

UNIVERSITI TEKNOLOGI MARA

**CORRELATION OF JUVENILE
HORMONE BISEPOXIDE III
CONCENTRATION WITH FLY
LARVAL DEVELOPMENTAL AGES,
AND MODEL DEVELOPMENT
TOWARDS TIME OF DEATH**

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ABSTRACT

Juvenile hormone (JH) is a sesquiterpenoid hormone found exclusively in insects and arthropods. This hormone regulates various physiological function in insects such as moulting, metamorphosis, reproduction, behaviour, and caste determination. In Diptera, juvenile hormone bisepoxide III (JHB III) has been identified as the primary homologs found in the larval stages and are well known to regulate larval moulting and metamorphosis. This study aimed to measure JHB III concentration patterns in three species of forensically important flies namely *Chrysomya megacephala*, *Chrysomya rufifacies*, and *Musca domestica*. The larval samples analysed were obtained from both laboratory colonies and from the field simulation study. Pearson correlation test was used to examine the relationship between JHB III concentration and larval developmental age. The potential application of predicting the minimum post-mortem interval (mPMI) using JHB III concentration was also explored. JHB III detection and quantification was performed using High-Performance Liquid Chromatography (HPLC). A fluctuating pattern of JHB III concentration was reported herein for *Ch. megacephala* and *Ch. rufifacies*. As for *M. domestica*, there was no clear pattern observed. The highest JHB III concentration was reported at 72 hours of larval development for both *Ch. megacephala* and *M. domestica*. While in *Ch. rufifacies* the highest concentration was at 120 hours. Correlations between larval developmental age and the JHB III concentration was reported in all three species of flies. However, the correlation was not statistically significant ($p > 0.05$). Based on the regression model, JHB III concentration was not a reliable predictor in estimating fly larval developmental age. In conclusion, the concentration of JHB III in fly larvae cannot be used to predict the larval age, or specifically, the mPMI; instead, the decline of the hormone at the end of larval-larval moult may be used to determine the initiation of metamorphosis.

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CHAPTER ONE

INTRODUCTION

1.1 Research Background

Juvenile hormones (JH) are a sesquiterpenoid hormone found in insects and arthropods. Seven JH homologs have been identified, including JH 0, JH I, JH II, JH III, JH III bisepoxide (JHB III), and JH III skipped-bisepoxide (JHSB III). This hormone is mainly produced and secreted by a pair of glands named corpora allata (CA), but it has been reported that male accessory glands may also secrete it. However, in the order of Diptera, only two homologs are present, which are JH III and JHB III. JH III have been identified in *Drosophila melanogaster* (Linnaeus) (Diptera: Drosophilidae) (Bownes & Rembold, 1987; Sliter et al., 1987), *D. hydei* (Sturtevant) (Diptera: Drosophilidae) (Bührlen et al., 1984), and *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) (Baker et al., 1983). A compound discovered co-eluted with JH I and JH III by a radiolabelled product of *in vitro* biosynthesis of corpus allatum- corpus cardiacum complex of *Phormia regina* (Meigen) (Diptera: Calliphoridae) in high-performance liquid chromatography (HPLC) by Liu et al., (1988) led to the discovery of JHB III by Richard et al., (1989). JHB III was identified in *D. melanogaster* (Richard et al., 1989), *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae) (Lefevre et al., 1993), *Calliphora vomitoria* (Linnaeus) (Diptera: Calliphoridae) (Cusson et al., 1991; Duve et al., 1992), *Musca domestica* (Linnaeus) (Diptera: Muscidae) (Richard et al., 1989), *Neobelliria (Sarcophaga) bullata* (Parker) (Diptera: Sarcophagidae) (Richard et al., 1989), *Calliphora vicina* (Robineau-Desvoidy) (Diptera: Calliphoridae) (Richard et al., 1989), and *P. regina* (Yin et al., 1995). These findings have demonstrated that JH III is not the only homolog found in the Diptera.

In dipterans, JH involves in reproduction (Bylemans et al., 1998; Saunders et al., 1990; Richard et al., 1989; Wu et al., 1987), metamorphosis (Lefevre et al., 1993; Richard et al., 1989; Bownes & Rembold, 1987; Bührlen et al., 1984), and behaviour (Teal et al., 2000). During moulting and metamorphosis of *D. hydei* and *D. melanogaster*, concentration of JH was reportedly changing in each larval moulting and pupation (Bownes & Rembold, 1987; Bührlen et al., 1984). The changing pattern of JH during moulting can be helpful in the identification of larval age in dipterans,