H12 Blow Flies and Nicotine: An Entomotoxicology Study

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After attending this presentation, attendees will understand the potential capabilities of entomotoxicology and how the presence of nicotine, a common and powerful drug, can affect the survival and the developmental rate of blow flies and, as a consequence, have an effect on the Postmortem Interval (PMI) estimation.

This presentation will impact the forensic science community by providing data that will be potentially useful in adding a new component to estimating the minimum time since death of remains exposed to drugs.

Entomotoxicology is a branch of forensic entomology which studies the potential uses of insects for detecting drugs, other toxic substances, and gunshot residues in decomposing tissues. In fact, insects developing on a cadaver present an alternative and appropriate substrate for analysis of toxicological substances, especially in the absence of tissues or biological fluids. The main focus of forensic entomology is determining the time elapsed since death of a deceased human or animal using arthropods, but in recent studies it has been demonstrated that such time frames may be severely compromised by drugs and toxins. Therefore, entomotoxicology also investigates the effects of these substances in cadavers, on arthropod development, and morphology. While the detection of drugs, metals, pesticides, and alcohol has been reported in entomotoxicological studies, there is no research concerning the detection, analytical quantification, and the effect of nicotine on the necrophagous entomofauna.

Nicotine is a volatile and water-soluble alkaloid present in the tobacco plant (*Nicotiana* species, Solanales: Solanaceae). Nicotine has acute toxicity and it is considered one of the most deadly poisons known to man: rapid administration of large doses of nicotine may cause death within a few minutes. Nicotine, regardless of the mode of administration, can be readily absorbed across the epithelium of the lung, the nose, and through the skin and mucosa. Therefore, there is a potential for poisoning from ingestion, injection, inhalation, and skin and rectum absorption of nicotine from nicotine-containing products.

Nicotine can be found in tobacco products (e.g., cigarettes, cigars, pipes, and refill solutions for e-cigarettes), in products for Nicotine Replacement Therapy (NRT), toothpastes, and insecticides. In humans, the median lethal dose (LD_{50}) of nicotine is 0.5mg/kg-1.0mg/kg, which means that the fatal dose of nicotine can be estimated in 30mg-60mg of nicotine in adults and 10mg in children. The nicotine content in an average cigarette is 10mg-20mg and the nicotine concentration in e-cigarette refills can reach approximately 22mg/mL; however, despite most of the everyday tobacco products containing a considerable amount of nicotine, only a small percentage can be absorbed by the body.

The literature reports a number of accidental/sudden, suicidal, and homicidal cases whereby nicotine (alone or mixed with other drugs) was used. To note, recipes are readily available on the internet on how to extract pure nicotine from smoking tobacco for suicidal purpose.

In the current research, the presence and the effects on larvae of the necrophagous blow fly *Calliphora vomitoria* L. (Diptera: Calliphoridae) were examined when reared on substrates (beef liver) spiked with three different concentrations of nicotine that could cause death in a human (2ng/mg, 4ng/mg, 6ng/m).

A method was developed and validated for Gas Chromatography/Mass Spectrometry (GC/MS) to determine nicotine in larvae reared on spiked liver. Statistics were calculated to determine the linearity of method, coefficient of determination (R2>0.99), the evaluated detection limit (LOD=0.25ng/mg), the quantification limit (LOQ=0.86ppb), extraction recovery percentage, precision, selectivity, and carry over. All parameters were satisfied.

The results demonstrated the following: (1) GC/MS can detect both nicotine and its metabolite cotinine in *C. vomitoria* immatures; (2) the presence of the three scheduled nicotine concentrations in the food substrate did not modify the developmental time of *C. vomitoria*; (3) during the pupation period, larvae reared on substrates containing nicotine died dependent on the concentration of nicotine present; and, (4) the resultant lengths of larvae and pupae exposed to both concentrations of nicotine were significantly shorter than the control.

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Results of this research will improve the general knowledge in the field of forensic entomology/entomotoxicology as well as provide another tool that may be useful in an investigation; however, this research underlines the need for further studies concerning nicotine and blowflies, such as: (1) the effects of lower and higher nicotine doses on blowfly development; (2) how nicotine mixed together with other drugs affects blow flies; and, (3) nicotine metabolite pathways and their effects on blow flies.

Entomotoxicology, Nicotine, Calliphora Vomitoria

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