K45 The Detection and Quantification of Fentanyl in *Phormia Regina* (Calliphoridae) and Its Effects on Growth and Developmental Rate

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Learning Overview: After attending the presentation, attendees will understand the detection of fentanyl in black blow fly (*Phormia regina*) (Meigen) larvae using Supported Liquid Extraction (SLE) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Attendees will also learn how the presence of fentanyl in the food substrate of larvae affects their growth and development throughout larval stages.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing entomological data on *Phormia regina* to increase accuracy of minimum Postmortem Interval (mPMI) determinations as well as provide a quick method of extraction and detection for fentanyl in blow fly larvae.

As a primary colonizer of carrion, *Phormia regina* plays a vital role in both nutrient recycling and ecosystems via carrion decomposition. They are currently used by forensic scientists in establishing mPMI estimation during criminal cases, but recent research has that larvae can be further used for the detection of drugs and toxins when traditional matrices are unavailable.[1,2]

Fentanyl is a rapid-onset synthetic opioid with extremely high potency.[3] Illegally, fentanyl has been increasingly used as an adulterant mainly due to the ease of manufacturing and availability of its precursors to be shipped internationally.[4] Due to the increase in accidental deaths by overdose, fentanyl is now routinely screened for in toxicological analysis. Death from fentanyl can be rapid, and by the time a body is discovered, significant time may have passed. When sampling traditional body fluids becomes difficult due to their absence, external factors, or the presence of skeletonization, insects may serve as reliable alternative specimens for toxicological analyses.[1]

There have been several studies that have confirmed the reliability of entomological specimens for qualitative analysis; however, quantitative extracts remain questionable and unreliable. In addition, the presence of toxins has been shown to increase or delay development of entomological specimens, which can be critical in a criminal investigation. It has become critical to understand the detection and effects of fentanyl on different matrices due to its increased use and high contribution to overdosing.

The purpose of this study was to further analyze the question currently poised for forensic entomologists/toxicologists: is there is any correlation between concentrations of toxins in human postmortem tissue to those concentrations detected in blow fly larvae and can they be used reliably as samples for toxicological investigations? Using *Phormia regina*, this study evaluated LC/MS/MS to quantify and qualify fentanyl accurately as well as obtain some preliminary information pertaining to the effects of fentanyl on larvae development.

A maximum of 100 individual pupae received from the University of New Haven in West Haven, CT, and University College of St. Mary in Omaha, NE, were placed inside a mesh enclosure with an ambient temperature of 26–28°C. Chunks of beef liver were provided to the adults to facilitate oviposition. Eggs were collected and placed on homogenized ground liver pre-dosed with fentanyl. The doses were a high-end therapeutic level (50ng/mL), a toxic level (125ng/mL), a lethal level (250ng/mL), and beyond lethal level (500ng/mL).

The larvae were monitored, and sample larvae were taken every day to confirm the lifecycle stage. Triplicate larvae samples were collected at the 2nd instar, 3rd instar, and post-feeding life stages. The larvae were homogenized, extracted, and analyzed using LC/MS/MS. Fifteen random specimens were selected and used as a representative set every day for each dose to monitor life stage duration. These larvae were also measured to obtain a growth curve.

During the trial, fentanyl was detected in 2nd and 3rd instar larvae fed on toxic and lethal level dosed meat while detection remained minimal or nonexistent in the post-feeding larvae of any dose. There was no correlation found between the feeding substrate and the larvae samples of any stage of the life cycle. The development rate to 2nd instar was delayed and correlated with the increase of fentanyl, thus prolonging the total time for development from two to four days.

With the increase of fentanyl throughout the Eastern coast of the United States and the prominent presence of *Phormia regina* in decomposition, this study aimed to find results that will lead to the use of larvae as alternative matrices. This study demonstrated that fentanyl was detectable in 2nd and 3rd instar larvae in toxic and lethal doses. Further, it demonstrated that fentanyl delayed development in *Phormia regina* by up to four days when exposed to high levels of lethal dosing, effecting accurate PMI. It was also concluded that there is no correlation between spiked food substrates and the concentration found in the analyzed insect.

Reference(s):

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