K11 Expanding Frontiers in Postmortem Toxicology: Drug Tracing in Different Postmortem Matrices During Human Decomposition Using Ultra High-Performance Liquid Chromatography-High-Resolution Mass Spectrometry (UHPLC-HRMS)

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Learning Overview: After attending this presentation, attendees will have a better understanding of a highly sensitive UHPLC-HRMS approach for detecting drugs postmortem throughout soft tissue decomposition.

Impact on the Forensic Science Community: This presentation will positively impact the forensic science community by revealing novel aspects of inter-individual toxicological differences in serum, fly larvae, decomposition fluid, and soil from human donors during the early and late stages of decomposition.

Decomposition rates of donors placed in identical environmental conditions appear to be verifiably different, therefore the focus of this study is on intrinsic drivers of human decay that may have the potential to alter decomposition trajectories.[1,2] The ultimate goal is to elucidate how drugs impact human decomposition rates and, ultimately, Postmortem Interval (PMI) estimation. Entomotoxicological experiments by Goff et al. revealed an impaired insect development for certain drugs of abuse; however, thus far it is completely unknown how drugs, particularly prescription and end-of-life medications, impact patterns of human decomposition.[3] This presentation provides insights on the analysis of drugs in several compartments of the highly dynamic postmortem biochemical environment from decomposing bodies through primary microbial and insect decomposers.

This research was conducted on human donors, obtained through the Body Donation Program of the Forensic Anthropology Center (FAC), and decomposed in an outdoor forested setting at the University of Tennessee Anthropology Research Facility (ARF) in Knoxville, TN. Serum, soil, fly larvae, and decomposition fluid samples were obtained from human subjects at various time points during soft tissue decomposition, flash frozen and analyzed for their toxicological composition. Decomposition fluid was collected at various anatomical positions from around the body; larvae were collected at each instar as well as during the post-feeding stage. Hourly records of temperature and relative humidity allowed for the calculation of Accumulated Degree Hours (ADH), a metric that combines temperature and time to determine specific sampling points. Termination of sampling occurred after the bodies completed active decay.

Aliquots of all specimens were extracted using a procedure adapted from Lu et al.[4] Samples were injected onto a Raptor Biphenyl column (100mm x 2.1mm) with 2.7-µm particle size in combination with a guard column (5mm x 2.1mm, 2.7-µm). Afterward, the eluent was introduced into a Q Exactive™ Plus Hybrid Quadrupole-Orbitrap via positive electrospray ionization. A full scan Mass Spectrometry (MS) analysis (70–1,050*m/z*) was performed, and samples analyzed for 12 minutes with a resolution of 140,000. Commercial drug standard stocks of 15 drugs from major drug classes were purchased for quantitative analyses. Spectral features were manually selected based on mass accuracy (± 5ppm mass tolerance) and retention times (≤ 2min) using an adapted drug database from Restek®.[5]

Initial toxicological screenings of nine donors revealed that both parent drugs and their drug metabolites are traceable postmortem over time from the initial serum sample to later samples of decomposition fluid and primary decomposers, to ultimately the local soil matrix. Moreover, it was possible to assign each donor a unique toxicological profile, which showed time-dependent changes and variability in detected drug intensities for each of the four analyzed matrices throughout the decomposition period. The current results show, first, the ability and, second, the unexplored potential of drug detection in a series of different matrices after death. Furthermore, they seem to reveal first signs of a possible direct relationship of drug-induced effects on decomposer physiology. Ongoing "big data" analysis will combine toxicological screening with metabolomics and lipidomics data sets to identify potential biochemical biomarkers of decomposition and create an overall picture of how postmortem metabolite signatures relate to perimortem toxicological loadings.

Reference(s):

1. Dautartas, Angela, et al. *Journal of Forensic Sciences* 63.6 (2018): 1556-4029.
2. Hayman, Jarvis, and Marc Oxenham. *Australian Journal of Forensic Sciences* 48.2 (2016): 171-185.
3. Goff, M.L., and Wayne D. Lord. *Forensic entomology: The utility of arthropods in legal investigations* (2001): 331-340.
4. Lu, Wenyun, et al. *Analytical Chemistry* 82.8 (2010): 3212-3221.
5. Big Pain Assays Aren't a Big Pain with the Raptor Biphenyl LC Column. *Big Pain analysis: www.restek.com/bigpain.*

Forensic Chemistry, Human Decomposition, Drug Analysis

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