there could have been a fault with the incubator that allowed the temperature to fluctuate a little or a lot, since I did not check the temperature of the incubator when I went to water the seeds, then this may well have happened. So, as already mentioned in the plan, temperature is a major factor in germination and it would affect the results by speeding up the germination, so more seeds germinated more quickly than expected, or it could slow down germination, if temperature is low.

- Limitations

The concentration of inhibitor solutions were recorded onto a table prior to the experiment being carried out. It shows a range of 10 concentrations which is adequate for the results to be reliable. However, if the experiment needed to be more reliable (i.e. more statistically significant results on the chi-squared test, the more results there are the more reliable the conclusion is). A range of 20 or more concentrations would provide a very legitimate set of results.

The presumably temperature fluctuations provide limitations to the reliability of the results as it brings in another factor affecting germination.

In errors, it was stated that temperature must be kept constant, this was not the only factor that should have been kept constant; light intensity, amount of water, and amount of oxygen available are also key factors that germination

The amount of water each Petri dish received could have been a limitation on the growth of the seeds, as perhaps it was not a sufficient amount for all the seeds to . Since the incubator was not placed by a window, it was on the opposite side of the room where no windows were, then the affect was not as severe as it would have been if it were right by the window because then it would get maximum sunlight and then no sunlight, but this way it has got some sunlight then no sunlight.

Reliability depends on the number of times the experiment is repeated and the range of the data. This investigation is interpreting the results of 6 of the germination experiment, my own results included. The two sets are compared on the graph (individual – my results, and group – the average of the 6 results), so a full range of data is available and it is reliable.

There is a range of 6 concentrations of inhibitor, and there are 50 seeds for each concentration so the range of concentrations is acceptable and the number of seeds means the reliability is very good as well.

For this experiment to go on further the range of concentrations used could be wider; say, 0. 10, 20, 30, 40, 50% etc., then there would be even more results to make the graph more stable and reliable. Also, instead of just investigating concentration, temperature could be brought in and then there could be all the different concentrations in different temperature conditions as well, like 10 C, 20 C, 30 C. Then it would be interesting to see whether the highest concentration and the highest temperature (obviously not high enough to denature the enzymes in the seeds) would allow the seeds to germinate, because a high temperature should speed up the reaction but the inhibitor should slow it down.

If the experiment noted the results for longer than 6 days then perhaps the seeds in the higher concentrations would germinate as well because the inhibitor may have just induced dormancy for a few days. But since it was just 6 days then not all of them had a chance. And the concentrations that did allow germination within the 6 days (mostly 0%, 10% and 25%) could have fully germinated all their seeds if there were a few more days on the experiment. However, only 6 days was the appropriate time limit.

Also one or two of the Petri dishes got mould, one was 50% and the other was 100%. But I had 2 batches of each concentration so there was still seeds for each concentration except 50% and 100% only had 50 seeds (one Petri dish) and the others had 100 seeds (two Petri dishes) representing them. So the mould affects the results because it makes 2 concentrations less reliable than the other ones. It was not possible to make another batch because then they would only have 1 or 2 days to germinate because the 2 mouldy ones contracted it near the end of the experiment. The mixture of heat and moisture must have been a good breeding ground, and because of that then they had to be removed quickly to avoid contamination of everyone else's Petri dishes (the incubator was shared with the rest of the class' experiments).

- Anomalies

These were mentioned earlier as evidence that things have gone wrong with the experiment. Usually it's a lone result not in sync with the others. So the hypothesis, that the results support, states

that "*in* h*ig*h *concentration conditions of inhibitor* th*en* th*e seeds will germina*te *very slowly or none* at a*ll – showing* a *relationship between inhibitor concentration and germination*" a*nd so* a*nomalies would be results* that *do not follow* th*is* st*atement*. Th*e* ta*ble of results does not show* a*ny obvious* a*nomalies b*ut th*e graph does show one result* that *is not in sync with* th*e others* (*it is indicated on* th*e graph*). It *is for individual d*ata *day* 6. Th*is is to be expected bec*aus*e* th*e individual d*ata *is* just *my experiment a*n*d so if something goes wrong it is much more significant* th*an if* it h*appened to one of* th*e group d*ata *experiments bec*aus*e* th*e averages are take*n. S*o it is one* Pe*tri dish of* 10% *concentration therefore it ca*n *be presumed* that *something* h*appened to* th*e* Pe*tri dish during* th*e* 6 *days which altered* th*e seeds germination process*. Th*is could be d*ue t*o one of* th*e* '*error*' *possibilities* st*ated earlier* (*e.g. due to light intensity cha*n*ging*), *or somebody may* ha*ve moved* th*e* Pe*tri dish by* a*ccident*, *when getting to theirs* a*nd* th*e seeds got moved* a*bout in* th*e dish* a*nd cramped into one spot therefore competition* (*for* wa*ter*) *would be fiercer* tha*n if* th*ey were spread o*ut. A*lso some of* th*e seeds may* ha*ve* just *got* a *smaller dose of* th*e inhibitor* tha*n* th*e* o*ther seeds*, a*nd therefore* th*e variety of germination would be greater* tha*n in o*th*er* Pe*tri dish*es (*see* "*intraspecific competition*" *in* a*nalysis of results*).

Th*ere* a*re not* a*ny o*th*er* a*nomalies on* th*e results* ta*ble or on* th*e graph*, th*ey* a*ll follow* th*e* h*ypothesis*.

Th*e validity of* th*is experiment is* qu*ite* h*igh bec*aus*e of* th*e* h*igh reliability* a*nd wide range of concentrations etc. but it is not* 100% auth*entic bec*aus*e like* a*ll experiments* th*ere* a*re uncertainties*; th*ings* cha*nging in or* a*round* th*e experiment* that *could affect* th*e outcome*.