A164 The Detection of Chemical Weapon Nerve Agents in Bone: An Anthropological Approach to Skeletal Toxicology

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Learning Overview: After attending this presentation, attendees will understand skeletal toxicological methods and their prospective role in forensic anthropology casework and human rights investigations.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating that chemical weapon exposure can be detected from skeletal remains, potentially expanding the ability to collect evidence from atrocities even after prolonged post-incident intervals, and by disseminating anthropologically informed models and methods that improve interpretation of skeletal toxicology results in general.

Forensic anthropologists aid forensic pathologists' determination of Cause Of Death (COD) from skeletonized remains when death is due to physically traumatic causes but cannot provide biological evidence for chemical CODs. Official COD in these cases is often left undetermined. The lack of reliable toxicological tests for skeletal material represents a significant gap in forensic science, especially considering both the opioid epidemic and recent chemical weapon attacks.

Studies show drugs can be detected from skeletal remains, even after years of decomposition.[1,2] Contrary to popular assumption, compounds are most readily detected following acute exposures (e.g., overdoses) rather than chronic abuse.[3] However, the absence of known incorporation mechanisms has hindered application of study results. A model for the incorporation of xenobiotics of forensic interest into the human skeleton, newly proposed as part of this research, suggests drugs and other analytes enter bone via equilibrium-based exchange between vascular fluids, bone interstitial fluid, and the separate water layer bound to each bone crystal.[4] Although most are poor substitutes for bone minerals and rapidly exchange back into vascular fluids, select compounds may adsorb onto mineral surfaces and/or fully substitute into the lattice structure.

Based on their unique chemical properties, Nerve Agent Metabolites (NAMs) may adsorb in relatively elevated quantities. To assess this possibility, methods for the extraction and semi-quantitative detection of NAMs EMPA, iBuMPA, IMPA, CMPA, and PMPA (corresponding to VX, Russian VX, sarin, cyclosarin, and soman) from bone were developed using liquid chromatography/mass spectrometry. A quadrupole time-of-flight mass analyzer was used to identify and evaluate the effects of endogenous bone compounds, while a triple quadrupole was used to achieve lower Limits Of Detection (LODs) of NAMs. Instruments were operated using reversed-phase chromatography and negative electrospray ionization. Bone samples were prepared using rapid demineralization and carbon-based solid-phase extraction. Fortified human femoral bone was used for validation. There were no interferences from the matrix or standards; however, ionization suppression was extremely high, ranging from -11.0% to -87.6% at low analyte concentrations. Citrate was the main source of suppression but could not be fully removed from the samples due to shared chemical properties with NAMs. Mean extraction efficiency was 86.7%–100.5% for low concentration preparations, with Relative Standard Deviations (RSDs) of 2.46%–5.73%. Due to high suppression, this equates to mean total recoveries of 12.8%–77.9%, with moderate RSD values (<20%). LODs were as follows: EMPA=350pg/g, IMPA=20pg/g, iBuMPA=7.5pg/g, CMPA=10pg/g, PMPA=5pg/g. Despite high suppression, these LODs are notably lower than most published for other biomatrices, facilitating detection of trace levels and potentially increasing tolerance for diagenetic loss.[5-9]

The proposed model of xenobiotic incorporation and the validated method were tested using femora from 12 mini pigs exposed percutaneously (high-dose exposure; *n*=6) or intramuscularly (low-dose exposure; *n*=6) to VX *in vivo*. EMPA was not detected in intramuscularly exposed animals. EMPA was detected in 6/6 trabecular samples and 5/6 cortical samples from percutaneously exposed mini pigs; concentrations were highest in animals that died due to their exposure. This is believed to be the first time *in vivo* nerve agent exposure has been detected from bone. Further, detected concentrations and diaphyseal-to-epiphyseal concentration ratios reflected animal exposure history (e.g., dosage level/route and post-exposure survival duration). The results are limited but promising, indicating NAMs interact with bone as a pharmacokinetic compartment and can be extracted from bone postmortem. Importantly, the results suggest toxicological testing of human skeletal remains from mass graves is warranted, although additional studies are needed to maximize testing efficiency.

Further, this research expands the foundation of skeletal toxicology broadly by providing a model for xenobiotic incorporation into bone and specifically by demonstrating a total mineral dissolution sample preparation method with the potential to increase extraction efficiency and lower LODs for multiple drug classes and other analytes. These advances may promote discussions between forensic toxicologists and forensic anthropologists and the resolution of overdose deaths.

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