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Impact of paracetamol on *Calliphora vicina* larval development

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Abstract This paper demonstrates that blowfly larval development is slightly impacted by paracetamol if present in the rearing foodstuff particularly during days 2–4 of development. Since paracetamol and similar analgesic drugs are involved in a large number of overdose deaths each year this information is of importance where blowfly growth is used to estimate the minimum postmortem interval.

Keywords Forensic entomology · Postmortem interval · Development rates · Entomotoxicology · 4-Acetamidophenol

Introduction

The reliability of entomological evidence in estimating a postmortem interval (PMI) can depend on the toxicology of the decedent's tissues. It has been demonstrated that certain toxins, such as illegal narcotics, present in a decedent's tissues can not only be detected in the larvae feeding on the corpse but can also affect their rate of development (e.g. Goff et al. 1989, 1991, 1993; Introna et al. 1990; Goff and Lord 2001; Gagliano-Candela and Avenaggiato 2001; Introna et al. 2001; Pien et al. 2003). An additional complication is that the rate of development has recently been shown to be influenced by the type of tissue being consumed (Kaneshraja and Turner 2004).

Misuse of analgesic drugs, such as paracetamol were responsible for over 1,000 deaths in the United Kingdom in 2000 (Office of National Statistics 2002). This paper addresses the question, does paracetamol have any significant effects on blowfly development which might influence PMI estimation?

Methods

Small groups of approximately 20 *Calliphora vicina* larvae were raised on paracetamol-liver mixtures at a constant temperature of 20°C and wet weights (in mg) were measured on a daily basis. The paracetamol-liver mixtures were created by homogenizing known weights of fresh pork liver with known amounts of powdered pure paracetamol (4-acetamidophenol) in a blender (MSE Atomix). Paracetamol was used at five concentrations: 1,000 mg/kg, 500 mg/kg, 250 mg/kg, 100 mg/kg, and 0 mg/kg. This order of magnitude of concentration spans the 250 mg/kg value found by Sadler et al. (1997) for the investigation of analgesics, to represent the drug concentration expected in a human fatality caused by a paracetamol overdose.

Fresh *Calliphora vicina* eggs were placed on the paracetamol-liver mixture and reared at a constant 20°C. The day larvae emerged from eggs was designated as day 1. *Calliphora vicina* adults were kept in laboratory cultures on granulated sugar and water. Fresh liver was provided as an oviposition stimulus and site. On a daily basis (from day 1 to pupariation – between 8 and 10 days from oviposition), 10 maggots were randomly taken from each paracetamol-liver concentration. Growth was measured using wet weight (Wells and LaMotte 1995) rather than the more usual length which requires the larvae to be killed in boiling water. The larvae were washed in distilled water, dried carefully on tissue paper, and weighed individually and then returned to their paracetamol-liver mixture. The experiment was replicated 3 times providing a dataset of 30 measurements per day per paracetamol concentration. Statistical tests were applied using the SAS package StatView v 5.

Results

The data were tested for homogeneity of variance using Bartlett's test which indicated that the variances were not equal. As a consequence Welch's test was used in place of standard ANOVA followed by Scheffé's post hoc testing. This showed that there was no significant difference between the various paracetamol dose rates but, with one exception, larvae feeding on any of the concentrations of paracetamol differed significantly from the control (Table 1). Since there was no significant difference between larvae feeding on different levels of paracetamol, the data from the four concentrations (1,000 mg/kg, 500 mg/kg, 250 mg/kg, and 100 mg/kg) were combined and plotted against the growth curve of the control (0 mg/kg) (Fig. 1).

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Table 1 Table of p-values for Scheffé's post-hoc multiple comparison test (following Welch's test) in which each treatment is compared with all others

Dose	<i>p</i> -Value results Scheffé's test Significance level 5%				
	1,000 mg/kg	500 mg/kg	250 mg/kg	100 mg/kg	0 mg/kg
1,000 mg/kg		0.81	0.99	0.65	0.05
500 mg/kg			0.58	0.99	0.02
250 mg/kg				0.41	0.15
100 mg/kg					0

There is no significant difference between the various paracetamol concentrations and with one exception (250 mg/Kg) larvae feeding on paracetamol differed significantly from the control.

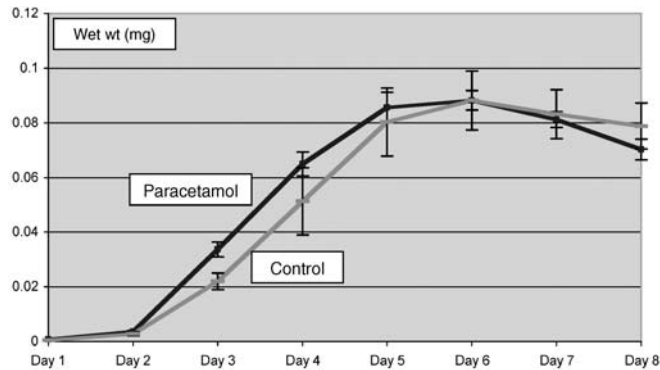


Fig. 1 Larval growth in terms of wet weight, of *Calliphora vicina*. Because larvae feeding on different concentrations of paracetamol demonstrated no significant difference, the data have been combined and labeled paracetamol. The control plot is of larvae feeding on a concentration of 0 mg/kg

The graph suggests that ingestion of paracetamol or its metabolites slightly accelerates larval growth rates during days 2–4, which may generate a difference of approximately 12 h in a PMI estimate calculated during this time-frame. Otherwise the differences in growth rates are not significantly different from the controls.

Conclusion

Paracetamol does appear to slightly affect blowfly larval development, particularly during days 2–4 of development where growth was accelerated in comparison to the control group. The results overall, however, do not indicate a significant difference in growth regarding the concentration of paracetamol ingested by the larvae. There are several difficulties in understanding the role paracetamol may play in this study. The drug is rapidly broken down to n-acetylimidoquinone which, in excess, causes irreversible liver damage. Whether this by-product toxicity spreads beyond the liver to other tissues and if it affects blowfly growth are unknowns.

These initial findings suggest paracetamol, or its breakdown product, does slightly influence blowfly larvae de-

velopment, however, more work is necessary, particularly in the area of establishing what levels and for how long paracetamol remains in a corpse, to determine whether this needs practical consideration in a forensic context.

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