

The Effect of Mephedrone Upon the Development of *Calliphora vomitoria*

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Introduction

Forensic Entomology is the application of insects to a criminal case. Medico-legal entomology specifically looks at insect growth on a human being, usually on a dead body, in an attempt to calculate the time of death.

Blowfly larvae are one of the first organisms to inhabit a dead body, and grow at a very predictable rate. When these larvae are collected from a body, they are examined microscopically, in order to determine their life stage. This can then be used to calculate the time of colonisation, and therefore the approximate time of death.

As portrayed by Goff et al. (1989 and 1991), drugs, such as heroin and cocaine, can affect the growth of the maggots, causing the time of death to be incorrect. In recent years the use of psychoactive drugs, or 'legal highs' has risen dramatically. Several of these have been found to have lethal properties, including Mephedrone, which could also affect the growth of the maggots.

In order to calculate the time of death, in cases where drugs are involved, the maggots must either be reared to adult-hood, or research should be done with a range of different drugs, and the effect quantified.

Materials and Methods

Upon arrival from WormsDirect UK, six-hundred *Calliphora vomitoria* maggots were separated into pots, and reared on pig's liver, into pupa. These were then placed into plastic tubs, with water, baby milk powder and caster sugar, where the adult flies emerged. Pig's liver was placed in the tub, to allow the adult flies to lay their eggs.



Figure 1. Maggots



Figure 2. Equipment to Make Solutions



Figure 4. Meat Soaking in Solution



Figure 6. Pot with Sawdust



Figure 8. Sample Tubes



Figure 10. Tub with Pupa

12mg of Mephedrone Hydrochloride was weighed into three separate vials, by a laboratory technician. Three solutions of each concentration of 2mg/L, 1mg/L, 0.5mg/L and 0.25mg/L, were made up using distilled water and 22mL of yellow food colouring. Six controls, with no Mephedrone Hydrochloride were also used; three with 22mL of food colouring, and three with an additional 22mL distilled water. 30g of minced beef was then soaked in each of the concentrations for one hour. The food colouring was used in order to ensure the solutions were absorbed by the minced meat.



Figure 3. Bottles of Solutions



Figure 5. Meat Soaking in Solution



Figure 7. Pots in Incubator

Each of the samples of meat were placed into lidded pots, with airholes, and a nylon gauze covering the top. Thirty eggs were placed in each pot, and the pots were placed in the incubator at 27°C and a humidity of approximately 65%.

One sample was taken from each pot each day, and placed in boiling water. This was then removed and preserved in ethanol. Each sample was then measured using a ruler and microscopically examined to determine its life stage.



Figure 9. Microscope

All the remaining samples were reared into pupa, and placed into separate tubs. It was then recorded how long it took for the adults to emerge from each batch, and whether or not they laid fertile eggs.

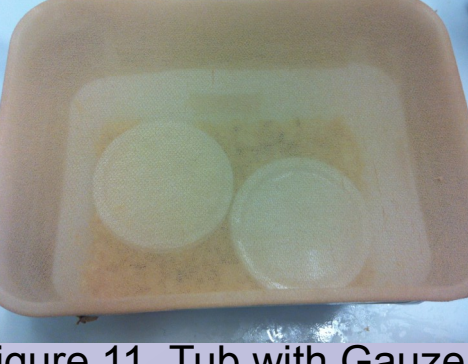


Figure 11. Tub with Gauze

Mephedrone and It's Use

The Mephedrone Hydrochloride used in this experiment was purchased from the United Kingdom Home Office.

The structure of Mephedrone can be found to the right. It has similarities to the structure of several amphetamines, including ecstasy.

Mephedrone used to be a popular legal high in the UK, and was sold over the internet as 'bath salts', but it was then linked with several deaths, and was controlled in March 2010. Since then it has been available to the general public from dealers on the black market.

Mephedrone, or 4-methylmethcathinone, is in the group cathinones. It is a schedule 2, class B controlled drug (Misuse of Drugs Act, 1971), and is usually administered through snorting or swallowing the substance.

Mephedrone is a stimulant, so causes alertness and euphoria in users, and is often combined with different amphetamines and alcohol.

Figure 12. Structure of Mephedrone

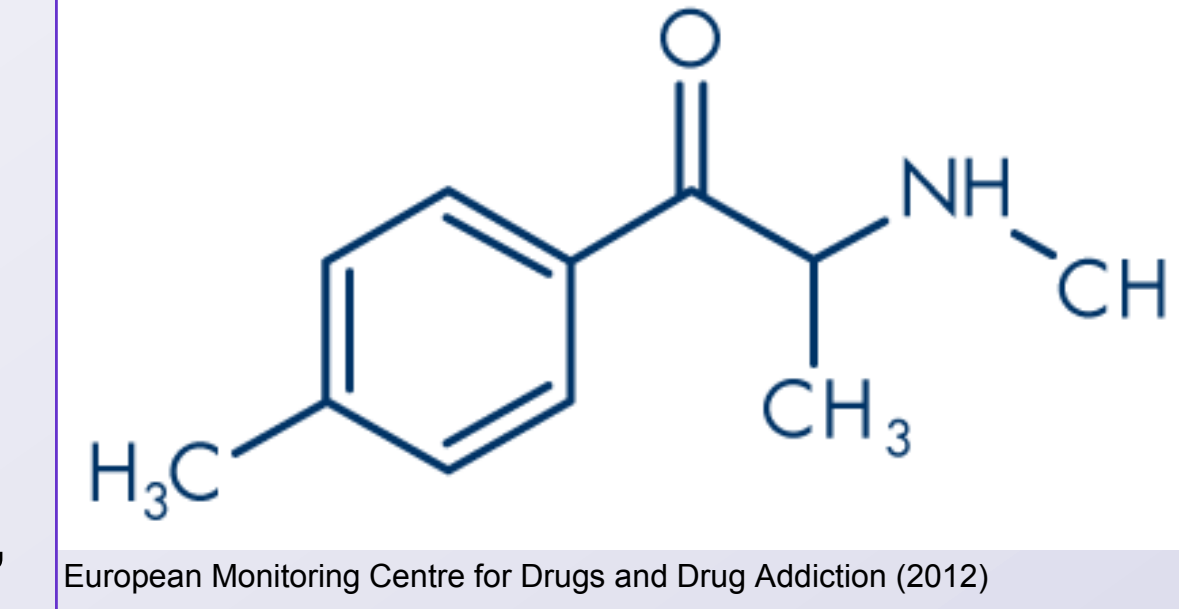


Figure 13. Microscopic Photograph of All Larvae Samples Taken on Day 6 (February 6th 2013)

Larvae Samples from top to bottom: 2mg/L, 1mg/L, 0mg/L (with food colouring), 0mg/L (no food colouring)

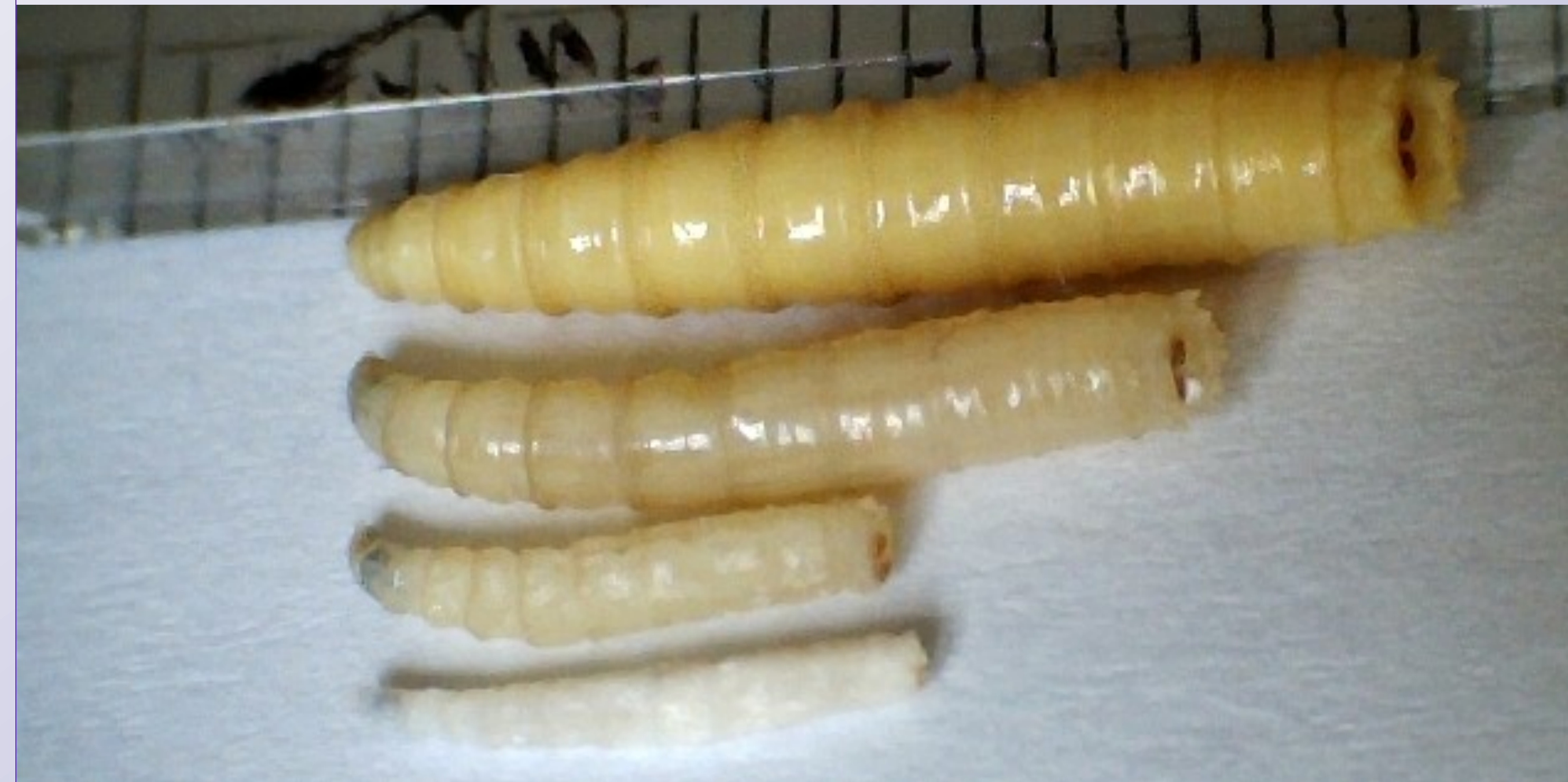


Figure 15. Life Stage Over Time of Maggots Reared Using Different Concentrations

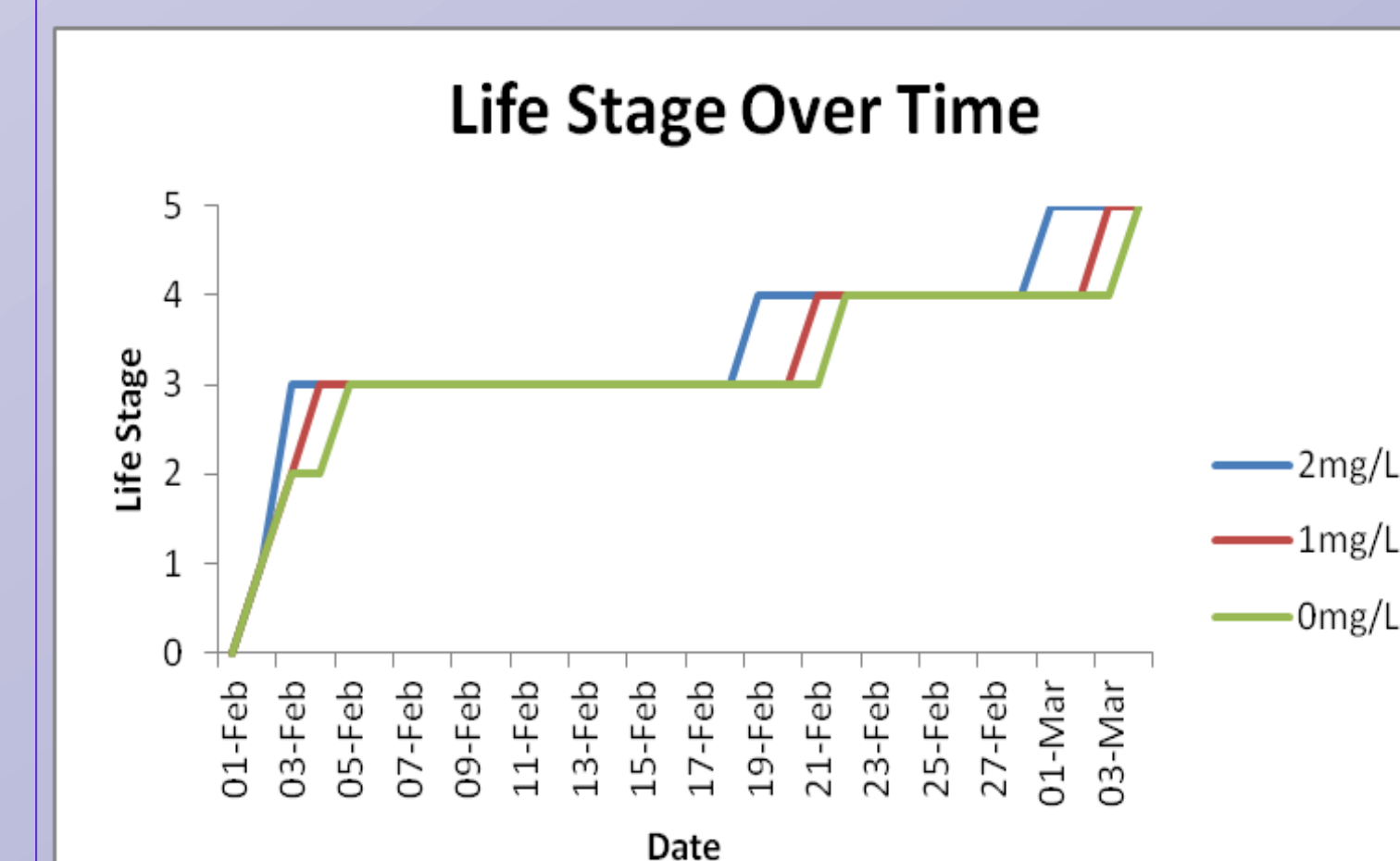
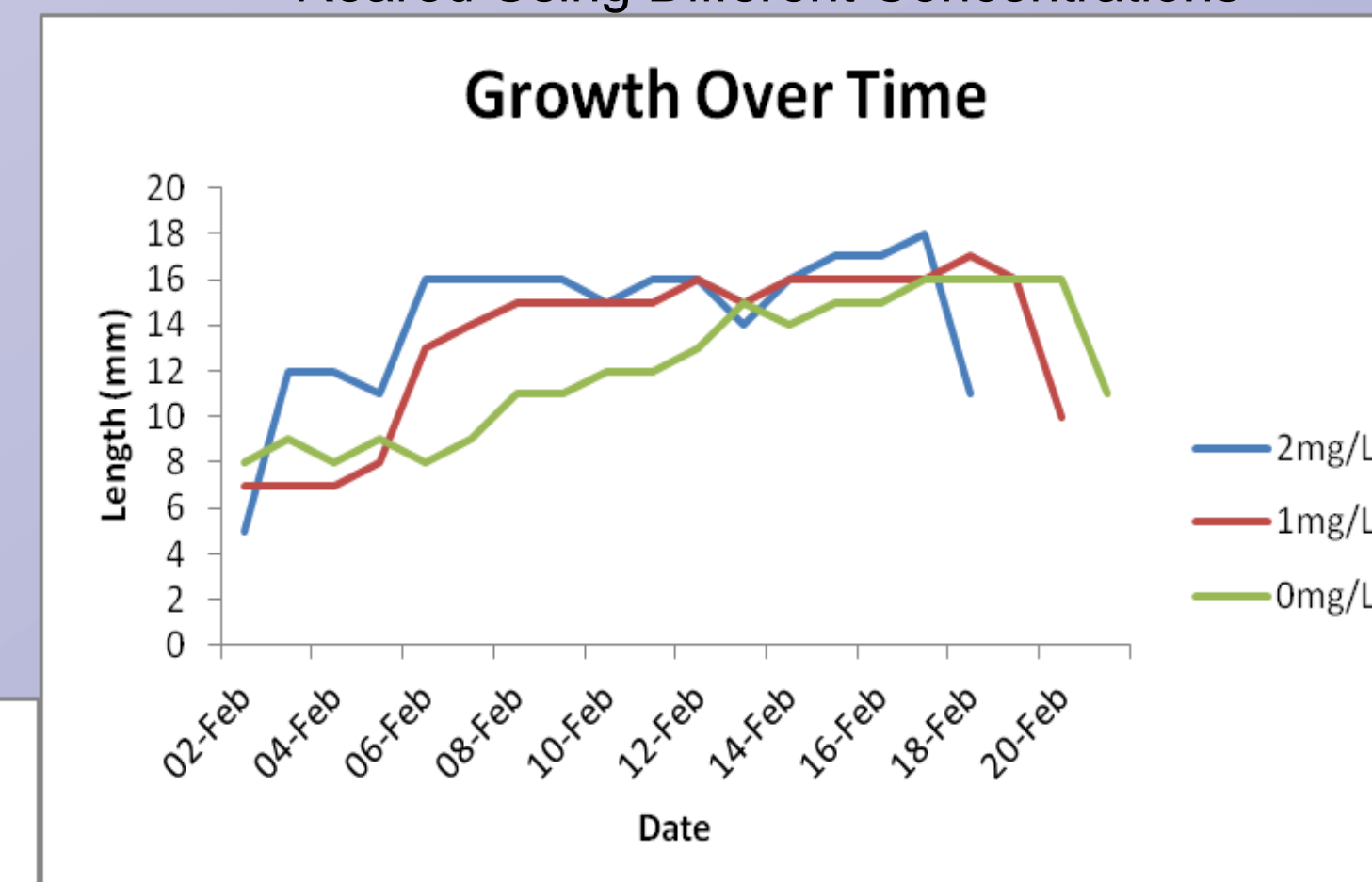


Figure 14. Growth Over Time of Maggots Reared Using Different Concentrations



Results and Discussion

Four of the batches were successful, growing from eggs through to adults, and laying fertile eggs. For reasons unknown, some batches did not make it through all the life stages.

The photograph to the left (figure 13) illustrates the samples taken on day six. The 2mg/L sample had reached 16mm by this point, whilst the 1mg/L sample was 13mm, and both controls were 8mm.

Of the four batches that were successful, the length of each maggot was recorded to the nearest millimetre, and the data for each concentration were compared to one another. As can be seen in figure 14, the larvae grown using 2mg/L of Mephedrone grew at an accelerated rate, compared to the 1mg/L batch and the negative control. Whilst figure 14 does show that the larvae grew at an accelerated rate with higher doses, it should be noted that all samples reached a similar peak; the maggots with the higher concentration simply reached their peak faster and therefore pupated sooner, which would affect the time of death estimation.

The posterior spiracles of the maggots were examined under a microscope, in order to determine the 'instar' of the maggot. This was recorded, and all the batches compared to each other, as can be seen in figure 15. This shows that the maggots feeding on mephedrone may reach the third instar two days earlier than otherwise realised.

Further Research

The method used in this experiment means the effect of any metabolites present have not been properly tested. In reality, when someone dies from a drug overdose, there would be metabolites present. As mentioned previously, Goff tested the effects of cocaine and heroin, but he used live rabbits, which were injected and euthanized. It was not ethically viable to complete an experiment like this in the UK, but without the animal intermediary to metabolize the drug, the results, while interesting, will probably not be applicable to actual cases.

When a maggot grows, it will eliminate the drugs from its system at an unknown rate. At the end of the third instar, maggots stop feeding and move away from their food source, to go and pupate. During this time however, they continue to eliminate any drugs, which means the concentration in these maggots will be considerably lower than those still feeding. This means that correlating the concentration present in the maggot, to that in human tissue is very difficult, and it has not yet been possible to accurately quantitate the blood concentration from the larvae concentration; this would possibly be the most beneficial advancement in entomotoxicology.

Conclusion

The presence of mephedrone appeared to accelerate the growth of the larvae. This may affect the post-mortem interval calculations, as the time of death may be as much as two days later, when looking at the instar, or as much as eleven days later when comparing the lengths of the maggot.

More research is needed in the area of forensic entomotoxicology, so that by demonstrating that mephedrone has this effect, it may help to determine the time of death more accurately in the future, as cases of death by mephedrone overdose become more prevalent.

References

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