Senior seminar research proposal

Introduction Forensic entomology is used to determine such crimes as murder, suicide, and other criminal acts by examining various insects instead of using human tissues. This forensic tool is used to determine the postmortem interval of a corpse and the cause of death of a corpse when all other forms of human evidence (human blood, tissues, hair, etc.) are not present at the scene. Forensic entomologists prefer using insects to determine these factors of death because the insects produce similar results as human test materials such as human blood or tissues which yield the best analytical results for the forensic entomologist.

The use of forensic entomological evidence has been accepted and used in many courts around the world (Anderson, 1999). The use of this tool in court can support or refute a suspect’s alibi and improves the criminal investigation against the suspect (Anderson, 1999). Even though forensic entomology is an efficient tool to use in criminal investigations there are some drawbacks to this analytical tool. Such disadvantages include improper collection of entomological evidence and improper analysis of insects after collection, resulting in incorrect entomological results and a possible false conviction of a suspect.

The proposed research of this paper focused on insects being affected by different concentrations of ethanol during natural insect development and also focused on the detection of ethanol in insects using gas chromatography mass spectrometry (GCMS) to determine if insects were significantly affected by ethanol exposure. The research also focused on the exposure of ethanol to the insects and how this exposure affected PMI (postmortem interval) determination. Forensic entomology is a commonly

used tool to determine cause and time of death by examining various characteristics of insects that are collected at the crime scene.

Such characteristics include size of the insect(s) and the life cycle stage of the insect collected. Forensic entomology becomes the most accurate and sometimes the only tool available for determining time of death, especially after 72 hours (Anderson and VanLaerhoven, 1996). Forensic entomology can also determine if a body has been moved from place to place, determine where the death occurred, determine the presence of various types of drugs and toxins present (if any), wound location, and determine who the suspect and victim are due to the presence of certain insects on the body (Grisales, et al. 2010). Background/Literature Review There are five levels of decomposition identified by a forensic entomologist when conducting an entomological investigation which include fresh, bloated, active, advanced, and remains (Grisales, et al. , 2010). These stages of decomposition are important to a forensic entomologist because insects appear on a corpse periodically throughout the decomposition cycle which therefore determines the time of death of a corpse. During these levels of decomposition insects begin to reach the corpse either by flying (adult flies) or by burrowing through the ground (pupae).

Some insects can also reach the corpse by hatching from eggs (larvae) that were laid on the corpse after death. The fresh stage of decomposition involves a drop in body temperature and the appearance of very few flies on the corpse. The insects that are collected are typically larvae and are collected from the mouth of the corpse. The bloated stage of decomposition involves a significant increase in the body weight of a corpse due to rainfall

exposure. The insects that are collected at this stage of decomposition are larvae found on the back, head, ears, and anus of the corpse (Grisales, et al. 2010). The active decay stage of decomposition involves fly larvae feeding on a corpse which significantly lowers the body weight of the corpse and an increased amount of fly larvae found in the intestines/ organs of the corpse (Grisales, et al. , 2010). The advanced stage of decomposition involves the fluctuation of body temperature depending on the location of the corpse and an even bigger decrease in body weight due to increased consumption of body tissues by fly larvae (Grisales, et al. , 2010).

There is an increase in adult flies that are collected at this stage of decomposition. In the last stage of decomposition, remains, there is no continued change of the corpse and the presence of insects found decreases (Grisales, et al. , 2010). The stages of decomposition and the insects present at these stages are also used to determine the postmortem interval (PMI) of a corpse. PMI, or postmortem interval, provides important detail of situations that occurred before time of death in cases of homicide and untimely death (Byrd and Castner, 2001).

Arthropods that are found on the corpse can determine the length of egg to fly transformation then to the recovered developmental stage (Gennard, 2007). The best arthropods to use are the oldest ones that were yielded from eggs when the larvae were first deposited on the corpse because they have the longest feeding time on the corpse which allows them to fully develop and retain any evidence from the corpse much longer; this will produce a more accurate PMI. Such information can help to identify both the criminal

and the victim by eliminating the suspects and connecting the deceased with other individuals.

The predictable physical and chemical consequences of death are usually the most reliable PMI indicators, which means knowing what an insect should look like at which developmental level to determine when a dead body became a corpse (Henssge et al. 1995). PMI is also associated with the succession rate of various insects which is another tool that is used to determine the time of death of a corpse. The succession rate includes information about the time elapsed between death and the presence of a particular arthropod or insect species and stage (Byrd and Castner, 2010).

A dead body will go through very recognizable decomposition stages (physical, biological, and chemical changes) that attract various types of arthropods in each decomposition stage that are needed to determine the succession rate (Monthei, 2009). The most common succession rate estimated by an investigator is the age of larvae and the time interval between death and the arrival of larvae on the corpse (Byrd and Castner, 2010). The knowledge of what insects are supposed to be present and absent based on the season also helps to determine PMI.

Calculation of PMI involves five different arthropod life stages (eggs, larvae, pupae, adult, and carcass) for flies when collecting insects from corpses. The egg stage of the fly is characterized by the laying of between 150-200 eggs, with the exception of some flies lying between 2000-3000 eggs, found on the body in cluster form within areas that provide protection, moisture, andfood(Gennard, 2007). Fly eggs are typically shiny and white; the eggs have a similar appearance to that of a grain of rice.

The reason for specific placement of eggs on a decomposing body is the fact that the body contains a high quality of nutrients which are used as a feeding source for the fly eggs to grow, as well as the influence of growth on other species of insects that feed on the decomposing body. The larval stage of the fly species is characterized into three stages (L1, L2, and L3) which correspond to the number of slits present on the backs of the larvae (Gennard, 2007). These slits are used by entomologists to determine what stage of life the larvae are in when collected.

In the third larval stage, where larvae are the largest, the larvae stop feeding on the corpse and begin looking for a place to begin pupariation. Pupariation is the final stage of development inmetamorphosisinto the adult stage and is generally called the larval post-feeding stage (Gennard, 2007). Pupariation typically doesn’t take place on the corpse because insects in this stage prefer to be in a cool, dark place or underground, if possible. In some instances of fly larvae in pupariation, the insects were shown to pupate on the corpse.

The adult stage, or the end of the life cycle, is initiated by the arthropod pushing itself out of the puparium case and once the adult fly is free from the puparium case, the insects make their journey upward through the soil. Once the adult fly has reached the top of the soil the insects release their waste and expand their wings to make their way to other corpses or decomposing material. The adult stage may be the final stage of the life cycle but the carcass of these adult flies can also be collected as evidence along will the carcasses of arthropods in each life stage.

Entomotoxicology Entomotoxicology is the analysis of insects and insect remains for the presence of toxins that may have been present in the corpse before death (Goff and Lord, 1994). Detection of various toxins and controlled substances in insects found on decomposing human remains has contributed to the determination of both cause and manner of death by determining what times of toxins were present in the corpse before death (Lord 1990, Goff and Lord 1994, Nolte et al. , 1992).

Entomotoxicology also serves as an alternative analysis tool to determine the presence of toxins or cause of death when certain specimens aren't available for collection, such as human blood or tissues. The toxicological analysis of insect biological materials is conducted in the same manner as the toxicological analysis of human biological materials, making cause of death determination more accurate (Definis- Gojanovic, 2007). Many different species of arthropods, such as flesh flies and blowflies, are used when conducting an entomotoxicological analysis and these arthropods are used to determine the PMI or time before death.

The use of entomotoxicology has many advantages, which includes determination of time before death (PMI) and identification of suspect, but this analytical tool also has several disadvantages. One disadvantage includes the issue of recording the exact temperature of the insects because if the information is incorrect then the outcome of the PMI will also be incorrect. Another disadvantage of this analytical tool is the fact that it is fairly new in the forensicsciencefield so if an individual isn't educated on how to use the technique then valuable entomological evidence related to the case may be lost.

Lastly, if proper collection of entomological evidence isn’t conducted properly, the loss of highly important evidence relating to time of death and cause of death could be lost. Cause of death is usually determined by various types of toxins that are detected in the insect after an entomotoxicological analysis was conducted. Issues with Determination of PMI The determination of PMI can be affected by multiple factors but only two will be discussed in this research proposal which include: temperature and ethanol. Temperature involves the rise or fall of temperature to such a high or low that affects the growth or succession rate of insects.

Air temperature and exposure to sunlight will raise the corpse temperature which will also increase the insect succession rate. Temperature can also be influenced by such weather conditions as rain, sun, snow, and wind which can greatly affect the amount of entomological evidence collected (insects) and the outcome of a legal investigation (Sharanowski et al. , 2008). Alcohol, or ethanol, is one of the oldest abused drugs in the world that is readily available and the most commonly abused drug in Western societies (Stripp, 2007).

Ethanol is a product of fermentation due to yeast cells acting on sugars from fruits and grains that produces a clear, volatile liquid that is soluble in water (Stripp, 2007). Once ethanol enters the blood stream orally it travels in the blood into other tissues. The ethanol travels to tissues with greater water content because these tissues will receive greater ethanol distribution. The ethanol concentration will be different in both the corpse and the insects due to the different water amounts found in each species (insects and corpse).

The rate at which ethanol is eliminated from the body is another important factor to a forensic entomologist because this can determine the time at which the individual started drinking. The focus of this experiment will involve different concentration levels of ethanol and the effects on the growth rate of the fly species Sarophagidae (flesh flies). Proposed Research The broad, long-term objectives that this research paper is focused on determining whether ethanol can affect the growth rate of entomological evidence and how much ethanol can be detected in the insects.

This research is also being conducted in an attempt to make a comparison to the other research experiments to see if the results concerning growth rate of insects exposed to such drugs as morphine or heroin are similar or different to the growth rate results of insects exposed to ethanol. This research paper includes four specific aims that was accomplished as a way to make the broad objective a more manageable piece that could was easier to manipulate. Specific aim one included determining whether ethanol could be found in both test fly species after feeding on the ethanol infused beef liver.

Specific aim two involved determining which concentrations of ethanol produced the most significant changes in the flies. Specific aim three involved tracking the growth rate of the fly species that were exposed to the ethanol infused beef livers (test subjects) and those who were not (control). Specific aim four involved observing any change in the growth rate data from the fly species test subjects that were exposed to three specific concentrations of ethanol. These specific aims will be used in sequential

order as a way of getting closer to solving the broad objective. Experimental Methods Overview

The research experiment hypothesized there would be significant changes in the growth rate of the fly species when exposed to the three specific concentrations of ethanol. The research experiment also predicted that there would be obvious physical changes in the flies when exposed to beef livers infused with specific concentrations of ethanol at different stages of life. The experimental design constructed for this experiment involves the fly species Sarophagidae (flesh flies) feeding on beef livers infused with ethanol over a period of eight to twenty-one days which is the general life cycle for flesh flies.

The three beef livers had varying concentrations of ethanol (25 ug, 50 ug, and 100 ug) injected into them that the flesh flies were exposed to. The control group for the experiment was hand massaged with deionized water as a way to keep liquid consistency amongst the groups. At the end of the experiment, the insects were collected into a test tube and then exposed to GCMS or gas chromatography mass spectrometry in an attempt to determine the presence of ethanol in the test subject (insects). The insects were also analyzed for any type of stunted or heightened growth in regards to a normal fly life cycle.

This analytical test was able to show that there was ethanol present in the fly species. Experimental Variables The independent variable in this experiment is the specific concentration of ethanol that is distributed amongst the three beef livers. The dependent variable in this experiment is the growth rate of both species after exposure to ethanol as well as the specific ethanol

concentrations. The controlled group was the fly larvae that were not exposed to ethanol. The other controlled variables of this experiment were the 45 degrees Farenheit (temperature) the beef livers were maintained at and the amount of beef used (8 oz. as the feeding substrate for the fly species. The experimental groups in this experiment were three larvae groups exposed to ethanol and the control group was a larvae group not exposed to ethanol. Procedures/ Measurements The following experimental design was adapted from a previous research study conducted by George et al. , 2009. Three beef livers weighing 8 oz. each were prepared using the corresponding ethanol concentrations for the three experimental groups of the fly species tested (Experimental Group Two- 25 ug, Experimental Group Three- 50 ug, and Experimental Group Four- 100 ug). 0 mL of deionized water was distributed evenly into the control group (EG 1) to maintain liquid consistency amongst all groups. A cluster of fly larvae for the three experimental groups was collected and distributed evenly amongst the three experimental groups (2-4) as well as the control group. The beef livers were contained in a small plastic tub and refrigerated at a temperature of 45 degrees Farenheit when the beef livers were not being used to discourage spoiling of beef liver as well as to avoid evaporation of ethanol.

The growth rate of the test subjects from both fly species exposed to ethanol is measured (any alteration in growth rate is the factor that is being measured) over a period of three weeks. At the end of three weeks, or longer if necessary, all of the perished insects from the experiment were collected for analysis using GCMS (gas chromatography mass spectrometry) to

determine the presence or absence of ethanol in the insects. Material List Deionized water Ethanol solution Fly larvae of Sarophagidae (flesh flies)

Four beef livers (8 oz. each) Graduated cylinder GCMS machine Microscope Pipette Plastic tubs Refrigerator References Anderson, G. S. (1999). Wildlife Forensic Entomology: Determining Time of Death in Two Illegally Killed Black Bear Cubs. Journal of Forensic Sciences, 44(4): 856-859 Anderson, G. S. and Van Laerhoven, S. L. (1996). Initial Studies on Insects Succession on Carrion in Southwestern British Columbia. Journal of Forensic Sciences, 41: 617-625 Brown, G. , Fuke, C. , Pounder, D. J. , Robertson, L. and Sadler, D. W. (1997). Barbiturates and Analgesics in Calliphora vicina Larvae. Journal of Forensic Sciences, 42(3): 481-485 Byrd, J. H. and Castner, J. L. (2001). Forensic Entomology: The Utility of Arthropods in Legal Investigations. CRC Press: Boca Raton, FL Definis- Gojanovic, M. , Britvic, D. , Kokan, B. , and Sutlovic, D. (2007). Drug Analysis in Necrophagous Flies and Human Tissues. Arh Hig Rada Toksikol, 58: 313-316 George. K. A. , Archer, M. S. , Green, L. M. , Conlan, X. A. , and Toop, T. (2009).

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