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### **K23 Detection, Quantification, and Relative Distribution of Ketamine, Norketamine, and Dehydronorketamine in Skeletal Tissue of Dosed and Buried Rat Remains at Different Stages of Decomposition**

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After attending this presentation, attendees will be informed of the potential to detect ketamine in buried skeletal tissue from decomposing rat remains using a passive methanolic extraction, solid phase extraction, and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method. In addition, attendees will understand the effect of postmortem interval on drug detection, quantification, and relative drug distribution.

This presentation will impact the forensic science community by demonstrating the effects of burial and postmortem interval on levels of ketamine, its metabolites, and their relative distribution in skeletal tissue, providing toxicologists with a reason to analyze buried and decomposing remains.

During a death investigation in which decomposition has occurred, skeletal remains may be the only available source of toxicological information. Recent literature has explored drug detection in skeletal tissue, but implications of drug measurements in bone as well as drug distribution remain poorly understood. Additionally, few studies have been published on buried remains and remains at different stages of decomposition.

The goal of this study was to detect and quantify ketamine in buried skeletal tissue from different stages of decomposition and to determine what bones are best suited for toxicological analysis. Rats were dosed daily for ten days at three levels: 20mg/kg ( $n=13$ ), 30mg/kg ( $n=13$ ), and 40mg/kg ( $n=13$ ). Control rats ( $n=3$ ) remained untreated. Rats were euthanized in two groups: one hour after the last injection ( $n=21$ ) and ten days after the last injection ( $n=15$ ). One rat from each dose group (0mg/kg, 20mg/kg, 30mg/kg, and 40mg/kg) was left unburied and tested for drugs. The remaining rats were buried in the New Jersey Pine Barrens and exhumed at different stages of decomposition (Fresh, Early Decomposition, Advanced Decomposition, and Skeletonization). Accumulated Degree days (ADD) were used to calculate the decomposition stage (77, 188, 293, 793, and 3104 ADDs). Rats were dissected and organs, skin, and hair were removed. Bones were cleaned of remaining tissue using dermestid beetles. By 3,104 ADDs, rats were fully skeletonized and not introduced to the beetles. Bone types were separated (pelvis, vertebrae, upper limbs, and lower limbs) and sampled (500mg) to be ground using a Biotage® Bead Ruptor 24. Samples underwent passive methanolic extraction and solid phase extraction. Ketamine, norketamine, and dehydronorketamine were detected in skeletal tissues using LC/MS/MS with ketamine-D4 as the internal standard. Drug levels were compared across decomposition stages and bone types.

Consistent with the hypothesis, drugs were not detected in the control samples. Ketamine, norketamine, and dehydroketamine were detected in skeletal tissues of the unburied subjects and most buried subjects that were dosed. Detected levels were higher in rats that received higher doses. Ketamine and its metabolites were detected in most bone types, with higher levels in long bones as a result of there being more marrow and blood flow. Due to the ability to detect ketamine in skeletal tissues, toxicologists should attempt drug screening, even at advanced stages of decomposition.

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#### **Ketamine, Bone, LC/MS/MS**