K7 A Suspected Case of Attempted Homicide by Rodenticides Administration: How Hair Analysis Can Help Us in Solving the Mystery

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Learning Overview: After attending this presentation, attendees will recognize the importance of applying segmental hair analysis in some cases of suspected intoxication.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by highlighting how forensic hair analysis could be pivotal for the chronology of exposure to toxins.

A 72-year-old man presented to the local hospital with hematuria and abdominal pain. Abnormal coagulation parameters (Prothrombin Time-International Normalized Ratio [PT-INR] between 16.14 and 19.07) were measured. During hospitalization, his PT-INR result was hard to stabilize, ranging from 2.72 to 12.18, despite numerous vitamin K infusions. Eventually, his blood tests resulted positive for anticoagulant rodenticides, so the case was reported to the public prosecutor's office. The man had been hospitalized for similar symptoms many times in the previous months. The patient's hair sample was taken 19 days after hospitalization. Analyses of blood, collected during hospitalization, hair, and material seized at the man's house were carried out.

Methods: 500µL blood samples were acidified with 1mL HCl (0.1M) and extracted using a Liquid-Liquid Extraction (LLE) procedure. Hair samples were extracted in 1mL methanol; then, the organic solvent was evaporated and reconstituted in 100µL methanol. Warfarin-D5 was used as an internal standard for both procedures. The seized material was diluted 1:10 with methanol and directly injected in the Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) system. A C18 column (100×2. mm i.d., 2.6µm particle size) was used in reversed phases, the mobile phases consisted of 0.1% formic acid in bidistilled water (phase A) and 0.1% formic acid in acetonitrile (phase B). The triple quadrupole was operated in Multiple Reaction Monitoring (MRM) negative mode. The following transitions were selected for identification and quantification: *m/z* -443.1/-135.0 and -443.1/-293.4 for difenacoum (declustering potential: -98V; collision energy: -43eV); *m/z* -541.4/-161.1 and -541.1/-117.1 for flocoumafen (declustering potential: -55V; collision energy: -40eV); *m/z* -312./-255.2 for warfarin-D5 (declustering potential: -55V; collision energy: -30eV).

Results and Discussion: Blood positivity was confirmed in all samples, with difenacoum at a concentration between 17.0 and 51.9ng/mL, while flocoumafen was found between 23.0 and 140.0ng/mL. In the proximal hair segment (1cm), difenacoum was detected in traces (Limit Of Quantitation [LOQ]=5pg/mg) while flocoumafen was measured at a concentration of 19.0pg/mg; in the intermediate segments (1-2 and 2-3cm), both difenacoum and flocoumafen were absent; in the distal segment (3-5cm), difenacoum was found in significant amounts (140.0pg/mg), while flocoumafen was lacking. Of all the seized material, only two contained rodenticides: a specimen similar to a red cereal (which contained difenacoum) and a number of blue pills (which contained flocoumafen) found at the man's house.

Conclusions: The presence of difenacoum and flocoumafen both in blood and in hair samples indicates that the man was poisoned with both of the molecules in the days immediately preceding his hospitalization, while there was a complete lack of exposure in the previous months. Yet, difenacoum was administered, probably multiple times, at least four months before hospitalization. Per research, there are few cases reported in literature about detection of rodenticides in keratin matrices; moreover, this case, for the first time, proved that segmental hair analysis of anticoagulant agents could represent important additional information for the interpretation of the case.

Rodenticides, Hair Testing, Intoxication

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